PREVALENCE OF GASTROINTESTINAL HELMINTHES AND ASSOCIATED RISK FACTORS IN MULE AND HORSE IN AND AROUND INJIBARA

Marie Teshager Tsehay[1], Banchgizie Fetene Aklilu[1] and Hana mulu sewagegn[2]

[1] Banja woreda Livestock and Fisheries Office, Animal health Department
Gmail: teshagermarie@gmail.com

ABSTRACT: A cross sectional study was conducted from November 2024 to April 2025 in and around Injibara town to determine the prevalence of gastrointestinal (GIT) parasites in mules and horses. A total of 324 equines (110 mule and 214 horses) were randomly selected and examined during the study period. Qualitative parasitological techniques like floatation and sedimentation were deployed to confirm eggs of nematode and trematode respectively. The overall prevalence of gastrointestinal parasites in equine was 72.22% in the study site. The species wise prevalence gastrointestinal parasites in horses and mule were 59.8% and 40.2% respectively. Among gastrointestinal parasite eggs identified *Strongly* type egg (20.51%) was the predominant followed by *Ascaris*s pp.(12.8%) *Parascaris* spp (3.42%), and relative prevalence of *Fasciolosis* was 5.13%. In mixed infection *strongly* and *Ascaris*. Were the common identified species with relative prevalence of 40.17%; 17.09% in two and three mixed parasite infection respectively. However, *Trichuris* spp. (0.85%) was the least recovered from Equine fecal sample of the study area. Majority of the hypothesized risk factors such as species, sex, and age were found to be statistically significant with (P<0.05). However, according to this data body condition score was found to be statistically insignificant with (P>0.05). Thus, the present study revealed that gastro-intestinal helminthes are among the major health problem of horse and donkey in the area. Finally, Strategic de worming using broad spectrum anthelmintic drugs and rotational grazing program implementation were recommended.

[Marie Teshager Tsehay, Banchgizie Fetene Aklilu and Hana mulu sewagegn. **PREVALENCE OF GASTROINTESTINAL HELMINTHES AND ASSOCIATED RISK FACTORS IN MULE AND HORSE IN AND AROUND INJIBARA.** Cancer Biology. 2026;16(3):20-27]. ISSN: 2150-1041 (print); ISSN: 2150-105X (online). http://www.cancerbio.net 04. doi:10.7537/marscbj160326.04

Key words: Gastrointestinal helminthes; mules and horse; prevalence

1. INTRODUCTION

The equine population of the world is estimated to be 122.4 (40 million donkeys, 15 million mules', 43.3 million horses and 24.1million zebras and camel) (Abayneh *et al*., 2002). In the distribution pattern, 98% of all mule, 97% of all mules and 60% of all horses are found in the developing countries. In the developing world, there are estimated 110 millions of equines (FAOSTAT, 2008). The number of equines in Africa is in the range of 17.6 million comprising 11.6 million donkeys, 2.3 million mules and 3.7 million horses (Bela, 2006).

Ethiopia has a large numbers of equines. It has a total of 9.83 millions equine populations. From those numbers, donkeys accounts 7.04 million while horses and mules are 2.03 and 0.4 million respectively (CSA, 2014/15). Ethiopia is home to the oldest feral horse population in Africa and the only wild horses left in east Africa, the Kundudo. One of the oldest recorded breeds, the Oromo bloodlines come from Ethiopia & later spread along the coast of the Red Sea. They were first imported into England in 1861, where they quickly became prized for several of their unique characteristics. Arsi and Bale provinces of Oromia region are known with their densely populated equine population, mainly with Oromo breed horses (Equinest, 2012).

Equids (donkeys, mules and horses) play an important role as working animals in many parts of the world, employed for packing, riding, carting and ploughing. Equine power is vital for both rural and urban transport system which is cheap and provides the best alternatives in places where the road network is insufficiently developed, the terrain is rugged and mountainous and in the cities where narrow streets prevent easy delivery of merchandise (Getachew *et al.*, 2008). There is one equine for every four people in the agricultural sector and for every five persons of the total population (Williams *et al.*, 2000).

Parasitic helminthes are one of the most common factors that constrain the health and working performance of mule and horses world widely. They cause various degrees of damage depending on the species, nutritional and the immune

status of equines. They decrease the performance and productivity in the animals mainly, in the reduction of body weight or failure to gain weight or even increase the mortality in acute case (Mezgebu *et al.*, 2013).

Equine endo-parasites divided into three categories: nematodes, or roundworms; cestodes, or tapeworms; trematodes, or flukes. Parasites are assigned to these categories according to their morphology, or structure. Growth and life cycles of parasites within each group are generally distinct from those of the other groups. The roundworms are by far the most economically important internal parasites of equines. Internal parasites continue to be a significant threat to the health of equines. Even under proper management equines will become infested with internal parasites. Internal parasites of equines are of veterinary importance in many countries, where current methods of control rely almost entirely on the use of antihlementics. (Chapman *et al.*, 2002).
In Ethiopia, various studies disclosed that *strangles*, *Parascaris quorum*, boot stomach worms, lungworms, tapeworms and liver flukes to be the most prevalent gastrointestinal parasites of equines (Shivered *et al.*, 2001). Among gastrointestinal helminthes, strongylosis is the most common diseases of horses throughout the world and cause death when control measures are neglected (Urquhart *et al.*, 1996). The disease process caused by strangles can be produced by migrating larvae and by adult worms. Larval of *Strongylus vulgaris* are the most pathogenic, causing arthritis, thrombosis and thickening of artery wall (Soulsby, 1986).

Gastro intestinal parasites of equines include large strongyles, small *Strongyle*, *Ascaris* and pinworms were common in Ethiopia (*Oxyurisequi*) (Worku and Afera, 2012). Despite the huge numbers and the increasing importance of equines in the Ethiopian economy, awareness of the health problems affecting and welfare is limited for most parts of the country more over no adequate researches on equine helminthes, were done in and around Injibara town .
Therefore the objective of this study was;

- To determine the Prevalence of helminthes of horses and mules in the study area.
- To assess associated risk factor of equine GIT parasites.

2. MATERIALS AND METHODS

2.1. Study Area

The study was conducted in and around Injibara from September 2025 to February 2026. Injibara is a town in northern Ethiopia serving as the administrative center of the Awi Zone in the Amhara Region. It is located at coordinates $10^{0}52^{`}$ N $36^{0}56^{`}$ E, roughly 447 km from Addis and 122 km from Bahir Dar; Situated at 2560 above sea level, it features a mountainous landscape near Zengena Crater Lake. The major types of animal population were cattle, sheep and goat, horses, mule, donkeys, and poultry (ICALFO, 2024).

2.2. Study Animals

Animals considered in this study were equine species (Horses and mule) of different age, sex and body condition. A total of 324 equines (214 horses and 110 mules) were included in the study to investigate the prevalence of gastro intestinal parasites.

2.3. Study Design

A cross sectional study design was conducted between September 2025 to February 2026 in a randomly selected horses and mule to determine the prevalence of gastro intestinal helminthes parasitic infections. Information about species, sex, age and body conditions of the study animals were gathered appropriately. The ages were determined using owners information and dentition as (<1) years) (1-5) years), (5-6year) and (>10years). The body conditions were classified as poor, medium and goods categories species (Svendsen, 2008). Donkeys were rarely found in the area, they were not considered in the study.

2.4. Sampling Method and Sample Size Determination

A simple random sampling technique was employed to select 324 equines **(**mule and horses) which were brought to Injbara Veterinary Clinic and mule from market and fields. The required sample size was calculated based on the expected prevalence of 70.4% which is reported by Alemayehu and Etaferahu (2013) in Wollo Zone absolute desired precision of 5% and at 95% confidence interval according to the formula provided by Thrusfield (2005).

$$n = \frac{1.96^2 P_{exp}(1-P_{exp})}{d^2}$$

Where: n =sample size

P_{exp} = maximum expected prevalence

d = 5% desired precision at 95% confidence interval

1.96 = the value of Z for 95% CI

Hence, the required total sample size was 324 animals.

2.5. Study method

2.5.1. Sample collection and transportation

The fecal samples were collected from equines (Horses and mule) of randomly selected from which were brought to Injibara Veterinary Clinic, market. Fecal samples were collected directly from the rectum using arm length rubber gloves and placed in 28 ml glass, screw-corked universal bottles half filled with 10% formaldehyde (Zajac and Conboy, 2012). Each sample was labeled with the animal number and species corresponding to, date and place of collection with inedible pen. The samples were transported to Banja woreda veterinary clinic Veterinary Parasitology Laboratory for further processing.

2.5.2. Coprological examination

All fecal samples were grossly examined for the presence of parasites. Fecal examination was done by different qualitative fecal examination techniques. All collected fecal samples were processed using the simple flotation, sedimentation and direct fecal examination technique. The procedure given a qualitative faces examination was made to search for nematodes, cestodes and trematodes eggs (Urquhart *et al*., 1996).

Questionnaire Survey

A Semi structured questionnaire was designed to collect information about common equine production or rearing constraints in the study area such as Equine holding pattern, management practices, feeding, diseases and health disorders.

2.6. Data Analysis

The collected data were coded and inserted in Microsoft spreadsheet excel and analyzed by SPSS (version 20). Based on the type of data, the association between risk factors and distribution of parasites were analyzed using bivariate and multivariate statistical analysis of logistic regressions, the chi square test and analysis of variance. The strengths of associations were determined using estimates of odds ratio. Statistical significance will be set at $P<0.05$ according to Thrusfield (2005).

3. RESULTS

Fecal samples were collected from 324 equines (214 horses and 110 mule) and examined. The result revealed that 72.22% (234/324) of equines were found harboring one or more species of gastrointestinal helminthes at varying extent (table 1).

Among the identified gastrointestinal parasite eggs, *Strongyle*egg was the predominant followed by *Ascaris* spp, *Fasciola* and *Parascaris* spp with 20.51%, 12.8%, 5.13% and 3.42%) respectively. In mixed infection, *Strongyle* and *Ascaris* were the common identified species with relative prevalence of 40.17%, 17.09% for two and three parasite mixed infection respectively. However, *Trichuris* spp. (0.85%) was the least recovered from Equine fecal sample of the study area (table 2).

Table 1. Overall prevalence of GIT Helminthes in equine species

Species	No. examined animals	Positive	Prevalence	X2	P-value
Horse	214	140	59.8	7.2677	0.007
Mule	110	94	40.2		
Total	324	234	72.22		

Table 2. Prevalence of GIT Helminthes Species in Equine

Species	Relative percentage%
Nematodes	
Stronglus	20.52
Ascaris	12.82
Parascaris	3.42
Trichuris	0.85
Trematode	
Fasciola	5.13
Mixed in fection	
Two parasite	40.17
Three parasite	17.09
Total	**100.00**

The present study revealed that prevalence of parasite was 59.8% in horse and 40.2% in mule. The Prevalence of parasite in male was found higher than females 62.4% and 37.6% respectively. Older animals >10 years were more likely to be infected compared with younger ones <1year and 1-5 years aged animals. According to this data the overall prevalence of gastrointestinal helminthes in different body condition scores of the study animals was statistically insignificant (P>0.05) and highest prevalence was recorded in animals with good (53.85%) followed by equines with medium (27.35%) and poor body conditions scored animals (18.80%). All of the hypothesized risk factors such species, sex, age and body condition score were found to be statistically significant with ((P<0.05)) except body condition score which is found to be statistically insignificant with (P>0.05) (table 3).

Table 3. The prevalence of gastrointestinal parasites in Equine across different risk factors

Variables	No. of animals examined	No. of animals found positive (%)	χ^2	*P-value*
Species				
Horse	214	140 (59.8)	7.2677	0.007
Mule	110	94 (40.2)		
Sex				
Male	218	146 (62.4)	4.5769	0.032
Female	106	88 (37.6)		
Age				
<1	16	16 (6.84)	10.2109	0.037
1-5	68	58 (24.79)		
5-10	182	124 (52.99)		
>10	56	36 (15.38)		
BSC				
Good	172	126 (53.85)	0.0978	0.952
Medium	90	64 (27.35)		
Poor	62	44 (18.80)		

5.1 Questionnaire Survey

A Survey questionnaires distributed for 162 equine owners revealed that major equine health problems in the study were *Epizootic lymphangitis* (54.3%), GIT *Helminths* (27.8%) and *Equine colic* (17.9%) (table 3).

Table 4. Common Equine diseases of the study area

Diseases	Frequency	Percent
Epizootic lymphangitis	88	54.3
GIT helminthes	45	27.8
Equine colic	29	17.9

The respondents complained that major problems of equine production in the study area were feed shortage, diseases and scarcity of grazing land (table 5).

Table 5. Major Equine production constraints in the study area

Major Constraints	Frequency	Percent
Feed Shortage	114	70.37
Disease	47	29.01
Scarcity grazing land	1	0.62

4. DISCUSSION

The current study revealed overall prevalence of Equine gastrointestinal helminthes was 72.22% which is comparatively similar with Tesfu *etal*. (2014) in Hawasa, Regassa and Yimer (2013) in South wollo zone reported 72.7% and 70.4% respectively. Whereas, this finding is lower than Yoseph *et al*. (2001) in Wonchi high lands, Fikru *et al*. (2005) who reported 100% and 98.2% respectively. This difference might be due to the differences in the study areas, deworming strategy and accessibility to veterinary clinic. In addition, it may also be due to the variation in sampling time, as seasonality affects the occurrence of the parasites.

The species wise prevalence of GIT parasites with 59.8% in horses and 40.2% in mule recorded this result is higher than 29.79% of horse and 37.48% of donkey by (Sawsan *et al*., 2008). However, it is slightly in agreement with 44.5% in mule and 48.2% in horses reported from East Shoa, Oromia (Wubit and Bariisoo, 2016). This variation could be due to the variation in management and ecological condition. Additionally, accessibility of equines to grazing land, deworming habit of the cart pulling horses and donkey giving supplementary feed to these animals affect its occurrence such as, temperature, altitude and humidity (Muleta, 2005).

The present result revealed higher prevalence of GIT helminthes in horses than mule which is in line with reports of (Tesfu *et al*., 2014) and this might be due to horses are exposed to a complex of gastrointestinal parasitic infections which compromises their health and welfare. These parasites have a high prevalence and are an important cause of morbidity and mortality (Matthews *et al.*, 2004).

The prevalence of gastrointestinal parasite species identified in this study *Strongyle* type egg (20.51%) was the predominant followed by *Ascaris* spp. (12.8%) *Parascaris*s pp (3.42%) and *Fasciolosis* was 5.13%. Considering a single gastrointestinal nematode infection, *Strongyle* spp. egg was prevalent and dominant and the most encountered nematode species examined from all samples collected from the study areas. This agrees with previous studies by (Saeed *et al*., 2010), (Wannas *et al*., 2012) (Mezgebu *et al*., 2013) and (Tesfu *et al*., 2014).
The prevalence of *Parascaris* was 3.42% this disagrees with (Tolossa and Ashenafi, 2007), (Getachew *et al*., 2008) in the Ethiopia, (Mahfooz *et al*., 2008) in Pakistan,(Uslu and Guclu, 2007) in Turkey, (Fikru *et al*., 2005) in Ethiopia highlands and (Aftab *et al*., 2005) in Lahore-Pakistan who reported 11.7%,16.2%, 12%, 10.81%17.1% and 10.92%, respectively. These finding are relatively higher than the present finding, this could be due to variation in management condition of the study animals used.

The prevalence of fasiola in the present study was recorded 22.84%.There was no significant difference between species, sex and body condition score however was statically significant in different age groups (young, adult and old having 50%, 21% and12% respectively).This investigation is greater than other reports in the central highlands of Ethiopia, which was reported byYoseph *et al*. (2001), Mulate (2005).The difference might be due to the differences in ecological conditions for the development of intermediate snails and the parasite.Permanent dampness, suitable luminosity, basic pH of soil and water, and temperature contribute to the multiplicity of snails and the parasite (Hammami and Ayadi, 1999).

With regard to risk factors, species and sex were statistically significant ($p<0.05$) while age and body condition were not statistical significant ($p>0.05$). This might be attributed the small sample size.

6. CONCLUSION AND RECOMMENDATIONS

Equines have crucial importance in the livelihood in and around Injibara town, particularly for carting, packing and transportation materials and goods. In this study, 5 types of helminthes parasites (mixed infection, *strongyle*, *Parascarisquorum*, Fasciola, and *Trichuris*) eggs were common with high prevalence. Among the GIT parasites, *strongyle* species was predominantly encountered in the study site. It was also observed that risk factors like species and sex are important for the occurrence of the disease with high prevalence in the study area. The major equine health problems in the study were *Epizootic lymphangitis* (54.3), GIT *Helminths* and equine colic. Feed shortage, disease and scarcity of grazing land are among the main constraints which hinders equine rearing and availing equine in the study site.

In light of the aforementioned conclusion, the following recommendations were forwarded.

- Improvement of housing and feeding managemental system for equines should be exercised.
- Regular deworming and promotion of equine husbandry practices by concerned organs.
- Strategic de worming using broad spectrum anthelmintic drugs and rotational grazing program should be implemented reduce pasture contamination and disease burden.

6. REFERENCES

Abayneh, T.F., B. Gebreab, G. Zekarias and A. Tadess, (2002): The potential role of mule inland fillage in central Ethiopia. Bulletin of animal'shealth and production in Africa, **50**: 172-178.

Aftab,J.,M.S. Khan, K. Pervez, M. Avais and J.A. Khan, (2005): Prevalence and Chemotherapy of Ecto-and Endoparasites in Rangers Horses at Lahore- Ecto-and Endoparasites in Rangers Horses at Lahore- Pakistan, Int. *Jornal Agri. Biol*., 7: 5.

Alemayehu, R. and Etaferahu, Y, (2013): Gastrointestinal Parasites of Equine in South Wollo Zone, North Eastern Ethiopia. Global Veterinarian, **11** (6): 824-830.

Ayele, G.G. Feseha, E. Bojia and A. Joe,(2006): "Prevalence of Gastrointestinal Parasites of Mule in Dugda Bora Dis-trict, Ethiopia," Livestock Research for Rural Develop- ment, Vol. **18**, No. 10, , pp. 14-21.

Belay, M. (2006). Preliminary study on helmenthosis of equines in south and north wollo zones. Veterinary Parasitology, **140**: 289-295.

Chapman, M., T. French and R. Klien,(2002):Gastrointestinal helminths of ponies in Louisiana: a comparison of species currently prevalent with those present 20 years ago. Journal of parasitology, **88:** 1130-1134.

CSA (Central Statistical Authority), 2014/15. Federal democratic republic of Ethiopia, Agricultural Sample Survey, Report on Livestock and livestock characteristics. Addis Ababa, **2**: 16-17.

Demelash, B. and Moges, W, (2006): Causes and factors associated with occurrence of external injuries in working equines in Ethiopia. Internal *Journal of applied Research in Veterinary Medicine*, **4** (1): 1-7.

Equinest., (2012): Horse breeds from Ethiopia. Online: www.info[at]the equinest.com.

FAOSTAT, Statistical Year Book: The Statistics Division Food and Agricultural Organization of the United Nations, (2008):http://www.faostat.fao.org.

Fikru, R. D. Reta, S. Teshale and M. Bizunesh, (2005): "Preva-lence of Equine Gastrointestinal Parasites in Western Highlands of Oromia, Ethiopia," Bulletin of Animal Health and Production in Africa, Vol. **53**, No. 3,,pp. 161-166. http://dx.doi.org/10.4314/bahpa.v53i3.32704

Getachew, M.G., A. Feseha, S.W. Trawford and J. Reid, (2008): A survey of seasonal patterns instrongylefaecal worm egg counts of working equidsof the central midlands and lowlands, Ethiopia. *Tropical Animal Health and Production*, **40**: 637-642

Hammami, H. and Ayadi, A., (1999):Ecology of Lymnaeatruncatula Muller, intermediate host of Fasciola hepatica Linne in the microclimate of Tozeur (southeast of Tunisia). Bulletin de la Société de PathologieExotique**92** (5):302-4.

ICALFO, 2024: Injibara City Adiminstration Livestock and Fisheries office, Injibara, Ethiopia.

Mahfooz, A., M.Z. Masood, A. Yousaf, N. Akhtar, and M.A. Zafar, (2008): Prevalence and anthelmintic efficacy of abamectin against gastrointestinal parasites in horses. *Pak. Vet. J*.**28**: 76–78.

Mezgebu, T., K. Tafesse and F. Tamiru,(2013). Prevalence of Gastrointestinal Parasites of Horsesand Mule in and around Gondar town,Ethiopia. Open *Journal of Veterinary Medicine*,**3**: 267-272.

Mulate, B., (2005): Preliminary study on helminthosis of Equines in south and north Wollo Zones.*Journal of Veterinary Association*.**9**(2): 25-37.

Ragassa, A. and E, Yimer, (2013): Gastro-intestinal Parasites of Equine in South WolloZone, Northeastern Ethiopia. Global Veterinaria, 11, 824-830.

Saeed, K., Z. Qadir, K. Ashraf, and N. Ahmad , (2010): Role of intrinsic and extrinsic epidemiological factors on strongylosis in horses. *Jornal. Anim. Plant. Sci*. **20**: 277–280.

Saeed, K. Z. Qadir, K. Ashraf, and N. Ahmad,(2010) "Role of intrinsic and extrinsic epidemiological factors on strongylosis in horses," *Journal of Animal and Plant Sciences*, vol. **20**, no. 4, pp. 277–280.

SawsanM., A. I., T Hassan, H. I. Seri, Hidaia B. Zolain, (2008): Field investigation of gastro-intestinal nematodes in horses and mule in South Darfur State, Sudan. 13 scientific congress Faculty of Veterinary Medicine Assiut University. Egypt.23-25 November, 723-729.

Shiferaw,Y., Gebreab, F,, Abebe, W, (2001): S urvey on Helminthosis of Equine in Wonchi. Ethiop. Vet. J**, 5** (1): 47-61.

Shiferaw,Y., Gebreab, F., Abebe, W, (2001): S urvey on Helminthosis of Equine in Wonchi. Ethiop. Vet. J**, 5** (1): 47-61.

Soulsby, E., J., (1986): Helminthes, Arthropods and Protozoa of domesticated animals. **7** the h edition Lea and Febiger Philadelphia, USA. pp. 357-504.

Soulsby, E., J., (1986): Helminthes, Arthropods and Protozoa of domesticated animals. **7** the h edition Lea and Febiger Philadelphia, USA. pp. 357-504.

Svendsen, E., (2008): The profession hand book of the mule. 4 ed. England, sidmouth Devon:whittetbooks, pp: 401.

Tesfu, N., B. Asrade, R. Abebe and S. Kasaye, (2014): Prevalence and Risk Factors of Gastrointestinal Nematode Parasites of Horse and Mule in Hawassa Town, School of Veterinary Medicine, Hawassa University. *Jornal Veterinary ScienceTechnology*, 5: 5.

Thrusfield, M., (2005): Veterinary Epidemiology, Third Edition, Blackwell Science Ltd., UK,

Tolossa, Y.H. and H. Ashenafi,(2013) Epidemiological study on Gastrointestinal Helminthes of horses in Arsi-Bale highlands of Oromiya Region, Ethiopia, Ethiop. Vet. J., **17**: 51-62. http://dx.doi.org/10.4314/evj.v17i2.4.

Urquhart, G.M.,J.Armour, A.M. Dunn andF.W Jennings, (2003). Veterinary parasitology.**3** ed.pp: 4-78

Uslu, U. and F. Guçlu,(2007): Prevalence of end parasites in horses and mule in turkey, Bull *Vet Inst Pulawy*, 51: 237-240.

Wannas, H .Y. K. A. Dawood, and G. A. Gassem,(2012)."Prevalence of gastro-intestinal parasites in horses and mule in al diwaniyah governorate," *AL-Qadisiya Journal of Veterinary and Medical Science*, vol. **11,** no. 1, pp. 148–155.

Williams, C.M.S. Richard, R. Damaee and B. Grieve, "Pasrasitolgy and Vector Biology," (2000): HardcourtAcademic Press, UK,.

Worku, S., B. and Afera, (2012): Prevalence of equinenematodes in and around Kombolcha, South WolloEthiopia. REDVET Rev. electrón. Vet., **13**: 1-13.

Wubit T., Bariisoo, M., (2016): Prevalence of Strongyle Infection and Associated Risk Factors in Horses and Mule In and Around Batu Town, Eastshoa, Oromia Regional State, Ethiopia School of Veterinary Medicine, Jimma University, Advances in Life Science and Technology Vol.**47**,

Yoseph,.S.Feseha.Gand Abebe.W, (2001): "Survey on Helmin- thosis of Equines in Wonchi," Ethiopia *Journal of the Ethiopian Veterinary Association*, Vol. **5,** , pp. 47- 61.

Zajac, M.A. and A.G. Conboy, (2012): Veterinary clinical Parasitology 8 Ed., (London, Blackwell publishing, and UK), pp: 67-72.

7. ANNEX

Annex 1.**Flotation technique**

Step1: 40g of NaCl was dissolved in 100 ml distilled water

Step 2:3 grams of fecal sample was measured and transferred into mortal

Step 3:10ml of salt solution (flotation fluid) was added to the fecal sample and mixed using a pestle

Step 4: The mixture was strained with a sieve into a beaker

Step 5: The filtered preparation was filled to two test tubes until 2.5 cm was remained to the top

Stepe6:Two test tubes were placed opposite to each other on a centrifuge, and centrifuged at 1500revolutions per minute for about 3 minutes

Step7: The tubes were withdrawn and flotation solution was added to the top of the tube until it had bulged creating a reverse meniscus

Step8: Cover slip was place on top of the tubes and allowed to stand for about 10 minutes.

Step9: Cover slip was carefully lifted up with the adherent drop of fecal flotation solution and placed on a clean microscopic slide
Step10:The entire cover slip area was examined at 10X objective lens magnification then switched to 40X.

Annex 2. **Sedimentation technique**
Step 1: 3 g of feces was mixed with 42 ml of tap water using Mortar and pestle.
Step 2: The suspension was filtered through a Sieve into a beaker
Step 3: The filtered material was poured into centrifuge tubes (not filled to the top) and Centrifuged at 1500 rpm for 3 minute and the supernatant was decanted
Step 4: using a pipette some of the sediment was removed from the top layer and a drop was placed on a slide for examination.
Step 5: The sediment was stained by adding one drop of methylene blue and a cover slip was Placed over the drops and examined under a microscope at 10 x magnification power.
Annex: 3. **Direct smear technique**
Step 1: Small amount of feces was placed on microscopic slide
Step 2: A drop of water was added on the feces and emulsified thoroughly
Step 3: The debris was avoided and the mixture of water and feces were spread on the slide
Step 4: The cover slip was placed on the smear and examined first under low power (10x) and then By high dry power (40x) microscope. For identification of parasite egg