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Full Length Research Paper

Prevalence of bovine tuberculosis and assessment of Cattle owners' awareness on its public health implication in and around Mekelle, Northern Ethiopia

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A cross-sectional study was conducted from September, 2010 to July, 2011 on 480 cattle in and around Mekelle town, northern Ethiopia, to determine the prevalence of bovine tuberculosis (BTB) using comparative intradermal tuberculin (CIDT) test and to assess cattle owners' awareness on its public health implication using a questionnaire survey. The individual animal and herd bovine tuberculin positivity prevalence were 54/480 (11.3%) (95% CI: 8.4 to 14.1%) and 24/120 (20%) (95% CI: 12.7 to 27.3%) at cut-off > 4 mm, respectively. Cattle kept in intensive type of production (odds ratio (OR) = 3.7), in larger herds with more than 10 cattle (OR = 11.3) and under poor management condition (OR = 4.3), were more likely to be infected with bovine tuberculosis. On the basis of animal characteristics, female (OR = 4.8), exotic (OR = 6.1) and cross bred (OR = 6.6), and cattle with poor body condition (OR = 2.7) were more reactive to tuberculin test than male, Zebu breed and good body conditioned animals, respectively. Out of the 54 CIDT positive cattle, 4 were slaughtered and tuberculous lesions were detected from the organs and carcasses of those cattle. One hundred and twenty household cattle owners or members of these households were interviewed, of these only 37 (30.8%) and 18 (15%) respondents had recognized or had heard about BTB and aware of zoonotic importance of BTB, respectively. The result of this study revealed poor awareness of cattle owners on BTB and its transmission; likewise, the study suggests that the prevalence of BTB in the study area is moderate and strong tuberculoid like lesions found from CIDT tested slaughtered animals. Further molecular characterization of cattle TB isolates present in the area are warranted.

Key words: Bovine tuberculosis, cattle, comparative intradermal tuberculin (CIDT), Ethiopia.

INTRODUCTION

Cattle are considered to be the main hosts of *Mycobacterium bovis* (*M. bovis*) although isolations have been made from many other livestock and wildlife species and transmission to humans poses a public health problem (Ayele et al., 2004; Mamo et al., 2009, 2011; Office International des Epizooties (OIE), 2010; Hiko and Agga, 2011). Bovine tuberculosis (BTB) along with other diseases, become a serious problem in intensive dairy farms (Asseged et al., 2000; Radostits et al., 2007) and seriously affects the productivity of the livestock industry in developing countries (Cosivi et al., 2010; Radostitis et al., 2007).

The standard method for BTB detection in live animal is the comparative intradermal tuberculin (CIDT) test based on delayed hypersensitivity reactions. The CIDT test includes bovine and avian purified protein derivatives (PPD) and is used mainly to differentiate between animals infected with *M. bovis* and those sensitized to tuberculin due to exposure to other mycobacteria or related genera (OIE, 2010). In developing countries, the occurrence of BTB in human is widely distributed in those areas where control measures are not applied or are applied sporadically and pasteurization of milk is rarely practiced (Cosivi et al., 1998).

In Ethiopia, BTB is endemic in cattle; prevalence varies from 0.8 to 50% depending on the geographical location, breed and the husbandry practices (Berg et al., 2009; Demelash et al., 2010; Regassa et al., 2010; Tschopp et al., 2010; Firdessa et al., 2012). However, there is lack of adequate data on the epidemiology and public health implication of this disease since most of the studies conducted in Ethiopia have concentrated around the central part of the country.

As the dairy industry in Ethiopia has expanded in recent years and is expected to continue doing so, significant number of high productive exotic and cross bred animals are likely to be traded from the urban areas around the capital to the rural areas where dairy cattle numbers are still relatively low (Firdessa et al., 2012). Consequently, the communities of the study area were observed with a great tendency to own crossbred (Zebu × Holstein) and exotic (Holstein) breeds with intensive production system to implement the program. However, milk pasteurization has not been practiced in the area; as a result people residing in the study area have been consuming raw milk.

Despite the existence of potential risk factors in the study area, the occurrence of BTB has not yet been investigated.

The present study therefore was designed to determine the prevalence of BTB using CIDT test and to assess cattle owners' awareness on its public health implication in and around Mekelle town, northern Ethiopia.

MATERIALS AND METHODS

Study area and population

The study was conducted in and around Mekelle town, northern Ethiopia. Mekelle is the capital of Tigray regional state and located at 783 km north of Addis Ababa, at a latitude and longitude of 13° 29′N and 39° 28′E, respectively with an elevation of 2,084 m above sea level. Climatic condition of the area is characterized by semi-arid weather with bimodal rainfall patterns, with an average annual rainfall of 479 to 650 mm. The annual average temperature is 20.9°C, with an annual mean humidity 75.4% (Bureau of Planning and Economic Development of Tigray Region, 1998). Cattle belonging to Mekelle town and its surroundings were the study animals. Extensive and intensive types of cattle production systems practiced in the study area and the cattle breeds reared in the area are zebu, exotic (Holstein) and cross-bred (Zebu × Holstein).

Design, sample size and sampling method

A cross-sectional study was conducted from September, 2010 to July, 2011 on 480 cattle in and around Mekelle town, northern Ethiopia, to determine the prevalence of BTB using CIDT test and to assess cattle owners' awareness on its public health implication using a questionnaire survey. The sample size for tuberculin testing was calculated using the cluster sampling formula described by Bennett et al. (1991). We assumed an intra-class correlation coefficient (rho) of 0.2, an expected prevalence of 46.8% (Ameni et al., 2003) and standard error of 2.9%. The total sample size calculated for 120 households/farms was n = 480 cattle.

A list of 138 households/farms owning dairy cattle was obtained from Mekelle town Urban Agricultural unit and these households/farms were used as sampling frames. The sampling method involved was a two-stage cluster sampling where households/farms and individual animals were considered as primary and secondary units, respectively and both the primary and secondary units were selected using the simple random sampling. Four animals above 6 months of age were randomly selected per household/farm and the selected risk factors (Table 1) considered for data collection at animal and herd levels were recorded before PPD injection. Temporary unique identification numbers were given for each tested animal. Body condition scoring was done according to Nicholson and Butterworth (1986). The management of the farm was categorized as described by Ameni et al. (2003) on the basis of housing condition (neatness, waste disposal, nature of the floor, presence of confinement), feeding (concentrate plus hay), possession of an exercise yard, contact with other herds and provision with clean water.

Study methodology

Tuberculin skin testing

The CIDT test was performed using both bovine and avian mycobacterium purified protein derivative (PPD) obtained from the Veterinary Laboratories Agency, Addlestone, Surrey, UK. Two injection sites were chosen in the middle third of the side of the neck, one above the other, separated at least 12 cm. The hair was

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Table 1. Risk factors considered for data collection during tuberculin testing/PPD injection in cattle of the study area.

No.	Risk factor	Category
1	Sex	Male, female
2	Age	<2, 2≤x ≤ 5, 5<x≤ 9, > 9
3	Breed	Local, cross bred, exotic
4	Body condition score	Good, medium, poor
5	Lactation status	Lactating, Non lactating
6	Parity class	Heifer, Parity 1 and 2, Parity 3-5, Parity ≥ 6
7	Pregnancy status	Pregnant, non pregnant
8	Farming system	Intensive, extensive
9	Management condition	Good, medium, poor
10	Owners' health(TB) status	Sick with TB, Not sick with TB
11	Herd size	≤5, 6 -10, >10
12	Presence of other livestock	Present, absent
13	Presence of wildlife	Present, absent

shaved around the sites to a radius of about 2 cm. Skin folds at both sites were measured with a caliper and the measurements were recorded. An aliquot of tuberculin containing 2,500 IU/0.1 ml bovine PPD was injected into the skin intradermally at the lower injection site and similarly, tuberculin containing 2,500 IU/0.1 ml avian PPD was injected at the upper site. After 72 h, the thickness of the same skin fold at both sites was measured and recorded. Bovine and avian positive reactors were obtained using the formula: $[(Bov_{72}-Bov_0) - (Av_{72}-Av_0)]$ and $[Av_{72}-Av_0) - (Bov_{72}-Bov_0)]$, respectively. Bov_0 and Av_0 indicated skin thickness before injecting bovine and avian tuberculin, and Av_{72} and Bov_{72} were the corresponding skin fold thickness 72 h post-injection. The tuberculin test results were interpreted based on OIE (2010) recommended cutoff, > 4mm. Increase in skin fold thickness of > 4 mm was regarded as positive reactor and negative if the increase in skin thickness at the bovine site of injection was less than the increase in the skin fold thickness at the avian site of injection. Increase in skin fold thickness of > 1 mm with visible reaction at avian site than at the bovine site was considered as positive for *Mycobacterium avium* spp.

Questionnaire survey

All herd owners of tuberculin tested animals were interviewed using pre-tested structured questionnaires to assess the knowledge and awareness of the communities of the study area regarding BTB and its transmission associated with feeding habits and other factors such as education level.

Data entry and analysis

The data collected from the study areas were entered into MS excel spread sheets and analyzed using STATA (11.0) statistical software. Individual animal prevalence was defined as the number of positive reactors per 100 animals tested. The farm level prevalence was calculated as the number of herds with at least one-reactor animal per 100 herds tested. The effects of different potential risk factors were computed using univariate analysis. A statistically significant association between the result and the risk factors was said to exist if the calculated $P < 0.05$ and the 95% confidence interval (CI) for odds ratio (OR) does not include 1.

RESULTS

Herd level prevalence and risk factors

From a total of 120 households/farms tested, 24 were positive; each one exhibited at least one tuberculin reactor animal with a herd prevalence rate of 20% (95% CI: 12.7 to 27.3%). The effect of the risk factors (herd size, farming system, management conditions, owners health status, presence of other livestock and presence of wildlife nearby the farm) on the herd prevalence are presented in Table 2.

Animal level prevalence and risk factors

The prevalence of bovine tuberculin positivity at animal level was 54/480 (11.3%) (95% CI: 8.4 to 14.1). On univariate analysis, female (OR = 4.8; 95% CI: 1.5 to 15.8), exotic (OR = 6.1; 95% CI: 2.2 to 17.4) and cross-bred (OR = 6.6; 95% CI: 1.8 to 24.8) breeds and poor body condition (OR = 2.7; 95% CI: 1.2 to 5.9) cattle were five, six and three times more at risk for BTB infection than male, Zebu breed and good body conditioned animals, respectively (Table 3). Seven of the 480 cattle were positive for avian tuberculin, with a prevalence rate of 1.5% (95% CI: 0.4 to 2.5).

Necropsy findings of tuberculin reactor cattle

Out of the 54 CIDT reactor cattle, four animals (three dairy

Table 2. Univariate analyses of risk factors for CIDT test result at farm/herd level.

Risk factor	No. of herd		OR (95% CI)	P-value
	Tested	Positive (%)		
Type of farming				
Intensive	84	21 (25.0)	3.67 (1.0-13.2)	0.036
Extensive	36	3 (8.3)	1	
Herd size				
≤5	49	4 (8.2)	1	0.000
6-10	45	7 (15.6)	2.1 (0.6-7.6)	
>10	26	13 (50.0)	11.3 (3.1- 40.4)	
Owners' health status				
Sick with TB	13	5 (38.4)	2.8 (0.9-9.8)	0.078
Not sick with TB	107	19 (17.7)	1	
Management				
Good	6	1 (16.7)	1	0.030
Medium	75	5 (6.7)	0.4 (0.03-3.7)	
Poor	39	18 (46.2)	4.3 (1.5-40.2)	
Presence of other livestock				
Present	82	20 (24.4)	2.7 (0.9-8.7)	0.077
Absent	38	4 (10.5)	1	
Presence of wildlife				
Present	57	13 (22.8)	1.4 (0.57-3.4)	0.465
Absent	63	11 (17.4)	1	

cows and one bull) with strong bovine tuberculin positivity were slaughtered for postmortem examination.

After detailed postmortem examination, gross pathological tuberculous lesions were detected from organs and carcasses of all (100%) the slaughtered cattle (Figure 1).

About 57% of the lesions were found in the thoracic cavity, followed by retropharyngeal (14.3%) and mesentric (14.3%) lymph nodes (Table 4).

Cattle owner awareness on public health importance of BTB

One hundred and twenty household cattle owners or members of these households were interviewed. Of these, 37 (30.8%) of the respondents reported that they had the knowledge of or had heard about BTB and only 18 (15%) respondents became aware of zoonotic importance of BTB (Table 5). Out of the total interviewed households (120), 13 (10.8%) had TB cases in their families or farm workers and in five of the thirteen households, both PPD-positive cattle and human tuberculosis cases were found but were statistically non significant (P > 0.05) (Table 2). Moreover, the 120 household cattle owners were interviewed regarding their milk drinking and meat eating habits and house sharing with their animals (Table 6). Several demographic characteristics and other factors were considered to investigate their possible association with BTB recognition of the respondents. The awareness of the respondents regarding cattle infection with BTB and the transmission of BTB from cattle to man improved as the educational background of the respondents increased (data not shown).

DISCUSSION

In the present study, the prevalence of bovine tuberculin positivity at animal and herd levels were 11.3 and 20%, respectively. The finding was in agreement with the findings of Ameni and Erkihun (2007) who reported 11

Table 3. Univariate analysis of host risk factors for CIDT test result at animal level in cattle rose in and around Mekelle town.

Risk factor	No. (%) of animals		OR (95% CI)	P-value
	Examined	Positive		
Sex				
Male	97	3 (3.1)	1	0.004
Female	383	51 (13.3)	4.8 (1.5-15.8)	
Age (years)				
<2	52	6 (11.5)	1	0.292
2≤x≤ 5	207	26 (12.5)	1.1 (0.4-2.8)	
5<x≤ 9	120	16 (13.3)	1.2 (0.4-3.2)	
>9	101	6 (5.9)	0.5 (0.2-1.6)	
Breed				
Local	145	4 (2.7)	1	0.001
Cross bred	38	6 (15.8)	6.6 (1.8-24.8)	
Exotic	297	44 (14.8)	6.1 (2.2-17.4)	
Body condition				
Good	134	9 (6.7)	1	0.036
Medium	184	19 (10.3)	1.6 (0.7-3.7)	
Poor	162	26 (16.0)	2.7 (1.2-5.9)	
Lactation				
Lactating	212	31 (14.6)	1	0.321
Non lactating	170	19 (11.2)	0.7 (0.4-1.4)	
Pregnancy				
Pregnant	205	28 (13.6)	1	0.722
Non pregnant	177	22 (12.4)	0.9 (0.5-1.6)	
Parity class				
Heifer	118	16 (13.9)	1	0.094
Parity 1 and 2	116	9 (7.8)	0.5 (0.2-1.2)	
Parity 3-5	119	22 (18.5)	1.4 (0.7-2.8)	
Parity ≥6	32	3 (9.4)	0.6 (0.2-2.4)	

and 15% prevalence of BTB at animal and herd level, respectively in Adama town. Similarly, Regassa et al. (2010) had reported 11.6% BTB prevalence at animal level in Hawassa town and its surroundings, Southern Ethiopia. This agreement could be due to the similarity in study subjects, herd size and production systems. In the present as well as in the aforementioned studies, majority of the tested herds were smallholders found in and around urban areas with smaller herd size and with similar herd compositions. However, the herd and animal prevalence recorded in this study were lower than that of the previous study carried out by Tsegaye et al. (2010) who reported herd and individual animal bovine tuberculin positivity prevalence rates 53.6 and 34.1%, respectively. Moreover, Elias et al. (2008) has reported higher prevalence of bovine tuberculin positivity than the present study with animal and herd prevalence of 23.7 and 43.4%, respectively. This difference could be because the studies conducted by Elias et al. (2008) and Tsegaye et al. (2010) had been on large herd sizes, Holstein breeds and intensive production system.

Several studies have indicated that as herd size increases, the risk of cattle within the herd showing a positive reaction also increases (O'Reilly and Daborn, 1995;

Table 4. Distribution and frequency of tuberculous lesions in four slaughtered CIDT test positive cattle.

Infected organ	Frequency	Percent (%)
Retropharyngeal LN	2	14.3
Bronchial LN	3	21.4
Mediastinal LN	3	21.4
Lungs	1	7.1
Visceral surface of rib	1	7.1
Mesenteric LN	2	14.3
Prescapular LN	1	7.1
Supramammary LN	1	7.1
Total	14	100

LN=lymph nodes.

Table 5. Knowledge of cattle owners about BTB and its transmission to humans.

Statement	Number of interviewed	Owners who know (%)
Know BTB can affect animals	120	37 (30.8)
Know BTB is Zoonotic	120	18 (15)
Know milk is vehicle for *M. bovis*	120	18 (15.0)
Know meat is vehicle for *M. bovis*	120	14 (11.7)
Know BTB can transmit by inhalation of cough spray	120	18 (15)
Know close-contact can facilitate BTB transmission	120	4 (3.3)

Table 6. Summary on milk consumption and meat eating habits, and housing status of the respondents.

Habit of respondents	Number of respondents	Present (%)
Milk drinking		
Raw milk	11	9.2
Boiled milk	43	35.8
Both raw and boiled milk	66	55
Yoghurt milk consumption		
Consume	113	94.2
Do not consume	7	5.8
Meat eating habit		
Cooked meat	96	80.0
Both cooked and row meat	24	20.0
House sharing		
Sharing	65	54.2
Not sharing	55	45.8

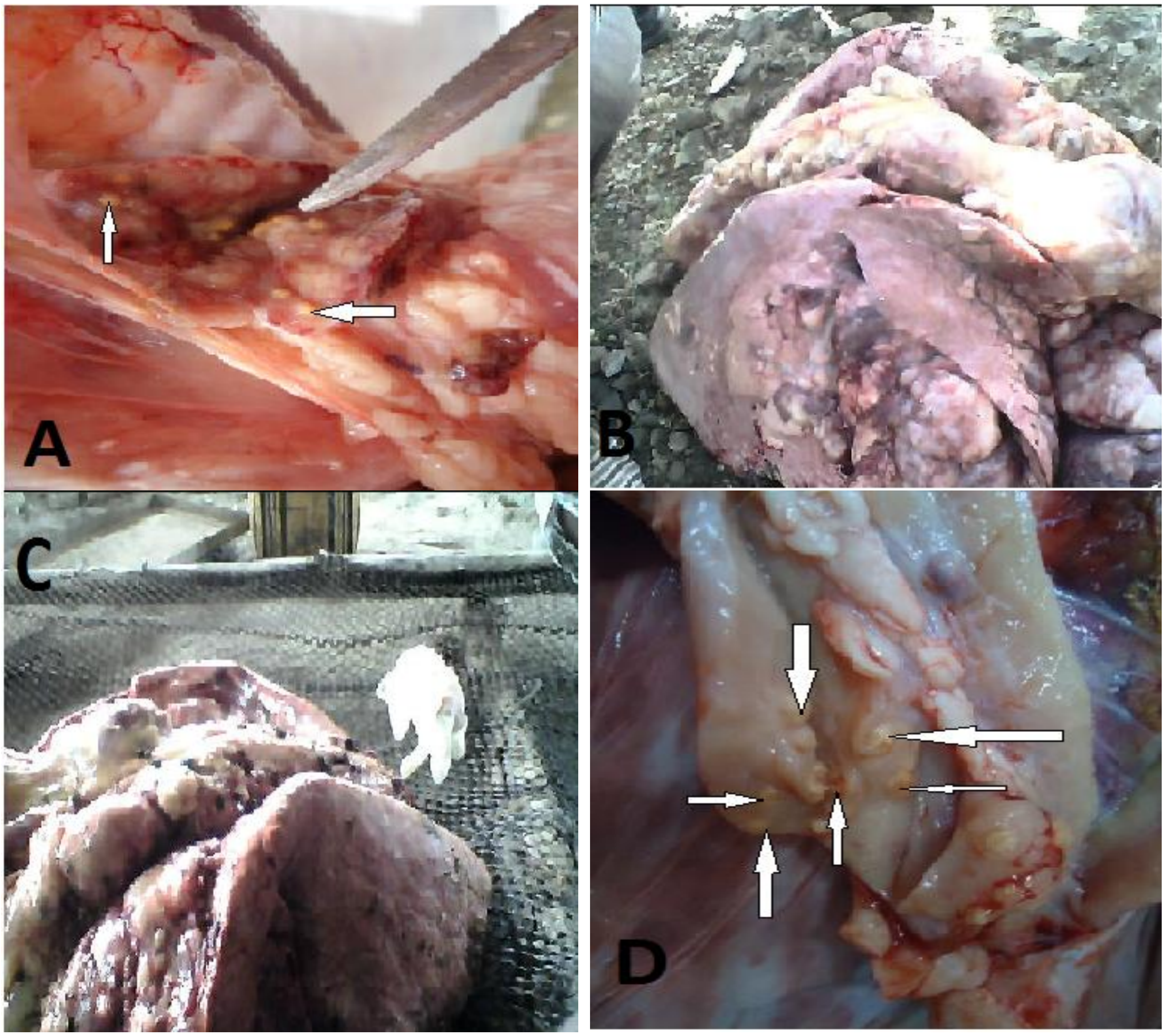

Figure 1. Tuberculous lesions in bronchial lymph nodes and Diaphragmatic view of lung in the sacrificed CIDT test reactor cattle; (A) Tuberculous granulomas in bronchial lymph node (indicated by arrow), (B) Lateral view of lung: extensively damaged with coalesced tubercle lesions and swollen associated lymph nodes, (C) Diaphragmatic view of lung; extensively damaged with coalesced tubercle lesions and swollen associated lymph nodes and (D) Mediastinal lymph node with tuberculous granuloma.

Barwinek and Taylor, 1996; Cook et al., 1996; Asseged et al., 2000). In the present study, the herd tuberculin test results showed a statistically significant association (OR = 11.3) with herd size; also, the proportion of reactors increased parallel to an increasing herd size. This finding is consistent with previous reports (Ameni and Erkihun, 2007; Elias et al., 2008; Tsegaye et al., 2010) and might be due to the risk that an individual animal may introduce tuberculosis infection into a herd and the lateral spread of infection within the herd may also be favored in large herd sizes. Moreover, this study revealed that intensive production system (OR = 3.7) had strong association with prevalence of bovine tuberculin positivity which was in line with the previous reports of Ayele et al. (2004) and Elias et al. (2008). This could be due to the fact that intensive farming system promotes close contact between animals, thereby favoring the spread of the infection.

Similarly, herd tuberculin results showed a statistically significant association with herd management conditions, signifying that poor managerial inputs increase the risk of tuberculosis (Griffin et al., 1993). Previous studies (Ameni et al., 2003; Elias et al., 2008) had similarly documented higher infection rates in farms under poor management conditions. It can therefore be generalized that the status of BTB could be improved by adopting sanitary measures that improve hygiene conditions on farms.

In this study, the prevalence of bovine tuberculin positivity had significant association with sex, as female animals (OR = 4.8) had a higher odds for tuberculin reactivity when compared to male animals, which was in agreement with the findings of Inangolet et al. (2008). This could be because cows are confined in a barn and kept long for production purpose which may facilitate infection and acquisition of the disease. Moreover, dairy cows experience greater production stress and gathering of cattle during milking increases the risk of transmission as shown by bovine TB transmission modeling in New Zealand (Barlow, 1997).

The finding of lower prevalence in Zebu breed is in line with previous reports (Kiros, 1998; Kazwala et al., 2001; Regassa et al., 2008). The reason could be that genetically improved cattle may suffer more severely from deficient housing and malnutrition and thus be more prone to infection than Zebu breeds. In contrast to earlier reports of Ameni and Erkuhin (2007) and Regassa et al. (2008), poor body conditioned (OR = 2.7) animals were more at risk of getting infected with BTB than good body condition animals. This matches to the established fact that an animal's resistance to tuberculosis is reduced by a shortage of feed and/or unbalanced diet, attributable to a deficiency of proteins, minerals and vitamins in the diet (Griffin et al., 1993). Previous studies (Collins, 1994; Elias et al., 2008) had similarly reported higher bovine tuberculin reactivity prevalence in animals with poor body condition compared to those with good body condition scores.

In agreement with the reports of Ameni et al. (2001, 2003), this study confirmed the presence of human tuberculosis case in households owned PPD reactor cattle. According to Seifert (1996), tubercle bacilli can survive in acidic milk for 15 days and in milk products such as cheese and butter for weeks. In this survey, more than half (55%) of the respondents consume both raw (butter milk) and boiled milk and 94.2% of the interviewed persons had the habit of yoghurt consumption.

The zoonotic risk of BTB is often associated with consumption (ingestion) of dairy products based on unpasteurized milk infected with *M. bovis*. Also, aerosol transmission from cattle-to-human should also be considered as a potential risk factor. Ethiopian milk consumers generally prefer raw milk (as compared to treated milk) because of its taste, availability and lower price (SNV, 2008). The disease transmission may be cyclical: cow-to-man-to-cow (Cosivi et al., 1998), underlying the existence of higher risk of dissemination of mycobacteria among the cattle and human populations.

Further, there is a need to use novel diagnostic techniques to monitor the spread of infection in large areas for developing nations (Liu et al., 2011; Cui et al., 2013; Li et al., 2014). Wadhwa et al. 2012 have described a bead based microfluidic assay for mycobacterial infections. Similarly, an ethanol votex ELISA for bovine tuberculosis in wild animals showing very high diagnostic sensitivity and specificity has been developed (Wadhwa et al., 2012). Such techniques should be tested to diagnose bovine tuberculosis in livestock. Molecular epidemiological, phylogenetic analysis and mathematical modeling should be carried out at the time of new outbreaks to understand the origin, efficacy of current vaccines and design control strategies (Kumar et al., 2014).

CONCLUSION AND RECOMMENDATION

The result of this study revealed that the communities of the study area lack awareness regarding BTB and its routes of transmission and this may allow circulation of the agent between the communities and animals. A test-and-segregation policy of tuberculin positive animals can be suggested and pursued since no compensation scheme for elimination of infected animals is currently in practice in Ethiopia.

Accordingly, government authorities should encourage farmers to regularly test their animals to keep their herds free from BTB. Likewise, milk should be strictly pasteurized before selling and consumption. In general, the communities of the study area should be aware regarding BTB and its routes of transmission. Moreover, the study suggests that the prevalence of BTB in the study area is moderate and the strong tuberculoid lesions found from CIDT tested slaughtered animals call for further molecular characterization of cattle TB isolates present in the area.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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Full Length Research Paper

Caseous lymphadenitis in goats from Borena Range Land South Ethiopia slaughtered at Luna Export Abattoir

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A study was conducted on 400 goats in Luna Export Abattoir originated from district area of Borena Range land (that is Yabello and Negella) from November, 2008 to April, 2009 to estimate the prevalence of caseous lymphadenitis (CLA) in goats. Detail post mortem examination and bacteriology were applied to undertake this study. On the bases of post mortem and bacteriological results, the prevalence of caseous lymphadenitis was 15% (60/400), out of which 42 (70%), 13 (21.7%) and 5 (8.3%) were cutaneous form, visceral form and generalized form, respectively. There is statistical significance difference (P < 0.05) among the three age groups. The study revealed that as age increases the prevalence also increases. Statistical significant difference was also recorded between goats having poor and good body condition score (P < 0.05). Moreover, goats with poor body condition score seem to be more infected (24.7%) than those goats with good body condition (12.5%). In conclusion, this study has indicated the occurrence of CLA in high frequency in goats of Borena range land. Therefore, further extensive research should be conducted over all the country to determine prevalence and economic significance of the disease.

Key words: Goats, Borena range land, Luna Export Abattoir, caseous lymphadenitis, post mortem examination, bacteriology, prevalence.

INTRODUCTION

Caseous lymphadenitis (CLA) is a chronic, recurring and highly contagious bacterial infection caused by *Corynbacterium pseudotuberculosis* which can manifest itself as cutaneous or visceral disease of sheep and goat. *Corynbacterium pseudotuberculosis* (*C. pseudotuberculosis*) produces an exotoxin, phosphlipase D, which is leukotoxic and damages endothelial cells, propagating the spread of the organisms from the site of infection to the regional lymph nodes and visceral organs/ structures. The cell wall of the bacterium is characterized by a high lipid content helping it to resist destruction by phagocytes and allowing for continued chronic infection (Nicastro, 2004).CLA is an endemic infection in regions with large sheep and goats' population (East, 1998). CLA also cause suppurativeorchitis in rams and sporadic disease in horses and cows (ulcerative lymphangitis), water buffalo, wild ruminants, primates, pigs and fowl (Merck, 1997).

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In majority of infected animals, there was no overt clinical disease or impairment of health other than visible abscessation but the disease is of considerable economic importance to sheep and goat industries (Radostits et al., 1994). Economic losses result from reduced weight gain, reproductive efficiency and wool and milk production, as well as from condemnation of carcass and devaluation of hides/skins and also from culling of infected animals (Merck, 2005). The bacterium is spread when the infected lymph nodes of a diseased animal rupture and drain, infecting naive animals that come in contact with the purulent infectious exudates. The draining exudates or pus, containing bacteria, enter through superficial skin cuts, abrasions or via mucus membranes (ingestion). Shearing, castration, docking, head butting and licking of another animal's draining abscess increase the chance of infection (Nicastro, 2004). Environmental contamination happens when the draining lymph nodes contaminate the hay, straw, shavings and/or soil. The bacteria can survive over 24 h in dips and for months in moist dark conditions, such as a shearing shed (Augustine and Renshaw, 1986).

Shearing is a risk factor for the disease in sheep as it creates skin wound but in goats, shearing is not a risk factor, other than with Angeras. The difference in abscess distribution in goats compared to sheep, with a predominance in the head, neck and sternum in goats, suggests that fomites and trauma form browse and use of common neck collars are probable risk factors (Radostits et al., 1994).

Diagnosis is based mainly on clinical signs for cutaneous form (that is, enlarged lymph nodes). Confirmation of diagnosis is through aspirates of enlarged nodes with cytology, gram stain and culture. Culture is definitive when supported by biochemical characterization of bacteria (Lloyd, 1998). Radiographs and Ultrasound may also be useful demonstrating abscesses within organ and internal lymph nodes. Internal abscess are often recognized during postmortem or slaughter (Matthews, 1999) and can be a cause of carcass condemnation (Lloyd, 1998). Serology is available for CLA. The enzyme linked immunosorbent assay (ELISA) test followed with an immunoblot analysis which is considered more sensitive and specific in goats (Laak et al., 1992).

Treatment for CLA is not curative. Once an abscess has drained, it will tend to recur. *C. pseudotuberculosis* is susceptible to penicillin; however, these antibiotics cannot penetrate the wall of abscesses. The best way to control this infection in a flock is not to treat individuals but to cull those showing clinical signs with confirmed diagnosis (East, 1998). Decreasing transmission of disease from infected to susceptible animals on farm and working to eliminate the disease from the environment are the two main goals in preventive control program. CLA is not only economically important disease but has also zoonotic importance. The disease in human is rare but well documented and it causes a suppurative glaucomatous lymphadenitis (Nicastro, 2004).

As the environment and management system in which the animal is kept plays an enormous role in the occurrence and severity of CLA infection (Radostits et al., 1994), goats in Borena area are reared largely in the hand of nomadic pastoralists where the management system is traditional (Tamrat and Asfaw, 2003). The disease is highly contagious and the causative agent can survive in feces, straw, hay and wood for several weeks (Radostits et al., 1994). On the other hand, the common behavior habit amongst goats of frequent licking, as well as of rubbing their heads and necks against fence posts and sheds, allows the rapid spread of CLA.

Recent epidemiological surveys have examined the prevalence of CLA in different countries (Al-Rawashdeh and al-Qudah, 2000; Ben et al., 2002; Connor et al., 2000). Among flocks surveyed in Australia, the average prevalence of CLA in adult sheep was 26% (Paton et al., 2003). Forty-five percent of the farmers interviewed in a study in the United Kingdom had seen abscesses in their sheep; however, this could be an overestimation of CLA prevalence since few farmers had investigated the causes of the abscesses (Binns et al., 2002). Twenty-one percent of 485 culled sheep examined in Canadian slaughterhouses had CLA (Arsenault et al., 2003). This disease remains an important subject of veterinary concern throughout the world. But, there was no study conducted on the prevalence of the disease in goats in Ethiopia. Hence, for an effective goat disease control program, it is pertinent to have a record of common diseases prevalent in an area. Slaughter houses provide excellent opportunities for detecting diseases of both economic and public health importance. Accordingly, the study was designed to determine the prevalence and occurrence of CLA in Borena goats in Luna export abattoir thereby determining lesion distribution and to generate base line data for future studies in Ethiopian goat population.

MATERIALS AND METHODS

Study area and abattoir

The study was conducted in goats slaughtered at Luna Export Abattoir. The abattoir is found at Modjo town, Lume district, East Shoa Zone of Oromia Regional State, Central Ethiopia at a distance of 70 km South East of Addis Ababa. The origin of goats slaughtered at the abattoir was from Borena pastoral area/range land in Borena zone of Oromia Regional State, South Ethiopia. Borena is found in the southern part of the country at about 570 km from Addis Ababa.

Study animals and study population

Goats originated from different districts of Borena area were sampled at Luna Export slaughter house. As there is no previous study on CLA in Ethiopia, expected prevalence (50%), precision (5%) and confidence level (95%) was used to calculate the sample size so as to determine the prevalence of the disease in the study area and the formula described by Thrusfield (2005) was used. Although, the required sample size was 384, by adding a few more

Table 1. Distribution of CLA lesions in different tissue of slaughtered goats.

Anatomical site	Number of CLA lesions	Percentage (%)
Parotid lymph nodes	20	23.2
Submandicbular lymph nodes	7	8.2
Retropharyngeal lymph nodes	7	8.2
Prescapular lymph nodes	12	13.9
Prefemoral lymph nodes	8	9.3
Inguinal lymph nodes	2	2.3
Popliteal lymph nodes	1	1.2
Lung	8	9.3
Bronchial lymph nodes	4	4.6
Mediastenal lymph nodes	7	8.2
Sternal area (Carcass)	2	2.3
Liver	1	1.2
Hepatic lymph nodes	3	3.5
Mesentric lymph nodes	4	4.6
Total	86	100

animals, a total of 400 goats were examined to increase precision.

Study design

Abattoir inspection

A cross sectional study was conducted in goats admitted to Luna Export Abattoir from Borena pastoral area. Goats were selected randomly and selected goat was first identified (given identification) using tag body condition, age and at the same time ante mortem examination was carried out. Immediately after the animal was killed, detail post mortem inspection of every organ, carcass and lymph nodes was carried out by visualization, palpation and incision.

Sample collection

Pus, several milliliters by scraping from the wall of abscess was collected aseptically using screw caps that are clearly marked with the tissue enclosed, with animal identification and the date of collection, and refrigerated at 4°C until transported to Alklilu Lemma Institute of Pathobiology. Sample collection was carried out according to the techniques recommended by Quinn et al. (2004).

Bacteriological examination

Pus from 60 infected goats was processed for laboratory isolation of the causative agent (*C. pseudotuberculosis*) and the result interpreted in accordance with Quinn et al. (2004). The pus was subjected to culture on blood agar. The cultures were then incubated at 37°C for 24 to 48 h. The colonies were differentiated based on their shape, size, color and presence of hemolysis. Pure culture was then made from these colonies using gram stain CAMP test and biochemical test (catalase test and sugar fermentation (O-F test)), the isolated bacteria were finally identified.

Data analysis

Data entry was made in Microsoft Excel Spreadsheet. Descriptive statistics was used to summarize the generated data on the prevalence. The effect of age on the occurrence of diseases was assessed by chi-square (x^2) test. A confidence level of 95% and P < 0.05 was set to interpret the statistical association.

RESULTS

Post mortem findings

In this study, a total of 400 goats were examined out of which, 60 (15%) suspicious CLA lesions were detected at post mortem examination. Of these, 42 (70%), 13 (21.7%) and 5 (8%) had cutaneous, visceral and generalized forms of the diseases, respectively. Macroscopically, the most common lesions seen in the affected lymph nodes and to lesser extent in internal organs were caseous abscess filled with greenish yellow pus. When palpated they were soft and pasty but in some findings, the pus was firm and dry on cross sectional cutting. The pus has a characteristic of laminates or “onion ring” appearance. The range and frequency of anatomical sites affected with CLA (cheesy gland lesion) is displayed in Table 1.

Overall, superficial lymph nodes were affected more frequently than visceral ones. Parotid lymph node was most affected followed by pre scapular lymph nodes. From a total of 400 goats examined, 15% (n = 60) were found to have CLA. The prevalence was 12.2% in Yabello and 17.8% in Negele districts. But there was no statistically significant difference in occurrence of CLA between the two study districts. Similarly no statistical significant difference was observed among different forms of the disease between the study areas. Table 2 presents the results of postmortem finding in goats from the two study areas.

Statistical significant difference was recorded among

Table 2. Prevalence of CLA in Goats from Yabello and Negelle districts.

Site	Number of animal inspected	Positive (%)	Cutaneous form (%)	Visceral form (%)	Generalized form (%)
Yabello	198	24 (12.2)	18 (9)	4 (2)	2 (1)
Negelle	202	36 (17.8)	24 (11.9)	9 (4.5)	3 (1.5)
Total	400	60 (15)	42 (10.5)	13 (3.3)	5 (1.3)
Chi-square (X^2)		2.548	0.544	1.886	0.183
P value		0.110	0.451	0.170	0.669

Table 3. Summary of CLA cases by age, and body condition.

Parameter	Number of animal examined	Positive	Negative	Positive (%)	X^2	P value
Total	400	60	340	15	-	-
Age						
<1 year (young)	149	7	142	4.7		
1 ½ -2 ½ years (yearling)	135	19	116	14.1	31.125	0.000
≥2 ½ years (adult)	116	34	82	29.3		
BCS						
Poor	81	20	61	5	7.482	0.006
Good	319	40	279	10		

different age groups, the highest being in adult (29.5%) followed by yearling (14.1%) and young 4.7% ($P < 0.05$). The study has also revealed a statistically significant difference in cutaneous form of the disease, the highest being in adult (22.4%) followed by yearling (9.6%) and young (2.7%). But no statistical significant association was recorded ($P > 0.05$) for visceral and generalized forms of the diseases. The highest being in adult (29.5%) followed by yearling (14.1%) and young 4.7% ($P < 0.05$). Statistical significant difference was also recorded between goats having poor and good body condition score ($P < 0.05$). In this study, goats with poor body condition score seems to be more infected (24.7%) than that of animals with good body condition (12.5%) (Table 3).

Bacteriological analysis

All 86 samples collected with CLA lesions were subjected to culture in blood agar and all culture yielding bacteria. The vast majority of colonies was small, white, dry and surrounded with narrow zone of hemolysis. Few of the colonies were from medium sized to large white mucoid. The gram stained smears from pure culture colonies have shown pleomorphic gram positive rods. Smear from the pus revealed that the rod was arranged at sharp angles to each other which look like “Chinese letters”. Zones of hemolysis interacting with the Beta hemolysis of *Staphylococcus auerous* were observed on all of the samples by CAMP test. The bacteria were grown only on the inoculated straight line which revealed that the organisms were non-motile. Upon catalase test, immediate effervescence was observed on all of the isolates which indicate the bacteria were catalase positive. During oxidation fermentation test all of the isolates utilized carbohydrate both aerobically and anaerobically which indicate that the isolated organisms were facultative anaerobes. The results of bacteriological analysis are depicted in Table 4.

DISSCUSSION

In the present study, the post mortem findings and bacteriological analysis performed have shown that the caseous lesion in the lymph nodes and other organs affected were due to CLA. 15% prevalence of caseous lymphadenitis is recorded in this study in Luna Export Slaughter house based on post mortem examination and bacteriology. This result indicates a high infection rate of CLA in goat population in Borana rangeland. This might be due to the environment and management system in which the animals are kept together which plays an enormous role in the occurrence and severity of CLA infection. The disease is highly contagious and the causative agent can survive in feces, straw, hay and wood

Table 4. Summary of Bacteriological findings.

Test conducted	Result
Colony characteristics	Small, white, dry, surrounded with narrow zone of hemolysis
Gram staining	From pure culture colonies have shown pleomorphic gram positive rods. Pus revealed that the rod arranged at sharp angles to each other which look like “Chinese letters”
CAMP test	Beta hemolysis
Catalase test	Positive
O-F test	Facultative anaerobes

for several weeks (Radostitis et al., 2007). Goats in Borana area are reared largely by nomadic pastoralists where the management system is traditional (Tamrat and Asfaw 2003). These pastoral area are featured by thorny bush encroachment which frequently cause cutaneous trauma that favor entrance of the bacteria. On the other hand, the common behavioral habit amongst goats of frequent licking as well as rubbing their heads and necks against bushes and sheds, allows the rapid spread of CLA.

Caseous lymphadenitis (CL) is characterized by abscess development in subcutaneous tissues, lymph nodes and internal organs. An American study found that 42% of culled mature sheep had abscesses compatible with CL and the most-frequently affected site (both for abscesses and CL) was the thoracic cavity (Stoops et al., 1984). In the present study, lesions were most frequently observed in superficial lymph node (66.3%) followed by lung and associated lymph nodes (22.4%). To a lesser extent, lesions were found in liver, hepatic lymph nodes, mesenteric lymph nodes and carcass on sternal area. This indicates that most CLA infections are acquired by skin abrasion and much greater proportion of affected goats having lesions in the head, related to possibly a high rate of superficial injury during browsing. The possible contribution of such browsing behavior in the thorny bushes to occurrence of CLA was previously described (Radostitis et al., 2007).

The study has shown that the cutaneous formed the diseases found in 42 (10.5%) goats which is much greater than visceral form 13 (3.3%). Out of 60 carcass of positive goats for CLA infection; 5 (1.3%) were infected with generalized form of the disease and were totally condemned. The visceral form was prevalent in respiratory tract (22.4%). This may be due to the fact that the organism could be acquired via inhalation of droplets from infected animals. Besides, the cutaneous form of CLA may spread through hemotogenous or lymphatic route which produces visceral form of the diseases (Radostitis et al., 2007).

The study revealed that no statistically significant difference ($P > 0.05$) exist between the study area. This may be due to pastoral way of keeping goat in the area which result in constant moving and mixing of goat between the two sites. However, a statistical significant association of age with caprine caseous lymphademitis infection ($P < 0.05$) was observed. Especially the cutaneous form of the diseases was significantly associated ($P < 0.05$) with age. As age increased, the prevalence of CLA was increased. This increase in prevalence of the diseases with age is due to the increasing probability of head butting, trauma from broloses, use of common collar and exposure to infected fomites (Radostitis et al., 2007). There is no statistical significant association ($P > 0.05$) of age with visceral and generalized form of CLA. There is also statistically significant difference between the goats with poor and good body condition ($P < 0.05$) in which goats with poor body condition are more infected by the disease condition than the ones with good body condition.

CONCLUSION AND RECOMMENDATION

Prevalence of CLA recorded in this study was based on post mortem examination and bacteriology. The result has indicated the presence of high infection (prevalence) of CLA in the animal/ goats population of Borena range land. Most of the CLA lesions were detected in lymph node head, shoulder and thigh area that signify cutaneous route of transmission. Detection of CLA by bacteriologic examination was successfully conducted. Therefore, caseous lymphademitis should be considered as serious diseases because of its impact on the economy and health of goat and sheep.

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Conflict of Interest

The authors declare that they have no conflict of interests.

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Full Length Research Paper

Outbreaks of Peste des petits ruminants in two different localities in Sudan

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This report describes the details of outbreaks of Peste des petits ruminants (PPR) which occurred during 2008 in Khartoum and River Nile States in Sudan. An outbreak was reported in a sheep flock at the southern part of the State (Soba) in Khartoum, with 100% morbidity and mortality rates. In ElDamer city at River Nile State, the morbidity rate was 31.4% while mortality rate was 17.1% in the total animal population of 3,500. PPR antigen was detected in seven samples from both outbreaks using immunocapture enzyme-linked immunosorbent assay (IcELISA). The PPR ELISA results were confirmed by reverse transcription polymerase chain reaction (RT-PCR). Using cELISA, only one serum was sent from Khartoum and 6 out of 13 sera from ElDamer were positive for PPR antibodies.

Key words: Peste des petits ruminants (PPR), outbreaks, sheep, Sudan.

INTRODUCTION

Peste des petits ruminants (PPR) disease is caused by a virus that belongs to the genus Morbillivirus of the family paramyxoviridae (Murphy et al., 1999). The disease is characterized by onset of depression, fever, discharges from the eyes and nose, sores in the mouth, disturbed breathing, cough, foul smelling diarrhoea and death (Roeder and Obi, 1999). PPR was first reported in West Africa and is now known to be reported in different Asian countries including Oman (Furley et al., 1987), Lebonon (Lefevre et al., 1991) and India (Shaila et al., 1989).

In Sudan, El Hag Ali and Taylor (1984) reported the first outbreak of PPR. Since then many outbreaks of PPR in Sudan had been reported; in Darfur State (Rasheed, 1992), El Hilalia area of Central Sudan (Hassan et al., 1994), in Khartoum (Zeidan, 1994). Intisar (2002) reported the antigenic prevalence of PPR in Khartoum, Gezira, River Nile, Kordofan and White Nile states. This report describes the occurrence of PPR outbreaks in Khartoum and River Nile State which was until recently considered as PPR free zone.

MATERIALS AND METHODS

Area of outbreaks

They include Soba city at Khartoum and ElDamer city at River Nile states, the former is the capital of Sudan and the second is bordering Khartoum to the North.

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Table 1. Detection of PPR antibodies in sheep sera collected during PPR outbreaks in Khartoum and River Nile States, Sudan during February 2008.

Area	Number tested	Number positive	Number negative	Percentage positive
Khartoum	1	1	0	100
River Nile (El Damer)	13	6	7	46.1
Total	14	7	7	50

Description of the outbreaks

In Soba area at the south of Khartoum State during the last week of January, 2008, a total of 20 sheep aged 9 to 15 months were brought to a farm for breeding, a week later, respiratory signs, diarrhoea and weakness appeared and within 2 weeks all the 20 animals died. On post mortem (PM), tissue samples (lung, spleen, lymph nodes) were collected and serum were collected from the last surviving ones immediately before death and sent to the Central Veterinary Research Laboratory for diagnosis. In ElDamer city at River Nile State during April, 2008, a flock of sheep (n = 3500) showed cases of diarrhoea, off food, respiratory signs and death. The affected ones were 1,098 (31.4% morbidity rate) and dead ones were 600 (17.1% mortality rate); the case fatality rate was 54.6%. Tissue samples (lung, spleen, lymph nodes of 6 animals) and 13 sera were collected and sent to the Central Veterinary Research Laboratory at Khartoum for examination. In both outbreaks, signs of pneumonia and gastroenteritis were observed on post-mortem examination. All animals in both outbreaks were not previously vaccinated against PPR.

Detection of PPR antigen

A total of 21 samples from both outbreaks were tested for PPR antigen using immunocapture enzyme-linked immunosorbent assay (IcELISA) as per the instructions of the manufacturer (CIRAD EMVT, Montpellier, France, distributed by BDSL, UK). It is a solid phase immunocapture ELISA (ICE) based on a technique described by Libeau et al. (1994).

Detection of PPR using RT-PCR

To confirm the results detected by IcELISA, clear PPR positive samples from both outbreaks (n = 7) were examined for molecular based confirmation by using RT-PCR. RNA was extracted using QIAGENE RNA extraction Kit, the procedure was applied as per the procedure supplied by the manufacturer. The PPRV nucleic acid was detected in the clinical tissue material by RT-PCR using two sets of primers following the method described by Kwiatek et al. (2007). Reagents used for PCR were obtained from Invitrogen, Germany. First the Pan-morbillivirus primers located in the middle of the gene (Nad1 and Nad2) allowed the amplification of the nucleoprotein (N) gene of all morbilliviruses and gave a product of 222 bases. The second pair of primers (NP3 and NP4) designed by Couacy-Hyamann et al. (2002) on highly conserved sequence of PPRV N gene gave a 351 bp product amplification. Amplification was done on a thermal cycler following the described conditions: reverse transcription 30 min at 50°C, initial PCR activation during 15 min at 95°C then 40 cycles of amplification corresponding to 30 s at 94°C/30 s at 60°C/1 min at 72°C and final extension during 10 min at 72°C.

Detection of PPR antibody

Sera collected during both outbreaks (n = 14) were tested for PPR antibodies using cELISA manufactured by CIRAD EMVT, Montpellier, France, distributed by BDSL, UK. The test is based on the competition between antibodies in sera and monoclonal antibody (MAb) to bind to the antigen (Libeau et al., 1995).

RESULTS

Detection of PPR antigen

PPR antigen was detected using IcELISA in 7 samples (lungs) of the two outbreaks.

Detection of PPR by molecular assay RT PCR

PPR was confirmed by RT PCR assay in all the seven lung samples positive for PPR antigen during the outbreaks (Figure 1).

Detection of PPR antibodies

Using cELISA, PPR antibodies were detected in 7 of 14 sera, the details are presented in Table 1.

DISCUSSION

PPR is known to be one of the most serious viral diseases affecting small ruminants; continuous outbreaks of the disease are reported in many countries. PPR has been existing in Sudan since the first reported outbreak in 1971 (El Hag, 1973) which was first diagnosed as rinderpest and later confirmed to be PPR (El Hag Ali and Taylor, 1984). During the last two decades, PPR was reported in different localities in Sudan; in sheep in Western Sudan (Rasheed, 1992); in sheep and goats in Central Sudan (Hassan et al., 1994) and in sheep and goats in Khartoum State (Zeidan, 1994; El Amin and Hassan, 1998). Until recently, River Nile State was known to be a PPR free area, however the disease was detected and the virus was isolated from River Nile State as well as from

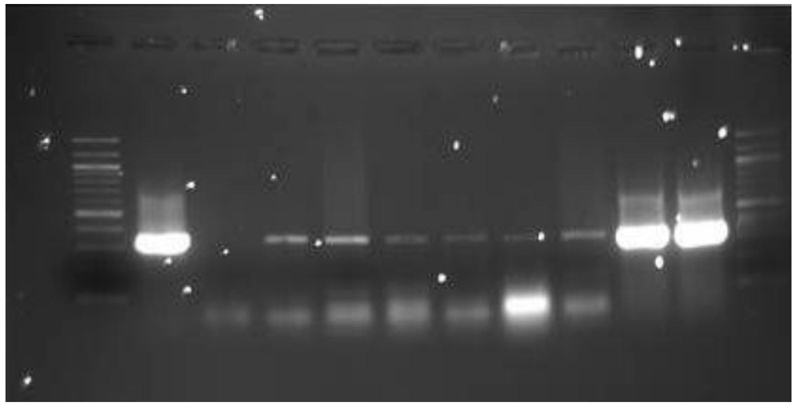

Figure 1. Detection of PPRV using PCR. Amplification is generated by primers NP3 and NP4 designed by Couacy Hymann et al. (2002) on highly conserved sequence of PPRV N gene giving a PCR product of 351 bp.
Lanes 1 and 12: DNA molecular weight marker (#100 bp ladder, Eurobio); Lanes 2, 10 and 11: Positive control (PPR 75-1 vaccine strain); Lane 3: Negative control; Lane 4: sample No 1 (Khartoum); Lanes 5 to 9, samples No 2 to 6 (ElDamer).

from different parts of Sudan [Gezira, White Nile (Central), Kordofan (Western), Khartoum] between 2000 and 2002 (Intisar, 2002).

PPR which was restricted to African countries have spread to Asia. Kwiatek et al. (2007) described an outbreak of PPR in 3 districts in Tajikstan. Similarly, Ahmad et al. (2005) reported an outbreak of PPR in goat flock in Pakistan. By using cELISA they found 35 sera tested for PPR antibodies to be positive. A severe outbreak of PPR was reported in 70 adult sheep and goats in Al-Hasa province of Saudi Arabia. Thirty out of seventy animals in the herd were affected (43% morbidity rate). The case mortality rate was 100% (Housawi et al., 2004); this picture is close to that seen in River Nile State in this report but less severe than that noticed in Khartoum. In Africa, During August, 2008 an outbreak of PPR was reported in Morocco; the outbreak has largely affected sheep, with 133 outbreaks in 29 provinces (FAO, 2008). In Ethiopia, PPR antibody seroprevalence was 3% in camels, 9% in cattle, 9% in goats and 13% in sheep (Abraham et al., 2005), although Ethiopia is bordering Sudan, this is considered a very low prevalence compared to our results.

The reported outbreaks in this study were the most sever ones in Sudan, especially in Khartoum, in which the morbidity and mortality rates were 100%, this is far higher than the previous reports in Sudan and is comparable to the previous report by Nussieba et al. (2008) who detected PPR antigen in 92.5% of 40 tissue samples of sheep in the Sudan during an active outbreaks, and most of samples (n = 32) were from the same flock at Khartoum. This report points to the existence of virulent PPR virus in Khartoum and River Nile States; the characterization and sequence analysis of PPR virus of the two outbreaks revealed the existence of Lineage IV PPR for the first time in Sudan (Kwiatek et al., 2011).

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Our thanks are expressed to our colleagues at River Nile State and Pathology Department of Central Veterinary Research Laboratory for sample collection.

Conflict of Interest

The authors declare that they have no conflict of interests.

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Full Length Research Paper

Rabies in animals and humans in and around Addis Ababa, the capital city of Ethiopia: A retrospective and questionnaire based study

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Reliable data is required on diseases like rabies by policy makers and professionals. This study was therefore aimed at assessing the situation of rabies in and around Addis Ababa using retrospective data obtained during 2008 to 2011. Besides, a questionnaire was used to generate information on factors associated with the occurrence of rabies. A total of 935 brain samples from different species of animals were examined for rabies virus antigen during 2008 to 2011, of which 77.6% (n = 726) of them were tested positive. The highest proportion, 87.2% (n = 633) of the positive cases, was recorded in dogs followed by cats, 5.1% (n = 37). Between the years 2008 and 2011, a total of 1,088 dogs were examined for rabies, of which 801 (73.62%) were confirmed to be rabid. The proportion of rabid female dogs (87.5%) was higher than that of males (73.44%), and dogs 3 to 12 months old were diagnosed with rabies more frequently (76.6%) than dogs belonging to other age category. The highest proportion of rabid dogs was recorded in dogs whose ownership was not known followed by ownerless dogs. Rabies cases were confirmed both in vaccinated and non-vaccinated dogs. The number of confirmed rabies cases was higher during September and lower during November. Significant variation was seen among years in occurrence of rabies. The study shows that the principal vector of rabies in Addis Ababa and its surroundings, but most likely in entire Ethiopia, is the dog. Effective rabies management and control based on confirmed cases is recommended.

Key words: Dog, Ethiopia, post-exposure prophylaxis, rabies.

INTRODUCTION

Rabies is a deadly zoonotic disease with world-wide occurrence and is transmitted mostly by carnivores to humans and livestock. It is known to cause large number of deaths in humans and animals each year. It has been responsible for estimated annual human mortalities of 31,000 and 24,000 in Asia and Africa, respectively, with

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people mostly at risk of dying due to rabies being those who live in rural areas of these continents (Knobel et al., 2005). Deaths due to rabies occur despite the availability of effective vaccines which can prevent the development of fatal rabies cases (World Health Organization (WHO), 2005). In Ethiopia, rabies remains to be one of the most feared infectious diseases and has been diagnosed for several years at the Ethiopian Health and Nutrition Research Institute (Fekadu, 1972). However, systematic recording and organization of the results of diagnosis was rarely carried out. Available data during the years 2001 to 2009 at the institute showed that 35 to 58 annual human deaths were recorded (Deressa et al., 2010) mostly in Addis Ababa, the capital city of the country.

Previous reports showed that dogs were the dominant species responsible for transmission of rabies virus to humans and livestock in the country. The demographic characteristics of dogs biting humans and livestock have not been fully elucidated. Besides, the rabies status of dogs biting humans has not been known. It has been a common practice to provide post-exposure vaccines to humans bitten by dogs irrespective of their rabies status. This increases the risk of complications associated with the Fermi type vaccines, as this vaccine accounts for 88% of the vaccines used throughout. The Fermi type vaccine is produced at the Ethiopian Health and Nutrition Research Institute. Imported cell culture vaccines are used in 12% of the cases. No immunoglobulin has been available for use in treating rabies in the country to our knowledge. During the 2008 to 2011 fiscal years, the administration of the capital city has launched spraying of female dogs, vaccination of owned dogs, killing of stray dogs and public awareness, using the available mass media. Besides, the expanding construction in the city is thought to reduce the number of stray dogs. Therefore, analysis of retrospective data and current information on rabies in animals and humans with special focus on the capital city, Addis Ababa, is important to understand the epidemiological situation of rabies. This will be crucial for effective planning of rabies management, prevention or control programs. In this study we investigated the prevalence of confirmed rabies cases during the years 2008 to 2011. In addition, we identified the demographic characteristics of animals responsible for human rabies and tried to quantify the number of humans exposed to rabid animals.

MATERIALS AND METHODS

Study areas

The samples diagnosed at the Ethiopian Health and Nutrition Research Institute orginated from various parts of the country but the majority of the samples were from the capital city, Addis Ababa and its vicinity. Therefore, the focus of this study was Addis Ababa and areas within about 100 km radius of the city. The city covers an area of 530 km^2 and is divided into 10 sub-cities. Laboratory work was done at Ethiopian Health and Nutrition Research Institute, which is the only rabies diagnostic center in the country. The study area is depicted in Figure 1. The dog population in the city is estimated to be 250,000 to 350,000 (UAECP, 2011).

Study design

Retrospective data was collected from record books of rabies in animals and humans at the Ethiopian Health and Nutrition Research Institute. The data on rabies cases in animals covered the period from September, 2008 to February, 2011. The brains of animals biting humans have been submitted to the diagnostic laboratory by the public and government veterinarians for confirmation of rabies. The laboratory utilizes the fluorescent antibody test (FAT) to confirm the presence of rabies virus in the samples. Those dogs that were available for quarantine or killed after bite were tested. The data on rabies in humans was a one year data from September, 2010 to September, 2011. Recorded information on rabies including species of animal, sex, age, vaccination status and the type of vaccine used in humans were obtained from the records when available.

A questionnaire was developed and used to collect data during the period from March to September, 2011 in addition to the retrospective data. Among those individuals who submitted the samples, volunteers were interviewed. They were briefed about the purpose of the study and asked for their consent before the interview commenced. The questionnaire was carried out by interviewing individuals who submitted samples for rabies diagnosis. Whenever additional information was required, the owner of the animals or anyone who had enough information about the case was asked. The questionnaire includes information like dog ownership and number of humans exposed to suspected or confirmed cases. The dog ownership was classified into three groups. Owned and restricted dogs: those dogs that were completely under the control of their owners. They were fully restricted to owners' premises. Owned and roaming: this group were owned but let to roam for varying periods of time in a day. Unknown ownership/ownerless: these were dogs to which no one is claimed as owner; they roamed freely and were known by the community in the area. Responses about the dog ownership were cross-checked from the victims who were exposed to the animals if they know about the ownership of the dog before the exposure. Rabies vaccination status was checked in the vaccination certificate.

Statistical analysis

The data collected were subjected to statistical package for social sciences (SPSS) version 20 for analysis. Descriptive methods such as chi-squared tests were used to test the presence of association among categorical variables such as occurrence of rabies and location. Logistic regression analysis was used to identify predictor of rabies.

RESULTS

Rabies in animals

A total of 935 brain samples from different animal species

Table 1. Demographic characteristics of rabid dogs diagnosed at Ethiopian Health and Nutrition Research Institute, Addis Ababa during the years 2008 to 2011.

Characteristic	No. tested	No. positive	Percent
Sex			
Male	241	177	73.44
Female	72	63	87.5
Sub total	313	240	76.7
Age			
≤ 3months	19	11	57.9
>3≤ 12 months	47	36	76.6
>12 months	126	91	72.22
Sub total	192	138	71.8
Dog ownership			
Owned and restricted	81	52	64.19
Owned and roaming	28	24	85.71
Ownership unknown/ Ownerless	30	29	96.67
Sub total	139	105	75.54
Vaccination status			
unvaccinated	428	310	73.43
vaccinated	16	8	50
Sub total	444	318	71.62

were examined for rabies virus during 2008 to 2011, of which 726 (77.6%) were found to be positive. Rabies virus was more frequently detected in brain samples from carnivores (94.5%) than other animal species. The proportion of rabid dogs that were positive for the virus during this time period was 87.2%. This was followed by cats (5.1%) while the proportion of rabies cases in other domestic animal species was 5.8%. Only 2.1% of the rabies cases diagnosed were attributed to wild animals.

The demographic characteristics of rabid dogs

Since dogs remain the most important animal species diagnosed with rabies, further analysis of demographic characteristics of rabid dogs including sex, age and ownership and vaccination status was carried out (Table 1). During the years 2008 and 2011, 87.19% of the dogs examined were confirmed to be rabid. The proportion of rabid female dogs (87.5%) was higher than that of males (73.44%) and dogs 3 to 12 months old were diagnosed with rabies more frequently (76.6%) than dogs belonging to other age category. The majority of rabies cases were diagnosed in dogs whose ownership was not known or which were ownerless. The proportion of dogs diagnosed with rabies was 96.67%% in dogs categorized into this group. Rabies was diagnosed in both vaccinated and unvaccinated dogs but a higher proportion was observed in unvaccinated dogs.

Spatio- temporal distribution of rabies

During the years 2008 to 2011, a total of 881 brain samples were tested for rabies virus from various regions of the country of which 687 (78%) were from Addis Ababa, 169 (9.2%) were from Oromia and 17 (1.9%) from Southern Nations, Nationalities and People’s region. Eight samples (0.9%) originated from Amhara, Tigray and Somali regions. None were submitted from the remaining administrative regions. In Addis Ababa, the number of confirmed rabies caes ranged from 34 in Lideta to 140 in Kolfe Karanyo (Figure 2). When the distribution of confirmed rabies cases in the vicinity of Addis Ababa was considered, the highest was from Oromia special zone followed by east Shewa zone (Figure 3). Rabies cases were diagnosed throughout the year even though slight variations in the distribution of confirmed cases were being in September and the lowest being in November (Figure 4). However, the variation in the confirmed rabies

Table 2. Species of animals responsible for PEP in humans in and around Addia Ababa, Ethiopia during 2010/2011 (n = 2337).

Species	No. of humans exposed	Percent
Dog	2046	87.54
Humans	93	3.98
Cats	61	2.60
Bovines	55	2.35
Donkeys	35	1.49
Monkeys	15	0.64
Foxes	12	0.51
Hyenas	11	0.47
Horses	3	0.12
Goats	2	0.10
Sheep	2	0.10
Cheetah	2	0.10

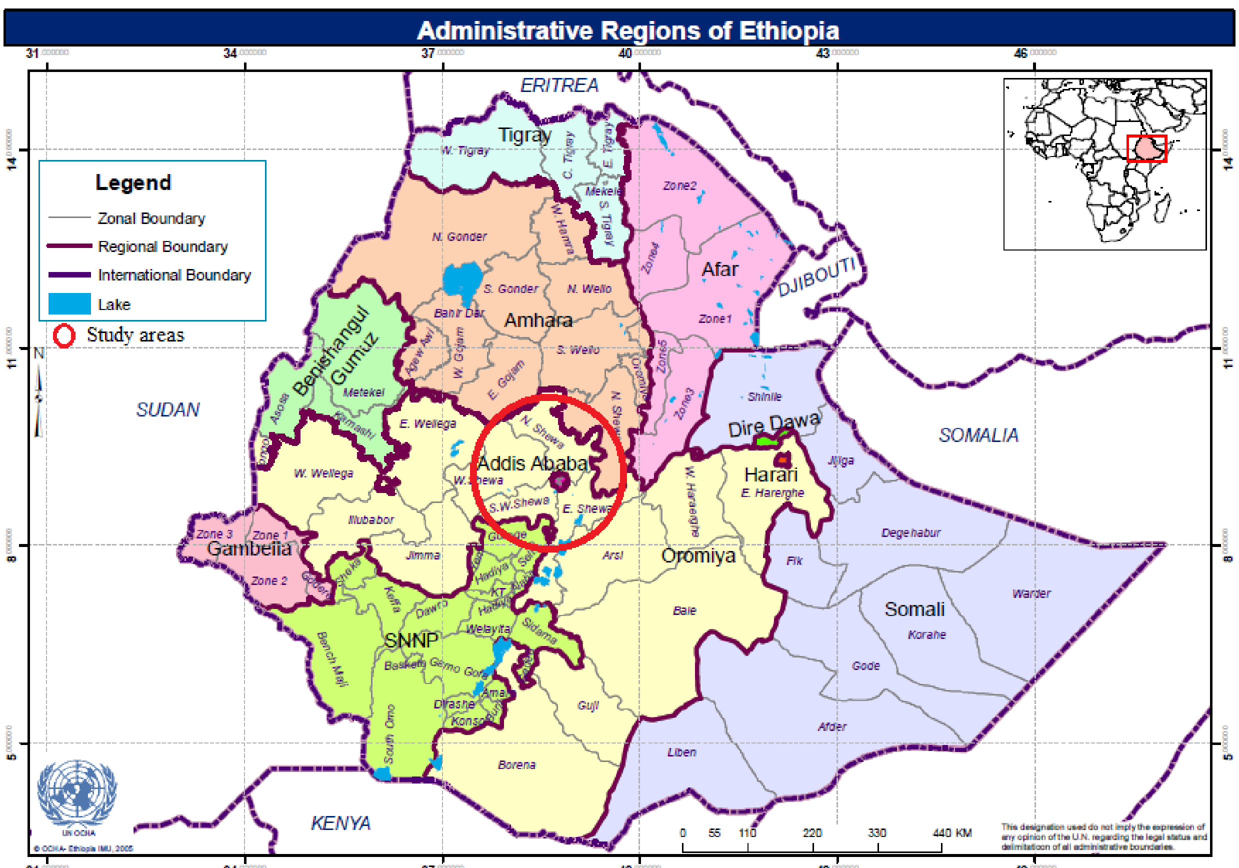

Figure 1. Map of Ethiopia showing the study area.

cases among months was only marginally statistically significant ($p = 0.05$). Statistically significant difference ($p = 0.03$) was observed in the distribution of confirmed rabies cases among the three years (Figure 5).

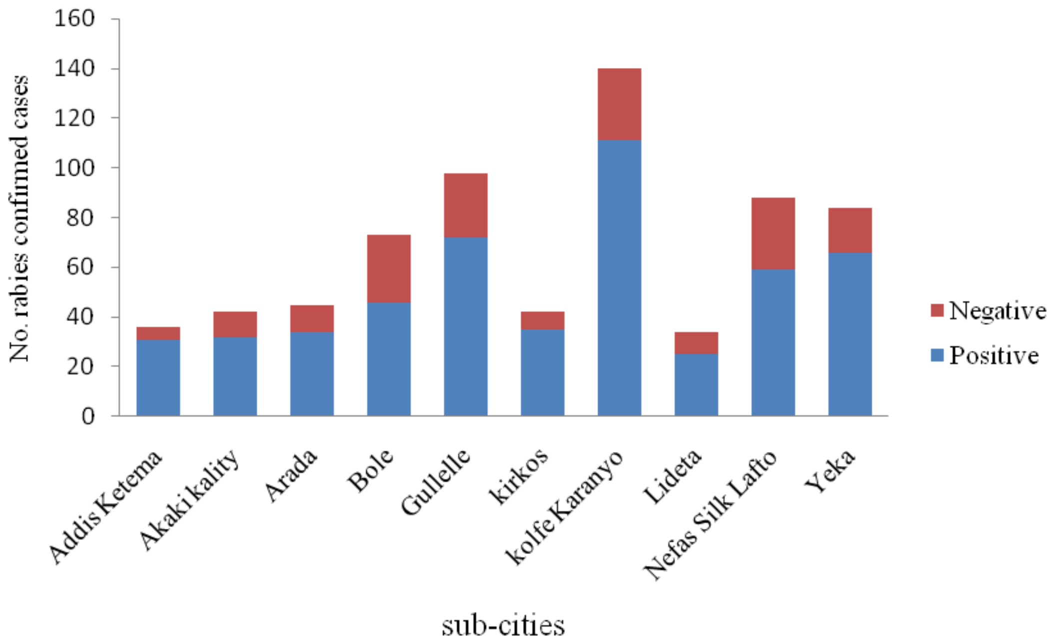

Figure 2. Spatial distribution of rabies confirmed cases in Addis Ababa sub-cities, Ethiopia during 2008 to 2011.

Administration of post-exposure prophylaxis in humans

Retrospective data showed that post-exposure prophylaxis (PEP) was provided to humans following a bite incident irrespective of the rabies status of the biting animals. A total of 2,337 people received post-exposure prophylaxis in 2010/2011 against rabies as a result of exposure to rabid or rabies suspected animals and humans. The greater proportion of post-exposure prophylaxis was given to humans exposed to rabid or rabies suspected dogs. The majority, 72.3% (1653/2286), of the post-exposure prophylaxis was given to humans bitten by animals with an unknown rabies status. Some of the PEP vaccines were prescribed while the dogs were under quarantine and observation for the development of clinical rabies. The majority of the PEP (78%) was given to humans bitten by animals in which their rabies status was unknown while 28% (633/2286) of the PEP was given to humans bitten by animals in which rabies was confirmed (Figure 6). During the year 2010 to 2011, 2,337 people were provided with PEP after being bitten by different species of animals and humans suspected or confirmed to be rabid (Table 2). The most important animal species responsible for PEP was dogs. Post-exposure prophylaxis due to exposure to suspected humans was considerable while other domestic and wild animals were responsible for the PEP to a lesser extent. Nerve tissue vaccine (NTV) produced from rabies virus infected brain of sheep was the most common (81%) vaccine type used for PEP in and around Addis Ababa. This vaccine is locally produced at Ethiopian Health and Nutrition Research Institute. The use of cell culture vaccine for PEP was limited (19%). No immunoglobulin has been available for use in treating rabies in the country to our knowledge. Active human rabies search during the period of March to September, 2011 was carried out at the Ethiopian Health and Nutrition Research Institute. The result showed that a total of 271 persons were recorded to be exposed to 95 rabid animals. Two hundred fifty seven (94.83 %) of them were exposed to 88 rabid dogs, 9 (3.32 %) persons were exposed to 2 rabid bovines, 4 (1.48 %) persons were exposed to 4 rabid cats and 1 person was bitten by a rabid donkey. In general, the ratio of humans exposed to rabid animals was 2.85.

DISCUSSION

The overall 77.6% prevalence of rabies virus in the samples submitted from geographically limited areas by few people who have awareness showed that rabies is wide spread in the country. If submission of all potential rabies cases were made, the number of rabies cases could even be higher than this. Hence, rabies remains endemic in Central Ethiopia and represents a serious

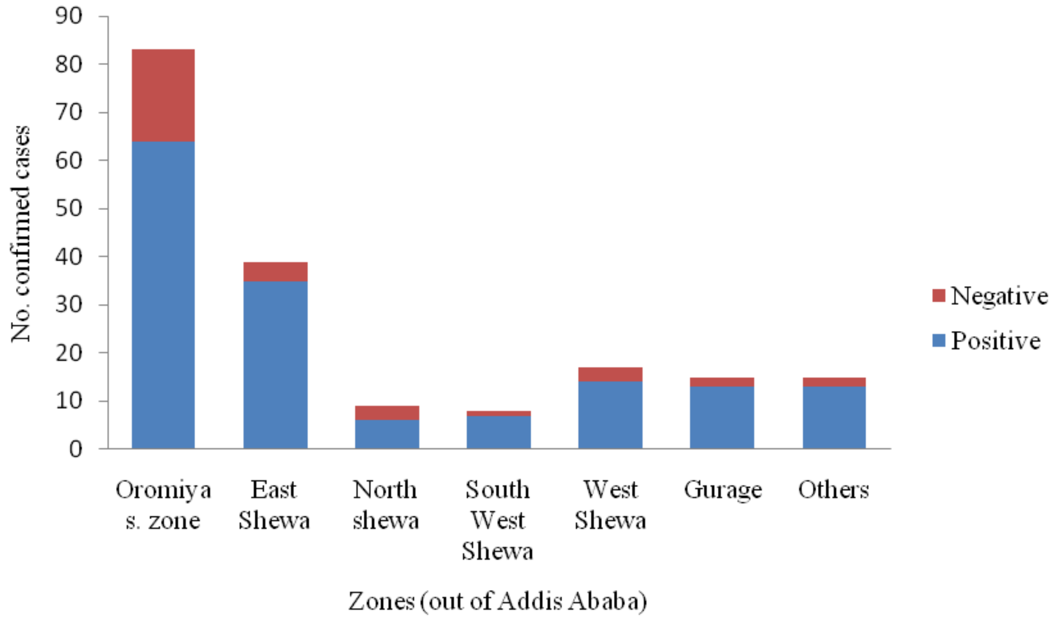

Figure 3. Distribution of confirmed rabies cases in areas surrounding Addis Ababa, Ethiopia during 2008-2011

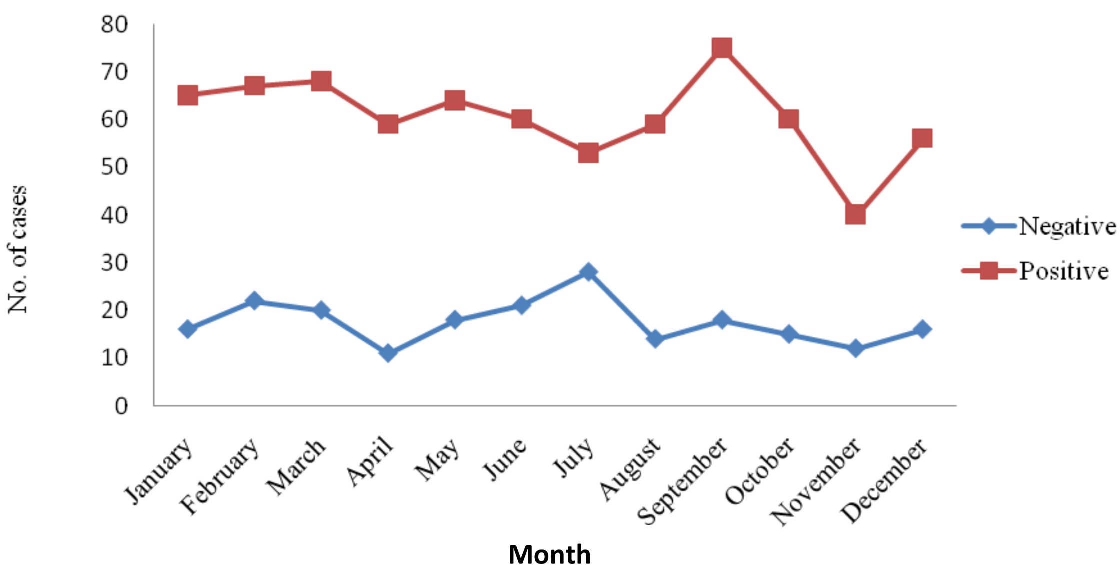

Figure 4. Distribution of rabies confirmed cases by months in Ethiopia during 2008 to 2011.

veterinary and public health problem. Domestic carnivores were the principal animals responsible for maintenance and transmission of rabies in the area even though several animal species were involed. This shows that no emphasis has been given to rabies by the relevant veterinary, medical and public authorities in contrast to European and American countries where rabies due to domestic carnivores has been controlled and wildlife rabies is a problem (Warrell, 2004; Blanton et al., 2010).

Similar to the finding in this study, Jemberu et al. (2013)

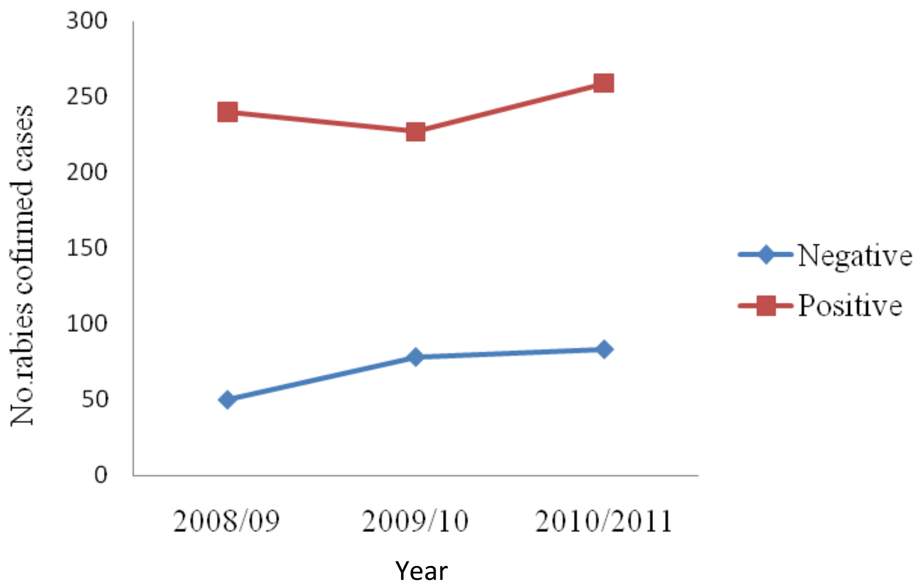

Figure 5. Occurrence of confirmed rabies cases by years in Ethiopia during 2008 to 2011.

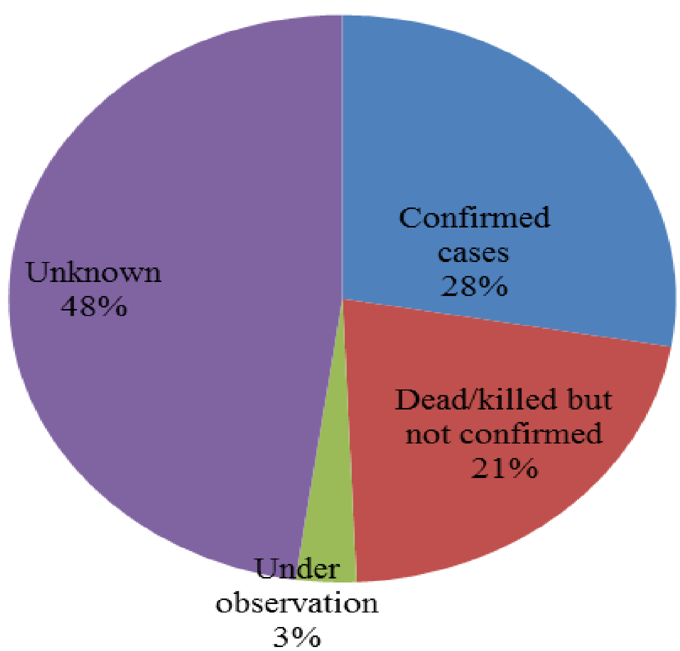

Figure 6. Proportion of people receiving PEP in relation to the rabies status of biting animals in and around Addis Ababa, Ethiopia during the year 2010/2011.

(2013) reported the preponderant occurrence of rabies in dogs than other animals. The involvement of herbivores and humans to limited extent in the transmission of rabies to humans was also made by these authors. In contrary to the results of this study, Jemberu et al. (2013) based their study on clinical observation without laboratory confirmation. The findings of this study are in consent with reports from other African countries in which 80 to 95% of rabies cases were due to dogs (Edelsten, 1995; Kitala et al*.,* 2000; Tefera et al., 2002; WHO, 2005). The proportion of confirmed rabies cases reported in this study is higher than the reports of Kretzmann (1993), Dlamini (1995), Kitala and McDermott (1997) and Kayali et al. (2003) from various African countries. However it is lower than the reports of Dürr et al. (2008).

Few confirmed rabies cases were observed in wildlife and domestic animals other than carnivores. This makes the role of wildlife in the maintenance and transmission of rabies to be elusive under Ethiopian conditions. This is due to the fact that most of the samples submitted to the diagnostic laboratory were from the capital city and its surroundings where the wildlife population is low. People also traditionally link rabies with dogs and rarely submit brain samples from other animal species including wild-life. This underestimates the potential role of wildlife in the transmission of rabies but wildlife transmitted rabies has been documented in Southern Africa (WHO, 2005). Rabies transmitted by wildlife species could be a future challenge in the country when rabies transmitted by domestic carnivores is controlled.

The number of samples examined from male dogs was higher than that from female ones but higher proportion of samples from female dogs were diagnosed with rabies virus than those from male dogs. The higher proportion in female dogs could be due to the possible contact of few females by several males, especially during estrus and the probability of being bitten by several dogs. But our finding is in contrary to the previous reports made else-where in the world (Brooks, 1990; Eng et al*.,* 1993; Kayali et al*.,* 2003*,* Dürr et al., 2008). Larger proportion of dogs in all age groups examined was positive for rabies virus but rabies cases were more prevalent in dogs older than 3 months. Dogs older than 3 months usually wander outside the home compound and have chances of being bitten by rabid dogs. The lower incidence of rabies among younger dogs could also be due to protection by maternal antibodies to some extent if the dam is vaccina-ted. In consent to our observation, Foggin (1988), Brooks (1990) and Kayali et al. (2003) reported higher rabies cases in dogs older than 3 months while correspondingly lower proportion of dogs younger than 3 months were found to be rabid elswhere in Africa. The confirmation of rabies in all age groups, however, shows the neccessity of including dogs of all age group during vaccination campaign. Exclusion of puppies from the campaigns has been suggested to reduce the level of herd immunity and could culminate in rabies cases during the interval between campaigns (Sudarshan et al., 2006; Kammer and Ertl, 2002).

Both vaccinated and non-vaccinated dogs were diagnosed with rabies even though the proportion of rabid dogs was higher in non-vaccinated ones. The detection of rabies virus in brain samples from vaccinated dogs could be due to vaccine failure. There has been frequent power disruption, lack of cold chain during transportation of vaccines and lack of storage facilities in the country. Thus, administration of the product does not necessarily guarantee the desired protection. Therefore vaccine failure as a result of miss-handling and inappropriate storage could occur frequently.

The existence of Lagos bat virus in dogs (Mebatsion et al., 1992) in the country could be another reason for the occurrence of rabies in vaccinated dogs as vaccination is not protective against this genotype. Highest proportion of confirmed rabies cases was observed in dogs whose ownership was not known or in ownerless dogs. Owned dogs that roam freely were also more prone to rabies than owned and restricted dogs. The higher proportion of rabies in dogs to which no owner is attributed is due to absence of vaccination and restriction in this group of dogs. Their free roaming nature can easily expose them to rabid animals so that they can be rabid more frequently. Our result is in agreement with the previous reports of Foggin (1988) and Kitala and McDermott (1997). In contrary to our observation, Dürr et al*.* (2008) and Kayali et al. (2003) reported higher proportion of ra-bies cases in owned dogs than ownerless ones in Chad.

Slight fluctuation in the occurrence of rabies was observed among the months of the year. The highest incedence was recorded in September while the lowest was observed in November. This peak occurrence probably coincides with breeding season of dogs which is from June to September. The decline in November could be due to mass destruction of unconfined dogs in response to increased movement during mating and increased rabies cases. The lower cases of rabies during the year 2009 and 2010 are due to discontinued killing of unconfined dogs. During the years 2008 to 2011, the administration of the Addis Ababa city has launched a rabies control activity including vaccination, birth control and removal of unconfined dogs. Public awareness by using television, radio and pamphlets to strengthen the control activities were also focused during the years. These activities together with the expanding construction in the city have reduced the number of dogs to 250,000 from about 500,000. Despite these control activities, dogs remain to be the main vector of rabies.

Rabid dogs were shown to be responsible for most of the rabies cases in humans. Our finding agrees with the reports of Kayali et al. (2003), yimer et al. (2002) and Eng et al. (1993). This shows that control activities need to be strengthened in developing countries to minimize the extent of rabies transmitted by dogs. Even though human-to-human transmission is extremely rare (Haaheim et al., 2002; Carter and Saunders, 2007), indi-viduals in close contact with rabies suspected humans received PEP in fear of acquiring the disease.

The majority of PEP in humans was administered without rabies diagnosis and only in 28% of cases PEP was administered after confirmation of the cases. The main reason for this low rate of confirmation before adminstration of PEP is the lack of diagnostic laboratories and simple and easier diagnostic tests. But it could also be due to lack of awareness by the community to collect and submit samples to the central laboratory. This has led to indescriminate administration of PEP to all rabies suspected human cases. This is a risk in Ethiopia where Fermi type (nerve tissue) vaccine is widely used. Fermi type vaccine was known for its post vaccination reactions and its use for humans was turned down by World Health Organization (WHO) in 1973 (WHO, 1973). The wide spread use of this vaccine in the absence of confirmation is a serious problem for the community. It can be assumed that the situation in rural areas of the country might be worse than the situation observed in Addis Ababa and its surroundings where cell culture vaccines are available in a few private pharmacies and the central diagnostic laboratory is located. This is in agreement with the observation of Jemberu et al. (2013) who have reported that 84% of people exposed to rabid dogs or other animals rely on traditional treatments.

Conclusion

The study shows that the principal vector of rabies in Addis Ababa and its surroundings, but most likely in entire Ethiopia, is the dog. Effective rabies management and control based on confirmed cases and the use cell cultured derived vaccine is recommended.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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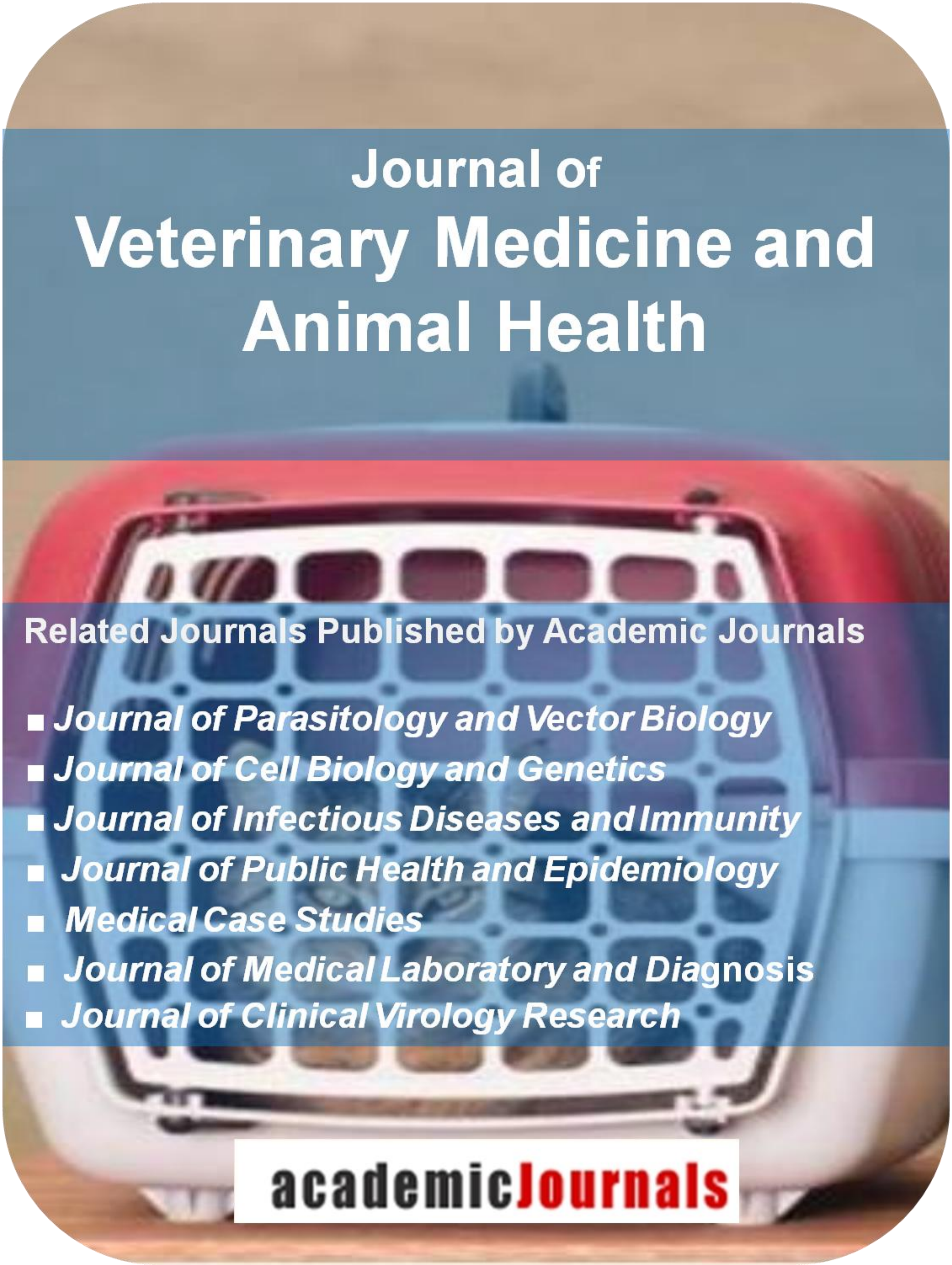
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