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Charnley AK (1992). Mechanisms of fungal pathogenesis in insects with particular reference to locusts. In: Lomer CJ, Prior C (eds), Pharmaceutical Controls of Locusts and Grasshoppers: Proceedings of an international workshop held at Cotonou, Benin. Oxford: CAB International. pp 181-190.

Jake OO (2002). Pharmaceutical Interactions between *Striga hermonthica* (Del.) Benth. and fluorescent rhizosphere bacteria Of *Zea mays*, L. and *Sorghum bicolor* L. Moench for Striga suicidal germination In *Vigna unguiculata*. PhD dissertation, Tehran University, Iran.

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Full Length Research Paper

Management practices and welfare problems encountered on working equids in Hawassa town, Southern Ethiopia

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A cross sectional study was conducted between November, 2008 and April, 2009 to evaluate (observe) management practices and welfare problems of working equids at Hawassa town, Southern Ethiopia. Six hundred working equids were screened for various lesions on the skin. Sixty animal owners or users were interviewed about management practices. Among studied (observed) equids, 90% were draught, and 10% were pack animals. Fifty two percent (52%), 45 and 3% revealed a thin, medium and good body condition score, respectively. Lesions resulting from limb tethering (94.5%), lameness (89.2%), lesions affecting the lips (88.5%), girth/belly (81%), wither/spine (78.7%) and breast/shoulder (62.8%) were most frequently observed. Tail/tail base (79%), ribs/flank (81%), breast/shoulder (84.5%) and hind quarter (70.7%) lesions were significantly associated with pack type of work ($p < 0.05$), whereas lip lesion (89.7%) and lameness (91%) were associated with draught type of work ($p < 0.05$). Poor/thin body condition significantly correlated with wither/spine lesions ($p < 0.05$). According to respondents, the average daily working time was 7.9 ± 0.2 h with an average burden of 70 kg of goods and 3 persons. The average water supply at a time amounted to 5.75 ± 2.7 liters. Among respondents, 53.3% provided water three times per day, and 41.6% of them only two times/day. The average amount of provided feed was 12.2 ± 3.4 kg twice daily. Shelters were provided for all working equids at home, but only for a few experienced individuals were provision of shelter to equids at work sites. In addition, rubber shoeing was found to be of poorest quality, thus leading to high slip hazard. In conclusion, even though owners/users take care of their animals, a great number of lesions associated with work type and body condition were noted. This finding shows that working equids experience multiple welfare problems in the study area.

Key words: Equids, work type, management, welfare problems, Hawassa, Ethiopia.

INTRODUCTION

Livestock is a backbone component of the agricultural sector in Ethiopia. It provides meat, energy for crop production, manure for soil fertility, raw material for the leather industry, serves as live bank and is used for transportation. Equids are mainly kept for transportation purposes in different agro-ecological zones of Ethiopia. Their meat and milk are consumed only in a few areas. In some regions of North West Kenya and South West

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Ethiopia, donkey meat is a delicacy and the milk believed to treat whooping cough (Fred and Pascal, 2006).

People in most peri-urban centers either own or rent horses, mules or donkeys to transport goods, people and even water (Mohammed, 1991). Despite their use, the husbandry practices of working equines are poor. Some hobbling methods cause discomfort and inflict wounds (Alujia and Lopez, 1991; Mohammed, 1991). In addition, inappropriate harnesses or yokes that may be heavy and ragged, long working hours and insufficient food, have a negative effect on the animals' health and welfare (safety) (Aluijia, 1998). Working equines are also suffering from lack of shelters that would protect them from sun, rain and insect parasites at market or working sites. Only few owners provide water (4.38%) and feed (10.5%), and almost no one provides shelters at the working site (Solomon and Rahmeto, 2010). Food is preferably allocated to bovines than to equines. In many cases, local communities, professionals and institutions pay more attention to the maintenance of cattle, because it provides meat and milk (Pearson, 1992).

The misuse, mistreatment and lack of general and veterinary care contribute in no small amount to the early death of working equids. In stark contrast, the life expectancy of equids reaches up to 30 years in countries where animal welfare is in practice (Svendsen, 1981; Fred and Pascal, 2006). The term "fit and feeling good" is to illustrate that animal welfare includes both emotional and physiological components. Physical wellbeing includes health and is compromised by injury and disease, whilst emotional wellbeing can be achieved by minimizing negative feelings such as fear, pain and distress, and by maximizing positive feelings such as happiness and comfort. A third component which overlaps with the previous two is instinctiveness. In the context of working animal welfare, this can be described as expression of normal behavior (Webster et al., 2004).

In Hawassa town, there are 3,000 working equids which are used for the transportation of goods and people, and experience various management and health care practices. The herein presented study has been conducted in order to assess these practices and identify major welfare problems.

MATERIALS AND METHODS

Study area

The study was conducted in Hawassa city, the capital of Southern Nations, Nationalities and People Regional State (SNNPRS), Ethiopia. The town consists of four sub-cities and 14 kebele's, which are the smallest administrative units in Ethiopia. The city is located 275 km south of Addis Ababa. It lies in central Ethiopia with an elevation of 1,800 meters above sea level, and exhibits a long and a short rainy season. The long rainy season extends from June to September, whereas the short rainy season ranges from mid February to the end of April. The remaining months are dry periods. According to the municipality of Hawassa, the city harboured about 3,000 working equids in 2008/09. These animals were engaged in different types of work such as the transportation of people, water, building materials, and goods by carts and pack. Working equids mainly comprised horses and donkey, whereas only a few mules were found in the records. Topographically, the city lies in a plain which allows the use of carts, whereas the western part of the city adjoins Lake Hawassa. The maximum annual temperature does not exceed 30°C and minimum temperatures range between 11.2 and 19.2°C.

Study design and animals

The type of study employed was a cross-sectional investigation on working equids in Hawassa city. The study was conducted from November, 2008 until April, 2009. Randomly selected animals were enrolled in the study (n = 600). Equids were selected regardless of work type, body condition, age and species, until approximately 20% of the total population were represented (N = 3000). Sixty animal owners/users were invited to complete questionnaires on behalf of equid welfare issues in the study area.

Examination

Co-workers (assessors) were trained in regard to animal examination procedures and collection of data. Then two trained assessors and handlers examined each selected animal with the owner's consent. One assessor performed the examination whilst the other recorded the data, and vice versa. The examination was carried out at the working site during daytime. Animals were allowed to stand for 5 to 10 min whilst being held by a lead rope attached to the head collar. Initially, general information was recorded for each animal including work type, sex, species and age. Subsequently, the parameters body condition score and mucous membrane color change were assessed. Lesions were recorded with regard to number, severity, size and anatomical location. The examination of each animal took between five and ten minutes without causing major interruption of routine work (Dennison et al., 2006).

Type of work

Animals were categorized as draught, pack, riding and other type of working equid. "Draught" animals were equids used for the transport of goods and people by cart. "Pack" animals were equids used for the transport of goods by pack. "Riding" animals are those used by owners for non-tourist riding, whereas the category "other work" included foals and non-functional animals (Pritchard et. al., 2005).

Body condition

The body condition of the selected animals was scored based on the criteria described by Carroll and Huntington (1988) and as cited by Pritchard et al. (2005). Body condition assessment was done by examining the animal from all sides without touching it. The equids' body condition was scored as 0 to 5 (0 = very thin; 1 = thin, 2 = fair, 3 = good, 4 = fat and 5 = very fat). However, for the purpose of data analysis, body condition 0 to 5 were assigned to three distinct

Table 1. Species of working equines, work types and body condition score proportion.

Species	Work type proportion		Body condition score category proportion		
	Draught	Pack	Thin (=1)	Medium (=2)	Good (=3)
Horses (n=384)	378 (0.98)	6 (0.02)	235 (0.61)	147 (0.38)	2 (0.01)
Donkeys (n=208)	161 (0.77)	47 (0.23)	76 (0.37)	117 (0.56)	15 (0.07)
Mule (n=8)	3 (0.38)	5 (0.62)	3 (0.38)	5 (0.62)	0
Total (600)	542 (0.90)	58 (0.10)	314 (0.52)	269 (0.45)	17 (0.03)

groups: Categories 0, 1 and 2 were grouped as "thin or poor", category 3 was defined as "medium" and body condition scores 4 and 5 were categorized as "good".

Lesions and abnormalities

Lesions of any size and severity at the external corners (commissures) of the mouth, where the bit would lie, were considered as lesions of the lips. Scars, hairless skin and broken skin were also regarded as labial lesion. Absence of lesions was scored with‘0’ and presence of lesions with‘1’. Lesions on the limbs were considered as being caused by tethering/hobbling, if any kind of hair loss, scars, healed or fresh lesion were present along the limb (Pritchard et al., 2005; Dennison et al., 2006). Mucous membrane of the mouth was assessed based on the color of the mucosa of the upper gum. Pale, yellow, white, or purple color were considered as abnormal, whereas pinkish was taken as normal. Eye abnormalities were scored with "1" if excessive lacrimation, blindness, opec colour, or other clinical aberrations were observed. Apparently healthy eyes received score '0". Wounds of the skin and deeper tissues were assessed according to the area, depth and location. Only substantial tissue abrasions were considered as true lesions and were recorded. Tissue abrasions of at least 2 × 2 cm^2 (quadratic lesion), 1 × 4 cm^2 (rectangular lesion) or 2.3 cm in diameter (circular lesion) were quoted as substantial lesions (Dennison et al., 2006). The observed animals were deamed positive for ectoparasites when they were found to harbor at least one external ectoparasite of any species (tick, lice, flea, mites or nits).

Statistical analysis

The raw data collected were managed into Microsoft excel and then descriptive statistics and 95% confident interval were used to summarize the proportion. Each observation were compared with assumed risk factors, to analyze the association of risk factors with each observation. The stata-9 (Stata corp. 4905 Lakeway Drive college station, TX77845, USA) software and level of significance was considered when "P < 0.05”.

RESULTS

Among the studied equids (EQUINES: horses; EQUIDS: horses, mules, donkeys), 64% were horses, 35% were donkeys, and 10% were mules. Most horses were kept for draught (carting) purposes (98%) followed by donkeys and mules. Draught type of work included transportation of people and goods using handmade carts. The majority of horses revealed a thin body condition (61%). In general, 90 and 10% of working equids were involved in draught and pack type of work, respectively. From these, 52% revealed a poor body condition as shown in Table 1.

Proportion of lesions and abnormalities in relation to species, work type and body condition

All working equids were analyzed in relation to the assumed risk factors or problems encountered. Comparisons made between risk factors and species, work type and body condition score are shown in Tables 2, 3 and 4. Species comparison was only made between donkeys and horses, with mules being omitted due to their small number. As shown in Table 2, limb tethering, lameness, lip lesions, girth/belly lesions and wither/spine lesions were observed for the vast majority of animals. Neck, tail/tail base and breast/shoulder lesion were significantly associated ($\chi^2 > 3.84$; $P < 0.05$) with donkeys as compared to horses. On the other hand, lesion of wither and spine, lameness and eye(s) abnormality significantly predominated ($\chi^2 > 3.84$; $P < 0.05$) in horses. There was also a significant difference regarding the presence of ectoparasites between these two species.

As shown in Table 3, only a few factors were significantly associated with work types. Lip lesions, lameness and abnormal gait showed a significant association with draught type of work. Tail/tail base, ribs/flank, breast/shoulder, hindquarter lesions and ectoparasites were significantly associated with pack animals. A further analysis was made in regard to the risk factors and the type of work on species level. Wither/spine, tail/tail base, ribs/flank and breast/shoulder lesion were significantly more associated ($P < 0.05$) with pack donkeys than draught donkeys; whereas lip lesion, abnormal gait and lameness were predominantly seen in draught donkeys (Table 4).

However, it was difficult to compare draught horses with pack horses because of the diverging sample size. Yet, the proportions of the few lesions seen in draught horses were very high. Even so, wither/spine, lip lesion, limb/tethering lesion were more often associated with

Table 2. Lesions and health parameter of working horses and donkeys

Parameter	Total	Donkey	Horse	Species difference	
	(n=600)	(=208)	(=384)	χ2	P-value
Lesion on skin and/or deep tissue					
Ear lesion	4.8	5.7	4.1	0.77	0.38
Head lesion	6.7	7.7	5.2	1.46	0.22
Neck lesion	10	14.4	7.6	7.1	0.01
Wither/spine lesion	78.7	73.6	81.5	5.1	0.02
Tail/tail base lesion	55.7	62.5	51.3	6.84	0.01
Back sore lesion	60.8	56.7	62.5	1.87	0.17
Ribs/flank lesion	57.5	59.6	56.3	0.63	0.43
Breast/shoulder lesion	62.8	67.8	59.9	3.59	0.06
Girth/belly lesion	81	82.7	80.2	0.54	0.46
Hind quarter lesion	58.2	62.5	55.5	2.74	0.19
Observation of health					
Lip lesion	88.5	86.5	89.3	1.02	0.31
Abnormal m/m	49.8	48.1	50.5	0.32	0.57
Limb tethering lesion	94.5	94.2	95.1	0.18	0.67
Firing lesion	49.3	54.3	46.9	2.99	0.08
Ectoparasites	29.3	34.6	26.3	4.5	0.03
Abnormal gait	75.7	79.8	73.7	2.75	0.09
Eye(s) abnormality	61.3	50.9	66.1	13.05	0
Lameness	89.2	84.1	92.2	9.2	0

*Eight of them were mule and omitted from statistical analysis to minimize biasness

draught horses than other factors (Table 4). Wither and spine lesion occurred significantly more frequently in thin equids that is, with a percentage of 81.8 when compared to others ($\chi^2 > 3.84$; $P < 0.05$ as shown in Table 5).

Watering and feeding management

According to 99% of the respondents, the average labour time per equid and day amounted to 7.9 ± 0.2 h, with an average of 70 kg and three travelers being transported over an average distance of 25 ± 2 km. The mean working span of carthorses amounted to 4.4 ± 0.8 years. Experience of provision of water was 100% although the amount of water given to a single working equid per day varied according to respondents. The average amount of water per supply was 5.75 ± 2.7 L. The absolute amount of water per supply varied between 3, 5, 6, and above 7 L, according to 11.6, 56.6, 15 and 16.6% of respondent, respectively. The majority of owners provided water 3 times per day (53.33%) followed by two times per day (41.6%). A minority (3.3%) of respondents supplied their equids with water one time per day.

Feed mainly consisted of cereal and other plant by-products such as wheat bran, maize residue, chopped sugarcanes, and green grass. According to animals' owners, 28.3% fed concentrates like chopped sugar-canes and cereal by-products, whereas 65% fed mixtures of concentrates and green grass. Only 6.6% of respondents reported to solely provide green grass. The average amount of feed given per day was 12.02 ± 3.4 kg. The majority of the respondents fed at different frequencies. Thirty-three percent of respondents provided feed once a day, whilst 25 and 42% of the respondents fed twice and thrice daily, respectively. According to the majority (99%) of the respondents, the average daily expense for feed and supplements per equid was 19.4 ± 7.3 ETHB, which is equivalent to 1.5 USD. The mean purchase price for feed shows seasonal variations in function of the availability of grass.

Housing, harness and shoeing management of working equids

Obtained data indicated that 100% of respondent provided shelter at home, whereas only 35% provided shelters of various quality at the working site. Shade is

Table 3. Lesions and health parameter of working equids in relation to the type of work.

Parameter	Draught	Pack	Significance difference	
	(=542)	(=58)	χ2	P-value
Lesion on skin and/or deep tissue				
Ear lesion	4.8	5.1	0.02	0.89
Head lesion	6.3	10.3	1.39	0.24
Neck lesion	9.9	12.1	0.25	0.61
Wither/spine lesion	77.7	88	3.28	0.07
Tail/tail base lesion	53.1	79.3	1 4.54	0
Back sore lesion	60.5	63.8	0.23	0.63
Ribs/flank lesion	54.9	81	14.55	0
Breast/shoulder lesion	60.5	84.5	12.88	0
Girth/belly lesion	80.1	89.7	3.12	0.07
Hind quarter lesion	57	70.7	4.13	0.04
Observation of health				
Lip lesion	89.7	77.6	7.51	0.01
Abnormal m/m	49.1	56.9	1.28	0.26
Limb tethering lesion	94.1	98.3	1.76	0.18
Firing lesion	48.7	55.2	0.87	0.35
Ectoparasites	28	41.4	4.49	0.03
Abnormal gait	76.8	65.5	3.59	0.05
Eye(s) abnormality	61.3	62.1	0.01	0.9
Lameness	91.1	70.7	22.69	0.0

usually provided by trees surrounding the market/working site. The floor of this natural shelter usually consists of tamped soil.

The type of harness used by the respondents was made of products like rubber adjusted with nails. According to 30% of the respondents, working equids also wear rubber shoes. Farriers use to change these rubber shoes at different time intervals. According to 21% of the respondents, the horse shoes at 3 week-intervals, whereas 18.0 and 25% respondents changed the horse shoes every second week or after more than a month, respectively depending on the activity of their animals. The costs for shoeing amounted to 3 ETHB or 0.2 USD. The method of shoeing consisted in covering the entire sole including the frog with an adjusted piece of tyre rubber. The nails used were traumatic and excessively long, penetrating the hoof wall beyond the white line that leads to damage of the sensitive portion of the hoof.

DISCUSSION

The objective of this study was to address equids management and welfare problems caused by inadequate management. Once the risks associated with issues have been identified, methods of decreasing or eliminating the effects of these risks can be incorporated into specific interventions that will be planned and implemented (Dennison et al., 2007).

In Hawassa town, 100% of equids are kept to transport people and goods in order to assure their owners' daily income. This observation is in agreement with reports by Blackeway (1994), Pritchard et al. (2005) and Dinka et al. (2006), describing that equids are mainly kept for transport purposes and only rarely as source of meat or milk. The working equid population of Hawassa mainly consists of an almost equal number of donkeys and horses which indicates that these species are fully integrated in the owners' daily life. The relative small number of encountered mules may be explained by their sometimes difficult behavior which makes them less attractive as working equids despite their sturdy nature and endurance.

The study revealed different welfare problems: most of them were lesions at different body sites of investigated equids. Donkeys showed a significant association with neck and tail/tail base lesions ($P < 0.01$; $\chi^2 > 3.84$). This might be due to several risk factors; such as frequent beating and trauma induced by the movement of the crupper, when the owner tries to accelerate the speed of

Table 4. Proportion of lesions and health parameters for different types of work in donkeys and horses.

Parameter	Donkey			Horse		
	Draught (n=161)	Pack (n=47)	P-valve	Draught (n=378)	Pack (n=6)	P-valve
Lesion on skin and/or deep tissue						
Ear lesion	16.4	4.2	0.61	4.2	0	1
Head lesion	8.7	6.4	0.7	5.3	0	0.56
Neck lesion	14.9	12.7	0.71	7.6	0	1
Wither/spine lesion	70.2	85.1	0.04	81.2	100	0.24
Tail/tail base lesion	57.1	80.8	0	51.3	50	0.94
Back sore lesion	56	59.6	0.65	62.4	66.7	0.83
Ribs/flank lesion	53.4	80.9	0	56.1	66	0.6
Breast/shoulder lesion	63.4	82.9	0.01	59.5	83.3	0.24
Girth/belly lesion	81.4	87.2	0.35	79.9	100	0.22
Hind quarter lesion	61.5	65.9	0.58	55	83.3	0.17
Observation of health						
Lip lesion	90.7	72.3	0	89.2	100	0.39
Abnormal m/m	45.9	55.3	0.26	50.5	50	0.98
Limb/tethering lesion	93.2	97.8	0.22	94.9	100	0.57
Firing lesion	54.7	53.2	0.85	46.3	83.3	0.41
Ectoparasites	31.6	44.7	0.09	26.5	16.6	0.58
Abnormal gait	85.1	61.7	0	73.5	83.3	0.58
Eye(s) abnormality	49.9	55.3	0.49	65.9	83.3	0.66
Lameness	89.4	65.9	0	73.5	83.3	0.41

the donkeys. Further analysis indicated that lesions of the wither/spine, tail/tail base, ribs/flank, breast/shoulder showed significant association with pack type of work; whereas lip lesions, lameness and abnormal gait were associated with draught type of work. Similar finding were reported by Dennison et al. (2007) where pack donkeys had a significantly higher proportion of tail/tail base lesions than draught animals. It is also supported by Blackeway (1994), Pritchard et al. (2005), Swan (2006) and Solomon and Rahmeto (2010) that the chance of tail/tail base lesion occurrence is very high when pack animals frequently cope with long distances. In addition, it was reported that lip lesions predominantly occur (79.4%) in horses and for draught type of work and less frequently develop in donkeys and more general in pack animals.

Within the horse group, it was difficult to compare the effects of draught and pack type of work because of the uneven ration between draught (n = 378) and pack horses (n = 6). However, it could be shown that the draught type of work is likely to induce lameness, wither/spine, lip lesion and hobbling lesion, in 73.5, 81.2, 89.2 and 94.9% of horses, respectively. This finding is suggestive for a direct correlation between health problems and the type of work. Especially lip lesions were signifcantly associated with the bit type used for leading/braking of draught animal. Lameness was associated with continuous movement in various landscapes and on bumpy roads. Nawaz et al. (2007) reported that among the bit characteristics jointed bar, sharp projection, dirt and rusty bar, ring bar connection was found to be related to lip lesions. This finding is supported by Dennison, et al., (2007), Blackeway (1994), Pritchard et al. (2005), Swan (2006) and Solomon and Rahmeto (2010).

Observations of lesion in relation to work type in donkeys were also analyzed. Accordingly, tail/tail base, ribs/flank, breast/shoulder and hindquarter lesions were significantly associated with pack type of work ($P < 0.01$; $\chi^2 > 3.84$). The causes for the development of these lesions are complex and multifactorial. Environmental factors, the type of harness material used (natural or synthetic), the fit of the harness, the behaviour of the owner, the frequency of work and the load all contribute to the onset of health problems. In general, bumpy roads and rugged land-scapes, a loose fit and synthetic harness materials, frequent beating and overwork may induce lesions and lameness. This finding is in agreement with reports by Blackeway (1994), Pritchard et

Table 5. Proportion of lesions and health parameter when compared with the body condition score category.

Body condition score category	Poor (=315)	Medium (= 268)	Good (=17)	Significance difference	
				$\chi 2$	*P-value*
Lesions of the skin and/or deep tissue					
Ear lesion	3.5	6.7	0	4.09	0.12
Head lesion	6.1	7.4	5.9	0.46	0.79
Neck lesion	10.2	10.4	5.9	0.36	0.84
Wither/spine lesion	81.8	76..2	58.8	6.8	0.03
Tail/tail base lesion	52.9	59.9	70	4.4	0.11
Back sore lesion	60.5	62.8	35.3	5.1	0.07
Ribs/flank lesion	57.6	57.9	47.1	0.8	0.67
Breast/shoulder lesion	63.7	60.9	76	1.85	0.39
Girth/belly lesion	78.9	82.9	88.2	2.04	0.36
Hind quarter lesion	53.8	62.1	76.5	6.47	0.06
Health parameter					
Lip lesion	88.2	88.1	100	2.27	0.32
Abnormal m/m	52	45.6	47.1	1.14	0.57
Limb tethering lesion	94.6	94.1	100	1.09	0.58
Firing lesion	48.1	50.6	52.9	0.44	0.8
Ectoparasites	28.3	30.5	29.4	0.32	0.85
Abnormal gait	75.5	74.7	94.1	3.28	0.19
Eye(s) abnormality	64.1	58.4	58.8	1.99	0.34
Lameness	88.2	90.7	82.4	1.77	0.41

al. (2005) and Swan (2006), where pack animals coping with long distances frequently develop lesions.

Fifty-two percent (n = 314) of studied animals had a poor body condition score and of these, 90% (n = 284) were engaged in draught type of work whereas only 9.5% (n = 30) were pack animals. Among the latter, wither/spine lesion were significantly associated with thin or poor body condition score ($P < 0.05$; $\chi^2 > 3.84$). Pritchard et al. (2005) found a lower prevalence (1.8%) of wither and spine wounds in pack animals, but did not compare the frequency of wounds with the body condition score.

It has been assumed that the type of work promotes the occurrence of certain lesions at different body sites. In the herein presented study, pack animals were found to be more likely to suffer from tail and tail base, ribs/flank, breast/shoulder and hindquarter lesions. In contrast, draught animals significantly suffered from lip lesions and lameness ($\chi^2 > 3.84$; $P< 0.05$). Animals with thin or poor body condition score were found to be more often affected by wither and spine lesion than equids in good body condition. However, Shaw et al. (2007) reported that the interaction with the body condition is difficult to explain but concluded that fatter donkeys wearing metal shafts were less likely to suffer from breast and shoulder lesions. Longer and smooth shafts were found to be less dangerous than shorter and protrusions surfaces.

The current study showed that the average amount of feed given per day was 12.02 ± 3.4 kg and that the frequency of feeding per day varied in the study area, with 33, 25 and 42% of respondents providing feed once, twice and thrice per day, respectively, and average daily cost amounting to 19.4 ± 7.3 ETHB or 1.5 USD. Dinka et al. (2007) reported that the amount of feed given per individual and day was 7.4 ± 0.39 kg, with 46, 24 and 24% respondents feeding once, twice and thrice daily daily, and with costs amounting to 6.8 ± 0.2 ETHB or 0.5 USD. Except for the costs, both studies are in agreement. The costs may vary according to the availability and the type of feed in the study areas. Besides, working equids in Hawassa are not allowed to graze. It was also noted that the watering system in Hawassa is superior as far as the users provide water along the road side whilst equids are at work.

On the other hand, although all studies agree that owners/users provide shelters for their animals at their home, only 35% of them provide shelters at the working place, that is a shadowy place under the trees or a closed shelter. Similar findings were reported by Solomon and

Rahmeto (2010), where almost all users and owners had no experience in providing shelters at working sites. Poor management associated with a lack of shelter at the working site seems to constitute a countrywide problem, exposing the animal to sun, rain, insect bites and resulting stress. Poor management was also noted in relation to shoeing, where rubber is used to cover the entire sole including the hoof frog. This puts the animals' health at risk. Equids may slip and fall on muddy or sloppy grounds. Moreover, microorganisms and parasites may damage the entirely covered sole and deposit their larvae, especially when the weather is humid.

Conclusion

Although animal users and owners are trying to improve maintenance issues, the working equids of Hawassa are still suffering from multiple welfare problems. It is hence imperative to increase the awareness of owners and users in regard to these unresolved issues. Further investigations on the risk factors associated with equid welfare are warranted as to improve the situation of these working animals. The herein presented findings may help in initiating training programs aiming at accustoming owners and users to improved harness material, adequate bits, alternative shoeing procedures and correct behaviour.

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REFERENCES

Aluijia AS (1998). The welfare of working equids in Mexico. Appl. Animal Behaviour Sci. Elsevier 59:19-29.

Alujia AS, Lopez F (1991). Donkeys in Mexico. In: Fielding D, Pearson RA (Eds). Donkeys, Mules and Horses in Tropical Agricultural Development pp 1-7. CTVM: Edinburgh.

Blackeway SJ (1994). The welfare of Donkeys. In: Network UK, the welfare of Donkeys-htm. Accessed on 5/2/2008.

Dennison TL, Khan GS, Khan AR, Pritchard JC, Whay HR (2007). A comparative study of the welfare of equines working in the brick kilns of Multan and Peshawar, Pakistan. In: Pearson RA, Muir CJ, Farrow M (2007) (Eds). The Future for Working Equines; The fifth International Colloquium on Working Equines. Proceeding of an International Colloquium held at the Addis Ababa University, Ethiopia, 30th October to 2nd November 2006. Pp: 153-160. The Donkey Sanctuary, Sidmouth, Devon, EX10 ONU.

Dinka H, Shelima B, Abalti A, Geleta T, Mume, T, Chala R (2007). Socio-economic importance and management of carthorses in the mid rift valley of Ethiopia. In: Pearson R A, Muir C J, and Farrow M 2007 (Eds). The Future for Working Equines. The fifth International Colloquium on Working Equines. Proceeding of an International Colloquium held at the Addis Ababa University, Ethiopia, 30th October to 2nd November 2006. pp: 181-188. The Donkey Sanctuary, Sidmouth, Devon, EX10 ONU.

Fred O, Pascal K (2006). Extension Approaches to improving the welfare of working equines. Kenya Network for Dissemination of Agricultural Technologies (KENDAT), pp: 1-28. Nairobi, Kenya.

Nawaz S, Shah Z, Gondal JI, Habib M, Shaw A (2007) The Influence of Cart and Bit Characteristics on presence, size and severity of lip lesions in draught equines in MARDAN- PAKISTAN. In: Pearson RA, Muir C J, Farrow M 2007 (Eds). The Future for Working Equines. The fifth International Colloquium on Working Equines. Proceeding of an International Colloquium held at the Addis Ababa University, Ethiopia, 30th October to 2nd November 2006. Pp: 181-188. The Donkey Sanctuary, Sidmouth, Devon, EX10 ONU.

Mohammed A (1991). Management and breeding aspects of donkeys around Awassa, Ethiopia. In: Fielding D, Pearson RA (Eds). Donkeys, Mules and Horses in Tropical Agricultural Development Pp 185-188. CTVM: Edinburgh UK.

Pearson RA (1992). Management and husbandry of working animals with particular reference to their welfare. In: Proceedings of the Cairo International Meeting on Working Animals, April 13-16, 1992 Cairo, Egypt.

Pritchard JC, Lindberg AC, Main DCJ, Whay HR (2005). Assessment of the welfare of working horses, mules and donkeys using health and behavior parameters. Prev. Vet. Med. 69, 265-283.

Shaw A, Kamu A, Lewa A, Murithi E, Ochieng F (2007). Determination of risk factors contributing to priority welfare issues in working equines: An illustration using breast and shoulder lesions in Kenyan draught donkeys. In: Pearson RA, Muir CJ, Farrow M 2007 (Eds). The Future for Working Equines. The fifth International Colloquium on Working Equines. Proceeding of an International Colloquium held at the Addis Ababa University, Ethiopia, 30th October to 2nd November 2006. Pp: 181-188. The Donkey Sanctuary, Sidmouth, Devon, EX10 ONU.

Solomon M, Rahmeto A (2010): Observations on major welfare problems of equines in Meskan district, Southern Ethiopia. http://www lrrd.org/.lrrd22/03/meku22048.htm.

STATA-9, (2007): Stata corp. 4905 Lakeway Drive college station, TX77845, USA.

Svendsen E (1981). Down Among the donkeys. Pan books, London.

Swan WJ (2006) Improving the welfare of working equine animals in developing countries. Appl. Anim. Behav. Sci. Elsevier, 100: 148-151.

Webster AJF, Main DCJ, Whay HR (2004). Welfare Assessment: indices from clinical observation. Animal welfare, 13: 93-98.

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Full Length Research Paper

Brine shrimp toxicity and in *vitro* antimicrobial activity of *Piliostigma thonningii* (Schum.) Milne-Redh. [Leguminosae-Caesalpinioideae] from Kenya and Malawi against some pathogens of human and veterinary importance

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Many microorganisms are responsible for causing serious diseases of bacterial origin. Development of drug resistance in animal and human pathogens against commonly used antibiotics has necessitated a search for new antimicrobial substances from other sources including plants. The present study reports on the antimicrobial and brine shrimp lethality of *Piliostigma thonningii* leaves collected from two geographical regions, Kenya and Malawi. Both aqueous as well as organic extracts from leaves of *P. thonningii* were screened for antibacterial activity against bacteria of human and veterinary importance using agar well diffusion and evaluated for acute toxicity using brine shrimp bioassay. Except for chloroform extract of *P. thonningii* from Malawi, all of the plant extracts demonstrated remarkable antibacterial activity against the five test bacteria at concentrations tested (250 µg/ml) in agar well diffusion method. In brine shrimp bioassay, all the crude extracts from Kenya and Malawi exhibited varying degrees of toxicity against *Artemia salina* larvae. Nevertheless, further evaluation of the *in vivo* toxicity and *in vivo* antibacterial activity of the crude plant extracts should be carried out.

Key words: *Piliostigma Thonningii*, brine shrimp bioassay, antibacterial activity, crude plant extract, Kenya, Malawi.

INTRODUCTION

In developing countries, diseases of bacterial origin is a serious problem, presenting a serious public issue of a significant segment of the population as uncovered by either private or health care systems. In economic crisis, high cost of industrialised medicine, inefficient public access to medical and pharmaceutical care, in addition to the side effects caused by synthetic drugs are some of the factors contributing to central role of medicinal plants in health care systems (Susan et al., 2007). The rapid development of multi-drug resistant strains of bacteria has increased the occurrence of bacterial infections that cannot be treated with conventional antimicrobial agents (Sieradski et al., 1999; Alanis, 2005). Because of the aforementioned reasons, lots of efforts are being made to discover new antimicrobial agents from various sources such as micro-organisms, animals and plants (Tomoko et al., 2002). Plants comprise the largest component of the diverse therapeutic elements of traditional health care practices both in humans and animals. Nearly all cultures and civilizations from ancient times to the present day

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have used herbal medicines which are antimicrobial sources to cure infections (Lino and Deogracious, 2006). Plant derived drugs against bacterial pathogens represent a vast untapped source of medicines and is effective in the treatment of infectious diseases, while, simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials (Parekh et al., 2005). Herbs are invaluable source of modern drugs, with more than 50% of modern drugs being derived from plants (Newman et al., 2005).

World Health Organization (WHO) has compiled an inventory of more than 20,000 species of medicinal plants which are used for a variety of applications (Buckingham, 1996; Hoffmann et al., 1993). Some of these species have been tested for antimicrobial properties, while majority are not yet evaluated. Documentation of toxicity and antimicrobial properties of medicinal plants is necessary in order to build a comprehensive database from which it may be possible to search for new leads in drug development when the need arises. On the other hand, the development of drug resistance in pathogens against commonly used antibiotics has necessitated a search for new antimicrobial substances from other sources including plants. Screening of medicinal plants for phytochemicals and antibacterial activities is important for finding potential new compounds for therapeutic use.

Piliostigma thonningii (Schum.) Milne-Redh. [Leguminosae-Caesalpinioideae] is a plant used for medicinal purposes in many African countries. Different parts of the plant have been used traditionally for the treatment of various diseases in humans and animals (Djuma, 2003). Its roots and twigs have been used in treatment of dysentery, wounds, respiratory ailments, snake bites, hookworms and skin diseases (Asuzu and Onu, 1994). The leaves of *P. thonningii* are used to treat wounds, chronic ulcers, diarrhoea, toothache and gingivitis, cough, and bronchitis (Watt and Breyer-Brandwijk, 1962). The bark, leaves or root extracts are taken as cough medicine, whereas the leaf extract as menorrhagia medicine. Alkaloids, flavonoids, saponins and tannins have already been isolated from the leaves of *P. thonningii.* The leaves from the tree posess antibacterial, antimicrobial and antioxidant activities (Alfred Maroyi, 2013). The dry leaf powder has been reported to contain alkaloids, saponins, flavonoids and tannins (Ighodaro et al., 2012). Besides this carbohydrates, glycosides, flavonoids, tannins, saponins, balsams, volatile oil and terpenes have also been isolated from the leaves of *P. thonningii* (Egharevba et al., 2010). Few studies have been done to evaluate the pharmacological activity and safety of the leaves of *P. thonningii* despite their rich phytochemical composition.

Despite this medicinal usefulness, little information is available regarding the comparative antimicrobial and brine shrimp lethality effects of *P. thonningii*. The current study was therefore designed to investigate the antimicrobial activity and acute toxicity of crude extracts from leaves of *P. thonningii* collected from two different phytogeographical zones, Kenya and Malawi.

MATERIALS AND METHODS

Collection of plant materials

The fresh leaves of *P. thonningii* were collected from Bunda College of Agriculture forest located thirty kilometres South West of the City of Lilongwe in Malawi and Imenti North District in Meru County in Kenya during December, 2010 based on ethno pharmacological use according to interviews with local communities and traditional health practitioners. Consent to collect the plant material was given by the study communities. The taxonomic identities of the plants were confirmed by Mr. Musembi J.K of the Department of Land Resource Management and Agricultural Technology (LARMAT), College of Agriculture and Veterinary Medicine, University of Nairobi. The voucher specimen numbers “WMD, 01” and “WMD, 02” from Kenya and Malawi, respectively were deposited in the Herbarium of LARMAT, University of Nairobi.

Preparation of the extracts

The method described by quality control for medicinal plant materials was employed according to WHO (1998). In this method, the leaves were cleaned with distilled water to remove dust, external pollutants, sediments, additives, insecticides and parasites, air dried at room temperature and ground into fine powder using a laboratory hammer mill with a one millimetre sieve pore size. Aqueous extraction was performed by soaking 100 g of dry powder of the *P. thonningii* leaves in distilled water (1000 ml) and boiled at 60°C in a water bath for one hour. This was followed by filtration of the suspension through cotton wool plugged in a funnel and the filtrate was collected in bottles. The filtrate was further separated by centrifugation at 3,000 rpm for three minutes. The supernatant was decanted in falcon tubes and kept in a deep freezer for 24 h which was then lyophilized. The lyophilized dry powder was then collected in falcon tubes with screw tight covers and kept at -20°C until used.

Organic extracts were prepared by cold maceration. 100 g of ground plant material was dissolved in 1000 ml of solvent. It was incubated at room temperature for 48 h and stirred periodically. The sample was filtered using Whatman paper No.1 and the filtrate was concentrated *in vacuo* set at 40°C and then transferred into an oven set at 40°C for further evaporation of the solvents. The methanolic and [chloroformic and methanolic mixture (1:1)] crude extracts were further taken for lyophilisation so as to remove the water component from methanol and subsequently obtain a dry powdered material. Percentage yield of plant extracts (both aqueous and organic) were calculated. The dried plant extracts were collected in airtight falcon tubes and kept at - 4°C until used.

Preparation of test extracts

Samples of aqueous crude extracts of *P. thonningii* leaves were prepared by dissolving 0.1 g of the crude extract in 10 ml of distilled water making a stock solution of 10,000 µg/ml. Samples of organic crude extracts of *P. thonningii* leaves were prepared by dissolving 0.1 g in 1 ml of dimethyl sulphoxide (Sigma chemical CO., St. Louis, MO, USA) followed by subsequent dilution to lower concentration of dimethyl sulphoxide (DMSO), to < 1% to avoid carry over (solvent) effect (Dorin et al., 2001). The positive control drug, cyclophosphamide was prepared by dissolving 0.1 g of the crude extract in 10 ml of distilled water making a stock solution of 10,000 µg/ml. Cyclophosphamide was used in this experiment as positive control while dimethyl sulphoxide and distilled water were used as negative controls.

Hatching of brine shrimp

Brine shrimp eggs were incubated and hatched at 37°C in a shallow rectangular container (14 × 9 × 5 cm) containing 225 millilitres of artificial sea water which was prepared by dissolving 33 g of commercial salt mixture [consisting of: sodium chloride at a concentration of 24.6 g/l; potassium chloride at a concentration of 0.67 g/l; calcium chloride at a concentration of 1.36 g/l; magnesium sulphate at a concentration of 4.66 g/l and sodium bicarbonate at a concentration of 0.18 g/l]. The mixture had a pH of 8.0] in a litre of distilled water followed by filtration through Whatman filter paper No. 1. The container had two unequal compartments with 2 mm holes in between the two compartments. The eggs were sprinkled into the larger compartment which was darkened, while the smaller compartment was illuminated. A few granules of yeast were added as source of energy for the *nauplii*. After 48 h, the phototrophic *nauplii* were collected by pipette from the lightened side, having been separated by the divider from the shells.

Brine shrimp bioassay

Ten larvae of brine shrimp (*Artemia salina* L.(Artemiidae) were transferred into test tubes using disposable pipettes. 5, 50, and 500 µl representing the three concentrations 10, 100 and 1000 µg/ml of the plant extract were transferred into test vials. Five replicates were used for each dose level per sample. Filtered brine solution at 3.3% was transferred to all test vials containing plant extract to make 5 ml volume. The vials were incubated for 24 h and percentage deaths were calculated at the various dose levels and the control. In cases where control deaths occurred, the data was corrected using Abbott's formula: Percentage death = [(Test – control / control)] × 100 (Abbotts, 1925).

Statistical analysis/LC_{50} determinations

Median lethal concentrations (LC_{50}s) were determined from the 24 h counts using probit analysis method described by Finney (Finney, 1971). In cases where data was insufficient for this technique, the dose response data were transformed into a straight line by means of a logit transformation (Hafner et al., 1977); the LC_{50} was derived from the best fit line obtained by linear regression analysis.

Agar well diffusion test and determination of antibacterial activity of *P. thonningii* crude extracts

Briefly, 1 ml of the test culture (10^7 CFU/ml) was inoculated into a sterile plate with 20 ml Muller Hinton molten agar and the plate was shaken for even spread and proper mixing of the organisms and agar. When the agar was solidified, six wells of 8 mm in diameter and 7 mm depth were made on the surface of the agar plates using a sterile borer. Stock solution of each plant extract was prepared at concentration of 250 mg/ml in the same solvent used for their extraction. Each of the four wells out of the six wells was filled with 0.350 ml of the plant extract. The fifth and the sixth wells were filled with 0.350 ml of distilled water and 13.13 µg equivalent of gentamycin in 0.350 ml of distilled water as negative and positive control, respectively. The plates were then incubated at 37°C for 24 h, the zones of inhibition were measured with a calliper and the ruler in millimetre, and the results were tabulated according to Yitbareck Habtamu et al. (2010). The test was conducted in duplicate for all the five bacteria of veterinary importance. Mean inhibition zones and significant differences between means of aqueous and organic extracts of *P. thonningii* were calculated by Genstat 13th Edition.

The antibacterial activity was tested using agar well diffusion method according to Lino and Deogracious (2006) and Sahm and Washington (1990). Aqueous, methanol, chloroform and chloroform + methanol crude extracts of *P. thonningii* from Kenya and Malawi were screened against five bacteria [(4 standard bacteria and 1 clinical isolate (*Streptococcus agalactiae*)] using agar well diffusion. The test bacteria which included *Bacillus cereus, Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Streptococcus agalactiae* isolate were all obtained from the department of Public Health Pharmacology and Toxicology, Faculty of Veterinary Medicine, University of Nairobi.

Statistical analysis

Data were analyzed using descriptive statistics. Means were calculated from three separate experiments. The results of the antimicrobial screening of the leaves were compared to a reference antibiotic, gentamycin.

RESULTS

Percentage yield of crude plant extracts

P. thonningii crude extracts showed variation in percentage yield in both aqueous and organic solvents. The highest yield was recorded for methanolic extract of Kenyan *P. thonningii* (12%) and the lowest yield was observed for the chloroformic extract of Malawian *P. thonningii* which was 1.2% (Table 1).

Brine shrimp toxicity bioassay

Results of the toxicity of different extracts against brine shrimp larvae are shown in Table 2. Aqueous extracts and organic extracts of the Kenyan *P. thonningii* displayed toxicity range of LC_{50} between 63 and 991.3 µg/ml while aqueous and organic extracts of the Malawian *P. thonningii* displayed toxicity range of LC_{50} between 128.4 and 540 µg/ml (Table 2). The results obtained have shown that the aqueous extracts of the Kenyan *P. thonningii* are more toxic than the chloroform + methanol extract with LC_{50} values of 63 and 991.3 µg/ml, respectively. Aqueous extract of the Kenyan *P. thonningii* was as toxic as that of the control drug, cyclophosphamide (LC_{50} value of 95 µg/ml) with an LC_{50} value of 63 µg/ml (Table 2).

Antibacterial screening

The test bacteria were screened for antibacterial activity using agar well diffusion method.

Agar well diffusion

The growth inhibition zones are tabulated in Table 3. The results indicated that out of 8 crude extracts of *P. thonningii* from Kenya and Malawi, 7 extracts had antibacterial activity at concentration of 250 µg/ml and

Table 1. Yield of leaf extracts of *P. thonningii* (%) using aqueous and organic extraction methods.

Voucher specimen No.	Initial sample weight (g)	Type of extraction	% yield (w/w)
WMD 01	100	Aqueous	9.4
WMD 02	100	Aqueous	9.4
WMD 01	100	Methanol	12.0
WMD 02	100	Methanol	8.3
WMD 01	100	Chloroform	3.1
WMD 02	100	Chloroform	1.2
WMD 01	100	Chloroform + Methanol	12.0
WMD 02	25	Chloroform + Methanol	3.9

WMD 01*: P. thonningii* from Kenya. *WMD 02:* P. thonningii *from Malawi.*

Table 2. LC_{50} values for *P. thonningii* screened against brine shrimp larvae (*A. salina* Leach).

	Parameter	Percentage death after 24 h			Value	
Zone	Extract Type	10 µg/ml	100 µg/ml	1000 µg/ml	LC_{50}	Remarks
KE	Aqueous	20	48	100	63.0	Toxic
	Methanol	8	36	100	109.6	Toxic
	Chloroform	16	42	84	121.4	Toxic
	Chloroform/methanol	38	44	50	991.3	Non-toxic
MA	Aqueous	12	24	100	128.4	Toxic
	Methanol	8	32	62	408.3	Toxic
	Chloroform	2	24	60	540.0	Toxic
	Chloroform/methanol	56	44	58	476.3	Toxic
Controls	Cyclophosphamide	20	52	80	95.0	Toxic
	Distilled water	0	0	0	0.0	Non-toxic
	DMSO	0	0	0	0.0	Non-toxic

KE = Kenya *P. thonningii* leaf extract; MA = Malawi *P. thonningii* leaf extract; DMSO = Dimethylsulphoxide

one crude extract was not effective against all the test bacteria. The largest zone (34 mm) of inhibition was observed for aqueous extract of the Kenyan *P. thonningii* against *B. cereus* while the Malawian *P. thonningii* recorded (30 mm) (Table 3). The smallest zone (14 mm) was observed for methanol extract of the Kenyan *P. thonningii* and Malawian *P. thonningii* against *E. coli*. For the methanol extract, the largest zone (33 mm) inhibition was observed in the Kenyan *P. thonningii* against *S. agalactiae e* while the Malawian *P. thonningii* recorded (28 mm) against the same bacteria (Table 3). For the chloroform extract, the largest zone (26 mm) of inhibition was observed in the Kenyan *P. thonningii* against *E. coli* while the Malawian *P. thonningii* recorded no activity against all tested bacteria.

DISCUSSION

The current study was designed to evaluate the safety of crude leaf extracts from *P. thonningii* collected from Kenya and Malawi using brine shrimp bioassay; and also to investigate the antibacterial activity using agar well diffusion technique. Brine shrimp bioassay is a simple method for natural product research. The procedure determines median lethal concentration values of active compounds and extracts in the brine medium. This method is rapid, reliable, inexpensive and convenient as an in-house bioassay tool. Babajide et al. (2010) considered LC_{50} values of < 10 µg/ml as very active, of < 700 µg/ml as active and of > 700 µg/ml as none active. Meyer et al. (1982) also considered an LC_{50} value of lower than 1000 µg/ml of an extract in brine shrimp lethality bioassay as toxic. The classification of toxicity in brine shrimp bioassay described by Padmaja et al. (2002) was used in the current study where LC_{50} > 1000 µg/ml was considered to be non toxic, LC_{50} (500 to 1000 ug/ml) was considered to be weakly toxic, LC_{50} (100 to 500 µg/ml) was considered to be moderately toxic and LC_{50} (0 to 100 µg/ml) was considered to be strongly toxic.

The results showed that aqueous and organic extracts of the Kenyan *P. thonningii* and Malawian *P. thonningii* displayed toxicity as follows: aqueous extract of Kenyan *P. thonningii* leaves was strongly toxic with LC_{50} of 63

Table 3. Antibacterial activity of aqueous and organic extracts of *P. thonningii* using agar well diffusion method.

Organisms	Mean zones of inhibition (mm)									
	Aqueous		Methanol		Chloroform		Chloroform/ Methanol		Controls	
	KE	MA	KE	MA	KE	MA	KE	MA	Gentamycin	Distilled water
E. coli	19±0.6	19±1.0	14±0.6	14±0.6	26±1.2	-	16±0.6	15±1.0	22±0.6	-
B. cereus	34±1.0	30±0.6	24±0.8	21±0.6	20±1.0	-	20±0.6	22±1.0	18±0.8	-
P. aeruginosa	26±1.2	29±0.8	23.5±0.6	24±0.8	19±0.6	-	22±0.6	24±1.2	20±1.0	-
S. aureus	28.5±0.8	31±1.2	33±0.6	28±0.6	-	-	26±1.0	28±1.2	32±1.2	-
S. agalactiae	30±0.6	31±1.2	26.5±0.8	23±0.6	26±1.2	-	25±0.6	26±0.8	16±1.0	-

KE= Kenya *P.thonningii* leaf extract; MA=Malawi *P. thonningii* leaf extract; (-) No zone of inhibition detected. Values are means ±SD from three replicate experiments.

µg/ml while aqueous Malawian *P. thonningii* was moderately active with LC_{50} of 121.4 µg/ml, methanol and chloroformic extracts of the Kenyan *P. thonningii* and Malawian *P. thonningii* were both moderately active with LC_{50} values of 109.6 and 403.3 µg/ml for methanol extracts and 121.4 and 476.3 µg/ml for chloroformic extracts, respectively. The chloroform + methanol extracts of the Kenyan *P. thonningii* was weakly toxic while Malawian *P. thonningii* was moderately active with LC_{50} of 991.3 and 476.3 µg/ml, respectively.

Observed differences in LC_{50} values in brine shrimp bioassay could be attributed to phytogeographical origins of the studied *P. thonningii* and also the reported carbohydrates, glycosides, flavonoids, tannins, saponins, balsams, volatile oil and terpenes in the leaves of the plant (Egharevba et al., 2010; Ighodaro et al., 2012; Maroyi, 2013). A study by Baratta et al. (1999) using pods of *P. thonningii* from Guinea showed that ethanol extract of the pods were toxic to brine shrimp with an LC_{50} of 26 µg/ml. Elsewhere, studies have shown that new compounds (2-phenoxychrome and C-methylflavonols) isolated from leaves of *P. thonningii* had virucidal activity (Ibewuike et al., 1996). These compounds could be responsible for the observed toxicity in brine shrimp bioassay.

All the extracts of *P. thonningii* from Kenya and Malawi showed antibacterial activity against the five test bacteria with the exception of chloroform extract from Malawi at the test concentration (250 µg/ml) suggesting that none of the reported bioactive compounds (Egharevba et al., 2010; Ighodaro et al., 2012; Maroyi, 2013) resides in the chloroform extract from Malawi. It is not uncommon phenomenon for certain extracts to show preferential activity against selected micro-organisms (Shail et al., 2008). Chloroform extracts of the Kenyan *P. thonningii* exhibited stronger activity to *E. coli* than gentamycin at the same test concentration (250 µg/ml). Aqueous extracts from Kenya and Malawi have shown to be more potent and efficacious than gentamycin against *B. cereus* and *S. Agalactae.* It has also been observed that higher zones of inhibition were recorded in Gram positive bacteria (*B. cereus, S. aureus* and *S. agalacitae*) and lower zones of inhibition were recorded in Gram negative bacteria (*E. coli* and *P. aeruginosa*). This is in agreement with earlier studies that established that gram positive bacteria are much more susceptible to drugs than gram negative bacteria (Cos et al., 2006). Ibewuike et al. (1997) observed that ethanolic leaf extract of *P. thonningii* had antibacterial activity against *S. aureus*, *E. coli*, *B. subtilis* and *P. aeruginosa.* Similarly, a concentration of 20 mg/ml of methanolic stem bark extract of *P. thonningii* showed antibacterial activity against *B. subtilis*, *Corynebacterium pyogenes*, *E. coli*, *Proteus vulgaris*, *Shigella dysenteriae* and *S. aureus* (Akinpelu and Obuotor, 2000). These reports are in line with the results obtained from the current study. It has also been shown that aqueous and organic extracts from Kenya and Malawi exhibited antimicrobial activity in agar diffusion method at the concentration tested (250 µg/ml). The study has also shown that aqueous and organic extracts of *P. thonningii* had activity in the brine lethality bioassay regardless of the phytogeographical origins. The differences that have been observed among the extraction methods of *P. thonningii* in recovery percentages and LC_{50} can be attributed to differences in two different geographical regions. However, it was established that there was no significant differences between the means of inhibition zones of aqueous Kenyan *P. thonningii* and aqueous Malawian *P. thonningii* on the five test bacteria ($p > 0.05$), no significant difference between the mean of inhibition zones of methanol extract of Kenyan *P. thonningii* and

methanol of Malawian *P. thonningii* on the five test bacteria ($p > 0.05$) and no significant difference between the means of inhibition zones of (Akinpelu and Obuotor, 2000) methanol/chloroform extract of Kenyan *P. thonningii* and methanol/chloroform extract of Malawian *P. thonningii* on the five test bacteria was observed. The *in vitro* findings are not always dependable, plants which are effective *in vitro* might not work when used *in vivo* while other extracts showing little or no effect *in vitro* might also be effective when evaluated in animals due to various factors that affect or favour the release of active principles in animal bodies (Gessler et al., 1995). Therefore, further detailed *in vitro* and *in vivo* evaluation of efficacy and safety of *P. thonningii* from both ecological zones should be carried out.

The fact that the aqueous extracts of *P. thonningii* leaves collected from both Kenya and Malawi exhibited higher growth inhibition activity against *B. cereus* than the positive control, gentamycin is interesting and lends support to the traditional use of this plant against bacterial infections, but *in vivo* tests are required to support this. After detailed *in vivo* antibacterial evaluation and thorough toxicological studies, this plant may find use as an antibacterial agent especially in rural communities where the conventional drugs are unaffordable or unavailable and the health facilities inaccessible.

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REFERENCES

Abbott WS (1925). A method of computing the effectiveness of an insecticide. J. Econ. Entomol.18:265-267.294.

Akinpelu DA, Obuotor EM (2000). Antibacterial activity of Piliostigma thonningii stem bark. Fitoterapia. 71(4):442-443.

Alanis AL (2005). Resistance to Antibiotics: Are we in the Post-Antibiotic Era? Arch. Med. Res. 36:697-705.

Alfred M (2013). Traditional use of medicinal plants in South-Central

Zimbabwe: review and perspectives. J. Ethnobiol. Ethnomed. 9:31.

Asuzu IU, Onu OU (1994). Anthelmintic activity of the ethanolic extract of *Piliostigma thinningii* bark in *Ascaridia galli* infected chickens. Fitoterapia. 65: 291-294.

Babajide TO, MabulselaWT, Green IR, Ameer F, Weitz F, Iwuoha EI (2010). Chemica screening and biological activity studies of five South African Indigenous medicinal plants J. Med. Plant. Res. 2:1924-1932.

Baratta MT, Ruberto G, Tringali C (1999). Constituents of the pods of *Piliostigma thonningii,* Fitoterapia. 70:205-208.

Buckingham J (1996). Dictionary of National Products Release 4:2, Chapman hall, London.

Djuma (2003). Djuma Game Reserve copyright (Co 1998- 2003).

Dorin DE, Le Roch K, Sallicandro P, Alano P, Parzy D, Poullet P, Meijer L, Doering C (2001). Pfnek-1, a NIMA related kinase from the human malarial parasite *Plasmodium falciparum*; biochemical properties and possible involvement in MAPK regulation. Eur. J. Biochem. 268:2600-2608.

Egharevba HO, Folashade KO (2010). Prelimnary phytochemical and proximate analysis of the leaves of *Piliostigma thonningii* (Schumach.) Milne-Redhead. Ethnobot. Leaflets. 14:570-77.

Finney DJ (1971). Probit analysis, 3rd Ed., Cambridge.

Gessler MC, Nkunya MHH, Chollet J, Heinrich M, Tanner M (1995). Tanzanian medicinal plants used traditionally for the treatment of malaria: in vivo antimalarial and in vitro cytotoxic activities. Phytother. Res. 9:504-508.

Hafner EE, Heiner, Noack E (1977). Arzneim-Forsch. 27:1871.

Hoffmann JJ, Timmeman N, MacLaughin R, Punnapayak H (1993). Potential Antimicrobial activity of plants from the South Western United States: Int. J. Pharmacol. 31:101-115.

Ibewuike JC, Ogunandaini AO, Ogungbamila FO, Matin MT, Gallard JF, Bohlin L, Paris Y (1996). Pilostigima a 2- phenoxychromone and C-methylflacanols from *P.thonningii*. Phytochem. 43:687-690.

Ibewuike JC, Ogungbamila FO, Ogundaini AO, Okeke IN, Bohlin L (1997). Antiinflammatory and Antibacterial Activities of C-methylflavonols from *Piliostigma thonningii.* Phytother. Res. 11:281-284.

Ighodaro OM, Agunbiade SO, Omole JO, Kuti OA. (2012). Evaluation of the chemical, nutritional, antimicrobial and antioxidant-vitamin profiles of *Piliostigma thonningii* leaves (Nigerian species). J. Med. Plants. Res. 6(7):537-543.

Lino A, Deogracious O (2006). The *in vitro* antibacterial activity of *Annona senegalensis*, *Securidace longipendiculata* and *Steanotaenia araliaceae*-Ugandan medicinal plants. Afr. J. Health Sci. 6:31-35.

Meyer BN, Ferrigin NR, Putmanet JE, Jacobsen IS, Nichols DE, McLaughlin JL (1982). Brine shrimp: A convenient bioassay for active plant constituents. J. Med. Plants. Res. 45:31-34.

Newman D J, Cragg GM, Snader KM (2003). Natural Products as Sources of New Drugs over the Period1981- 2002. J. Nat. Prod. 66:1022-1037.

Padmaja R, Arun PC, Prashanth D, Deepak M, Amit A, Anjana M (2002). Brine shrimp lethality bioassay of selected Indian medicinal plants. Fitoterapia 73:508-510.

Parekh J, Jadeja D, Chanda S (2005). Efficacy of aqueous and methanol extracts of medicinal plants for potential antibacterial activity. Tur. J. Biol. 29: 203-210.

Sahm DF, Washington DA (1990). Antibacterial susceptibility test Dilution Methods: In: Manuals of Clinical Microbiology. Lennette EH, 5th Edition, American Society for Microbiology, Washington D.C pp.1105-1116.

Shail LJ, McGaw, Adorogba MA, Madee LK, Eloff JN (2008). Triternoids with antibacterial activity from *Curtivia dentate* (*Burm.F*) C.A S.M leaves. J. Ethnopharmacol.119(2):238-244.

Sieradski K, Roberts RB, Haber SW, Tomasz A (1999). The development of vancomycin resistance in a patient with methicillin resistant *Staphylococcus aureus* infection. New Engl. J. Med.340:517-523.

Tomoko N, Takashi A, Hiromu T, Yuka I, Hiroko M, Munekazu I, Totshiyuki T, Tetsuro I, Fugio A. Iriya I, Tsutomu N, Kazuttito W (2002). Antibacterial activity of extracts prepared fromtropical and and sub tropical plants on methicillin resistant *Staphylococcus aureus*. J. Health. Sci. 48:273-289.

Watt JM, Breyer-Brandwijk MG (1962). Medicinal and Poisonous of Southrn and Eastern Africa, P.640.E and S.Livingstone.London.

World Health Organization, WHO Geneva (1998). Quality Control Methods for Medical Plant Materials.

Yitberek H, Tedesse E, Alegne W, Takele S (2010). *In vitro* Antimicrobial Activity of Selected Ethiopian Medicinal Plants against some important bacteria of veterinary importance. Afr. J. Microbiol. Res. 4:1230-1234.

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Full Length Research Paper

Assessment of anthelmentic resistance in gastirointestinal nematodes of small ruminants, Dale district, Southern Ethiopia

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The anthelmintic resistance status of gastrointestinal nematodes of small ruminants owned by smallholder farmers in the Dale district, Southern Ethiopia, was investigated. A faecal egg count reduction test (FECRT) was conducted in traditionally managed and naturally infected goats and sheep. For this study, 60 sheep and 60 goats of both sexes, aged from 6 to 18 months, and with a faecal egg count (FEC) of more than 150 eggs/g of faeces were selected for the test from 5 neighboring kebeles. Both sheep and goats were grouped into four treatment groups: albendazole, tetramisole, ivermectin and control groups. In sheep, the percentage reduction in FECs (95% confidence intervals) for albendazole, tetramisole and ivermectin were 95.0% (86.5 to 98.2%), 97.5% (93.2 to 99.1%) and 96.7% (91.0 to 99.1%), respectively. In goats, the percentage reduction in FECs (95% confidence intervals) for albendazole, tetramisole and ivermectin were 96.6% (88.3 to 99.0%), 97.7% (90.6 to 99.4%) and 97.1% (91.0 to 99.1%), respectively. All the anthelmintics were found to be effective, but resistance to albendazole was suspected. Based on the findings, it was concluded that development of anthelmintic resistance could be prevented by avoiding frequent dosing and under dosing, while strategic deworming should be practiced by both animal health workers and animal owners.

Key words: Anthelmintics, resistance, faecal egg count reduction, small ruminants, Dale, Ethiopia.

INTRODUCTION

Sidama Zone contributes to approximately 7% of the total small ruminant population of the Southern Regions of Ethiopia, and these small ruminants are mainly kept by resource poor smallholder farmers. There are about 432,947 and 253,447 sheep and goats, respectively, in the Sidama Zone (CSA, 2012). However, the productivity of this huge small ruminant population remains marginal due to prevailing diseases, poor nutrition and husbandry systems, and lack of effective veterinary services (Gizaw et al., 2010; Assefa, 2007). Gastrointestinal nematodes (GINs) constitute one of the greatest disease threats for grazing livestock worldwide. Infection with helminth parasites results in both clinical and sub-clinical diseases causing low productivity due to stunted growth, insufficient weight gain, delay of puberty, anemia, poor feed utilization and mortality (FAO, 2002; Nahed et al., 2003) hindering optimization of the economical benefits from small ruminants (Tembely et al., 1997).

The control of parasitic helminths in domestic animals relies largely on the use of anthelmintic drugs (Taylor et al., 2002). However, increasing reports of parasitic populations that have developed anthelmintic resistance (AR) have become increasingly common, and this phenomenon severely threatens the beneficial

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exploitations of this control strategy (Waller, 1997). In fact, AR in gastrointestinal nematodes of sheep and goats has been reported in different parts of the world (Waller, 2007), making it a seriously increasing problem (Wolstenholme et al., 2004).

In Ethiopia, various anthelmintics have been used in different parts of the country for the treatment of sheep and goats helminth parasites (Biffa et al., 2006; Asmare et al., 2005). The use of anthelmintics has been practiced for a long time, and constitutes a considerable share of the costs spent by the country in the control of helminthosis (Biffa et al., 2006). Also, smuggling and misuse of veterinary drugs involving anthelmintics is a wide spread practice in the country (Maingi et al., 1997). Some of these drugs, particularly albendazole and tetramisole, have been continuously imported and distributed to every corner of the country under different trade names and by different manufacturers (Kumsa and Wossene, 2006). There was a complaint by the Regional Animal Health Officers and some animal owners with regard to the effectiveness of available anthelmintics, especially albendazole. Moreover, some researcher reported existence (Kumsa and Abebe, 2008; Kumsa and Abebe, 2008) and some others absence of anthelmintic resistance (Sheferaw and Asha, 2010; Asmare et al., 2005) in the region.

Therefore, the objective of this study was to investigate the existence of GIN resistance for albendazole, tetramisole and ivermectin, in naturally infected sheep under field conditions.

MATERIALS AND METHODS

Study area

The study was conducted in the midland area of Dale district, Sidama Zone in Southern Ethiopia, from November 2011 to May 2012. The area is characterized by a bimodal rainfall, and receives a total annual mean rainfall of 1314 mm (Improving Productivity and Market Success of Ethiopian Farmers, 2005). It is located at 6.45N and 38.23E (Gonfa, 1996). The annual mean maximum and minimum temperature are 25.4 and 14.5°C, respectively. The main livestock species in the district are cattle, goats, sheep and equines with estimated population of 2056994, 31443, 30152 and 19233, respectively (CSA, 2012).

Study animals and study design

The study population was sheep and goats in the Dale district, especially Awada and its surroundings, which were kept by smallholder farmers under backyard management system. Before the actual experiment, screening was done to identify sheep and goats naturally infected with GINs. During the screening examination, fecal samples for 135 and 111 sheep and goats were collected, respectively and the result was recorded using the owner's code of identification, for ease of identification. Sheep and goats with more than 150 eggs per gram (EPG) of feces and aged 6 to 18 months were eligible for inclusion in the field experiment on anthelmintic efficacy, following guidelines by Coles et al. (1992).

Accordingly, 60 animals of each species were selected, and for each species were grouped into four treatment groups (n=15): albendazole, tetramisole, ivermectin and control (i.e. left untreated). On day 0, fecal samples were collected from each animal enrolled in the study, and then the animals were either treated with an anthelmintic or left untreated. The manufacturer of the anthelmintics used and dose rate are described in Table 1. The expiration date of albendazole and tetramisole is august 2015, and that of ivermectin is January 2014. Fecal samples were collected again 10 to 14 days post-treatment from all animals included in the study, and the changes in the EPG were determined. All fecal samples were analyzed using a modified McMaster technique as described by Ministry of Agriculture, Fisheries and Food (1984) and Coles et al. (1992), with a minimum detection limit of 50 EPG (Cole et al., 2006).

Data analysis

The effectiveness of different anthelmintics was evaluated by computing the mean faecal egg counts reduction for each treatment group. Computation of the arithmetic mean, percentage of reduction and 95% upper and lower confidence limits; and the findings were interpreted as described by Coles et al. (1992).

RESULTS

The fecal samples collected during the screening indicated that 91 and 92.6% of the sheep and goats sampled, respectively were shedding GIN eggs in their feces (Table 2). The mean fecal egg count (per gram of faeces) was 546.4±64.5 and 619.9±43 for sheep and goats, respectively (Table 3).

The mean pre and post treatment faecal egg counts (EPG) and the percentage of faecal egg count reduction (FECR) and the lower and upper 95% confidence limit for each groups of anthelmintic drugs tested was summarized in Table 4. The percentage reduction of faecal egg count (95% confidence intervals) for albendazole, tetramisole and ivermectin were, 95.0% (86.5 to 98.2), 97.5% (93.1 to 99.1) and 96.7% (91.0 to 100.0), respectively in sheep. The percentage reduction of faecal egg count (95% confidence intervals) for albendazole, tetramisole and ivermectin were, 96.6% (88.3 to 99.0), 97.7% (93.2 to 99.4) and 97.1% (91.0 to 99.1), respectively in goats.

DISCUSSION

The coprological screening of the studied goats and sheep confirmed the common occurrence of GINs in goats and sheep of Dale district (92.6 and 91.0% of the goats and sheep sampled were infected, respectively). This result is comparable to the previous report by Kumsa and Abebe (2008) conducted on an agricultural farm in Hawassa College.

In this study, anthelmintic resistance was considered to be present if the percentage reduction in faecal egg

Table 1. The study animals included the four treatment groups, and the name of the manufacturer, dose and route of the anthelmintics used in a field study to determine anthelmintic efficacy in small ruminants in the Dale district, Southern Ethiopia (2011-2012).

Animal	Group	Treatment	Manufacturer	Dose and route
Sheep	S-I	Albendazole 300 mg	Ashish Life Science PVT, Ltd. India	7.5 mg/kg (Oral)
	S-II	Tetramisole 600 mg	Ashish Life Science PVT, Ltd. India	15 mg/kg (Oral)
	S-III	Ivermectin 1% (W/V)	Laboratorios Microsules Uruguay, SA	300 mcg/kg (S/C)
	S-IV	Control	-	-
Goats	G-I	Albendazole	Ashish Life Science PVT, Ltd. India	7.5 mg/kg (Oral)
	G-II	Tetramisole	Ashish Life Science PVT, Ltd. India	15 mg/kg (Oral)
	G-III	Ivermectin	Laboratorios Microsules Uruguay, SA	300 mcg/kg (Oral)
	G-IV	Control	-	-

Table 2. The number of animals included, and number of animals that had positive fecal egg counts, in a field study to determine anthelmintic efficacy in sheep and goats in the Dale district, Southern Ethiopia (2011-2012).

Species	Category	No. of animals examined	No. of positive animals (proportion, %)	Standard error	95% CI
Sheep	Male	34	30 (88.2)	0.06	77.1 - 99.4
	Female	77	71 (92.2)	0.03	86.1 - 98.3
	Total	111	101 (91.0)	0.03	85.6 - 96.4
Goat	Male	62	56 (90.3)	0.04	82.8 - 97.8
	Female	73	69 (94.5)	0.03	89.2 - 99.8
	Total	135	125 (92.6)	0.02	88.1 - 97.0

Table 3. The mean gastrointestinal nematode egg count (per gram of faeces) in sheep and goats in the Dale district, Ethiopia (2011-2012).

Species	Category	No. of animals examined	Mean egg count ± Standard error	95% CI
Sheep	Male	34	555.9 ± 164.5	229.8 - 881.9
	Female	77	542.2 ± 59.0	429.2 - 659.2
	Total	111	546.4 ± 64.5	419.4 - 673.4
Goat	Male	62	584.0 ± 64.0	457.3 - 710.6
	Female	73	650.4 ± 58.3	535.2 - 765.7
	Total	135	619.9 ± 43.0	535.1 - 704.7

counts was less than 95% and the lower limit of the 95% confidence interval was less than 90% (Coles et al., 1992). If only one of these criteria is met, anthelmintic resistance is suspected. Based on this criterion, the FECR percentage and the lower confidence limit obtained from Dale district smallholder sheep and goat production system revealed the absence of a significant level of GIN resistance to tetramisole and ivermectin. This finding is in line with other studies conducted in various parts of Ethiopia, on the efficacy of the most commonly used anthelmintics in small ruminants (Asmare et al., 2005; Kumsa and Abebe, 2008; Kumsa and Nurfeta, 2008; Kumsa and Wossene, 2006; Sheferaw and Asha, 2010; Tadesse et al*.,* 2009). These studies also reported the absence of anthelmintic resistance to albendazole, tetramisole and ivermectin in small ruminants in Ogaden. However, the lower and upper 95% confidence limits of the FECR percentage following albendazole treatment were 86.5 to 98.2% and 88.3 to 99.0% for sheep and goat, respectively. This finding revealed that albendazole was suspected for resistance in this region of Ethiopia.

Table 4. The faecal egg count reduction percentage following treatment with albendazole, tetramisole and ivermectin, in sheep and goats in the Dale district, Ethiopia (2011-2012).

Treatment group	Animal species	EPG		Reduction (%)	95% CL (UCL – LCL)
		Pre-treatment	Post-treatment		
Albendazole	Sheep	756.7 ±171.5	20.0 ± 9.5	95.04	98.2 - 86.5
	Goat	550.0 ± 42.5	20.0 ± 11.8	96.6	99.0 - 88.3
Tetramisole	Sheep	723.3 ± 89.0	10.0 ± 7.2	97.5	99.1 - 93.2
	Goat	1063 ± 187.9	13.3 ± 9.1	97.7	99.4 - 90.6
Ivermectin	Sheep	743.3 ± 197.8	13.3 ± 9.1	96.7	100.0 - 91.0
	Goat	976 ± 323.4	16.7 ± 7.0	97.1	99.1 - 91.0
Control	Sheep	436.3 ±44.6	403.3 ±45.4	-	-
	Goat	623.3 ±75.3	583.3 ±72.6	-	-

UCL: Upper confidence limit; LCL: lower confidence limit.

CONCLUSION AND RECOMMENDATION

Most worm control strategies rely heavily on the use of anthelmintics. However, the regular and indiscriminate use of anthelmintics increases the risk of development of resistant parasite populations. The current findings indicated that albendazole was suspected for development of resistance, while ivermectin and tetramisole were found to be effective. To prevent further development of anthelmintic resistance in this area, the following practices are recommended: i) avoid frequent and unnecessary treatments anthelmintics, opting instead for strategic deworming and, ii) avoid under dosing of animals (Cole et al., 1992). Further studies, are needed to determine the anthelmintic resistance status of the different species of GINs in other neighboring areas of Ethiopia. Moreover, studies are needed to be conducted based on a comparative efficacy on drugs from reliable source and drugs used by the owners from unreliable sources such as imported drugs or smuggled drugs to arrive at a proper conclusions and further advice.

REFERENCES

Asmare K, Gelaye E, Ayelet G (2005). Anthelmintic resistance test in gastrointestinal of small ruminants in southern Ethiopia. Bulletin of Anim. Health Prod. Afr. 53:89-95.

Assefa E (2007). Assessment of Production and Marketing System of Goats in Dale District, Sidama Zone. MSc thesis, Hawassa University. pp. 134.

Biffa D, Jobre Y, Chaka H (2006). Ovine helminthosis, a major health constraint to productivity of sheep in Ethiopia. Anim. Health Res. Rev. 7:10-118.

Coles GC, Bauer FHM, Borgsteed S, Geerst TR, Klei TR, Taylor MA, Waller PJ (1992). World Association for the advancement of Veterinary Parasitology (WAAVP). Methods for detection of anthelmintic resistance in nematodes of veterinary importance. Veterinary Parasitology. 44:35-44.

Coles GC, Jackson F, Pomroy WE, Prichard RK, von Samson-Himmelstjerna G, Silvestre A, Taylor MA, Vercruysse J. (2006).The detection of anthelmintic resistance in nematodes of veterinary importance, Review. Vet. Parasitol., 136:167–185.

CSA (2012) Central Statistical Agency of the Federal Democratic Republic Of Ethiopia. Agricultural Sample Survey of 2011/12 (2004 E.C). Volume II. Report on Livestock and Livestock Characteristics (Private Peasant Holdings), Central Statistical Agency, Addis Ababa, Ethiopia.

FAO (2002). Biological control of nematode parasites of small ruminant in Asia. In final proceedings of FAO technical cooperation project in Malaysia TCP 0065(7), (Eds food and agricultural organization of the United Nations, Malaysia, pp. 104.

Gizaw S, Tegegne A, Gebremedhin B, Hoekstra D (2010). Sheep and goat production and marketing systems in Ethiopia: Characteristics and strategies for improvement. Improving Productivity and Market Success (IPMS) of Ethiopian Farmers Project, International Livestock Research Institute (ILRI), Addis Ababa, Ethiopia. pp. 49.

Gonfa L. (1996). Climatic classification of Ethiopia, Metrological research report series. Addis Ababa, Ethiopia. pp. 45.

Improving Productivity and Market Success of Ethiopian farmers (2005). Dale pilot learning Woreda diagnosis and program design. Addis Ababa, Ethiopia. pp. 76.

Kumsa B, Abebe G (2008). Multiple anthelmentic resistance on a goat farm in Hawassa, Trop. Anim. Health Prod. 6:100-112.

Kumsa B, Nurfeta A (2008). Comparative efficacy of albendazole, tetramisole and ivermectin against gastrointestinal nematodes in naturally infected sheep in Hawassa, Southern Ethiopia. Revue de Méd. Vét. 159(12):593-598.

Kumsa B, Wosene A (2006). Efficacy of Albendazole and Tetramisole against Heamonchus contortus in Experimentally Infected Lambs. International Journal of Applied Research in Vet. Med. 4:94-98.

Maingi N, Bjorn H, Thamsborg SM, Bogh HO (1997). A survey of anthelmintic resistance in nematode parasites of goats in Denmark. Vet. Parasitol. 66:53–66.

Ministry of Agriculture, Fisheries and Food (1984). Manual of Veterinary Investigation Laboratory Techniques, Vol. 2, London. pp. 162-184.

Nahed J, L´opez Q, Mendoza G, Aluja A, Trigo FJ (2003). Epidemiology of parasitosis in the Tzotzil sheep production system. Small Ruminant Research, 49:199–206.

Sheferaw D, Asha A (2010). Efficacy of selected anthelmintics against gastrointestinal nematodes of sheep owned by smallholder farmers in Wolaita, Southern Ethiopia. Ethiop. Vet. J. 14 (2):31-38.

Tadesse A, Asmare K, Bekele J, Abebe G (2009). Study on anthelmintic resistance nematodes at goat farm of Hawassa college agriculture

southern Ethiopia. Ethiop. Vet. J. 13(2):49-57.
Taylor AM, Hunt RK, Goodyear LK (2002). Anthelmintic resistance detection methods. Vet. Parasitol. 103:183-194.
Tembely S (1997). Development and survival of infective larvae of nematode parasites of sheep on pasture in a cool tropical environment. Vet. Parasitol. 79:81–87.
Waller PJ (1997). Sustainable helminth control of ruminant in developing countries. Vet. Parasitol. 71:195-207.
Waller PJ (2007). Nematode parasites of small ruminant livestock-global perspectives, impact and coping with the problem of anthelmintic resistance. World Situation of Parasite Resistance in Veterinary Medicine. V International Seminar of Animal Parasitology, Merida, Yucatan, Mexico, 76–84.
Wolstenholme AJ, Fair-Weather I, Prichard R, Von Samson-Himmelstjerna G, Sangster NC (2004). Drug resistance in veterinary helminthes. Trends Parasitol. 20:469-476.

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Full Length Research Paper

Foot and mouth disease sero-prevalence in cattle in Kenya

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A cross sectional study was conducted on serum from 39 counties in Kenya in order to determine the prevalence of foot and mouth disease in bovine species. The study utilized serum samples at Foot-and-Mouth Disease (FMD) laboratory including Somali Ecosystem Rinderpest Eradication Coordination Unit (SERECU) project collected in the year 2010. From the serology results, the national prevalence of foot and mouth disease in bovines was 52.5% (CI = 95). Of the 3709 samples subjected to Nonstructural protein (NSP) enzyme linked immunosorbent assay (ELISA) screening test, 1,947 of those were interpreted as positive representing 52.5% (1947/3709) while the other 1,762 samples turned negative representing 47.5% (n = 1,762). There was significant association between seropositivity and age groups (p = 0.002) and vaccination status (p = 0.048) but no association between the seropositivity and sex (p = 0.063).

Key words: Cattle, foot and mouth disease (FMD), Kenya, seroprevalence.

INTRODUCTION

Foot and mouth disease (FMD) is a highly contagious acute viral infection of cloven hoofed animals including domesticated ruminants and pigs and more than 70 wildlife species, and is one of the most important economic diseases of livestock (Coetzer et al., 1994; Broonsvoort et al., 2004). The disease is characterized by fever, loss of appetite, salivation and vesicular eruptions in mucosa of the mouth, skin of the inter-digital spaces and coronary bands of the feet and teats. It is also characterized by high morbidity and low mortality (Coetzer et al., 1994). It is caused by virus of genus *Aphthovirus*, in the family Picornaviridae (Belsham, 1993), of which seven distinct serotypes O, A, C, SAT1, SAT2, and SAT 3 and Asia 1 are known. The disease is endemic in Kenya, and five of these serotypes have been in circulation that is, O, A, C, SAT1 and SAT2 (Vosloo et al., 2002), although no type C has been recorded in the world since 2004 (FAO, 2005, 2006).

Foot-and-mouth disease is one of the major Trans-boundary animal diseases (TADs) that impact negatively on trade in livestock and livestock products in the region. In order to control and/or eradicate this disease in the targeted areas, a good understanding of disease epidemiology is important and this can only happen if the disease is traced and regular, and effective surveillance is done together with vaccination regimes being put in place (FAO, 2005, 2006).

FMD is a global disease that through the years has affected most of the countries. FMD is endemic in most countries in sub Saharan Africa (Vosloo et al., 2002) with six of the seven serotypes reported to occur in East Africa namely O, A, C, SAT 1, SAT 2 and SAT 3 thus

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complicating the epidemiology and control of the disease in the region. Serotype SAT 3 has been recorded only in Uganda (Vosloo et al., 2002). Infection with any one serotype does not confer immunity against the other serotypes. Within serotypes, many strains can be identified by biochemical and immunological tests. The disease spreads rapidly by movement of infected animals or mechanically on formites such as clothing, shoes, vehicles, and veterinary instruments. The reasons for the rapidity of spread to fully susceptible populations is due to the highly infectious nature of the virus, the production of high titer in respiratory secretions and the large volumes of droplets and aerosols of virus shed by infected animals, the stability of virus in such droplets, the rapid replication cycle with very high virus yields and the short incubation period (Sellers, 1971).

Nonstructural protein (NSP) enzyme linked immunosorbent assay (ELISA) test is useful because it is able to discriminate animals that have been infected from those that have been vaccinated. Such test would be able to detect continued viral circulation and would therefore be extremely useful for serological surveys with a view to eradication. As NSP ELISA detects antibodies to the non-structural proteins of FMDV and is therefore able to differentiate between vaccinated and convalescent animals where purified vaccine is used. Previous serology results indicated a very high FMD prevalence in counties within Western and North Rift areas of Kenya in both bovines and porcines. Some counties such as Uasin Gishu, Baringo, Elgeyo Marakwet, Tranzoia, Bungoma, Pokot and Kakamega recorded 100% prevalence. The counties of Mandera, Moyale and Nyeri had the lowest seroprevalence of 8.3, 7.5 and 5.3%, respectively.

The aim of this study was therefore to determine the prevalence of antibodies against foot and mouth disease virus in Kenya using NSP ELISA, to determine the prevalence of antibodies in each county and lastly to determine the prevalence of FMD in disaggregated units including age and sex.

MATERIALS AND METHODS

Sample source

The serum samples were obtained from the collection at the Embakasi laboratory assembled from various activities including the Somali Ecosystem Rinderpest Eradication Coordination Unit (SERECU) project. The samples were collected throughout the country, with the unit of sampling being the computer generated divisions in every district, in the year 2010 between April and May. All the 4,262 samples were individually verified and entered into a data sheet with the details below; animal laboratory identification, location and coordinates of the source (district and county), species, sex (either male or female), age of the animal (stratified as follows < 1 year, 1 to 2 years and > 2 years) and vaccination history (either vaccinated, non-vaccinated or unknown).

Study area

Serum samples analyzed were collected from the 39 counties out of the 47 counties. The counties that did not feature included; Kiambu, Murang'a, Nairobi, Nyamira, Busia, Kirinyaga, Kericho and Kisii (Figure 1).

Sampling method

The sampling unit was considered to be the county. The sample size was calculated using the following formula (Dohoo et al., 2003).

$$n = \frac{(1.96)^2 p(1-p)}{L^2}$$

Where L is the required precision, (+ or - error around the estimate) and was assumed at 95%, p is the anticipated prevalence or proportion of attribute. The anticipated prevalence used was 50%. The sample size obtained was 384 and in order to improve on the precision, the sample size was increased by four fold to 1,383 samples. The sample size was also informed by the number of test kits that were available. On average, 34 samples were randomly selected from each county. Some counties had fewer samples as to enable random selection. In that scenario, all the samples were considered for analysis. For counties that had slightly above the average number of samples required, systematic random selection was done.

Serological tests

All the 3,709 bovine samples were subjected to FMD screening test; NSP-ELISA (AniGen® FMD NSP Ab ELISA). The AniGen® Foot and Mouth Disease Virus Ab ELISA kit was designed to detect FMDV specific antibodies in bovine serum. The test was useful because it was able to discriminate animals that had been infected (wild virus induced antibodies) from those that had been vaccinated with purified vaccine (vaccine induced antibodies). Some of the test materials including NSP-ELISA test (AniGen FMD NSP Ab ELISA/Anigen/South Korea), chemical (washing and stopping solutions) and biological (negative and positive control, enzyme conjugate and substrate) reagents were availed by the FMD Reference Laboratory while others were sourced from Pirbright through the assistance from National Council for Science and Technology.

Briefly, the test sera, negative and positive reference sera were added to all the 96 well ELISA plate coated with 3ABC antigen. Following addition of the diluted enzyme conjugate and incubation for 90 min at 37°C, the plates were washed 3 times with washing buffer. After the last washing, tetramethylbenzidine (TMD) ready to use substrate was added and plates were incubated at room temperature for another 15 min. The reaction was terminated by adding 1 M sulphuric acid stopping solution. The optical density of the samples were measured at 450 nm and the result was expressed as an index derived by dividing the absorbance value of the test serum by that of the cut-off control (OIE, 2004). A sample with a percentage Inhibition (PI) value of above 50 (that is, ≥ 50.0) on AniGen FMD NSP Ab Elisa was regarded as a positive result while a sample of PI value of less than 49 (that is, < 50.0) was regarded as a negative result.

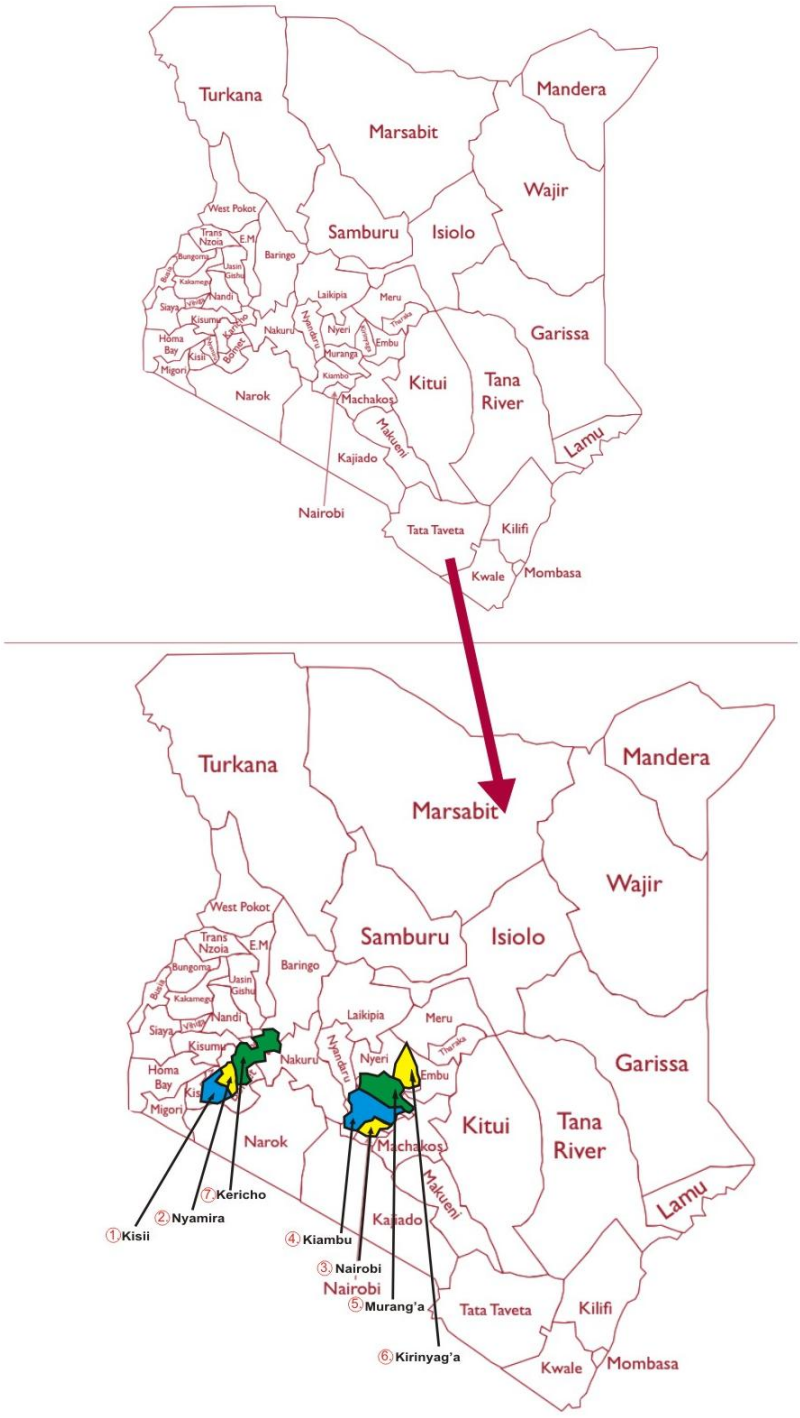

Figure 1. Map showing the new 47 administrative units of Kenya and the missing counties.

Data analysis

The results of both the NSP Elisa were then entered in an Excel spreadsheet (Microsoft Corp) with the following information; sampling location, age, sex, species, vaccination history, AniGen results. The data was imported to statistical package for social sciences (SPSS 20 version) for analysis. Descriptive statistical analysis was then done to determine the proportion of positive samples and

Table 1. The overall county prevalence.

No. of counties sampled	No of animals sampled	Results			
		Positive	Negative	Prevalence	95%CI
39	3709	1947	1762	52.5	49.97-55.03

Table 2. The seropositivity & distribution of different serotypes within sexes (M/F).

Number of serotypes			Sex		Total
			Female	Male	
	0	Count within sex	98	49	147
0		% within sex	22.0	19.0	41.0
		% of total	13.3	6.6	19.9
1	1		83	52	135
		% of total	11.2	7.0	18.2
2	2		123	45	168
		% of total	16.7	6.1	22.8
3	3		112	40	152
		% of total	15.2	5.4	20.6
4	4		70	27	97
		% of total	9.4	3.7	13.1
5	5		32	7	39
		% of Total	4.3	1	5.3
Total count			518	220	738
% of Total			70.2	29.8	100.0

serotype distribution across the country and at county level. Graphs were drawn using Microsoft excel.

RESULTS

NSP results (AniGen)

Of the total of 3,709 bovine serum samples subjected to NSP screening 70.4% (n = 2611) were females while 29.6% (n = 1098) were males. Of the 738 serum samples that were positive on NSP and subjected to LPBE, 29.8% (220/738) were males while the rest 70.2% (518/738) were females. 78% (171/220) of all males tested positive for at least one serotype where as 22% (49/220) tested negative (Table 2). Samples from adult animals (> 2 years) accounted for 44.0% (n = 1635). Those of 1 to 2 years accounted for 28.4% (n = 1,052) while serum samples belonging to age group less (< 1 year) were 1,022 representing 27.6% of the total samples. Of the total positive animals that tested positive to at least one serotype of FMD virus 181/591 (30.6 %) were of the age group <1 years. Those of between 1-2 years were 26.6 % (157/591) whereas as 253/591 of cases 42.8% were those of >2 years age (Table 3). Of the 3,709 subjected to NSP screening test, 1,947 of those were interpreted as positive representing 52.5% while the other 1,762 samples turned negative representing 47.5% (Table 1).

County prevalence

The national bovine FMD prevalence stood at 52.5%. From among the counties that had the highest FMD prevalence are those in Western part of the country and included: Baringo, Elgeyo Marakwet, Uasin Gishu, Nandi, Tranzoia, Bungoma, Kakamega and West Pokot counties, all of which had 100% seropositivity (Table 4). Bovine sera from Narok, Embu, Turkana, Migori, Garissa and Bomet counties showed high seropositivity of > 70%. Serum samples from Kajiado, Lamu, Tana River, Siaya, and Kisumu counties had medium to high seropositivity of between 50 and 70%. Counties that had low seropositivity of between 30 and 50% included Laikipia, Machakos, Kwale, Taita Taveta, Samburu, Tharaka Nithi, Nyandarua, Meru, Isiolo and Wajir. The counties that had low

Table 3. The seropositivity and distribution of different serotypes within age groups.

Serotype level	<1 year	1-2 years	>2 years	Total
0	46	46	55	147
1	49	41	45	135
2	52	40	76	168
3	50	37	65	152
4	22	25	50	97
5	8	14	17	39
≥ 1 serotype	181	157	253	591
% level	30.60	26.60	42.80	80.00
TOTAL	227	203	308	738
% Total	30.80	27.50	41.70	100

seropositivity of < 30% included Kitui, Makueni, Malindi, Kilifi, Marsabit and Nakuru. The counties of Mandera, Moyale and Nyeri had the lowest seroprevalence. 30 counties had seropositivity of more than 30% (> 30%). This accounted of 76.9% of the total serum samples (Table 4).

DISCUSSION

Serology results indicated a very high FMD prevalence in counties within Western and North Rift areas of Kenya. Some counties such as Uasin Gishu, Baringo, Elgeyo Marakwet, Tranzoia, Bungoma, Pokot and Kakamega recorded 100% prevalence. This may be attributable to the sub-optimal vaccination carried out in these areas, the presence of highly susceptible breeds in sedentary system, and the very high intensity of exposure because of the closeness to the national park (Mt. Elgon) with subsequent interaction with wild life (bufallos). The African Buffalo have been identified as the major carrier wildlife species of SAT serotypes in southern Africa and serological analyses suggest they are the major species infected in East Africa (Vosloo et al., 2002). In Pokot county for example, there is unrestricted high herd mobility, continuous contact and intermingling of different herds at water points and communal grazing areas.

Bovine sera from Narok, Embu, Turkana, Migori, Garissa and Bomet counties showed high prevalence of ≥ 70% (Table 4). The high seroprevalence in these areas may still be attributable to the sub-optimal vaccination together with cross border movement. Narok, Garissa and Turkana counties lie within borderlands where intense unrestricted border movements in search of pasture and water occur. These counties also harbor very vibrant livestock markets where contact among cattle from different sources is high therefore acting as one of the key factors of exposure.

Serum samples from Kajiado, Lamu, Tana River, Siaya, and Kisumu counties had medium to high FMD prevalence of between 50 to 70% (Table 4). Counties which had medium seropositivity of between 30 to 50% included Laikipia, Machakos, Kwale, Taita Taveta, Samburu, Tharaka Nithi, Nyandarua, Meru, Isiolo and Wajir. The moderately high seroprevalence may be associated with the sub-optimal vaccination carried out in these areas together with the livestock production system in which people are largely pastoralists and therefore high incidence of exposure. The distance to the national parks with subsequent mixing with wild ungulates may also explain the relatively high prevalence. In some counties like Taita Taveta, Machakos, Laikipia, Tana River and Kajiado, several animal ranches exist. Animals bought straight from the markets are taken here for fattening. These animals trek from markets for long distances and therefore disseminate the virus along the way.

Other counties had low prevalence of < 30% and included: Kitui, Makueni, Malindi, Kilifi, Marsabit and Nakuru. The counties of Mandera, Moyale and Nyeri had the lowest seroprevalence of 8.3, 7.5 and 5.3%, respectively. Very robust vaccination regime is carried out in the counties of Nyeri, Nakuru, Kitui and Makueni explaining the low prevalence of FMD in these areas. The indigenous type of cattle kept in Moyale, Kilifi, Mandera, Marsabit, Malindi, Kitui and parts of Makueni are resistant to the FMD virus infection.

The counties that make up the Somali ecosystem

Table 4. FMD prevalence in different counties.

Range	Counties	% Prevalence
Very High	Baringo	100
	Elgeyo Marakwet	100
	Uasin Gishu	100
	Nandi	100
	Trans nzoia	100
	Bungoma	100
	Kakamega	100
	West Pokot	100
High	Narok	90.4
≥70%	Embu	82.9
	Turkana	80
	Migori	75.6
	Garissa	72.9
	Bomet	70
Medium	Kajiado	67.6
50-70	Lamu	65.7
	Tana River	65.7
	Siaya	62.1
	Kisumu	51.1
Low	Laikipia	49.2
30-50	Machakos	43.2
	Kwale	42.2
	Taita Taveta	40.2
	Samburu	40
	Tharaka Nithi	40
	Nyandarua	37.5
	Meru	35
	Isolo	33.9
	Wajir	33
Very Low	Kitui	28.8
<30%	Makueni	26.8
	Malindi	26.7
	Kilifi	25.3
	Marsabit	18
	Nakuru	22.7
	Mandera	8.3
	Moyale	7.5
	Nyeri	5.3

namely Mandera, Wajir and Garissa had a mean prevalence of 38.1%, which was slightly lower than the national mean prevalence. The prevalence was highest in Garissa at 72.9% and lowest in Mandera at 8.3%. The above prevalence findings in the Somali ecosystem slightly varied with the findings of Chepkwony et al. (2012) which found mean prevalence of 45.3%. Garissa county had the highest FMD seroprevalence prevalence at 72.9% as compared to the other counties within SES. The main market for cattle in the SES is located in the town of Garissa and receives animals from Somalia, Ethiopia and other parts of the SES. About 1,500 to 4,000 are sold every wednesday and then trucked to Nairobi or Mombasa for the beef market or to the coastal ranches.

During trekking, animals meant for the market interact with local cattle and this may explain why the prevalence rates are high. There is need to enforce this practice of preventing interaction

Association between seropositivity, sex and age

The number of females sampled were higher at 70.2% (n = 518) compared to males 29.8% (220/738). There was no significant association between seropositivity and sex ($p \leq 0.05$). This finding was consistent with previous findings elsewhere (Esayas et al., 2009; Megersa et al., 2009), where sex appeared not to have a significant effect on seropositivity of FMD. On the contrary, in their report on the incidence of FMD among dairy cattle in northwest of Ethiopia, Hailu et al. (2010) documented a higher incidence rate in female (16.63%) cattle compared to that of males (1.37%).

The study revealed a significant association between seropositivity and age. There was great variation in seropositivity among the three age groups. The significantly higher seroprevalence in adults > 2 years compared to that of calves < 1 year and mid age 1 to 2 years was consistent with other previous findings largely done in Ethiopia which includes studies by Rufael et al. (2008) in Borena pastoral area and Molla et al. (2010) in Gamo Gofa and Sidama zones. On the other hand, the results were not in agreement with the findings of Esayas et al. (2009) who carried out a research in Bench Maji zone, southern of Ethiopia and documented no significant association. The high seropositivity of adults > 2 years may be associated with high frequency of exposure in addition to movement of animals especially in pastoral areas in search of water and pasture and intermingling with wildlife especially the African bufallo. In high potential counties, in some instances with sub = optimal vaccination cover, adult dairy animals kept for long time because of milk and with history of previous infection act as chronic carriers. Young cattle are herded around

homesteads and hence have less chance of exposure.

Conclusion

FMD is endemic in Kenya with variation in prevalence across several counties. Movement of the herds, sometimes across the borders, in search of water and pasture is one of the major factors that contribute to contact of cattle and subsequent spread of FMD. The movement and intermingling of domestic and wild animals is a risk factor for FMD and should be addressed in programmes to prevent and control FMD in the affected counties. It is recommended that sero-prevalence surveys be undertaken for wildlife and small stock so as to determine the role that these animals may play in the transmission of FMD in Kenya. Such surveys should be part of a systematic disease surveillance and data collection. It is recommended that strategic vaccination programmes using quadrivalent vaccines would reduce the risk posed by cattle obtained from these areas and which are transported and marketed freely for beef production.

REFERENCES

Belsham GJ (1993). Distinctive features of foot-and-mouth disease virus, a member of the Picornavirus family; aspects of virus protein synthesis, protein processing, and structure. Prog. Biophys. Molecul. Biol. 60:241-260.

Broonsvoort BM, Hamman SM, Tanya VN, Kitching RP, Morgan KL (2004). Risk factors for herdsman-reported foot and mouth disease in the Adamawa province of Cameroon. Prev. Vet. Med. (66):127-139.

Chepkwony EC, Gitao GC, Muchemi GM (2012). Seroprevalence of Foot and Mouth Disease in the Somali Eco-System in Kenya. Int. J. Anim. Vet. Adv. 4(3):198-203.

Coetzer JAW, Thomson GR, Tustin RC (1994). In Infectious diseases of livestock with special reference to Southern Africa. Infect. Dis. Livest.. Vol II, pp 825-852.

Dohoo I, Martin W, Stryhn H (2003). Veterinary Epidemiologic research. AVC Inc. Canada. pp. 41.

Esayas G, Gelagay A, Tsegalem A, Kassahun A (2009). Seroprevalence of foot and mouth disease in Bench Maji Zone Southwestern Ethiopia. Afr. J. Microb. Res. 5(21):3559-3563.

FAO (2005-2006) Empres watch, Foot and Mouth disease situation worldwide and major epidemiological Events, Retrieved from http://www.fao.org/docs/eims/upload/225050/focus-ON-1-07-en.pdf.

Hailu M, Mengistie T, Negussie H, Alemu S, Asaminew T (2010). Incidence of foot and mouth disease and its effect on milk yield in dairy cattle at Andassa dairy farm. Northwest Ethiopia. J. Agric. Biol. 1:969-973.

Megersa B, Beyene B, Abunna F, Regassa A, Amenu K, Rufael T (2009). Risk factors for foot and mouth disease seroprevalence indigenous cattle in Southern Ethiopia: the effect of production system. Trop. Anim. Health Prod. 41:891-898.

Molla B, Ayelet G, Asfaw Y, Jibril Y, Ganga G, Gelaye E (2010). Epidemiological Study on Foot and Mouth Disease in Cattle: Seroprevalence and Risk Factor Assessment in South Omo Zone, South-Western Ethiopia. Trans. Emerg. Infect. Dis. 57:340-347.

Rufael T, Catley A, Bogale A, Sahle M, Shiferaw Y (2008). Foot and Mouth Disease in Borana pastoral system, southern Ethiopia and implications for livelihoods and international trade. Trop. Anim. Health. Prod. 40:29-38.

Sellers RF (1971). Quantitative aspects of spread of foot and mouth disease. Vet. Bul. 41:431-439.

Vosloo W, Bastos ADS, Sangare O, Hargreaves S.K, Thomson GR (2002). Review of the status and control of foot and mouth disease in sub-Saharan Africa. OIE Sci. and tech. Rev., 21(3):437-447.

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Full Length Research Paper

Sero-epidemiology of camel brucellosis in the Afar region of Northeast Ethiopia

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Camel brucellosis represents a major public health concern, which affects social and economic development in developing countries. A cross-sectional study was conducted in three selected districts of Afar region of Ethiopia to determine seroprevalence of camel brucellosis. A total of 1152 camels from 168 camel herds were included in the study. All serum samples were consequently tested and confirmed serologically using Rose Bengal Plate Test (RBPT) and Complement Fixation Test (CFT). Risk factors analysis was also conducted using multivariable and univariate logistic regression analysis. As a result, 58 (5.0%) were RBPT reactors in which 47 (4.1%, 95% CI: 2.9 to 5.3%) were confirmed to be positive using CFT and at least one reactor camel was found in 37 (22.0%) of the total herds sampled. The statistical analysis indicated that herd size (OR=0.64; 95% CI: 0.42 to 0.98, P=0.04) and contact with other ruminants (OR=0.62; 95% CI: 0.47 to 0.82, P=0.001) were the major risk factors for the presence and transmission of the disease between animals. In addition, pluriparous (4.7%), abortive (5.7%), pregnant (6.6%) and lactating (4.1%) camels were found with higher seropositivity which contributed in transmission of the disease to calves, other ruminants as well as to humans, but this was not a statistically significant association (P>0.05). In conclusion, camel brucellosis is prevalent in this area of study and there is a need for planning and implementation of joint programs by stakeholders in prevention and control of the disease as well as raising public awareness in decreasing the distribution of the disease in the area.

Key words: Camel brucellosis, complement fixation test (CFT), Ethiopia, Rose Bengal plate test (RBPT), risk factors, seroprevalence.

INTRODUCTION

Brucellosis is an infectious disease of domestic and wild animals with serious zoonotic and economic implication in humans. The disease is an important public health problem in many parts of the world (Pal, 2007; Hadush and Pal, 2013). The disease in dromedary camels can be caused by *Brucella abortus, Brucella melitensis* and *Brucella ovis* (Seifert, 1996). Different studies showed that *B. abortus* and *B. melitensis* are the most frequently isolated from milk, aborted fetus and vaginal swabs of diseased camels (Radwan et al., 1992; Gameel et al., 1993; Agab et al., 1994; Abou-Eisha, 2000; Hamdy and Amin, 2002) and the transmission of brucellosis depends on the *Brucella* species being prevalent in other animals sharing their habitat and on husbandry (Musa et al., 2008).

Camels are not known to be primary hosts of *Brucella*, but they are susceptible to both *B. abortus* and *B. melitensis*. Consequently, the prevalence depends upon

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the infection rate in primary hosts being in contact with them. Brucellosis may spread from camels to humans, especially via milk. Therefore, the zoonotic risks from camel milk must be considered in view of the traditional African and Arabian preference for raw milk consumption (Cooper, 1991). Groups at high risk for brucellosis are animal health workers, butchers, farmers, and those who habitually consume raw camel milk and come in contact with these animals (Chukwu, 1987).

The uncontrolled movement of camel from infected herds or area to *Brucella* free herds or areas is the major obstacles in brucellosis eradication program (Radostits et al., 2007). Other management factors influencing inter-herd transmission are proximity to infected herds, water ways, and scavengers. Vaccination level, herd size, population density, methods of housing, and use of maternity pens also influence the probability of exposure to the infection (Crawford et al., 1990).

The disease can generally cause significant loss of productivity through late first calving age, long calving interval time, low herd fertility and comparatively low milk production, as in cattle may also happen in camels. The disease can also have an impact on export and import of animals constraining livestock trade (Radostits et al., 2007).

Africa hosts 80% of the world population of dromedary (16.5 million) of which 63% are attributed to East Africa (Wilson, 1998). Camels are a subset of huge livestock resources in Ethiopia with the population estimated to be over one million. This number ranks the country third in Africa after Somalia and Sudan and fourth in the world. The arid and semi-arid areas of the country that constitutes more than 60% of the total area are suitable for camel production. The eastern and southern parts of the country, namely, Afar, Somali and Borena are the major areas where camel husbandry is widely practiced. In these areas, the livelihood of the pastoral communities is certainly ensured by dromedaries (Teka, 1991; Wossene, 1991).

Therefore, the present study was contemplated to determine the seroprevalence and associated risk factors of camel brucellosis in selected districts of Afar regional state of Ethiopia.

MATERIALS AND METHODS

Study areas

Afar regional state is located in the Great Rift Valley, comprising semi-arid range land in Northeastern Ethiopia. According to regional estimates, the livestock population of Afar is about 10.12 million Trap Logic Unit (TLU) and out of this, about 859,580 (8.5%) are camels. The Afar Regional State has five administrative zones, which are further subdivided into 32 districts. Pastoralism and agro-pastoralism are the two major livelihood ways practiced in the region. The population of the region is estimated to be about 1.2 million of which 90% are pastoralists and 10% agro-pastoral (CSA, 2007). This study was conducted in three purposively selected districts of zone one namely, Mille, Chifra and Dubti. This study was conducted in the pastoral areas of the districts. Pastoralist association (PA) is the lowest administrative unit within the districts considered during the study. Accordingly, six PAs each from Mille and Chifra and five PAs from Dubti district were randomly selected.

Study design

A cross-sectional study design was conducted to determine the prevalence of *Brucella* infection in camels in the selected districts and to identify the potential risk factors associated with the sero-positivity. Camels above six months of age with no history of vaccination against brucellosis were selected. Camel's history such as sex, age, herd size, body condition score and contact with other ruminants was recorded.

Sampling methods

About 30% of the PAs in each of the districts were considered representative to the districts and included in the study on the basis of feasibility and affordability or cost. Hence, six PAs each from Mille and Chifra, and five PAs from Dubti were selected randomly. Multi-stage cluster sampling technique was used in this study by considering PAs as primary units, camel herds found in each PAs as secondary units and selected camel herds as tertiary units. Cluster sampling was the suitable method for this study as constructing sample frame for random sampling was not possible in pastoral production system. Since there was no previous year's prevalence of brucellosis in the districts, the average expected prevalence was assumed to be 50% for the areas within 95% confidence interval (CI) at 5% desired accuracy and the total sample is 384 (Thrusfield, 2005). However, cluster sampling can lead to an increased sampling variance and a large sample size would be required to reduce variance to acceptable levels. Therefore, the sample size was increased by three folds and a total of 1152 camels from 168 herds were considered for the study.

Samples collection

Approximately 6 to 8 ml of blood sample was collected from jugular vein of each camel using plain vacutainer tubes. The collected blood samples were allowed to clot at room temperature and serum was separated from clotted blood by decanting to plastic criovials. Separated sera were stored at –20°C for further serological testing.

Serological tests

All sera samples collected were initially screened by Rose Bengal Plate Test (RBPT) using RBPT antigen (IVRI, Indian Veterinary Research Institute, Izatnagar, U.P., India). Sera and antigen were taken from refrigerator and left at room temperature for half an hour before the test to maintain to room temperature and processed following the test procedure recommended by Alton et al. (1975) and OIE (2004). The result was recorded as ++++ (coarse clumping and clearing), +++ (clumping and some clearing), ++ (visible fine agglutination), + (weak fine agglutinations using magnifying glass) in case of positive reactions, and 0 (no agglutinations) in negative reactions.

Sera that tested positive to the RBPT were further tested using Complement Fixation Test (CFT) for confirmation using Standard *B. abortus* antigen S99 (CVL, New Haw Weybridge, and Surry KT15 3NB, UK). Preparation of the reagent was evaluated by titration and performed according to protocols recommended by World Organisation for Animal Health (OIE, 2004). Sera with strong reaction, more than 75% fixation of complement (3+) at a dilution of

1:5 or at least with 50% fixation of complement (2+) at a dilution of 1:10 and above were classified as positive and lack of fixation/complete hemolysis was considered as negative. Samples were considered positive for brucellosis if they were positive for both RBPT and CFT.

Data management and statistical analysis

Descriptive and analytic statistics were computed using software SPSS® Version 20. Logistic regression and Chi-square test (χ^2) were employed to identify possible risk factors associated with seropositive camels. The degree of association was computed using odds ratio (OR) signified by 95% confidence intervals (Thrusfield, 2005).

RESULTS

A total of 1152 sera sample were collected from 168 camel herds with no previous history of vaccination against brucellosis from three districts. Out of 1152 tested samples, only 58 (5.0%) were found positive by RBPT and further confirmation with CFT showed that 47 (4.1%, 95% CI: 2.9 to 5.3%) were positive out of the 58 RBPT reactors. Further analysis of the confirmed positive samples (n=47) revealed that Mille district had slightly highest prevalence for brucellosis (n=20) (5.2%, 95% CI: 3.0 to 7.4%) followed by Dubti (n=15) (3.9%, 95% CI: 1.9 to 5.9%) and Chifra (n=12) (3.1%, 95% CI: 1.3 to 4.9%), respectively.

The Chi-square analysis revealed that it was only herd size (χ^2=8.043, P=0.018) and contact with other ruminants (χ^2=13.397, P=0.004) that showed statistically significant (P<0.05) association with seropositivity of camel brucellosis than the other risk factors considered during the study.

The univariable logistic regression analysis of the putative risk factors indicated statistically significant difference on seroprevalence of brucellosis between camels in contact with cattle (OR=3.546, 95% CI: 1.358 to 9.259, P<0.05) and camels in contact with small ruminants (OR=2.324, 95% CI: 1.221 to 4.424, P<0.05) than camels with no contact with any other ruminant. Camels in contact with both cattle and small ruminant (OR=1.854, 95% CI: 0.862 to 3.989, P>0.05) showed no statistically significant difference (Table 1).

Multivariate logistic regression analysis of risk factors determined herd size and contact with other ruminants (P<0.05) as the major risk factor for the occurrence of camel brucellosis seropositivity when compared with the rest risk factors considered in the analysis. When these two major risk factors are compared, contact with other ruminants was highly associated with the occurrence of seropositivity of the disease in camels than herd size with statistically highly significant association (P=0.001) in the study areas (Table 2).

Advance in herd size and contact with other ruminants were significantly associated with the infection (P<0.05) and have an effect on seropositivity when other factors which were not statistically significant (P>0.05) were removed (Table 3).

Out of the total 1152 camels examined, 810 were she-camels in which 598 were at the age of puberty with 28 (4.7%) of them seropositive to *Brucella* infection. Similarly, out of 256 male camels which were at age of puberty, 12 (4.7%) were seropositive (Table 4).

DISCUSSION

Previous serological surveys in Ethiopia showed that the disease is prevalent in different camel rearing areas of the country (Domenech, 1977; Richard, 1980; Teshome et al., 2003; Zewolda and Wereta, 2012). A study on camel husbandry practice in eastern part of the country by Getahun and Kassa (2000) indicated abortion rates and stillbirths of 9 and 4.3%, respectively, for which brucellosis is more likely to be incriminated. Hence, a cross-sectional study was conducted in three selected districts of Afar region to determine the prevalence of brucellosis in camels and to assess the associated risk factors in these areas.

In the present study, an overall seroprevalence of 4.1% (95% CI: 2.9 to 5.3%) was recorded in camels using both RBPT and CFT. This finding is in agreement with the results recorded by Teshome et al. (2003) and Domenech (1977) in Borena with prevalence of 4.2 and 4.4%, respectively and with Gameel et al. (1993) who recorded a prevalence of 4.1% in Libya. However, the result of this study is lower than the observation recorded by Richard (1980), Teshome et al. (2003) and Zewolda and Wereta (2012) with prevalence of 5.5, 5.7 and 7.6%, respectively in Afar region. It is also much lower than 6.0 to 38.0% reported by Wilson et al. (1990) in Kenya and 8.0% by Osman and Adlam (1987) in Sudan. But the observation of current investigation is higher than 0.4 to 2.5% reported by Bekele (2004) in Borena in which the variation could be due to the difference in sample size used and agro-ecology. The differences could also be due to variations in animal management and production systems. Kenya and Sudan are characterized by mixed farming (Wilson et al., 1990; Schwartz and Dioli, 1992) in which fewer animals are raised and they are kept separately, whereas in the camel-rearing areas of Ethiopia, large numbers of different species of animals are raised on communal pastures and watering areas.

Since brucellosis is considered as disease of herd importance, in this study higher herd level seropositivity of 22.0% was found than 16% recorded by Bekele (2004) in Borena. This could be due to the presence of high number of camels in the herds and mixing of aborting camels with normally parturient camels. Even though, brucellosis was detected in all the three districts with slight variation in prevalence, it was not statistically significant difference (P>0.05). This could be attributed to the similarity in agro-ecological conditions and livestock management system in the districts.

Table 1. Univariable logistic regression analysis of the putative risk factors.

Risk factor	Category	OR	95% CI	P value
Districts	Mille*	-	-	-
	Chifra	1.175	0.620-2.226	0.621
	Dubti	0.786	0.394-1.569	0.495
Sex	Male*	1.122	0.626-2.009	0.699
	Female			
Age	≤4 years*	1.041	0.597-1.815	0.888
	>4 years			
Body condition score	Good*	-	-	-
	Fair	2.075	0.888-4.853	0.092
	Poor	1.430	0.633-3.233	0.390
Herd size	≤10 camels*	-	-	-
	11-20 camels	1.948	0.989-3.837	0.054
	>20 camels	0.608	0.294-1.259	0.180
Contact with other ruminants	No contact*	-	-	-
	With cattle	3.546	1.358-9.259	0.010
	With cattle and SR	1.854	0.862-3.989	0.114
	With SR	2.324	1.221-4.424	0.010
Parity	No parturition*	-	-	-
	Primiparous	0.477	0.095-2.390	0.368
	Pluriparous	0.834	0.382-1.817	0.647
Reproductive problems	No RP*	-	-	-
	Abortion	0.837	0.244-2.868	0.777
	Still birth	1.444	0.576-3.621	0.433
	RFM	0.897	0.286-2.809	0.852
Physiological status	Heifer*	-	-	-
	Dry	0.477	0.095-2.390	0.368
	Lactating	0.545	0.191-1.555	0.256
	Pregnant	0.594	0.233-1.516	0.276

SR: Small ruminant; RP: Reproductive problems; RFM: Retained fetal membrane. *Reference category; OR: Odds ratio; CI: Confidence interval.

In contrary to the established fact, no significant difference was observed in the prevalence of brucellosis between sexes. The number of breeding males kept by the pastoralists in the camel herds of the present study was very small on which random sampling method was applied and this predictably bias the statistical analysis. Even though Hirsh and Zee (1999) have reported that male animals are less susceptible to *Brucella* infection due to the absence of erythritol, other authors (Waghela et al., 1978; Abu-Damir et al., 1984; Abbas et al., 1987) reported equal distribution of *Brucella* antibodies between both sexes. On the contrary, Bekele (2004) from Ethiopia, Yagoub et al. *(*1990) and Agab et al. (1994) from Sudan, and Ajogi and Adamu (1998) from Nigeria revealed the likelihood of occurrence of infection is higher in female than male animals. Relatively higher susceptibility of she-camels could be due to the fact that they have more physiological stresses than the males (Walker, 1999).

Camels produced under extensive production system reach maturity at 3 to 4 years of age (Wilson, 1998). Tefera and Gebreab (2001) recorded age at puberty and first calving to be 4 and 5 years, respectively for females

Table 2. Multivariate logistic regression analysis of risk factors.

Risk factor	OR	95% CI	P value
Districts	0.854	0.593-1.231	0.398
Sex	0.961	0.503-1.836	0.904
Age	1.146	0.615-2.136	0.668
Body condition score	0.724	0.466-1.126	0.152
Herd size	0.640	0.421-0.975	0.038*
Contact with other ruminants	0.619	0.470-0.815	0.001*
Parity	1.126	0.602-2.104	0.711
Reproductive problems	0.952	0.680-1.332	0.733
Physiological status	1.285	0.802-2.060	0.297

OR: Odds ratio; CI= Confidence interval.*Herd size and contact with other ruminants were statistically found the risk factors for the occurrence of camel brucellosis (P<0.05) at 95% level of confidence.

Table 3. Putative effects of advance in herd size and contact with other ruminants on seroprevalence.

Risk factors	OR	95% CI	P value
Herd size	0.656	0.450-0.957	0.029
Contact with other ruminants	0.681	0.527-0.879	0.003

OR: Odds ratio; CI: Confidence interval.

Table 4. Status of seropositivity to camel brucellosis before and after age of puberty

Age	No. examined			CFT positive (% per sex)		
	Male	Female	Total	Male	Female	Total
Before puberty (<3 years)	86	212	298	2 (2.3)	5 (2.4)	7 (2.4)
After puberty (≥3 years)	256	598	854	12 (4.7)	28 (4.7)	40 (4.7)
Total	342	810	1152	14 (4.1)	33 (4.1)	47 (4.1)

whereas males had age of 5 years at puberty in Eastern Ethiopia. Wossene (1991) also reported the same age for puberty and first calving in Ogaden female dromedaries. Accordingly, age was classified as 'before and after puberty' in order to see the distribution of the disease in immatured and sexually matured camels and camels of 3 years and above are considered matured (at age of puberty) and less than 3 years considered sexually immatured for this study. Accordingly, out of the she-camels examined, 598 with seropositivity of 28 (4.7%) were at age of puberty (that is three years and above) and 212 with seropositivity of 5 (2.4%) were immatured (less than three years of age). Likewise, out of male camels examined, 86 with seropositivity of 2 (2.3%) were immatured and 256 with seropositivity of 12 (4.7%) were at age of puberty in which they can mate and used for breeding in the herds. This indicated that more seropositivity to camel brucellosis was seen in adults than in young camels as it is a disease of sexually matured animals. Hence, the presence of seropositive breeding males and she-camels were considered as risk factors playing a role in the transmission of the disease to other animals in the study districts.

Although no statistically significant difference (P>0.05) was observed between the two age groups, slightly higher seroprevalence was found in those groups with age of greater than 4 years (4.4%) than those groups with age of less than and equal to 4 years (3.6%). Sexually matured animals are more prone to *Brucella* infection than sexually immatured animals of either sex (Radostits et al., 2007). On the other hand, it is also true that younger animals tend to be more resistant to infection and frequently clear an established infection, although latent infections can occur (Walker, 1999; Quinn et al., 2004). This may be due to the fact that sex hormones and erythritol, which stimulate the growth and multiplication of *Brucella* organisms, tend to increase in concentration with age and sexual maturity (Radostits et al., 2007).

Immunity against various infections can be depressed

due to different reasons in which stress and feed play a greater role. Underfed animals are expected to have a decreased immunity that is manifested by poor body condition (Faye and Bengoumi, 2006; Radostits et al., 2007). Therefore, body condition of the camels was considered during the study to see the distribution of the infection in different body condition scores. But, high seropositivity was found in camels with good (5.7%) and fair (3.6%) body condition score than camels with poor (3.3%) body condition score and the difference was statistically not significant (P>0.05). This illogical finding could be due to the condition that majority of the camels sampled (81.4%) were with good and fair body condition score and only 18.6% of the total samples were with poor body condition.

This study revealed that herd size was significantly associated with brucellosis in camels (P<0.05). Consequently, herd size was statistically identified to be the second major risk factor for brucellosis to occur in relation to other factors (P=0.04). This is in accordance with the findings of Bekele (2004) and Zewolda and Wereta (2012) in Borena and Afar, respectively.

As herd size increases, the chance of contact between animals increases leading to more chances of infection (Abbas and Agab, 2002), which is particularly more important during calving or abortion when most of the *Brucella* contamination occur (Gameel et al., 1993; Agab et al., 1994). Thus, herd size and density of animal population together with poor management are directly related to infection rate (Abbas et al., 1987; Abou-Eisha, 2000; Wernery and Kaaden, 2002).

High number of camels, cattle and small ruminant diversification were noticed in the study districts. Such animal species distribution and diversification is common to other areas and has economic and ecological advantages (Wilson et al., 1990; Getahun and Kassa, 2000). However, it increases the chance of brucellosis and other disease transmission from other infected ruminants to dromedaries (Andreani et al., 1982; Radwan et al., 1992). In the present study, there was highly statistically significant difference (P<0.05) in the prevalence of the disease in the camel population which had contact with other ruminants. It is considered as the first major risk factor (P=0.001) for the occurrence of brucellosis when compared with other factors and even when compared with herd size. Those camel herds which usually made close contact on pasture with cattle were 3.6 times more at risk of being seropositive to the disease than those with no contact and those mixed with small ruminant were 2.3 times more at risk than those camels not mixed with other ruminants.

There was no statistically significant association (P>0.05) between parity and the seroprevalence of the disease. The seropositivity of she-camels with the history of single parity and more than one parity were 3.9 and 4.7%, respectively which is slightly higher than those with no parturition (2.7%). Higher seropositivity was recorded in she-camels which gave birth to more than one calf than those with single parity. This is therefore, in consistent with the previous study by Bekele (2004) and Zewolda and Wereta (2012) where higher reactors were recorded in camels with more than one parity, compared to other group of camels. The possible explanation for this is that because the repeated exposure of the she-camels to parturition and other physiological stress increases the probability of acquiring *Brucella* infection.

This study also illustrated that there was no statistically significant difference (P>0.05) in distribution of *Brucella* infection among the different reproductive problems and physiological statuses of the she-camels considered. Among the she-camels with the history of reproductive problems, abortion (5.7%) and retained fetal membrane (4.1%) were found with slightly higher seropositivity to *Brucella* infection. Moreover, the pregnant (6.6%) and lactating (4.1%) she-camels showed higher seroprevalence of brucellosis. Therefore, the pregnant she-camels during delivery time and the lactating she-camels excreting the organisms through milk were the risk factors for transmission of the infection to calves, other animals and even to human beings (Abbas and Agab, 2002).

Conclusion

The seroprevalence recorded in the present study revealed that brucellosis is a widespread and established disease in the three camel rearing districts. The risk factors identified for the presence and transmission of the disease from animal to animal were sex, age, body condition, herd size, contact with other ruminants, parity, reproductive problems and physiological status. However, according to the statistical analysis, advance in herd size and contact with other ruminants were found to be the major risk factors for the transmission of the disease from camel to camel as well as from area to area. Moreover, higher seropositivity was recorded in female, matured, pluriparous, pregnant, abortive and lactating camels which contributed for transmission of the disease. Traditional husbandry and poor management practices, mixing with other animals and unrestricted movement of camels were thought to support spread of the disease in the study area. Therefore, a strategic plan should be developed to support in decreasing the chance of contact of animals at different situations and to keep only few healthy and fertile camels per herd together with immunization campaigns, and public health education on modern animal husbandry and disease prevention techniques should be imparted continuously.

REFERENCES

Abbas B, Agab H (2002). A review of camel Brucellosis. J. Prev. Vet. Med. 55:47-56.

Abbas B, El-Zubeir AE, Yassin TT (1987). Survey for certain zoonotic diseases in camels in Sudan. Rev. Elev. Med. Vet. Pays. Trop. 40:231-233.
Abou-Eisha AM (2000). Brucellosis in camels and its relation to public health. Asuit. Vet. Med. J. 44:54-64.
Abu-Damir H, Kenyon SJ, Khalafalla AE, Idris OF (1984). *Brucella* antibodies in Sudanese camels. Trop. Anim. Health. Prod. 16:209-212.
Agab H, Abbas B, El-Jack AH, Maoun IE (1994). First report on the isolation of *Brucella abortus* biovar 3 from camel (*Camelus dromedarius*) in the Sudan. Rev. Elev. Med. Vet. Pays. Trop. 47:361-363.
Ajogi I, Adamu NB (1998). Camel brucellosis in semiarid zones of Nigeria. In: Proceeding of ARC Onderstepoort, OIE International Congress. August, 1998. Berg En-Dal, South Africa, P. 16.
Alton GG, Jones LM, Pietz DE (1975). Laboratory Techniques in Brucellosis. 2nd ed. Geneva: WHO, pp. 23-124.
Andreani E, Prospori S, Salim AH, Arush AM (1982). Serological and bacteriological investigation on brucellosis in domestic ruminants of the Somali Democratic Republic. Rev. Elev. Med. Vet. Pays. Trop. 35:329-333.
Bekele MB (2004). Sero-epidemiological study of brucellosis in camels (C*amelus dromedarius*) in Borena lowland pastoral areas, Southern Ethiopia. MSc Thesis. Addis Ababa University, Faculty of Veterinary Medicine, Debre Zeit, Ethiopia.
CSA (Central Statistical Agency) (2007). Human and animal population census in Afar region. Addis Ababa, Ethiopia.
Chukwu CC (1987). Brucellosis in Africa Part II: Importance. Bull. Anim. Health. Prod. Afr. 35:92-98.
Cooper CW (1991). The epidemiology of human brucellosis in a well defined urban population in Saudi Arabia. J. Trop. Med. Hyg. 94:416–422.
Crawford P, Huber D, Adams S (1990). Epidemiology and surveillance. In: Nielson K, Duncan R (eds.). Animal brucellosis. CRC Press, Boca Raton, Florida, pp. 131-151.
Domenech J (1977). Brucellosis of dromedaries in Ethiopia. Rev. Elev. Med. Vet. Pays. Trop. 30:141-142.
Faye B, Bengoumi M (2006). Assessment of body condition and body composition in camel by barymetric measurements. J. Camel Pract. Res. 13:67-72.
Gameel SE, Mohammed SO, Mustafa AA, Azwai SM (1993). Prevalence of camel brucellosis in Libya. Trop. Anim. Health. Prod. 25:91-93.
Getahun T, Kassa B (2000). Camel husbandry practices, households and herd characteristics in eastern Ethiopia. In: Proceedings of the Ethiopian Society of Animal Production (ESAP). August 2000, Addis Ababa, Ethiopia, pp. 168-179.
Hadush A, Pal M (2013). Brucellosis - An infectious re-emerging bacterial zoonosis of global importance. Int. J. Livest. Res. 3:28-34.
Hamdy ME, Amin AS (2002). Detection of *Brucella* in the milk of infected cattle, sheep, goats and camels by PCR. Vet. J. 16:299-305.
Hirsh DC, Zee YC (1999). Veterinary microbiology. Blackwell Science, Cambridge, Massachusetts, pp. 196-203.
Musa MT, Eisa MZ, El-Sanousi EM, Abdel-Wahab MB, Perrett L (2008). Brucellosis in camels (*Camelus dromedarius*) in Darfur, Western Sudan. J. Comp. Pathol. 138:151-155.
OIE (2004). Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. 5th Ed. Office International des Epizootics. Paris, pp. 409-438.
Osman AM, Adlam AM (1987). Brucellosis in domestic animals: Prevalence, diagnosis and control in Sudan. Rev. Sci. Tech. Off. Int. Epiz. 6:67-72.
Pal M (2007). Zoonoses. 2nd Ed. Satyam publishers. Jaipur, India, pp. 98-99.
Quinn PJ, Carter ME, Markey B, Carter GR (2004). Clinical Veterinary Microbiology, (Eds.). Mosby, Edinburgh, pp. 168-172; 261-267.
Radostits M, Blood C, Gay C (2007). Veterinary Medicine: A text book of the disease of cattle, sheep, goats, pigs and horse. 10th ed. Bailliere Tindall. London, pp. 984-988.
Radwan AI, Bekairi SJ, Prasad PV (1992). Serological and bacteriological study of brucellosis in camels in central Saudi Arabia. Rev. Sci. Tech. Off. Int. Epiz. 11:837-844.
Richard LK (1980). A seroprevalence study of camel brucellosis in camel-rearing areas ofEthiopia. Trop. Anim. Health Prod. 35:381-389.
Schwartz HJ, Dioli M (1992). The one-humped camel in eastern Africa. A pictorial guide to diseases, health care and management, Verlag Josef Margraf Scientific Books (eds.). Verlag, Weikersheim, P. 282.
Seifert SH (1996). Tropical Animal Health, 2nd Ed. Dordrecht: Dordrecht Kluwer Academic Publishers, pp. 358-362.
Tefera M, Gebreab F (2001). A study on productivity and diseases of camels in eastern Ethiopia. Trop. Anim. Health Prod. 33:265-274.
Teka T (1991). The dromedaries in eastern African countries. The Nomadic People 29:3-9.
Teshome H, Molla B, Tibbo M (2003). A seroprevalence study of camel brucellosis in three camel-rearing regions of Ethiopia. Trop. Anim. Health Prod. 35:381-389.
Thrusfield M (2005). Veterinary Epidemiology. 2nd ed. Blackwell Science Ltd., London, pp. 178-198.
Waghela S, Fazil MA, Gathuma JM, Kagunya DK (1978). A serological survey of brucellosis in camels in north eastern province of Kenya. Trop. Anim. Health Prod. 10:28-29.
Walker RL (1999). Veterinary Microbiology. Blackwells Science. Cambridge, Massachusetts, pp. 196-203.
Wernery U, Kaaden OR (2002). Infectious diseases of *Camelids*. Blackwell Science Inc. London, pp. 99-116.
Wilson RT (1998). Camels. Macmillan Education Ltd. London, P.134.
Wilson RT, Araya A, Melaku A (1990). The one humped camel: Analytical and Annotated bibliography, 1980 –1989. Technical paper series No 3. United Nation Sudano – Sahelian Office (UNSO): New York, USA, p. 300.
Wossene A (1991). Traditional husbandry practices and major health problems of camels in the Ogaden, Ethiopia. The Nomadic People 29:21-30.
Yagoub IA, Mohamed AA, Salim MO (1990). Serological survey of *Brucella abortus* antibody prevalence in the one-humped camels (*Camelus dromedarius*) from eastern Sudan. Rev. Elev. Med. Vet. Pays. Trop. 43:167-171.
Zewolda SW, Wereta MH (2012). Seroprevalence of *Brucella* infection in camel and its public health significance in selected districts of Afar region, Ethiopia. J. Environ. Occupat. Sci. 1:91-98.

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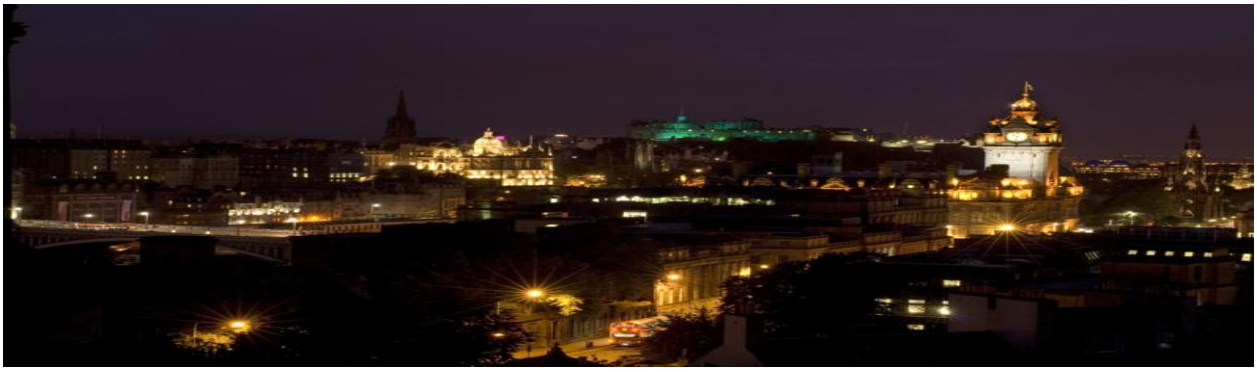

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