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Retrospective Study

Assessment of the Reasons for Culling and its Relation to Age at Culling in Dairy Cows in and around Mekelle City, Tigray, Ethiopia

Gutema D. Idesa, DVM[1*]; Sultan Aman, DVM[2]

[1]*Animal Products, Veterinary Drug and Feed Quality Assessment Center, Addis Ababa, Ethiopia*
[2]*Arsi Zone Livestock and Fishery Resource Development Bureau, Oromiya, Ethiopia*

[*]***Corresponding author***
Gutema D. Idesa, DVM
Animal Products, Veterinary Drug and Feed Quality Assessment Center, Addis Ababa, Ethiopia; Tel. +251967749293; E-mail: gutemadin@gmail.com

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ABSTRACT

Background
Culling is defined as the departure of cows from the herd because of sale, slaughter, salvage, or death. Culling is an important cost for dairy farms. At the same time, culling is a way to increase herd productivity and profitability, as keeping diseased and unproductive cows might result in lower herd milk production and deteriorated reproduction. In order to maximize profitability, the proportion of voluntary culling (selling for dairy purposes or culling due to low production) should be highest among the total culling rate. Previous studies indicate an ascending trend in the proportion of involuntary culling. This observational study used registry data of all cows from herds with ≥5 cow-years in 2016-2017.

Objective
This study was conducted to assess the reason for culling a dairy cow and its association with age at culling in and around Mekelle using a structured questioner format and direct observation. Visits were performed to each farm to collect data directly from owners or animal attendants and using direct observation.

Materials and Methods
The cross-sectional study was conducted in and around Mekelle city dairy farm from November, 2016 to April, 2017.

Results
The most common causes of culling were disease and economic reasons. The most common causes of voluntary culling were economic reasons (17.39), low milk yield (20.29%), and aging (8.70%). The common causes of involuntary culling were diseases (34.78%), injury (7.25%), and infertility (5.80%). In this study rates of culling increased with parity. The highest rate of culling was in animals between parity 2 and 5, increased from (14.49%) to (21.74%) and slightly decreased from parity 6 to 7, i.e. (13.04)% to (8.70)%, respectively. The highest rates of culling were between 3 and 5-years, (17.39)% and (23.19)%, respectively.

Conclusion
This study indicated animals were culled at premature age because of involuntary culling, which is economically not feasible, diseases were the most common causes of involuntary culling and voluntary culling was the most type of culling in dairy cattle farms and animals mostly culled for decreased production and economic reasons.

Keywords
Culling; Dairy cattle; Milk yield; Parity.

INTRODUCTION

Ethiopia is believed to have the largest livestock population in Africa. The livestock sector has been contributing a considerable portion to the economy of the country and still promising to rally around the economic development of the country. The Ethiopian total cattle population is estimated to be about 56.71 million. Out of this the female cattle constitute about 55.45% and the remaining 44.55% are male cattle and 98.66% of the total cattle in the country are local breeds and the remaining are hybrid and

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exotic breeds that accounted for about 1.19% and 0.14%, respectively. The livestock sector plays a vital role as a source of food, income, services, and foreign exchange to the Ethiopian economy.[1]

Moreover, Ethiopia has diverse animal genetic resources and its relatively large livestock population is well adapted to and distributed among diverse ecological conditions and management systems.[2] Despite the huge number of cattle and their economic importance, productivity is low due to the constraints of disease, nutrition, poor management, and poor genetic potential. These constraints result in poor reproductive performance of dairy cattle.[3] Through intensive management practices and progress in genetics, the dairy industry has been rapidly changing over the years. To meet the increasing demand for a growing population, the smaller number of cows is producing more milk. Trends also show a decrease in the number of farms, but an increase in herd size per farm. These changes have created many new challenges in the industry, one of which is the decline in fertility and reproductive efficiency.[4]

Maintenance and optimization of a dairy herd profit and avoidance of economic losses are a continuous challenge to dairy herd farmers especially when dairy cattle are reared under stressful conditions. To achieve this goal, farmers have to imply good dairy management practice for their herd by improving the overall health indices and increasing milk yield and reproductive performance. One of these practices is culling. Culling is the removal and disposal of an individual from the herd due to sale or death. It is classified as either voluntarily, when the farmers have the choice to remove the animal for example for low milk yield or aging, or involuntary when the farmers have no choice to remove certain individuals from the herds for example due to infertility or infectious diseases.[5] Culling is one of the important management practices to be adopted in dairy herds to maximize profit and minimize economic losses. However, culling will not be effective when it is made in non- systematic and non-programmed models.[6]

The decision to remove a cow from the herd is based on economic considerations. Optimum herd profitability is achieved by minimizing the proportion of the herd culled for health (involuntary culling) reasons and by maximizing the proportion culled for voluntary or economic reasons.[7] A high number of involuntary culling indicates potential health and welfare problemsin a herd. The rate of profitable culling is varying with regard to many considerations. Farmers should make strategies to minimize the rate of involuntary culling atexpense of voluntary culling which, the latter, is important and is used as a positive economic tool to make a balance between inputs and outputs of a farm.[8]

Identifying causes for culling is important and can help define the management status of a herd. Involuntary culling, which is often due to diseases or poor reproductive performance, is one of the factors which negatively affect the profitability of a dairy herd particularly when it is being ata high rate.[9] Several studies were conducted to identify the extent and possible reasons forculling worldwide. However, there are no studies conducted so far in the study area that showed the extent and possible reasons for culling bothvoluntary and involuntary culling. Therefore, the objective of this study was: to assess the reasons for culling and its association with age at culling.

MATERIALS AND METHODS

Study Area

The study was conducted in and around Mekelle, the capital city of Tigray Regional State of Ethiopia. Mekelle is found at 39° 33'E East and 13° 32'N north of the equator which is 783 kms away from Addis Ababa, the capital city of Ethiopia. The altitude of the area ranges from 2000-2200 meters above sea level. The mean annual rainfall of the study area is 579-650 mm. The annual minimum and maximum temperature is 11.8 °C and 24.9 °C, respectively in 2013.

Study Population

The study populations were dairy cattle kept in intensive dairy farms in and around Mekelle.

Study Design

A cross-sectional study design was conducted using a structured questionnaire and direct observation methods of collecting data to collect data on the possible reasons for culling a dairy cow and its relation to age.

Methods of Data Collection

Structured questionnaire was prepared and used to collect information from 69 dairy farm owners in one visit interview and the reason for the culling of their dairy cattle was studied. The questionnaires were checked for clarity of the questions prior to the interview. Prior to the interview, respondents were briefed on the objective of the study by using the local language. Following that, the actual questions and questionnaires were presented. Accordingly, information about the disease, age, decreased milk yield, injury, aggressiveness, infertility, and financial need were collected.

Data management and Statistical Analysis

The data collected was entered into a Microsoft Excel sheet (version-2010) and the data were analyzed using SPSS statistics version-20. Descriptive statistics such as frequency and percentage were used to summarize the collected data regarding reasons forculling.

RESULTS

Respondent's Background

Most of the respondents were with a primary level of education 30 (43.48%), followed by those who attended secondary education 25 (36.23%). Of the total respondents, only 7 (10.14%) attended level of education above secondary and the remaining were illiterate 7 (10.14%) (Table 1).

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Table 1. *Respondent's Educational Background*

Variables	Category	Frequency	Percent
Educational status	Illiterate	7	10.14
	Primary education	30	43.48
	Secondary education	25	36.23
	Above secondary	7	10.14
	Total	69	100

Farms Background Information

Most of the farms in and around Mekelle were established between 6 and 10-years 38 (55.07%). A significant number of farms were established between 1 and 5-years 21 (30.43%). The rest 10 (14.49%) were established 10 years ago (Table 2).

Table 2. *Duration of the Farm Since Establishment in Years*

Variables	Category	Frequency	Percent
When the farm was established	1-5 yrs	21	30.43
	6-10 yrs	38	55.07
	>10yrs	10	14.49

In this study 24 (34.78%) farms had 6-10 heads of dairy cows and 5 (7.25%) had 30-100 heads of dairycows (Table 3).

Table 3. *Number of Dairy Cow Feads per Farm*

Variables	Category	Frequency	Percent
Numbers of dairy cows	1-5	15	21.74
	6-10	24	34.78
	11-15	9	13.04
	16-20	8	11.59
	20-25	1	1.45
	26-30	7	10.14
	30-100	5	7.25

Parity

In this study culling rate of dairy cows increased with increasing parity. The highest rate of culling was in cows with parity 4 and 5, followed by cows with parity 2 and 3 which accounted for 10 (14.49%) to 15 (21.74%), respectively (Table 4).

Table 4. *Parity Level of Dairy Cows*

Variables	Category	Frequency	Percent
Parity	Parity 0	1	1.45
	Parity 1	2	2.90
	Parity 2	10	14.49
	Parity 3	11	15.9
	Parity 4	15	21.7
	Parity 5	15	21.7
	Parity 6	9	13.0
	Parity 7	6	8.70

Age at Culling

The highest rates of culling were recorded in cows at the age of 5 years (23.19%) followed by cows at the age of 4-years (21.74%). In this study, most of the animals were culled at premature and productive age (Table5).

Table 5. *Age at Culling*

Variables	Category	Frequency	Percent
Age at culling	2-years	8	11.59
	3-years	12	17.39
	4-years	15	21.74
	5-years	16	23.19
	6-years	11	15.94
	7-years	3	4.35
	8-years	4	5.80

Reason for Culling

In this study the most frequent reasons for culling were disease conditions (34.78%) followed by decreased milk yield (20.29%) and financial needs (17.39%) (Table 6).

Table 6. *Reason for Culling*

Variables	Category	Frequency	Percent
Reason for culling	Disease	24	34.78
	Age	6	8.70
	Decreased milk yield	14	20.29
	Injury	5	7.25
	Aggressiveness	4	5.80
	Infertility	4	5.80
	Financial needs	12	17.39

Disease condition was the most common cause that contributed to culling. Culling due to mastitis increased linearly with parity and reached nearly 21.74% at 6-years of age. Lameness accounted for 7.25% of the disposals, which increased with age from 0% in three-year-old animals to 33.33% in seven-year-old animals. Rates of culling due to decreased milk yield and financial needs were 20.29 % and 17.39 %, respectively. Further 5.80% and 5.80% of the recorded culling was for aggressiveness and infertility reasons, respectively. In terms of the cow's age, this type of culling was highest at the age of three-years (5.80%) which then became 0% in older ages (Table 7).

DISCUSSION

To make the right decision to remove an animal from the herd, many factors should be taken into considerations. The most important factors considered in culling decisions are age, health status, fertility status, stage of lactation and level of milk production, as well as the value of the replacement animal and its cost.[10] The probability of a cow being culled differs, depending on the age of

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Table 7. *The Degree of Association between the Reason for Culling and Age at Culling Using Chi- square (2) Test*

Ageat Culling	Reason for Culling							
	Disease	Age	Decrease Milk Yield	Injury	Aggressiveness	Infertility	Financial Need	Total
2-years	5	0	0	0	2	0	1	8
	62.50	0.00	0.00	0.00	25.00	0.00	12.50	100.00
3-years	3	0	1	0	2	3	2	12
	25.00	0.00	8.33	0.00	16.67	33.33	16.67	100.00
4-years	8	0	0	2	0	1	5	15
	53.33	0.00	0.00	13.33	0.00	0.00	33.33	100.00
5-years	7	0	5	1	0	1	3	16
	43.75	0.00	31.25	6.25	0.00	0.00	18.75	100.00
6-years	1	0	8	1	0	0	1	11
	9.09	0.00	72.73	9.09	0.00	0.00	9.09	100
7-years	0	2	0	1	0	0	0	3
	0.00	66.67	0.00	33.33	0.00	0.00	0.00	100.00
8-years	0	4	0	0	0	0	0	4
	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100.00
Total	24	6	14	5	4	4	12	69
	34.78	8.70	20.29	7.25	5.80	5.80	17.39	100
Pearson chi^2 (36)=115.032 pr=0.00								

the animal; in a study by, the risk of culling was highest in cows between 3 and 5-years of age and also in cows over 7-years of age. This age dependency of culling rate is in agreement with the result of the present study, in which most cows are culled at the age of 3-5-years.[11]

The rate of overall culling in this study is near to that reported by Elimam et al[12] in Sudan (11.95%),[13] in Ireland (19.6%), and Mohammadi et al[14] in Iran (13.1%); however, this rate is slightly different from rates reported by others,[7] reported (25.1%), including death cases, as overall culling rate in dairy cows in Shiraz, Southern Iran. In this study, death cases were not considered, the fact which might contribute to the low culling rate in this study.

The proportions of voluntary and involuntary culling in this study constituted 71.8% and 28.2%, respectively. These proportions were in agreement with reports by who reported that voluntary culling was the most prevalent type of culling in New South Wales, Australia and which is in agreement with the results of Rajala-Schultz et al[15] who reported that a total replacement percentage was 26% with the highest frequency of voluntary culling in Finish dairy herds. However, Mohammadi et al[14] reported an overall culling rate of 13.1% with 98.5% for involuntary culling and 1.5% for voluntary culling in 23 Holstein dairy herds in Iran. It also reported 74% for involuntary culling and 26% for voluntary culling.[7]

The increased percentage of voluntary culling, in general, is considered as a sign of good management practice. However, the high rate of voluntary culling in this study can be explained by the non-systematic and non-programmed culling practice in these farms as the owner's just cull cows to be sold to maintain the financial needs. More than half of all culling was associated with health disorders. The morbidity of a health disorder plays a significant role in culling decisions. In addition, indirect effects of diseases on culling are manifested through decreased milk yield and/or fertility of a cow. Many diseases can reduce milk production[15] and it might be the low yield that triggers the decision to remove the cow rather than the disease occurrence itself.

In the present study, the reproductive status of a cow was the most important factor in the farmer's culling decisions. This is in agreement with several other studies, which have indicated that a failure to conceive at first service or a longer period of days open increases the risk of culling reported that conceiving decreases the risk of culling.[5] In contrast to other studies, reasons for involuntary culling such as infectious diseases constituted a considerable proportion of culls in this study. This is likely to be related to the diseases present in the herd orthe region, as there is a strong relationship between the existing diseases in a herd and the culling rate.[16]

CONCLUSION AND RECOMMENDATIONS

The most common cause of culling in the present study was diseases and economic reasons. Most cows were removed from the herd at premature age because of disease; some of them are because of infertility and injury. It can also be concluded that voluntary culling was the most prevalent type of culling in dairy cattle farms in and around Mekelle during the period from November to April 2017 and animals were mostly culled for low milk yield and economic reasons.

Based on the above conclusion the following

recommendations are forwarded:

• More detailed epidemiological studies are needed to plan and implement healthcare programs.

• These programs targeted toward diseases that lead to culling would be prerequisite for a profitable farming.

• Further studies are needed to critically evaluate and describe the strategies of culling in dairy farms in and around Mekelle by studying the reproductive and productive characteristics of culled animals.

STATUTORY DECLARATION

We declare that this thesis presents the work carried out by ourselves and does not incorporate without the acknowledgement of any material previously submitted for a degree or diploma in any university; and to the best of our understanding, it does not contain any materials previously published or written by another person except where due reference is made in the text; all substantive contributions by others to the work presented including jointly authored publications, is clearly acknowledged

The Mekelle University, College of Veterinary Medicine ethical review committee had critically reviewed and concludes that there was no ethical misconduct. The approval of ethical committee was taken for conducting this study and followed all the animal ethics and welfare guidelines.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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Review

Review on Molecular Diagnosis of Cestode and Metacestode in Cattle

Ziyad M. Bilal, DVM[1*]; Kedir S. Musa, DVM[2]

[1]*Department of Veterinary Medicine, Bedano Woreda Veterinary Clinic, East Hararghe, Ethiopia*
[2]*Department of Veterinary Medicine, Haramaya University, College of Veterinary Medicine, P. O. Box 138, Dire Dawa, Ethiopia*

[*]***Corresponding author***
Ziyad M. Bilal, DVM
Department of Veterinary Medicine, Bedano Woreda Veterinary Clinic, East Hararghe, Ethiopia; E-mail: ziyadmohammedbilal@gmail.com

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ABSTRACT

Cestode infestations in animals are the most important parasite of livestock and humans because most of these parasites are zoonotic causing cysticercosis and hydatidosis in man and it causes economic and production losses in livestock. Diagnosis of Taenia *Spp* by microscopic observation lack sensitivity and specificity and detection by enzyme-linked immunosorbent assay (ELISA) technique form cross-reaction. The molecular diagnostic can be best to detect in adult and larval stage in definitive and intermediate host based on the amplification of deoxyribonucleic acid (DNA) of target gene with the primer using a different technique of polymerase chain reaction (PCR) such as multiplex PCR. Conventional PCR, real-time PCR, nested PCR, and PCR-restriction fragment length polymorphism (RFLP) are highly sensitive for the diagnosis of cestode and metacestode. Those diagnoses are used for differentiation of *Taenia* species and differentiation of *Taenia* and *Echinococcus* species. As compared to other diagnostic techniques most molecular methods have higher sensitivity and specificity but due to the relatively higher cost, few are commercially available. Most of the molecular diagnostic tests developed to date are generally applicable for laboratory research purposes. The developments in the genomic and proteomic analysis should be used for further understanding of parasite-animal host interaction to find additional targets for diagnosis.

Keywords
Cestode; Molecular test; Metacestode; Veterinary importance.

Abbreviations
Bp: Base pair; DNA: Deoxyribonucleic acid; ELISA: Enzyme-linked immunosorbent assay; gDNA: Genomic DNA; AMP: Loop-mediated isothermal amplification; NAD: Nicotinamide adenine dinucleotide; NADH: reduced form of NAD; PCR-REA: Polymerase chain reaction restriction enzyme analysis; PCR: Polymerase chain reaction; RFLP: Restriction fragment length polymorphism; rRNA: Ribosomal ribonucleic acid; REA: Restriction enzyme analysis; SSCP: Single-strand conformation polymorphism.

INTRODUCTION

Taeniidae is the largest family of flatworms (tapeworms) representing the order cyclophyllidae. It comprises numerous tapeworms with medical and veterinary importance.[1] Tapeworms 4154(Cestode) of the family Taeniidae are transmitted from the definitive host such as carnivores to the intermediate hosts in1uding herbivores or omnivores and human beings *via* oral-fecal cycle.[2] *Taenia saginata* and *Taenia solium* are the two taeniids of greatest economic and medical importance, causing bovine and porcine cysticercosis and taeniasis in humans.[3] Echinococcosis, also called hydatidosis, is a zoonosis and in humans, it occurs as a result of infection by the larval (metacestode) stages of taeniid cestodes of the genus *Echinococcus*. It is characterized by long-term growth of Metacestode (larval) stages (hydatid cysts) in internal organs (mainly the liver and lungs) of intermediate host animals.[4]

The diagnosis of taeniasis is based on the detection of eggs by microscopic observation of fecal samples. This technique lacks both sensitivity and specificity since the eggs of most members of the family Taeniidae are morphologically indistinguishable.[5] Similarly, differentiation of *T. solium* and *T. saginata* is based on the morphological characteristic of the scolex or gravid proglottids. Recovery of scolices after treatment is uncommon for

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T. solium and in many cases, both the scolex and proglottids can be recovered only after special treatment.[6] Detection of *T.solium* coproantigen by the enzyme-linked immunosorbent assay (ELISA) technique is used. The method is more sensitive than microscopy but cross-reacts with *T. saginata*.[7]

Molecular approaches[8] have attempted DNA differential diagnosis of taeniasis and cysticercosis by Multiplex PCR. Also, the presence of Metacestodes in animals is detecting by Multiplex PCR with cytochrome-c oxidase subunit 1 gene yield evident differential products unique for *T. saginata*, *T. asiatica*, and *T. solium* reviewed by.[9]

Molecular methods have also been used to determine species or genotypes of taeniids using 'pure' parasite DNA obtained from adult worms or Metacestodes from intermediate hosts.[10] However, the potential of these approaches to identify or differentiate among species of taeniid eggs in faecal or environmental samples had not been evaluated. Several polymerase chain reaction (PCR) assays have been developed for the specific identification of *E. multilocularis* from such samples.[11] The specific identification of *E. granulosus* eggs using monoclonal antibodies has been described but this method has not been utilized in further epidemiological studies.[10] Therefore, the aim is to explore the developments of the molecular diagnostic test for cestodes and metacestodes of veterinary importance.

CESTODES AND METACESTODES OF VETERINARY IMPORTANCE

Cestodes are a large diverse group of platyhelminths that share two common features: as adults, they have an elongate body, and they lack an alimentary canal. Thus, adult tapeworms is almost invariably found in the definitive host intestine where they absorb nutrients directly across their tegument.[12]

Taenia Solium/Cysticercus Cellulosae

T. solium causes Cysticercosis in pigs and as for *T. saginata*, humans are the obligate definitive host. Unlike *T. saginata*, the eggs from the adult *T. solium* that are present in the faeces of a tapeworm carrier can infect not only the natural animal intermediate host (pigs) but are infective the person who might accidentally ingest the eggs. In humans the cysticerci may encyst in the brain, causing neurological disease.[13]

Taenia Saginata/Cysticercus Bovis

Taeniasis is a cestode (tapeworm) infection of humans, with the adult phase of the worm residing in the intestine. Man is the definitive host of the cestode *Taenia saginata* (beef tapeworm). The intermediate host of the larval form of *T. saginata* is mainly domestic cattle. Beef tapeworm is transmitted when terminal segment (gravid proglottids), which each contain up to 100,000 eggs of *T. saginata* are detached from the segment chain (strobila), one by one and are passed in the feces of an infected person and the excreta is deposited in pastures or grazing area where the eggs are ingested by cattle.[14] Cysticercus Bovis is caused by the metacestode stage of *Taenia saginata*, a zoonotic tapeworm of cattle and humans. Adult tapeworm develops in humans who consume undercooked beef infected with viable Metacestodes.[15]

Taenia Hydatigena/*Cysticercus Tenuicollis*

Cysticercus tenuicollis is the metacestode of canine tapeworm Taenia hydatigena, which has been reported in domestic and wild ruminants, pigs, monkeys.[16] Metacestodes are found attached to the omentum, mesentery, and occasionally on the liver surface, however, unusual location of *C.tenuicollis* have been described as lungs, kidneys, brain, ovaries, uterine tubes, uterus, cervix, and vagina. An aberrant location of *C.tenuicollis* vesicle inside the chorioallantoic membrane of a goat foetus was reported.[17] Pathogenicity of adult parasites is not high for the definitive hosts. However, a large number of developing cysticerci migrate contemporaneously in the liver of intermediate hosts, producing "hepatitis cysticercosa" a condition whose gross pathology resembles acute fasciolosis and which is often fatal.[18]

Echinococcus/Hydatid Cyst

Cystic Echinococcosis or cystic hydatidosis is a chronic helminthic zoonotic disease with a cosmopolitan distribution[19] and is especially prevalent in the sheep-raising countries. The causative organism, the dog tapeworm *Echinococcus granulosus* is transmitted cyclically between canines and numerous herbivorous livestock animals, which can serve as intermediate hosts. In herbivorous animals and in people who become infected by accidentally ingesting *E. granulosus ova*, the cystic larval form (Hydatid Cyst) develops and can cause serious morbidity.[20] Likewise, the disease often has severe consequences for health.

DIAGNOSTIC TECHNIQUES FOR CESTODES AND METACESTODES

Despite its many limitations, visual inspection of carcasses remains the most common method of diagnosing *T. saginata* cysticercosis. Studies showed the failure of detection during meat inspection as high as 80%.[21] Diagnosis of cestodes and metacestodes is by physical imaging methods such as ultrasonography. However, the diagnostic potential of such techniques is sometimes limited by the atypical appearance of the visualized lesion that may also be insufficient in providing information about the involved species or about the viability of the parasite.[22] Immunodiagnosis is a useful complementary diagnostic tool for the identification of infection and disease.[23] Nevertheless, infections with different taeniid species and antigenic cross-reactivity between these related parasites and the low level of specific antibody response to infection problems with poor specificity and sensitivity of serological tests.[24] To overcome the above diagnostic problem a molecular technique has been developed and adapted to advance laboratory diagnosis for cestode and metacestode.[25]

MOLECULAR DIAGNOSIS OF CESTODES AND METACESTODES

Bovine and Porcine Cysticercosis

The most widely used approach for DNA identification of Taenia

taxa has been to target the nucleotide sequences of fragments of selected genes using pairs of concerned PCR primers..[15] The variable segment between the primers is PCR amplified for a particular Taenia sample and then directly sequenced. The *mt cox1*, *nad1*, *cob*, and *12S rDNA* genes, and *nuclear 28S rDNA* and *ITS1/ITS2 rDNA* have proven particularly valuable markers amenable to this approach.[26] Given that different DNA markers have different rates of evolution and conserved sites, the results obtained from analyzing the same samples may be inconsistent. The mitochondrial cytochrome c oxidase subunit 2(cox2) gene has been widely used in studies of evolution and genetic diversity in many species.[2]

Conventional Polymerase Chain Reaction

Using primer designed to hybridize with the region of the 18S and 28S ribosomal gene of DNA taken from *T. solium* (eggs, cysts, immature and mature worms) and *T. saginata* (eggs and mature worms).[27] PCR detection of swine cysticercosis genomic DNA extracted from swine sera using different protocols showed higher specificity (100%) with no cross-reaction to trichinellosis and toxoplasmosis. sensitivity was lower Cox1 PCR (23%), T3/T4PCR(32%), and Nested PCR (64%) then ELISA-based detection of antibody from serum in the same study.[28]

Restriction Enzyme Analysis

DNA based differentiation of *T. saginata* and *T. solium* has been described and includes the use of probes.[29] PCR with species-specific primers and PCR followed by restriction enzyme analysis (PCR-REA).[30] However, these methods require pure parasite DNA, which means that the DNA has to be extracted from a single proglottid and adequately cleaned, because these primers may amplify any eukaryotic DNA, causing cross-amplification. A few reports must describe the use of DNA-based techniques to differentiate *T. solium* from *T. saginata* from the fecal sample[31] but they still lack sensitivity. Nonetheless, nowadays studies describe a; copro-PCR; for the simultaneous detection of the human tapeworm *T. solium* and *T. saginata*. Nkouawa[32] reported stool PCR and loop-mediated isothermal amplification (LAMP) can distinguish between *T. saginata* and *T. solium*.

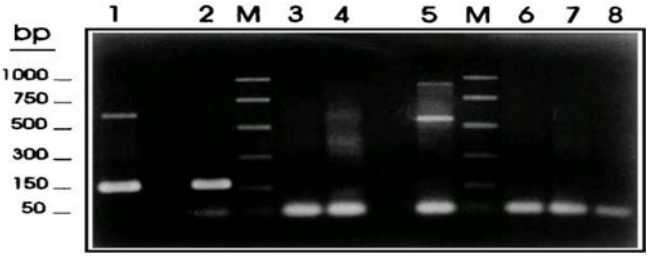
Real-Time Polymerase Chain Reaction

Newly developed a Real-time PCR to allow the sensitive and specific diagnosis of targets the COI gene of *T. saginata* by using *TsagF* oligonucleotide primers the technique yield 131 bp. When the results were compared with the reference A multiplex PCR the assay was less sensitive but offered the advantages of faster turnaround time and reduced contamination risk.[15]

Multiplex Polymerase Chain Reaction

As regards the identification of taeniid cestode parasites, multiplex PCR using taeniid species-specific and Taenia solium genotype specific-primers yielded differential products unique for *T. saginata*, *T. asiatica*, and Asian and American/African genotypes of *T. solium* with the molecular size of 827,269,984, and 720 bp, respectively (Figure 1).[33]

Figure 1. *Multiplex PCR Amplification Products for Differential Detection of T. saginata, T. solium, and E. granulossus*[34]



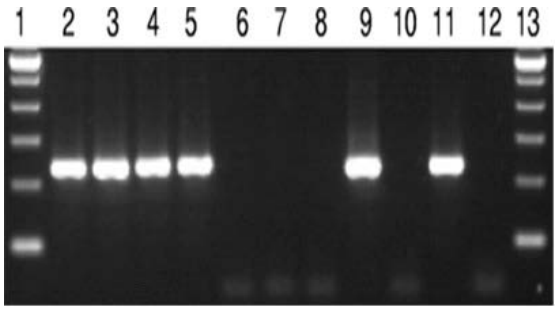
Gonzalez et al[34] used Multiplex PCR for differential detection of *T. saginata*, *T. solium*, and *E. granulossus* based on the *PTs7S35F1*, *PTs7S35F2*, and *PTs7S35R1* primers derived from the *T. saginata* genomic sequence HDP2. Samples of genomic DNA (1ng) from *T. saginata* (lane 1), *T. solium* (lane 2), *T.taeniformis* B (lane 3), *T. taeniformis* M (lane 4), *E. granulosus* (lane 8). Promega PCR molecular markers were used (lanes M).

Samples of genomic DNA (1 ng) from *T. saginata* (lane 1), *T. solium* (lane 2), *T.taeniformis* B (lane3), *T.taeniformis* M (lane4), *E.granulosus* (lane 5), a calf (lane 6), and a human (lane 7) were amplified by the multiplex PCR based on the *PTs7S35F1*, *PTs7S35F2*, and *Ts7S35R1* primers derived from the *T.saginata* genomic sequence HDP2. A negative control without DNA was also included (lane 8). The reactions were carried out as described in Materials and Methods. The amplification products were fractionated on a 2% agarose gel and were stained with ethidium bromide. Promega PCR molecular markers were used (lanes M).

Nested-Polymerase Chain Reaction

Mayta et al[35] reported Tso31 nested-PCR amplification using DNA extracted from different sources. Electrophoresis was performed using 5 micros (1) of the amplification product. Lane 1 and 13, 100-bp ladders; lane 2 to 8, DNA from a contaminated sample with 100, 50, 20, 10, 5, 2, and 1 *T.saginata* proglottid; lane 9, DNA from a *T. solium* proglottid; lane 10, DNA from a *T. saginata* proglottid; lane 11, DNA from a *T. solium*-positive stool sample; lane 12, DNA from a *T. saginata*-positive stool sample (Figure 2).

Figure 2. *Nested PCR Amplification Products using DNA Extracted from Different Sources*[35]



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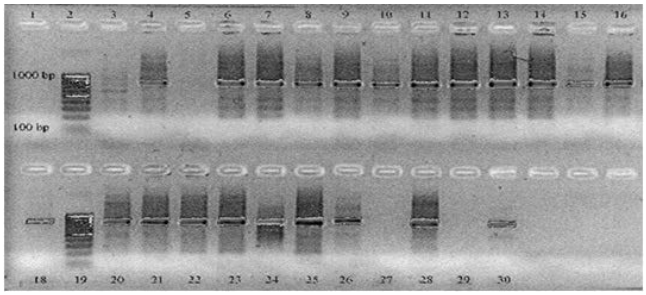
Restriction Fragent Length Polymorphism

Molecular approaches include restriction fragment length polymorphism (RFLP) analysis, PCR-linked RFLP analysis (PCR-RFLP), and direct comparison of PCR-amplified DNA sequences.[36] Sequence data of mitochondrial NADH dehydrogenase subunit 1 (mt-ND 1) and cytochrome c oxidase subunit 1 (mtCO1) genes of genus Taenia (*T. taeniaeformis, T. hydatigena*, *T. pisiformis*, *T. ovis, T. multiceps*, *T. solium*, and the Asian Taenia) is available on gene bank.[37]

Geysen et al[38] performed PCR-RFLP by amplification of two sequential rounds. In the first round, primers ITMTnR and TaenF were used to amplify 846 nucleotides. A second round was performed using ITM TnR and nTAE to amplify a sequence of 766 nucleotides. lanes 4, 6 to 18 and 20 to 26 display a band of approximately 800 bp and are positive PCR results. Lane 3 and 5 are PCR-negative sample results. Lane 27 and 29 are negative control samples (Milli instead of extracted DNA) and lanes 28 and 30 are positive control samples (*T. crassiceps* DNA). Lanes 2 and 19 are DNA size markers (Figure 3).

Figure 3. *PCA Results of T.saginata Lesions. Amplification was Performed in Two Sequential Rounds*[38]



In the first round, primers ITMTnR (*5CTCAATAATC-GAGGGTGACGG3*) and Taen F (*5GTTTGCCACCTCGAT-GTTGACT*) were used to amplify 846 nucleotides. A second round was performed using ITMT n R and TAE (*5CGTGAGCCAG-GTCGGTTCTTAT3*) to amplify a sequence of 766 nucleotides. Lane s 4, 6 to 18, and 20 to 26 display a band of approximately 800 bp and are positive PCR results. Lanes 3 and 5 are PCR-negative sample results. Lanes 27 and 29 are negative control samples (MilliQ instead of extracted DNA) and lanes 28 and 30 are positive control samples (*T. crassiceps* DNA). Lanes 2 and 19 are DNA size markers.

***Echinococcus*/Hydatid Cyst**

Conventional polymerase chain reaction: Genetic variation in *Echinococcus* has been investigated using sequences from both the nuclear and mitochondrial genomes. The advent of the polymerase chain reaction (PCR) has provided a highly sensitive approach that is now widely used for Echinococcus identification purposes.[39] Loop-mediated isothermal amplification assay primers (F3 and B3) for amplification mitochondrial NADH-1 gene of EG-complex Hydatid Cyst cattle strain of genotype 5(G5), was used as a target for using conventional PCR. Visualization of the 200-bp specific DNA PCR products on ethidium bromide-stained agarose gels.[40]

Conventional PCR targeting the *E.granulosus* specific DNA sequences of the NADH1 gene was performed on all serum, urine, and *Hydatid Cyst* fluid samples. The resultant 450 bp amplification products were observed on a 1.2% agarose gel following electrophoresis.[41] Boufana et al[42] described conventional PCR assay based on the amplification of a fragment within the NADH dehydrogenase subunit 1 (ND1) mitochondrial gene were optimized for the detection of *Echinococcushi quicus*, Echinococcus granulosus, G1, and *Echinococcus multiloclaris* DNA-derived from parasite tissue or canid fecal samples.

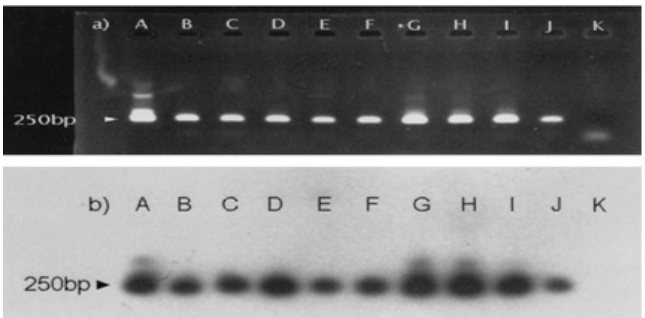
Real-time polymerase chain reaction: A real-time PCR for the differentiation of the G1 and G2/G3 genotype of *Echinococcus granulosus* has been developed it has been suggested to offer several advantages over conventional PCR for the detection of parasitic infections, including increased sensitivity and specificity, reduced reaction time, and a quantitative estimate of the amount of bDNA in the sample (which May relate to both the infectiousness of the sample and the possible burden of infection.[43]

Restriction fragment length polymorphism: Azab et al[44] developed restriction fragment length polymorphism (RFLP) analysis using conventional southern blotting. without loss of resolution or accuracy, by linking RFLP analysis with PCR targeting the nuclear ribosomal DNA (rDNA) internal transcribed spacer 1(ITS-1) region. The random amplified polymorphic (RAPD)-PCR(RAPD-PCR) has also been used under careful conditions for distinguishing the four recognized Echinococcus species and genetically distinct forms of *E. granulosus*.

Nested Polymerase Chain Reaction

Complete sequences of the mitochondrial (Mt) genomes of the horse and sheep strain of *E. granulosus* and *E. multilocularis*, and the availability of mt DNA sequences for several other *E. granulosus* genotypes, has provided additional genetic information that can be used for more in-depth strain characterization and taxonomic studies of these parasites. While Nested PCR on mitochondrial 12S rRNA gene shows 100% specificity when it was tested against *E. multilocularis* and *E. granulosus* isolates.[45]

Figure 4. *Nested PCR Amplification of DNA from Nine Positive Fox Fecal Samples*[46]



Nested PCR products from 60 positive fecal samples were randomly selected and underwent hybridization with the specific probe *E. multi.* 1. with all samples a hybridization signal was obtained. *E. multilocularis* metacestodes yielded the same characteristic band of 250 bp (Figure 4).

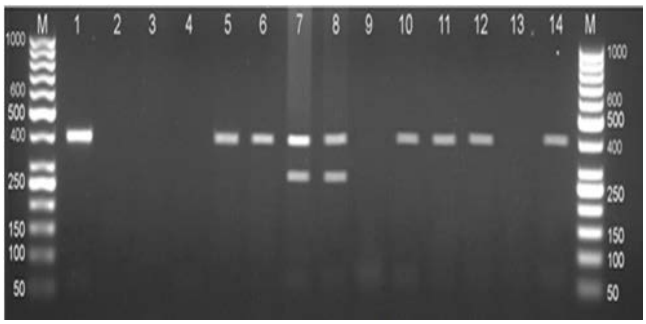
Ethidium bromide staining of 10 l of PCR products after 1.5% agarose gel electrophoresis showed the specific 250 bp band (a). The reaction products were analyzed by Southern transfer and hybridized with internal oligonucleotide *E. multi.* 1. labeled at the 5 end with digoxigenin(b). Lanes A, positive control; lanes B, C, D, E, F, G, H, I, and J, positive fox fecal samples; lanes K, negative control.[46]

Multiplex Polymerase Chain Reaction

Stieger et al[47] introduced Multiplex PCR amplify partial sequences of the mitochondrial genes for NADH dehydrogenase subunit 1 (nad 1) for detection of *E. multilocularis*, and the small subunit of ribosomal RNA (rRNA) for detection of *E. granulosus* and *Taenia spp* amplification products were visualized by 2%(W/V) agarose gel electrophoresis, and the 395,117, and 267 bp expected fragments were examined for the presence of *E. multilocularis*, *E. granulosus*, and *Taenia spp*, respectively. DNA isolation from Fox excrement was performed according to a novel procedure involving lysis in KOH, phenol-chloroform extraction, and purification steps on a matrix (prep-A-Gene). The target sequence for amplification was the *E. multilocularis* U1 snRNA gene.[48]

Beiromvand et al[48] reported by sequencing of *E. multilocularis* positive samples. Multiplex PCR showed 30 of 85 captured specimens (35.3%) to be infected with *E. multilocularis* and 14 (16.5%) infected with *Taenia spp*. by amplification of 395 bp fragment of nad1 and 267 bp fragment of rRNA, respectively (Figure 5).

Figure 5. *Multiplex PCR Amplification of Mitochondrial genes of E.multilocularis and Taenia spp*[48]



DNA extracted from the liver of Small mammals. Lane M, 50 bp DNA ladder (Fermentas; Cat No SM0373); Lane 1, positive control, a standard DNA of *E. multilocularis* (395 bp); Lane 2, negative control; Lane 5, 6, 10-12 *E. multiloclaris*; Lane 7 and 8, mixed infection of *E. multilocularis* (395 bp) and *Taenia spp*. (267 bp); Lane 3,4,9, and 13 negative samples.

Single Strand Conformation Polymorphism

Single-strand conformation polymorphism (SSCP) has been used to rapidly a large number of Echinococcus isolates. Another useful mutation scanning method is dideoxy fingerprinting (ddF), which is a hybrid between SSCP and conventional dideoxy sequencing. The technique has been used reproducibly for the direct display of sequence variation in the *Cox1* gene to type and differentiate all of the Echinococcus genotypes examined.[49] Bartholomei-Santos et al[50] isolate and characterize microsatellites using eight different oligonucleotides containing particular repeats as probes. Microsatellite DNA analyses have identified polymorphisms within the *E. multilocularis* endemic region.

CONCLUSION

In conclusion, the infection of cestode and metacestode of veterinary importance such as Echinococcosis and Cysticercosis contribute to a high-level of human and livestock production losses and morbidity. That is, *Taenia saginata*, *Taenia solium*, and *Echinococcus cause* production loss in bovine, sheep, goat, and pig respectively. As compared to other diagnostic techniques most molecular methods have higher sensitivity and specificity but due to the relatively higher cost, few are commercially available. Most of the molecular diagnostic tests developed to date are generally applicable for laboratory research purposes. The developments in the genomic and proteomic analysis should be used for further understanding of parasite-animal host interaction to find additional targets for diagnosis.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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Original Research

Study on Prevalence of Major Gastrointestinal Nematodes of Sheep in Wayu Tuka and Diga District, Oromia Regional State

Adamu R. Chali, DVM[1]; Fedhiko T. Hunde, DVM[2]*

[1]*Mekelle University, College of Veterinary Medicine, Mekelle, Ethiopia*
[2]*Veterinary Drug and Feed Administration and Control Authority (VDFACA), Addis Ababa, Ethiopia*

Corresponding author
Fedhiko T. Hunde, DVM
Veterinary Drug and Feed Administration and Control Authority (VDFACA), Addis Ababa, Ethiopia; E-mail: fedhikot@gmail.com

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ABSTRACT

Aim
This study was devised to be conducted to determine the prevalence and identify the major gastrointestinal nematode parasites based on fecal examination.

Methods
A cross-sectional study was carried out to determine the prevalence and associated factors with ovine gastrointestinal nematode infestation by fecal examination of 384 sheep from Wayu Tuka and Diga district, Eastern Oromia regional state.

Results
Out of the total 384 sampled sheep, 169 (44.0%) had been infected with gastrointestinal nematode parasite. Haemonchus species were the most frequently (20.8%) recovered nematode eggs followed by Trichostrongylus (13.0%) and Nematodirus (10.2%). There was no significant difference ($p>0.05$) in prevalence between age groups and sex. Sheep with poor body condition had a significantly higher prevalence of gastrointestinal nematode parasite ($p<0.05$) than those sheep in moderate or good body condition. There was no significant association between the gastrointestinal nematode infection in animals of different ages and sex groups.

Conclusion
The study shows that the gastrointestinal nematode parasite was a major important health problem and impact on the production of sheep in the study area. Therefore, a detailed study should be conducted to identify the parasite at the species level and special consideration should be taken on the management of sheep in poor body condition to reduce the burden of gastrointestinal nematodes.

Keywords
Diga; Eastern Wollega; Gastrointestinal nematodes; Prevalence; Sheep; Wayu Tuka.

INTRODUCTION

Small ruminants are the most numerous of man's domesticated livestock and are especially important in more extreme climates of the world. Over two-thirds of the total populations of small ruminant occur in developing countries where they often provide a major contribution to farming enterprises.[1] Historically, the livestock sector was subsistence-oriented and dominated by smallholders, and even today livestock isconsidered a more secure source of income generation for poor and landless farmers, particularly true for sheep and goats.[2] Because of relatively low inputs needed such as startup capital, feedstuffs, and maintenance expenditures as compared to large ruminants.[3]

Sheep and goats are under sober coercion of clinical and sub-clinical gastrointestinal helminths infestation in undeveloped countries, which reduces the productive and reproductive potential of animals due to reducing voluntary feed intake and feed conversion efficiency of the animals, especially the ineffective use of absorbed nutrients leads to retarded growth.[4] When comparing the population size and importance of small ruminants, the country has little benefited from this enormous resource owning to a multitude of problems, the disease is the most important. Disease alone accounts for mortality of 30% in lambs and 20% in adults.[5]

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Globally, parasitic diseases of animals continue to be a major constraint for undeveloped countries. Parasitic diseases remain the main constraint to animal production systems all over agro-ecological zones throughout the world. The productive and reproductive potential of domesticated livestock is adversely impaired by clinical and sub-clinical helminths diseases. It has well-recognized that in resource-poor regions of the world, helminth infections of sheep and goats are major factors responsible for economic losses through the reduction in productivity and increased mortality of animals. They are responsible for suppressing the immune system of animals, enhancing the susceptibility of the animals to other diseases.[6] Among helminths, gastrointestinal nematodes significantly affect the production of sheep and goats due to reduce appetite of animals, loss of body condition, anemia, hypoproteinemia, Impaired digestive absorptive efficiency, other pathogenic complications, and even death of animals.[7]

Several factors influence the diseases of gastrointestinal nematode parasites in small ruminants. These include weather conditions, husbandry practices, and the physiological status of the animals and for sustainable and normal control of gastrointestinal nematodes of sheep, comprehensive knowledge of epidemiology is a prerequisite.[8] In a developed country, the greatest component of impact by the gastrointestinal nematode parasites is probably found in the cost of control. But their impact is greater in sub-Saharan Africa in general and Ethiopia inparticular because of suitable ecological factors for diversified hosts and parasite species.[9]

Ethiopia has the highest livestock population in Africa. Small ruminants are among the major economically important livestock in Ethiopia; sheep and goats are of great importance as major sources of livelihood and contribute to the sustenance of landless, smallholder, and marginal farmers especially to the poor in the rural areas throughout the developing countries in which they are playing an important role in the livelihood of resource for poor farmers and provide a vast range of products and services such as meat, milk, skin, hair, horns, bones, manure, security, gifts and for many purposes.[10]

Shoats are very important in rural areas of Ethiopia due to their ease of management, short generation cycles, and high reproductive rates which lead to high production efficiency and a significant role in the provision of food and cash income generation for the farmers. These animals serve as a living bank for many farmers, closely linked to the social and cultural life of resource-poor farmers, and provide security during the cultivation of crops became difficult.[11] Therefore, they form an important economic and ecological niche in all agricultural systems throughout the country.[12]

The livestock population of Ethiopia is estimated to be 56.71 million cattle, 29.33 million sheep, and 29.11 million goats.[13] When the large livestock population of Ethiopia is compared with its economic benefits, it remains less because of prevailing diseases, poor nutrition, poor animal production systems, reproductive inefficiency, management constraints, and awareness of owners.[12]

Gastrointestinal nematode parasite infection is one of the major important health problems of animals in the world. These nematode infections affect the health of millions of animals, causing a huge economic loss in livestock farming.[14] In Ethiopia, gastrointestinal nematode parasite infections have a greater impact on livestock due to the availability of a wide range of agro-ecological-factors suitable for diversified hosts and parasite species.[15] Nematode parasites of small ruminants are primarily parasites of the gastrointestinal tract. The most important species are those found in the abomasum and small intestine. This includes; *Haemonchus*, *Trichostrongylus*, *Nematodirus*, *Cooperia*, *Bunostomum*, *Ostertagia*, and *Oesophagostomum*.[16]

In many parts of the Eastern Wollega Zone, the gastrointestinal nematodes parasite remains an important disease problem of sheep in the area. Hence, this study was devised to be conducted in WayuTuka and Diga district of Eastern Wollega Zone, Oromia regional state, to determine the prevalence and identify the major gastrointestinal nematode parasites based on fecalexamination.

MATERIALS AND METHODS

Study Area

The present study was conducted from November 2016 to April 2017 in Wayu Tuka and Diga district which are found in East Wollega Zone Oromia Regional state. Wayu Tuka and Diga District are located at about 319 km and 343 km distance from Addis Ababa, altitude of the study area ranges from 1300-3140 and 2250 m.a.s.l. respectively. According to Wayu Tuka Agricultural Office, the district has various topographic features. The rainfall distribution of the area is unimodal. In general, the mean annual temperature and mean annual rainfall are 18.8 °C and 2,067 mm, respectively. Diga receives the average annual rainfall of approximately 1250 mm. The annual temperature varies from 14 °C to 32 °C with an average of 22.6 °C.[17]

Study Animals

The study animals include all grazing sheep of different age groups and both sexes in Wayu Tuka and Diga district of Eastern Wollega Zone, Oromia that are kept under traditional extensive production management system. A total of 384 sheep were randomly selected and examined for gastrointestinal tract nematodes considering different age groups (<1, 1-2 and >2-years), sex group (male and female), and body condition groups (poor, medium, and good) as described by Gatenby et al[18] and Russel.[19] Natural pastures from communal grazing lands were the principal sources of feed for sheep and other livestock throughout the year. Mostly, a large number of different livestock including sheep are grazed together on communal grazing pasture.

Study Design

A cross-sectional study was undertaken to determine the prevalence of sheep gastrointestinal nematodes by qualitative fecal examination. Individual animals were carefully identified and sex, age, and body condition score were recorded. These risk factors were assessed for the presence of possible association with the

presence of gastrointestinal nematode.

Sampling Method and Sample Size

A random sampling strategy was followed to collect feces from the individual animals. The sample size was determined based on the formula described by Tsedeke.[11] The sample size was calculated using 50% expected prevalence, 5% absolute precision, and 95% confidence interval and the calculated total sample size was 384.

$$N = \frac{1.96^2 p\exp (1\text{-}p\ \exp)}{d^2}$$

Where n=number of sample size
P exp=expected prevalence and d=desired absolute precision
1.96=constant from normal distribution at a given confidence level.

Laboratory Procedure

Fecal samples were collected directly from the rectum of each animal and placed in universal bottles and then transported to Wollega University Laboratory. In the laboratory, the samples were subjected to floatation techniques for screening of study animals for nematodes parasite presence. A simple test tube floatation technique is performed with the purpose of a qualitative test for detection of nematodes eggs. Eggs of different nematodes were identified on the basis of morphological appearance and size of eggs.[20]

Equipment required; fecal sample, tea strainer, measuring cylinder, test tube and test tube rack, pistol, and mortar, stirring rod, beaker, digital balance, floatation fluid, microscope, slide, and coverslip. Procedures; 3 gram of feces was measured and placed in a mortar and crushed by pistol, 50 ml of floatation fluid was added and mixed thoroughly, the suspension was poured into beaker through a tea strainer, from beaker the fecal suspension was poured into test tube supported by test tube rack, the coverslip was placed on the top of the test tube, the test tube was left for 20-minutes, the cover slip was lifted off after 20-minutes and placed on the slide then examined under microscope 10x magnification.[21]

Data Managemnt and Statistical Analysis

All collected data were entered into a Microsoft Excel spreadsheet. Data were analyzed using STATA 13 statistical software.[22] Pearson's chi-square test was employed as a test of the association if there is the relationship between the factors and the prevalence of gastrointestinal nematodes. A statistically significant association was said to exist when the calculated *p*-value is less than 0.05 ($p<0.05$) at a 95% confidence level.

RESULTS

In this study, the overall prevalence of gastrointestinal nematodes in the study area was 44.0% from two districts. During the study period, some factors were taken to identify the association of gastrointestinal nematodes parasite in sheep. The overall demographic study animals are summarized in the following table (Table1).

Prevalence of Gastrointestinal Nematodes in Male and Female Sheep

During the, a higher prevalence of major gastrointestinal nematode infection was observed in female animals as compared to male while the overall prevalence was 44.0%.

However, the difference in prevalence between the two sexes was not statically significant ($p>0.05$) (Table2).

Table 1. *Distribution of Study Animals by Demographic Data*

Districts	Factors	Wayu		Tuka	
		Frequency	Percent	Frequency	Percent
Sex	Male	83	43.2	61	31.8
	Female	109	56.8	131	68.2
Age	<1-year	49	25.5	50	26.0
	1-2-year	68	35.4	53	27.6
	>2-year	75	39.1	89	46.4
Body condition	Poor	69	35.9	65	33.9
	Medium	65	33.9	62	32.3
	Good	58	30.2	65	33.9
Agroecology	Midland	192	100.0	0	0.0
	Highland	0	0.0	192	100.0
Kebele	Gute	96	50.0	0	0.0
	Warababo	96	50.0	0	0.0
	Haro	0	0.0	96	50.0
	Soyama	0	0.0	96	50.0
Genus	*Haemonchus*	34	17.7	46	24.0
	Nematodirus	18	9.4	21	10.9
	Trichostrongylus	26	13.5	24	47.4

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Table 2. *Prevalence of Gastrointestinal Nematodes in Male and Female Sheep*

Sex	No. of Examined	No. of Positive	Prevalence	χ^2	*p*-value
Male	144	61	42.4%	0.2543	0.614
Female	240	108	45.0%		

Prevalence of Gastrointestinal Nematodes in Sheep Based on their Age

The study revealed that the proportion of the prevalence was greater than in less than one year, followed by between one up to two years and two years respectively. There was no statistically significant difference (p>0.05) in the prevalence of gastrointestinal nematode between the ages (Table 3).

Table 3. *Prevalence of Gastrointestinal Nematodes in Sheep Based on their Age*

Age	No. of Examined	No. of Positive	Prevalence	χ^2	*p*-value
<1-year	99	46	46.5%	0.5105	0.775
1-2-year	121	54	44.6%		
>2-year	164	69	42.1%		

Prevalence of Gastrointestinal Nematodes of Sheep Encountered in the Study Area

The dominant gastrointestinal nematodes parasite genus found during the study period were *Haemonchus*, *Trichostrongylus*, and *Nematodirus*. There was statically significant between genes of the parasite (p<0.05) (Table 4).

Table 4. *Prevalence of Gastrointestinal Nematodes of Sheep Encountered in the Study Area*

Genus of Parasite	No. of Examined	No. of Positive	Prevalence	χ^2	*p*-value
Haemonchus	384	80	20.8%	0.001	0.001
Trichostrongylus	384	50	13.0%		
Nematodirus	384	39	10.2%		

Prevalence of Gastrointestinal Nematodes in Sheep Based on Body Condition

Prevalence was significantly higher in animal with poor body condition when compared to that of medium and good body condition animals. There was statically significant difference in prevalence of gastrointestinal nematode parasite between body conditions of animals (p<0.05) (Table5).

Table 5. *Prevalence of Gastrointestinal Nematodes of Sheep based on Body Condition*

Body Condition	No. of Examined	No. of Positive	Prevalence	χ^2	*p*-value
Poor	134	96	71.6%	70.159	0.001
Medium	127	47	37.0%		
Good	123	26	21.0%		

Prevalence of Gastrointestinal Nematodes in Sheep Based on Agroecology

Out of all the sheep examined in different agroecology, samples from high land were a relatively high prevalence of gastrointestinal nematode. But the distribution of the parasite was not significantly different between midland and highland (Table 6).

Table 6. *Prevalence of Gastrointestinal Nematodes in Sheep Based on Agroecology*

Agroecology	No. of Examined	No. of Positive	Prevalence	χ^2	*p*-value
Midland	192	78	40.6%	1.7860	0.181
Highland	192	91	47.4%		

Prevalence of Gastrointestinal Nematodes Sheep on Different Site of the Study Area

During the study period, samples from Haro and Soyama revealed higher gastrointestinal nematode prevalence, and lower prevalence nematode were recorded in samples from Gute and Warababo. The chi-square (χ^2) test value indicated that there was no statistically significant difference (p>0.05) in prevalence of gastrointestinal nematode infection of sheep between these sites (Table 7).

Table 7. *Prevalence of Gastrointestinal Nematodes in Sheep on Different Site of Study Area*

Study Site	No. of Examined	No. of Positive	Prevalence	χ^2	*p*-value
Gute	96	40	41.7%	2.3990	0.494
Warababo	96	38	39.6%		
Haro	96	48	50.0%		
Soyama	96	43	44.8%		

DISCUSSION

The gastrointestinal nematodes of sheep are one of the major important parasitic diseases that reduced the productivity of sheep raised by farmers using traditional husbandry management system in Wayu Tuka and Diga district. The result of the present study which was conducted based on qualitative fecal examination during the study using fecal floatation method revealed an overall prevalence of gastrointestinal nematodes parasitic infection in sheep was (44.0%). This finding was found to be higher than that reported by Aga et al[23] who reported (24.7%) prevalence of gastrointestinal nematodes in sheep which was conducted in Western Oromia, Ethiopia.

The overall prevalence of present study findings are in agreement with Khan et al[24] who observed the overall prevalence of gastrointestinal helminthiasis, prevalence, and associated determinants in domestic ruminants of district Toba Tek Singh, Punjab, Pakistan as (44.2%).[25] Reported nematodes prevalence (47.7%) in North Gondar zone of Northwest Ethiopia, which is almost close to the present study. However, Kenea et al[26] who showed a higher prevalence of (73.1%) of gastrointestinal nematodes of sheep in three districts of Kaffa and Bench Manji

zones,[27] reported a higher prevalence (65.0%) of gastrointestinal nematode than the present study.[28] Reported (83.6%) and even higher (91.3%) by Tefera et al[29] in South-Western Ethiopia, respectively. This difference could be due to the sample size considered, the climatic condition of the study area and types of techniques utilized, and lack of intervention with anthelmintic.

In the present study, the prevalence of gastrointestinal nematodes was higher in females (45.0%) as compared to males (42.4%). In this study, no significant variation was observed in males and females despite slightly higher infection noticed in female sheep. The absence of statistical association between sex and prevalence of gastrointestinal nematode is in agreement with that of Regassa et al[9]; Assefa et al[30]; Keyyu et al[31]; yet, it is in disagreement with other reports including Maqsood et al[32] and Urquhart et al[33] who found higher infections in female animals than males with a significant difference between them. It is assumed that sex is a determinant factor influencing the prevalence of parasitism[32] and females are more prone to parasitism during pregnancy and per-parturient period due to stress and decreased immune status.[9,31,33]

The present study revealed that higher prevalence of gastrointestinal nematode parasites in sheep of less than 1-year age (46.5%) followed by 1-2-years (44.6%) and greater than two years age (42.1%) groups. These findings were in line with the study of Shimelis et al[25] who recorded a higher prevalence of gastrointestinal nematodes in sheep of less than one-year <1 (69.2%), followed by 1-2-years (50.8%) and >2-years (37.0%) animals. This might be due to low immunity in younger than the older ones. These findings were also similar to other researchers[24,27,33,34] also stated that a significant immunity develops with age against a few parasites in adult stock. Pointed out the two age groups of sheep most commonly affected are weaned lambs and yearlings. These were agreed with the report of Hamdullah et al,[35] who recorded a higher prevalence of gastrointestinal nematodes parasites in sheep of less than one year age (60.8%) followed by >2-years (43.3%) and 1-2-years (41.3%) age groups.

It disagrees with the report of Tasawar et al[36] who reported a higher prevalence of gastrointestinal nematodes in older age animals than younger. The low nematode prevalence in younger sheep might bedue to grazing on (nearby home) low contaminated pastures and supplemental feeding (barley grain, green wheat, etc.). While higher nematode prevalence in adult sheep might be due to grazing on the larger areas of pastures being contaminated with various flocks and different stress conditions like climate, long daily traveling, and gestation, etc. They added that the sheep over 18-months of age are less commonly affected because of immunity, resulting from the previous infection. In contrast,[28] stated that there was no significant difference in sheep nematodes prevalence between age and month. Young animals also get infected with internal parasitic ova from the contaminated pastures being spread for the female animals during the gestation period because of the higher parasitic load at thisstage.

Three genus of gastrointestinal nematode parasites were recovered from the gastrointestinal tract during the study period. Among these, *Haemonchus* was highest (20.8%) Inprevalence followed by *Trichostrongylus* (13.0%) and *Nematodirus* (10.2%) respectively. These findings are in agreement with Lateef et al[27] who recorded the highest prevalence of *Haemonchus* (61.5%) followed by *Trichostrongylus* (46.1%) and *Ostertagia* (33.0%). Similarly, Asif et al[37] reported that *Haemonchus* was higher inprevalence (80.6%) followed by *Trichuris* (32.3%) and *Nematodirus* (29.0%), respectively.[38] Recorded the prevalence of *Haemonchus* (28.9%), *Trichuris* (40.0%), and *Nematodirus* (11.1%) in sheep.[39] Recorded the genera of nematodes *Haemonchus* and *Trichostrongylus* as (33.0%) and (29.0%), respectively Abunna et al[28] observed the prevalence of *Haemonchus* (78.1%) and *Trichostrongylus* species (90.4%) in sheep.[40] Recorded genera-wise prevalence in sheep for strongly type eggs, Strongyloides, *Trichuris* as (39.8%), (17.5%) and (7.8%), respectively. Kantzoura et al[41] Reported prevalence in sheep for *Nematodirus* as (1.1%) and *Trichuris* as (2.9%).[42] Recorded prevalence of *Haemonchus* and *Trichuris* (6.5%) and (5.7%), respectively. While Al-Shaibani et al[34] recorded a higher prevalence of *Haemonchus* (24.6%) which was found to be predominant of gastrointestinal nematode parasites while *Trichostrongylus* (18.0%) was the next most prevalent species. while Khan et al[43] reported the same parasites in upland districts of Baluchistan.

Differences in the occurrence of different gastrointestinal nematode parasites in the present and other studies carried out in different locations might be due to different ecologies, temperatures and pastures.[44] Reported that the occurrence of gastrointestinal nematodes was higher and lower in arid conditions of upland Baluchistan as compared to the semi-humid, subtropical climate of Punjab province.[45] Pointed out that warm, wet weather provides favorable conditions for the translation of eggs to larvae in the majority of helminths. The areas having severe summer and dry winter reduced the parasitic burden on the local livestock. It was observed in the present study that most of the flocks were sedentary and they were under strict confinement during grazing which leads to a high-risk of helminths infection.[46] In thepresent study among three genus of gastrointestinal nematodes. *Haemonchus* was the most prevalent nematode. It might be due to its biotic potential which justified the percentage of infection.[39]

Trichostrongylus was the next most prevalent nematode parasite in the present study. These findings are in contrast to those of Busetti et al[47] mentioned that *Trichostrongylus* populations were high in autumn and reached their peaks in June to July, while the highest larval availability was in autumn. *Trichostrongylus* species has the capability to developed and survive at a lower temperature.[48]

The prevalence of *Nematodirus* was (10.5%) in the present study. These findings are close to the findings of Asif et al[37] who reported prevalence in sheep for *Nematodirus* as (11.1%) and Kantzoura et al[41] reported lower prevalence (1.1%). Some particular parasites, *Nematodirus*, have no obvious seasonal pattern of occurrence, and chill temperature requirement for larval development are important factors for their prevalence during a different period of year. Similarly, some climatic factors like warmness and moisture favor development and allow the accumulation of large numbers of infective stages on the rangelands.[33]

In this study, a significant difference was observed in prevalence of nematode infection in relation to body condition where a higher prevalence of gastrointestinal nematodes parasites were recorded in poor and moderate body as compared to animals with good body condition. Difference in body condition score is statistically significant ($p<0.05$) with gastrointestinal nematode prevalence such that shedding of nematodes eggs increased with poor body condition (71.6%) than in medium (37.0%) and good body condition (21.0%). This finding was agrees with Gonfa et al[49]; Radostits et al.[50]

CONCLUSION AND RECOMMENDATIONS

Gastrointestinal nematode parasites are the major health constraints in sheep production and contributing loss in productivity and economy. The present study was based on fecal examination for detection of gastrointestinal nematode eggs; it has provided the current prevalence and associated factors. It suggested that ovine gastrointestinal nematodes are of the major problem in the study area. There was significant difference within body condition of animals and genus of the parasite. However, significant difference was not recorded within age, sex and agro-ecology.

Based on the above conclusion the following recommendations are forwarded:

- Detailed study should be conducted to identify the parasite at species level.
- Special consideration should be taken on the management of sheep in poor body condition to reduce burden of gastrointestinal nematodes.
- Further investigation is needed to study on the association of prevalence of gastrointestinal nematodes within age, sex and agro-ecology in the area.

ETHICAL CONSIDERATION

The case report was done under super vision of Mekelle University, College of Veterinary Medicine ethical review committee and had critically reviewed and concludes that there was no ethical misconduct. The approval of ethical committee was taken for conducting this study and followed all the animal ethics and welfare guidelines.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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ANNEXES

ANNEX 1. *Estimation of Age of Sheep*[18]

Prominent Incisors	Age of Sheep
None	less than 1-year
One pair	1-year
2 pair	1-year to 10-months
Above 3 pair	more than 2-year

ANNEX 2. *Body Condition Score of Sheep*

Condition Score	Indications in Number	Body Condition
Starving	0	Extremely thin, nearly dead, no muscle between skin and bone
Very thin	1	Spinous process sharp sticks up. Thoracic processes are sharp and fingers easily pushed under thin end. There is hollow between the ends of each process, lion muscles are shallow.
Thin	2	Spinous process fell less sharp; fingers can be pushed under the thoracic process with the little pressure. lion muscles are moderate depth
Moderate	3	Spinous process only sticks up very slightly; they are smooth and rounded. Firm pressure is needed to detect each one separately. Thoracic processes are smooth and well covered, firm pressure is required to push, lion muscles are full.
Fat	4	Spinous process can just be felt with firm pressure as herd line and level with the flesh on either side. The ends of the thoracic process cannot be felt, lion muscles are full.
Very fat	5	Spinous process cannot be felt at all. Thoracic process cannot be felt lion muscles are very fully developed.
Body condition of animals was classified in to three as poor, medium and good BCS 1 and 2 poor; BCS 3 medium; BCS 4 and 5 good. Source[19]		

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Review

Hepatic Diseases in Canine and Feline: A Review

Kassahun A. Negasee, DVM, MSc*

Department of Animal Health, Awi Zone Livestock Office, Amhara Region, Ethiopia

Corresponding author
Kassahun A. Negasee, DVM, MSc
Specialist in Veterinary Medicine, Department of Animal Health, Awi Zone Livestock Office, Amhara Region, Ethiopia; E-mail: kassahunayana@gmail.com

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ABSTRACT

Dogs and cats are belonging to canine and feline family respectively. The liver is the largest gland in the body and is located in the cranial abdomen between 3rd and 4th ribs in dogs and cats. This review is mainly focused on: to understand the anatomy and physiology of liver, the liver diseases pathophysiology, to diagnose the liver diseases, managemental and ameliorative methods of liver diseases. The dual blood supply to the liver is hepatic artery and portal vein. The function of liver includes the regulation of digestion and metabolism, the synthesis of hormones and proteins, immune response and filtering of toxins from the blood stream. Any problem that affects the liver is liver disease. Inflammation of liver is hepatitis. Hepatitis caused by infectious, non-infectious, auto-immune and reactive. It can be acute and chronic. The most encountered liver diseases in dogs and cats are hepticlipidosis, cholangiohepatitis, portosystemicshunt, cholelithiasis, choledocholithiasis, cholecystitis, pneumobilia and hepatic neoplsia. The clinical symptoms of liver diseases include jaundice, hepatic encephalopathy, gastro intestinal disorders and non-specific signs include polyuria/polydipisia. The liver disease diagnosed based on history, liver function tests, medical imaging. The latest imaging procedures are endoscopic retrograde cholagio pancreatography (ERCP) and computed tomography (CT). For confirmatory diagnosis liver biopsy and histopathological interpretation is required. Therefore, based on diagnosis appropriate treatment should be selected: bile stasis is treated urodeoxycholic acid (URDA), fluid therapy include sugar and salt solution for replacement fluid loss, gastrointestinal protectors include ranitidine, cimitidine and lactulose, albumin to treat hypoalbuminimia, antioxidant for scavenging free radicals include vitamin E, Selenium, S-adenosyl-L-methionine (SAMe), diuretic fursimid for treatment of ascites and supplementation low protein diets. This laparoscopic technique for removal of gallstones and endoscopic retrograde cholagiopancreatography (ERCP) to treat gall stones on bile ducts and to widen the slipped ducts and liver transplantation for cirrhotic dogs and cats. Early accurate diagnosis and managing any predisposing factors that affects the health of dogs and cats are important for controlling the liver diseases.

Keywords
Canine; Feline; Liver disease; Hepatitis; Hepatocyte; Kuppfer cells.

INTRODUCTION

Dogs and cats are domestic animals belonging to different species; canine and feline families respectively. There are many differences between a dog and a cat including their physical features, nature and character.[1] Both dogs and cats are our wonderful and lovable pets; canines and felines are friends more than anybody for human beings because of their keen observations, patience and easily adapt with people in the home.[2]

The dogs and cats like other animals for maintaining their life physiology have different organs and tissues among that is the digestive tract. Their function is digests food, absorption and eliminates wastes from the body.[3] The liver is the main principal largest glandular digestive tract organ located under a rib cage on the right side in the abdominal cavity.[4] It consists of hepatocytes, hepatic stellate cells or Ito cells and sinusoidal cells include kuppfer cells and endothelial cells.[5]

The major functions of the liver are carbohydrate, protein and fat metabolism, detoxification, secretion and storage. Thus, maintaining a healthy liver is a crucial factor for the overall health and well-being of life of animals.[6] It is vital and complex organ of the body, it become susceptible to many adverse effects including drugs, chemicals, infectious agents, autoimmune disease, and reactive hepatitis and there is also idiopathic occurrence.[7]

The liver disease includes hepatocellular reversible and irreversible injury (necrosis), porto systemic shunt, neoplsia (primary hepatic and secondary) and hepatic fibrosis or cirrhosis.[7] Pets

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with liver disease can present with arrangement of clinical conditions from severely ill to asymptomatic. Some vague signs can be depression, weight loss, jaundice, gastro-intestinal and neurological symptoms.[8]

The liver disease diagnosed by using history and physical examination, liver function tests and medical imaging techniques. However, for identification of specific hepatopathies definitive diagnosis of liver disease the histopathological examination is required to set recommended treatment.[9,10] The hepatic diseases are managed by reducing predisposing factors, providing intravenous fluid to rehydrate, administering gastro-intestinal protectants to prevent ulcerations, avoiding the use of sedatives, supplementation of low protein diets and Zinc for regulation of protein and nitrogen metabolism, cholecystectomy and antibiotics are also used for management of hepatic diseases.[10,11] Therefore, the objective of this review is to understand the anatomy and physiology of liver, the liver diseases pathophysiology, to diagnose the liver diseases, manage mental and ameliorative methods of liver diseases.

THE LIVER ANATOMY AND PHYSIOLOGY

The Liver Anatomy

The liver, that lies across the stomach and duodenum and derived from the embryonic gut.[3] The liver is the largest gland in the body and is located in the cranial abdomen between 3rd and 4th ribs in dogs and cats.[12] Its gross anatomical divisions comprise the right, left, caudate and quadrate lobes with their own blood supply and biliary drainage. The portahepatis transmits the hepatic artery, portal vein and right and left hepatic ducts (the portal triad), together with lymphatic and autonomic nerves. The venous drainage of the liver directly into the inferior vena cava comprises the right, left and middle hepatic veins, together with the small accessory hepatic veins.[13] The anatomy of the liver differs between dogs and cats. Dogs have two separate ducts a pancreatic duct and a bile duct. In cats, however, the bile duct joins the pancreatic duct; this may allow the reflux and mixing of gastric secretion with both pancreatic secretions and bile.[14]

The internal structure of the liver is made small hexagonal functional units known as lobules. Each lobule consists of a central vein surrounded by hepatic portal veins and hepatic arteries. These blood vessels are connected by many capillary-like tubes called sinusoids which extends from the portal veins and arteries to meet the central vein like spokes on a wheel. Each sinusoid passes through liver tissue containing two main cell types including Kupffer cells and hepatoctes.[10,15]

Kupffer cells are a type of macrophages that passes through the sinusoids. Hepatocytes are liver cells which are cuboidal epithelial cells that line the sinusoids and make up the majority of cells in the liver.[14,15] Canaliculi, tiny bile collection vessels run parallel to the sinusoids on the other side of the hepatocyte and drain into the bile ducts of the liver.[15]

Physiology of the Liver

The liver plays an active role in the process of digestion through the production of bile.[16] When food containing fats reaches the duodenum, the cells of the duodenum release the hormone cholecystokinine to stimulate the gallbladder to release bile. Bile travels through the bile ducts and is released into the duodenum where it emulsifies large masses of fat.[15]

The worn out red blood cells called bilirubin, present in the bile is the product of liver's digestion. Kupffer cells in the liver catch and destroy old, worn out red blood cells and pass their components on to hepatocytes.[16] They metabolized by hepatocytes into hemoglobin (the red oxygen-carrying pigment of red blood cells) into the components heme and globin. Globin protein is further broken down and used as an energy source for the body. The iron-containing heme group cannot be recycled by the body and is converted into the pigment bilirubin and added to bile to be excreted from the body.[15-17] Bilirubin gives bile its distinctive greenish color. Intestinal bacteria further convert bilirubin into the brown pigment stercobilin, which gives feces their brown color.[14,15]

The hepatocytes have the important metabolic jobs that support the cells of the body because all of the blood leaving the digestive system passes through the hepatic portal vein. The liver is responsible for metabolizing carbohydrates, lipids, and proteins into biologically useful materials.[15,17]

Blood passes from the digestive organs through the hepatic portal circulation, the hepatocytes of the liver monitor the contents of the blood and remove many potentially toxic substances before they can reach the rest of the body. Enzymes in hepatocytes metabolize many of these toxins such as alcohols and drugs into their inactive metabolites. The liver also metabolizes and removes hormones from circulation produced by the body's own glands to maintain normal homeostasis of the body.[15,19]

The liver is storage organs of many essential nutrients, vitamins and minerals obtained from blood passing through the hepatic portal system.[16] Glucose is transported into hepatocytes under the influence of the hormone insulin and stored as the polysaccharide glycogen,[17] this maintain the blood glucose homeostasis.[15,16] The liver cells also absorb and stores fatty acids from digested triglyceride's,[15] vitamins such as vitamins A, D, E, K, and B[12,17] the minerals iron and copper-in order to provide a constant supply of these essential substances to the tissues of the body.[16,17]

The liver is responsible for the production of several vital proteins components of prothrombin, fibrinogen and albumins. Prothrombin and fibrinogen proteins are coagulating factors involved in the formation of blood clots. Albumins are proteins that maintain the isotonic environment of the blood so that cells of the body do not gain or lose water in the presence of body fluids and it is carrier protein for carrying different chemicals, hormones, nutrients and cells for normal functioning of the body.[16]

The liver functions as an organ of the immune system through the function of the Kupffer cells that line the sinusoids. Kupffer cells are a type of fixed macrophage that form part of the mononuclear phagocyte system along with macrophages in the spleen and lymph nodes. The liver macrophages Kupffer cells play

an important role by capturing and digesting bacteria, fungi, parasites, worn-out blood cells and cellular debris.[16,19]

THE LIVER DISEASES IN DOGS AND CATS

Any disorder that damages the liver is liver disease. Dogs and cats suffering from liver disease can be in serious danger, since the liver performs a number of important functions throughout the body.[19] Liver disease is a problem occasionally occurred in older animals acquired diseases and in younger animals with both acquired and congenital disease.[20] Hepatitis is familiarly termed as inflammation to the liver. It can be incurred by both infectious and non-infectious ways.[21] It is very likely that more than one causative infectious agent may cause hepatitis in small animals.[22]

The Liver Diseases Ethiopathophysiology

Infectious hepatitis: The liver of small animals (dogs and cats), other animals and humans beings can be affected by a number of infectious agents like viruses, bacteria, parasites, fungus and protozoa. The liver cells affected by a number of infectious agents and results inability of the liver to function properly.[22]

Viral hepatitis: Inflammation of the liver by virus is named as viral hepatitis. It is very specific for each felines and canines. Infectious canine hepatitis is an acute liver infection in dogs caused by canine adenovirus type-1.[23] Infection with canine herpes virus causes an acute, rapidly fatal illness associated with hepatic necrosis vasculitis and/or immune-mediated mechanisms.[24] Viruses that cause feline leukemia and feline infectious peritonitis can results in feline viral hepatitis, as the viruses destroy liver tissues. These pathogens not only destroy liver tissue but also affect other organs of the body.[25]

Parasites diseases: Infection with protozoan parasites Toxoplasma gondi and *Leishmaniainfantum* in cats and dogs cause chronic hepatitis by affecting kupper cells and hepatocytes in immune compromised patients.[26] Infection with liver fluke *Platynosomumconcinnum* can cause acute and chronic cholangitis. Since the fluke infecting emerges from the intestine and migrate into the common bile duct, gallbladder, or hepatic ducts and cause hepatic bile duct damage.[27]

Bacterial diseases: Leptospirosis is caused by *Leptospira Interrogans Serovars IcterohemorrhagicAe* and canicola which is the most common pathogenic bacteria that affect the liver of small animals. The disease produces an acute multisystem disease affecting the liver, kidney and other organs. It is known to cause hepatitis[28] due to direct leptospiral cytotoxic effect on endothelial hepatocytic membranes.[29] Infections in dogs and cats most commonly occur in immune compromised. It is an infectious disease characterized by necrosis of liver since the bacteria resides in cytoplasm of hepatocytes and results liver swelling with multiple areas of hepatocellular necrosis with infiltrates of neutrophils and mononuclear cells.[30]

Mycotic infection: The most common mycotic infections associated with liver dysfunction in small animals' are candidiasis is caused by *Candidiaalbicans*, *histoplasmosis* by *Histoplasmacapsulatom*; aspergilosis by *Aspergilus fumigates*.[31] Fungal infections occurred in immune compromised patients. The spores from lungs or the intestines, or it may spread to other parts of the body through the bloodstream or lymphatic system, causing a generalized or systemic infection in different organs including liver.[20]

Non Infectious

Wilson's disease: Wilson's disease is anautosomal recessive inherited disease of copper metabolism. When the hepatic storage capacity of Cu is exceeded, parenchymal inflammation followed by cell death with Cu release into the plasma causing hemolysis. This causes an inability to excrete copper into the biliary tract, and results the formation and release of free copper which is toxic and since has the potential to create reactive oxygen species cause hepatocyte damage and subsequent chronic hepatitis and cirrhosis.[32]

Drugs and toxins The liver is the major site of drug metabolism and is therefore a common target of adverse drug reactions. Hepatotoxicity implies chemical-driven liver damage. Drug-induced hepatotoxicity is a significant cause of acute liver failure. Certain medicinal agents, when taken in overdoses and sometimes even when introduced within therapeutic ranges, may injure the organ.[19] Other chemical agents, such as those used in laboratories and industries, natural chemicals and herbal remedies can also induce hepatotoxicity. Chemicals that cause liver injury are called hepatotoxins.[6,19]

Drugs can cause liver injury in several ways, three main types are usually referred dose-dependent (or intrinsic), dose-independent (or idiosyncratic metabolic) toxicity and drug allergy (or idiosyncratic immunological). These drugs have inherent ability to cause hepatic injury either by directs hepatocellular damage or by disturbance of hepatocellular homeostasis, which results in liver cell death.[33]

Drugs that cause liver damage in small animals (dogs and cats) are anticonvulsants drugs include primidone; phenytoin and phenobarbital have hepatotoxic effects, especially in long-term treatment. Antifungal ketoconazole and antibiotic (trimethoprim-sulfa), antihelminths (mebendazole, diethylcarbamazine- oxybendazole, and thiacetarsamide), inhalation anesthetics (halothane and methoxyflurane) and analgesics (acetaminophen, naproxen, and phenylbutazone) cause toxicity on liver cells, Also certain environmental toxins (pesticides, herbicides, cleaning agents and plant toxins) have hepatotoxic effects in dogs and cats as well as other animals.[22] The other hepatotoxicity include plant aflatoxins, cyanotoxins and mushroom toxins have direct hepatotoxic effect liver cells and cause acute liver failure.[34]

Autoimmunity Hepatitis

Autoimmune disorders results from an exaggerated reaction of the immune system directed against the body's own tissue. In artificial insemination by husband (AIH), the liver cells are no longer recognized as 'belonging' to organisms are there attacked by the immune system resulting in chronic inflammation of the liver.[35] The immune reaction may be related to defects in the immunological control of auto-reactivity; with consequent loss of self-tolerance to liver auto-antigens. The aetiology of AIH remains unknown

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or idiopathic, but evidence suggests a coalescence of genetic susceptibility and environmental risks, female dogs and cats are more susceptible.[36]

Reactive Hepatitis

Reactive hepatitis is an inflammatory disorder of the liver induced by an extra hepatic process. It is associated with disorders of many other organs apart from the liver including gastrointestinal, respiratory diseases, heart failure, diseases of the urinary and reproductive system. Different inflammatory mediators cytokines such as Interleukin-1 (IL-1), Interleukin-6 (IL-6) and Tumor Necrosis Factor- (TNF-) are released as lipopolysaccharide (LPS) can activate kupffer cells in the liver parenchyma. A consequence of this activation is the release of pro-inflammatory that induces leukocyte migration and therefore induces reactive hepatitis results excessive damage of liver cells.[37]

Endocrine Disorder

Endocrine diseases are imbalances in hormone levels. Hormone imbalances can affect pet's health in many ways. Endocrine diseases develop when the body produces too much hormone or too little hormone. Diabetes mellitus, hyperadrenocorticism (Cushing's disease), and hyperthyroidism can all cause impaired liver function because of their effects on the organ.[38]

Hyperthyroidism is one of the most common endocrine disorders which cause liver dysfunction. It is characterized by increased secretion of thyroid hormones T3 and/or T4. Excess T3 and/or T4 induce apoptosis of hepatoctes which is programmed cell death and cause liver dysfunction it is more common in cats but rare in dogs.[39]

Another endocrine disorder is Cushing's disease (hyperadrenocorticism). It is excessive production of cortisol hormone produced by the abnormalities adrenal glands. It is a common disease of adrenal hyper function that is seen most commonly in the dogs and rare in cats.[40] In liver, cortisol induces enzymes activity and also decreases insulin from pancreas to then leading to hyperglycemia in Cushing's dogs and cats. Excessive liver metabolism causes the liver overload and the liver become hepatomegaly.[18]

Bile Duct Obstruction

Obstruction of the common bile duct is associated with a number of diverse primary conditions, including inflammation (e.g, pancreatitis, duodenitis, duodenal foreign body, etc.), cholelithiasis, gallbladder mucocele, cholecystitis, neoplsia, bile duct malformations, parasitic infection the bile duct and extrinsic compression, and fibrosis.[19]

Complete bile duct obstruction results cholestasis which is an impairment of bile flow from the liver to the duodenum. Bile cannot enter the distal "stagnant loop" of the ductal system or gallbladder (cystic duct occlusion). Increased ductal mucin contributes to duct distention. In this the biliary tree becomes colonized by bacteria cause cholangitis and ascending infection of the liver, inadequate antibiotic penetration into bile duct and results liver dysfunction from liver cell damage.[41]

General Classification of Liver Diseases

All diseases of hepatic parenchyma, vasculature or biliary tract are included as liver diseases.[2] Hepatitis is inflammation of the liver, it can be acute and chronic. The most common liver diseases encountered in small animals include acute and chronic hepatitis.[38]

Acute hepatitis: Acute hepatitis is morphologically characterized by combination of inflammation hepatocellular apoptosis and necrosis and in some instances, regeneration. This can result in sudden death of the animal even within 48-hours after the start of the disease.[28]

Chronic hepatitis: Chronic liver disease is also known as chronic hepatitis refers to a long-term pathological process of continuous destruction of liver parenchyma and its gradual substitution with fibrous tissue, which ultimately results in liver cirrhosis associated with a fatal outcome.[42] It has structurally abnormal nodules (micro- or macro-nodules), it is considered irreversible, and usually it is idiopathic in origin. It is a regularly diagnosed condition in dogs, is less frequently encountered in cats but more frequently in dogs.[43] Chronic liver dysfunction or injury is a serious health problem worldwide in animals and human beings. Chronic liver disease involves a wide range of liver pathologies that include hepatic lipidosis, neoplsia, fibrosis or cirrhosis, cholangitis complex.[44]

Liver Diseases Pathophysiology

Portosystemic shunt: A portosystemic shunt (PSS) is a congenital malformation within the blood supply to the liver. Blood coming from the digestive system is shunted from the portal circulation, around the liver effectively bypassing it. The implication of PSS is that toxins, such as ammonia, which would typically be removed by the liver can, accumulate in the systemic circulation leading to clinical signs including stunted growth and neurological symptoms.[19,45]

Hepatic lipidosis: Hepatic lipidosis (HL) is the accumulation of fat in the liver cells as fat is mobilized from the body stores for energy source. HL occurs in cats that become anorexic and lose significant amounts of weight.[46]

Biliary Tract Abnormalities Dogs and Cats

Cholangitis complex: Cholangitis (cholangiohepatitis) is inflammation of the biliary system and liver. It is the most common primary hepatic disorder in felines. The two main forms of cholangitis neutrophilic are cholangitis and lymphocytic cholangitis. Neutrophil cholangitis is thought to be caused by an ascending infection of the biliary tract and stemming from the intestine. Feline anatomy is thought to predispose to developing this condition. Pancreatitis and/or inflammatory bowel disease may occur along side neutrophilic cholangitis and may predispose to it. Lymphocytic cholangitis is thought to be immune-mediated; however, the aetiology is not known.[11,47]

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Cholelithiasis: Cholelithiasis is medical condition resulting from the formation of stones in the gallbladder.[48] Choleliths (gallbladder stones) are thought to form due to imbalances influxes between bile salts and cholesterol that maintain a liquid composition to bile. Resultant change to a thicker or congealed form of bile provides scaffolding for the deposition of cholesterol, bilirubin, or calcium salts resulting in the formation of choleliths. Other factors thought to promote cholelith formation include increased levels of gallbladder motility, bile stasis and biliary inflammation.[49]

Pneumobilia: The presence of gas in the biliary system is pneumobilia. It is a common finding in dogs and cats that have recently undergone biliary surgery or endoscopic biliary procedure, infection by gas forming bacteria (*emphysematous cholangitis*).[50]

Choledocholithiasis: It is condition when a gallstone lodged within any duct of the bile system. The ducts typically involved are the common bile duct, the cystic duct and the common hepatic duct. Gallstones usually form in the gallbladder.[51]

Cholecystitis: Cholecystitis means painful inflammation of the gallbladder most cases are caused by gallstones. Gallstone becomes stuck in the cystic duct (this is the tube that drains bile out from the gallbladder into the bile duct). Bile then builds up in the gallbladder which becomes stretched (distended). The walls of the gallbladder become inflamed. In some cases the inflamed gallbladder becomes infected. An infected gallbladder is more prone to lead to complications.[52]

Hepatic Neoplsia

Neoplsia can occur in the liver either as a primary disease or due to metastases. The categories of hepatic tumors in cats and dogs are hepatocellular, bile duct and mesenchymal.[18] In dogs, malignant tumors are more common, whereas benign neoplsia particularly cystic bile duct adenoma is more frequent in cats. Metastasis to the liver is most common from tumors of the spleen, pancrease, and gastrointestinal tract since the liver is rich in blood supply. Animals may have increased liver enzyme activities detected on serum biochemistry.[53]

Mostly Encountered Clinical Symptoms of Liver Diseases Pathophysiology

Hepatic encephalopathy: Hepatic encephalopathy can be acquired and congenital neurological changes that occur with liver failure as the liver becomes less able to remove toxins, drugs and metabolites from the blood.[18] Ammonia is absorbed in the intestine and most of it is taken up by liver, where it is mostly converted to urea. An increased ammonia concentration in systemic circulation indicates the liver unable to adequately metabolize ammonia. It can also be caused by a defect in the urea cycle, or anomalies in portal circulation causing blood from the intestine to bypass the liver, as described on figure.[54] It results abnormal mentation and neurologic dysfunction occurs as a result of exposure of the cerebral cortex to absorbed intestinal toxins that have not been removed from the portal circulation by the liver.[18,19]

Portal hypertension and ascites: Chronic liver disease increases resistance blood flow to the liver and results portal hypertension and the back flow of blood, lymphatic fluid and increased pressure in the portal system. From increased pressure in portal system there is leakage of blood from capillaries and causes abnormal fluid accumulation in abdomen cavity is as cites from low albumen production in the liver, organ failure like liver and heart.[28,55]

Jaundice (Ictrus): One of the most common symptoms of liver disease is jaundice which is yellowish tinge to the skin, in the eyes gums and ears. The liver is responsible for excreting bilirubin, by-product of red blood cell breakdown. When the liver isn't functioning this bilirubin builds up in the blood and leads to the yellowish appearance. The causes of jaundice are classified as pre-hepatic, hepatic, or post-hepatic in origin. Pre-hepatic jaundice occurs when red blood cell breakdown or hemolysis, produces bilirubin faster than the liver can metabolize it. Hepatic jaundice results from primary and seciseases within the liver that interfere with the liver cells' ability to metabolize bilirubin or excrete it normally into the biliary tract. Post-hepatic jaundice can result from obstruction to the flow of bilirubin-containing bile within the bile duct or from injury that causes leakage from the gallbladder or bile duct.[20,46]

Diagnosis of liver disease: Diagnosis of liver diseases can be difficult because the symptoms of the organ may be ambiguous or may easily interfere with the symptoms of other diseases. However, liver disease can be diagnosed by using history and clinical symptoms, liver function tests, medical imaging and tissue analysis or biopsy.[2]

Clinical symptoms: The liver has a large reserve capacity for many of the functions it carries out which results in relatively specific clinical indications of hepatobiliary diseases, such as icterus, ascites, coagulopathy or increases bleeding time, neurological signs and abdominal pain.[41] In addition, early indications of a hepatobiliary disease, such as apathy, anorexia, polyuria, polydipsia, vomiting, weight loss, and anemia are highly non-specific and may occur as a result of diseases affecting many other organ systems.[56]

Liver Function Tests Other than Liver Enzymes

Liver function tests are helped to check livers health and detect liver damage. These tests known as liver chemistries to help to determine the health of liver by measuring the levels of proteins and bilirubin, bile acids, copper, cholesterol, glucose and complete blood count.[57]

Billirubin: An elevated level of bilirubin presence in the blood and urine indicates jaundice may become clinically evident as serum bilirubin level rises above the normal level in dogs 0.1-0.3 mg/dL and 0.1-0.4 mg/dL in cats.[56,58]

Bile acid: Bile acid concentrations elevation in dogs and cats are suggestive of hepatobiliary disease. Bile acid concentrations elevation from the normal range 25-30 μmol/L and 25 μmol/L in dogs and cats respectively are suggestive of hepatobiliary disease, i.e. decreased functional mass, alterations in portal circulation (or cholestasis).[59]

Albumin: Albumin is produced by hepatocytes it is released into

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the hepatic interstitial and subsequently into the sinusoids and hepatic veins. Hypoalbuminaemia is occurred on animals where the level of albumen in the serum is less than the normal in dogs 2.7-4.4 mg/L and 2.5-3.9 mg/L in cats.[58] But hypoalbuminaemia usually is not specific to liver disease and can occur due to many other diseases the most important are protein-losing enteropathy, vasculitis, large exudative skin injuries and blood loss.[60]

Urea: Reduced blood urea nitrogen below normal range 6-25 mg/dL in dogs and 14-36 mg/dL in cats indicates liver insufficiency.[59] In the case of hepatic insufficiency (e.g., due to liver atrophy caused by a portosystemic shunt, PSS), urea levels may be reduced, as the liver cells are no longer capable of producing sufficient urea.[61]

Cholesterol

Cholesterol is one of several fatty substances (lipids) that circulate in pet's blood. Hypercholesterolemia may occur in conjunction with diseases associated with a cholestasis, there is a whole range of differential diagnoses for increased cholesterol values, while there are only a few causes of reduced cholesterol values (protein-losing enteropathy, certain malignant tumors and severe malnutrition).[62] The elevation of cholesterol from the normal level 3-6.6 mmol/L in dogs and 1.8-4.2 mmol/L in cats usually indicates liver disease.[59]

Liver Function Test Based on Liver Enzymes

Elevated liver enzymes may indicate inflammation or damage to liver cells. Inflamed or injured liver cells leak higher amounts liver enzymes into the bloodstream, which can result in elevated liver enzymes on blood tests. The elevated liver enzymes most commonly found are Alanine transaminase (ALT), Aspartate transaminase (AST), Alkaline phosphatase (ALP), Gamma-glutamyltranspeptidase (GGT).[63]

Alanine aminotransferase: Alanine aminotransferase is an enzyme specific to the liver that is found in the cytoplasm of the liver cells. It is almost exclusively found within hepatocytes so serum ALT increase indicates high hepatocellular injury in dogs and cats (there is only a minor contribution from skeletal muscle and red blood cells). ALT can also increase mildly with muscular injury and gastrointestinal disease. The magnitude increase may relate to severity of liver damage but does not predict reversibility.[64] In dogs and cats the normal range of ALT is 5-107 µ/L and 10-100 µ/L respectively.[65]

Alkaline phosphatase: It is primarily an indicator of cholestasis liver disease. It also increases with severe bone destruction and due to steroid induction. Hepatic ALP is found mainly in liver canalicular cell membranes and increases with biliary disease, especially with cholestasis.[66] In dogs and cats the normal range of ALP is 10-150 µ/L and 6-102 µ/L respectively.[59]

Gamma glutamyltransferase: Gamma glutamyltransferase (GGT) is found in many tissues, but serum GGT originates mainly in the liver. It is predominantly found in the intrahepatic biliary epithelial cells. Increases in GGT are most frequently observed in cholestatic liver diseases. In cats GGT often elevates in hepatic lipidosis where GGT elevation is uncommon in other liver diseases. In dogs GGT activity in liver disease is higher specificity and lower sensitivity for detection of hepatobiliary disease.[66] In dogs and cats the normal range of GGT 0-14 µ/L and 1-10 µ/L respectively.[61,64]

Diagnostic imaging in liver diseases: Radiography and abdominal ultrasound are the most frequently used imaging modalities for assessment of the hepatobiliary system in dogs and cats, but alternative imaging techniques are now being used more frequently used are computed tomography and Endoscopic retrograde cholangiopancreatography.[65]

Ultrasonography: Abdominal ultrasonography (US) is considered the most practical diagnostic imaging procedure for detecting a hepatobiliary disease. US well-suited for liver imaging because it shows the alterations in the tissue structure as differences in echogenicity. This allows comparing liver tissue to other soft tissues, as well as detecting heterogeneous structures within the liver parenchyma.[2] Hepatic lipidosis in cats could be diagnosed slightly more accurately than other diffuse hepatic diseases.[60]

Radiography: Radiography may be used in liver diagnostics mostly to evaluate the size and shape of the liver and possibly to locate the lesions more specifically.[2] Abdominal radiographs allow assessment of hepatic size, shape, opacity and location in most patients. Radiography may also allow identification of extra hepatic abnormalities that affect the liver.[66]

Computed tomography (CT): It distinguishes mass lesions, detect changes in structure of hepatic parenchyma and the biliary system, identify choleliths, detect abnormal hepatic perfusion (involving the portal vein, hepatic artery or hepatic vein), and portal thrombi, and can give detail the extent of traumatic hepatobiliary injuries.[2]

Endoscopic retrograde cholangiopancreatography: Endoscopic retrograde cholangiopancreatography (ERCP) is advanced medical imaging a procedure that uses an endoscope and X-rays to look bile duct and pancreatic duct by inject a contrast medium, so they can be seen on radiographs. It is the latest technological method used primarily to diagnose and treat conditions of the bile ducts and main pancreatic duct, including gallstones as seen figure , inflammatory strictures (scars), leaks (from trauma and surgery) and cancer.[67]

Liver Biopsy

The liver biopsy is the most accurate means to determine the necroinflammatory activity process.[62] Liver biopsy and histology is the final examination in the diagnostic tree and provides a diagnosis of the lesions prognosis and for the selection of appropriate treatment, since liver biopsy is the only definitive way to obtain a specific diagnosis.[67]

It is obtained through the skin with a special needle and ultrasound guidance, or it may be obtained surgically under general anesthesia.[66] Liver biopsy provides main histologic features in favors of liver disease lesion are: a histologic picture includes

granulomas; substantial numbers of eosinophils, sharply stamped-out perivenular necrosis, ground-glass-like hepatocytes, unusual vascular lesions, veno-occlusive lesions can be detected on eosine and hematoxylin staining procedures.[62,67]

Therefore, the diagnosis is usually based on pharmacological history; the relationship between drug intake and the onset of clinical signs. Another diagnosis is done by detecting the pathogen in clinical specimens and/or demonstrating an increase in antibody.[68] Direct detection of the toxins in gastric content is confirmatory diagnosis.[32]

TREATMENT AND MANAGEMENT OF LIVER DISEASES

Hepatic disease is often treatable and has a predictable prognosis when a definitive diagnosis is made. Anti-inflammatory drugs, antioxidants, diuretics, appropriate low protein diets, fluid therapies, antibiotics and protectants are mostly used for the treatment of liver disease and the latest advanced instrumental methods liver disease treatment.[52,67]

Antioxidants and Choleretics

Oxidation is a significant mechanism of hepatocellular damage; therefore providing antioxidants are free radical scavengers include vitamin E, zinc, silymarin (milk thistle), N-acetyl cistine (NAC) and SAMe. SAMe which increases hepatic and red blood cell glutathione levels is widely available as a nutraceutical in dogs and cats. It is helpful for treating toxic hepatopathies in humans and there is evidence for best efficacy the use of both SAMe and silymarin in dogs with acute toxic hepatopathies treatment.[18,28]

Choleretic stimulates bile flow indicated in all biliary stasis cases. The best choice choleretis is ursodeoxycholic acid (UDCA) and Metronidazole relies upon hepatic clearance. They are widely used in both human and veterinary. They displace toxic bile acids also important in immune modulating and encourage antioxidant activity. UDCA synergistic action with SAMe and vitamin E. It should be avoided in dogs with complete biliary obstruction, which is uncommon in dogs due to its potential to cause gallbladder rupture.[14,28,67]

Diuretics and Gastro Protectants and Dietary Management

It is important to check blood albumin levels, and if low, dietary control by supplementing the animal with a high biological value protein including cottage, cheese and fish are advised.[28] The administration of blood products, e.g. canine plasma or human albumin solutions and also thiazide diuretics or furosemide can be initially used in combination with spironolactone to 'speed up' diuresis.[14] Therapeutic paracentesis, which can cause a significant drop in blood albumin levels due to inability of the diseased liver to make up for the loss, should be avoided unless the ascites are life-threatening.[60,67]

Portal hypertension is common in dogs with chronic hepatitis and leads to gut-wall oedema, which is at risk of ulceration. Perforation of GI ulcers causing septic peritonitis is a common cause of death in patients with chronic PH. Anorexia predisposes a patient to gastro intestinal ulceration; therefore, ensuring adequate enteral nutrition is important and supplementation ranitidine, Cimetidine and omeprazole are commonly.[63,66] Medications such as lactulose is required to reduce gut absorption of ammonia if the liver is too damaged to break.[67] Zinc supplementation has been shown to reduce inflammation reduce copper absorption form the gut and to protect the liver.[18,62]

D-Penicillamine (DPA) is the most commonly used as chelator to treat hepatic copper accumulation and treatment is most effective in early stages of disease. A low-copper/high-zinc diet can help to prevent accumulation or reaccumulation of hepatic copper in dogs with complex forms of copper-associated hepatitis 14. 2, 2, 2-tetramine tetrahydrochlodide may be more useful in acute hepatitis.[68] Zinc gluconate or acetate can be used prophylactically, especially in young dogs known to have copper storage disease, to reduce the absorption of copper from the gastrointestinal tract (GI) and prevent the development of copper-associated hepatitis.[28,67]

Antibiotics and Antifibrotics

Ampillicin, cephalosporins, enrofloxacin, metronidazole and clindamycin and chloramphenicol are good choices antibiotics for treating bacterial liver diseases.[10] Colchicines are a specific antifibrotics used in dogs with moderate to marked fibrosis.[18,67]

Fluid Therapy

Animals severely affected by liver disease, particularly those that are vomiting may require a period of hospitalization and intravenous fluids to help to flush out toxins from the blood stream and replace fluid lost in vomiting.[68] Intravenous fluid include glucose, sodium salts and blood products as needed depending are required for treatment of liver disease in dogs and cats as well as human beings.[34]

Latest Advanced Liver Disease Treatment

Cholecystectomy: Cholecystectomy is surgical removal of the gallbladder. It is a common treatment of symptomatic gallstones and other gallbladder conditions. It is a laparoscopic cholecystectomy, and surgical on the gallbladder.[67]

Endoscopic retrograde cholangiopancreatography: Endoscopic retrograde cholangiopancreatography (ERCP), is an endoscopic procedure that can remove gallstones or prevent blockages by widening parts of the bile duct where gallstones frequently get stuck. ERCP is often used to retrieve stones stuck in the common bile duct with gallstone pancreatitis or cholangitis.[65]

Liver transplantation: A liver transplant is a surgical procedure that removes a liver that no longer functions properly (liver failure) and replaces it with a healthy liver. Liver transplant is usually reserved as a treatment option due to end-stage of chronic liver disease usually cirrhosis.[63,68]

CONCLUSION AND RECOMMENDATIONS

In pet liver disease is a broad term referring to any disorder that

damages the liver. The liver performs a number of important functions throughout the body including the regulation of digestion and metabolism, the synthesis of hormones and proteins, immune response and filtering of toxins from the blood stream. The liver disease is mostly termed as hepatitis which is inflammation of the liver; it is acute and chronic. Infectious hepatitis can be occurred by virus, bacteria, fungus and parasites while noninfectious hepatitis is occurred by toxicity, endocrine disorders and bile duct obstruction, autoimmune hepatitis and reactive hepatitis. The development of the liver disease results neurological signs, ascites, vomiting, diarrhea, polyuria, polydipsia, weight loss, stunted growth, abdominal pain and changes the body fluids and as diseases progresses the liver is unable to function well and the dog and cat will die.

Based on the above conclusions the following recommendations for liver disease dogs and cats are forwarded: early accurate diagnosis of liver diseases of dogs and cats by using clinical symptoms and advanced diagnostic techniques. Based on diagnostic methods proper management and amelioration of the liver diseases of dogs and cats for maintaining good immunity and health must be required. For life threatened liver disease dogs and cats cholelithiasis surgical removal of gallbladder (cholecystectomy) or by Laparoscopy and common bile duct stone removal by ERCP. For cirrhotic liver dogs and cats liver transplantation is highly recommended.

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CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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Original Research

Host Related Risk Factors of Bovine Trypanosomosis and Vector Density in Halu District of Ilubabor Zone, West Ethiopia

Dagim Bekele, DVM[1*]; Ahimadin Beshir, DVM[2]

[1]National Institute for Control and Eradication of Tsetse Fly and Trypanosomosis, Kaliti Tsetse Fly Mass Rearing and Irradiation Center, Addis Ababa, Ethiopia
[2]Bedele Tsetse Fly Control and Eradication Center, Ethiopia

Corresponding author
Dagim Bekele, DVM
National Institute for Control and Eradication of Tsetse Fly and Trypanosomosis, Kaliti Tsetse Fly Mass Rearing and Irradiation Center. P.O. Box: 19917, Addis Ababa, Ethiopia; Tel. :(+251)0915884053; E-mail: dagimbekele4@gmail.com

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ABSTRACT

Background
Trypanosomosis is disease caused by unicellular parasites, trypanosome, found blood and other tissue of vertebrates; including livestock, wild life and people. It is a serious disease in domestic livestock causing a significant negative impact on food production and economic growth in many parts of the world, particularly in sub-Saharan Africa. Its epidemiology and impact on livestock production are largely determined by the prevalence and distribution of the disease and its vectors in the affected area.

Aim
To assess the host related risk factors of bovine trypanosomosis and apparent density of tsetse flies in four peasant associations of the study area. In relation to the host risk factors, the prevalence of bovine trypanosomosis was highest in those animals with poor body condition.

Results
The overall 5.32% prevalence of bovine trypanosomosis was recorded from 432 blood sample collected from randomly selected animals using Buffy coat method. *Trypanosoma congolense* was the dominant species 14 (60.87%). However, it was not statistically significant between sex of animals ($p>0.05$). The mean packed cell volume (PCV) value of the infected animals was lower (20.65%±2.85) compared to non-infected animals (25.74%±4.80). There was statistically significant difference ($p<0.05$) in the PCV values of infected and non-infected animals. Moreover, animals with different body condition exhibited statistically significant variation ($p<0.05$) in the prevalence of trypanosomosis. Overall an apparent density of the flies was 2.42 f/t/d by using mono-pyramidal and biconical traps. It indicated that, *G. morsitance submorsitance, G. pallidipes* and *G. tachinoides* were tsetse flies species caught.

Conclusion
Finally, this work showed that trypanosomosis is an important disease affecting the health and productivity of cattle in the district. Hence, due attention should be given to this sector so as to improve livestock production and agricultural development in the area.

Keywords
Halu; PCV; Trypanosomosis; Prevalence.

INTRODUCTION

African trypanosomosis is one of the major constraints of animal production in sub- Saharan African countries including Western and Southwestern parts of Ethiopia.[1] Vector borne trypanosomosis is excluding some 180, 000-200, 000 km^2 of agriculturally suitable land in the west and southwestern parts of the country.[2]

Trypanosomosis is disease caused by unicellular parasites, trypanosome, found blood and other tissue of vertebrates; including livestock, wild life and people.[3,4] It is a serious disease in domestic livestock causing a significant negative impact on food production and economic growth in many parts of the world, particularly

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in sub-Saharan Africa. Its epidemiology and impact on livestock production are largely determined by the prevalence and distribution of the disease and its vectors in the affected area.[5]

This disease is transmitted mainly by tsetse flies (cyclically), biting flies (mechanically) and by other means of transmission. The most important species that infected cattle include *Trypanosoma congolense*, *T. brucei* and *T. vivax*. Mechanical transmission is particularly important in relation to *T. vivax* and *T. evansi* particularly on the fringe of tsetse areas. It can also transmitted by biting. Trypanosomosis is prevalent in two main regions of Ethiopia i.e. the North West and the South West regions. In Ethiopia, trypanosomosis is one the most important disease limiting livestock productivity and agricultural development due to its high prevalence in the most arable and fertile land of south west part of the country following the grater basins of Abay, Omo, Ghibe, Didessa and Baro with a high potential for agriculture.[6]

The economic burden of trypanosomosis is not only due to the direct losses resulting from mortality, morbidity and infertility of the infected animals but also due to the indirect losses like exclusion of livestock and animal power based crop production from the huge fertile tsetse infested areas. In Ethiopia, about 5.5 million heads of cattle are exposed to the risk of trypanosomosis. Nevertheless, in Halu district the magnitude of trypanosome infection and the distribution of its vectors are not well-known except complaints from farmers of the area.

Therefore, the objective of the study was

- To determine the prevalence of bovine Trypanosomosis.
- To identify vector species and their apparent density.
- To assess the risk factors associated with the disease and collecting baseline data to control the vectors.

MATERIALS AND METHODS

Study Area

The study area is located in Oromia regional state, Illiabbabor zone and lies at 035°29 to 035°35E longitudes and 08°14 to 08°37 latitude and north of equator. Altitude of the area ranges from 500 to 1800 m.a.s.l.. The climatology alternates with long summer rain fall (June-September), short rainy seasons (March-April) and winter dry seasons (December-February). The district has 32 °C maximum temperature and 15 °C minimum temperature and 1000 mm to 1800 mm rain-fall. The study was conducted in 4 peasant associations (PAs), namely Halu Gamachisa, Inago, Kidana Mirat and Walkitesa. There are river basins which flow throughout the year from the district to Gabba River system, namely Ibu, Aba Mamo, Kaso and Uka River other seasonal rivers which are tributers of Gabba and Uka Rivers. The different vegetation type which are found in the district, include *combretum Spp*, *Pillistigamathonningi*, *Acacia Spp* and *Ficassycomors*. Wild games like buffalos, bush pig, kudu, warthog, hippo and crocodiles are the most commonly found in the study area. Agriculture is the main stay of livelihood of people with a mixed farming system and livestock plays an integral role for agriculture.[7]

Physiology of the Liver

Invariable all age group of Local Zebu cattle maintained under extensive system of management are used for this study without sex difference. Agriculture is the main livelihood of the society with mixed farming system and livestock play an integral role for agriculture.

Study Design

Cross-sectional study was conducted to determine the prevalence of bovine trypanosomosis and apparent density of vectors (tsetse population).

Sample Size and Sampling Method

The simple random sampling technique was applied to collect from the ear vein. The sample size can be determined based on the study type and sampling method for investigation, 95% confidence interval, 5% desired absolute precision and 50% average prevalence.[8]

STUDY METHODS AND PROCEDURES

Entomological Survey

For the entomological study, tsetse flies and other flies were collected from selected sites of the study area. The altitude levels, Peasant Associations, numbers of traps, tsetse species caught, other biting flies, days and vegetation types were recorded during the sampling period. The different fly catches in each trap were counted and identified; the species of tsetse flies and other biting flies were identified based on their morphological characteristics such as size, color and wing venation structure.[9]

Determination of Packed Cell Volume

The capillary tubes were placed in micro-haematocrit centrifuge with sealed end outer most. The tube was loaded symmetrically to ensuring good balance after screwing the rotators cover and closing the centrifuge lid, the specimens were allowed to centrifuge at 12,000 revolutions per minute for 5-minutes. Tubes were then placed in a haematocrit and readings were expressed as a percentage of red blood cells to the total volume of whole blood. Animals with packed cell volume (PCV)<24% were considered to be anemic.[10]

Buffy Coat Technique

A small blood was collected from an ear vein using heparinized microhaematocrit capillary tube. The haematocrit tube with whole blood sample end was sealed with haematocrit clay. The tube was centrifuged at 12000 revolutions per minute for five minutes. After centrifugation trypanosome were usually found in or just above the buffy coat layer. The capillary tube was cut using a diamond tipped pen 1 mm bellow the buffy coat to include the upper most layers of the red blood cells and 1 mm above to include the plasma. The content of capillary tube was expressed on to side, homogenized on to clean side and covered with cover slip. The slide was under

40× objective 10× eye piece for the movement of the parasites.[11,12]

Data Management and Analysis

The prevalence was calculated as the number of infected individuals divided by the number of total examined and multiplied by 100. For the analysis of data statistical software program (statistical package for the social sciences (SPSS) 20.0) was used. Descriptive statistics were used to summarize data. The association between the prevalence of trypanosome infection and risk factors were assessed by logistic regression, whereas the two group mean comparison (*t* test) was used to assess the difference in mean PCV between trypanosome positive and negative animals. The density of fly population was calculated by dividing the number of flies caught by the number of traps deployed and the number of days of deployment and expressed as Flies/Trap/Day.

RESULTS

Entomological Survey

A total of 290 tsetse flies were caught during study period. The overall apparent density of tsetse flies was 2.42 f/t/d. Three tsetse species have been identified. 150 (51.72%) were *Glossina morsitance submorsitance*, 80 (27.57%) were *Glossina pallidipes* and 60 (20.69%) were *Glossina tachinoides*. From total tsetse flies trapped, females occupied larger proportion and out of 290 tsetse flies caught, 214 (73.79%) flies were female while the rest 76 (26.21%) were male (Table 1).

Parasitological Findings

Out of 432 cattle examined, 23(5.32%) were found to be infected with trypanosomes. The prevalence of Trypanosomosis was significantly high in Halu Gamachisa followed by that of Inago and Walkitesa. The highest prevalence on the basis of species was *T. congolense* followed by *T. vivax* (Table 2).

Risk Factor Variable

The prevalence of trypanosomosis was higher in males as compared to female animals. However, the difference was not statistically significant ($p>0.05$). The prevalence of trypanosomosis between body condition scores was highest in poor and it was statistically significant ($p<0.05$).

Haematological Findings

The mean PCV value of the infected animals was lower (20.65%±2.85) as compared to the mean PCV value of non infected animals (25.74%±4.80) and statistically significant difference was observed between the PCV value of infected and non-infected animals ($p<0.05$) (Table 3).

Table 1. *Tsetse Distribution in Different Peasant Associations in Halu District*

PAs	No of Trap Deployed	*G.pallidipes*		*G.tachinoides*		*G.m.submorsitance*		Total	FTD
		M	F	M	F	M	F		
Halu Gamachisa	15	4	23	6	14	12	31	90	3
Inago	15	2	10	5	8	13	26	64	2.13
Kidane mirat	15	5	16	2	12	10	24	69	2.3
Walkitesa	15	3	17	3	10	11	23	67	2.23
Total	60	14	66	16	44	46	104	290	2.42

PAs: Peasant associations, FTD: Fly per trap per day, F: Female, M: Male

Table 2. *The Prevalence of Trypanosomosis in Different Area with Respective Species*

Peasant Association	Number of Animal Examined	Number of Infected Animals	*Trypanosome spp.* Identified		Prevalence (%)
			T.c	T.v	
Halu Gamachisa	168	15	8	7	8.93
Inago	96	3	2	1	3.13
Kidane mirat	72	2	2	-	2.7
Walkitesa	96	3	2	1	3.13
Total	432	23	14(60.87%)	9(39.13%)	5.32

Table 3. *The Mean Packed Cell Volume of Examined Cattle in Halu District*

Group	Observations	Mean PCV	SE	SD	95% CI
Negative	409	25.74	0.23	4.80	25.27---26.20
Positive	23	20.65	0.59	2.85	19.41--- 21.88
Total	432	25.46	0.23	4.85	25.01---25.92

SD= Standard Deviation, SE= Standard Error, PCV=Packed cell volume

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Prevalence of Trypanosomosis According to Sex and Body Condition

The prevalence of trypanosomosis was higher in males as compared to female animals. However, the difference was not statistically significant (p>0.05). The highest prevalence was observed in the poor body condition animals and the variation in prevalence between the different body condition group was statistically significant (p<0.05).

DISCUSSION

The present study revealed that from a total of 432 randomly selected cattle's in the study area, 23(5.32%) animal were found positive for trypanosomes. This finding was lower than the previously reported infection rate of 18.5% in Arba-Minch Zuria district,[13] 11.7% in Abay Basin North Western Ethiopia,14 20.4% in Wolyta and Dawero Zone of Southern Ethiopia,[15] 16.9% in Sayo, district, Kellem Wollega, Western Ethiopia[16] and 29% prevalence in Gawo-Dale, West Oromia.[17] The lower prevalence in the current study might be due to the use of prophylactic and trypanocidal drugs, application of relatively designed method of tsetse fly control and expansion of cultivation land in the area which indirectly affects its vectors.

Out of the 5.32% overall prevalence of trypanosome infection, 3.24% were due to *T. congolense* and 2.08% were due to *T. vivax*. The finding of this study showed that the total trypanosome positive animals 60.87% were found to be infected with *T. congolense* and 39.13% were infected with *T. vivax*. The higher proportion of *T. congolense* in this study was in agreement with the previous results of Abebe et al[18] for tsetse infested areas of Ethiopia (58.5%); NTTICC[19] Frat Adanhegn Peasant Association (62.5%); Muturi[20] at Mereb Abaya, South Ethiopia (66.1%) and Terzu[21] in selected sites of Southern region (63.4%). Moreover, the results of Tewelde et al[22] at Kone (75%); Duguma et al[23] (85.2%) in Southwestern Ethiopia and Lelisa et al[24] in Hawa Gelan, Kellem Wollega, west Ethiopia (84%) had also shown higher results of *T. congolense*. These suggest that the major cyclical vectors or Glossina species are more efficiently transmitters of *T. congolense* than *T. vivax* in East Africa[25] and also due to the high number of serodems of *T. congolense* as compared to *T. vivax* and the development of better immune response to *T. vivax* by infected animals. According to Getachew,[26] *T. vivax* is highly susceptible to treatment while the problems of drug resistance are higher in *T. congolense* and *T. congolense* is mainly confirmed in the blood, while *T. vivax* and *T. brucei* also invade the tissues. *T. congolense* and *T. vivax* are the most prevalent trypanosomes that infect cattle in tsetse infested and tsetse free areas of the Ethiopia, respectively.

Even though higher infection rate was registered for males as compare to the females, the difference was not statistically significant (p>0.05). This result is in agreement with the previous researches reported by Adane et al[27] and Abebayehu et al.[28] This might be due to the fact that both sexes have virtually similar exposure to flies in grazing areas.

The prevalence of Trypanosomosis under different body condition groups indicated statistically significant difference (p<0.05) with higher infection rate in poor body conditioned than medium and good body conditioned cattle. Similar findings were reported in Abay (Blue Nile) base areas of Northwestern, Ethiopia[29]; in Bure district, western Ethiopia.[30] On another hand disagreement with the study in Metekel and Awi zone of North West Ethiopia.[31] On one hand, the disease itself results in progressive emaciation of the infected animals; nevertheless, on the other hand non-infected animals under good body condition are with good immune status that can respond to any foreign protein better than those non–infected cattle with poor body condition which can be immune compromised due to other diseases or malnutrition, since malnutrition and concurrent infections depress the immune responsiveness in some cases.[32]

The present study indicated that the difference between mean PCV values of parasitaemic (20.65%+2.85) and aparasitaemic (25.74%+4.80) cattle of the study area was statistically significant (p<0.05). This result was in agreement with the previous work done in Bilo Nopha district, South West Ethiopia[33] and three highland districts bordering LakeTana, Ethiopia.[34] Being intracellular blood parasites, trypanosomes result in lowering PCV of cattle because they lyse and destruct the red blood cells. The appearance of trypanosomosis in negative animals with PCV values of less than the threshold values (25%) may be due to the inadequacy of detection method used or delayed recovery of aneamic situation after current treatment with trypanocidal drugs or due to be anaemic by other complicative cause like malnutrition. Parasitaemic animals with PCV values greater than 25% might be thought of recent infection. Trypanosome infection and mean PCV values obtained in this study in the parasitaemic animals was found to be highly associated. Different authors in southern, northwestern and southwestern Ethiopia[35,36] also reported similar results. The mean PCV can be affected by many factors including helminth parasites infections, nutritional deficiencies and blood parasites, other than trypanosomosis, however, these factors are likely to affect both trypanosomosis positive and negative animals.[37,38]

The risk of trypanosomosis is also influenced by apparent density of the tsetse flies and type of vector prevailing in the area. In this study, the entomological findings revealed that three species of Glossina (*Glossina pallidipes*, *G. tachinoides* and *G. morsitance* sub-morsitance) out of five reported in Ethiopia. The overall apparent density of *Glossina species* was 2.42 Flies/Trap/Day. These findings lower than the previous report 11.9 Flies/Trap/Day from Hewa-Gelan district, Oromia region, West Ethiopia,[39] 4.3 Flies/Trap/Day from Lalo-Kiledistrict, KellemWollega Zone, Western Ethiopia.[40] The result also higher than the previous report 1.15 Flies/Trap/Day for tsetse in East Wollega zone[41] and 1.35 Flies/Trap/Day in southern rift valley of Ethiopia.[42] Higher percentage of female (73.79%) tsetse flies was caught than males (26.21%) that are in line with various reports from different parts of Ethiopia.[43,44] This could be adhered to longer lifespan of female tsetse flies than males.[45-47]

CONCLUSION AND RECOMMENDATIONS

Our study revealed that *T. congolense* and *T. vivax* were the prevailing

species of trypanosomes in the study area. In relation to the host risk factors, the prevalence of bovine trypanosomosis was highest in those animals with poor body condition than animals with good and medium body condition. Finally, bovine trypanosomosis is an important disease and a potential threat affecting the health and productivity of cattle in the district. Hence, the necessary strategic control of bovine trypanosomosis including integrated and sustainable vector control should be strengthened to improve livestock production and agricultural development in the area.

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CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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