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Review

Review on the epidemiological features of equine Endoparasites in Ethiopia

Dagim Berhanu Gebresilassie[1*] and Bamlaku Andarge[2]

[1]Department of Animal and Range Sciences, College of Agriculture and Natural Resources, Madda Walabu University, P. O. Box 247, Bale Robe, Ethiopia.
[2]College of Veterinary Medicine, Haramaya University, P. O. Box 138, Dire Dawa, Ethiopia.

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Equines contributed a lot to the livelihood of Ethiopian people in terms of income generation, public transport, ploughing, threshing and ambulatory service for sick humans and animals, however various health problems hamper their contributions and of which endoparasitism is the major one. A number of epidemiological studies conducted in different part of Ethiopia point out the burden and type of endoparasites predominantly affecting horses, donkeys and mules, together or independently for each species. So the current study review is conducted on findings of different investigators with the objective of reviewing the epidemiology and identifies the gaps in the epidemiology of equine endoparasites infection in the country. With these objectives, both published and unpublished original works of numerous investigators were collected and reviewed. With this regard, a number studies conducted in different part of Ethiopia reported a high prevalence of endoparasitism of equines, which may be as high as 100%. In addition, numerous species of endoparasite were identified at the different prevalence in different parts of the country. These studies also indicate that species, age, sex and body conditions of animals were found to be an intrinsic host factor, while climatic condition and management were identified as extrinsic risk factor contribute for the epidemiology of endoparasitism in equines. Limited accessibility of information on agro-ecological based data and lack of detailed study on the general epidemiological status of endoparasitism in different parts of the country were identified as gaps for research area. Finally, further epidemiological study on the spatial and temporal distribution of endoparasites infection in equines is recommended.

Key words: Endoparasite, equines, Ethiopia, prevalence, risk factors.

INTRODUCTION

There is an estimated number of 59 million horses, 43.4 million donkeys and 11million mules in the world (Food and Agricultural Organization, 2011). According to Central Statistical Authority (CSA) (2017) survey report, Ethiopia's horse, donkey and mule population is estimated to be 2.16 million, 8.4 million and 0.41 million, respectively.

Equines, especially in developing countries have a

*Corresponding author. E-mail: dagim.berhanu2010@yahoo.com. Tel: +251921704837.

Table 1. The overall prevalence of equine endoparasites in a different part of Ethiopia.

Equine species	Study area	Type of parasite	Overall prevalence (%)	Sources
Donkey	Hawassa town *	Helminth parasites	96.9	Nuraddis et al. (2011)
Horse	Hawassa town*	Helminth parasites	97.9	Tilahun et al. (2014)
Horse	Kombolcha*	GIT nematode	52.1	Samuel and Berihun (2012)
Donkeys and Horses	Gondar town*	GIT parasites	92.71	Tola et al. (2013)
Donkeys, Horses and Mules	Dessie and Kombolcha**	GIT parasites	70.4	Alemayehu and Etaferahu (2013)
Donkeys and Mules	Bahir Dar*	Strongyles	83.85	Basaznew et al. (2012)
Donkeys and Mules	Bahir Dar*	GIT helminths	83.6	Bewketu and Endalkachew (2013)
Horse	Arsi-Bale highlands	GIT helminths	84.4	Yacob et al. (2013)
Donkeys, Horse and Mules	Jimma town*	Lungworm	13.80	Tihtina et al. (2012)
Donkeys, Horses and Mules	Jimma town	Intestinal nematode parasites	72.25	Bamlaku (2011)
Donkey	Dugda Bora**	GIT parasites	100	Ayele et al. (2006)
Donkey	Sululta and Gefersa**	Endoparasite	99.5	Zerihun et al. (2011)

* In and around, ** District(s).

diversified role in the livelihood and health of human being. They are mainly used for pulling carts, public transport, for ploughing, threshing and ambulatory service for sick humans and animals. The recent work done on livelihood contribution of working equines in Ethiopia has disclosed the contribution of these animals in monetary terms (Berhanu and Yoseph, 2011).

Equines power in both rural and urban transport system is cheap and viable, providing the best alternative in places where the road network is insufficiently developed, and the terrain is rugged and mountainous, and in the cities where narrow streets prevent easy delivery of merchandise (Feseha et al., 1991). In addition to the transport service they provide, equines have an enormous role as a therapeutic tool for human (Schultz et al., 2007).

Although equines are often described as hardy and resistant animals, they do suffer from a number of health problems (Svendsen, 1986; Marquardt et al., 2000). Among which the most common entities leading to ill-health, suffering and early demise and finally death are infectious diseases and parasitism (endoparasites), which resulted in considerably reduced animals work output, reproductive performance and most of all their longevity (Feseha, 1998; Tamador et al., 2011).

Endoparasites are those parasites that live within the body of the host (Heinemann, 2001). Numerous internal parasites are known to infect equines. These include round worms, flukes, tapeworm, protozoan's and fly larvae that infest and damage the intestine, respiratory system and other internal organs (Pereia and Vianna, 2006; Taylor et al., 2007; Alemayehu and Etaferahu, 2013).

A number of epidemiological studies conducted in different part of Ethiopia point out the burden and type of endoparasites predominantly affecting horses, donkeys and mules, together or independently for each species. So the current paper review is conducted both on published and unpublished findings of different investigators on the epidemiology of endoparasites of equine. The objectives of this review paper are:

(1) To review the epidemiology equine endoparasites infection in Ethiopia.
(2) Identify the gaps in the epidemiology of equine endoparasites infection in Ethiopia.

LITERATURE REVIEW

Prevalence of equine endoparasites

In the past decade, a number of studies have been done with the objective of determining the prevalence of equine endoparasitism in different parts of Ethiopia. Accordingly, different investigators have reported from 14 to 100% overall prevalence of endoparasite infection in equines (Table 1).

The study conducted by Getachew et al. (2010) during the periods 1996 to1999 revealed a high prevalence of polyparasitism in donkeys. The presence of high infection prevalence of endoparasitism in Ethiopian equine population in different parts of the country might be associated with lack of effective veterinary services and intervention program, immuno-suppression due to stress and malnutrition, poor awareness of animal welfare and poor management system in those areas where many equines were allowed to graze on the same grazing land throughout the year which facilitates contamination between animals (Ayele et al., 2006; Nuraddis et al., 2011; Zerihun et al., 2011).

Relatively lower infection prevalence of internal

parasitism was also reported in some areas. Different investigators justified variation in the prevalence between the areas and between equine species as it occurs due to variation in season of the year, agroecology, management and difference in sample size (Ayele et al., 2006; Nuraddis et al., 2011; Zerihun et al., 2011; Basaznew et al., 2012; Tihtina et al., 2012; Yacob et al., 2013) (Table 1).

Species of parasite involved in equine parasitosis in Ethiopia

The result of different studies and observations conducted in the last two decades has shown that polyparasitism being a major health hazard limits the overall performance of equines (Getachew et al., 2010; Basaznew et al., 2012).

Equines harbor several parasite that prevail in the gastrointestinal tract (GIT) and other internal organs including round worms (Family: Strongylidae, Spiruridae, Oxyuridae, Trichostrongylidae and Ascarididae), tapeworm (Family: Anopiocephalidae), intestinal and liver fluke (Family: Paramphistomatidae and Fasciolidae), protozoan parasites and flies (Family: Oestridae) that infest and damage the gastro intestinal tract and respiratory system depending on the age and natural defense of the individual equine (Pereia and Vianna, 2006; Taylor et al., 2007; Getachew et al., 2010).

According to Lichtenfels (1975) and Lichtenfels et al. (2008), helminths of equids include 83 species in 29 genera of nematodes, 5 species in 2 genera of trematodes and 4 species in 3 genera of cestodes. Different investigators identified the species of internal parasites found in Ethiopian equines populations using macroscopic and microscopic fecal examination, and fecal culture techniques (Getachew et al., 2010; Ayele et al., 2006; Zerihun et al., 2011).

Getachew et al. (2010) reported a total of 42 species of parasites, consisting of 33 species of nematodes, 3 trematodes, 3 cestodes and 3 arthropods that were recovered from the necropsied equine (donkeys). These included 24 strongylids, 2 anoplocephalids, 1 anoplocephaloid (paranoplocephalid), 2 fasciolids, 1 paramphistomatid, 1 ascarid, 1 strongyloidid, 1 dictyocaulid, 1 trichstrongylid and 1 oxyurid. In addition, 1 atractid (cosmocercid), 1 onchocercid, 2 spirurids (habronematids), 2 gasterophilids and 1 oestrid species were also identified**.** Adult *Strongylus vulgaris* were recovered from colon and caecum, and its larval form from the cranial mesenteric artery and its branches. The same researchers also identified seventeen species of cyathostomins in 7 genera.

Strongyles (red worm) infestation in equines

The red worms (strongyles) are nematode parasites, which are commonly found in the large intestines of horses and other Equidae. They belong to the subfamily Strongylinae (large strongyles) and Cyathostominae (small strongyles). The large strongyles include *S. vulgaris*, *S. edentatus* and *S. equinus*, which, migrate extensively throughout the body, and *Triodontophorus* species, which do not have a migratory life cycle (Radostitis et al., 2006). Members of the genus *Triodontophorus* are frequently found in large numbers in the colon and contribute to the deleterious effects of mixed infection with other strongyles (Taylor et al., 2007).

The large strongyles are among the most distractive parasites of equines. All of them are bloodsuckers as an adult worm in the caecum and colon, and their larvae undergo migration that inflicts greater damage especially in foals and yearlings (Bowman, 2003). Grossly they are robust dark red worms that are easily seen against the intestinal mucosa. Microscopically species differentiation is based on the size and presence, and shape of the teeth at the base of the buccal capsule (Urquhart et al., 1996).

The study conducted in different towns (Gondar and Jimma) and districts (Dugda Bora, Sululta and Gefersa, Dessie and Kombolcha) revealed strongylosis as an important disease of equines in Ethiopia. Those studies also showed a higher prevalence in donkeys as compared to other species of equine (Table 2).

A high prevalence of strongyles has been observed in working donkeys in Ethiopia, and it is attributed to lack of anthelmintic treatment and/or immuno-suppression due to stress and malnutrition (Getachew et al., 2010). However, Samuel and Berihun (2012) reported 32.6% prevalence of strongyles infection in cart horses by a cross-sectional study conducted in and around Kombolcha town. The low prevalence of strongyles in carthorse was due to regular deworming.

Diverse species of large strongyles are known to infect equines population of the country (Getachew et al., 2010). The difference in the prevalence of each species was observed from place to place and between host species (Table 3). The cyathostomins (small strongyles) comprises of more than 40 non-migrating species of which only 10 species occur more commonly. They parasitize the large intestine of equines and it is common to find as many as 20 to 25 of these species infecting individual host at the same time (Bowman, 2003).

Feseha et al. (1991) recorded cyathostomes (in 85 to 92%) as the major helminthic parasites of equines in central Ethiopia. Other studies also reported a higher prevalence of cyathostomins infection in equines (Table 4).

***Parascaris* equorum**

Ascariosis in equines is exclusively caused by *Parascaris equorum*. It is a very large whitish nematode, up to 40 cm in length, cannot be confused with any other intestinal

Table 2. Prevalence of strongylosis in different species of equine in Ethiopia.

Host species	Prevalence (%)	Sources
Donkeys	100	Ayele et al. (2006)
	99.5	Zerihun et al. (2011)
Mules	67.3	Alemayehu and Etaferahu (2013)
	66.28	Bewketu and Endalkachew (2013)
Horses	66.67	Tola et al. (2013)
	63.72	Bamlaku (2011)

Table 3. Prevalence of large strongyles species in different host.

Host species	Prevalence of large Strongyles species (%)					Source**
	S. vulgaris	*S. edentatus*	*S. equinus*	*Triodontophorus species*	*O. robustus*	
Donkeys	100	64.3	-	50	-	1
	91.3	0.97	9.7	12.6	31.1	2
	51.8	30.8	12.3	29.7	-	3
	100	73.8	-	52.8	-	4
Horses	44.7	36.8	16.3	33.2	-	5
	38.46	30.77	-	7.7	-	7
Mules		65.09*		33.72	-	6

*Overall prevalence of strongyles species, **Ayele et al. (2006); Getachew et al. (2010); Nuraddis et al. (2011); Zerihun et al. (2011); Tilahun et al. (2014); Bewketu and Endalkachew (2013); Yacob et al. (2013).

Table 4. Prevalence of cyathostomins in different species of equine.

Host	Prevalence (%)	Sources
Donkeys	100	Ayele et al. (2006)
	100	Zerihun et al. (2011)
	99	Getachew et al. (2010)
Horses	35.1	Yacob et al. (2013)

parasites of equines.

Typical *Parascaris* egg has a thick shell containing a single cell inside (Urquhart et al., 1996). The eggs are very resistant to adverse conditions, like drying or freezing and the larvae rarely hatch and infection usually takes place through ingestion of the eggs (Soulsby, 1982). For this reason, no significant difference was observed in the infection prevalence of ascariosis between dry and wet season (Ayele et al., 2006).

The Infection is common throughout the world and is the major cause of unthriftiness in young foals (Clayton and Duncan 1979; Wintzer, 1996). Adult worms are common in young equids and infrequent in adults (Taylor et al., 2007). However, the high prevalence of *P. equorum* in an adult may be observed irrespective of the age of equines if the animals cannot develop immunity as a young or they might have been immuno-compromised as adults (Getachew et al., 2010).

Heavy infection can cause respiratory signs (from migrating larvae), ill thrift, colic, diarrhea, and intestinal obstruction that may be fatal (Taylor et al., 2007). The work of different researchers demonstrated a high prevalence of equine ascariosis in many parts of Ethiopia, even though low prevalence was also observed in some parts of the country. Those studies also indicate donkeys as a number one host of *P. equorum* followed

Table 5. Prevalence of equine ascariosis in a different area by host species.

Equine species	Study areas	Prevalence (%)	Sources
Donkeys	Dessie and Kombolcha**	70.8	Alemayehu and Etaferahu (2013)
	Sululta and Gefersa**	53.2	Zerihun et al. (2011)
	Hawassa town*	52.8	Nuraddis et al. (2011)
	Dugda Bora**	50	Ayele et al. (2006)
	Gonder town*	42.29	Tola et al. (2013)
	Bahir Dar*	13.68	Bewketu and Endalkachew (2013)
Horses	Dessie and Kombolcha**	58.5	Alemayehu and Etaferahu (2013)
	Hawassa town*	55.8	Tilahun et al. (2014)
	Gonder town*	43.81	Tola et al. (2013)
	Arsi-Bale highlands	11.7	Yacob et al. (2013)
Mules	Bahir Dar*	10.46	Bewketu and Endalkachew (2013)
	Dessie and Kombolcha**	67.3	Alemayehu and Etaferahu (2013)

* In and around, ** District(s).

Table 6. Prevalence of ascariosis in relation to age group.

Host species	Prevalence by age group (%)			χ^2 value	P-value	Sources
	Young **	Adult **	Old **			
Donkeys	48.5	17.1	34	2.10	0.34	Nuraddis et al. (2011)
	45.45	10.81	5.71	34.374	0.000*	Bewketu and Endalkachew (2013)
Horse	28.3	20.8	50.9	2.07	0.34	Tilahun et al. (2014)
Mules	17.91	6.66	5.263	0.022	0.022*	Bewketu and Endalkachew (2013)

*Statistically significant ($p<0.05$) among age groups; ** Young <3 years, Adult 3-10 years and Old >10 years.

by mules and horses (Table 5).

Due to lacks of well-organized immune system, young animals are known to have a high chance of infection than an adult; however insignificant variation in age group based on prevalence of *P. equorum* was reported in donkeys and horses because of the compromised immune system as a result of workload, poor husbandry and health care service (Table 6).

Oxyuris equi

Pinworms (*Oxyuris equi*) are an annoying but not life-threatening parasite of equines. They provoke irritation of the perianal region of equines causing them to rub and bite their tail. This can result in hair loss and sometimes physical damage to the tissue of the area. The parasite is ubiquitous but of greater prevalence in areas of high rainfall (Hendrix, 1998).

The mature females are large grayish white, opaque worms, with a very long tapering tail that may reach 10 to 15cm in length, whereas the mature males are generally less than 1.2 cm long. The L_4 stages of this parasite are 5 to 10 mm in length, having tapering tails and are often attached orally to the intestinal mucosa. Egg of *O. equi* is ovoid, yellowish, thick shelled and slightly flattened on one side with a mucoid plug at one end. Eggs contain a morula or larval stage when shedding in faeces (Taylor et al., 2007).

The study conducted by Yoseph et al. (2001) revealed 32.4% prevalence of equine oxyurosis in Wonchi, and similarly different studies reported high prevalence of *O. equi* in different parts of the country, while relatively lower prevalence ware also observed in others. The difference in prevalence might be related to the climatic condition of the study areas (Ayele et al., 2006). Table 7 summarizes

Table 7. Prevalence of oxyurosis in different host and area.

Host	Study area	Prevalence (%)	Sources
Donkeys	Hawassa town*	31.8	Nuraddis et al. (2011)
	Dessie and Kombolcha**	4.5	Alemayehu and Etaferahu (2013)
	Dugda Bora**	3	Ayele et al. (2006)
Horses	Hawassa town*	34.2	Tilahun et al. (2014)
	Dessie and Kombolcha**	3.8	Alemayehu and Etaferahu (2013)
	Arsi-Bale highlands	1.8	Yacob et al. (2013)
Mules	Dessie and Kombolcha**	4	Alemayehu and Etaferahu (2013)

*****In and around, **District(s).

Table 8. Prevalence of *Dictyocaulus arnfieldi* by host species and area.

Host species	Study area	Prevalence (%)	Sources
Donkeys	Jimma town*	35.3	Tihtina et al. (2012)
	Dugda Bora District	32	Ayele et al. (2006)
	Bahir Dar*	22.17	Bewketu and Endalkachew (2013)
	Hawassa town*	3.6	Nuraddis et al. (2011)
Horses	Jimma town*	4.3	Tihtina et al. (2012)
	Hawassa town*	3.7	Tilahun et al. (2014)
Mules	Jimma town*	29.3	Tihtina et al. (2012)
	Bahir Dar*	8.14	Bewketu and Endalkachew (2013)

*****In and around.

the prevalence of oxyurosis in a different part of Ethiopia with respect to equine species.

Dictyocaulus arnfieldi

Infestations with *Dictyocaulus arnfieldi* were identified more commonly in donkeys than in horses, and the former is considered to be the more normal host. Patent infections may persist in donkeys throughout their lives but in horses are generally confined to foals. These animals, therefore, provide the most important sources of pasture contamination, nevertheless, a small proportion of infected adult horses shed low numbers of eggs and this may be sufficient to perpetuate the lifecycle even in the absence of donkeys and foals (Radostitis et al., 2006). Studies conducted in some parts of Ethiopia showed a high prevalence of equine lungworm infection in donkeys followed by mules and horses, respectively (Table 8). Even though *D. arnfieldi* is thought to be more common in donkeys than in horses (Radostitis et al., 2006), a high prevalence was reported in the horse as compared to donkeys in some parts of Ethiopia with unjustified reason (Nuraddis et al., 2011; Tilahun et al., 2014).

Strongyloides westeri

Strongyloides (threadworm) is unique among the nematodes of veterinary importance, being capable of both parasitic and free-living reproductive cycles. The parasitic phase is composed entirely of female worms in the small intestine, and these produce larvated eggs by parthenogenesis, that is, development from an unfertilized egg.

After hatching, larvae may develop through four larval stages into free-living adult male and female worms, and this can be followed by a succession of free-living generations. However under certain conditions, possibly related to temperature and moisture, the L3 can become parasitic, infecting the host by skin penetration or ingestion and migrating via the venous system, the lungs and trachea to develop into adult female worms in the small intestine (Taylor et al., 2007).

Infections are very common, especially in warm and humid environments. *Strongyloides* infective larvae are not ensheathed and are susceptible to extreme climatic conditions. However, warmth and moisture favor development and allow the accumulation of large numbers of infective stages. A second major source of infection for the very young animal is the reservoir of

larvae in the tissues of their dams and this may lead to clinical strongyloidosis in foals in the first few weeks of life. Successive progeny from the same dam often show heavy infections (Taylor et al., 2007).

In Ethiopia, different investigators reported strongyloidosis in equines at different levels of prevalence, and their results are summarized in Table 9. The difference in the prevalence report in the country between different investigators might be due to variation in the management system, sample size and sampling method used (Nuraddis et al., 2011). However, the difference between host species was not justified.

Fasciola species

Fasciolosis is an economically important disease of domestic livestock. Most commonly *F. hepatica* and *F. gigantica* are implicated as the aetiological agents of fasciolosis. *F. hepatica* has a worldwide distribution but predominates in temperate zones while *F. gigantica* is found on most continents, primarily in tropical regions (Dalton, 1999). The amphibious snails of the genus *Lymnaea*, most commonly *Lymnaea truncatula*, are intermediate hosts and release the infective form, the metacercaria, onto herbage (Taylor et al., 2007). Even though Dalton (1999) described equine fasciolosis as less economically importance on a global scale; higher infection was registered with *F. hepatica* and *F. gigantica* in donkeys from fasciolosis endemic area of Ethiopia (Getachew et al., 2010). This is attributed to the presence of wide marshy and swampy vast communal grazing areas, which is common in many parts of Ethiopia (Yacob et al., 2013). Getachew et al. (2010) also suggested a further epidemiological study on the prevalence of equine fasciolosis and the role of equines in the disease epidemiology (Table 10). There was an increasing trend in the prevalence of fasciolosis with age of equines, which can be explained from the fact that older animals might have a high risk for exposure to *Fasciola* than the young (Yacob et al., 2013).

Equine tapeworms

Several tapeworm species are found in horses, donkeys and other equines. Intermediate hosts for all species are forage mites of the family Oribatidae, in which the intermediate cysticercoid stages are found (Taylor et al., 2007). In Ethiopia, three species of tapeworms are identified by Getachew et al. (2010) including *Anoplocephala magna, A. perfoliata* and *Anoplocephaloides mamillana.* Varied prevalence of equine tapeworm infection was reported in Ethiopia, and it is summarized in Table 11. The low prevalence of *Anoplocephala* species in some report was justified with the seasonality of the intermediate host (oribatid mites), sporadic discharge of gravid segments in the faces and low sensitivity of fecal examination (Zerihun et al., 2011).

Gasterophilus species

Species of *Gasterophilus*, known as bots, are obligate parasites of horses, donkeys, mules, zebras, elephants and rhinoceroses. Nine species are recognized in total, six of which are of interest as veterinary parasites of equids. The burrowing of first- and second-stage *Gasterophilus* larvae in the tissues of the tongue and mouth may result in lesions. The presence of larval parasites in the stomach is difficult to identify except by observation of the larvae in feces, and adult flies are most active during late summer (Taylor et al., 2007). Ayele et al. (2006) reported a total prevalence of 20.9% *G. intestinalis* and *G. nasalis* in donkeys on gross fecal examinations. Tola et al. (2013) also reported 0.95% prevalence of *G. intestinalis* larvae in horses.

Other equine endoparasites identified in Ethiopia

Getachew et al. (2010) reported a range of internal parasites of donkey identified on necropsy of seven donkeys from Ada in addition to those discussed earlier. The result are displayed in Table 12.

Risk factors

Intrinsic host factors

Various studies with the objective of determining the risk factors associated with the infection of equine endoparasite were undertaken in Ethiopia. These studies indicated age, species, body condition and sex were intrinsic factors associated with equine endoparasitism (Ayele et al., 2006; Nuraddis et al., 2011; Bewketu and Endalkachew, 2013; Yacob et al., 2013).

According to Nuraddis et al. (2011), Zerihun et al. (2011), Samuel and Berihun (2012), Tihtina et al. (2012), Alemayehu and Etaferahu (2013) and Bewketu and Endalkachew (2013) age is one of the important factors, which influence the occurrence of some helminth parasites in equines. The statistically significant difference ($P<0.05$) was observed in the prevalence of *S. vulgaris*, *S. edentatus, O. equi,* and *P. equorum* with body condition.

Helminth parasites are more prevalent in animals with poor body condition than well-conditioned animals (Nuraddis et al., 2011). Similarly, Ayele et al. (2006), Zerihun et al. (2011) and Tihtina et al. (2012) found a statistically significant difference in the prevalence of endoparasites between different body condition score. It is advisable to train the owners in order to improve the management system, especially in terms of the level of nutrition so that the animal can have good body

Table 9. Prevalence of *Strongyloides westeri* in equines.

Study area	Prevalence (%)	Host	Sources
Hawassa town*	20	Donkeys	Nuraddis et al. (2011)
Hawassa town*	28.4	Horse	Tilahun et al. (2014)
Ada, Akaki, Boset and Bereh	11	Donkeys	Getachew et al. (2010)
Arsi-Bale highlands	0.7	Horse	Yacob et al. (2013)

*****In and around.

Table 10. Prevalence of *Fasciola* species in equines.

Study area	Prevalence (%)	Host spp.	Sources
Ada, Akaki, Boset and Bereh	80	Donkeys	Getachew et al. (2010)
Dessie and Kombolcha **	5.9	Mules	Alemayehu and Etaferahu (2013)
Arsi-Bale highlands	23.1	Horses	Yacob et al. (2013)
Bahir Dar	27.1	Mules	Gezahegn (2000)
Dessie and Kombolcha **	9.2	Horses	Alemayehu and Etaferahu (2013)

**District.

Table 11. Prevalence of equine tapeworm in Ethiopia.

Study area	Prevalence (%)	Host	Sources
Sululta and Dugda Bora**	2.8	Donkeys	Zerihun et al. (2011)
Bahir Dar*	23.12 and 16.86	Donkeys and Mules, respectively	Bewketu and Endalkachew (2013)
Dugda Bora**	7.4	Donkey	Ayele et al. (2006)

*In and around, **Districts.

Table 12. Other helminthic parasites and arthropod larvae identified on the postmortem of donkey from Ada.

Parasite	Location
Gastrodiscus aegyptiacus	Caecum
Probstmayria vivipara	Colon
Setaria equina	Peritoneal cavity
Rhinoestrus uzbekistanicus	Paranasal sinus
Habronema muscae	Stomach
Draschia megastoma	Stomach
Trichostrongylus axei	Stomach

Source: Getachew et al. (2010).

condition that confers some level of resistance against helminthes infection (Nuraddis et al., 2011).

According to Yacob et al. (2013), female horses were found to be more susceptible to P. equorum infection than their counter males. The prevalence of P. equorum was also higher in mares (15.7 %) than their counterpart stallions (9.5 %). This can be justified by the fact that mares have a close relation to their foals, which favors frequent recycling of the parasite between the dam and foal.

Female donkeys were found to have a significantly higher infestation of strongyles than their counterpart males as they might have lower immunity due to gestation, lactation and stresses occurred during this period (Sapkota, 2009; Bewketu and Endalkachew, 2013).

In addition to the aforementioned factors, species was also indicated as an important intrinsic factor and statistically, significant difference was reported in the prevalence of equine endoparasitism among different species (Tihtina et al., 2012; Alemayehu and Etaferahu, 2013; Bewketu and Endalkachew, 2013; Tola et al., 2013).

Extrinsic factors

Management is considered as an important factor contributed to the high prevalence of equine endoparasitism. This is demonstrated with the study conducted by Alemayehu and Etaferahu (2013), which indicates a variation in prevalence of endoparasitism between equine that was used for packing and cart pulling. Higher prevalence of parasitism was observed in equines used for packing and transportation than animals used for cart pulling, and this might be confounded by the difference in the management (care) given to these groups of animals. There is a habit of giving special care (for the equines used for cart pulling) such as deworming and supplementary feed. Moreover, the chance of grazing for these animals was less as they are on work, which actually reduces the chance of getting an infection, and cart-pulling equines feeding system also reduce exposure of equines for infection (Samuel and Berihun, 2012; Alemayehu and Etaferahu, 2013). The climatic condition of the area like rainfall and temperature also affect the development and survival of infective larvae in the external environment (Yacob et al., 2013).

CONCLUSION AND RECOMMENDATIONS

Equine contributed a lot to the livelihood of Ethiopian people in terms of different aspect including in income generation. However, various health problems hamper their contributions of which endoparasitism is the major one. Several studies conducted in different part of Ethiopia reported a high prevalence of endoparasitism of equines, which may be as high as 100%. In addition, several species of endoparasite were identified at the different prevalence, and also risk factors for infection were investigated at a certain level. The presence of polyparasitism with high prevalence and high infection intensity is an indication that is favorable environmental conditions for infection, survival and perpetuation of the parasites as they exist in Ethiopia. The lack of anthelmintic treatment, poor body condition, weak immune status and workload may also be a contributing factor. However, the information on the prevalence of equine endoparasitism in different areas and agro-ecological based data are still limited. In addition to this, detailed study on the pathogenicity, treatment and control strategies, and the immune response of equines to the infection of each parasitic species was barely available.

In line with the aforementioned conclusion, the following recommendations are forwarded:

(1) Further epidemiological studies should be conducted to reflect the spatial and temporal distribution of endoparasite infection in equine
(2) Detail studies on the pathogenicity, treatment and control strategies and the immune response of equines to the infection of each parasitic species should be conducted

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Evaluation of metacestode of *Taenia solium* antigens for detection of anti-cysticercal IgG among patients with epilepsy in three districts of Northern Uganda

Simon Peter Alarakol[1*], Moses Lotokome Joloba[2], Walter Onen Yagos[3] and Emmanuel Odongo Aginya[4]

[1]Department of Medical Biochemistry, Faculty of Medicine, Gulu University, P. O. Box 166, Uganda.
[2]School of Biomedical Sciences, Makerere College of Health Sciences, P. O. Box 7072, Kampala, Uganda.
[3]Medical Library, Faculty of Medicine, Gulu University, P. O. Box 166, Gulu, Uganda.
[4]Department of Microbiology and Immunology, Faculty of Medicine, Gulu University P.O. Box 166 Gulu, Uganda.

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Neurocysticercosis causes serious public health and socioeconomic problems in developing countries including Uganda. The aim of the study was to evaluate the metacestodes of *Taenia solium (T. solium)* antigens for detection of anticysticercal Immunoglobulin G (IgG) antibodies among patients with epilepsy in three districts of Northern Uganda. Three hundred (300) random samples were screened for anticysticercal IgG using indirect antibody Enzyme Linked Immunosorbent assay (ELISA). Samples positive for anticysticercal IgG were confirmed using western blot. The sensitivity and specificity of the ELISA method was 90 and 95.4% with positive predictive value and negative predictive value of 77.8 and 98.4% respectively.. The kappa value at 95% CI was 0.668 (0.545-0.791). The strength of agreement between ELISA assays and immunoblot was good. The finding indicates the ELISA based method using locally derived antigens can be used to screen most patients with epilepsy for exposure to T. *solium* antigens and the method can be employed in resource limited settings in most developing countries.

Key words: Evaluation, metacestode, Taenia solium antigen, ELISA, Neurocysticercosis, Northern Uganda

INTRODUCTION

Cysticercosis is a human parasitic disease caused by eating pork infected with *Taenia (T) solium* cysticerci that infect humans and free roaming pigs in developing countries. (Carabin et al., 2009; Abdo et al., 2010). Human cysticercosis can also be acquired by ingesting embryonated *T. solium* eggs in food or water contaminated with fecal matter of persons harbouring the adult tapeworm Zoli et al., 2003, Waiswa et al., 2009).

The eggs of the adult tapeworm hatch and release the first larvae onchospheres in the duodenum. These onchospheres penetrate the intestinal mucosal and enter into the blood circulation. Subsequently, the onchospheres invade striated muscles, brain, liver, and other organs where they form cysticerci (Del Brutto and

*Corresponding author. E-mail: alarakol@yahoo.com. Tel: +256772957280.

García, 2013). The most severe manifestation of the disease occurs when the metacestode (larval stage) of the parasite infect the human central nervous system (CNS), causing neurocysticercosis (NCC) (Morales et al., 2008; Winkler et al., 2009). This disease may be asymptomatic or may present with a number of nonspecific indications, such as seizures, headaches, focal neurological deficits, increased intracranial pressure, or cognitive decline (Del Brutto and García, 2014; Suzuki et al., 2007; Alarakol et al., 2018). Globally, approximately 50 million are infected with *T. solium* parasites and 50,000 people die of cysticercosis related diseases annually (Fleury et al 2006; Lescano et al., 2007). In developed countries particularly United States of America, cases of human cysticercosis have been reported among immigrants (Lescano et al., 2007). In Uganda, information about NCC is limited (Alarakol et al., 2017). Neuroimaging techniques such as computer tomography (CT) scans and magnetic resonance imaging (MRI) have been recognized as the gold standard for diagnosing NCC (Suzuki et al., 2007; Winkler et al., 2012). However, these techniques do not provide a definitive diagnosis of NCC due to the complex pathological processes of this disease in the CNS, which often mimic other infectious or non-infectious diseases (Mayta et al., 2008; Del Brutto and García, 2014). While these techniques are essential in the diagnoses of NCC, the costs of the equipment are immense and most health facilities in rural setting in developing countries are unable to bear. More so, serologic findings particularly enzyme linked immuno-sorbent assay (ELISA) based techniques are essential in the screening for NCC, however, their setbacks associated with false negatives and false positives make them unreliable (Marcello et al., 2006.) The Electroimmunotransfer blot (EITB) technique developed by Tsang et al. (1989) which had the sensitivity and specificity of 98 and 100% respectively provides an alternative to this limitation. Despite these efforts, many rural settings are unable to use this technique due to long procedures, sophisticated equipment and cost of reagents required to carry out these procedures. (Alarakol et al., 2017). The aim of this study was to evaluate *T. solium* antigens for serodiagnosis of human cysticercosis using antibody ELISA based model suitable for use in resource limited settings of Northern Uganda.

MATERIALS AND METHODS

Study area

The study was conducted in three rural districts of Northern Uganda between June 2014 to April 2015. This includes; the districts of Gulu (Latitude, 2.77° North, Longitude 32.31° East), Moyo (Latitude 3.37° North, Longitude, 31° 46 East) and Adjumani (latitude, 3.3833°N, longitude, 31.7667° E and 32.7833°N) covering an estimated 6,500 km^2.with an estimated population of 916,000 inhabitants(https://en.wikipedia.org/wiki/Adjumani_DistrictWikipedia, (http://www.ugandatravelguide.com/guludistrict.html),(https://en.wikipedia.org/w/index.php?title=Moyo_District&action=info).

Study design

A cross sectional study was conducted on sera samples of patients with epilepsy recruited and referred for further management and care at epilepsy Treatment Centers in Gulu Regional Referral Hospital (GRRH), Moyo Hospital (MH) and Adjumani Hospital (AH), Northern Uganda. Detection of anticysticercal Immunoglobulin G (IgG) was done using indirect antibody ELISA (Ab-ELISA). Positive Sera samples for anticysticercal IgG was confirmed using western blot analysis. The research protocol were approved by Gulu University Research Ethic Committee and Uganda National Council of Science and Technology (Ref: HS987).

Sampling and sample collection

One thousand three hundred eighty three (1383) people suspected of epileptic seizures at households' level were referred for further management and care at epilepsy Treatment Centres in the three hospitals; MH, AH and GRRH. These patients were initially identified from a large community based study conducted in the area as described by Alarakol et al. (2017). Patients presented to the hospitals or health facilities were recorded in the register using the patients` identification number. Simple random sampling was then conducted on the six hundred patients confirmed for epileptic seizures by neurologists. This was done to ensure each patient selected had an equal chance of being included in the study. A total of 300 patients confirmed for epileptic seizures were selected for the study. Blood specimens were collected from selected patients into plain vacuotainer tubes. These were allowed to clot at ambient temperature, later centrifuged and the sera were extracted and stored at -20°C for subsequent use (Alarakol et al., 2017). The sera samples were used in antibody ELISA assays done at Faculty of Medicine, Gulu University Uganda.

Preparation metacestode of *Taenia solium* antigens

Crude antigens for detecting anti-cysticercal IgG in patients with epilepsy was prepared from cysts collected from heavily infected pigs. The antigen was prepared as described by Gottstein et al. (1986). Briefly, 3.0 g of frozen whole cysts thawed in 50 ml falcon tubes on dry ice were added to 7 ml Phosphate Buffered Saline (PBS) containing 0.01% of NaN_3 (Invitrogen, Germany). This was homogenized on ice for fifteen minutes. The samples were freeze-thawed twice in liquid nitrogen for further denaturation. These were ultrasonicated in ice bath six times, at intervals of 30 seconds for a total of three minutes, and thereafter centrifuged at 4 degrees Celsius in ultracentrifugation at 13,000g for 45 min. The protein concentration of the supernatant was determined using Biuret's method and the characteristic purity evaluated using sera samples obtained from known NCC positive patients and confirmed with western blot. The antigens were stored at -80°C until use.

Detection of anticysticeral IgG using ELISA

The IgG from patient sera was screened using indirect Ab- ELISA as described by Sloan et al. (1995). Each ELISA plate was coated with antigens. The antigens were prepared in the Department of Infectious Diseases and Tropical Medicine (DITM), University of Munich, Germany. Briefly, 1.0 µg/ml of antigens was coated on to the 96 polystyrene microplate (Nunc® Maxisorp) wells and incubated overnight. The unbound antigens were washed four

times with Phosphate buffered Saline (PBS)/TWEEN (PH = 7.2) and this was followed by blocking with PBS/bovine serum albumen (BSA)/TWEEN for 1 h at 37°C. 10 µl of antisera were then added and incubated for 1 h at 37°C. The unbound sera components were washed and rabbit anti human horseradish-peroxidase polyclonal IgG (Invitrogen, Germany) specific for the first antibody in the sera was added to form antigen-antibody complex. The unbound antibody enzyme conjugate was washed and a substrate *o*-phenlydiamine (OPD) (Invitrogen) was added. This was followed by dark incubation of the samples for 30 min. The reaction was stopped by adding 0.5 M H_2SO_4 (BDH UK). This conjugate catalysed formation of colored substance which was measured photometrically at 492 nm using ELISA reader (Tecan Austria GmbH, Sunrise). Known positive and negative control sera were included for validation of the test results. The cut-off points were calculated as the mean of the optical density (OD) values obtained with 8 negative serum samples plus two standard deviations (SD). A sample was considered positive if the OD value was greater than the estimated cut-off point.

Positive control sera

The control positive sera were obtained from 15 patients with confirmed neucysticercosis using clinical, CT scan and/MRI and immunodiagnosis. The patients were referred to the hospital after presenting with convulsions which had not been previously investigated and none of them were on treatment program.

Negative control sera

The negative control samples were from 15 healthy individuals no history of seizures and were screened for other helminthes namely; *Taenia saginata*, *Echinococcus granulosus*, *T. hydatigena,* askarids, strongyloides. Additionally, they also underwent serodiagnosis with western blot

Polyacrylamide gel electrophoresis

The sera samples which were positive in Ab-ELISA were run on western blot for confirmation. This was preceded by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) analysis. Preparation of the immunoblot strips for serodiagnosis was done as previously described by Parija and Raman (2011) but with some modifications of the procedures. These were required in order to shorten the time of separation of the glycoprotein in Tris –Trisine buffers using high voltage and current. Briefly, 750 ug/ml of crude antigens were run on a SDS-PAGE in a 16.4% resolving and 4.0% stacking gel (in Tis-Tricine buffers) at 100V 150 mA 10W for 5 h. The antigens were separated under non reducing conditions by SDS-PAGE in a 10 × 8 cm Mini gel (Austria). The gels were stained with bromophenol blue and the molecular weight marker (ladder) (Invitrogen, Germany) placed at both ends.

Electroimmunotransfer blotting (Western blot)

The separated antigenic proteins on the gels were electronically transferred in wet 0.22 um pores size nitrocellulose membranes (USA, Billerica, MA) in a mini Transblot cells (BIORAD) at 150 V 100 mA 30 W for 1 h as described by Parija and Raman (2011). The two small portions on the nitrocellulose membrane branded with the molecular weight markers were cut and air dried. The larger portion of the nitrocellulose membrane containing separated antigenic proteins was placed on 5% BSA for 30 min to block nonspecific binding sites and thereafter air dried. The immunoblot strips of 4 mm were cut from the larger portion of the dried nitrocellulose membrane and stored at 4°C in fulcon tubes for serodiagnosis.

Electroimmunotransfer blot analysis

Western blot analysis was conducted as previously described by Parija and Raman (2011) with some modifications. Briefly, serodiagnosis with the stored immunoblot strips was done on all patients samples which were positive with antibody ELISA. The immunoblot strips were placed in Mini incubator Trays (Hindenerg, Germany) and incubated on 5% BSA for 30 min. This was followed by washing with Phosphate Buffered Saline (PBS)//TWEEN (PH= 7.2) five times with ELISA washer (Tecan Austria GmH, Hydrofolex). All subsequent steps were washed five times. The patient sera were added and incubated on a shaker (Hindenberg, Austria) for 70 min. This was followed by addition of a secondary enzyme alkaline phophatase linked Poly Rabbit anti-human IgG antibody (Invitrogen, Germany) and incubation on a shaker for 75 min. The last incubation and washing was done for 5 and 10 min, thereafter, the substrate diaminobenzidine (DAB) (Invitrogen, Germany) added. The strips developed the bands within 10 min on a shaker (Heinsburg, Germany). Thereafter, the strips were removed from incubator tray and placed in water to stop the reaction. These were then removed and air dried on glass plate. The presence of the antibody in the sera were confirmed when at least two specific bands of 8, 10, kDa were observed on the diagnostic region. The molecular weight of *T. solium* characteristic antigenic peptides were determined by comparing the bands in diagnostic region with the standard molecular weight markers placed alongside the strips. Major antigenic peptides were; 8, 10, 18, 32, 40, 50, 76 and 100 kDa. The bands outside the diagnostic regions were not considered as specific for *T.* solium because these were shared by other helminthes (Alarakol et al., 2017). The proportions of patients positive for *T. solium* antibody in Ab-ELISA and those in immunoblot were determined.

Data analyses

The data are presented as mean, standard deviation, frequency and variance. The data were analysed using Pearson's chi square to test for statistical level of significance between means of proportions (dependent and independent variables). The dependable variables included optical densities of the IgG and study locations while the independent variables included age and sex of the patients. The sensitivity was calculated by dividing the proportion of samples of people with NCC who have a positive antibody ELISA results. While the specificity was calculated by dividing the proportion of samples of people without the NCC who have a negative antibody ELISA results. Student-t test was used to test for differences between the means of proportions (optical density) for levels of significance. MacNemar chi-square ($\chi 2$) with Yates correction continuity factor was used to compare the differences between the proportions for independence. The probability value of $p<0.05$ were considered to be statistically significant. Data was also analysed to check the levels of agreement between tests using Kappa values.

RESULTS

Demographic characteristics

Out of 300, epileptic patients who presented to the health

Table 1. Demographic characteristics of study population.

Variable	Frequency	Percentage
Sex		
Male	170	56.7
Female	130	43.3
Age group		
10-19***	132	44.2
20-29**	78	26.2
30-39*	40	13.4
40-49	20	6.7
≤50	30	10.0

**; Age group 10-19 were more predominant in the present study, follows by ** ; 20-29 and *; 30-39; Number of males were significantly higher than female counterpart, *P*=0.00.

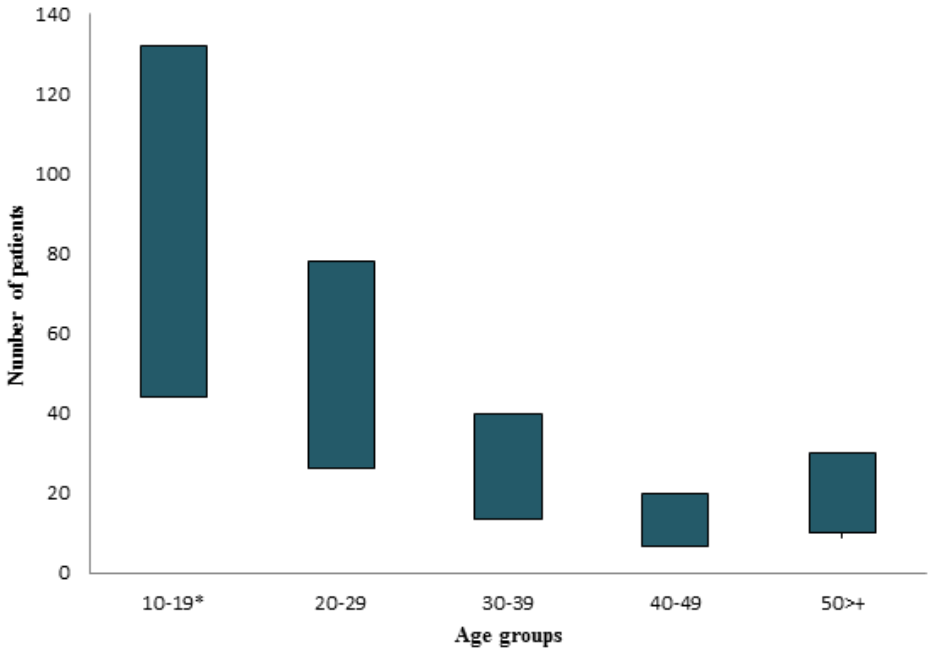

Figure 1. Graph showing variation of number of patients within different age groups. Majority of patients were in the age group 10-19** follows by 20-29 and 30-39. Males were more among age groups 10-19, 75(56.9%), 20-29, 37 (47.4%), 30-39, 24 (60%), 40-49, 13(65%) and 50>, 21(70%). While the females were more among aged groups 20-29, 37(47.4%).

facilities for medical care, 170 (56.7%) were males and 130 (43.3%) were females with the mean age of 25±13. The proportion of patients were more among age groups 10-19, 132(44.2%), 20-29, 78 (26.2%), 30-39, 40 (13.2%), 40-49, 20(6.2%) and 50>,30(10.0%) (Table 1). Males were more among age groups 10-19, 75(56.9%), 20-29, 37 (47.4%), 30-39, 24 (60%), 40-49, 13(65%) and ≤50, 21(70%) (Figure 1). While the females were more among aged groups 20-29, 37(47.4%).

Detection of anticysticercal IgG antibody using antibody ELISA

Out of 300 serum samples Gulu, 17 had representing 15.3% of patient samples positive for anticysticercal IgG. Adjumani and Moyo had 18(19.6%) and 10 (10.3%) of patients samples positive for anticysticercal IgG respectively. There was statistical significance difference observed between the positivity of patients' sera of Gulu

Table 2. Seropositivity of anticysticercal IgG antibody using antibody ELISA (N=300).

District	Number of patients screened	Antibody ELISA positive samples (n)	Percent (%)
Gulu	111	17	15.3
Adjumani	92	18	19.6
Moyo	97	10	10.3
Total	**300**	**45**	**15.0**

n = represents number of samples positive for anticysticercal IgG antibody, N = represents total number of patients screened in the study area. The positivity to anticysticercal IgG differed significantly between Gulu and Adjumani (*P*=0.00), Gulu and Moyo (*P*=0.26) when screened with ELISA.

Table 3. Seropositivity of IgG antibody using immunoblot assays.

District	Antibody ELISA positive samples[a]	8and 10 kDa positive samples[b]	Percent (%)[c]
Gulu	13	12	30.8
Adjumani	18	16	41.0
Moyo	15	11	28.2
Total**	**45**	**39**	**86.7****

[a], samples positive for anticysticercal IgG on Ab-ELISA; [b], total of samples positive for immunoblot 8 and 10 kDa, [c]: percentage of patients samples positive for immunoblot 8 and 10 kDa bands, ** Total number of antibody positive patients samples positive for 8 and 10 kDa.

and Adjumani (*P*=0.00). While no significant difference in the level of positivity was observed between those of Gulu, Adjumani and Moyo (*P*=0.26).Table 2.

Detection of anticysticercal antibody IgG using immunoblot assays

Table 3 shows forty five antibody positive sera analyzed by Immunoblot for specific *T. solium* glycoproteins. Of the 45 samples analyzed, 86.7% were positive on the diagnostic bands 8 and 10 kDa proteins. Adjumani had significantly (*P*=0.00), highest patients tested positive for 8 and 10 kDa proteins than Gulu 12 (30.8%) and Moyo 11(28.2%) respectively (Table 3). Eighteen, 18(40.0%) patients sera strongly reacted with higher molecular weight of 14 kDa, 21 kDa, 24 kDa, 38-42 and 50kDa proteins (Figure 2). Fourteen 14(31.1%) patients sera showed reactions below the diagnostic bands (below 8 - 10 kDa) (Figure 2).

Sensitivity and specificity of Ab-ELISA Vs. immunoblot

Ab ELISA findings were analyzed using immunoblot as the gold standard, the sensitivity (Se) and specificity (Sp) of local Ab- ELISA was 89.7 and 95.4% with PPV and NPV of 77.8 and 98.4% respectively (Table 4). The McNemar $\chi 2$ square test with Yates continuity Correction for small samples sizes revealed that the percentage of samples positive with local antibody ELISA differed significantly from local immunoblot, $\chi 2$ (1, n= 300) = 0.02, $P < 0.05$, the odd ratios with associated 95% confidence limits was 1.7 (1.1-2.5); ($P<0.01$). When analyzed for kappa values, the numbers of observed and expected agreements were 92.0% (n=300) and 75.1% (n=300) of the observations respectively. The kappa value at 95% CI was 0.668 (0.545-0.791). Therefore, the strength of agreement between local Ab ELISA assays and immunoblot was good (Table 4).

DISCUSSION

Diagnosis of NCC is a challenging construct that requires technical knowledge, skills, and robust equipment in its execution. However, these are mostly possible in fairly developed facilities in urban and peri-urban centres. Solving these inadequacies require development of a robust, simple and cheap technique that can be used in the screening of NCC in rural settings. The present study evaluated the diagnostic potential of the metacestodes of *T. solium* antigens for use in ELISA based format. Our findings indicate that Ab-ELISA format with locally derived antigens had sensitivity and specificity of 90 and 95.4%, respectively. This indicates that the ELISA based method can detect majority (90%) of the patients associated with NCC as positive for anti-cysticeral IgG, and with a specificity at 95.4%. These are important attributes, particularly when the ELISAs are used for screening purposes to identify previous exposures to T. *solium*

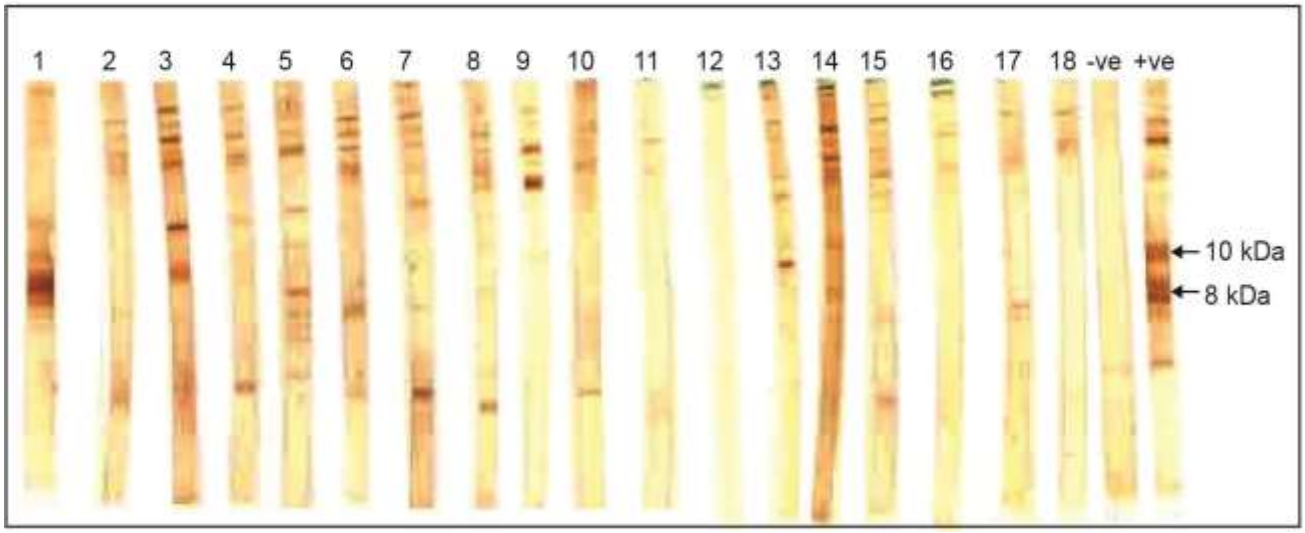

Figure 2. Immunoblot results for patients positive for 8 and 10kDa glycoproteins of *Taenia Solium*. Lane (+ve) (right); immunoblot containing known NCC sera (positive control). Lane (-ve); negative control; Lane 1, 3, 5, 6, 7, 14 and 17 are positive for 8 and 10 kDa glycoproteins

Table 4. Sensitivity and specificity of Ab- ELISA assays Vs. local immunoblot.

		Immunoblot**		Total
		Positive	Negative	
Ab-ELISA	Positive	35	10	45
	Negative	4	251	255
Total		39	263	300

** Immunoblot was the gold standard, sensitivity (Sn) and specificity (Sp); sn; 90.%, sp; 95.4%, PPV; 77.6, NPV; 98.4%, χ^2 =0.02, *P*<0.05.

infections. The ELISA based methods for detecting the presence of IgG and circulating antigens have long been described in previous studies (Decker et al., 2010). These serologic findings, together with neuroimaging techniques have been used in the diagnosis of NCC (Garcia et al., 2005). The latter have been used for confirmation of the NCC among suspected patients. The sensitivity was defined as the proportion of people with NCC who have a positive antibody ELISA results. While the specificity was defined as the proportion of people without the NCC who have a negative antibody ELISA results. Although, the present study used crude antigens of *T. solium* metacestodes, the sensitivity and specificity obtained from this ELISA method is comparable to the previous studies. Previous studies have reported on Ab-ELISAs with varying results. Mittal et al. (2001) reported the sensitivity and specificity of 10.4 and 70% (Sloan et al., 1995), 93 and 95% (Parija and Raman 2011), 91 and 96% for crude *T. solium* metacestodes. Minozzo et al. (2008) reported a sensitivity and specificity of ELISA at 96 and 90% using serum samples. Ito et al. (2006) on the other hand observed that the sensitivity of ELISA in confirming active NCC case with serology was 94%, of which 93% was from native antigens. Whereas Ito et al. (2006) reported high sensitivity and specificity with native antigens and chimeric recombinant antigens in both ELISA and immunoblot; in this study, the low sensitivity (90%) of locally derived antigens in Ab-ELISA format may be due to cross reactions with other helminthes in the patients' sera which interact with the antigenic epitope, hence blocking the true antibody the accessibility (Alarakol et al., 2017). Fleury et al. (2001) reported that un-purified antigens have moderate sensitivities and relatively poor specificities. While the research on the antigenic properties of cyst fluid and surface associated glycoprotein, and improved protein purification have resulted into much more reliable serological tools (Dorny et al., 2003); none of these have eliminated the issue of cross reactions. Previous studies have reported that most tests that employ crude *T. solium* metacestodes antigens lack sensitivity and specificity (Chung et al., 1999. In the present study, the locally derived antigens used in Ab-

ELISA might have had problems with cross reacting helminthes. This is evident from the results of the Se = 90% and Sp = 95.4%), (P>0.05). Purified antigens from crude metacestodes of *T. solium* have yielded good results in most studies especially the use of chimeric recombinant and the synthetic proteins in Ab- ELISA. It is not clear whether the purity of the antigens used in these Ab-ELISA formats significantly contributed to the low sensitivity and specificity in the ELISA formats. Therefore, more study is necessary to investigate other potential cross reacting parasites in the endemic areas. Further studies need to explore the use of purified antigens in the diagnosis of *T. solium* cysticercosis. This will widen the scope of searching for better antigens for use in the Ab-ELISA formats. The present study had some limitations which include the use of small sample size for evaluation of this ELISA based technique. However, larger sample size is required for better understanding of the performance of this technique for the screening of NCC in a rural setting. More so, there is need to include more healthy control samples before this can be introduce for routine screening of NCC in the rural communities.

In conclusion, This study has evaluated the ELISA based model using locally derived antigens of metacestodes of *T. solium*. The finding indicates that the ELISA based method using locally derived antigens can be used to screen most patients with epilepsy for exposure to *T. solium* antigens and the method can be employed in resource limited setting in most developing countries. Finally, more highly purified antigens need to be used in the Ab-ELISA format for better detection of exposure to *T. solium* antigens among patients with Neurocysticercosis.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interest

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Full Length Research Paper

Prevalence of intestinal helminths among undergraduate students of Obafemi Awolowo University Ile Ife, Southwestern, Nigeria

Salawu Saheed Adekola[1*], Ojo Olawale Mayowa[1] and Awosolu Oluwaseun Bunmi[2]

[1]Department of Zoology, Obafemi Awolowo University, Ile Ife Osun State, Nigeria.
[2]Department of Biology, Federal University of Technology, Akure, Ondo State, Nigeria.

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A cross section study of the prevalence and knowledge of intestinal helminths was investigated among the newly admitted undergraduate students of Obafemi Awolowo University, Ile Ife, Osun State, Nigeria between April and June 2016. Faecal samples from 767 subjects comprising 406 males and 361 females were collected and processed using modified kato-katz method. Eggs of four helminths, *Ascaris lumbricoides, Trichuris trichiura,* hookworms and *Taenia* spp. were observed with prevalence of 22.03 7.30, 5.08 and 1.43% respectively. 240 (31.3%) of the subjects haboured at least one parasitic infection and the prevalence of infection was higher among the male (31.52%) than the female (31.02%) although the difference in the rates between the two sexes was not statistically significant (P>0.05). The trend in the infection rate was observed to be both age and sex dependent. The lowest age group (16-20 years) recorded the lowest prevalence (26.88%) and the prevalence of infection increased with increase in age till it peaked at 26-30 years (42.85%) and dropped at the age 31-35 years (33.33%). The results of the questionnaire revealed that majority of students that were infected with the helminths have poor knowledge about causes, transmission, prevention and treatment of intestinal helminths which make it difficult to avoid exposure to the parasite.

Key words: Intestinal helminths, prevalence, *Ascaris lumbricoides*, University students, Nigeria.

INTRODUCTION

Intestinal helminths especially *Ascaris lumbricoides, Trichuris trichiura* and hookworms (*Ancylostoma deodenale* and *Necartor americanus*) has been reportedly rank among the commonest and most persistent human intestinal helminths in both male and female of all age groups globally (Mbuh et al., 2011). About 2 billion people have been reported to be infected with intestinal helminths globally, majority been children from the developing countries especially in sub-Sahara Africa of which ascariasis account for approximately 1.6

*Corresponding author. E-mail: salawuadekola@yahoo.com. Tel: +2348052237255.

billion while trichuriasis and hookworm infection account for about 800 million (de Silva et al., 2003). High prevalence of intestinal helminths reported in developing countries are due to some peculiar factors which include poverty, overcrowding, lack of sanitation (Ojurongbe, 2013), poor nutrition, (WHO, 2002), impoverished health services, poor environmental and personal hygiene (Naish et al., 2004). Morbidities associated with these helminths infections include malnutrition, impaired intellectual performance (Ojurongbe et al., 2014), anaemia, dysentery and abdominal complication (Salawu and Ughele, 2015). Studies on these pathogens in many developing countries had shown that children (≤18 years) are mostly vulnerable and approximately 90% of these children are either in elementary or secondary schools (Salawu and Ughele, 2015). A child is defined as an individual under the age of 18 years (UNCRC, 1990; Salawu and Ughele, 2015). Consequently, mostly newly admitted students in tertiary institution in Nigeria fall under this category with the mindset of the general public that they are matured enough to take good care of themselves hygienically (Ojurongbe et al., 2010). However, studies have shown that environments, location, school attended, feeding and behavioral habits, and upbringing are among the factors predisposing these children to intestinal helminthiasis. Also, numerous studies on intestinal helminthiasis have been carried out over the years in different parts of the country mostly among rural dweller (Akinseye et al., 2017) and school-age children in elementary schools (Aniwada et al., 2016; Adefioye et al., 2013; Ojurongbe et al., 2014; Salawu and Ugbele, 2015) with little studies on tertiary students (Ojurongbe et al., 2010; Dada and Ekundayo, 2015; Isibor et al., 2013; Afolabi et al., 2016). However, information about the newly admitted students who are the one bridging the gap between the secondary and the tertiary students on the prevalence and knowledge of intestinal helminthiasis is yet to be documented especially in Obafemi Awolowo University Ile Ife, hence this study.

MATERIALS AND METHODS

The study was carried out at Obafemi Awolowo University Ile Ife, Nigeria. The population of the subjects consisted mainly newly admitted undergraduate students. The purpose of the study was explained to the subjects and documented consents were obtained from all participants. The participants were assured of confidentiality. The survey was carried out generally among the students irrespective of their faculty or department.

Consent and ethical issues

Ethical clearance for the study was issued by the ethical committee of the Obafemi Awolowo University Health Centre, Ile Ife, where the questionnaire administration and sample collection took place.

Questionnaire administration

A well-structured and pre-tested questionnaire was administered to collect socio-demographic information on each student and their responses were recorded by ticking the appropriate boxes provided. The socio-demographic information contained in the questionnaire include students' bio-data, other important information on transmission, control and knowledge about soil transmitted helminths.

Collection of stool samples

All participating students were supplied with a clean pre-labelled, wide-mouthed screwed capped plastic universal bottle, a sheet of paper and a wooden spatula each. The students were instructed to pass their faeces on the sheet of paper provided and to use the wooden spatula to transfer about 5 g of early morning feaces to the bottle and ensured the bottle was tightly screwed. The samples were taken to the Parasitology Laboratory, Department of Zoology, Obafemi Awolowo University where they were fixed immediately by adding adequate 10% formalin and mixed thoroughly with a wooden applicator stick and examined for helminth ova by a simple thick smear technique using a 41.7mg Kato-Katz technique (WHO, 1994).

Statistical analysis

All statistical analyses were performed using SPSS for windows version 21.0. Differences in prevalence of each parasite infection among subgroup were determined by chi-square. Statistical difference was assigned at $P \leq 0.05$.

RESULTS

A total of 767 students comprising 406 (52.93%) males and 361 (47.06%) females were screened for intestinal helminths infection in this study. The eggs of intestinal helminths observed in the faecal samples were those of *Ascaris lumbricoides,* hookworms, *Trichuris trichiura*, and *Taenia* spp. with the overall prevalences of 22.03, 7.30, 5.08 and 1.43% respectively.

The population and STH's infected distribution of participating students within different faculties in Obafemi Awolowo University, Ile Ife is shown in Table 1. The prevalence of each intestinal helminth egg observed in the analyzed faecal sample infection among students is shown in Table 2 while Table 3 shows the age-gender distribution of intestinal helminths among the students in the study area.

In total, 240 (31.29%) students haboured at least one parasitic infection (Table 1). Prevalence of intestinal helminths was recorded in all the faculties with varying prevalence but higher prevalence were recorded among students in non-science based faculties (Social sciences, 40.29%) while lower prevalences were recorded among students with science based faculties (H/sciences 21.74%).

Table 1. Population and STH's distribution of participating students within different faculties of Obafemi Awolowo University, Ile Ife.

S/N	Faculty	Number Examined	Number infected	%infection
1	Administration	105	31	29.52
2	Agriculture	55	17	30.91
3	Art	209	64	30.62
4	Health Science	46	10	21.74
5	Education	44	16	36.36
6	Environmental design and management	79	26	32.91
7	Law	39	11	28.21
8	Science	65	17	26.15
9	Social Sciences	67	27	40.29
10	Technology	58	21	36.21
Total		767	240	31.29

Table 2. Prevalence of soil-transmitted helminths among students examined within different faculties of Obafemi Awolowo University Ile Ife.

S/N	Faculty	Number examined	% +ve for helminth eggs			
			A. lumbricoides	Hookworms	*T. trichiura*	*Taenia spp.*
			n(%)	*n(%)*	*n(%)*	*n(%)*
1	Admin	105	20(19.05)	6(5.72)	8(7.62)	0(0.00)
2	Agric	55	14 (25.45)	3(5.45)	2(3.64)	1(1.82)
3	Art	209	43 (20.57)	16 (7.65)	10(4.78)	4(1.91)
4	H/science	46	10 (21.74)	0(0.00)	0(0.00)	0(0.00)
5	Education	44	11(25.00)	4(9.09)	1(2.27)	2(4.45)
6	EDM	79	18(22.78)	7(8.86)	2(2.53)	2(2.53)
7	Law	39	9 (23.07)	2(5.13)	1(2.56)	0(0.00)
8	Science	65	12 (18.46)	6 (9.23)	6(9.23)	0(0.00)
8	Social Science	67	18(26.86)	8 (11.94)	5(7.46)	1(1.49)
10	Tech	58	14 (24.14)	4(6.89)	4(6.89)	1(1.72)
	Total	767	169(22.03)	56(7.30)	39(5.08)	11 (1.43)
	p value	p<0.05	p<0.05	p<0.05	p<0.05	p<0.05

Table 3. Prevalence of soil transmitted helminths (STH) with respect to age group and sex of students in the study area.

Age (years)	Sex					
	Male		Female		Total	
	N E	No (%) infected	N E	No(%) infected	N.E	No (%)infected
16-20	185	44(23.78)	239	70(29.28)	424	114(26.88)
21-25	173	67(38.72)	109	34(31.19)	282	101(35.81)
26-30	38	15(39.47)	11	6(54.54)	49	21 (42.85)
31-35	10	2(50.00)	2	2 (100.00)	12	4 (33.33)
Total	406	128(31.52)	361	112(31.02)	767	240(31.29)

NE=Number examined.

Table 4. Knowledge about the causes, transmission, prevention and treatment of STHs among the students in the study area.

Variable	Category	Response (%) N=767	Number infected (%) N=240
Heard about intestinal helminths	Yes	152(19.82)	41(17.08)
	No	615(80.18)	111(46.25)
Causes of intestinal helminths	Bacterial	66(8.60)	21(8.75)
	Virus	17(2.22)	5(2.83)
	Through sexual intercourse	13(1.69)	4(1.66)
	Through parasitic helminths	32(4.17)	8(3.33)
	No idea	639(83.02)	202(84.16)
Transmission	Through faecal-oral route	33(4.30)	9(3.75)
	Through injection	20(2.60)	6(2.50)
	Sexual intercourse	25(3.25)	6(2.50)
	Parasitic helminths	37(4.82)	13(5.42)
	No idea	652(85.01)	206(85.83)
Prevention	Adequate disposal of human waste	27(3.52)	8(3.33)
	Boiling of drinking water	12(1.56)	4(1.66)
	Proper washing of cloth	8(1.04)	6(2.50)
	Good Personal hygiene	75(9.77)	14(5.83)
	Avoidance of stool	48(6.25)	17(7.08)
	No idea	597(77.83)	191(79.58)
Treatment	Chemotherapy	24(3.13)	11(4.58)
	Vaccination	78(10.16)	16(6.66)
	No cure	15(1.95)	4(1.66)
	No idea	650(84.74)	209(87.08)

Generally, high prevalence of infection was recorded among male students (31.52%) than female students (31.02%) although with no significant difference ($p>0.05$). The trend in the prevalence values of the four observed parasites were both age and gender dependent. In all age groups within both sexes, increase in prevalence of infections was observed as the age increases (Table 3). Approximately, 27% of the younger age category (16-20 years) was found to be infected and the rate increased though the succeeding age groups reached the maximum of 42.85% among the older age group (26-30 years) but declined to 33.33% among the oldest 31-35 years. There was no significant difference in prevalence of infection within age group ($p>0.05$).

Considering the infection among the sexes within each age group, females had more infection rates than the males except at age group 21-25 years where otherwise.

Based on the questionnaire survey, all the students that took part in this study submitted their faecal samples and also completed the questionnaires. Table 4 shows that majority of the students lack knowledge about the causes, transmission, prevention and treatment of intestinal helminthiasis. Based on the knowledge of intestinal helminths among the students, approximately 80% of the respondents have not heard about intestinal helminths before while about 83% have no idea about the causes of intestinal helminthiasis. About 85% of the respondents do not have an idea about the transmission while majority also do not know about the prevention and treatment of intestinal helminthiasis. Also, comparing the rate of infection among the respondents, the results of this study shows that the respondents that do not have any knowledge about causes, transmission, prevention and treatment of intestinal helminths are heavily infected (Table 4).

DISCUSSION

The occurrences of intestinal helminthiasis due to *A. lumbricoides*, hookworms and *Trichuris trichiura* have been reported in tertiary students by various authors from

various parts of the country and it has been observed that the prevalences and intensities of these infections ranges depend on the location and the methodology employed in the study (Nwaneri et al., 2013). In this study, the most prevalence among the intestinal helminths is *A. lumbricoides* (22.03%) followed by hookworms (7.30%) and *Trichuris trichiura* (5.08%); this result is consistent with the reports of some authors who reported that intestinal helminthiasis among school children in Nigeria is very common and caused due to the triad of roundworm, hookworms and whipworms (Taiwo and Agbolade, 2000; Asaolu et al., 2002; Adeoye et al., 2007; Salawu and Ugbele, 2015)

In this study, an overall prevalence of 31.49% was recorded among the newly admitted undergraduate students of Obafemi Awolowo University Ile Ife Nigeria. This results is in line with 20.6% reported from tertiary students in Ede Osun State (Ojurongbe et al., 2010), 40.5% prevalence reported among students in FUTA (North gate area) Akure, Ondo State (Dada and Aruwa, 2015) and 21.1% in Ilorin (Babatunde et al., 2003) but contradicts overall prevalence of 13.3% reported among students in FUTA Akure (Afolabi et al., 2016) and 11.1% in Benin city (Wagbastoma and Aisien, 2005). The high overall prevalence of infection recorded in this study shows that intestinal helminthiasis is not limited to children and rural dweller but also tertiary students in urban centers and the high prevalence recorded could be attributed to the poor sanitation and eating habit, poor personal and environmental hygiene practices among the students in their various hall of residence.

In this study, the trend of infection among the students were both age and gender dependent. Prevalence of infection increase as the age of student increases. Students in age group 16-30 years were mostly infected while the older age group 31 years above was least infected. This agree with the submission of both Adanyi et al. (2011) and Salawu and Ughele (2015) who stated that as a child get older, the child tends to be more cautious and mindful of hygienic practices by minding what they eat and may not always get involved in playing in dirty environment, also they tend to spend more of their leisure time indoors.

A non-significantly high prevalence of infection was recorded among males compared to the females among the students studied. The results agrees with those of Ojurongbe et al. (2010); Aniwada et al. (2016) and Dada and Aruwa (2015) but contradicts the reports of Afolabi et al. (2016) and Adefioye et al. (2011) who reported otherwise. The prevalence of infection recorded among males might be due to the fact that male students are very playful and active outdoor, tend to less careful about what they eat than their female counterparts (Ojurongbe et al., 2010).

The result from the questionnaire shows clearly that majority of tertiary students have inadequate or poor knowledge about intestinal helminthiasis and some other parasitic pathogens in their environment. Adequate knowledge about the causes, transmission, prevention and treatment will make it possible to avoid practices that might exposed them to infection. Also, it is imperative for government, stakeholder in health sector and healthcare provider to embark on awareness and sensitization program on the need for the newly admitted university students to know about soil transmitted infections and diseases.

In conclusion, there is a need for the control measures which include deworming programs couple with inclusion of compulsory health education courses for all tertiary students so as to bring about reduction in the prevalence and ensures adequate control by the authority concerned.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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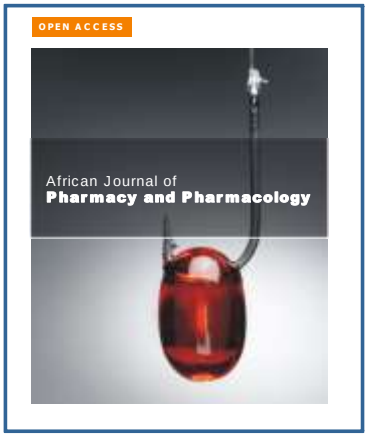

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