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Journal of Parasitology and Vector Biology

Full Length Research Paper

The prevalence of bovine trypanosomosis and associated risk factors in Mareka Woreda of Dawuro Zone, Southern Ethiopia

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A cross-sectional study was carried out to determine the prevalence of bovine trypanosomosis, to identify predominant trypanosome species and some associated risk factors,in purposively selected areas of Mareka district of Dawuro zone, southern Ethiopia from November 2015 to April 2016. For this purpose, a total of 384 blood samples were collected from cattle using systematic random sampling methodconsidering different age, body condition and coat color; as well as both sex groups of cattle. The packed cell volume (PCV) of each sampled animal was measured using hematocrit reader after centrifugation at 12,000 rpm for five minutes. Buffy coat technique was used to determine prevalence of trypanosomal parasites and species was further confirmed by Giemsa stained thin smear. The overall prevalence of bovine trypanosomosis was found to be 8.3% (32/384). The predominant trypanosome species were*Trypanosoma congolense* (62.5%) followed by *T. vivax* (37.5%) with significant statistical variation (P<0.05). The mean PCV was recorded as 21.03±3.297 in parasitaemic and 27.98±3.519 in aparasitaemic animals with results revealing significant statistical difference (P<0.05) between thetwo groups. From assessed risk factors; the age, body condition and coat color of animals showed statistically significant variation (P<0.05), butanimal location and sex were insignificant (P>0.05). In conclusion bovine trypanosomosis is one of the major livestock diseases posing continuous threats to the production and productivity of livestock sub-sector in the study area, thus it requires due attention to strengthen an integrated trypanosomosis and vector control.

Key words: Bovine, Ethiopia, Mareka, prevalence, risk factors, trypanosomosis.

INTRODUCTION

Livestock are of enormous importance in Africa, economically, for nutritional and agricultural purposes and socially (FAO, 2002). The size and diversity of Ethiopia's major agro-ecological zones render it suitable for the support of large numbers and class of livestock. Among the livestock population of Ethiopia, there are about 53.4 million of cattle; 25.5 million sheep and 22.78 million goats (CSA, 2011). Cattle are important components

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to nearly all farming systems and provide draught power, milk, meat, manure, hides, skins and other products. They are “Living banks” or “Living accounts” for rural and urban poor farmer or owners, because of they serve as a financial reserve for period of economic distress such as crop failure as well as other cash income. Despite the presence of huge numbers of cattle and their multipurpose; the country is not as such advantageous due to a multitude of problems. This comprises of: Diseases, age old traditional management system, inferior genetic make-up coupled with under nutrition and complicated by malnutrition as well as absence of well-developed market infrastructure. Among the diseases tsetse transmitted animal trypanosomosis has been still remain as one of the largest causes of livestock production losses in the country. As result 14 million heads of cattle, an equivalent number of small ruminants, nearly 7 million equines and 1.8 million camels are at risk of contracting tsetse- borne trypanosomosis at any one time (MoARD, 2004).

Trypanosomosis is a complex protozoan disease caused by unicellular parasites (trypanosomes) found in the blood and other tissues of vertebrates including cattle and man (Tesfaye, 2002). The most important trypanosome species affecting livestock in Ethiopia are *Trypanosoma congolense*, *Trypanosoma vivax* and *Trypanosoma brucei*, in cattle, sheep and goats, *Trypanosoma evansi* in camels and *Trypanosoma equiperdium* in horses (Getachew, 2005).

Researches on the socio-economic impacts of trypanosomosis have revealed that, over 3 million heads of various livestock species in Africa are lost per year by deaths due to the disease. Furthermore, over 35 million doses of trypanocidal drugs are bought annually to treat animals against trypanosomosis and more than 70 million heads are at risk of contracting the disease, so that total direct and potential losses attributable to the disease worth over 4.5 billion dollars per year (Bett et al., 2004). Even if tsetse transmitted animal trypanosomosis has been studied widely in Ethiopia, still remain as one of the largest causes of livestock production losses in the country (NTTICC, 2004). Furthermore, the disease causes direct loss through mortality estimated to amount 1.5 to 2 billion birr per year and indirect losses due to decreasing productivity, and restriction from international livestock trade in Ethiopia (Bizuayehu et al., 2012).

Therefore, trypanosomosis was considered to be an important disease of cattle in different part of the country in general (Shimelis et al., 2005; Bitew et al., 2011) and Dawuro zone in particular, which is within the tsetse belt, bounded by big rivers and tributaries such as: Omo, Ghibe and Gojab and Mareka woreda is under this zone. The previous study conducted in this particular study area (Mareka woreda) spotlighting on the impacts of tsetse challenge on herd composition and mortality, lactation and reproductiveperformance of cattle indicated that, the mortality rate in cattle for one year in the tsetse challenged areas was about 26.73 times higher than in the tsetse free area (Tigicho et al., 2012). However, studies have not yet been fully conducted on the determination of the prevalence and identification of trypanosome species affecting bovine in the study area. Therefore, the present study was accomplished with the general objectives to determine the prevalence of bovine trypanosomosis, identify the species of trypanosomes and assess the risk factors associated with the disease in Mareka district of Dawuro zone.

MATERIALS AND METHODS

Description of the study area

The study was carried out in Mareka district, in Dawuro Zone of SNNPR,Southern Ethiopia from November 2015 to April 2016. The area is located atabout 544 and 277 km south west of Addis Ababa; the capital city of Ethiopia and Hawassa; the administrative town of SNNPR respectively.Tarcha was the former town of the district, but the current town of district is Waka; while Tarcha became the administrative town of Dawuro Zone. The woredais bordered on the South by Loma Woreda, on the West by Gena Bosa Woreda, on theNorth by Tocha Woreda and the Gojeb River which defines its boundary with the Oromiya Region, and on the East by EsseraWoreda. The total land coverage of the woreda is 44050 ha of which 2000 ha (4.5%) is covered by forest, 11500 ha (26.1%) is grazing land, 28140 ha (63.9%) is cultivating land and the remaining 2410 ha (5.5%) comprises bushes, savanna, rivers, springs, stagnant waters and hills. According to the agro-ecological classification criteria the woreda is partioned into three agro-ecological zones; namely high land (Dega), midland (Woinadega), and lowland (kola) with their total land holds of 53, 30 and 17%, respectively. The study area's elevation ranges from 1000 to 2400 m above sea level. The mean annual rainfall ranges from 650 to 1100 mm and the rainfall distribution is bimodal with highest fall at wet season (April to September) and lowest fall at last half of dry season (February and march). The mean daily temperature ranges from 18 to23°C with the highest temperature share at dry season (November to march) and lowest temperature share at wet season. The predominant farming system in the areawas crop-livestock production system. The woreda has a total human population of 126022, of whom 65321 are men and 60701 women. The livestock population consists of:122,084 cattle, 47,438 sheep, 18,854 goats, 4,860 horses, 2,759 mules, 1,699 donkey, and 63,042 poultry and 2,750 traditional and 863 modern bee hives (MWOoA, 2015).

Study population

Local breeds of cattlewith different age groups, body conditions and coat colors as well as both sex groups that were kept under traditional extensive husbandry system with communal herding were considered as study population. The animals examined were categorized into different age groups as less than 2 years (young), between 2 up to 4 years (medium) and greater than 4 years (adult) according to their teeth dentition (Johnson, 2003). The body condition was estimated as per the recommendations of Macintosh (2007) for evaluating the body condition of the zebu cattle. The body condition of animals was recorded by classifying animals in the three groups as good, medium and poor based on the appearance of the ribs and dorsal spines. The examined animals were categorized into five groups according to their coat colour asred, white, mixed, black and gray coat colors to observe whether coat colour of animal have any influence on the disease prevalence (Wondewosen et al., 2012).

Study design

A cross-sectional study was conducted to determine the current prevalence of bovine trypanosomosis and to estimate the potential risk factors associated with the epidemiology.

Sampling method and sample size determination

The study area was selected by convenience sampling method based on previous information on lack of detailed study on bovine trypanosomosis. A total of 384 cattle were selected among cattle brought for deltamethrin pour-onusing systematic random sampling method. During sampling the age, sex, body conditions and coat colors of study animals were considered. The sample size was calculated using Thrusfield (2005) formula and 384 cattle were sampled.

$$N = \frac{1.96^2 [Pexp- (1-Pexp)]}{d^2}$$

Where, N is the required sample size, Pexp was the expected prevalence and d is the desired absolute precision. An expected prevalence of 50% was used, because of no previous studies were conducted in the area. In the overall study a 5% absolute precision at 95% confidence level was considered.

Parasitological survey and packed cell volume (PCV) determination

Blood sample collection

The format that includes locations, code number of animal, sex, age, body condition score, coat color, PCV, and parasitological result was prepared. After restraining the animals, 70% alcohol was used to clean ear of cattle then dried with cotton gauze, vein allocated by the tip of thumb, the vein was punctured by sterile lancet and blood sample was collected from marginal ear vein of cattle with heparnized capillary tubes up to ¾ of their length. One end sealed by sealer and placed on capillary holder on sealer by matching sample number with sealer number.

Packed cell volume (PCV) determination

For the measurement of PCV using a micro-hematocrit reader, the capillary tubes filled with blood were placed in micro- hematocrit centrifuge with sealed end outer most. The tubes were loaded symmetrically to ensure good balance. After screwing the rotary cover and closing the centrifuge lid, the specimens were allowed to centrifuge at 12,000 rpm for 5 min. After centrifugation, the capillary tubes were placed in a hematocrit reader. The length of the packed red blood cell column was expressed as a percentage of the total volume of blood; taking the PCV values 24 to 46% as normal for zebu cattle (Blood and Radostits, 2007; Samdi et al., 2011).

Buffy coat technique

After packed cell volume (PCV) determination thecapillary tubes were cut using a diamond tipped pen 1 mm below the buffy coat to include the upper most layer of the red blood cells and 3 mm above to include the plasma. The content of capillary tube was expelled on to slide and covered with cover slip. The slide was examined under × 40 objective for movement of parasite. Trypanosomes species were identified according to their movement in wet film preparations according to Paris et al. (1982).

Thin blood smear

For thin blood smear examination, a small drop of blood from a microhaematocrit capillary tube of buffy coat positive samples was applied to a clean slide and spread by using another clean slide at an angle of 45°. The smear was dried by moving it in the air and fixed for two minutes in absolute methyl alcohol; then it was flooded with Giemsa stain (1:10 solution) for 30 min. Excess stain was drained and washed by using distilled water. Then allowed to dry by standing up right on the rack and examined under the microscope (×100) oil immersion objective lens to confirm the trypanosome species based on their morphology (Murray et al., 1977).

Data analysis

The data collected were recorded properly in a format prepared for this purpose and were collected properly, handled carefully and analyzed systematically. For the analysis of data, statistical software program: SPSS-20 for windows version was used. Prevalence of bovine trypanosomosis was expressed as the number of parasitaemic animals through buffy coat microscopic study to the total animals examined (%). Hematological findings were expressed as percentage of the RBC to the total blood content (%). In all cases, a 95% CI were employed to extrapolate sample results to the target population in the study area. In order to compare trypanosomosis prevalence and the pooled data of mean PCV between aparasitaemic and parasitaemic animals of different factors, a combination of frequency distribution and student's t-test values and correlation were done to compare the relationship of PCV value with trypanosome infection rate.

RESULTS

Parasitological findings

From 384 randomly selected cattle, 32 (8.3%) were found to be positive for trypanosomosis using buffy coat technique. *Trypanosoma congolense* and *T.vivax* are the pre-dominant trypanosome species as indicated in Table 1.

The prevalence with respect todifferent risk factors like location, age, body condition, sex and coat color of cattle was determined. Although, different prevalence was found between both sex groups and among the three selected kebeles, no statistical significance (P>0.05) was observed in overall prevalence of trypanosomosis between sexes and among the kebeles. In contrast, statistically significant (P<0.05) prevalence of trypanosomosis was observed with respect to age, body condition and coat color of examined animals as shown in Table 2.

Hematological result

The mean PCV of individual animals was measured and recorded before buffy coat examination to assess degree

Table 1. Prevalence of trypanosome species among positive cases.

Trypanosome species	No. of positives	Prevalence (%)	Significance
T.congolense	20	62.5	X^2=384.000
T.vivax	12	37.5	P-value=0.000
Total	32	100	

Table 2. Prevalence of trypanosomosis in relation with different associated risk factors.

Risk factors	Category	Cattle examined	No. of negatives	No. of Positives	Prevalence (%)	Significance
Study kebeles	Tarcha Zuria	132	128	14	10.6	X^2=2.191 P=0.320
	Shina Gaburi	126	115	11	8.7	
	Shaba Yoyo	126	119	7	5.6	
Sex	Male	169	157	12	7.1	X^2=0.600 P=0.464
	Female	215	195	20	9.3	
Age	Young	48	47	1	2.1	X^2=6.493 P=0.025
	Medium	127	120	7	5.5	
	Adult	209	185	24	11.5	
Body condition	Poor	73	56	17	23.3	X^2=26.606 P=0.000
	Medium	163	154	9	5.5	
	Good	148	142	6	4.1	
Coat color	Red	134	122	12	9	X^2=21.519 P=0.001
	White	78	76	2	2.6	
	Mixed	64	58	6	9.4	
	Black	39	29	10	25.6	
	Gray	69	67	2	2.9	

of anemia. As a result, the mean PCV of parasitaemic animals were 21.03±3.297 and aparasitaemic animals were 27.98±3.519 with significant statistical variation (P<0.05) as represented in Table 3. In addition, the mean PCV of infected animals were measured and calculated in order to deduce severity of anemia in relation to trypanosome species. Accordingly, the mean PCV of *T.vivax* infected animals were lower (19.83±2.15) than that of *T.congolense* infected animals (21.75±1.65) as shown in Table 4.

DISCUSSION

The overall prevalence of bovine trypanosomosis obtained in present study (8.3%) was in accordance with previous results recorded as 7.8% at Wemberma district of West Gojjam zone, Ethiopia (Yehunie et al., 2012); 9.3% at Humbo Larena of Wolaita zone, South Ethiopia (Habtewold, 1993); 9.63% at Awi zone, North West Ethiopia (Kebede and Animut, 2009). But, lower than that of the results reported as 21.33% at Konta special woreda, Southern Ethiopia (Ataro et al., 2015); 14.2% at selected villages of Humbo district, Southern Ethiopia (Feyissa et al., 2011); 19.01% at Ghibe Valley (Shimelis, 2004); 27.5% at selected districts of Arba Minch, Southern Ethiopia (Abraham and Tesfaheywet,2012); 28.1% at tsetse infested Asosa district of Benishangul Gumuz Regional State (Shimelis et al., 2011). The low prevalence observed in present study might be due to anintegrated tsetse and trypanosomosis control program under taken by STEP or due to frequent use of trypanocidal drugs by owners. It might be also due to chronic stage of disease as parasitaemia reach its peak at early acute phase and become low or absent as disease progresses. The chronic phase is characterized by low and transient parasitaemia or complete absence of detectable parasites in the blood (Loses and Ikede, 2002).

This study result is higher than the results documented as 4.2% at South Achefer district, Northern Ethiopia (Denbarga et al., 2012); 4.43% at selected villages of

Table 3. The mean PCV of parasitaemic and aparasitaemic animals.

Conditions	No. of animals	PCV≤24	PCV≥25	PCV range	Mean±SD	Significance
Parasitaemic	32	27(84.4%)	5(15.6%)	14-27	21.03±3.297	T-test =10.35
Aparasitaemic	352	46(13.1%)	306(86.9%)	17-36	27.98±3.519	P-value=0.00
Total	384	73(19%)	311(81%)	14-36	27.40±3.991	

Table 4. The mean PCV of trypanosome infected animals in relation to trypanosome species.

Trypanosome spp	No.posetives	Mean PCV	Significance
T. congolense	20	21.75±1.65	T-test=1.634
T. vivax	12	19.83±2.15	P-value=0.113

Arbaminch, Ethiopia (Wondewosen et al.,2012); 5.43% at Mandura district, Northwest Ethiopia (Kumela et al., 2015); 4.86% at Didessa district (Gamechu et al., 2015); 6.9% at Chena district, Southwest Ethiopia (Bizuayehu et al., 2012). This might be due to the prolonged implementation of an integrated tsetse and trypanosomosis control program by STEP, wich was established earlier in those areas than current study site

The predominant trypanosome species in current study were*T. congolense* (62.5%) followed by *T.vivax* (37.5%) and this result is in consistent with previous reports at Daramallo district of South Western Ethiopia, which is 93% *T.congolense* and 5.3% *T. vivax* (Ayele et al., 2012); Lalo Kile District of Kelem Wollega zone with 75% *T. congolense* and 25% *T. vivax* (Efrem et al., 2013); upper Didessa Valley ofwestern Ethiopia with 81.42% *T. congolense*and 12.85% *T. vivax* (Mulugeta, 2014). In contrast to present study, *T.vivax* was highly predominant species than *T.congolense* as reported at Kindo Koisha district with 71 and 28.4%, respectively (Kidanemariam et al., 2002). The predominance of *T. congolense* in present study might be due to its high number of serodems as compared to *T. vivax* and the development of better immune response to *T. vivax* by infected animals as well as due to the presence of major cyclical vectors Glossina species (*G. pallidepes*) as current entomological result witnessed. Since the transmission of *T. congolense* is cyclical, it requires the presence of tsetse flies, where as *T. vivax is* most readily transmitted mechanically by biting flies (Abebe, 2005). The lower prevalence of *T. vivax* might be due to low distribution of mechanical vectors such as Tabanus and Stomoxys.

No statistically significant prevalence variation (P>0.05) was observed among study kebeles; this might be due to the study was conducted in the same agro-ecological zone (kola) with similar climatic conditions. But, the highest prevalence recorded at Tarcha zuria might be due to the existence of relatively highest apparent density of tsetse flies as present entomological survey revealed. The occurrence of trypanosomosis frequently corresponds with vector density which in turn dependent on those climatic factors such as; temperature, humidity and vegetation coverage of the area (Abebayehu et al., 2011).

The higher prevalence observed in female animals than male animals in current study was in agreement with previous reports of Konta special woreda; which is 20.7% in female and 17.3% in male animals with no significant statistical variation (Migbaru and Desta, 2015). Similar findings were also reported by Daya and Abebe (2008) plus Tadesse and Tsegaye (2010) and they suggested that the prevalence difference between male and female animals is due to "physiological difference between sex groups". The possible explanation for higher prevalence of trypanosomes in female animals in present study area might be also that female animals were more likely exposed to tsetse flies as they were always released to common grazing site of tsetse infestation, in contrast to male animals as they were kept around house after ploughing and have little chance to be exposed to tsetse flies.

The infection rate was assessed by categorizing animals into different age groups as young (1-2 years), medium (2-4 years) and adult (>4 years) since the age was assumed as one of potential risk factor. As a result the highest infection rate with statistical significance (P<0.05) was observed in adults (11.5%) than medium (5.5%) and young (2.1%) aged animals. This result is in line with the previous reports at Konta Special Woreda with significant variation (P<0.05) of 24.7, 16.7 and 4.8% in adult, medium and young aged animals respectively (Migbaru and Desta, 2015). However, the variation in infection rate could be due to the fact that adult animals travel long distance for grazing and draft as well as harvesting of crops to tsetse challenged areas. In addition to this; Suckling calves do not go out with their dams but graze at home until they were weaned off (Rowlands et al., 1995). Young animals are also naturally protected to some extent by maternal antibodies (Fimmen et al., 1999) this perhaps results in low

prevalence of trypanosome. Moreover, tsetse flies were less successful in feeding from young cattle aged up to 2 years. The lower feeding rate in young animals attributed to higher rate of defense movement; this in turn reduces the risk of contracting trypanosomosis (Torr and Mangwiro, 2000).

Significantly highest infection rate (P< 0.05) was recorded in poor body conditioned animals (23.3%) than medium (5.5%) and good (4.1%) body conditioned animals and was consistent with previous reports at Goro district (Bitew et al., 2011); Konta special woreda (Ataro et al., 2015); selected villages of Humbo district (Feyissa et al., 2015). It is difficult to conclude either poor body condition predisposes to trypanosome infection or trypanosome infection cause loss of body condition. The disease itself results in progressive emaciation of the infected animals; nevertheless, non-infected animals under good body condition have well developed immune status that can respond to any foreign antigen better than those non-infected cattle with poor body condition which can be immune compromised due to either haematopagus parasites, concurrent diseases or malnutrition (Collins, 1994).

Statistically significant variation (P<0.05) was observed between coat color of animals with respective prevalence of 9, 2.6,9.4, 25.6 and 2.9% in red, white, mixed, black and gray colored animals. Similar finding was reported at Konta special woreda as there was significant difference in prevalence among different coat colored animals with the highest prevalence in black hair-coat animals (33.39%) whereas the least prevalence rate was recorded in white hair-coat animals (8.06%) (Ataro et al., 2015). In contrast to this, there was report of slightly higher prevalence in cattle's having mixed skin color (7.25%) followed by 4.88% in red, 3.57% in black, 1.56% in white and 0% in gray skin color (Wondewosen et al., 2012). The possible reason for highest prevalence in black colored animals in current study might be due to the nature of tsetse flies to be attracted toward black color. Tsetse flies have a preference for dark surfaces (Green, 1993).

The mean PCV value of presently studied animals was significantly (P<0.05) varying between parasitaemic (21.03±3.297) and aparasitaemic (27.98±3.519) animals. This result was in agreement with the previous report from Eastern Wollega with lower mean PCV of 20.2±3.0 in infected animals as compared to non-infected animals of 26.5±5.1 (Yibrah, 2012). Likewise, Thrusfield (2005) stated that average mean PCV of parasitologically negative animals was significantlyhigher than those of parasitologically positive animals. Therefore, trypanosomosis may adversely lower PCV value of infected animals, even though other diseases such as helminthosis, thick borne disease and nutritional imbalances contribute to the low PCV values.

Anemia is one of the most important indicators of trypanosomosis in cattle (Stephen, 1986). The level of anemia or PCV usually gives a reliable indication of the disease states and reduced performance of infected animals (Trail et al., 1993). Even though significant difference is found in the study, PCV alone could not be used as diagnostic criteria for trypanosomosis, because there are other factors causing anemia such as worm infestation and nutritional deficiency (Radostits et al., 1994). The occurrence of parasitologically positive animals with PCV greater than 25% might be due to recent infection.

The mean PCV of trypanosome positive animals was also measured and calculated purposively in order to deduce severity of anemia in relation to trypanosome species. Accordingly, the mean PCV of *T. vivax* infected animals was lower (19.83±2.15) than that of *T.congolense* infected animals (21.75±1.65). Therefore *T.vivax* can cause more severe PCV reduction than that of *T.congolense*, this might be due to *T.vivax* can migrate and invade tissues and lymph nodes in addition to blood stream. *T. vivax* usually multiplies rapidly in the blood of cattle and is evenly dispersed throughout the cardiovascular system, whereas *T. congolense* tends to beaggregated in small blood vessels and capillaries of theheart, brain and skeletal muscle. Very acute infection with *T. vivax* in cattle causes parasitaemia and dis-seminated intravascular coagulation with hemorrhages, this perhaps result in PCV reduction (Murray and Dexter, 1988).

Conclusion

Bovine trypanosomosis,which accounts for an overall prevalence of 8.3% is the major livestock constraint in the study area and affects their health, production and productivity. The major species of trypanosomes encountered were *T.congolense* followed by *T.vivax.* Infection with trypanosomosis negatively affected PCV and body condition and it is an indication that trypanosome infection of cattle causes loss of body weight and production. Adult, poor body conditioned and black colored animals were most risky group for being affected by trypanosomosis.

RECOMMENDATIONS

Based on the aforementioned the following is recommended:

1. An integrated tsetse and trypanosomosis control action should be strengthened in the area in ordered to minimize direct and potential loss of livestock due to the disease.
2. The government should supply effective trypanocidal drugs and trained man poweras much as feasible to reduce effect of trypanosomosis.

3. Further research should be conducted on drug resistance and epidemiology of disease at different season of the year.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Seroprevalence of *Taenia solium* cysticercosis among people with epilepsy epileptic patients in three rural districts of Northern Uganda

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Taenia solium **pork tapeworm is one of the parasites that causes serious public health and socioeconomic problems in developing countries. In Northern Uganda, extreme level of poverty, lack of sanitation infrastructure and communities' practice of free range pig farming provide suitable condition for survival of *T. solium* in the area. Additionally, increased cases of epilepsy are of serious concern. The aim of the study was to determine the proportion of patients with epilepsy who are positive for metacestodes of *T. solium* antigens and anticysticercal IgG antibodies in three districts of Northern Uganda. Forty two thousand nine hundred three participants were screened for epileptic seizures. Three hundred random samples were screened for anticysticercal IgG and circulating antigens using indirect antibody and monoclonal antibody ELISAs. Samples positive for anticysticercal IgG were confirmed using western blot. The seroprevalence of anticysticercal IgG and circulating antigens among patients samples using indirect antibody ELISA and monoclonal antibody ELISA was 15% (95% CI = 14.5-15.5 and 9% (95% CI 8.5-9.5) respectively. Thirteen, 13% (95%CI = 12.5-13.5) of patient samples were positive for *T. solium* specific glycoprotein on immunoblot. There was no significance difference (*P* = 0.057) in seroprevalence of anticysticrecal IgG and circulating antigens between males and females. This finding indicates that *T. solium* infections occur among communities in three rural districts of Northern Uganda. There is a potential for proliferation of pork tape worm infections among the communities. Therefore, there is need for Health authorities to strengthen training of health workers and enforcement of public health education in the community on epilepsy associated with neurocysticercosis.**

Key words: Neurocysticercosis, seroprevalence, westernblot, enzyme-linked immunosorbent assay (ELISAs), rural communities, Northern Uganda.

INTRODUCTION

Taenia (T) solium, is one of the parasites that causes huge public health and socioeconomic burden in endemic

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regions (Basem et al., 2010). Taeniasis is acquired when human eat raw or undercooked pork containing cysticerci, the larva stage of the *T. solium* (Bueno et al., 2001; Basem et al., 2010). When ingested, the cysticerci migrate to intestine mucosal where they attach and become adults. These adults worm shed proglottids containing eggs in human feces and become the source of infections that can infect other human or pigs by direct or indirect contamination of food or water (Basem et al., 2010).

T. solium causes human cysticercosis when one ingests embryonated eggs in food or water contaminated with fecal matter of persons harbouring the adult tapeworm (Bueno et al., 2005). When the human central nervous system (CNS) is infected with larval stage of *T. solium*, neurocysticercosis may occur which commonly manifests as epileptic seizures (Carabin et al., 2006). Approximately 50 million people worldwide 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). *T. solium* infections are prevalent in rural areas in developing countries particularly in Central and South America, Asia and Sub Saharan Africa (Ngowi et al., 2004). In Eastern Africa, the seroprevalence to anticysticercal Immunoglobulin G (IgG) of *T. solium* has been reported among human population with varying results; in Tanzania 38% (Ngowi et al., 2004), Kenya 14% (Githigia et al., 2002), Burundi 39 and 44% (Nsengiyumva et al., 2002; Prado Jean et al., 2007), Democratic Republic of Congo, (2.6%) (Praet et al., 2010). In Uganda, information on human cysticercosis is still scanty. The diagnosis of human cysticercosis remains a challenge in most hospitals and other health facilities due to lack of skilled health professionals and specialized medical equipment such as Computed Tomography (CT) scans and Magnetic Resonance Imaging (MRI). In addition, there are no studies that have been done on immune response to *T. solium* cysticerci among rural communities in Northern Uganda.

The objective of the present study was to determine the proportion of patients with epilepsy who are positive for metacestodes of *T. solium* antigens and anticysticercal IgG antibodies in three districts of Northern Uganda.

MATERIALS AND METHODS

The study was conducted in the three rural districts of Northern Uganda from April 2012 to June 2013. This includes the districts of Adjumani, Moyo and Gulu. The three districts cover a total area of over 6,500 km^2 with an estimated population of 916,000 inhabitants (https://en.wikipedia.org/wiki/Adjumani_District Wikipedia), (http://www.ugandatravelguide.com/guludistrict.html),(https://en.wikipedia.org/w/index.php?title=Moyo_District&action=info).

These areas have high level of poverty, lack sanitation infrastructure and the communities practiced free range pig farming which provide suitable environment for survival of *T. solium* in the study population. The pigs are usually kept at night in pens. In addition, these areas have shortage of toilet/pit latrine as well as safe sources of water. The main sources of water in these areas are: spring /wells/, bore holes, tape water and protected wells (Nsadha et al., 2011).

Study design

A cross sectional study was done on epileptic patients in the three districts of northern Uganda. The patients were selected on the basis of the chronicity of epilepsy starting with those with most recent onset of seizuresusing simple random sampling. Serodiagnosis of anticysticercal IgG and circulating antigens was done using indirect antibody ELISA (Ab-ELISA) and Monoclonal antibody ELISA (MoAb-ELISA) respectively. Anticysticercal IgG was confirmed using immunoblot. Standardized laboratory questionnaires were designed and used to get information from patients in relation to their demographics, eating behaviors and ingestion of raw or under cooked pig meat. These were to relate social risk factors for *T. solium* cysticercosis to seropositivity.

The research protocol was approved by Gulu University, Faculty of Medicine Ethic Committee and Uganda National Council of Science and Technology (Ref: HS 987).

Community based sampling and patient selection

This study conducted community based surveys in the districts of Gulu, Adjumani and Moyo to identify people with epileptic seizures among the rural communities. This was preceded by initial visits to sensitize the local communities in the study areas. The health authorities, the local leaders and the veterinarians were involved in this exercise. They also helped in identifying areas in each district with high cases of patients with epilepsy and areas where pig farms are practiced. Forty two thousand nine hundred three people were randomly selected using multistage cluster sampling and screened for epileptic seizures through interviews. Interviews of household members were conducted after obtaining oral consents from the heads of households.

The sample size for this population was calculated based on formula by Kish Leslie (1965). Using the prevalence study conducted in a neighboring country, Tanzania with a prevalence of epilepsy at 12/1000 in an area with 45,000 participants and at a 95% confidence interval, the sample size was calculated. Households with suspected positive cases were identified and later visited by the researcher who administered in-depth questionnaires for confirmation of epileptic seizures. People suspected of epileptic seizures at household level were referred to hospitals or health facilities for further examination by the neurologists. Patients presenting 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.

Briefly, random number was generated from a sampling frame of 1383. These were then aligned to the patients' register. Each patient assigned random numbers was included in the study. Three hundred patients were randomly selected using random numbers. This was done to ensure that each patient constituting the sampling frame had equal chance of being included in the study. Patients who were not selected for further examinations were recommended for treatment for their epileptic seizures. Therefore, the three hundred patients randomly selected were sent for further investigations. The patients were bled through vein puncture of the arm and blood collected 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. The sera samples were used in antibody ELISA assays done at Faculty of Medicine, Gulu University.

Antibody ELISA assay in detection of *T. solium* anticysticercal IgG

The seroprevalence of IgG from patient sera was screened using indirect antibody ELISA as described by Sloan et al. (1995). Briefly, 1.0 µg/ml of antigens was coated on to the 96 polystyrene microplate (Nunc® Maxisorp) wells and incubated overnight. 10 µl of antisera were then added and incubated. The 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 substrate *o*-phenlydiamine (OPD) (invitrogen) was added. The reaction was stopped by adding 0.5 M H_2SO4 (BDH UK). The colored substance formed was measured photometrically at 492 nm using ELISA reader (Tecan Austria GmbH, Sunrise).

The amount of antibody present was then calculated. 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). In the present study, a sample was considered positive if the OD value was greater than 0.35 positive and negative controls were included in all the ELISA reactions for validation. Incubations were conducted for 1 h at 37°C for the subsequent ELISA reactions except for the dark incubation (30 min). Antigens preparations were done in the Department of infectious Diseases and Tropical Medicine (DITM), Munich, Germany at Professor Gisela Bretzel, Laboratory.

Sodium dodecyl sulphate polyacrylamide gel electrophresis (SDS-PAGE)

Preparation of the immunoblot strips for serodiagnosis was done as described by Parija et al. (2011) but with some modifications. 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 from naturally infected pigs were run on a sodium dodecyl sulphate polyacrylamide electrophoresis (SDS-PAGE) in a 16.4% resolving and 4.0% stacking gel (in Tis-Tricine buffers) at 100 V 150 mA 10 W 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 (BDH UK) 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 µm pores size nitrocellulose membranes in mini Transblot cells (USA) at 150 V 100 mA 30 W for 1 h. 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 was placed on 5% BSA for 30 min to block non-specific binding sites and thereafter air dried. The immunoblot strips of 4 mm were cut from the larger portion of the dried nitrocellulose membrane for serodiagnosis.

Electroimmunotransfer blot analysis

Serodiagnosis with the immunoblot was done on all patients samples which were positive with antibody ELISA. The dry immunoblot strips were fully submerged in Mini incubator Trays and incubated on 5% Bovine Serum Albumen (BSA) for 30 min for subsequent procedures. The patient sera were added and incubated for 70 min. Horse radish peroxidase labeled Rabbit anti human IgG antibody (invitrogen) was used as secondary IgG in a dilution of 1/10000 and diaminobenzidine (invitrogen) used as substrate. All incubation was followed by subsequent washing with PBS/T20. The presence of the antibody in the sera due to *T. solium* was confirmed when at least two specific bands of 8 or 10 kDa were observed on the diagnostic region (Figure 1). The molecular weight of *T. Solium* characteristic antigenic peptides were determined by comparing the bands in the diagnostic region with the standard molecular weight markers placed alongside the strips (Figure 1). Major antigenic peptides were: 8, 10, 18, 32, 40, 50 and 76 kDa. The bands outside the diagnostic regions were not considered as specific for *T. solium* because these were shared by other helminths.

Monoclonal antibody ELISA assays in detection of circulating antigens

The MoAb-ELISA was done as described previously by Brandt et al. (1992) in the laboratory of Dr. Chummy Sikasunge in the University of Zambia. Briefly, the polystyrene ELISA plate (Nunc® Maxisorp) was coated with 1.1 µl/ml concentrations of Moab. Two Moab (Institute of Tropical Medicine (ITM), Antwerp, Belgium) were used. The first MoAb B158C11A10 was diluted at 1.24 µg/ml in carbonate buffer (0.06M, pH 9.6) and used for coating the ELISA plate while the second biotinylated MoAb B60H8A4 diluted to (3.6 µl/ml) in (Phosphate Buffered Saline-Tween 20 PBS-T20) + 1% New Born Calf Serum (NBCS) and used as detector antibody. The coating of the first MoAb was carried out at 37°C on a shaker for 30 min and all other subsequent steps incubated for 15 min. The blocking was done by adding PBS-T20 + 1% NBCS per well.

Pre-treated sera at of 1/4 concentrations were added to the wells. These were followed by addition of a second biotinylated MoAb B60H8A4 also diluted to 3.6 µg/ml in PBS-T20/1% NBCS. Streptavidin horseradish peroxidase (Jackson Immunoresearch Lab, Inc.) diluted at 1/10000 in PBS-T20/1% NBCS was added to act as conjugate. This was followed by addition of the OPD solution and 30% H_2O_2. This was incubated without shaking in the dark at room temperature for 30 min. All procedures involved washing the plate in each step five times with PBS-T20.

Fifty microliters of 4 N H_2SO_4 was added to each well to stop the reaction. The plates were read using an ELISA reader at 492 nm. To determine the cut-off, the OD of each serum sample was compared with a series of 8 reference negative serum samples at a probability level of (P = 0.001). Thus, was calculated as the mean of the OD values obtained with known negative samples plus two SD. A sample was considered positive if the OD value was greater than the estimated cut-off point.

In the present study, the OD cut-off value was 0.233. Monoclonal antibody ELISA assays were done at Dr. Chummy Sikasunge's laboratory in University of Zambia.

Statistical analyses

The categorical data are presented as mean, standard deviation, median and interquartile range or percent frequency. Seroprevalence to anticysticercal IgG and circulating antigens was calculated by dividing number of Ab-ELISA and antigen positive samples by the total number of people screened. Data were analyzed using univariate and Multivariate logistic regression to test for statistical level of significance between variables. Student-t test was used to test for differences between the means of proportions for levels of significance. Comparisons between groups were made using Fisher exact test. The probability value of (P<0.05) were considered to be statistically significant. Statistical analysis of data

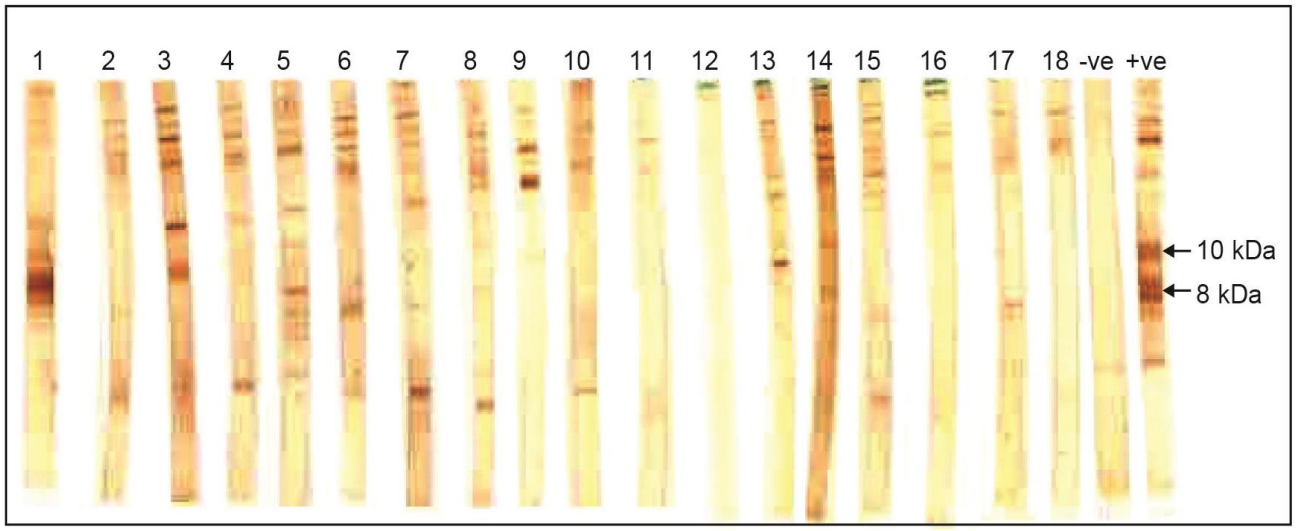

Figure 1. Immunoblot results for patients positive for 8 and 10kDa glycoproteins of Taenia Solium. Lane (+ve) (right); immunoblot containing known NCC sera (positive control). Lane (-ve); negative control; Lane 1, 3, 5, 6, 7, 14 and 17 are positive for 8 and 10 kDa glycoproteins.

Table 1. Age and sex distribution of the study subjects.

Age group	Sex	Percentage
	Male/female	%
10-19[a]	75/57	44.2
20-29	37/31	26.2
30-39	24/16	13.4
40-49	13/7	6.7
50>	21/9	10.0
Total	**170/130**	**100**

[a]Most males and females are in the age group of 10 to19.

was performed using the SATA 11.0

RESULTS

Study population

This cross sectional study included 170 (56.7%) males and 130 (43.3%) females with the overall mean age 25 ±13.2. The age range was from 12 to 77.

Age and sex distribution of study subjects

Our findings indicate there was a higher proportion of males than females among patients of group aged 10-19, 75 (56.8%), 30-39, 24(60%), 40-49, 13 (65%), >50, 21(70%) (Table 1). Conversely, there was a higher number of females than males among aged groups 20-29, 37(47.4%). However, the difference was not statistically significant, (P = 0.058).

This finding indicates age distribution was predominant among younger age group (10-19). Therefore, the younger people with epilepsy were more represented than older people.

Seroprevalence of anticysticercal IgG antibody using indirect antibody ELISA

Our findings from 300 serum samples screened among people with epilepsy for the presence of anticysticercal IgG indicate 15.0% (45/300) (CI 95%: 14.5-15.5) of epileptic patients positive for *T. solium* anticysticercal IgG in the three districts studied (Table 2). The districts of Gulu, Adjumani and Moyo had positivity to anticysticercal IgG of 17, 18 and 10 with a corresponding seroprevalence: 5.7(95% CI: 4.23-7.18), 6.0 (95%CI:

Table 2. Seroprevalence of anticysticercal IgG antibody using antibody ELISA.

Districts	Number of patients screened	Antibody ELISA positive	Seroprevalence, (N=300) n (95% CI)
Gulu	111	17	5.7(4.23-7.17)
Adjumani	92	18	6.0(4.53-7.47)
Moyo	97	10	3.3(1.83-4.77)
Total	**300**	**45**	15(14.5-15.5)

N; Total number of patients screened, n; number of samples positive for anticysticercal IgG; CI, confident interval.

Table 3. Seroprevalence of anticysticercal IgG antibody using immunoblot assays.

Districts	Immunoblot positive sample [a] (n=39)	Seroprevalence, (N= 300) n (95% CI) [b]
Gulu	12	40 (-1.4-1.51)
Adjumani	17	5.7(4.23-7.17)
Moyo	10	3.3(2.8-5.2)
Total	39	13(12.5-13.5)

N, total number of patients screened; n, number of sample positive for 8 and 10 kDa glycoproteins,; CI, confident interval; [a]Number of immunoblot samples positive for 8 and 10 kDa glycoproteins, [b]Seroprevalence of anticysticercal IgG on immunoblot at 95% confidence interval.

Table 4. Immunoblot blot kDa 8 and 10 seropostivity in relation to age.

Districts	Number of patients per age group[a]					
	10<19	Number(%) positive	20<39	Number(%) positive	40+	Number(%) positive
Gulu	58	3(7.7)	44	6(15.4)	9	3(7.7)
Adjumani	39	4(10.2)	33	7(17.9)	20	8(20.7)
Moyo	36	2(5.2)	41	3(7.7)	20	3(7.7)
Total	133	9(23.1)	118	16(41)	49	14(35.0)

[a]Frequency of seropositivity to IgG positive for 8 and 10 kDa glycoproteins in different age groups increased in the different age

4.53-7.47) and 3.3(95% CI: 1.88-7.17) respectively.

Seroprevalence of anticysticercal IgG antibody using immunoblot assays

45 antibody positive sera were screened using immunoblot for specific *T. solium* 8 and 10 kDa glycoprotein (Table 3). Our findings indicate that 86.7% (39/45) were positive on the diagnostic bands 8 and 10 kDa proteins (Figure 1). Twenty one patients, 53.8% (21/39)) sera strongly reacted with higher molecular weight of 14, 21, 24, 38-42 and 50 kDa proteins while twenty five, 64.1% (25/39) patients sera showed reactions below the diagnostic bands (below 8 to 10 kDa). The frequency of reactivity of patients sera to higher molecular weight glycoproteins and the bands below the diagnostic region (8-10 kDa) was not statistically significant (P = 0.26). The seroprevalence of anticysticercal IgG among the patient using immunoblot was 13%, (95% CI: 12.5-13.5).

Immunoblot blot kDa 8 and 10 seropostivity in relation to age

39 immunoblot positive samples were screened for specific *T. solium* 8 and 10 kDa glycoproteins in relation to age (Table 4). The frequency of seropositivity was 23.1, 41 and 35% among the age groups 10-19, 20-39 and ≥40 years old respectively. The highest seropositivity was in the age group 20-39. The influence of age on seropositvty significantly differed among the age groups (P = 0.001).

Our finding indicates that there was a statistically significant difference in the frequency of seropositivity to

Table 5. Immunoblot blot kDa 8 and kDa10 for seropositivity in relation to gender.

Districts	Number of patients (N=300)	Number of seropositivity (n) [a]	Male/Female[b]	Seroprevalence
Gulu	111	12	6/6	2.0/2.0
Adjumani	92	16	9/7	3.0/2.3
Moyo	97	11	6/5	2.0/1.7
Total	**300**	**39**	**21/18**	**7.0/6.0**

N; Total Number of patients screened, n; number of seropositivity, [a] Number of patients positive for 8 and 10 kDa glycoprotein, [b] data indicate more males positive for 8 and 10 kDa than females but this was not statistical significance (*P* = 0.38).

Table 6. Multivariate logistic regressions for some risk factors for *T. solium* cysticercosis

Risk factors for *T. solium*	No. of seropositive%	OR	95% CI	*P* value
Male[a]	21	1.8	(0.33-3.35)	0.01
Pork consumption[b]	170(57.0)	1.1	(0.2-6.9)	0.62
Free range pigs[c]	24(8.0)	1.7	(1.1-2.5)	0.01
No hand washing[d]	11(3.6)	1.4	(0.8-2.0)	0.04
Absence of toilet	6(2.0)	1.2	0.8-1.6)	0.001
Drinking of un boiled	3(1.0)	0.2	0.1-1.6	0.02

[a] Being male; [b]pork consumption, [c] free range rearing of pigs and [d] no hand washing were statistically significant (*P* = 0.001).

IgG positive for 8 and 10 kDa glycoproteins among the age group (*P* = 0.003). Thus older people are more likely to be affected with *T. solium* cysticercosis than the young ones.

Immunoblot blot kDa 8 and 10 for seropositivity in relation to gender

Of the 39 patients analyzed for kDa 8 and 10 seropositivity, 56.4% (22/39) were males (Table 5). Gulu, Adjumani and Moyo had 12, 16 and 11 patients positive with immunotblot respectively. Of the 12 patients (Gulu), 50% (6 /12) were males and 50% (6 /12) were females. Adjumani had 56.2% (9/16) males and 43.8% (7/16) females positive with immunoblot. While, Moyo had 54.5% (6/11) males and 45.5% (5/11) females positive on immunoblot. The seroprevalence of patients in relation to gender was 7% (95% CI: 6.5-7.5) and 6% (95% CI: 5.5-6.5) respectively. The seroprevalence in each district was; Moyo 2.0/1.7, Gulu, 2.0/2.0, Adjumani, (3.0/2.3) respectively. There was no statistical difference between males and females for risk of infection with cysticercosis. (OR= 1.10; CI= 0.17-6.90 (*P* = 0.62).

Some risk factors to *T. solium*

The aim was to find which of the risk factors had significant impact in the proliferation of *T. solium* parasites in the community. Our results indicate that of the 207 patients who consumed pork, 24 (11.6%) were positive for anticysticercal IgG on antibody ELISA assays (Table 6). The analysis with multivariate logistic regression model (Table 6) show significant positive association (*P* = 0.01) with pork consumption. The OR = 1.7 (1.1-2.5); (*P* = 0.01). Similarly, out of the 86 households who reared pigs, 11 (12.6%) had patients' positive for anticysticercal IgG. The risks of infections with *T. solium* was significantly higher among those 11 patients; OR = 1.4 (0.8-2.0), (*P* = 0.04). Additionally, the seropositivity to anticysticercal IgG was significantly higher in patients who do not wash their hands after visiting toilet/pit latrine (*P* = 0.001). As of groups with or without toilets, the risk of infections was significantly higher among patients without toilets. Three, 3 (42.9%) patients without toilets were positive for anticysticercal IgG. The OR = 1.2 (0.8-1.6); (*P* = 0.04). There was no statistical significance (*P* = 0.25) in seropositivity to IgG between those patients who drank and those who did not drink unboiled water. Risk of being male was highly significant (*P* = 0.001).

Seroprevalence of circulating antigens using monoclonal antibody ELISA

When three hundred sera samples from epileptic patients in three districts of Adjumani Gulu and Moyo were screened for the presence of *T. solium* metacestodes (Table 7), Gulu, Adjumani and Moyo had 5 (4.5%), 9 (9.8%) and 13 (13.4%) patient samples positive for metacestodes of *T. solium* with the seroprevalence of 1.7, 3.0 and 4.3 respectively. The overall seroprevalence

Table 7. Monoclonal antigen ELISA assay for *T. solium* cysticercosis.

Districts	Number of patients tested (N=300)	antigen ELISA positive samples n (%) (n=27)	Seroprevalence (95% CI) (n=9)
Gulu	111	5(1.66)	1.7 (0.23-3.17)
Adjumani	92	9(3.0)	3.0 (1.53-4.47)
Moyo	97	13(4.0)	4.3 (2.83-5.77)
Total	**300**	**27(8.99%)**	**9.0% (8.5-9.5)**

N; total number of patients screened, n; number of samples positive for metacestodes of Taenia solium antigen, CI; confidence interval.

was 9% (27/300) and 95% (CI: 8.5-9.5).

DISCUSSION

Seroprevalence studies on anticysticercal IgG indicate high rates of exposure to cysticercal antigen of *T. solium* parasites in several countries worldwide. While many of these studies are in other developing countries, there is still limited information on this subject in Uganda. Recent studies conducted by Nsadha et al. (2011) revealed increase in porcine cysticercosis in Northern Uganda. This is an indication of human exposure to *T. solium* in the population. This study investigated the proportions of people with *T. solium* cysticercosis among epileptic patients in Northern Uganda. Three hundred patients were sampled and their sera analyzed using ELISA, MoAb-ELISA and immunoblot. The seroprevalence of *T. solium* anti-cysticercal IgG on Ab-ELISA and western blot were 15 and 13.0% respectively while the seroprevalence of circulating antigens was 9.0%. This indicates a possible exposure to this parasite and occurrence of active infections. In addition, the result reflects porcine cysticercosis reported earlier by Nsadha et al. (2011). While this is the first report on prevalence of human cysticercosis in the three districts of Northern Uganda, limited studies in these areas hamper comparison of the present data. However, several seroprevalence studies have been reported in other countries with varying results. Jin-Mei et al. (2010) conducted a study among rural village communities in Lyte, in the Philippines and reported a seroprevalence of anti-cysticercal IgG of 24.6%. A similar study done among epileptic patients in a rural village in the West and North West Province of Cameroon using Antibody ELISA showed a seroprevalence of *T. solium* specific antibody at 44.6% (Zoli et al., 2003). In addition, Parija et al. (2011) conducted a study to assess the immuno response among patients with epileptic seizures in India and they reported a seroprevalence of anti-cysticercal IgG of 16.3%. Similarly, in Malaysia a study conducted to determine exposure to cysticerci among rural population revealed a seroprevalence of 2.2% (Azian et al., 2006). Garcia et al. (2003) on the other hand carried out a study on prevalence of *T. solium* cysticercosis in endemic areas of Peruvian highlands and reported a seroprevalence of anti-cysticercal IgG ranging from 7.1 to 26.9% (mean = 13.9%). While the results in the current study among the epileptic patients is nearly comparable to the previous studies reported by Garcia et al. 2003 (13.9%); Githigia et al. (2002) (14%) and Parija et al. (2006) (16.3%), the current seroprevalence of anti-cysticercal IgG is higher than those reported by Basem et al. (2010) (6.5%); Prado et al. (2007) (3.6%); Azian et al. (2006) (2.2%); Ito et al. (2003) (8.6%); Chung et al. (2005) (2.97%) and Sutisna et al. (1999) (1.6%). On the other hand the current seroprevalence of anti-cysticercal IgG is lower than those reported by Jin-Mei et al. (2010) (24.6%); Zoli et al. (200) (44.6%), Wandra et al. (2003) (47.9%). A much higher seroprevalence of anti-cysticercal IgG of 79.0% was reported by Ferrer et al. (2003) in rural village in Mexico. There are many reasons for high seroprevalence to *T. solium* cysticercosis. These may be due to low socioeconomic status of the populations in endemic areas where pork is consumed (Parija et al., 2011). Infected persons may also act as a source of infection by contaminating the food and water through defecation (Parija et al., 2011; Waiswa et al., 2009). In addition, consumption of undercooked infected pork, absence of toilet facilities and allowing pigs to feed on human fecal matter may result in high seroprevalence of anti-cysticercal IgG.

Relations between seroprevalence of anti-cysticercal IgG, gender and age

The present study found more males (7%) affected by *T. solium* cysticercosis than the females (6%). A higher seropositivity may be due to the male traditional roles of providing food and out sourcing meat for family demands. Furthermore, with two decades of war which occurred in Northern Uganda, all livestock were depleted leaving pigs as the only animals for consumption. This resulted in increased pork sales as more males got involved in pork business. Several authors have reported higher seroprevalence of anti-cysticercal IgG among males than females in many seroprevalence studies. Parija et al. (2011) reported higher seroprevalence of anti-cysticercal IgG, among males 2.0% (4/202) than females 0.99% (2/202) in South India. This was attributed to males being more involved in outside activities and consumption of

improperly cooked food than females in South India. In addition, Garcia et al. (2003) reported that more males are likely to be infected because of their adventurous occupations. In contrast, Basem et al. (2010) reported a higher seroprevalence of anti-cysticercal IgG in women (8.5%) than men (3.0%). Similar results were reported by Oliveira et al. (2006) (15 and 11.3%), Moore et al. (1995) (1.8 and 0.71%) in both women and men.

The present study has found the seroprevalence of anti-cysticercal IgG higher among older patients aged 20-39 and 40-49 years old, respectively. However, the anti-cysticercal IgG antibody titers did not differ significantly among these age groups (P = 0.33). This may be because older patients have similar immunogenic challenges to *T. solium* cysticerci variants as compared to the young ones. In addition, antibody titre may be influenced by the status of the cysticerci (active or inactive). Previous studies implicate inactive cysticerci or calcifications for late onsets of seizures among older epileptic patients. For instance, patients with inactive cysticerci are known to have reduced antibody titres compared to those with active cysticerci. In the present study, the difference in antibody titres between inactive or active cysticerci is not statistically significant (P = 0.24). The seroprevalence of anti-cysticercal IgG of 13.0% in this study indicates a high rate of *T. solium* cysticercosis among epileptic patients in the three districts of Northern Uganda. While this shows the extent of exposures of patients to *T. solium* infections the seroprevalence of anti-cysticercal IgG should be interpreted with caution as previous studies have reported possibility of overestimation (Garcia et al., 2001). However, many studies have reported the influence of age on seropositivity to *T. solium* cysticercosis among the population. Willingham et al. (2003), while conducting a hospital-based survey in Vietnam found a higher prevalence of cysticercosis in older age group. A similar finding was reported by Wandra et al. (2003) in Irian Jaya, Indonesia. Garcia et al. (2003) reported that the peak incidence of cysticercosis is between 30 and 50 years of age while antibodies disappear within 1 to 3 years in 30 to 40% of the seropositive people in endemic countries, reflecting a transient anti-cysticercal IgG antibody reaction after exposure or self-cure. In the present study the high seroprevalence of anti-cysticercal IgG reflects the current levels of endemicity of *T. solium* cysticercosis which is distributed among the patients.

Immunoblot assays for 8 and 10 kD proteins of *T. solium*

In this study 45 antibody positive serum samples were analyzed by immunoblot for specific *T. solium* glycoproteins. Of the samples analyzed, 86.7% (39/45) were positive for the diagnostic bands of 8 and 10 kD proteins. This result indicates that the 86.7% (39/45) serum samples analyzed contain the glycoproteins components of *T. solium* parasites, a manifestation of the presence of *T. solium* cysticercosis among the epileptic patients. Additionally, the presence of 8 and 10 kD proteins of the anti-cysticercal IgG show evidence of exposure to *T. solium* parasites. The present finding is in agreement with the results reported by Minozzo et al. (2008) who found that the bands between 9 and 12 kDa had 38% sensitivity and 79% specificity. In this study, 53.8% (21/39) sera strongly reacted with higher molecular weights of 14, 21, 24, 38-42 and 50 kD proteins.

This is in contrast with the results of sensitivity (97%) and specificity (98%) reported by Chung et al. (1999). Sixty four, 64% (25/39) patient's sera showed reactions below the diagnostic bands (below 8 to 10 kDa). While Handcock et al. (2004) showed that the band 50 kDa had a higher reactivity. In the present study, the diagnostic bands 8 and 10 kDa have high sensitivity and specificity. Higher molecular weights bands are known to present strong cross reactions with other helminths hence are not allowed for use in diagnosis (Chung et al., 1999; Handcock et al., 2004). In this study it appears that antibodies from other helminths recognized many interacting sites particularly those with high molecular weights. The high frequency of these reactions may be due to the presence of other parasites in the patient's sera. Molinari et al. (2002) reported that samples from patients with viable cysts had higher absorbance than samples from calcified or transitional stages. In addition, cross reacting helminths such as *T. saginata, T. hydatigena, Echinococcus granulosus, Ascaris suum* have been implicated in provoking the immune systems leading to production of antibodies which can interact with the surface protein on the epitopes. While this may not be directly linked with consumption of infected pork, it may explain the diverse views on the presence of other parasites in the study area. In addition, the bands which were recognized in the lower side of the diagnostic bands equally require more investigations.

In conclusion, this study showed that *T. solium* infections occur among communities in the three rural districts of Gulu, Adjumani and Moyo in Northern Uganda. Therefore, there is a potential for proliferation of pork tape worm infections among the communities in this region. This is the first report on human cysticercosis in Northern Uganda and can be used as a baseline data for future management of the disease. However, further studies need to investigate for presence of other parasites which induce the antibodies which interact with the lower kDa glycoproteins below the diagnostic regions. In addition, there is a need to investigate the incidence of human cysticercosis among communities in the three districts of Gulu Adjumani and Moyo. Finally, more highly purified antigens need to be used in the Ab-ELISA format for better understanding the seroprevalence of human cysticercosis in the area.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Experimental studies on the reproductive biology of *Hyalomma truncatum* (Acari: Ixodidae) in Maiduguri, Nigeria

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Experimental studies were conducted between July and September, 2014 on the reproductive biology of *Hyalomma truncatum* in Maiduguri. Engorged adult female ticks were collected from the body of slaughtered cattle and morphologically identified to the species level. For further study, they were incubated in desiccators at 28°C and 85% relative humidity (RH) to determine their pre-oviposition and oviposition periods. The eggs collected from ovipositing females were counted under a stereoscopic microscope to obtain the mean daily and total egg counts as well as the average number of eggs/gram of weight of female and the percentage of body mass conversion. Batches of eggs laid were also incubated under similar laboratory conditions to determine their incubation periods. The results showed that the mean±SE values of pre-oviposition, oviposition and incubation periods for *H. truncatum* were 7.25±0.78, 10.40±1.37 and 19.90±0.97 days, respectively, while the mean±SE total egg count per head and average number of eggs per gram of weight of female *H. truncatum* were 5645.7 ± 939.14 and 8838.5±1204.1, respectively. A strong correlation was observed between pre-oviposition weight and mean egg count ($r = 0.637$, $p<0.05$), as well as between the convertible blood mass and mean egg count ($r = 0.779$, $p<0.05$). Also, female *H. truncatum* converted 53.3 to 74.3% of their total body mass (g) during oviposition. In conclusion, this study has revealed that the pre-oviposition weight of engorged female *H. truncatum* influenced their mean egg counts, oviposition pattern and efficiency of body mass conversion (%).

Key words: Biology, convertible blood mass, egg count, *Hyalomma truncatum*, Maiduguri, oviposition pattern.

INTRODUCTION

In Nigeria, 90% of the cattle population are raised under traditional pastoral husbandry system of Fulani herders in the northern region (Opara and Ezeh, 2011; Lorusso et al., 2013). Under this system, cattle are extensively grazed and become exposed to infestation with three important genera of Ixodidae; *Amblyomma*, *Hyalomma*

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and *Rhipicephalus*, including the sub-genus *Boophilus* (Opara and Ezeh, 2011; Obadiah and Shekaro, 2012; Lorusso et al., 2013). Ixodid ticks are currently recognized as the most economically important species of ecto-parasites infesting livestock in the tropics and sub-tropics (Soulsby, 1982; Lorusso et al., 2013). The direct effects of tick infestation in cattle leads to decreased productivity (Jongejan and Uilenberg, 2004; Lorusso et al., 2013). Additionally, they serve as vectors for pathogenic protozoa, virus, bacteria and rickettsia which constitute serious threats to livestock and man (Rajput et al., 2006). *Hyalomma marginatum* is the main vector of Crimean-Congo haemorrhagic fever virus, a widespread deadly disease of man (Geevarghese and Dhanda, 1987; Hassan, 2001). Several *Hyalomma* spp. are also incriminated in the transmission cycle of babesiosis, theileriosis and anaplasmosis to livestock in the tropics and subtropics (Soulsby, 1982).

The life cycle of Ixodid ticks is generally influenced by a combination of intrinsic and extrinsic factors (Shah-Fischer and Say, 1989). Temperature and humidity are recognized as the most important extrinsic factors affecting the life cycle of *Ixodidae* (Khalil and Hagras, 1988; Shoukry et al., 2000; Durrani et al., 2008), and they result from simultaneous environmental factors such as latitude, altitude, sunlight, rainfall and local wind patterns (Shah-Fischer and Say, 1989). The duration of egg incubation, female pre-oviposition and oviposition periods are all influenced by temperature, humidity and moisture conditions (Knight et al., 1978; Dipeolu, 1983; Khalil and Hagras, 1988; Shah-Fischer and Say, 1989; Shoukry et al., 2000; Durrani et al., 2008).

Several authors working in different geographical regions of the world have published data on the reproductive biology of various species of *Hyalomma* (Dipeolu, 1983; Ammah-Attoh, 1984; Khalil and Hagras, 1988; Shoukry et al., 2000; Durrani and Shakoori, 2009), but to date, there is no report on reproductive biology of *Hyalomma* species infesting livestock in the semi-arid zone of North-eastern Nigeria, hence the need for the experimental study.

MATERIALS AND METHODS

Study design

Experimental studies were conducted from July to September 2014 to determine some developmental parameters and ovipositional behaviour of *Hyalomma truncatum* at 28°C and 85% relative humidity. The period of this study corresponds to peak rainfall and humidity in Maiduguri, and is also known to favour bionomics of Ixodid ticks under natural conditions in Nigeria (Dipeolu, 1983).

Tick collection and identification

Engorged female ticks were collected by forceps from cattle during slaughter at the Maiduguri central abattoir and taken to the Veterinary Parasitology Postgraduate Research Laboratory, University of Maiduguri, where morphological identification was carried out to the species level with the aid of a stereoscopic microscope (MOTIC SM2-140 SERIES). *Hyalomma* species were recognized by their large sizes (5 to 6 mm), brown scutum and conscutum, striated integuments, and the presence of festoons in both sexes. *H. truncatum* was distinguished by the presence of smooth, shiny and dark conscutum bearing a large depressed area in the caudal part of males (Walker et al., 2003).

Determination of developmental parameters and egg counts

The first experiment was conducted to determine pre-oviposition period, oviposition period, daily mean egg counts and total mean egg counts of *H. truncatum*. Individual ticks were weighed using a Metra® Precision Electronic Balance (Model JA103P) and placed into separate test tubes (Pyrex®:15 x 125 mm) loosely capped with cotton wool. The test tubes were set up in vertical positions on a perforated plastic rack in a desiccator maintained at 28°C and 85% RH for the commencement of egg laying (Durrani et al., 2008). The eggs laid by female ticks were collected at 10:00 h daily and counted under a stereoscopic microscope (MOTIC SM2-140 SERIES). Counting was aided by drawing vertical lines on a Petri dish to serve as a counting chamber. The daily batches of eggs collected were incubated under similar conditions like the first experiment to determine their incubation periods (Durrani and Shakoori, 2009).

Determination of oviposition patterns

To determine the oviposition patterns of engorged female *H. truncatum*, ovipositing females under experimental conditions were divided into three groups based on their pre-oviposition weights. Thus, females weighing 0.1-0.4, 0.5-0.8 and 0.9-1.1 g were categorized as groups I, II and III, respectively. Daily mean egg counts of each group was calculated throughout the oviposition period and plotted on a line graph to show the oviposition patterns.

Determination of egg laying efficiency

The body mass conversion (%) through oviposition was calculated using the formula of Dipeolu et al. (1991) viz:

Body mass conversion (%) = pre-oviposition weight - post-oviposition weight x 100

The number of eggs per gram live weight of ovipositing females was also calculated using the formula of Linthicum et al. (1991). No. of eggs/g = Total number of eggs laid by female ticks in cluster ÷ total weight of female ticks in the same cluster.

Statistical analyses

Data generated from the various experiments were summarized as mean±SEM. The relationships between pre-oviposition weight and weight loss through oviposition (convertible blood mass) on one hand and the number of eggs laid on the other hand, were estimated using one-way linear regression analysis model (Y=A+BC) (Freedman, 2005), and $p<0.05$ was considered significant.

RESULTS

The mean ± SEM values of some parameters on the

Table 1. Mean ± SEM values of some developmental biology parameters of *H. truncatum* under experimental conditions.

Parameter	Mean ± SEM (range)
Pre-oviposition weight (g)	0.61±0.04 (0.3-1.1)
Post-oviposition weight (g)	0.25±0.04 (0.1-0.60)
Pre-oviposition period (days)	7.25±0.78 (00-15.00)
Oviposition period (days)	10.40±1.37 (00-19.00)
Number of eggs/gram female weight	8838.5±1204.1 (00-17635)
Total egg count	5645.7±939.14 (00-14108)
Ova incubation period (days)	19.90±0.97 (17.00-26.00)

g= gram, SEM = standard error of the mean.

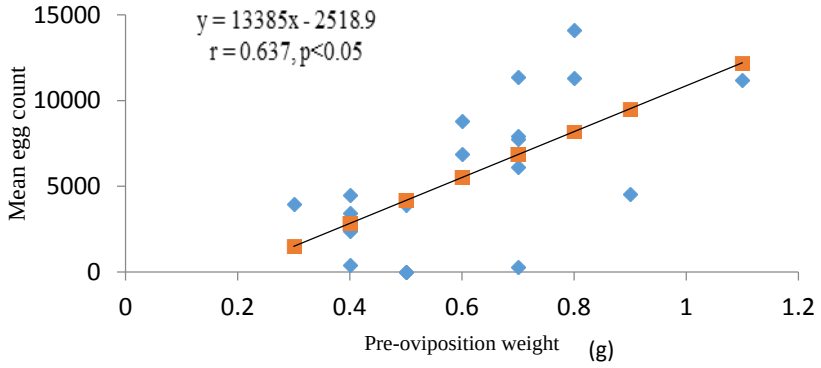

Figure 1. Correlation between pre-oviposition weight and mean egg count of *H. truncatum*.

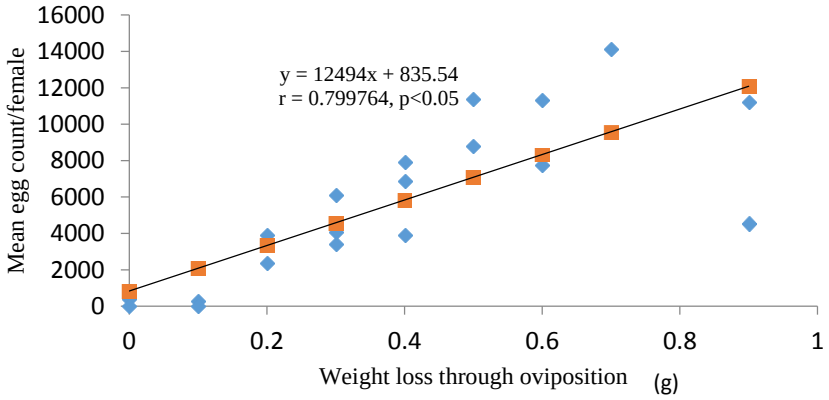

Figure 2. Correlation between convertible blood mass and mean egg count of *H. truncatum*.

biology of *H. truncatum* at 28°C and 85% RH are presented in Table 1. The mean±SEM values for pre-oviposition, oviposition and ova incubation periods of engorged female *H. truncatum* recorded in this study were 7.25±0.78, 10.40±1.37 and 19.90±0.97 days, respectively, while the mean egg/gram of engorged female weight and total egg counts were 8838.5±1204.1 and 5645.7±939.14, respectively.

A strong correlation (r = 0.637, p<0.05) was observed between pre-oviposition weight and mean total egg count of engorged female *H. truncatum* (Figure 1), as well as between the convertible blood mass and their mean total egg count (r= 0.779764, p<0.05) (Figure 2).

The mean daily egg count of *H. truncatum* is presented in Figure 3. The daily egg count increased progressively within the first 5 days to attain a plateau on day 6,

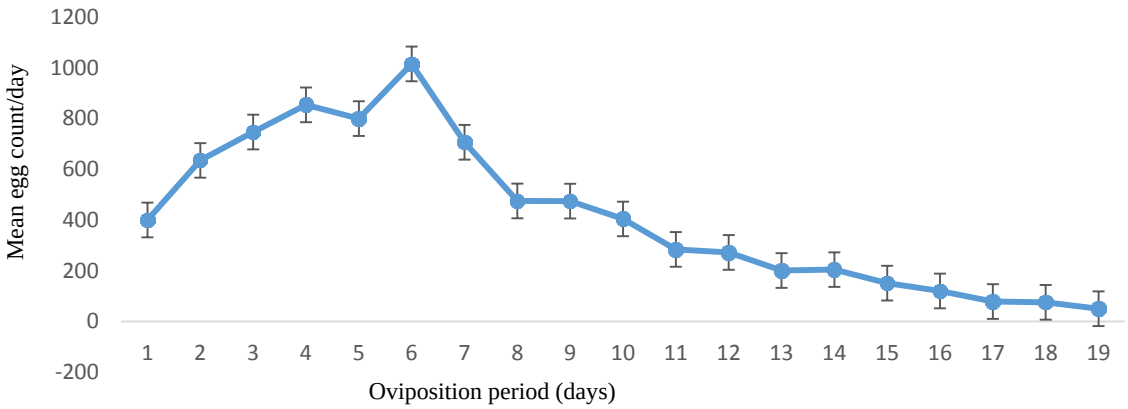

Figure 3. Total mean daily egg counts of *H. truncatum* under laboratory conditions.

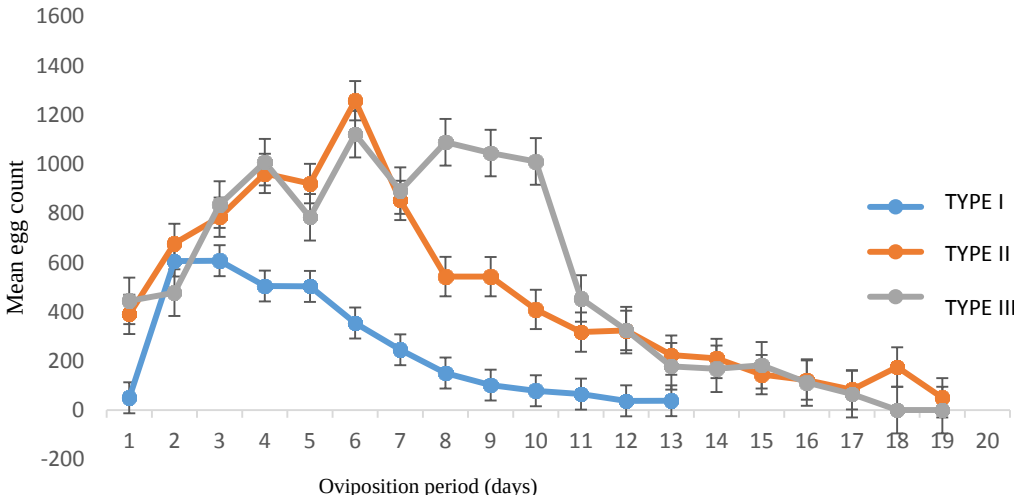

Figure 4. Oviposition patterns of three weight groups of *H. truncatum* under laboratory conditions.

followed by a regressive phase from day 7 forwards, with the lowest counts recorded on day 19. The three patterns of oviposition observed in female *H. truncatum* of different weights are presented in Figure 4. Type I was observed in group I ticks and is characterized by an initial high onset, and the early attainment of peak egg laying on day 3, with a short oviposition period of 13 days. By contrast, type II oviposition pattern observed in group II ticks was characterized by an initial low onset of egg laying, attainment of peak oviposition on day 6, and had a longer oviposition period of 19 days. Type III pattern was identified in group III ticks, and had a similar pattern of onset as group II, attaining peak egg laying on day 6, but having a lower mean egg count on the day of peak oviposition.

The efficiency of body mass conversion (%) through oviposition by three weight groups of female *H. truncatum* is presented in Figure 5. Ticks weighing 0.1-0.4, 0.5-0.8 and 0.9-1.2 g converted an average 53.3, 57.2 and 74.3% of their total body mass during oviposition, respectively.

DISCUSSION

The duration of developmental periods of *H. truncatum* recorded in this study are comparable with previous reports. The pre-oviposition period of *H. truncatum* in this study was comparable with the earlier reports of Knight et al. (1978). The mean oviposition period recorded in this study is also comparable with previous reports elsewhere for various *Hyalomma* species by Ammah-Attoh (1984), Khalil and Hagras (1988) and Durrani and Shakoori (2009). However, our pre-oviposition period does not agree with Linthicum et al. (1991), Al-asgah (1992) and Chen et al. (2011) who reported 11.9±0.8, 10.6±0.56 and

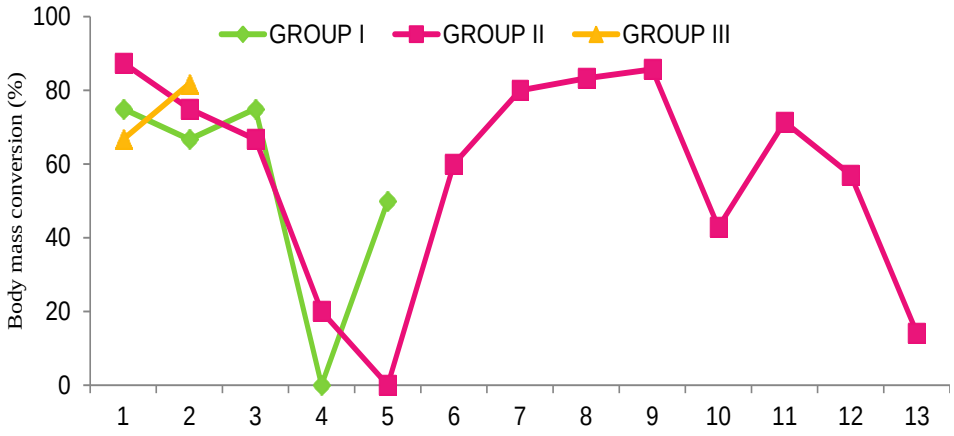

Figure 5. Percentage body mass (g) conversion by three weight groups of *H. truncatum* under laboratory conditions.

12.2±2.05 days, respectively. Similarly, the oviposition period recorded in this study differs from previous reports by Soulsby (1982), Dipeolu (1983) and Chen et al. (2011) who reported 37-59, 15 and 39.7±1.59 days, respectively. Furthermore, the results also disagreed with Shoukry et al. (2000) who reported a higher mean oviposition period (20.96±0.42) in *Hyalomma schulzei* at 28°C. The variations observed from these different studies may be associated with differences in laboratory conditions, especially temperature and humidity under which the ticks were reared. Temperature is widely recognized as the most important extrinsic factor that regulates the bionomics of Ixodid ticks (Soulsby, 1982; Khalil and Hagras, 1988; Shah-Fischer and Say, 1989; Dipeolu et al., 1991; Durrani et al., 2008). Other extrinsic factors known to affect the bionomics of Ixodid ticks include photoperiodic regime (light: day), rainfall and vegetation (Ouhelli et al., 1982; Shah-Fischer and Say, 1989; Adejinmi and Akinboade, 2012).

The mean ova incubation period of *H. truncatum* recorded in this study is not comparable with previous reports on various species of *Hyalomma* by Knight et al. (1978) who reported a mean ova incubation period of 29.3±2.96 for *Hyalomma marginatum*. Soulsby (1982) reported ova incubation period of 34-66 days for the genus *Hyalomma*. Ammah-Attoh (1984) reported a mean ova incubation period of 33 (18-50) days for *H. marginatum rufipes* in Nigeria. Shoukry et al. (2000) reported a mean ova incubation period of 29.3±2.96 days for *Hyalomma schulzei* in Egypt, while Durrani and Shakoori (2009) also reported a mean incubation period of 15 (10-20) days. The observed differences could be attributed to variations in temperature, humidity and other important ecological conditions under which the different studies were conducted. However, the result of this study is comparable with that of Biu et al., (2012) who reported a mean incubation period of 20.60±3.04 (8-12) for *Rhipicephalus sanguineus* in Maiduguri. The total mean egg count recorded in this study falls within the expected normal range of 2,000 to 20,000 eggs in a single batch for female hard ticks (Soulsby, 1982; Walker et al., 2003). However, the total mean egg count in this study is lower than previously reported by Knight et al. (1978) for *H. marginatum*, and those of Linthicum et al. (1991) who reported a mean total egg count of 6701 for *H. truncatum*, Al-asgah (1992) who reported a total mean egg count of 10259±728 for *Hyalomma schulzei*, but was higher than that reported by Ammah-Attoh (1984) with a mean egg output of 4899 (114-1038) for *H. marginatum rufipes* in Nigeria. The discrepancy in findings may be associated with species differences in fecundity (Shah-Fischer and Say, 1989). Moreover, these studies were conducted under different experimental and ecological conditions which have been reported to regulate the fecundity and rate of development of hard ticks (Soulsby, 1982; Shah-Fischer and Say, 1989; Durrani et al., 2009), and could account for the observed differences in mean egg counts recorded in these studies.

The strong positive correlation observed between pre-oviposition weight of engorged female *Hyalomma* and their total mean egg count in this study were comparable with the findings of Dipeolu et al. (1991) who reported a direct relationship between egg production and weight of engorged *Amblyomma variegatum* females. This finding also was comparable with the reports of Shoukry et al. (2000) who reported a positive correlation between weight of replete *H. schulzei* females and the number of eggs laid. Thus, irrespective of species, the pre-oviposition weight of engorged females significantly contributes to the number of eggs produced by Ixodid ticks. In the other side, the weight of female ticks was reported to be proportional to their degree of engorgement and determines their fecundity (Soulsby, 1982; Dipeolu et al., 1991). The most important intrinsic

factor influencing propagation of a hard tick is the degree of engorgement of the females, which determines the pre-oviposition weight (Shah-Fischer and Say, 1989; Dipeolu et al., 1991). Furthermore, the strong correlation observed between weight loss during oviposition and total mean egg count of engorged female *H. truncatum* in this study was comparable with previous reports by Dipeolu et al. (1991) and Shoukry et al. (2000), and suggests that female ticks become depleted as they lay eggs, a significant part of the lost weight being converted into egg mass.

The average efficiency of body mass conversion (%) recorded in different weight groups of ovipositing females in this study falls within the range of values previously reported for some *Hyalomma* species elsewhere (Khalil and Hagras, 1988; Linthicum et al., 1991; Al-asgah, 1992), and the high body mass conversion (%) efficiency recorded in this study (53.3-74.3%) may suggest that the experimental conditions were favourable for oviposition. Moreover, the three different oviposition patterns observed in this study indicates that the oviposition behaviour of *H. truncatum* depends on their pre-oviposition weights. Also, this finding suggests that female *H. truncatum* with different pre-oviposition weights have different capacity to discharge eggs once in their life time. In the current study, there were two patterns of oviposition behavior between groups I and II-III. This agrees with Dipeolu et al. (1991) who observed two patterns of oviposition behaviour in two different weight groups of *Amblyomma variegatum*.

Conclusion

This study revealed that *H. truncatum* is a highly prolific species of Ixodidae, and represents a serious threat to livestock and control methods. Also, pre-oviposition weights of the engorged females influenced some bionomical parameters, including the mean egg counts, oviposition pattern and efficiency of body mass conversion (%).

RECOMMENDATION

For effective population control of *H. truncatum*, measures to reduce reproductive efficiency such as the use of effective repellents, anti-feedent compounds and the destruction of their habitats are recommended. For bionomical studies or bioassays involving adult *H. truncatum*, females weighing 0.5 to 0.8g are the most suitable. Studies are ongoing in our laboratory to evaluate the effects of some plants extracts on the reproductive efficiency of *H. truncatum* as potential candidates for population control.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Distribution and antimicrobial resistance of *Salmonella* serotypes in minced beef, calves and humans in Bishoftu and Addis Ababa, Ethiopia

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This study was conducted to determine the prevalence and antimicrobial profile of *Salmonella* in 102 beef, 384 stool and 107 calf faecal samples. The beef samples were collected from 34 randomly selected supermarkets in Addis Ababa, stool samples were collected from Bishoftu General Hospital and calf faecal samples were from randomly selected dairy farms in Bishoftu. Of the total 102 minced beef, 384 stool and 107 faecal samples examined, 9.8, 3.4 and 1.9%, respectively, were positive for *Salmonella.* Twenty-five *Salmonella* isolates comprising of 14 different serotypes were identified. Among the different serotypes, *S.* Typhimurium was predominant (28%) followed by *S.* Uganda (20%) and *S.* Bovismorbificans (8%). The other serotypes identified were *S.* Anatum, *S.* Blockley, *S.* Braenderup, *S.* Enteritidis, *S.* Hadar, *S.* Havana, *S.* Livingstone, *S.* Mikawasima, *S.* Muenchen, *S.* Saintpaul and *S.* Typhimurium var. Copenhagen totally comprising 44%. *Salmonella* Mikawasima was reported for the first time in Ethiopia. Assay of antimicrobial resistance revealed that 20% of the isolates were resistant to three or more of the 24 antimicrobials checked. Resistance to 15 antimicrobials was recognized. The most common resistance was to nitrofurantion, streptomycin and tetracycline. Most of the antimicrobial resistant *Salmonella* isolates were from the meat samples. Result of the present study indicate that *Salmonella* isolates are diverse in serotype with significant antimicrobial resistance in the samples tested which could be potential sources of drug resistant *Salmonella* infections.

Key words: Addis Ababa, Bishoftu, calf, Ethiopia, human, minced beef, *Salmonella.*

INTRODUCTION

Domestic animals harbour *Salmonella* in their gastrointestinal tracts and Salmonellae are often excreted in faeces by healthy animals with no apparent signs of illness (Loneragan et al., 2012). *Salmonella* frequently contaminates raw foods of animal origin through faecal contact during production and slaughter. Humans generally become infected by eating undercooked or contaminated food (Majowicz et al., 2010) and beef is often contaminated with *Salmonella* (Guo et al., 2011). Most of the time, invasive non typhoid salmonellosis in human is related to *S.* Typhimurium and *S.* Enteritidis (Reddy et al., 2010). As chopping of meat facilitates additional microbes to adapt, minced beef samples are frequently contaminated with high number of microbes.

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Salmonella is often present in minced beef in large amount (Tegegne and Ashenafi, 1998).

Antibiotics are usually applied for the control of diseases in humans and animals. As a result of antibiotic use in food animals, however, drug-resistant pathogens are increasing (Alexander et al., 2009) and this limits therapeutic options both in veterinary and public health practices. Sub-therapeutic and/or prophylactic doses and indiscriminate use of antibiotics in veterinary medicine result in on-farm selection of resistant *Salmonella* which may then pass to humans (Hoelzer et al., 2011; Pui et al., 2011).

Earlier investigations in Ethiopia have demonstrated the presence of *Salmonella* in humans, animals and animal products (Alemayehu et al., 2003; Mache, 2002). *Salmonella* Mishmarhaemek was the predominant isolates in cattle (Tadesse and Tessema, 2014). *Salmonella* Concord, *S.* Typhi, *S.* Typhimurium and *S.* Paratyphi were the dominant serotypes isolated from human and *S.* Concord was reported to be the most common serotype to be resistant to third generation cephalosporins (Tadesse, 2014). *Salmonella* Dublin was the most frequent serotypes isolated from beef (Tadesse and Gebremedhin, 2015). Similarly, other studies in Ethiopia have demonstrated the presence of drug resistance among other *Salmonella* isolates (Haimanot et al., 2010; Beyene et al., 2011; Reda et al., 2011) and *S.* Kentucky was the most frequently reported serotype resistant to ciprofloxacin in animal (Molla et al., 2006; Aragaw et al., 2007).

Real-time investigations on serotype diversity and antimicrobial resistance profile of *Salmonella* in animals, animal products and humans give full understanding of the disease. Therefore, the aim of this study was to determine the prevalence, distribution and antimicrobial resistance profile of *Salmonella* serotypes in calves, minced beef samples and humans in some areas of Bishoftu and Addis Ababa.

MATERIALS AND METHODS

Study area and study design

A cross-sectional survey of *Salmonella* in minced beef originating from Addis Ababa supermarkets, calf faeces from Bishoftu commercial and smallholder dairy farms and human stool samples from out-patients of Bishoftu General Hospital was undertaken from October, 2010 to May, 2010. The study involved a total of 593 samples consisting of 102 minced beef, 107 calf faecal samples and 384 human stool samples.

Sample collection

The meat samples were purchased randomly once a week as sold for consumers (usually in a refrigerated display cases at 4°C) from34 randomly selected supermarkets. The faecal samples were collected from all calves under 6 months of age from randomly selected commercial and smallholder dairy farms in Bishoftu. The stool samples were collected randomly from out-patients in collaboration with the medical personnel in the hospital. Samples were identified by sample number, date of sampling, source and sample type. Minced beef samples were collected in a plastic material with which meat was distributed to the consumer or pre-packed in polyethylene bags. The faecal and stool samples were collected using sterile universal culture bottles. Samples were then taken to the Microbiology Laboratory of the College of Veterinary Medicine and Agriculture, Addis Ababa University, Bishoftu using icebox and kept chilled until microbiological analysis was done.

Isolation and identification

Salmonella isolation and identification was carried out in line with the guidelines of the International Organization for Standardization (ISO, 2002) and Quinn et al. (1999), and steps that include primary enrichment in non-selective liquid medium (pre-enrichment), secondary enrichment in selective liquid media, plating out on selective and non-selective media and final confirmation by biochemical and serological characterization were employed.

Primary enrichment in non-selective liquid medium (pre-enrichment)

The chilled samples were left for 3 to 5 h at 20 to 22°C before being processed. Twenty-five grams of minced beef, 10 to 25 g of faecal samples and 4 to 5 g of stool samples were added to buffered peptone water (BPW) (OXOID, Hampshire, England) in 1:9 ratio (1 gram of sample to 9 ml of BPW). The mixture was homogenized using a laboratory blender at high speed for 2 min. The enrichments were then incubated aerobically at 37°C for 18 to 24 h.

Secondary enrichment in selective liquid media

For this purpose, Rappaport-Vassiliadis magnesium chloride/malachite green (RV) (OXOID, Hampshire, England) and selenite cystine (SC) (DIFCO, Becton, Dicknson and Company, USA) broth media were used. From the incubated pre-enrichment culture, 0.1 ml (aliquot) was taken and mixed with 10 ml RV broth and was incubated aerobically at 42°C for 18 to 24 h. Another 1 ml from pre-enrichment culture was mixed with 10 ml SC broth and incubated aerobically at 37°C for 18 to 24 h.

Plating out and identification

Plating out was done on brilliant green-phenol red-lactose-sucrose (BPLS) agar (MERCK, Darmstadt, Germany) and xylose lysine desoxycholate (XLD) agar (MERCK, Darmstadt, Germany) plates. A loopful from each of the two enrichment broth cultures was streaked onto the two plating out media. The plates are then incubated aerobically at 37°C for 18 to 24 h. Then the plates were examined for the presence of *Salmonella* colonies. Presumptive *Salmonella* colonies with characteristic appearance on both solid media were then streaked onto Rambach agar (MERCK, Darmstadt, Germany) and were incubated aerobically at 37°C for 24 h. Characteristic colonies for *Salmonella*, which appear red on Rambach agar, were then transferred onto nutrient agar (MERCK, Darmstadt, Germany) and incubated aerobically at 37°C for 24 h.

Biochemical characterization

Colonies suspected to be *Salmonella* were further tested

Table 1. List of antimicrobials used and their concentrations.

Antimicrobial	Abbreviations	Breakpoints and concentrations[a]	
		Susceptible at ≤ μg/ml	Resistant at ≥ μg/ml
Amikacin	AMK	16	ND[b]
Ampicillin	AMP	ND	32
Amoxicillin/clavulanic acid	AMC	ND	64/16[c]
Apramycin	APR[d]	ND	32[e]
Carbadox	CRB[d]	ND	30[f]
Cephalothin	CEF	ND	32
Ceftriaxone	CRO	8	ND
Ceftiofur	CTF	ND	8
Cefoxitin	FOX	ND	32
Chloramphenicol	CHL	ND	32
Ciprofloxacin	CIP	0.125[g]	ND
Florfenicol	FLO[d]	ND	16[h]
Gentamycin	GEN	ND	16
Kanamycin	KAN	ND	64
Nalidixic acid	NAL	ND	32
Neomycin	NEO[d]	ND	16[e]
Nitrofurantoin	NIT	ND	64[i]
Spectinomycin	SPT[d]	ND	64[e]
Streptomycin	STR[d]	ND	32[e]
Sulfisoxazole	SUL	ND	512
Sulfamethoxazole/trimethoprim	SXT	ND	76/4
Tetracycline	TET	ND	16
Tobramycin	TOB	ND	8
Trimethoprim	TMP	ND	16

[a]The breakpoint concentrations to determine susceptible, intermediate and/or resistance were those specified by the NCCLS standards M31-A and M100-S12.[b] ND, not done. [c]The strains were considered resistant when growing on agar plates with amoxicillin/clavulanic acid at 64/16 μg/ml. [d]There are no interpretative standards specified by the NCCLS standards M31-A and M100-S12 for apramycin, carbadox, florfenicol, neomycin, spectinomycin and streptomycin. [e]Strains were considered to be resistant to apromycin, neomycin, spectinomycin and streptomycin at 32,16,64, and 32 μg/ml, respectively. [f]The strains were considered to be resistant to carbadox, a veterinary growth promoter for pigs, at 30 μg/ml. [g]A 0.125 μg/ml of ciprofloxacin concentration determines reduced sensitivity to ciprofloxacin. [h]Strains were considered to be resistant to florfenicol at the level of 16 μg/ml. [i]Strains were considered to be resistant to nitrofurantoin at 64 μg/ml; human urinary tract isolates are considered to be resistant to nitrofurantoin at 128 μg/ml.

biochemically using triple sugar iron (TSI) agar slants (BBL, USA), lysine decorboxylase test using lysine decarboxylase broth (DIFCO, Becton, Dicknson and Company, USA), urease test using urea broth (MERCK, Darmstadt, Germany) and citrate utilization test using Simmon's citrate agar (DIFCO, USA). The TSI agar was inoculated; lysine decarboxylase test, urease test and citrate utilization test were conducted according to Quinn et al. (1999).

Serological characterization

Colonies that exhibited typical reactions for the battery of the biochemical tests were further confirmed by agglutination test by *Salmonella* polyvalent O antiserum (DIFCO, Becton, Dicknson and Company, USA) for the presence of *Salmonella* antigen. Before slide agglutination was performed, a loopful of colonies were suspended in a drop of normal saline on a microscope slide and examined for auto-agglutination. Then, a drop of *Salmonella* polyvalent O antiserum was placed on a clean slide, to which a loopful of colony from nutrient agar was transferred, mixed and rocked for one minute and examined for agglutination.

Finally, *Salmonella* isolates were sent to Salmonellosis Reference Laboratory, Ontario, Canada, for serotyping, phage typing and antimicrobial resistance investigation.

Serotyping and phage typing

The somatic (O) and flagellar (H) antigens of *Salmonella* isolates were determined using slide agglutination test (Ewing, 1986) and micro-technique method (Shipp and Rowe, 1980), respectively. The antigenic formula of Le Minor and Popoff (1997) was used to name the serotypes. Phage typing of *Salmonella* Enteritidis was performed according to Ward et al. (1987 using typing phages obtained from the International Centre for Enteric Phage Typing, Central Public Health Laboratory, Colindale, UK.

Antimicrobial susceptibility test

All *Salmonella* isolates were tested for susceptibility against 24 antimicrobial agents in accordance with the National Committee for Clinical Laboratory Standards (NCCLS, 1999) guidelines. The list and concentrations of antimicrobials used were shown on Table 1.

Table 2. The prevalence of *Salmonella* in faecal, stool and minced beef samples.

Sample type	Number of samples examined	Number (%) of samples positive for *Salmonella*
Calf faeces	107	2 (1.9)
Human stool	384	13 (3.4)
Minced beef	102	10 (9.8)
Total	**593**	**25 (4.2)**

Table 3. *Salmonella* serotypes isolated by source.

Source	Serotype (number)	Antigenic structure	Phage type
Faeces (n=107)	*S*. Typhimurium (2)	4,5:i:2	911
Stool (n=384)	*S*. Typhimurium (5)	4,5:i:2	
	S. Typhimurium var. Copenhagen (1)	4:i:2	
	S. Anatum (1)	10:eh:6	
	S. Havana (1)	23:fg:-	
	S. Enteritidis(1)	9,12:gm:-	
	S. Muenchen (1)	6,8:d:2	
	S. Mikawasima (1)	6,7:y:z15	
	S. Saintpaul (1)	4:eh:2	
	S. Uganda (1)	10:1,z13:5	
Minced beef (n=102)	*S*. Uganda (4)	10:1,z13:5	
	S. Bovismorbificans (2)	6,8:r:5	
	S. Braenderup(1)	6,7:ehz15	
	S. Livingstone (1)	6,7:d:1,w	
	S. Hadar (1)	6,8:z10:x	
	S. Blockley (1)	6,8:k:5	

In this study, *Salmonella* isolate was considered resistant if it was resistant to at least one antimicrobial drug and multiple resistant if it was resistant to two or more antimicrobial drugs tested.

Moreover, isolates with intermediate resistance were considered susceptible to that antimicrobial. Standard and reference strains, which include *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa*, ATCC 27853, *Staphylococcus aureus* ATCC 29213, and *Enterococcus faecalis* ATCC 29212 were used.

RESULTS

Prevalence and distribution

Of the total 593 faecal, stool and minced beef samples examined, 25 were found contaminated with *Salmonella* (Table 2).

Serotyping

Twenty-five *Salmonella* positive samples representing 14 different serotypes were identified from 593 samples examined. The most common serotypes identified in this study were *S. typhimurium* (28%) and *S*. Uganda (20%) followed by *S. bovis morbificans* (8%). *Salmonella* serotypes less commonly isolated include *S. anatum, S. blockley, S. braenderup, S. enteritidis, S. hadar, S. havana, S. livingstone, S. Mikawasima, S. Muenchen, S. Saintpaul and S.Typhimurium var.* Copenhagen (Table 3).

S. typhimurium was the most dominant serotype detected in this study. It was isolated from 2 faecal samples and from 38.5% of the stool samples. However, this serotype was not recovered from minced beef samples. *S. typhimurium var. Copenhagen, S. anatum, S. Havana, S. enteritidis, S. muenchen, S. mikawasima and S. Saintpaul were detected only in stool samples, whereas S. bovismorbificans, S. braenderup, S. Livingstone, S. hadar and S. Blockley* were detected only from meat samples.

Nine different serotypes from human stool samples and 6 different serotypes from minced beef samples were isolated, whereas *S*. Typhimurium was the only serotype detected from calf faecal samples. Of the *Salmonella* serotypes isolated from human out-patients, *S*.

Table 4. Distribution of resistant *Salmonella* serotypes by source.

Source	*Salmonella* isolates		
	Serotype tested (Number)	**Resistant (%)**	**Resistant serotype**
Faeces	*S*. Typhimurium (2)	-	-
Minced beef	*S*. Uganda (4) *S*. Bovismorbificans (2) *S*. Braenderup (1) *S*. Livingstone (1) *S*. Hadar (1) *S*. Blockley (1)	3 (30)	*S*. Blockley *S*. Braenderup *S*. Hadar
Stool	*S*. Typhimurium (5) *S*. Typhimurium var. Copenhagen (1) *S*. Anatum (1) *S*. Havana (1) *S*. Enteritidis (1) *S*. Muenchen (1) *S*. Mikawasima (1) *S*. Saintpaul (1) *S*. Uganda (1)	2 (15.4)	*S*. Enteritidis S. Typhimurium var. Copenhagen
Total	**25**	**5 (20)**	**-**

typhimurium (38.5%) was dominant. *Salmonella* Uganda was a predominant serotype isolated from minced beef representing 40% of the isolates followed by *S*. bovismorbificans (20%). One serotype, *S*. mikawasima, was reported in Ethiopia for the first time. This new finding might underline the consequence of human movement as infection with *S.* mikawasima is increasing in European countries.

Antimicrobial resistance of *Salmonella*

Five isolates (20%) belonging to 5 different serotypes were found multidrug resistant (MDR), that is, resistant to 3 to 9 antimicrobials tested. Thirty percent (3/10) and 15.4% (2/13) of *Salmonella* isolates from meat and stool samples, respectively, were found resistant to 3 or more ampicillin, amoxicillin/clavulanic acid, chloramphenical, florfenicol, nitrofurantoin, streptomycin, spectinomycin, sulfisoxazole and tetracycline. *S.* Blockley was resistant to ciprofloxacin, kanamycin, nalidixic acid, neomycin, nitrofurantoin, streptomycin and tetracycline. *S.* Braenderup was resistant to ampicillin, spectinomycin, streptomycin, sulfisoxazole, sulfamethoxazole/trimethoprim, tetracycline and trimethoprim. *S.* Enteritidis was resistant to ciprofloxacin, nalidixic acid and nitrofurantoin, whereas *S*. Hadar was resistant to nitrofurantoin, streptomycin and tetracycline (Table 6). All isolates belonging to *S*. Anatum, *S*. Bovismorbificans, *S*. Havana, *S*. Livingstone, *S*. Mikawasima, *S*. Muenchen, *S*. Saintpaul and *S*. of the antimicrobials checked. With regard to sources of the five resistant *Salmonella* isolates, minced beef accounted for 60% (3/5) and human stool accounted 40% (2/5). *Salmonella* isolates from calf faeces were susceptible to all the 24 antimicrobials used (Table 4).

Nine of the 24 (37.5%) antimicrobials used were effective against all *Salmonella* isolates with the exceptions of carbadox, cephalothin, ceftiofur, florfenical and kanamycin which showed intermediate resistance pattern to the isolates (Table 5).

Among multidrug resistant isolates, resistance to nitrofurantoin, streptomycin and tetracycline was most often observed. Of the multiple resistant serotypes, *S*. Typhimurium var. Copenhagen was predominant (resistant to 9 antimicrobials) followed by *S*. Blockley and *S*. Braenderup each resistant to 7 antimicrobials. *S.* Typhimurium var. Copenhagen was resistant to Typhimurium were susceptible to all 24 antimicrobials. Similarly, all isolates of *S*. Uganda were susceptible to all antimicrobials with the exception of one isolate from stool which showed intermediate resistance to cephalothin ceftiofur, florfenicol and kanamycin. *Salmonella* Blockley also showed intermediate resistance to carbadox (Table 6).

DISCUSSION

Prevalence and distribution of *Salmonella*

Salmonella contamination rate of 9.8% in minced beef

Table 5. Resistance pattern of *Salmonella* isolates to the tested antimicrobials.

Antimicrobials[a]	Number of resistant *Salmonella* serotypes by source		
	Faeces	Stool	Minced beef
Ampicillin	-	1	1
Amoxicillin/clavulanic acid	-	1	-
Carbadox	-	-	1*
Cephalothin	-	1*	-
Ceftiofur	-	1*	-
Chloramphenicol	-	1	-
Ciprofloxacin	-	1	1
Florfenicol	-	1*	-
Kanamycin	-	1*	1
Nalidixic acid	-	1	1
Neomycin	-	-	1
Nitrofurantoin	-	2	2
Spectinomycin	-	1	1
Streptomycin	-	1	3
Sulfisoxazole	-	1	1
Sulfamethoxazole/trimethoprim	-	-	1
Tetracycline	-	1	3
Trimethoprim	-	-	1

[a]Resistance to Amikacin, Apramycin, Ceftriaxone, Cefoxitin, Gentamycin and Tobramycin was not observed; *Intermediate in resistance.

Table 6. Multiple resistance pattern of *Salmonella* isolates.

Salmonella serotype	Number of serotypes tested	Multiple resistant	Antimicrobial resistance pattern	Remark
S. Anatum	1	-	-	
S. Blockley	1	1	CIP, KAN, NAL, NEO, NIT, STR, TET, CRB*	
S. Bovismorbificans	2	-	-	
S. Braenderup	1	1	AMP, SPT, STR, SUL, SXT, TET, TMP	
S. Enteritidis	1	1	CIP, NAL, NIT	Phage type 911
S. Hadar	1	1	NIT, STR, TET	
S. Havana	1	-	-	
S. Livingstone	1	-	-	
S. Mikawasima	1	-	-	
S. Muenchen	1	-	-	
S. Saintpaul	1	-	-	
S. Typhimurium	7	-	-	
S. Typhimurium var. Copenhagen	1	1	AMP, AMC, CHL, FLO, NIT, SPT, STR, SUL, TET	
S. Uganda	5	1*	CEF*, CTF*, FLO*, KAN*	
Total	**25**	**5**	**-**	

* Intermediate in resistance.

was in agreement with the findings of previous study undertaken in Ethiopia (Ashenafi, 1994). This was also consistent with the 8% prevalence of *Salmonella* from minced meat reported from Egypt (WHO, 1988) and 1.8 to 20% report of *Salmonella* from various countries (D'Aoust, 1989). However, the current finding was lower than the 14.4% (Ejeta et al., 2004), 40% (Molla et al., 1999a) and 42% (Tegegne and Ashenafi, 1998)

prevalence rates reported in Ethiopia. The difference could be due to improvements of sanitation in the supermarkets, seasonal influence or variations between sample sizes and number of supermarkets included. Methodology of isolation employed such as variation in media used for isolation, sampling procedures and one versus multiple picks of suspect colonies for confirmation could also contribute to the difference.

The 3.4% *Salmonella* isolation rate from human stool samples was lower than 4.5% (Ashenafi and Gedebou, 1985) and 7.9% (Mache et al., 1997) reported from Ethiopia and 15.3% reported from Culcutta, India (Saha et al., 2001). Samples examined by all the aforementioned studies originated from diarrhoeic out-patients. This and the slightly different methods employed to culture *Salmonella* may have attributed to the differences observed.

In this study, the prevalence of 1.9% *Salmonella* in calf faeces was in agreement with 2% faecal shedding of *Salmonella* reported (Williams et al 1978). However, this finding was significantly lower than 6.7% reported from apparently healthy cattle in Botswana (Miller, 1971). The low estimate in this study might be due to low proportion of *Salmonella* carriers in the study population and the low and intermittent nature of faecal shedding of carrier animals.

S. typhimurium was the most dominant serotype isolated from human out-patients in this study and this serotype was the most reported serotype in human salmonellosis in many countries (Fisher, 2004). It is worth mentioning that *S*. mikawasima was isolated for the first time in Ethiopia. Report of *S*. mikawasima for the first time might underline the risk of introduction of salmonellosis as a result of traffic of people, livestock and foodstuffs across national boundaries.

The identification of six different serotypes from ten isolates from minced beef samples is indicative for the potentially widespread presence of *Salmonella* serotypes in cattle and minced beef in Ethiopia. *S. typhimurium* was detected from apparently healthy calves and this would be regarded as a greater concern because of its demonstrable significance both in animal and human health.

All *Salmonella* isolates in this study from human stool samples belong to non-typhi serogroups. This was not consistent with earlier studies undertaken in Addis Ababa indicating *S.* Typhi a predominant serotype isolated (Afeworki, 1985; Messele and Alebachew, 1981). This was so because most of the samples in the aforementioned studies were blood samples.

Antimicrobial resistance of *Salmonella*

The current 15.4% multidrug resistant (MDR) *Salmonella* isolates from minced beef was lower than previous reports in Ethiopia (Tibaijuka et al., 2002; Molla et al., 2004), but much higher than the one reported in USA (Fluckey et al., 2007). The differences could be as a result of the varied serotypes identified in the respective studies and variation of antimicrobial usage in the study population.

Unlike other reports (Molla et al., 1999b; Gebre-yohannes et al., 1987), no *S*. Typhimurium isolate in this study was found resistant to any of the antimicrobial drugs tested. This might indicate the survival of some non-resistant *S.* Typhimurium strains circulating in the population.

On the other hand, the 30% (3/10) *Salmonella* resistant isolates from minced beef in this study and absence of resistance in Nyeleti et al. (2000) was contrasting. This is an indication for the emerging of drug resistant *Salmonella* through time.

All *S*. Uganda serotypes from minced beef were susceptible to all of the antimicrobials used. However, one strain of *S.* Uganda from human stool sample showed intermediate resistance to kanamycin, cephalothin, florfenical and ceftiofur. This might be due to frequent usage of these drugs in public health than in veterinary medicine.

Resistance was not detected against amikacin, apramycin, carbadox, cefoxitin, ceftriaxone, ceftiofur, cephalothin, gentamycin and tobramycin with the exceptions of carbadox, cephalothin, ceftiofur, florfenical and kanamycin with intermediate resistance. Resistance was low to amoxicillin/clavulanic acid, chloramphenicol, florfenicol, kanamycin, neomycin, sulfamethoxazole/trimethoprim and trimethoprim. The absence or low levels of resistance to the aforementioned antimicrobials were possibly because of their narrow prescriptions in Ethiopia.

Conclusion

The present study demonstrated that Salmonellae are present in minced beef, calves and humans in Bishoftu and Addis Ababa. In general, the detection of 14 different serotypes from 25 *Salmonella* isolates and the isolation of *Salmonella* Mikawasima for the first time in Ethiopia indicate the occurrence of widespread *Salmonella* serotypes in the country. The finding of 5 multidrug resistant *Salmonella* serotypes in this study signifies how treatment of clinical salmonellosis is becoming difficult both in animals and humans. Moreover, resistance to nitrofurantoin, streptomycin and tetracycline was an indication of their easy admittance and broad practice in livestock and humans. This justifies the need for strict intervention measures to make sure prudent utilization of antimicrobials and monitoring of drug resistance.

CONFLICT OF INTERESTS

The author has not declared any conflict of interests.

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Full Length Research Paper

Studies on the prevalence, cyst viability, organ distribution and public health significance of bovine cysticercosis in Ambo municipality abattoir, Western Shoa, Ethiopia

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A cross-sectional study was conducted on cattle slaughtered in Ambo municipal abattoir with the aim of determining the prevalence, cyst viability, organ distribution and public health significance of bovine cysticercosis. Of the 600 carcasses inspected, 93 (15.5%) were infected with cysticerci. A total of 122 cysts were collected of which 95 (77.87%) were viable while others 27 (22.13%) were degenerated. The anatomical distribution of cysticerci were highest in the shoulder muscles 41(85.4%) followed by masseter muscles 11(78.6%), thigh muscles 17 (77.3%), heart 14 (73.68%), tongue 7 (70%), liver 4 (66.67%) and 1(50%) in the intercostals mussels. The prevalence of cysticercosis varied significantly (P<0.05) with sex, age, breed and origin of animals. Interview was conducted on 180 residents using a structured questionnaire to know the public health significance of the disease. Forty eight (60%) out of 80 respondents from Ambo town, thirty four (68%) out of 50 respondents from Guder woreda and twenty three (46%) out of 50 respondents from Dendi woreda had contracted *the disease* at least once in their life time and maximum infestation frequency was two times per year. The infestation varied significantly (P<0.05) with sex, age, place of respondents, habit of raw meat consumption, religion, marital and educational stutas.

Key words: Ambo, bovine cysticercosis, cyst viability, Dendi, Guder, prevalence, public health, organ distribution.

INTRODUCTION

Ethiopia is one of the countries in Africa with huge livestock resources that play a crucial role in the livelihoods of the majority of Ethiopians. The cattle population for the country is estimated at 50.8 million out of which females constitute about 54.87% and the remaining 45.13% are males (CSA, 2012). Despite the

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huge resources, Ethiopia livestock productivity remains a below being adequate. The major biological constraints contributing to low productivity includes the low genetic potential of the animals, poor nutrition and prevailing disease like parasitosis (CSA, 2012).

Among the many prevalent livestock diseases parasitism represent a major drawback to livestock development in the tropic in general and *Cysticercus bovis*, which is the larvae stage of the human tapeworm, *Taenia saginata* causes significant economic losses to the beef industry and public health hazard (FAO, 2011).

Bovine cysticercosis refers to the infection of cattle with metacestodes of the human tapeworm (Ambachew and Yitagel, 2015). Ingested eggs develop into cysticerci, which can often be detected during meat inspection at the routinely inspected localization sites of the parasite, including, the skeletal muscle, heart and diaphragm. The matured cyst is grayish white vesicle about one centimeter in diameter. It is filled with fluid, in which the scolex is usually visible, like the adult parasite, it has no hooks and rostellum (Gebretsadik et al., 2016). Several authors reported that the metacestode of *T. saginata* is common in organs like the tongue, heart, masseter muscle, thigh muscle, shoulder muscle, liver, diaphragm, intercostals and kidney (Radostits et al., 2007).

The adult tape worm, *T. saginata* occurs in the small intestine of the final host, man and the metacestode (*C. bovis*) is found in cattle that serve as main intermediate host (Endiras, 2011). The epidemiology of the disease is associated with the cattle rearing system, age of cattle, meat inspection practice, and habit of consumption of raw and under cooked meat. Low awareness and poor hygiene and sanitary infrastructures may facilitate transmission of the disease between animals and human beings in the rural areas.

As per an estimate, 50 million cases of such infestations occur worldwide with 50,000 people dying from this problem annually (WHO, 2015). In humans, the disease is called taeniasis which is accompanied by symptoms like nausea, abdominal discomfort, epigastric pain, diarrhea, excessive appetite or loss of appetite, weakness, loss of weight and intestinal blockage (Brown and Neva, 2010; Pal, 2012; Deressa et al., 2012). Sometimes, the mobile gravid segments may make their way to unusual sites such as the appendix and bile tract and may cause serious disorders.

Cattle infected with C*. bovis* show no symptoms, however, heavy infestation may cause myocarditis or heart failure (Gracey et al., 2011). Cysticerci can remain alive in cattle from weeks to years. Infection in human is a public health problem as the infection is acquired through consumption of raw or undercooked beef. The economic losses result from condemnation and down grading of carcasses and due to treatment of carcasses to make it fit for human consumption (Deressa et al., 2012). Inadequate health education and low availability of taenicides are the major obstacles for the control of such infections (Pawlowski, 2013). Due to these reasons, taeniasis is more common in developing countries including Ethiopia where meat is an important component of human diet and traditionally, it is consumed raw on several occasions. About 45% of Ethiopia's domestic meat consumption comes from cattle, but this income is affected due to various unimproved animal health problems, among which, *T. saginata/C. bovis* is one (FAO, 2011).

The prevalence rate is excess of 20% in developing countries, where as in developed countries the prevalence of cysticercosis is low usually less than 1% (Pawlowski, 2013). Even though, *T. saginata* has a worldwide distribution, its prevalence is particularly higher in Sub-Saharan Africa (WHO, 2015). Taeniasis *c*aused by *T. saginata* is a well-known helminthic zoonotic disease in Ethiopia with prevalence ranging from 10 to 70% (Jemal and Haileleuil, 2011). However, to my knowledge in the last decades the prevalence and distribution *C. bovis* was not report for implementing control and prevention program throughout the country. Hence, the research work is aimed to determine the prevalence, cyst viability, organ distribution and public health significance of bovine cysticercosis at Ambo municipality slaughter houses.

MATERIALS AND METHODS

Study area

The study was conducted in Ambo town which was found in Western Showa zone of Oromia regional state. Ambo is located 112 km West of Addis Ababa, and the area is found at a longitude of 370 32' to 380 3' E, and latitude of 80 47' to 90 20' N and the altitude range is from 1900 to 2275 meters above sea level. The climatic condition of the area is 23% highland, 60% mid altitude, and 17% lowland. It has an annual rainfall and temperature ranging from 800 to 1000 mm and 20 to 29°C respectively (AAB, 2015).

The rainfall is bi-modal with the short rainy season from February to May and long rainy season from June to September. Agriculture is the main occupation of the population of the area. The agricultural activities are mainly mixed type with cattle rearing and crop production under taken side by side (AAB, 2015).

Study animal

Postmortem inspection was conducted on 600 cattle slaughtered at Ambo municipal abattoir for the presence of *C. bovis* which originate from neighboring marketing areas such as East Showa from Dendi woreda, West Showa from Toke kutaye (Guder) and Ambo areas. From those animals that daily came to the municipal abattoir, study animals were selected and routinely inspected for cysticercosis.

Sampling and sample size determination

Sampling was conducted using simple random sampling method. The study was cross sectional abattoir survey which includes cattle brought from different livestock markets to Ambo municipality abattoir. The total numbers of cattle required for the study was

calculated based on the formula given by Thrusfield (2007). In this study, 50% prevalence was considered to calculate the sample size using the following formula.

$N=1.96^2 \times Pexp\ (1\text{-}pexp)/d^2$

Where n=required sample size; Pexp=expected prevalence (50%); d=desired absolute precision; $N= 1.962 \times 0.5(1.0.5)/ (0.05)^2 = 384$ animals.

Actually 600 animals were sampled and inspected during the study period.

Cyst identification

During the study period, 600 cattle carcasses were examined for the presences of *C. bovis* following the meat inspection was made as the procedures of Ethiopia Ministry of Agriculture (MOA) Meat Inspection Regulation (1972), for the detection *of C. bovis.* Before inspecting the animals ante-mortems inspection was carried out and the number of each animal was recorded. It comprised data of carcass identification number, age, sex, breed and number of cyst found on the suspected predilection site. During meat inspection of each organ of an animal was strictly and separately examined to avoid mixing up of organs. The tongue, heart, liver, masseter muscles, triceps, thigh muscles, diaphragm and intercostals muscles of all slaughtered beef cattle were assessed by visual inspection, palpation followed by incisions for the detection of *C. bovis*.

Cyst evaluation (viability test)

The cyst which was found at meat inspection was removed with the surrounding tissue and taken to laboratory for viability test. The viability of the cyst were examined by placing them in a normal saline solution with 40% ox-bile and incubated at 37°C for 1 to 2 h. A cyst was regarded as viable if the scolex evaginated during this period (Gracey et al., 2011).

Questionnaire survey for public health significance

In order to assess the extent of human taeniasis, 180 voluntary respondents were randomly selected from three distinct *woredas'*; namely, Ambo, Guder and Dendi, and interview were made individually using semi-structured questionnaire. The potential risk factors like age, habit of raw meat consumption, religion, sex, marital status and educational level was recorded.

Methods of data collection and variable used

In order to conduct the study, ante mortem and postmortem examinations and laboratory characterizations of the cysts was employed. Data on species, sex, age, and origin of the study animals was collected during ante mortem examination of the animals. Data on infestation status of the animals, organs affected, cyst number and distribution were collected or recorded from postmortem examination results. Information on characteristics of each of the cysts examined in the laboratory was obtained and recorded.

Data management and analysis

The data collection from the study area was recorded in the format developed for this purpose and later on entered into Micro Soft office Excel 2007 spreadsheet computer program and analyzed using SPSS statistical software version 17.0. The prevalence of cysticercosis was calculated as the number of cattle found to be infected with *C. bovis* against to total number of examined animals expressed as a percentage of the total number of cattle slaughtered. Chi-square (x^2) test was applied to compare the infection status with regard to the hypothesized risk factors like age, sex, and cyst characteristics. A statistically significance association between variables and considered to exist if the completed P-value is less than 0.05.

RESULTS

Abattoir survey (postmortem examination)

Out of 600 cattle inspected in Ambo municipality abattoir 93 (15.5%), CI (12.6-18.4) were found infected with *Cysticercus bovis*. Male animals 76 (12.66%) had higher cysts of *C. bovis* than female animals 17 (2.83%). Although more males than females were examined, the prevalence of infections showed significant difference (OR=36.85, P= 0.001). However, more light infections and apparently lower prevalence was observed in animals originated from Ambo than those from Guder and Dendi areas (Table 1).

The infection of cattle with *C. bovis* was 34 (16.9%) in younger (<5years) and 59 (14.8%) in older (>5years) animals (OR=2.8), P=0.001). Out of 600 cattle carcasses examined, 598 (99.7%) were local breeds and infected with *C. bovis* while 2 (0.3%) cross breeds cattle carcasses failed to reveal infection.

Anatomical distributions of cyst

Seventy nine organs were found to harbor the cyst. The variation in the anatomical distribution of cysticerci were as follows shoulder, masseter, thigh, heart, tongue, liver, intercostals and diaphragm muscles were the major predilection sites with respective prevalence value of 29.11, 21.5, 16.5, 12.66, 11.4, 5.06, 2.5 and 1.3%, as presented in Table 2. The shoulder muscles were predominantly affected site in the study area.

Viability test

Out of 122 cysts encountered, 95 (77.87%) were viable while other 27 (22.13%) were non-viable (degenerative) cysts. Viability test showed that shoulder had the highest relative frequency proportion of viable cysts 41(85.4%) followed by masseter 11(78.6%), thigh 17(77.3%), heart 14(73.68%), tongue 7(70%), liver 4(66.67%) and intercostals mussels 1(50%) (Table 3).

Among 122 total cysts of carcasses, 16 (13.1%) cysts were found in female animals with 10 (62.5%) cysts were alive while 106 (86.9%) cysts were found in male animals with 89 (83.9%) cysts were alive (Table 4).

Table 1. Analysis of potential risk factors associated with the occurrence of viable cysts in carasses inspected at the Ambo municipal abattoir.

Risk factors	No. of animals examined	Infected	Chi square	*P*-value
Sex				
Male	521	82 (15.74%)	39.676	0.001
Female	79	11 (13.9%)		
Age				
<5 Years	201	34 (16.9%)	3.199	0.00
>5 Years	399	59 (14.8%)		
Breed				
Local	598	93 (15.5%)	41.134	0.000
Cross	2	-		
Origin of animals				
Ambo	116	9 (7.76%)	12.35	0.0235
Guder	273	55 (20.14)		
Dendi	211	29 (13.74%)		

Table 2. Number of organs infected and distribution of *C. bovis* on different organs at Ambo municipality abattoir.

Organs examined	No. of organs infected	Prevalence (%)	Total no. of cysts examined in each organ	Percentage (%)
Tongue	9	11.4	10	8.2
Masseter muscles	13	16.5	15	12.3
Shoulder muscles	23	29.11	48	39.34
Thigh muscles	17	21.5	22	18.0
Heart	10	12.66	18	15.6
Liver	4	5.06	6	4.9
Diaphragm m/s	1	1.3	1	0.8
Intercostal m/s	2	2.5	2	1.6
Total	**79**	**100.0**	**122**	**100.0**

Questionnaire survey on taeniasis

Survey was done at three distinct *Woredas'*; namely, Ambo, Guder and Dendi. Of the total 180 interviewed respondents who participated in this study, 48 (60%) out of 80 respondents in Ambo, 34 (68%) out of 50 respondents in Guder and 23 (46%) out of 50 respondents in Dendi *woredas'* had contracted *T. saginata* infection at least once in their life time and maximum infestation frequency was two times per year.

The majority of the respondents had an experience of raw meat consumption due to traditional and cultural practice. The multiple logistic regression analysis of the risk factors showed significance difference ($p<0.05$) in the prevalence of taeniasis with raw meat consumers, age, religion, sex, marital status, place and educational level of the respondents. Accordingly frequent raw meat consumers (OR=3.5, 95% CI [2.08-81.04], male individuals (OR=5.8, 95% CI [1.23-5.43], Christian (3.6) and old age (OR=3.65) had higher likelihood of acquiring taeniasis than Muslims, cooked meat eaters and female, respectively (Table 5).

DISCUSSION

Among 600 carcasses of cattle inspected in Ambo municipal abattoir, 93 carcasses were found to harbor viable or non-viable cysts of *C. bovis* with an overall prevalence of 15.5%. The infection prevalence of *T. saginata* cysticercosis was invariably high in those animals come from Guder and Dendi *woredas*'. This suggests wide occurrences of the disease throughout the study areas regardless of agro-ecological and socio-cultural differences.

The present finding on the prevalence of *C. bovis* is in agreement with earlier reports of 13.3% at Addis Ababa abattoir (Nigatu et al., 2009), 11.3% at Wolaita Soddo

Table 3. Proportion of viable and non-viable cysts in different organs.

Organs affected	Condition of cysts				
	Total no. of cyst	Viable	Percent (%)	Non-viable	Percent (%)
Tongue	10	7	70	3	30
Masseter muscles	14	11	78.57	3	25
Shoulder muscles	48	41	85.4	7	14
Thigh muscles	22	17	77.68	5	22.7
Heart	19	14	73.3	5	26.3
Liver	6	4	66.67	2	33.3
Diaphragm muscles	1	-	-	1	100
Intercostal muscles	2	1	50	1	50
Total	**122**	**95**	**62.35**	**27**	**37.75**

Table 4. Distribution of *Cysticercus bovis* cyst between male and female animals.

Predilection site	Female			Male			Total
	No. cyst	Viable	Non-viable	No. cyst	Viable	Non-viable	
Tongue	2	1	1	5	3	2	7
Masseter m/s	2	1	1	13	11	2	15
Shoulder m/s	3	2	1	45	39	6	48
Thigh m/s	3	2	1	18	16	2	21
Heart	3	2	1	10	8	2	13
Liver	2	1	1	14	12	2	16
Diaphragm m/s	-	-	-	1	-	1	1
Intercostal m/s	1	1	-	-	-	-	1
Total	**16**	**10**	**6**	**106**	**89**	**17**	**122**

municipal abattoir (Alemayehu et al., 2009) and 18.49% at Bahir Dar municipal abattoir (Nigatu, 2008), but it is far lower than the 30% prevalence report for the whole Ethiopia (Solomon, 2012) and greater than 2.7% in Gondar municipal abattoir (Ambachew and Yitagel, 2015).

Prevalence of this study is comparable to some reports from African countries, such as 20% in Senegal, 27% in Tanzania and 38 to 62% in Kenya (Over et al.,2013; Onyango-Abuje et al., 2011), and 6.2% in Namibia (Kumba et al., 2010). Likewise Opara et al. (2012) have reported comparable prevalence of 26.2% from slaughter animals in Nigeria. Conversely, lower prevalence was reported from developed countries, such as 0.26% in Croatia (Zivkovic et al., 2010), 0.48 to 1.08% in Germany (Abuseir et al., 2012) and 0.9% in Cuba (Suarez and Santizo, 2013).

Thus, *T. saginata* cysticercosis has more public health and economic significance in developing countries like Ethiopia compared with developed countries. Problems associated with poor sanitary infrastructure, low awareness and improper disposal of sewage are major factors for higher prevalence of cysticercosis in developing countries (Gebretsadik et al., 2016).

The highest prevalence *of C. bovis* in young cattle in the present study is in line with the works of Gracey et al. (2011) who described that in countries where *T. saginata* is common in cattle frequency ingest tapeworm ova. An active immunity develops and the prevalence, which decreases progressively with age. Adult cattle have also indicated that in Africa are resistant to re-infection because they acquire cysticercosis at a young age. With subsequent development of active immunity since the animals slaughtered are old, the majority of cysticerci from initial calf hood infection may degenerate or disappear (Deressa et al., 2012).

Minozzo et al. (2015) have demonstrated a wide distribution of *T. saginata* metacestode infection throughout bovine muscles showing inefficiency of routine meat inspection. Thus, higher prevalence in the present study might be attributed to the variation in the method and quality of meat inspection, human use of non-latrine as well as personal and environmental contamination of the study areas. Furthermore, the study animals were from outside of the town and managed under extensive farming system, where contamination of grazing fields by human excreta is common, especially in Guder *woreda.*

Table 5. Questionnaire survey for public health significance in three distinct woredas'.

Risk factor	Interviewed no.	Infested no.	Prevalence (%) (95% CI)	OR(95% CI)	P-value
Age (years)					
<30	48	17	35.4 (28.4-42.4)	3.65	0.022
>30	132	88	66.67(59.8-73.6)		
Sex					
Male	128	91	68.25 (61.4-75)	5.8	0.000
Female	52	14	26.9 (20.4-33.4)		
Religion					
Muslim	17	4	23.53(18.87-29.7)	5.3	0.001
Christian	163	101	61.96 (54.86-69)		
Place of respondents					
Ambo	80	48	60 (52.8-67)	1.8	
Guder	50	34	68 (61.2-74.8)	2.5	0.04214
Dendi	50	23	46 (38.7-53.3)		
Habit of raw meat consumption					
Not consume	75	36	48 (40.7-55.3)	3.5	0.0313
Consume	105	80	76.2 (69.9-82.4)		
Marital status					
Un married	80	42	52.5 (45.2-59.8)	1.5	0.040
Married	100	63	63 (55.9-70)		
Education					
Educated	83	45	54.2 (46.9-61.5)	1.3	0.023
Non educate	97	60	61.85 (54.7-68.9)		

More light infections were observed animals came from Ambo areas, perhaps due to the less habitual raw meat consumption among the local community and less conducive environment for the parasite. Secondly, the practical limitations to the number of incisions allowed in skeletal muscles might have reduced the efficiency of cyst detection. Thirdly, human used toilet.

The prevalence of *T. saginata* cysticercosis in sex, origin, local and crossbred of animals varied significantly (P< 0.05). One possible explanation for this significant difference between local and crossbred of cattle in the study area is might be due to the fact that; crossbred animals are kept indoor, they are fed only with feed guaranteed free from *cysticerci* eggs (this means that no feed from pastures or crops can be used, unless treated) and less exposed to human excreta than local breed. Jemal and Haileluil (2011) reported that the existence of difference in geographical isolates of the parasite and in the breed of cattle as a possible factor affecting the distribution and prevalence of *T. saginata* cysticercosis.

Sex-related distribution of *C. bovis* infection of the slaughtered cattle in this study showed that incidence rate was significantly (P=0.001, OR=36.85) higher in males than in females. The variations in prevalence rate might be because higher number of males (86.83%) of total slaughtered than number that were females (13.16%). This finding is in agreement with the report of Ahmed (Endiras, 2011).

During the study period, the most frequently affected organs with the highest number of cysts of *C. bovis* was recorded in shoulder muscles followed by masseter muscles, thigh muscles, heart, tongue and liver. The variations in anatomical distribution depend on a number of factors, such as blood kinetics and animals' daily activities. Any geographical and environmental factors affecting blood kinetics in the animal affect the distribution of oncospheres as well and hence the predilection sites during meat inspection (Gracey et al., 2011).

The finding of the current study is in agreement with the reports of Opara et al. (2012), Alemayehu et al. (2009) and Hailu (2010) who indicated that examination of the shoulder muscles is the most effective means of detection of bovine cysticercosis, while the heart and liver are described as the most frequently infected organ by Tembo (2014). Thus, there is no particular "predilection site" which could be acceptable for all cattle.

Viability test showed that shoulder muscles had the highest relative frequency proportion of viable cysts 41 (85.4%) followed by masseter 11(78.6%), thigh 17

(77.3%), heart 14 (73.68%), tongue 7 (70%), liver 4 (66.67%) and intercostal mussels 1 (50%). This observation goes parallel with the findings of Opara et al. (2012) who recovered higher proportion of cysts from shoulder muscles that had the highest proportion of viable cyst.

The explanation for this may lie in the fact that muscle activity receives more blood than a muscle at rest, and that the distribution of the cysts is controlled by the volume and intensity of the arterial blood (Gracey et al., 2011).

Human taeniasis was a widespread health problem in the study area with prevalence of 68% (34/50), 60% (48/80) and 46% (23/50) in Guder, Ambo and Dendi *woredas'* respectively. The occurrence of the disease had significant association (P=0.04214, OR=2.5) with habit of raw meat consumption. Thus, infection of human being by *T. saginata* is mainly due to the habit of eating raw '*kurt*' or semi-raw '*kitifo*' meat dishes in Ethiopia (Pal, 2012).

Taeniasis prevalence was higher among the Christian community than Muslims in the study area. Similar to the reports of Deressa et al. (2012), taeniasis prevalence was higher among the Christian community than Muslims. Because raw meat consumption is not common in Muslims as in Christians, and Christians also celebrate several annual festivals with the tradition of raw meat consumption (Pal, 2012).

This presentation revealed that males 91 (68.25%) were highly affected than females 14 (26.9%) (OR=5.8, P=0.001). This observation is similar to the finding of Ambachew and Yitagel (2015) who reported higher prevalence of taeniasis among males than females in Gondar. The difference in the rate of infection between males and females in the study area could be due to the fact that males enjoy eating raw beef with local drink ''*Tej* and *Catukela*", as it is traditionally described ''*Arada Tej*'' at Ambo town, "*Kubaya sefar Tej*" at Dendi woreda and "*Kub-lame* or *Catukela*" at Guder woreda. The second reason might be males have the access to eat raw tongue and rumen folds locally called ''*Milas-Sember"* during ''*Kircha*'', this is common in the study area. The third reason might be males provide and control the finance and hence, they can eat raw beef in the butcher house.

T. saginata was observed among old aged people (> 30 years) as compared to young age people (< 30 years). This agrees with Alemayehu et al. (2009) observation that the older people greater chance of eating raw beef and hence contracting taeniasis. Therefore, the two age groups might be due to the fact that older people have the finance to eat raw beef in the butchers house and generally the tradition of consuming ''*Arada-Tej"* and "*Catukela"* is common among older people and also might be it is the older males individuals who participates in ''*Kircha*'' and hence having the opportunity to eat raw beef particularly tongue and rumen fold ''*Milas-Sember"* in the field.

Based on the place of respondents at the three woredas' survey showed that Guder *woreda* was the highest infection rates (68%), followed by Ambo (60%) and less infection rates was observed in Dendi *woredas'* (46%). The difference of the infection rates in different areas was due to the fact that the difference of cultural, religion and use of latrine. The majority of the communities in Guder *woreda* were Christian with feeding habit of raw meat and non-use of sanitary latrine than in Ambo and Dendi *woredas'*.

Depending on the marital status, married peoples were more infected than unmarried ones. This might be due to the fact that married peoples have the finance to eat raw beef in the butcher's house than unmarried peoples. The results indicated that uneducated had higher prevalence than those of educated ones (OR=1.3, P=0.023). Most of the peoples in the study area were uneducated and with low level of awareness about this helminthic zoonotic disease.

The study showed that the existence of higher prevalence of cysticercosis throughout the edible organs together with deep-rooted tradition of raw meat consumption, which magnifies the public health hazards of taeniasis in the study area. Therefore, attention must be given to the revision of routine meat inspection, public awareness on improving personal and environmental hygiene.

CONFLICT OF INTERESTS

The author confirms you there is no any conflict of interest related with the research paper.

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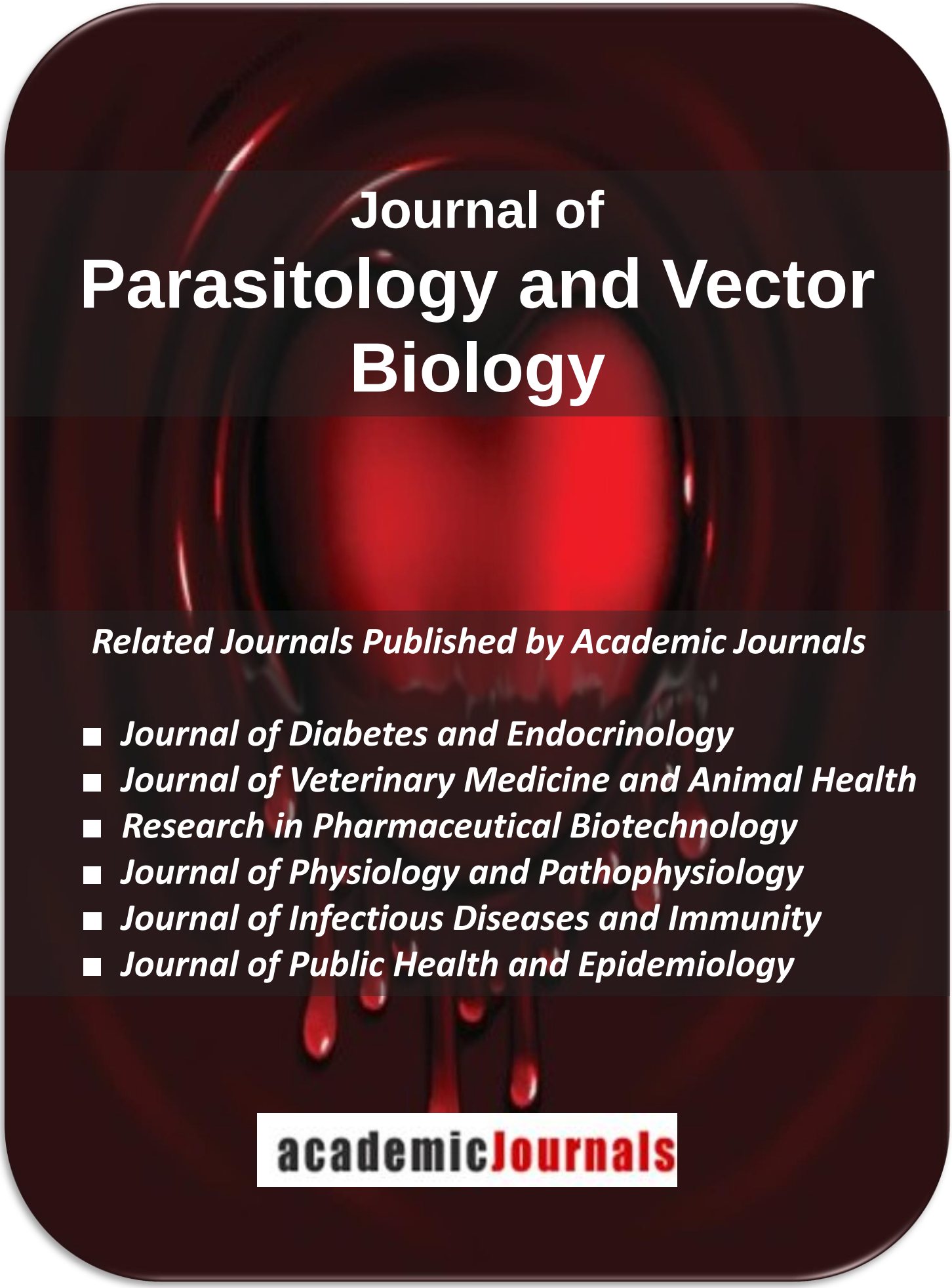

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