



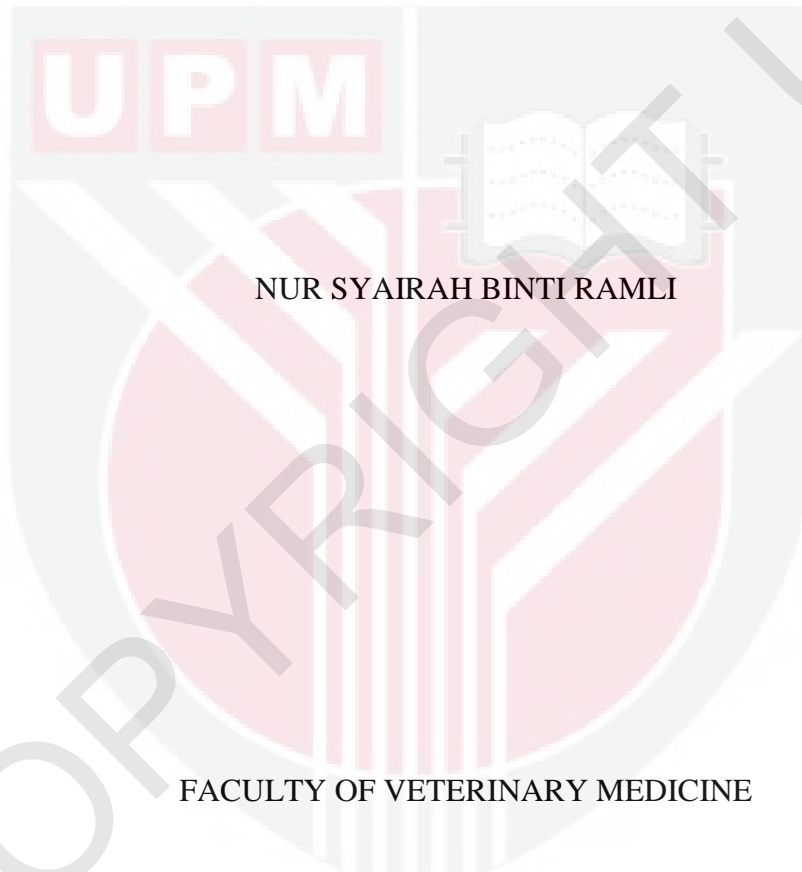
UNIVERSITI PUTRA MALAYSIA

***PREVALENCE RATE OF GASTROINTESTINAL NEMATODES AND
TOTAL WORM COUNT IN SHEEP***

NUR SYAIRAH BINTI RAMLI

**Ip
FPV 2015 56**

PREVALENCE RATE OF GASTROINTESTINAL NEMATODES AND TOTAL
WORM COUNT IN SHEEP



NUR SYAIRAH BINTI RAMLI

FACULTY OF VETERINARY MEDICINE

UNIVERSITI PUTRA MALAYSIA

SERDANG, SELANGOR

2015

PREVALENCE RATE OF GASTROINTESTINAL NEMATODES AND TOTAL
WORM COUNT IN SHEEP

NUR SYAIRAH BINTI RAMLI

A project paper submitted to the
Faculty of Veterinary Medicine, Universiti Putra Malaysia

In partial fulfillment of the requirement for the

DEGREE OF VETERINARY MEDICINE

Universiti Putra Malaysia

Serdang, Selangor Darul Ehsan

MARCH 2015

It is hereby certified that we read this project paper entitled “Prevalence Rate Of Gastrointestinal Nematodes And Total Worm Count In Sheep”, by Nur Syairah Binti Ramli and in our opinion it is satisfactory in terms of scope, quality, and presentation as partial fulfillment of the requirement for the course VPD 4999- Final Year Project.

DR. MURUGAIYAH MARIMUTHU

DVM (BOMBAY), MSC (UPM), PHD (UPM)

Faculty of Veterinary Medicine

Universiti Putra Malaysia

(Supervisor)

PROF. DR MOHAMMED ARIFF OMAR

BS (LSU), MS (OSU), PHD (Texas A&M)

Faculty of Veterinary Medicine

Universiti Putra Malaysia

(Co-supervisor)

DEDICATION

I would like to dedicate this project to

My source of inspiration and motivation

Ramli bin Abdul Rahman

Rosimah binti Kaharudin

My supporters and guidance

Prof Dr. Rehana Abdullah Sani

Mohamad Zamri bin Ahamd

Abdul Rashid Abdul Rahman

Maizatul Akmal Mokhtar

Norisal Nasai

My sweet and bitter

Mohammad Noorul Syazwan bin Yaacob

My friends

Veterinarian

ACKNOWLEDGEMENT

First of all, I am grateful to The Almighty God for establishing me to complete my thesis. There are a number of people without whom this thesis might not have been written, and to whom I am greatly indebted.

Beautiful grace upon my awesome family for all their incomparable love and support. To my parents, who have been my source of encouragement and inspiration throughout my life and actively supported me to complete my project and thesis.

My supervisor Dr Murugaiyah Marimuthu and co-supervisor Prof Dr Ariff who continue to guide my career path with ingenuity and finesse. It is very comforting to know that I am in such good hands. I tremendously appreciate their contribution in polishing my work until it shines and all the support both of you gave me.

Not to forget, I wish to record my appreciation to staff of Parasitology Laboratory Fakulti Perubatan Veterinar, Maizatul and Rashid as well as owner of Ladang Tok 4 Agus Salak Tinggi, and Zamri for his cooperation during the study.

Lastly, very special thanks to my love one, Mohd Noorul Syazwan Yaacob for his practical and emotional support as well as his encouragement along the way.

Alhamdulillah.

CONTENTS	PAGE
TITLE	I
CERTIFICATION	II
DEDICATION	III
ACKNOWLEDGEMENT	IV
CONTENTS	V
LIST OF TABLES	VII
LIST OF FIGURES	VIII
ABSTRAK	IX
ABSTRACT	XI
CHAPTER	
1.0 INTRODUCTION	
1.1 Justification	2
1.2 Study Objectives	2
2.0 LITERATURE REVIEW	
2.1 Nematode Infection in Small Ruminants	2

2.2	Major Nematode species in Sheep	4
2.3	Assessment of GIN Burden in Sheep	7
3.0 MATERIALS AND METHODS		
3.1	Sample Collection	10
3.2	Faecal Egg Count	10
3.3	FAMACHA Score	11
3.4	Total Worm Count	11
3.5	Statistical analysis	12
4.0	RESULTS	13
5.0	DISCUSSION	19
6.0	CONCLUSION	24
7.0	RECOMMENDATION	24
8.0	REFERENCES	25

LIST OF TABLES

TABLES	PAGE
Table 1: Prevalence of GIN eggs measured as EPG in two breeds of sheep	12
Table 2: Prevalence of GIN eggs measured as EPG in two age groups of sheep	12
Table 3: Correlation among EPG, FAMACHA score (FS)..... and severity of infection in sheep	14
Table 4: Correlation of TWC with EPG and FAMACHA..... score in 5 sheep	15

LIST OF FIGURES

FIGURES	PAGE
Figure 1: Prevalence rate of GIN based on the classification.....	13
of severity of infection in sheep	
Figure 2: Prevalence rate of GIN based on TWC.....	14
Figure 3: A scatter plot of TWC (<i>Haemonchus</i>) against EPG.....	16
Figure 4: A scatter plot of TWC (<i>Trichostrongylus</i>) against EPG	16

ABSTRAK

Abstrak kertas projek yang dikemukakan kepada Fakulti Perubatan Veterinar dalam memenuhi sebahagian keperluan bagi kursus VPD 4999- Projek Tahun Akhir

PREVALENS GASTROUSUS NEMATOD DAN JUMLAH BILANGAN CACING DALAM BIRI-BIRI

Oleh

Nur Syairah Binti Ramli

2015

Penyelia: Dr Murugaiyah Marimuthu

Prevalens parasit nematod gastrousus telah dikajikan dalam 50 ekor biri-biri yang ditenak di sebuah ladang pekebun kecil di Salak Tinggi, Selangor. Biri-biri terdiri daripada 27 kacukan Damara dan 23 kacukan Barbados Blackbelly yang dibahagikan kepada dua kumpulan umur: muda dan dewasa. Sampel najis telah diperiksa untuk kiraan telur nematod strongyle sebagai jumlah telur se gram najis (EPG) menggunakan teknik McMaster yang telah dimodifikasi. Tahap infestasi telah dikategorikan kepada ringan, sederhana dan berat berasaskan EPG (SI). Lima ekor biri-biri disembelih secara rawak untuk mengenal pasti nematod gastrousus dewasa dengan kaedah jumlah bilangan cacing (TWC). Skor FAMACHA (FS) telah digunakan untuk menentukan beban cacing

berdasarkan kepada anemia di kalangan haiwan ini. Kajian ini mendedahkan kadar prevalens EPG adalah 88%, di mana 84.1% daripada biri-biri tersebut adalah jangkitan ringan. Bilangan kacukan Barbados Blackbelly menunjukkan perbezaan yang signifikan ($p = 0.002$) untuk dijangkiti bagi EPG berbanding kacukan Damara. Terdapat perbezaan yang signifikan ($p = 0.004$) bagi EPG di kalangan kumpulan umur bagi kacukan Barbados Blackbelly tetapi tidak dalam kacukan Damara ($p=0.941$). Korelasi antara SI dan FS adalah signifikan ($r = 0.289$; $p = 0.042$). Berdasarkan TWC, *Haemonchus* sp adalah nematod yang paling dominan diikuti dengan *Trichostrongylus spp.* dan *Oesophagostomum spp.*. Korelasi antara EPG dan TWC bagi *Haemonchus* adalah positif ($r=0.854$; $p=0.066$) tetapi kolerasi tersebut adalah tidak signifikan. Berdasarkan analisis regresi, 73% daripada kebolehubahan dalam TWC bagi *Haemonchus* dapat dijelaskan oleh EPG. Oleh itu, kesimpulannya terdapat hubung kait yang baik antara skor FAMACHA dengan tahap infestasi nematod dan skor FAMACHA boleh digunakan untuk menilai beban nematod strongyle dalam biri-biri.

Kata Kunci: *Prevalens, nematod gastrousus, FAMACHA, EPG*

ABSTRACT

An abstract of the project paper presented to the Faculty of Veterinary Medicine in partial fulfillment of requirement of the course VPD 4999- Final Year Project

PREVALENCE RATE OF GASTROINTESTINAL NEMATODES AND TOTAL WORM COUNT IN SHEEP

By

Nur Syairah Binti Ramli

2015

Supervisor: Dr Murugaiyah Marimuthu

The prevalence of gastrointestinal (GI) nematode parasites was determined in 50 sheep raised in a smallholder farm in Salak Tinggi, Selangor. The sheep comprised of 27 Damara crosses and 23 Barbados Blackbelly crosses grouped into two age groups: young and adult. Faecal samples were examined for strongyle nematode egg count as egg per gram (EPG) using modified McMaster technique. Severity of infection was categorized into mild, moderate and heavy based on EPG. Five sheep were randomly slaughtered for adult GI nematodes identification by total worm count (TWC) method. FAMACHA score was used for investigation of worm load based on anaemic condition. The study revealed the prevalence rate of EPG was 88%, of which 84.1% of the sheep had mild infection. Barbados Blackbelly crosses were significantly different ($p=0.002$)

in EPG prevalence rate compared to Damara crosses. There was significant difference ($p=0.004$) in EPG between age groups for Barbados Blackbelly crosses but not for Damara crosses ($p=0.941$). Correlation between severity of infection and FAMACHA score was significant ($r=0.289$; $p=0.042$). From TWC, *Haemonchus* was the most predominant nematode followed by *Trichostrongylus* and *Oesophagostomum*. EPG and TWC for *Haemonchus* was positively correlated but not significant ($r=0.85$, $p=0.066$). From regression analysis, 73% of the variability in TWC for *Haemonchus* could be explained by EPG. Thus it can be concluded that FAMACHA score correlates well with severity of infection of nematode and can be used to assess the strongyle nematode burden in sheep.

Keywords: *Prevalence, gastrointestinal nematode, FAMACHA, EPG*

1.0 INTRODUCTION

Malaysia has a hot and humid climate that favors for parasite population. Climatic, geographical and farm management variation endorse marked differences in the helminth population (Domke *et al.*, 2013). Besides, outbreaks are most severe in warm and humid climates (Gadahi *et al.*, 2009). Gastrointestinal nematode (GIN) prevalence is influenced by farm management and climatic conditions such as quantity and quality of pasture, temperature, humidity and grazing behavior of the host (Pal and Qayyum, 1993).

GIN is actually a potential constraint on small ruminant productivity in terms of economic losses by deaths, decline in meat and milk production and diminished fertility (Vanessa *et al.*, 2014). Alternative steps are essential to control measures of local epidemiology and unnecessary drug treatments that cause detrimental effect like increased development of anthelmintic resistance that leaves residues in the meat and milk, as well as in the environment.

Among the parasitic diseases, endoparasites are of greatest importance in sheep and goats (Gadahi *et al.*, 2009). In Malaysia, *Haemonchus contortus* and *Trichostrongylus spp.* were reported to be the most prevalent and highly pathogenic endoparasite in livestock, particularly in small ruminants (Tan *et al.*, 2014). According to Rahman *et al.*, (1992), *Haemonchus spp.*, *Ostertagia spp.*, and *Trichostrongylus spp.* are the dominant species of nematodes commonly found in tropical goats. The pathogenicity of *H. contortus* is undeniable in livestock as its biotic potential and blood

sucking ability. The most prevalent GIN in Malaysia is similar to Brazil, which is *Haemonchus* sp (80.1%), followed by *Trichostrongylus* sp (13.2%) and *Oesophagostomum* sp (6.7%) (Vanessa *et al.*, 2014). In Malaysia, ova detection is normally performed by a floatation principle and observation under a light microscope (Tan *et al.*, 2014).

1.1 Justification

Current status of GIN prevalence in sheep in intensive smallholder farms is not widely studied. Furthermore, sub-clinical and clinical infections can lead to increase mortality and reduced fertility leading to a decline in meat and milk production.

1.2 Study Objective

The aim of the present study was to determine the prevalence of gastrointestinal nematodes (GIN) in non-grazing Damara and Barbados Blackbelly sheep at a Salak Tinggi smallholder farm.

2.0 LITERATURE REVIEW

2.1 Nematode infection in small ruminants

Infection with gastrointestinal nematodes (GIN) can have a detrimental effect on animal health under extensive and intensive livestock rearing. In Malaysia, haemonchosis is one of the major disease problems affecting sheep production (Sani *et al.*, 1995). Dorny *et al.* (1995) reported *Haemonchus contortus* and *Trichostrongylus*

spp. were the most commonly found GIN in small ruminants in Malaysia. There are various options to control GIN in small ruminants namely a combination of rotational grazing management and anthelmintic prophylaxis. This is the common way as a control measure throughout the world. Nevertheless, rotational grazing management is often impractical due to limited land area for pasture in Malaysia. This situation has led to the rapid development of resistance of GIN to various anthelmintics in livestock animals and also the practice of unnecessary drenching. All these factors have an increasing concern in the world (Domke *et al.*, 2013). Strongyle parasite has the ability to produce large numbers of eggs and survives in adverse climatic conditions through hypobiosis which causes extensive pasture contamination (Awizer *et al.*, 2014).

Both adults and young sheep can be infected with GIN. Furthermore, acute infection can cause sudden death. The main signs for production are weight loss in lambs due to diarrhea and reduced food conversion efficiency (FCE). Haemonchosis causes variable degrees of anaemia due to blood-sucking nature of the nematodes (Awizer *et al.*, 2014). Awizer *et al.* (2014) reported that GIN can cause loss of production and mortality in lambs and kids in situations of poor farm management.

Infection with GIN is widespread in tropical and subtropical areas in the world and this is associated with large economic losses (Vanessa *et al.*, 2014). Infected lactating ewes can cause significantly reduced milk production by *Haemonchus*. The most profound effect of parasitism is sub-clinical production loss in which the farmer is unlikely to be aware (Tan *et al.*, 2014). Reduction in live weight gain in sheep infected

with strongyle parasite was reported by Abbot *et al.* (1986). Poor carcass quality and wool, weight loss and lowered milk production are evident of subclinical infections with GIN (Nansen, 1986).

2.2 Major nematode species in sheep

The lifecycle of a nematode consists of four stages from egg to adult worm. Eggs are hatched in faeces of livestock animals. Third larval stage (L3) is most infective but does not cause harm to animals. With conditions of optimum humidity or moisture existed, L3 moves on to grasses and other herbage. L3 exsheathes and burrows into the gut wall when grass is infected with L3 and eaten by the sheep. Exsheathed L3, L4 and adults can contribute to general disease. L3 can survive for weeks to months on pasture without host.

Studies have been done on the prevalence of gastrointestinal (GI) strongyles in livestock animals in many countries. Rahman and Adanan (1992) reported the prevalence of worm egg counts and their nematode species in goats in Penang, Malaysia, in which their egg counts were generally moderately heavy, ranging from 500 to 2000 eggs per gram (epg). Common strongyles seen were *Cooperia spp.*, *Haemonchus spp.*, *Oesophagostomun spp.*, *Strongyloides spp.*, and *Trichostrongylus spp.*, of which *Haemonchus spp.* were predominant.

Gadahi *et al.* (2009) stated a prevalence rate of 63.50% for endoparasites in sheep and goats in Rawalpindi and Islamabad, Pakistan. Among the samples from

sheep, *Trichuris*, *Haemonchus*, *Coccidia*, *Nematodirus* and *Fasciola* were found with prevalence rates of 40.00, 28.88, 27.77, 11.11 and 4.44%, respectively.

Domke *et al.* (2013) did a survey on GIN in Norway and showed that there was a higher mean excretion of *Trichostrongyle* eggs in sheep than in goats at the individual level. *Trichostrongylus*, *Haemonchus* and *Nematodirus* of third stage larvae were reported as the most prevalent ones in the coprocultures from sheep, whereas larvae of *Trichostrongylus* and *Nematodirus* type dominated in goats. However, the most prevalent gastrointestinal nematode species found at necropsy were *Teladorsagia circumcincta*, *Haemonchus contortus* and *T. circumcincta* causing the highest worm burden in sheep and goats.

According to Getachew *et al.* (2007) use of various parasite control methods such as grazing management, use of biological agents, vaccines and selection of resistant animals, with or without moderate use of anthelmintics to control haemonchosis in sheep enables the enhancement of productivity and minimization of risks regarding drug resistance and consumer as well as environmental concerns.

Haemonchus spp. is among the most pathogenic helminth species of ruminants according to Love and Hutchinson (2003), and *Haemonchus contortus* is mainly a parasite of sheep and goats. Female worms are 18-30 mm long and 'barbers pole' in appearance of the white ovaries and uteri twisting structure around a red blood-filled intestine are easily recognized. Meanwhile, male worms are 10-20 mm long and uniformly reddish-brown. The cervical papillae are prominent and spine-like.

Both larval (L4) and adult worms have sharp lancet structures that pierce the mucosa, causing pettichia haemorrhage at sites of feeding on the abomasal mucosa which may be oedematous. According to Abbott *et al.* (2009) each adult worm is capable of removing about 0.05 ml of blood per day.

Trichostrongylus colubriformis and *T. vitrinus* live in small intestines occurring commonly in sheep. *Trichostrongylus axei* live in the abomasum. Intestinal *Trichostrongylus spp.* are small, slender, pale reddish brown worms without specially-developed head end (females 6-8 mm and males 6-7 mm long), and not readily seen at necropsy. The excretory pore is usually situated in a ventral notch near the anterior extremity. There is no buccal capsule. The common clinical signs are inappetence, diarrhea, weight loss and scouring. Mild infection causes poor growth rates, occasionally accompanied by soft faeces. Chronic infection of *T. colubriformis* is accompanied by reduced food conversion efficiency (FCE).

Oesophagostomum columbianum (nodular worm) and *O. venulosum* (large bowel worm) occur in sheep and goats. The adult worms have cylindrical buccal capsule, usually narrow and presence of leaf-crown structure. *O. columbianum* causes caseous nodules 0.5 – 1 cm diameter in small intestines and colon. Histologically, the nodules show eosinophilic granulomata. Clinical signs in heavy infection include variable diarrhea, emaciation, a humped back appearance, stiff gait and the tail is often held at angle from body.

2.3 Assessment of GIN burden in sheep

FAMACHA scores

FAMACHA is a practical on-farm system designed for small ruminant producers as a tool to improve the management of *Haemonchus contortus* infection. The system is based on a colour chart with five colour categories representing varying degrees of anaemia that are compared with the colour of the ocular membranes of sheep. A score of “3” represents a borderline value for anaemia, whereas the eye score values for anaemic animals are considered in the categories of 4 and 5. According to Burke *et al.* (2007), FAMACHA technique is a valuable tool for identifying anaemic sheep and goats in which the correlation between FAMACHA scores and pack cell volume (PCV) or faecal egg count (FEC) was high for sheep. It has high specificity, but sensitivity was low about 50% for sheep. The percentage of false negatives was less than 5% in sheep.

According to Loria *et al.* (2009), FAMACHA system showed a low sensitivity in detecting anaemic sheep and acted as an additional element that could be integrated with clinical examination in which the correlation between FAMACHA score with haemoglobin (Hb) and haematocrit (Hct) was significant and negative. Vatta *et al.* (2001) reported the method was applied in goats farmed under resource-poor conditions in South Africa and a test sensitivity of 85% and specificity of 55% were found.

Modified McMaster for detection of worm eggs count

Modified McMaster technique was developed for most nematode eggs and coccidia oocysts. It is used to estimate faecal egg count (FEC). The technique allows strongyle eggs to be floated up for counting with minimal debris since most of the debris will sink and remain out of focus. However, light infections may be missed since only small quantity of faeces is used. According to Rinaldi *et al.* (2009) FEC is the most common approach for the estimation of prevalence and intensity of these parasites. The study by Rinaldi *et al.* (2009) showed that the FEC was not affected by the time of sample collection but FEC was highly correlated with total GI strongyle worm burden in goats, especially *Haemonchus spp.* (Rinaldi *et al.* 2009).

Total Worm Count (TWC)

Total worm count (TWC) provides a more precise estimation of parasite burden than FEC. The technique involves the detection of adult and larvae of GIN present in the intestinal tract from abomasum to rectum of slaughtered animals. The adult and larval GIN are carefully washed out, counted and identified. Usually, varying number of parasites will be found in almost every grazing animal, regardless of the health state. According to McKenna (2008), processing only the first 10 m of small intestine is likely to result in a serious underestimation of the total number of worms present. Cutting, opening and rinsing of the GI tract is unnecessary if it had already been washed out by introducing water into it. Portions of the total number of both 5th and late 4th stage worms recovered using a 250 mm aperture sieve is generally high, thus a saving in time.

Standardization of techniques is required to allow accurate comparisons between laboratories via aliquot usage, in which assuming the worms are uniformly distributed in the whole volume of each subsample (Gaba *et al.*, 2006). Furthermore, the study reported usage of washings alone would allow a faster and a suitable estimation of the total worm burden for all strongyle species of the abomasum in both ewes and lambs.

According to Amarante (2000), the correlation between FEC and TWC was highly significant for *H. contortus* and there was a reduction in the sensitivity of FEC to predict the number of GIN in animals. However, it seemed to be adequate with FEC if the aim was to monitor parasitological status of the herd. According to Roberts and Swan (1981), the high relationship between FEC and total number of adult *H. contortus* is valuable for implementation of program for the diagnosis and control of haemonchosis in sheep flocks.

3.0 MATERIALS AND METHODS

3.1 Sample Collection

This study was conducted at a smallholder farm, Ladang Tok 4 Agus, Salak Tinggi, Selangor. The sheep were managed under an intensive system in which cut and carry system of Napier grass from communal and own pasture plots was practiced. Total number of sheep used in the present study was 50 comprising of 27 Damara crosses and 23 Barbados Blackbelly (BBB) crosses. The sheep were subsequently separated into two groups according to their age: young (less than 12 months) and adult (more than 12 months old). The sheep were randomly selected from several pens. Deworming program was not practiced in this farm. The sampling period was for two weeks in January 2015. The animals had not been treated against GIN for at least six weeks prior to sampling.

3.2 Faecal Egg Count (FEC)

Samples of faeces from individual animals were taken directly from rectum using gloves. These gloves were labeled prior to sampling. Defecation was stimulated by gentle massage of rectal wall with the index finger. These samples were kept in icebox immediately after sampling to prevent egg development and hatching. The faecal egg count was determined by a modified McMaster technique using saturated salt solution as flotation fluid to quantify strongyle nematode ova.

Assessment of the severity of GIN infection was made on 50 sheep based on the level of EPG using McMaster egg counting technique following the classification,

according to Chagas *et al.*, (2008): mild infection (EPG<500), moderate infection (EPG=500–1,500), and heavy infection (EPG>1,501).

3.3 FAMACHA system

The color of ocular membranes was classified into one of five categories according to FAMACHA eye color chart (Loria *et al.*, 2009): 1 = red, non-anaemic; 2 = red-pink, non-anaemic; 3 = pink, mildly anaemic; 4 = pink-white, anaemic; 5 = white, severely anaemic. FAMACHA score is a field diagnostic method used for rough indication of the presence of moderate to severe worm load. The FAMACHA score was recorded for the 50 sheep.

3.4 Total Worm Count (TWC)

Five adult lambs were slaughtered randomly by the farmer at the farm and adults GIN were examined. The animals were selected from the same flock as those sampled for FEC.

Upon slaughter, the abomasum, small intestine, and large intestine were ligated and removed soon from each animal and processed for worm recovery. Each organ was then opened longitudinally and the contents were washed with tap water thoroughly from mucosa and passed through a 100 mesh sieve. Subsequently, TWC from each sample was counted by species. The worms from each organ were collected and later examined by microscopy and identified according to their morphology (Jorgen and Brian, 1994).

3.5 Statistical Analysis

Data on FAMACHA scores, EPG and TWC were checked for normality distribution. Appropriate T-test or Mann–Whitney test was applied to compare differences between breed and age groups. Correlations between FAMACHA scores, EPG and TWC were performed using Pearson's or Spearman's correlation. All tests were performed with 5% significance level using SPSS 20.0 software for Windows.

4.0 RESULTS

The prevalence rate of EPG from total sample was 88% positive with GIN infection. The result was high compared to a previous study that showed intensive management to be lower between 14 to 72% (Nowosad *et al.*, 2000). This could be due to communal pasture system that allows other sheep to graze at the same pasture and cause the spread of the GIN infection. The prevalence of strongyle eggs in Damara crosses and Barbados Blackbelly crosses were 40.7% and 47.8%, respectively (Table 1). The breed factor was significantly different ($p=0.002$), where Barbados Blackbelly crosses had significantly higher prevalence of GIN infection than Damara crosses. For age category, there was significant difference between age groups ($p=0.004$) in EPG for Barbados Blackbelly crosses but not for Damara crosses ($p=0.941$; Table 2).

Table 1: Prevalence of GIN eggs measured as EPG in two breeds of sheep

Breed	No. of animals examined	No. of positive animals	Prevalence (%)	EPG (Mean \pm SE)
Damara crosses	27	22	40.7	305.56 \pm 144.838
Barbados Blackbelly crosses	23	22	47.8	310.87 \pm 47.357

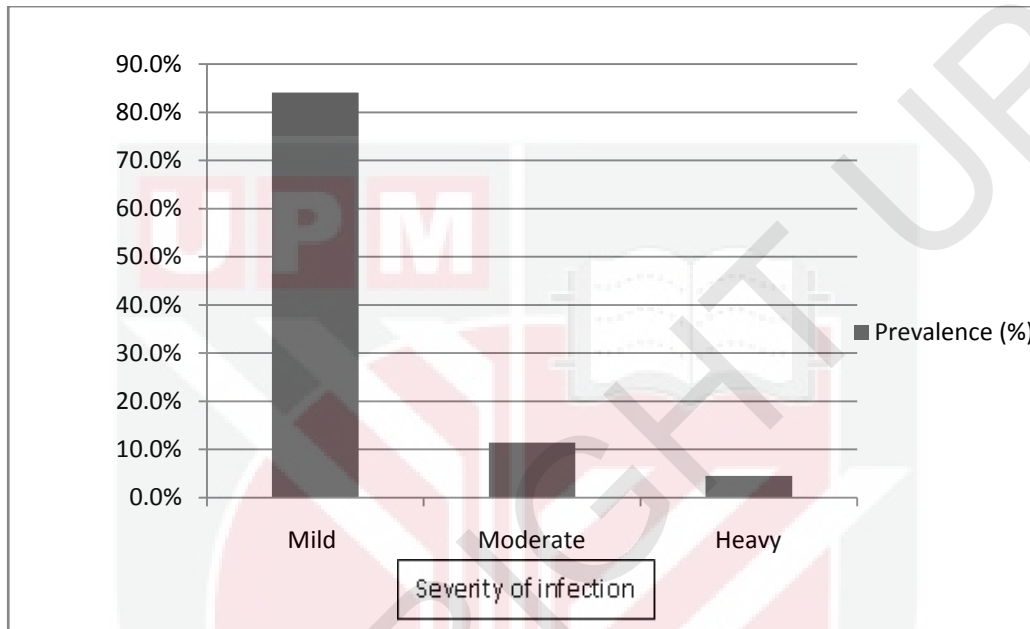
$p = 0.002$

Table 2: Prevalence of GIN eggs measured as EPG in two age groups of sheep

Breed	Age	No of animals examined	No. of positive animals	EPG (Mean \pm SE)	Prevalence (%)
Damara crosses	Young	12	10	341.67 \pm 242.92	22.5
	Adult	15	12	276.67 \pm 181.39	21.6
				p=0.941	
BBB crosses	Young	14	14	185.71 \pm 24.26	23.0
	Adult	9	8	505.56 \pm 80.56	20.4
				p=0.004	

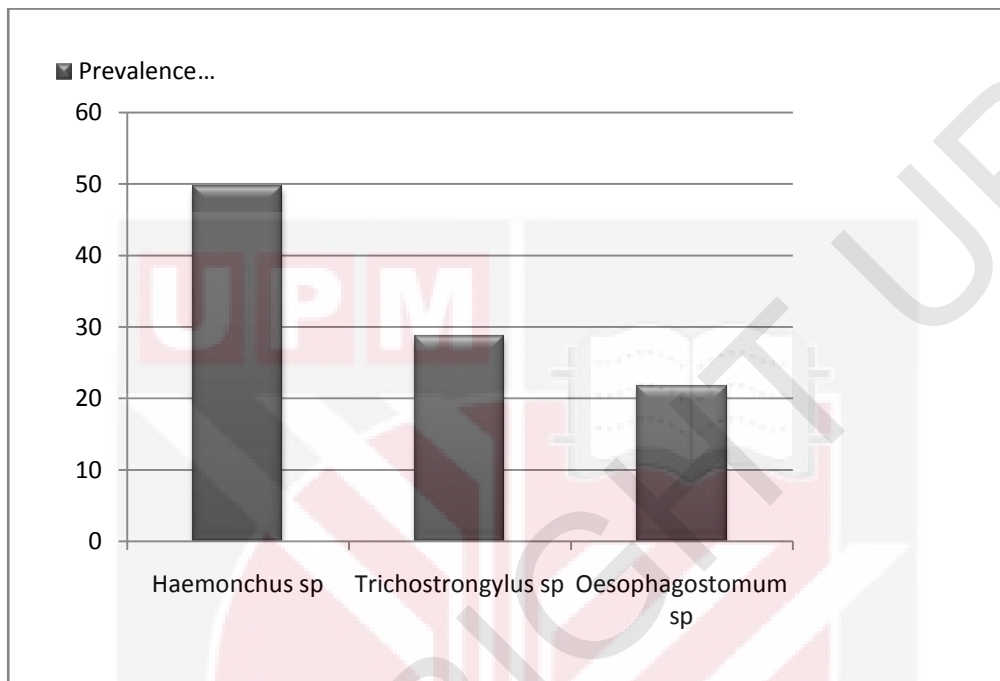
From the EPG analysis, there were 84.1%, 11.4%, 4.5% which could be categorized as mild, moderate and heavy infected, respectively (Figure 1).

Figure 1: Prevalence rate of GIN based on the classification of severity of infection in sheep



The prevalence of specific GIN based on total worm count (TWC) in five sheep slaughtered was 49.6% for *Haemonchus* sp, 28.7% for *Trichostrongylus* sp, 21.7% for *Oesophagostomum* sp (Figure 2).

Figure 2: Prevalence of GIN based on TWC



Correlation between EPG and FS was not significant ($r=0.237$; $p=0.097$; Table 3). This means increase of anaemia, would not necessarily result in increased EPG. Severity of infection was significantly correlated with FAMACHA score ($r=0.289$; $p = 0.042$).

Table 3: Correlation coefficients among EPG, FAMACHA score (FS) and severity of infection in sheep

Parameter	FAMACHA score	Severity of infection
EPG	0.237 ($p=0.097$)	0.776 ($p=0.000$)
FAMACHA score		0.289 ($p=0.042$)

The correlations between TWC and EPG for all nematode species were positive but not significantly different, in which strong relationship particularly for *Haemonchus* TWC with EPG was observed (Table 4). The correlations between TWC and FS for all nematode species were negatively correlated but not significantly different. From the regression analysis, 73% of the variability in TWC for *Haemonchus* could be explained by EPG. Scatter plots of TWC of *Haemonchus* and *Trichostrongylus* against EPG revealed higher relationship between TWC and EPG (Figures 3 and 4).

Table 4: Correlation coefficients of TWC with EPG and FAMACHA score in 5 sheep.

Parameter	GIT nematode species	Correlation coefficient, r	Significance value, p
EPG & TWC	<i>Haemonchus</i>	0.854	0.066
	<i>Trichostrongylus</i>	0.723	0.168
	<i>Oesophagostomum</i>	0.036	0.954
FS & TWC	<i>Haemonchus</i>	-0.164	0.792
	<i>Trichostrongylus</i>	-0.080	0.899
	<i>Oesophagostomum</i>	-0.707	0.182

Figure 3: A scatter plot of TWC (*Haemonchus*) against EPG

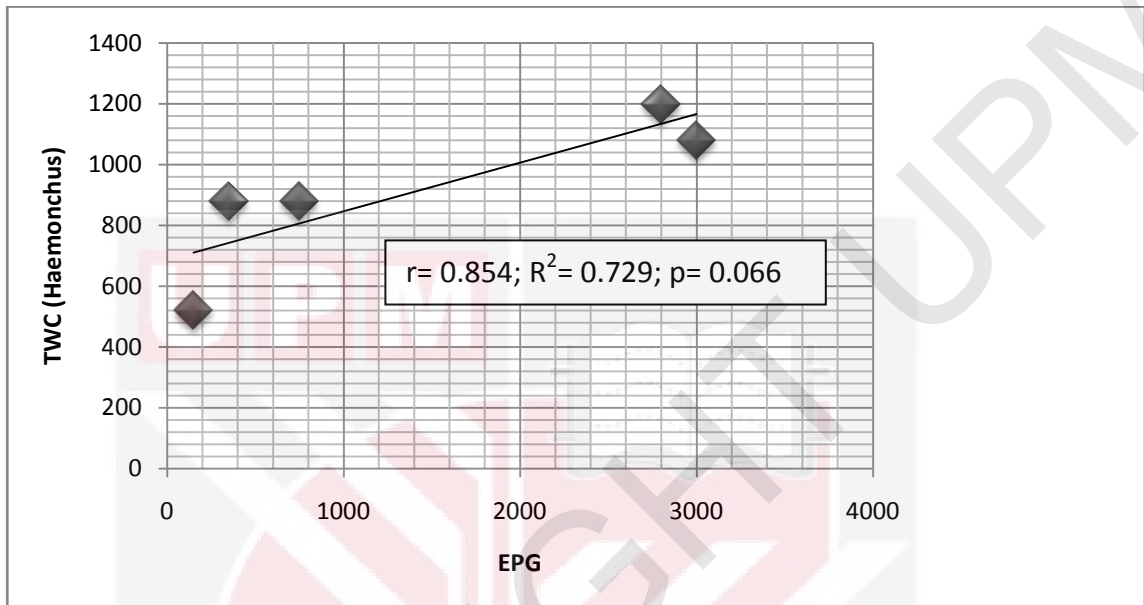
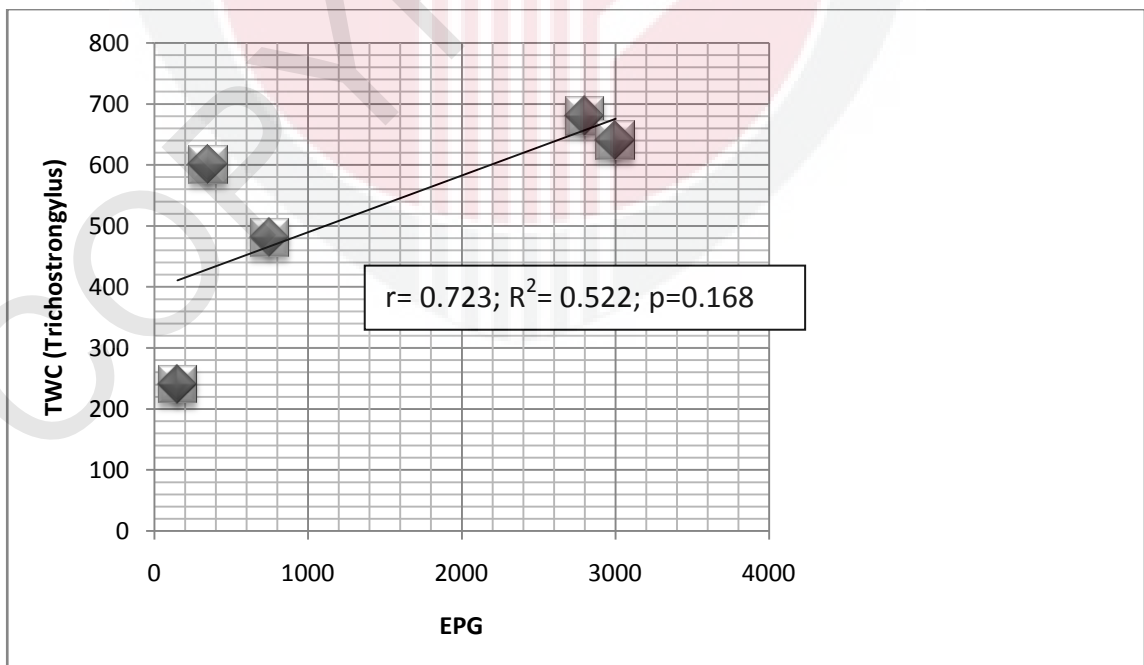


Figure 4: A scatter plot of TWC (*Trichostrongylus*) against EPG



5.0 DISCUSSION

The result showed 88% of total number of sheep sampled was positive for gastrointestinal nematode infection. Among the sheep tested positive, 84.1% had light infection to GIN. This could be attributed to the type of management practiced which involved communal pasture that also allowed other herds to graze on the same pasture possibly causing the spread of GIN infection (Nowosad *et al.*, 2000). Since all sheep were kept under intensive management system with zero grazing, there was less chance of infection from the ground. Wales (2005) suggested for better helminth control, more management of sheep should include intensive methods and alternatives should be explored in the pursuit of environmental friendly parasite control with minimal or no chemical dewormer usage.

In the present study, the sheep flock had mild infection of GIN. This could be due to lower stocking rates observed at the time of faecal collection. Thus less competition among the sheep leading to higher feed intake consequently lowers FEC (Dobson *et al.*, 1990). The non-grazing management practiced in this farm facilitated in reducing the worm load because a study had shown that sheep had grazing habit in which they tended to graze closer to the ground, thus promoting exposure to parasites (Zeryehun, 2012). The EPG of the sheep in present study was still mild infection, the reason could be due to strategic feeding, where the feed was given to the animals at noon. The hot day temperature would have killed the larvae because humidity and temperature influence their survival rate (Wales, 2005; Zeryehun, 2012; Rahman and

Adanan, 1992). Besides, constant removal of excrement from the floors of the pens contributed to the reduction in GIN infection. This is also important in controlling the parasite in the farm itself (Rahman and Adanan, 1992).

The number of Barbados Blackbelly crosses infected with GIN was higher than in Damara crosses. This finding was in agreement with other studies that stated Barbados Blackbelly sheep were reported to have natural *Trichostrongyle* infection (Zeryehun, 2012; Zajac, 1995). However, in contrast, this breed was more resistant to infection (Chandrawathani, 2004). Another reason for this situation can be attributed to nutritional stress. Lamy *et al.* (2009) reported Damara crosses had high level of adaptation to nutritional stress as corroborates by the present study that showed Damara crosses had lower GIN infection.

Generally, younger sheep were more susceptible to strongyle infection than mature animals (Getachew *et al.*, 2007; Peter and Stig, 2004; Wales, 2005; Zeryehun, 2012). It is believed that the lower resistance to disease in young ruminants is partly due to immunological immaturity, and ewes which are not exposed sufficiently to pathogens to develop immunity. However, in the present study, there was no significant relationship between age group and EPG in Damara crosses. BBB lambs were more susceptible to GIN infection as the EPG was higher compared to other adult group. This finding is similar to that of Zeryehun (2012), who reported that lambs up to six months of age were unable to mount vigorous immune response to GIN infection. Moreover, sheep offered ample amount of pasture were able to increase the nutritional plane and

accordingly increase the immunity of the animals, thereby reducing the fecundity which prevents the rise in EPG (Zeryehun, 2012). On other hand, if the animals were exposed previously to infection, this leads to alterations of immunological and physicochemical mechanisms will be occurred. So, there will be an enhancement of resistance to subsequent infection even though the animals were incapable of controlling the primary infection and this has been studied in several breeds such as Dorset, St. Croix and Black Belly (Getachew *et al.*, 2007). In contrast, Vanessa *et al.* (2014) reported older goats were five times more likely to develop GIN infection when compared to younger animals.

The FAMACHA scores showed that an increase in anaemia would not necessarily be due to increase EPG as the relationship was low. The present study showed no significantly correlation between FS and EPG and this could be due to mild infection. Sheep did not suffer phenotypic changes due to GIN infection and this could be due to the low EPG of less than 500 EPG (Vanessa *et al.*, 2014). Moreover, Vilela *et al.* (2008) reported that some goats had no signs of anaemia even though EPG was greater than 1,300. However, Burke *et al.* (2007) reported that the relationship of FS and EPG was significantly correlated. This is because GIN predominantly infected by *Haemonchus* sp, which sucks blood from the mucosa of GIT causing blood loss and development of anaemia in sheep (Love and Hutchinson, 2003). Thus, more blood loss would occur if the animal was heavily infected with this worm. Eye scores of four or greater was considered as anaemic. However, in this study, PCV value was not obtained to confirm the anaemic state as in other studies where they compared the FS with PCV,

haemoglobin and haematocrit value (Kaplan *et al.*, 2004; Burke *et al.*, 2007; Vatta *et al.*, 2001; Loria *et al.*, 2009). FAMACHA is one of the alternatives for the detection of GIN as a reduction of 75.6% in the treatment costs was observed, with no death reported by Vanessa *et al.* (2014).

Necropsy examinations confirmed the highest prevalence of the very prolific worm *Haemonchus* in the present study. This finding corroborates with those of other studies that showed *Haemonchus* was the most prevalent nematode in small ruminants (Rahman and Adanan, 1992; Vanessa *et al.*, 2014; Tiong *et al.*, 2014; Zeryehun, 2012; Altaif and Issa, 1983; Gadahi, 1992; Rinaldi *et al.*, 2009). In contrast, Kumsa *et al.* (2011) reported a higher prevalence of *Trichostrongylus* sp. *Hemonchus* may acquire resistance faster due to its high biotic potential and very prolific thus showing highest prevalence in the study (Zeryehun, 2012). According to Getachew *et al.* (2007), a mature female of *Haemonchus* species can produce 5,000-7,000 eggs per day while *Trichostrongylus* species produce only 100-200 eggs per day which shows its prolific ability. *Oesophagostomum* sp has a very long generation interval of 45 days that causes its prevalence to be much lower (Rahman and Adanan, 1992).

EPG and TWC were positively correlated for all nematode species with highest relationship with *Haemonchus* sp. This relationship could be useful in predicting the level of GIN infection in sheep flocks. The result was in agreement with the findings of Roberts and Swan (1981), Rinaldi *et al.* (2009) and Amarante (2000) reported a positive relationship between FEC and total GIN burdened with highest relationship with

Haemonchus sp. This depends on various factors which include fecundity of species, age of worm, volume of ingesta and host resistance (Rinaldi *et al.*, 2009). Fecundity of species depends on life cycle and prolific ability of worms. Age of worm is influenced as the higher mix in the population between genders of adult worms leading to higher burden. Ingesta volume is a risk factor in which ample amount of pasture offered to sheep leads to increase of faecal output and consequently causing reducing number of worms. The factor of resistance level against GIN infection influences the composition of worm burden in sheep, where Amarante *et al.* (1999) found that worm burden in Rambouillet sheep was higher than Florida Native sheep even though they grazed in the same paddock and remained in the same flock.

There was no significant relationship between FS with EPG and TWC in this study. This present finding was in contrast to other studies that stated there was strong relationship between FS with EPG (Kaplan *et al.*, 2004; Burke *et al.*, 2007; Loria *et al.*, 2009). This is influenced by many factors such as evaluation of color of ocular mucosa, resistant breed, and genetic differences of *Haemonchus* sp in different populations, which lead to different adaptation subsequently different pathogenic effect (Loria *et al.*, 2009). Evaluation of color of ocular mucosa also varies with physiological status such as stress causing vasoconstriction. Local resistant breeds would also show less phenotypic expression such as anaemia.

6.0 CONCLUSION

The Salak Tinggi farm had high prevalence rate of gastrointestinal nematodes in sheep but was mostly mildly infected. Barbados Blackbelly had higher GIN infection compared to Damara sheep. *Haemonchus sp* was the most prevalent gastrointestinal nematode species identified. FAMACHA score can be used to assess the strongyle burden in sheep and faecal egg count was a good predictor of total worm count for *Haemonchus sp*.

7.0 RECOMMENDATION

Future studies should include more farms of different management systems which are intensive, semi-intensive, and extensive system. Possibilities of using abattoir data for measurements of total worm count and faecal egg count could give a better indicator.

8.0 REFERENCES

- Abott, K.A., Taylor, M., Stubbings, L.A. (2009). Sustainable worm control strategies for sheep, 3rd ed. *SCOPS Publishing*.
- Amarante, A.F.T. (2000). Relationship between faecal egg counts and total worm counts infected with gastrointestinal nematode. *J. Vet. Parasitol*, 9:1: 45-50.
- Burke, J.M., Kaplan, R.M., Miller, J.E., Terrill, T.H., Getz, W.R., Mobini, S., Valencia, E., Williams, M.J., Williamson, L.H., Vatta, A.F. (2007). Accuracy of the FAMACHA system for on-farm use by sheep and goat producers in the southeastern United States. *Vet. Parasitol*. 147: 89–95.
- Burke, J.M., Miller, J.E., Terrill, T.H. (2009). Impact of rotational grazing on management of gastrointestinal nematodes in weaned lambs. *Vet . Parasitol*. 163: 67-72.
- Calvetea, C., Calaviaa, R., Ferrerb, L.M., Ramosb, J.J., Lacastab, D. (2012). Management and environmental factors related to benzimidazole resistance in sheep nematodes in Northeast Spain. *Vet . Parasitol*. 184: 193–203.
- Chandrawathani, P. (2004). Problems in the control of nematode parasites of small ruminants in malaysia: resistance to anthelmintics and the biological control alternative. *Doctoral thesis. Swedish University of Agricultural Science*.

Domke, A.V.M., Chartierb, C., Gjerdec, B., Leined, N., Vatne, S., Stuen, S. (2013).

Prevalence of gastrointestinal helminths, lungworms and liver fluke in sheep and goats in Norway. *Vet. Parasitol.* 194: 40– 48.

Gaba, S., Chadoeuf, J., Monestiez, P., Sauve, C., Cortet, J., Cabaret, J. (2006).

Estimation of abomasum strongyle nematode infections in sheep at necropsy: Tentative proposals for a simplified technique. *Vet. Parasitol.* 140: 105–113.

Gadahi, J. A., Arshed, M.J., Ali, Q., Javaid, S. B., Shah, S.I. (2009). Prevalence of

gastrointestinal parasites of sheep and goat in and around Rawalpindi and Islamabad. *Veterinary World*,2(2): 51-53.

Getachew, T., Dorchies P., Jacquet P. (2007). Trends and challenges in the effective

and sustainable control of *Haemonchus contortus* infection in sheep. *Vet. Parasitol.* 14: 3-14.

Joshua, P.A., Sweenya, Robertsona, D., Ryana, U.M., Caroline J., Woodgate, R.G.

(2011). Comparison of molecular and McMaster microscopy techniques to confirm the presence of naturally acquired strongylid nematode infections in sheep. *Molecular & Biochemical Parasitol.* 180: 62– 67.

- Kaplan, R.M., Bur, J.M., Terrill, T.H., Miller, J.E., Getz, W.R., Mobini, S., Valencia, E., Williams, M.J., Williamson, L.H., Larsen, M., Vatta, A.F. (2004). Validation of the FAMACHA© eye color chart for detecting clinical anaemia in sheep and goats on farms in the southern United States. *Vet. Parasitol.* 123: 105–120.
- Loria, A.D, Veneziano, V., Piantedosi, D., Laura, R., Mezzino, L., Giuseppe C., Ciaramella, P. (2009). Evaluation of the FAMACHA system for detecting the severity of anaemia in sheep from southern Italy. *Vet. Parasitol.* 161: 53–59.
- Love, S.C.J and Hutchinson, G.W. (2003). Pathology and diagnosis of internal parasites in ruminants. In gross pathology of ruminants, Proceedings 350, *Post Graduate Foundation in Veterinary Science, University of Sydney, Sydney*; Chapter 16:309-338.
- McKenna, P.B. (2008). Comparison of two worm counting procedures for the enumeration of abomasal and small intestinal nematode parasites of sheep. *Vet. Parasitol.* 157: 254–259.
- Rahmann, G., Seip, H. (2006). Alternative strategies to prevent and control endoparasite diseases in organic sheep and goat farming. *Ressortforschung für den Ökologischen Landba.*

Rinaldia, L., Colesb, G.C., Maurellia, M.P., Musellac, V., Cringolia, G. (2011).

Calibration and diagnostic accuracy of simple flotation, McMaster and FLOTAC for parasite egg counts in sheep. *Vet. Parasitol.* 177: 345–352.

Rinaldi, L., Veneziano, V., Morgoglione, M.E., Pennacchio, S., Santaniello, M.,

Schioppi, M., Musella, V., Fedele, V., Cringoli, G. (2009). Is gastrointestinal strongyle faecal egg count influenced by hour of sample collection and worm burden in goats? *Vet. Parasitol.* 163: 81–86.

Roberts, J.L., Swan, R.A. (1981). Quantitative studies of ovine haemonchosis.

Relationship between faecal egg counts and total worm counts. *Vet. Parasitol.* 8: 165-171.

Peter, J.W., Stig M.T (2004). Nematode control in ‘green’ ruminant production

systems. *TRENDS in Parasitol.* Vol.20: 10.

Vanessa, D.V. , Feitosa, T.F, Vilela, V.L.R, Azevedo, S.S, Net, J.L.D.A, Dayana F.D.M,

Ana, R.C.R, Athayde, A.C.R (2014). Prevalence and risk factors associated with goat gastrointestinal helminthiasis in the Sertão region of Paraíba State, Brazil.

Trop Anim Health Prod. 46: 355–361.

Vatta, A.F., Letty, B.A., Linde, M.J., Wijk, E.F., Hansen, J.W., Krecek, R.C. (2001).

Testing for clinical anaemia caused by *Haemonchus spp.* in goats farmed under resource-poor conditions in South Africa using an eye colour chart developed for sheep. *Vet. Parasitol.* 99: 1–14.

Wahab A.R. and Adanan C.R. (1992). Observations on the worm egg counts and their nematode species in goats from the North-East district of Penang Island, Peninsular Malaysia. *Pertanika* 15(3): 221-224.

Zeryehun, T. (2012). Helminthosis of sheep and goats in and around Haramaya, Southeastern Ethiopia. *J. Veterinary Medicine and Animal Health.* 4(3): 48-55.