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Cole (2000), Steddy et al. (2003), (Kelebeni, 1983), (Bane and Jake, 1992), (Chege, 1998; Cohen, 1987a,b;Tristan, 1993,1995), (Kumasi et al., 2001)

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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

Photoperiod as a factor for studying fluctuations of seminal traits in rams during breeding and non-breeding season

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The main purpose of this study was to evaluate the influence of the photoperiod on the seminal traits of crossbreed wool-producing rams throughout one year period. For the effect of photoperiod, two periods were considered: decreasing daylight length (summer and autumn) and increasing daylight length (winter and spring). For this study, 5 Baluchi × Moghani (BL × MG) and 5 Arkharmerino × Moghani (AM × MG) rams were used. Semen collections were performed from first of October, 2010 to the end of September, 2011. After a training period of 2 weeks, semen ejaculates were evaluated for volume, total sperm count/ejaculate (TSE), sperm concentration, semen color, wave motion, percentage of progressive motility, percentage of live and abnormal sperm, pH, methylene blue reduction time (MBRT) and semen index (semen volume × sperm concentration/ml × live sperm% × progressive motility%). Analysis of the yearlong data showed that semen samples with the best quality were collected in September to November ($P < 0.05$). Despite the fact that no statistical differences were found between the two genetic groups ($P > 0.05$), significant seasonal variations of semen traits were observed for all seminal traits except for progressive motility, percentage of live sperm and MBRT. Although there were significant seasonal changes in seminal traits of the crosses; the fresh semen showed adequate quality to be used for breeding purposes throughout the year.

Key words: Crossbred rams, photoperiod, seasonal variation, spermatozoa.

INTRODUCTION

Sheep production is a traditional economic activity used for meat and milk production in Iran. The most important limiting factor for economic development of sheep industry is seasonal breeding and lambing in the part of year. The Arkharmerino is a breed of sheep obtained by crossbreeding between wild Arkhar rams with ewes of the Novocaucasian MerinoPrécoce and Rambouillet breeds (Ernst and Dmitriev, 2007). These two genetic groups are developed targeting the improvement of local breeds (Baluchi and Moghani) for wool traits. There is no published information on the reproductive traits of Baluchi × Moghani or on Arkharmerino × Moghani genetic groups. The marked seasonality of breeding activity linked to annual cycle of daily photoperiod (Rosa and Bryant, 2003). Understanding of their sperm quality in non-breeding season will be helpful for developing sheep industry. In contrast to ewes and most horse mares that become anovulatory outside the breeding season,

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stallions and rams are not azoospermic during the non-breeding season despite a significant reduction in sperm production or quality (Aurich et al., 1996). Also, overall physiological and behavioral sexual variations are also less pronounced in rams than ewe (Rosa and Bryant, 2003). Therefore, yearlong comparative studies comprising breeding and non-breeding seasons in rams will be useful for understanding their reproductive physiology. Furthermore, due to the revolution in assisted reproductive technologies in domestic animals in Iran, there has been a growing interest and necessity to have more information concerning the reproductive physiology of farm animals (Talebi et al., 2009). The breeding season starts in most ovine breeds during summer or early autumn (Chemineau et al., 1992), and its length varies largely among breeds but in general it ends during the winter (Hafez, 1952).

Many other factors affect the semen characteristics, including nutrition, social environment, the presence of females, geographical location, age, testicle and body conformation, libido and management system, as reported in many studies (Nowakowski and Cwikla, 1994; Mandiki et al., 1998; Al-Ghalban et al., 2004; Zamiri and Khodaei, 2005; Zarazaga et al., 2005), but the photoperiod and breed are primary factors regulating the seasonal reproduction. Therefore, these parameters became preference for many researchers (Simplicio et al., 1982; Ibrahim, 1997; Karagiannidis et al., 2000; Kafi et al., 2004; Al-Ghalban et al., 2004; Barkawi et al., 2006; Talebi et al., 2009; Zamiri et al., 2010). In intensive management systems, a significant number of ewes are inseminated in non-breeding season (Colas et al., 1988, 1990). Therefore, a detection of semen characteristic of the crosses in non-breeding season is necessary.

Although information is available on the level of semen quality of ram (Mohamed, 1978; Haynes and Schanbacher, 1983; Nowakowski and Cwikla, 1994; Ibrahim, 1997) but the present study is the first report about semen characteristics of the Arkharmerino × Moghani and Baluchi × Moghani rams reared at the Northwest of Iran. The study includes the evaluation of quality of semen in breeding and non-breeding seasons. Additionally, information on reference values for semen characteristics, evaluation of the effects of photoperiod on ram semen characteristics at this latitude, along with the evaluation of putative differences on semen traits between the two genetic groups was another pursuit for this study.

MATERIALS AND METHODS

Location

This trial was performed at the sheep breeding Research Center, in Tabriz (38° 02' N, 46° 27' E and an altitude of 1567 m above sea level), East Azerbaijan, Iran. This experiment was carried out from October, 2010 to September, 2011, with the training period performed during early September, 2010.

Animals

Ten crossbreed and fertile rams consisting of 5 Baluchi × Moghani (BL × MG) and 5 Arkhar Merino × Moghani (AM × MG), aged of 3 to 6 years old and with a live weight of 74 to 88 kg were used in this study. The animals were maintained under natural photoperiod and equal levels of nutrition per day 20% concentrate (75% barley, 25% corn, soya, bran) and 80% alfalfa hay. The rams were separated of the herd and housed in a large cover shelter with an open precinct for walking freely. Levels of nutrition remained equal and all rams had free access to salty stones and were sent for drinking of fresh water twice or three times a day. Hoof trimming, shearing, crutching, dipping, disease prevention and other general management were checked up during the study. The temperature, relative humidity and photoperiod were recorded during the experiment (Table 1). The rams were trained (at beginning of September, for 15 days) to semen collection with an artificial vagina (AV) in the mating pen (210 cm length, 60 cm width, and 120 cm height). Both training and semen collections were performed in the presence of an anestrus teaser ewe with quiet temperament.

Semen collection

Ejaculation intervals of each ram were five days throughout the study. Semen collection was performed by AV. Collecting glass of AV was warmed at 37°C before the operation and was maintained at this temperature until processed. The fresh semen samples were immediately transferred to the laboratory (avoiding sunlight) and were analyzed.

Semen analysis

Seminal traits of the fresh semen were evaluated according to the procedure adopted by Evans and Maxwell (1987). Volume of ejaculates (SV) was measured in a conical tube graduated at 0.1 ml intervals. Semen pH was measured both by a pen type pH-meter (with 0.1 grades, model 8685, AZ Instrument, Taiwan) and a universal indicator paper (with 1.0 grade, Merck, Germany). Spermatozoa concentration was determined on a Thoma hemocytometer slide (depth 0.1 mm). Fresh semen was diluted using 0.1 M sodium citrate dehydrate 2.9% (pH = 6.7 to 6.9) plus one drop of formalin (1: 400), and the sperm counted under a light microscope (400× magnification). The overall number of sperm per ejaculate (TSE) was then calculated (volume × density). Wave motion (WM) of fresh semen was evaluated (100× magnification) (Evans and Maxwell, 1987). The assessment of the spermatozoa progressive motility was a visual scaled from 0 to 100% on basis of suspended droplet slide and on a heated (37°C) stage using phase-contrast optics (×400). It has been evaluated in increments of 5 or 10 percentage points. This slide showed individual spermatozoa with more lucidity, as it will be more comfortable for estimating spermatozoa progressive motility.

For sperm morphology (SAB) and sperm live/dead ratio (SL), semen was stained with eosin-nigrosin stain and examined microscopically (×400). From several areas of the slide, about 300 spermatozoa were counted for vitality and 200 for abnormality. Results were expressed as percentage. Metabolic activity of spermatozoa was measured by the methylene blue reduction time

(MBRT) method, based on color change from blue to colorless at 37°C. In a thin and transparent tube (1 mm diameter), 0.2 ml semen was added to 0.2 ml of methylene blue and time for color change recorded.

Semen index (SI) = semen volume × spermatozoa concentration/ml × live spermatozoa% × progressive motility% was calculated as an indicator of semen quality.

Statistical analysis

All statistical analysis was performed using the statistical analysis system (SAS, 1996). The MIXED procedure of SAS was used for analysis of the repeated measurement data. For semen volume, semen color (SC), abnormality and MBRT traits, the outlier data has been deleted. Mean values were compared with Tukey test. Values were considered to be statistically significant at $P \leq 0.05$. Pearson correlation coefficient was calculated to evaluate the relationship between quality and quantity of semen attributes.

RESULTS

Descriptive statistics for seminal parameters are shown in Table 2. Results of seasonal fluctuations of semen characteristics were surveyed for eleven traits and presented in Tables 3 and 4. Photoperiod influence on semen characteristics has been shown in Table 5. Semen quantity traits including semen volume, sperm concentration, TSE and semen color were significantly influenced by season of the year (Table 3). In AM × MG and BL × MG genetic groups, minimum and maximum values of semen volume were recorded at spring and autumn, respectively ($P < 0.01$). Semen volume increased from end of June and received the highest mean values at October, and decreased at the end of October gradually. This falling process followed during autumn and winter except for BL × MG in April (Figure 1).

The highest mean values of live spermatozoa were recorded in December (in BL × MG) and September (in MR × MG). The highest and the lowest percentage of live spermatozoa were recorded in September (71.56 ± 1.56) and June (64.66 ± 1.65), respectively in BL × MG genetic group. Monthly variations of percentage of live spermatozoa are shown in Figure 2. In AM × MG rams differences between the spring (the part of non-breeding season) and other season were significant ($P < 0.01$). In the BL × MG, a significant difference was found between the non breeding season (spring and winter) and the breeding season ($P < 0.01$). In both genetic groups sperm concentration, TSE and semen color were highest in winter and lowest in spring, and the peak for mean values of semen pH was registered in spring. In BL × MG, rams mean values of semen pH increased concurrently with the spring. The highest semen index was observed during autumn (October) and the lowest mean values in spring (June). In AM × MG rams there was significant difference in wave motion between breeding season (summer and autumn) and non-breeding season (Table 4). The results in the AM × MG and BL × MG rams demonstrated that individual progressive motility of spermatozoa was higher in the breeding season (autumn and summer). The spermatozoa abnormality occurred mostly as tail abnormalities.

In spite of these facts, semen quality from the viewpoint of sperm normality improved significantly during autumn in AM × MG and summer in BL × MG groups. Season and genetic group did not influence the rate of metabolic activity. Significant seasonal fluctuation was not observed with respect to methylene blue reduction time (MBRT) in the two genetic groups. The BL × MG group showed lowest MBRT in autumn and AM × MG group in summer. Correlation coefficients between various semen characteristics (Table 6) exhibited good correlation of live spermatozoa with motility ($r = 0.90$, $P < 0.01$) and sperm density and semen color ($r = 0.30$, $P < 0.01$).

Semen volume could be correlated with sperm concentration, color and TSE as shown by the positive correlation of 0.21, 0.24 and 0.39, respectively. The MBRT decreased over time and correlated with all of semen traits ($P < 0.01$). Percentage of abnormal spermatozoa was correlated with all the semen quantity traits except for TSE. The percentage of abnormal spermatozoa was significantly correlated with wave motion ($r = - 0.69$, $P < 0.01$), progressive motility ($r = - 0.88$, $P < 0.01$) and percentage of live spermatozoa ($r = - 0.92$, $P < 0.01$). Wave motion and individually progressive motility of semen samples showed a significant correlation with semen density ($r = 0.19$ and $r = 0.33$, respectively) and semen pH ($r = - 0.38$ and $r = - 0.39$, respectively). Moreover, semen pH showed the high negative correlation with semen concentration ($r = - 0.6$, $P < 0.01$).

DISCUSSION

At University of Tabriz, the interest arose for finding suitable sheep breed to produce more uniform wool from Arkharmerino breed that was imported from Kazakhstan, and to assess the influence of photoperiod on seminal characteristics. As expected, the summer and autumn with decreasing daylight length (breeding season) and winter and spring seasons with increasing daylight length (non-breeding season) affected the seminal indices of the crossbreed rams. This study is the first to report on the seasonal variations in seminal indices of the two crossbreed genetic groups of rams (Baluchi × Moghani and Arkharmerino × Moghani) in Iran. The effect of season and/or photoperiod on semen quality and quantity has been previously reported in different breeds of rams (Amir et al., 1986; Ibrahim, 1997; Karagiannidis et al., 2000; Kafi et al., 2004; Zamiri and Khodaei, 2005; Deldar Tajangookeh et al., 2007; Zamiri et al., 2010), including the Iranian genetic groups Ghezel × Baluchi and Arkharmerino × Ghezel (Moghaddam et al., 2012) and also

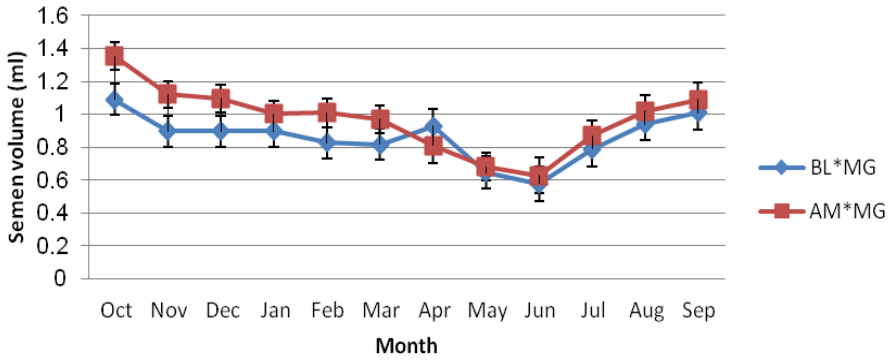

Figure 1. Monthly variations of semen volume in two genetic groups throughout the year.

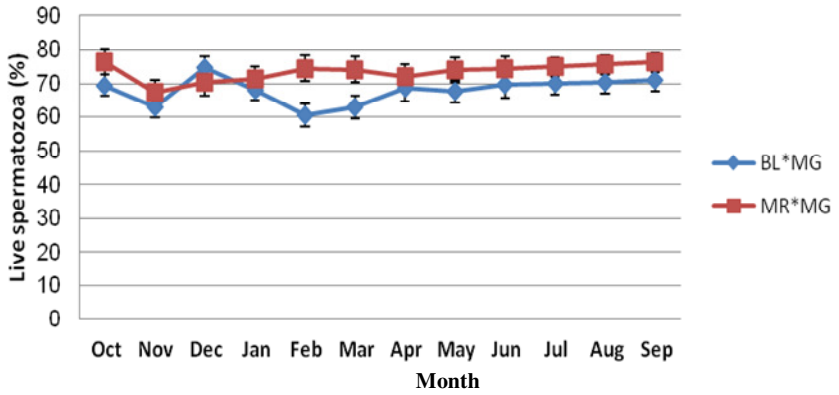

Figure 2. Monthly variations of live spermatozoa in two genetic groups throughout the year.

Table 1. Climatic data during the experiment (October, 2010 until September, 2011) at Khalat Poshan Research Center, University of Tabriz.

Month	Air temperature (°C)		Relative humidity (%)		Average
	Minimum	Maximum	Minimum	Maximum	Day length (h)
October	7.6	25.1	26.9	77.5	11.3
November	0.23	16.7	32.5	71.1	10.2
December	-4.08	12.4	34.7	67.9	9.6
January	-7.93	3.65	54.26	84.06	9.9
February	-7.85	4.2	51.33	85.1	10.9
March	-2.32	8.51	48.5	81.75	12
April	2.64	16.06	25.03	67	13.3
May	6.83	19.45	36.16	80.93	14.3
June	11.51	28.03	23	78.54	14.8
July	15.75	32.61	22.8	57.87	14.6
August	16.83	33.61	15.29	56.51	13.7
September	12.16	28.22	16.74	74.16	12.5

Table 2. Semen characteristics in Baluchi × Moghani (BL × MG) and Arkharmerino × Moghani (AM × MG) genetic group over the year.

Genetic group	Statistics	SV (ml)	WM (0-5)	PM (%)	SC (0-5)	TSE (×10^9)	Conc (×10^9)	SL (%)	SAB (%)	SI (×10^9)	pH	MBRT (s)
	n	334	334	334	334	334	334	334	332	334	333	331
	Mean	0.86	3.72	67.42	3.73	3.55	3.77	68.91	13.02	14769	6.54	119.39
BL × MG	SE	0.08	0.09	1.70	0.16	0.33	0.17	1.60	0.76	1823.50	0.09	3.10
	Minimum	0.45	2.00	40.00	2.00	0.916	1.95	45.00	4.00	722.40	5.70	65.00
	Maximum	1.40	5.00	85.00	5.00	18.90	5.68	90.00	26.00	43834.50	8.20	230.00
	n	334	334	334	334	334	334	334	330	334	334	331
	Mean	1.02	3.93	72.79	3.55	4.495	3.516	74.57	10.95	19472.29	6.57	111.70
AM × MG	SE	0.08	0.09	1.59	0.16	0.327	0.182	1.57	0.71	1767.06	0.09	3.17
	Minimum	0.45	2.00	45.00	1.00	0.985	0.960	50.00	3.00	1395.2	5.90	45.00
	Maximum	1.90	5.00	90.00	5.00	31.55	5.81	94.00	21.00	104058	7.80	220.00

SV: semen volume, WM: wave motion, PM: progressive motility, SC: semen color, TSE: total spermatozoa per ejaculate, Conc: spermatozoa concentration, SL: percentage of live spermatozoa, SAB: Percentage of abnormal spermatozoa, SI: semen index. MBRT: methylene blue reduction time.

2012) and also in other seasonal breeding animals such as buck (Barkawi et al., 2006; Karagiannidis et al., 1999) and stallion (Janett et al., 2003; Gamboa et al., 2010) with sperm production down regulated in the non-breeding season (Gerlach and Aurich, 2000).

In the present study, the photoperiod significantly affected some of the seminal characteristics. Among quality characteristics of sperm, a significant effect of season was recorded on percentage of abnormal spermatozoa, semen pH ($P < 0.05$) and semen index ($P < 0.01$). Sperm progressive motility, percent of live spermatozoa and MBRT did not have significantly seasonal variations. Moreover, photoperiodic effect also was observed clearly on semen quantity characteristics ($P < 0.01$). These seasonal variations in both semen quality and quantity were attributive mainly to changes in daylight length throughout the year (Chemineau et al., 1992).

Significant differences were not found on all traits between the two genetic groups. Significant differences among rams within each genetic group ($P < 0.05$) were found in some of seminal traits, but, non significant differences were found between the two genetic groups in any other traits in consistence with the previous report by Karagiannidis et al. (2000). The results on mean values of semen characteristics observed in our study were in agreement with those of other researchers (Zamiri et al., 2010; Gundogan, 2007; Kafi et al., 2004; Al-Ghalban et al., 2004; Karagiannidis et al., 2000). The semen volume of 0.60 to 1.6 ml, spermatozoa concentration of 2.6 to 5.5 × 10^9, abnormal spermatozoa of 4 to 29% and live or motile spermatozoa of 60 to 90% is on record (Karagiannidis et al., 2000; Kafi et al., 2004; Gundogan, 2007).

Therefore, it could be accepted that there is wide amplitude of semen characteristics in several breeds of ram. In the current study, mean values for the abnormal spermatozoa of the crosses (9 to 14%) were generally higher than the other researchers (Karagiannidis et al., 2000) for Chios and Friesian rams in Northern Greece, and by Gundogan (2007) for Akkarman and Awassi in Turkey. Zamiri et al. (2010) reported in Moghani breed a minimum spermatozoa abnormality of 7.9% in September, much lesser than the value observed in our study 11.42% in BL × GH rams in September and 8.91% in the AM × MG genetic group in November. Percentage of live spermatozoa in the two genetic groups was lower than the values recorded by Kafi et al. (2004) in South of Iran. The semen volume in the BL × MG (0.84 ± 0.09) did not coincide with results of Kafi et al. (2004) and was lower (1.03 ± 0.08) in AM × GH group than reported value (Kafi et al., 2004), making the comparison of seminal attributes often difficult. Thus, it is not surprising that wide variations

Table 3. Seasonal variations in seminal quantitative parameters (mean ± SE) of Baluchi × Moghani (BL × MG) and Arkharmerino × Moghani (AM × MG) rams.

Semen quantity	Season	BL×MG (Mean±SE)	AM×MG(Mean±SE)
Total spermatozoa/ejaculate (×10^9)	Spring	2.430±0.430^b	2.326±0.463^c
	Summer	3.210±0.298^a	3.730±0.290^b
	Autumn	3.636±0.316^a	4.336±0.285^b
	Winter	4.105±0.306^a	5.853±0.306^a
	Mean	3.345±0.337	4.061±0.336
Spermatozoa concentration (×10^9)	Spring	3.443±0.195^b	3.315±0.199^b
	Summer	3.625±0.177^{ab}	3.555±0.185^{ab}
	Autumn	3.796±0.176^a	3.482±0.176^{ab}
	Winter	3.952±0.180^a	3.676±0.179^a
	Mean	3.704±0.182	3.507±0.184
Semen volume (ml)	Spring	0.69±0.09^c	0.70±0.09^b
	Summer	0.90±0.08^a	1.02±0.08^a
	Autumn	0.96±0.09^a	1.15±0.09^a
	Winter	0.84±0.09^b	1.06±0.08^a
	Mean	0.84±0.09	0.98±0.08
Semen color (0-5)	Spring	3.351±0.171^c	3.326±0.177^b
	Summer	3.512±0.156^{bc}	3.419±0.173^{ab}
	Autumn	3.712±0.159^{ab}	3.566±0.158^{ab}
	Winter	3.979±0.161^a	3.671±0.161^a
	Mean	3.638±0.162	3.495±0.167

$^{a, b, c}$ Means in the column of each parameter with different superscripts differ significantly ($P < 0.05$).

have been reported in the seminal attributes of rams (Gundogan, 2007; Zamiri and Khodaei, 2005; Kafi et al., 2004; Karagiannidis et al., 2000). In BL × GH genetic group, the sperm concentration remained high (3.952 ± 0.180) during winter and low in spring (3.443 ± 0.195), summer (3.625 ± 0.177) and autumn (3.796 ± 0.176), a trend comparable to that reported by Karagiannidis et al. (2000) and Talebi et al. (2009). These findings confirmed the previous records of seasonal variations of sperm concentration in BL × MG rams at 38°N latitude. In both crosses, circumstance of seasonal fluctuations of semen color and sperm density was similar. In our study, most of the mean values for the semen characteristics of BL × MG and AM × MG rams, born and raised in northwest of Iran (38° 02' N, 46° 27' E), were almost similar to those reported by other authors (Barkawi et al., 2006; Zamiri et al., 2010; Gundogan, 2007) in similar temperate regions.

The quantity and quality attributes of seminal characteristics in the crossbreed rams differed in breeding and non-breeding seasons. Sperm concentration did not follow a quite similar trend to that of the ejaculate volume in this study and was comparable with the results obtained by Talebi et al. (2009). Mean values of MBRT in our study were quite different with reports of Galal et al. (1978). Also, the seasonal variations were different. BL × MG and AM × MG rams performed best in breeding season. In Egypt, Galal et al. (1978) recorded in their study on Merino, Ossimi and their crosses, the best metabolic activity in spring (76.8 ± 1.04 sec) and autumn (77.2 ± 1.04 s). While summer (102.2 ± 1.04 s) was greatest mean values in these breeds. On the contrary, Galal et al. (1978) did not observe significant difference in MBRT traits between several seasons of the year.

In the present study, the semen characteristics were generally better towards the end of summer (onset of improvement) and in the first two months of autumn than during the winter (onset of decrease in quality) and spring(usually was lowest quality and quantity). In both genetic groups, the progressive motility of sperms was lowest in winter and spring in contrast to the findings of Karagiannidis et al. (2000) at 40°N. The data suggest that summer and autumn with decreasing daylight length (breeding season) and winter and spring with increasing daylight length (non-breeding season) influenced the

Table 4. Seasonal variations in seminal qualitative variables (mean ± SE) of Baluchi × Moghani (BL × MG) and Arkharmerino × Moghani (AM × MG) rams.

Semen quality	Season	BL×MG (Mean±SE)	AM×MG (Mean±SE)
Wave motion (0-5)	Spring	3.60±0.10^{bc}	3.81±0.09^{b}
	Summer	3.92±0.09^{a}	4.00±0.09^{a}
	Autumn	3.72±0.09^{ab}	4.05±0.11^{a}
	Winter	3.51±0.11^{c}	3.75±0.09^{b}
	Mean	3.68±0.09	3.90±0.09
Progressive motility (%)	Spring	65.37±1.61^{b}	72.00±1.75^{a}
	Summer	70.51±1.54^{a}	74.67±1.61^{a}
	Autumn	69.89±1.50^{a}	74.89±1.50^{a}
	Winter	64.40±1.73^{b}	71.00±1.54^{a}
	Mean	67.54±1.70	73.14±1.59
Live spermatozoa (%)	Spring	65.91±1.68^{c}	73.33±1.67^{a}
	Summer	70.37±1.66^{ab}	75.73±1.66^{a}
	Autumn	70.96±1.51^{a}	75.26±1.51^{a}
	Winter	66.54±1.54^{bc}	72.28±1.51^{a}
	Mean	68.44±1.60	74.16±1.57
Abnormal spermatozoa (%)	Spring	12.71±0.76^{ab}	11.47±0.75^{a}
	Summer	11.86±0.70^{b}	9.67±0.67^{b}
	Autumn	12.65±0.74^{ab}	9.11±0.73^{b}
	Winter	14.04±0.75^{a}	10.84±0.77^{a}
	Mean	12.81±0.74	10.27±0.72
Semen index (×10^{9})	Spring	11346±1859.61^{b}	12123±1854.65^{b}
	Summer	16511±1881.39^{a}	19852±1769.43^{a}
	Autumn	17249±1813.56^{a}	22645±1827.49^{a}
	Winter	14023±1797.32^{ab}	20071±1769.46^{a}
	Mean	14782±1837.97	18672±1805.25
Semen pH	Spring	6.88±0.10^{a}	6.69±0.11^{a}
	Summer	6.42±0.09^{b}	6.60±0.10^{a}
	Autumn	6.37±0.11^{b}	6.57±0.09^{ab}
	Winter	6.51±0.10^{b}	6.51±0.09^{b}
	Mean	6.54±0.10	6.59±0.09
MBRT (s)	Spring	120.22±3.61^{a}	111.18±3.70^{a}
	Summer	110.74±3.77^{a}	106.27±3.11^{a}
	Autumn	116.69±3.81^{a}	108.33±3.20^{a}
	Winter	123.47±3.17^{a}	116.08±3.25^{a}
	Mean	117.78±3.59	110.46±3.31

$^{a, b, c}$ Means in the column of each parameter with different superscripts differ significantly ($P < 0.05$). Means within each column within each factor having the same letter did not differ significantly from each other ($P < 0.05$).

seminal characteristics of BL × MG and AM × MG rams. This indicated that the two test groups of rams were sensitive to photoperiod in respect of their reproductive behaviour.

Photoperiodic effects on seasonal breeders have been reported to be dictated by the latitude at which they are kept. At latitudes above 40°N, marked variations in seminal characteristics and increased sperm production with

Table 5. Effect of genetic group, season and ram on semen characteristics of ejaculates obtained during one year.

Effect	SV	WM	PM	Color	TSE	Conc	SL	SAB	SI	pH	MBRT
Genotype	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Season	**	*	ns	**	**	**	ns	*	**	*	ns
Ram	*	*	*	*	ns	*	*	*	*	*	*

*P < 0.05, **P < 0.01, ns: not significant different. SV: semen volume, WM: wave motion, PM: progressive motility, TSE: total spermatozoa per ejaculate, Conc: spermatozoa concentration, SL: Percentage of live spermatozoa, SAB: percentage of abnormal spermatozoa, SI: semen index. MBRT: methylene blue reduction time

Table 6. Correlation coefficients between various seminal traits of the rams.

	MBRT	pH	SI	SAB	SL	Conc	TSE	Color	PM	WM
SV	-0.21**	-0.20**	0.71**	-0.10	0.13*	0.21**	0.39*	0.24**	0.15**	0.21**
WM	-0.76**	-0.38**	0.50**	-0.69**	0.74**	0.19**	0.19*	0.37**	0.78**	
PM	-0.84**	-0.39**	0.59**	-0.88**	0.90**	0.33**	0.11*	0.34**		
Color	-0.67**	-0.56**	0.57**	-0.31*	0.30**	0.92**	0.34**			
TSE	-0.22**	-0.17*	0.41**	-0.05	0.09	0.29**				
Conc	-0.67**	-0.60**	0.61**	-0.28*	0.30**					
SL	-0.81**	-0.35*	0.57**	-0.92**						
SAB	0.80**	0.29**	-0.54**							
SI	-0.67**	-0.30**								
pH	0.52**									

*Significant at P < 0.05, **Significant at P < 0.01, Coefficients without symbol (* or **) are not significant. SV: semen volume, WM: wave motion, PM: progressive motility, TSE: total spermatozoa per ejaculate, Conc: spermatozoa concentration, SL: percentage of live spermatozoa, SAB: percentage of abnormal spermatozoa, SI: semen index. MBRT: methylene blue reduction time

decreasing daylight length have been observed (Zamiri et al., 2010). Seasonal variations, although less marked, were observed between 30°N and 40°N latitude, with higher sperm production during the summer and autumn (Corteel, 1977), although the crossbreed rams were capable for ejaculating overall the year. Seasonal breeding animals occurred in middle latitudes (Rosa and Bryant, 2003). High correlation between wave motion and sperm progressive motility with live sperm (r = 0.74, r = 0.90, P < 0.01, respectively) demonstrated that concurrent with improved sperm motility (as one of most important of semen quality indicators) increased the percent of live sperms which resembled the findings of Kafi et al. (2004). Significant correlation was found between motility indices and TSE in agreement with the results of Kafi et al. (2004).

A high negative correlation of MBRT with motility traits (r = - 0.76 to r = - 0.84, P < 0.01) was similar to findings of Chandler et al. (2000) but was inconsistent with the results of Kishk (2008). MBRT is an evaluator of the metabolic status of the spermatozoa (Salisbury et al., 1978). Thus, respiration rate of spermatozoa at the dense semen lead to rapid reduction of methylene blue. The observed high negative correlation between sperm concentration and MBRT (r = -0.67, P < 0.01), and between MBRT and percent of live sperm (r = - 0.81, P < 0.01) were similar to the findings of Kishk (2008). This can be attributed to the rate of release of hydrogen upon fructose utilization by the sperm cells. Thus, these samples might become acidic and not reliable for long-term storage.

Most relationship among semen traits with semen pH could be well correlated with concentration and color (r = - 0.60 and -0.56, P < 0.01) watery sample trending alkaline. Among the quantitative traits, a high correlation between MBRT with semen color and sperm concentration was observed. Karagiannidis et al. (2000) also reported a significant correlation between sperm concentration and abnormal sperm percentage (r = - 0.19, P < 0.05). The results of the present investigations suggest that ewes exhibiting estrus could be artificially inseminated by fresh semen throughout the year and consequently, reproductive performance of herd increased considerably. Seasonal fluctuations of environmental conditions markedly influenced reproduction of animals at higher latitudes and altitudes (Rosa and Bryant, 2003).

Robinson (1981) argued that breeds located between 35°N and have the tendency to breed at all times of the year. Evans and Maxell (1987) reported 30°N and 40°N latitudes for the breeds to follow this tendency. Latitudes above 35°N (Hafez, 1952; Goot, 1969) or higher than 40°N (Talebi et al., 2009; Zamiri et al., 2010) considerably influenced the seminal attributes. However, in some studies, for example in Jordan (at 31.5°N latitude and altitude of 350 m, in Damascus bucks) and Iran (34° 18' N, 47° 3' E Kermanshah, Iran, in Markhoz bucks) the photoperiod was found to have significant effect on breeding behaviour of sheep. In temperate latitudes (40 to 50°N), sperm production of rams is a continuous process, although the total number of spermatozoa produced per testis is usually higher in autumn than in spring (Dacheux et al., 1981).

The present study showed that the reproductive activity of the seasonal breeding animals for example, rams, may be improved by exploitation of photoperiod synchronized circannual reproductive rhythm (endogenous mechanisms) and exogenous factors. The pineal hormone melatonin has been established as the common link between photoperiod and reproduction (Gerlach and Aurich, 2000). Reproductive activity is not a direct function of day length, but is affected by the photo-periodic history of the animal, the direction of photoperiodic changes and the stage of the circannual rhythm at which a photoperiodic signal is received (Robinson and Karsch, 1987; Gorman and Zucker, 1995). Our study clearly showed linkage between photoperiod and reproduction of Baluchi × Moghani (BL × MG) and 5 Arkharmerino × Moghani (AM × MG) breeds located at 38°02' N, 46° 27' E and an altitude of 1567 m above sea level in Iran.

Conclusion

Semen evaluation does have an important role in artificial insemination programs or in flocks where single sire joining groups are used. This will be useful for identifying rams with poor performers. Thus, it will provide optimum breeding selection of males in herd. The semen characteristics of BL × MG and AM × MG rams in Northwest of Iran showed a significant seasonal variation in semen characteristics. The best semen is produced during late summer to second month of autumn (breeding season). Nonetheless, the magnitude of these seasonal effects should not prevent the animals from serving or semen collecting for artificial insemination throughout the year but it is necessary to perform semen evaluation on an individual basis for every ram used for artificial insemination or breeding.

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Assessment on the indigenous knowledge of pastoral community on contagious caprine pleuropneumonia in Borana and Guji lowlands, Southern Ethiopia

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Assessment on the indigenous knowledge of pastoral communities in Borana and Guji lowlands on contagious caprine pleuropneumonia was done in 2008 using participatory appraisal techniques and conventional disease investigation methods. The participatory appraisal techniques employed were matrix scoring and seasonal calendar. To triangulate the findings during the participatory appraisal, clinical and laboratory examinations were also employed. The matrix scoring and seasonal calendars were standardized and repeated with 20 informant groups. After clinical examinations, serum samples were collected from 9 goats which were infected with contagious caprine pleuropneumonia as per the perception of the informant groups. Disease matrix scoring depicted a good to perfect agreement among the informant groups (W=0.546 to 1.00, p<0.001), indicating that contagious caprine pleuropneumonia affects only goats and possesses high morbidity, coughing and high mortality. Moreover, it showed that there was a good to perfect agreement among the informant groups (W=0.880 to 1.00, p<0.001) depicting no circular movement, diarrhea, and skin damage. Similarly, it was indicated that there was a good agreement among the informant groups (W=0.910, p<0.001) that the occurrence of contagious caprine pleuropneumonia was high during cool dry season locally known as "Adoolessaa" and long dry season locally known as "Bona". Out of the 9 serum samples of goats, 6 (66.6%) samples showed sero-positivity. Most of the serologically positive goats showed symptoms of nasal discharge, coughing, diarrhea and dyspnea during clinical examination. During the matrix scoring, very few informants identified diarrhea as an indicator. All the informant groups identified nasal discharge and coughing as an indicator for contagious caprine pleuropneumonia. This study indicated that pastoral communities living in Borana and Guji lowlands have detailed indigenous knowledge about contagious caprine pleuropneumonia. Therefore, the combined use of participatory appraisal and conventional methods is very useful for the diagnosis and surveillance of the disease.

Key words: Assessment, Borana, Guji, contagious caprine pleuropneumonia, Ethiopia, participatory approaches.

INTRODUCTION

In Ethiopia, the presence of contagious caprine pleurapneumonia has been suspected for long period, especially in areas at the immediate vicinity of endemic regions of Kenya and Sudan. As indicated by Thiaucourt

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et al. (1992),contagious caprine pleuropneumonia has been confirmed to be present in Ethiopia since 1980s. Since its confirmation, most of the reports of the disease were from Oromia and Southern Nation, Nationalities and Peoples Regional States indicating the widespread nature of the disease in these two regions (MoARD, 2007). Moreover, different workers have also indicated the importance and widespread nature of the disease in different parts of the country (Gelagay et al., 2007).

Contagious caprine pleuropneumonia is becoming one of the major killer diseases of goats in Borana and Guji lowlands. The area is characterized by poor animal health service as control measures for various livestock disease in general and contagious caprine pleurapneumonia in particular is ineffective and inefficient. To suffice the purpose, the pastoralists living in the area have been using their indigenous knowledge.

Pastoralists have a rich indigenous knowledge about animal health problems affecting their herds (Catley et al., 2002). This rich indigenous veterinary knowledge is based on oral tradition, shared information and the lifelong experience of the individuals. The core of this knowledge is clinical, pathological and epidemiological observations that serve to organize disease information into recognizable entities.

Like other pastoralists, pastoral communities living in Borana and Guji lowlands have a good indigenous knowledge on livestock disease identification and management (Genene, 2005) which could be a good platform to design appropriate livestock disease control and prevention program. Similar reports were made by Shiferaw et al. (2009) indicating that Afar pastoralists have detailed knowledge about their livestock health. However, the indigenous knowledge of the pastoral community in Borana and Guji zone on contagious caprine pleuorpneumonia is not recorded. Therefore, this study was performed with the objectives of assessing the perceptions and indigenous knowledge of pastoral community about contagious caprine pleuropneumonia, so that the areas of complementarities between local indigenous knowledge and conventional animal health service approaches to control the disease can be identified.

MATERIALS AND METHODS

Description of the study area

The study area comprised of Borana and Guji lowland of Southern Ethiopia, covering an area of 95000 km^2. The Borana plateau gently slopes from the high mountain massif in the North 1650 m above sea level to the South bordering Kenya at 1000 m above sea level with slight variation due to central mountain ranges and scattered volcanic cones and craters (Coppock, 1994). The area borders Kenya to the South, Somali region to the East, the highland parts of Borana and Guji to the North, and Southern Nations and Nationalities People's Regional State to the West. This study was conducted in three selected districts, namely Teltale, Moyale and Liban.

Assessment of goat diseases

Two participatory approach techniques, matrix scoring and seasonal calendars were employed to assess and generate data on the indigenous knowledge of the local pastoral community on goat diseases in general and contagious caprine pleuropneumonia in particular. The guideline descriptions for the two techniques were given Catley et al. (2002, 2005). The assessment was conducted with 20 informant groups (7 groups from Teltale district, 6 groups from Moyale district, and 7 groups from Liban district). The group size varied from 20 to 30 people. Overall 400 to 600 pastoralists were contacted during the assessment. The level of agreement among the informant groups was determined by methods of Siegel and Castellan (1994).

The informant groups identified major goat diseases in their areas, out of which, the first five were selected for matrix scoring. Pair wise comparisons of the five goat diseases were conducted. During the pair wise comparison, list of reasons known as "indicators" in participatory methods were established. The identified indicators locally perceived clinical signs of the diseases. The five diseases placed along the x-axis in the matrix were scored against the list of clinical signs of the disease which were placed along the y-axis of the matrix. The diseases and clinical signs were represented by locally known objects so that the informants could easily identify them during scoring. For each clinical signs, informants were asked to score each disease by dividing piles of 25 stones (five for each disease). The informants were given the chance to change the score if they wished. The final score was recorded.

In the seasonal calendar, the local names of seasons in Borana and Guji lowlands were identified as long rainy ("Ganna"), short rainy ("Hagayya"), cold dry ("Adoolessa") and long dry ("Bonaa"). The seasons were represented by local materials on the top x-axis and the indicators to be scored against the season were placed along the y-axis. The diseases, rainfall and seasons were represented using locally known objects. Rainfall was chosen as the first event to be scored during the seasonal calendar scoring. The annual objective rainfall data of the study area during the past one year were collected from the local station. The informant groups were given a pile of 30 stones and were asked to divide the stones against the seasons to show the patterns of the indicators. The informants were given the chance to change the score if they wished. The final score was recorded.

Clinical and laboratory examination

Serum samples were collected from 9 goats which were infected with contagious caprine pleuropneumonia as per the perception of the community. Serological investigation was done using complement fixation test (CFT) according to Office International des Epizooties (OIE, 2000) standard test procedure using *Mycoplasma capricolum capripneumonia* antigen. The test was done at National Veterinary Institute (Debre Zeit, Ethiopia). Moreover, the sampled goats were clinically examined focusing on body temperature, respiration rate, presence of nasal discharge, presence of coughing, body condition and presence of diarrhea.

Data management and analysis

The data were stored in MS-Excel 2003 spread sheet and were analyzed by computing descriptive statistics. The level of agreement among the groups was assessed using Kendall's coefficient of concordance (W) using SPSS (2006) version 15.0. W values less than or equal to 0.26 at $p>0.05$ show weak agreement, W values greater than 0.26 and less than or equal to 0.38 show

Table 1. Summarized matrix scoring of major goat disease signs (n=20).

Disease sign, W and p-value	Diseases with their scientific and local names				
	Mange mite (Locally known as Cittoo)	Coenurosis (Locally known as Sirgo)	Contagious caprine pleuropneumoniaa (Locally known as Sombeessa)	Ticks (Locally known as Silmii)	GI parasites (Albaatii)
High morbidity, W=0.786*	5.5 (4-7)	3 (2-4)	9 (7-12)	3 (2-5)	5 (2-7)
Circular movement, W=1.00*	0 (0-0)	25 (25-25)	0 (0-0)	0 (0-0)	0 (0-0)
Coughing and nasal discharge, W=1.00*	0 (0-0)	0 (0-0)	25 (25-25)	0 (0-0)	0 (0-0)
High mortality, W=0.773*	3.5 (2-6)	5 (3-6)	11 (10-15)	2 (1-4)	3 (2-6)
Has drugs, W=0.846*	6 (4-7)	0 (0-0)	3 (2-5)	8.5 (4-12)	7 (5-15)
Loss of weight, W=0.898*	8.7 (7-11)	2 (0-5)	3 (0-4)	5 (4-7)	6 (5-9)
Diarrhea, W=0.880*	0 (0-2)	0 (0-0)	0 (0-1)	0 (0-7)	25 (22-25)
Skin damage, W=0.960*	15 (12-19)	0 (0-0)	0 (0-0)	8.5 (6-10)	0 (0-4)
Loss of milk yield, W=0.824*	11.5 (10-14)	1.5 (0-4)	2.5 (1-4)	4.5 (3-6)	4.5 (3-6)
Chronic disease, W=0.922*	8.5 (8-10)	3 (1-5)	0 (0-1)	8 (5-8)	6 (2-7)
Seasonal disease, W=0.918*	3.5 (3-5)	0.5 (0-2)	9 (8-10)	5 (4-6)	7 (4-9)
Has vaccine, W=1.00*	0 (0-0)	0 (0-0)	25 (25-25)	0 (0-0)	0 (0-0)
Affects both goat and sheep, W=0.546*	5 (2-8)	6 (3-11)	0 (0-0)	6 (4-9)	5.5 (2-8)

W=Kendall's coefficient of concordance. *Shows good agreement among the informant groups at p<0.001. The median score is outside the parentheses. The minimum and maximum scores are shown in the parentheses.

moderate agreement and W values greater than 0.38 at p<0.01 to p<0.001 show good agreement between the informant groups.

RESULTS

Matrix scoring

Matrix scoring done with the 20 informant groups showed that there was a good agreement among the informants (W=0.546 to 1.00). The agreement was significant (p<0.001). The matrix scoring findings were analyzed, summarized and presented pictorially (Table 1).

Seasonal calendars

Seasonal calendar with the informant groups showed that there was good agreement among the informant groups (W=0.912 to 0.981). The agreement among the informant groups was significant (p<0.001). However, in case of coenurosis, the agreement was weak (W<0.26) and statistically not significant (p>0.05). The matrix scoring findings were analyzed, summarized and presented pictorially (Table 2).

Clinical and serological examination

About 66.7% (6 out of 9) of the goats identified by the community as clinical cases of the disease were positive serologically. Of the serologically positive cases, 66.7% (4 out of 6) had history of coughing, 83.3% (5 out of 6) had nasal discharge and all the positive cases had respiration rate greater than or equal to 45 breaths/min. The summary of the clinical and serological finding was presented (Table 3).

DISCUSSION

Participatory appraisal methods have been used by veterinarians in Africa since late 1980s and examples of field research includes the use of matrix scoring to characterize cattle diseases in Southern Sudan and seasonal calendars to depict seasonal variations in disease incidence and vector population (Catley et al., 2004).

The participatory matrix scoring method has shown that there are many diseases affecting goat population in Borana and Guji lowlands and the pastoralists were able to describe, identify and prioritize these diseases. Accordingly, the major goat diseases identified during the scoring are contagious caprine pleuropneumonia, coenurosis, gastro-intestinal tract (GIT) parasites, mangemite and tick infestations. Observations during the matrix score showed that most cases of GIT parasite infections are manifested through the presence of diarrhea which could be an indicator for the presence of Peste des Petitis Ruminants. However, no attempts were made to further explore this observation. The clinical signs of contagious caprine pleuropneumonia such as high morbidity, high mortality, coughing and nasal discharge, abortion, availability of vaccine for the disease and others

Table 2. Summarized seasonal calendar of major goat diseases (n=20).

Indicator, W and p-value	Scientific and local Borana and Guji seasons			
	Long rainy season	Cool dry season	Short rainy season	Long dry season
	March–May (Locally known as Ganna)	June-August (Locally known as Adoolessa)	September-November (Locally known as Hagayya)	December-February (Locally known as Bona)
Mean seasonal rain fall (mm)	175.5 mm	21.4 mm	82.7 mm	11.6 mm
Rain fall, W=0.946*	17 (15-19)	2 (0-3)	10 (8-13)	1 (0-3)
Mange mite infestation, W=0.964*	1(0-4)	14.5 (9-20)	4.5 (3-7)	10 (6-13)
Coenurosis, W=0.013**	8 (5-9)	8 (7-12)	8 (5-8)	7 (5-10)
Contagious caprine pleuropneumonia, W=0.910*	3 (0-6)	9 (6-12)	4 (2-7)	12 (10-17)
Tick infestation, W=0.981*	16.5 (10-20)	8 (0-4)	3.5 (2-8)	2 (0-5)
Internal parasite, W=0.912*	14.5 (10-20)	4.5 (1-6)	8 (6-10)	3 (0-7)
Livestock movement, W=0.968*	1 (0-4)	8 (5-12)	6 (2-8)	14 (10-23)
Fly infestation, W=0.912*	13 (10-18)	6.5 (4-9)	8 (5-9)	2.5 (0-6)

W=Kendall's coefficient of concordance. *Shows good agreement among the informant groups at p<0.001, **Shows weak agreement among the informant groups at p>0.05. The median scores written outside of the parentheses. The minimum and maximum scores are shown in the parentheses.

Table 1. Summary of clinical and serological findings in participatory appraisal (n=9).

Variable	Case number								
	1	2	3	4	5	6	7	8	9
Clinical examination									
Coughing	+	-	-	+	+	+	+	-	+
Nasal discharge	+	+	+	-	-	+	+	+	-
Poor body condition	+	+	+	-	+	+	+	+	-
Diarrhea	-	+	+	+	-	+	+	+	+
Age in years	1	2	2	4	5	6	6	7	3
Sex	M	M	F	M	F	M	M	F	F
Rectal temperature in 0_C	39.5	39	40	39.5	41	39.5	40	40.5	41
Respiration (breaths/min)	45	38	50	40	52	54	42	50	51
Laboratory examination									
CFT result	+	-	+	-	+	+	-	+	+

+= Positive, - =Negative.

described by the informant groups were almost similar to the text book descriptions of the disease. This is in agreement with the results obtained previously (Mekuria and Asmare, 2009).

The seasonal calendar has identified the season of the year when the incidence of caprine pleuropneumonia is high. It is shown that the disease can occur at any season of the year; however, the incidence of the disease were found to be high during the cool dry season locally known as "Adoolessaa" and during long dry season locally known as "Bona". It was explained that the cold climate during the cool dry season and the frequent meeting at watering point and shortage of feed during the long dry season, were the major contributing factors for the high incidence of the disease during these seasons.

In both matrix scoring and seasonal calendar methods, it was observed that the scoring has achieved high level of agreement among the informant groups. During the scoring, the informants were seen to carefully count the stones allocated to each indicator. The scoring has showed that the community can prioritize goat diseases and score factors which they consider to be of relevance while distinguishing these diseases. Similar observations were reported previously (Gezahegn, 2006).

Although, matrix scoring was considered to be a useful method and hence its use should be combined and triangulated with other methods. Basic veterinary investigation methods such as clinical and post mortem examinations can be used to validate local diagnosis for some diseases (Catley et al., 2002). It has been observed that the cases identified by the communities as of caprine pleuropneumonia, 66.6% were found to be serologically positive. Majority of the serologically positive goats had nasal discharge, coughing, diarrhea and rapid breathing. These indicators have also been identified by the informant groups during the matrix scoring. But few informants identified diarrhea and rapid breathing as indicators for caprine pleuropneumonia even though they subsequently seemed to be associated with goats that were seropositive for caprine pleuropneumonia. However, the research methods did not show any clear indication about what proportion of goats seropositive for caprine pleuropneumonia could be recognized by local diagnosis. An answer to this question has implication for the use of local diagnosis in disease surveillance, and should therefore be the subject of further research.

In conclusion, the participatory appraisal has indicated that pastoral communities living in Borana and Guji lowlands have detailed indigenous knowledge about caprine pleuropneumonia. Therefore, the combined use of participatory appraisal and conventional methods is very useful for the diagnosis and surveillance of the disease.

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Full Length Research Paper

Trypanosome infection rate of *Glossina pallidipes* and trypanosomosis prevalence in cattle in Amaro Special District of Southern Ethiopia

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The survey was conducted in Amaro Special District, Southern Ethiopia in 2010. It was initiated to determine the trypanosome infection rate, population density of *Glossina* species and prevalence of trypanosomosis in cattle. *Glossina pallidipes* was the only species of tsetse found in the study area during the study period. A total of 202 flies were dissected. The overall infection rate of *G. pallidipes* was 6.93%, among which 1 (0.49%) was male and 13 (6.43%) female flies. The prevalence was significantly higher (χ^2 = 99.82; P = 0.00) in female flies than male flies. In determination of tsetse flies population density, flies were trapped using baited stationary traps, and other biting flies were estimated in relation to altitude levels and vegetation types. Higher proportion of tsetse flies was caught in the riverine vegetation type followed by savanna and bush areas. Blood samples from 561 randomly selected cattle of both sex and different age groups were collected and examined with conventional haematological and parasitological techniques. Out of the total examined animals, 74 (13.19%) cattle were infected with trypanosomes. Most of the infections were due to *Trypanosoma congolense* (78.37%) followed by *Trypanosoma vivax* (12.13%), mixed infections of *T. congolense* and *T. vivax* (8.1%) and the rest were *Trypanosoma brucei* (1.35%). There was no statistically significant difference (P > 0.05) in infection between male and female, and altitude levels. Mean packed cell volume (PCV) value of parasitaemic and aparasitaemic animals was significantly (P < 0.05) different. Diagnosis of trypanosomosis in tsetse or domestic livestock is a basic requirement for epidemiological studies as well as for planning and implementing control operations. Therefore, the results of this study should be used to define the strategy of disease control in places where tsetse and trypanosomosis challenge were reported.

Key words: Cattle, apparent fly density, Glossina pallidipes, Infection rate, Trypanosomosis, Southern Ethiopia.

INTRODUCTION

Trypanosomes are the parasite that causes trypanosomosis of humans and domestic animals (International Laboratory for Research on Animal Diseases (ILRAD), 1988; Connor, 1994). The most important species responsible for the disease complex commonly known as Nagana in livestock are *Trypanosoma brucei, Trypanosoma congolense* and *Trypanosoma vivax.* They are usually transmitted by tsetse flies. Tsetse flies ingest trypanosomes present in the blood or lymph while feeding on an infected host.

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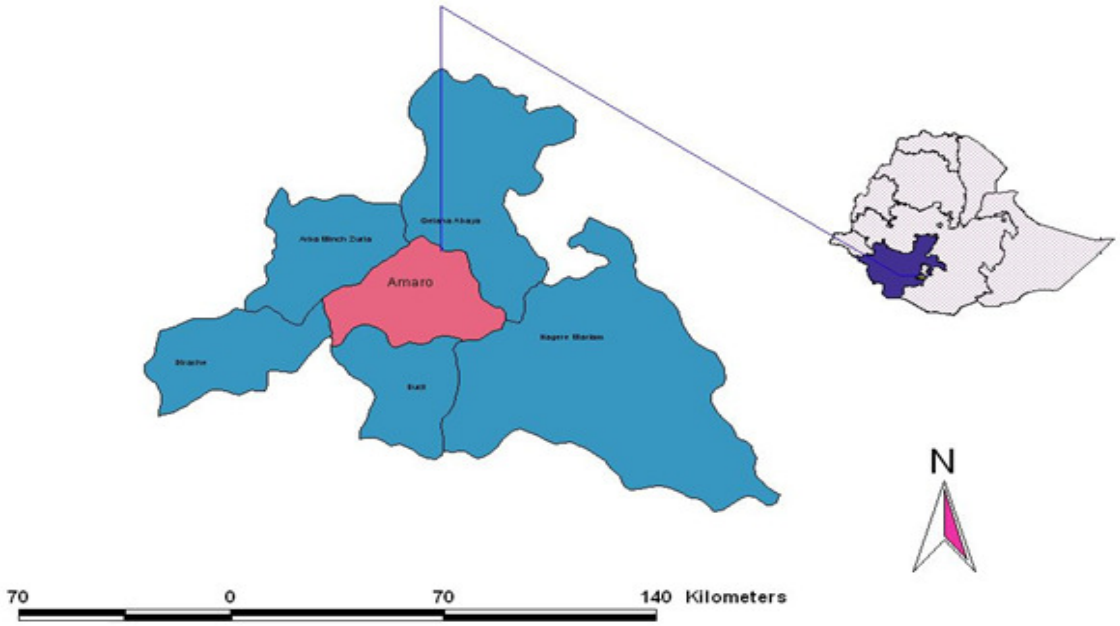

Figure 1. Location map of the study area.

There after the trypanosomes lose their glycoprotein surface coat and in the case of *T. brucei* and *T. congolense*, become elongated and multiply in the midgut before migrating forward to the salivary glands (*T. brucei*) and the proboscis (*T. congolense*). The entire process takes at least two or three weeks and the metacyclic trypanosomes are inoculated into the new host when the tsetse fly feed (Urquhart et al., 1995). In *T. vivax*, a similar process of cyclic development takes place except that it occurs entirely within the proboscis.

Tsetse challenge is determined by the product of relative tsetse density, trypanosome prevalence in tsetse and the proportion of meals obtained by the tsetse from a defined host (Leak et al., 1988). The occurrence and impact of trypanosomosis on the other hand depends on tsetse challenge, host distribution, livestock breeds, farming practices and control practices (Rogers et al., 1996). Therefore, it is prudent to study the infection rate in the tsetse flies to obtain a reasonable indication about the risk of trypanosomosis to domestic livestock and consequently a useful parameter for prioritizing the strategy in the disease control techniques.

Tsetse flies in Ethiopia are confined to the southern, western and southwestern regions between longitude 33° and 38° E and latitude 5° and 12°N. Tsetse infested areas lie in the low lands and also in the river valleys of Baro, Akobo, Didessa, Abay (Blue Nile), Ghibe and Omo (Langridge, 1976). Five species of tsetse flies are believed to be found in Ethiopia. These are *Glossina marsitans submorsitans, Glossina pallidipes, Glossina fuscipes fuscipes, Glossina tachinoides* and *Glossina longipennis* (Langridge, 1976; MOA, 1996; Ministry of Agriculture and Rural Development (MOARD), 2004). There are five economically important animal trypanosome species in Ethiopia. These are *T. congolense, T. vivax, Trypanosoma brucei brucei, Trypanosoma evansi* (Langridge, 1976) and *Trypanosoma equiperdum* (Dagnachew and Shafo, 1981).

In Ethiopia, few studies were conducted regarding trypanosome infection rate in tsetse fly while no studies were performed in the current study area. Therefore the objective of the present study is to determine the trypanosomes infection rate of *Glossina* species and prevalence of trypanosome in cattle in Amaro Special District of Southern Ethiopia.

MATERIALS AND METHODS

Study area

The study was carried out in Amaro Special District of Southern Ethiopia, from August to September, 2010. The study area was located between 5° 47` N, 37° 58`E, longitude, along the escarpment of Amaro Special and Gelana Districts. The elevation ranges from 1100 to 3400 meters above sea level. The climate condition of the area is some what unique which divided into three seasons: short rainy season (between May and early June), long summer rainy season (from September to November) and dry season (from late December to April). The annual mean minimum and maximum temperature is 13.0 to 15.5 and 26.1 to 28.4°C, with an annual rainfall ranging from 735 to 1200 mm. The dominant floras were wood grass, acacia, *Ficas sycomorus* and other bushes. Major faunas in the study area were bush pig, antelopes, warthog, and others. Amaro Special District is bordering Gelana District in the North and East, Burji Special District and Gamogofa Zone in the West (Figure 1).

Study design

A cross-sectional study was conducted to determine the trypanosome infection rate and population density of *G. pallidipes* and prevalence of trypanosomosis in cattle, in Amaro Special District of Southern Ethiopia.

Sample size and sampling method

The simple random sampling technique was used for the study of trypanosomes infection rates in *G. pallidipes,* and stratified sampling method in cattle based on the herd common characteristics of the population using simple random sampling method and sample sizes were allocated using proportional allocation under which the sizes of samples from different strata were kept. The sample size was determined based on the expected prevalence rate of 50% and absolute desired precision of 5% at confidence level of 95%. As a result, a total of 384 animals were needed to be sampled (Thrusfield, 1995). However, in case of stratified sampling, the subjects are not independent and hence larger sample size has been required (Martin, 1978).

Tsetse flies survey

A total of 82 monoconical (Challier and Laveissiere, 1973) standard traps were deployed in the study area for tsetse fly trapping. All the traps were baited uniformly with octenol (1-oct-3-nel), acetone and three weeks old cow urine (Brightwell et al.,). All odours were placed on the ground about 30 cm upwind of the trap. The poles of traps were greased to prevent fly predators, mainly ants. Traps were allowed to stay at the site of deployment for a period of 48 h before collection. Trap deployment sites were selected to represent all vegetation type/habitat that could be related to fly multiplication, behavior, feeding, and other related aspects. After 48 h of deployment, the catchments of each trap was sorted by fly species and then counted, identified and analyzed. The apparent density of the tsetse fly was calculated as the number of tsetse catch/trap/day (Leak et al., 1987).

Sex determination

Tsetse flies were trapped using monopyramidal traps which were deployed along riverside and cattle roots. The flies were collected from the trap and before dissecting them the number of each sex and species of tsetse flies were recorded. Tsetse flies were identified as male or female by examining the posterior end of the abdomen. The male fly has a lump on the ventral side of the abdomen (hypophgeum) at the posterior end but not in the female flies (Food and Agriculture Organization (FAO), 2000).

Age determination

In male tsetse, the age estimation was done according to the degree of wear or fraying observed on the hind margin of the wing. According to the degree of wear, flies were assigned to one or other of the six categories as described by Jackson (1946) and Challier (1965). After giving the wing fray category, the age was estimated using directions for estimating the mean age of a sample of tsetse flies, as mean wing fray was calculated as the sum of each category total divided by the sum of fly number for each category and finding the given value on the table as given in the FAO Training manual for tsetse control personnel (FAO, 2000). Female flies were age graded according to the contents of the uterus and the relative development of the four ovarioles. Ageing of the female tsetse flies using ovarian age determination was done by carrying out tsetse dissection and observing the contents of the uterus and the relative size of the follicles in each of the two ovarioles and in each of the two ovules that constitute each ovary. The sub-division of each of the age category was done as described by Saunders (1962) and followed as illustrated in the FAO Training manual for tsetse control personnel (FAO,1979).

Dissection of tsetse flies and Infection rate determination

Wings and the legs were removed from the flies. The dissection was carried out as described by the FAO Training manual for tsetse control personnel (FAO, 2000). Then, freshly killed tsetse flies were dissected under a dissecting microscope by using 0.9% normal saline. Trypanosome infections in the tsetse flies were identified using a compound microscope at a magnification of ×400, using the methods of Lloyd and Johnson (1924). Parasites found in the midgut, salivary glands and mouth parts were regarded as *Trypanozoon;* "*T. brucei*-type", those located in the mouth parts and midguts were *Nanomonas;* "*T. congolense*-type*"*, while those found in the mouth parts only was put in the group of *Duttonella*; *"T. vivax*-type infection", immature infections, when only the midgut was found infected. The Infection rate (IR) was calculated using the following formula:

$$\text{Infection rate (IR)} = \frac{\text{Number of tsetse flies infected}}{\text{Total Number of tsetse flies dissected over a given period}} \times 100$$

Trypanosomes prevalence in cattle

Study animals

The study animals used were all age and sex group of *Bovine* species of local zebu and Boran breed of cattle. All of them were kept under extensive management system together with other livestock species. A total of 561 cattle were selected from study population by simple random sampling methods technique according to Thrusfield (2005) and Martin (1978), with 95% confidence interval, 5% desired absolute precision, and 50% expected prevalence. The study animals were selected from six Peasant Associations (PAs) of Amaro Special District of Southern Ethiopia.

Parasitological examinations

Blood samples were collected directly from the ear veins of the study animals into heparinized capillary tubes. The blood samples were examined by the capillary microhaematocrit centrifugation method to estimate the packed cell volume (PCV) as an indicator of anemia. After determination of the PCV, the buffy coat (BC) was examined by dark ground/phase contrast microscope (Paris et al., 1982). For the purpose of species identification, a thin blood smear was prepared from the BC for those samples that were positive on

BC examination and stained with Giemsa stain and examined under a microscope using the oil immersion ×100 (Paris et al., 1982).

Data analysis

The data was entered into a Microsoft excel spread sheet to create a database and transferred to the Statistical Package for Social Sciences (SPSS) software programs of the computer before analysis. Descriptive statistics was utilized to summarize data. The SPSS version 16.0 software of the computer program were applied for the statistical analysis. The point prevalence was calculated for all data as the number of infected individuals divided by the number of individual sampled and multiplied by 100. The association between prevalence of trypanosome infection and the assumed risk factor was tested by chi-square, whereas student's *t* test was used to examine the differences in mean PCV value between parasitaemic and aparasitaemic animals.

RESULTS

Tsetse fly survey

From 82 traps deployed during the study period, a total of 4,714 flies were caught. Of these, 370 (7.84%) belong to *Glossina* species, the remaining 1853 (39.30%) were *Stomoxys*, 309 (6.55%), *Tabanus* and 2182 (46.28%) were Heamatopota belonging to biting flies, while *G. pallidipes* was the only tsetse species found in the surveyed areas. The overall apparent fly density (tsetse) was 2.25 flies/trap/days (F/T/D). The difference in apparent fly density at PA level was 6.6 and 0.85 at Jelo and Dano, respectively. The number of fly counted was significantly different ($P < 0.05$) among PAs, and between tsetse and other biting flies (Table 1).

Infection rate

A total of 202 tsetse flies were dissected during the study period. The overall trypanosome infection rate was 6.93%. More trypanosome infections were observed in female tsetse with an infection rate of 7.55% (Table 2). Overall 69.23% (or 9/13) of the trypanosome infections carried by the female tsetse were identified as belonging to the Duttonella group; these were classified as *T. vivax* and the 23.07% (3/13) were *Nanomonas;* "*T. congolense*-type" and the remaining 7.69% (1/13) were *Trypanozoon;* "*T. brucei*-type" infections. There was significant difference in the proportion of tsetse infected with trypanosomes between male and female flies ($\chi^2 = 99.82$; $P = 0.00$) and also an age related effect in the number of trypanosome infections detected by microscopy with number of infected flies older than 31 days being significantly higher than those aged < 20 days ($P < 0.05$).

Prevalence of trypanosomes in cattle

Out of a total 561 cattle sampled, 74 (13.19%) were found to be infected with trypanosomes (Table 3). The prevalence of trypanosomosis at Dorbade and Kore Biko peasant association was 20.37 and 7.01%, respectively. There was statistically significant ($P < 0.05$) difference in the prevalence of trypanosome infection between the two sites. According to the survey result obtained, *T. congolense* was the predominant species and found to be a major cause of infection in the study area followed by *T. vivax* and mixed infection of *T. congolense* and *T. vivax,* and lastly by *T. brucei*. When the proportional frequency of trypanosomes was considered, *T. congolense* appeared 58 times while *T. vivax,* mixed infection of *T. congolense* and *T. vivax* and *T. brucei* were 9, 6 and 1 times, respectively. In other words, *T. congolense* being a major cause of infection exceeded the other trypanosome species by 78.37%. The overall trypanosome prevalence in the surveyed areas of the district comprised 13.19%, with a range of 8.77 to 20.37% while the overall mean PCV-value appeared to be 25.78% in a parasitaemic and 22.96% in parasitaemic animals. Of 292 males and 269 females examined, 39 (13.35%) and 35 (13.01%), respectively were infected with trypanosome, but there was no significant difference ($P > 0.05$) between two sexes (Table 4). Age was categorized into three groups from randomly selected animals during blood sample collection. Out of 561 animals sampled, 95 (16.9%), 165 (290.4%) and 301 (53.6) were under age group 2 to 4 years, 5 to 7 years and > 7 years, respectively (Table 4). In each group, 12 (12.63%), 22 (13.33%) and 40 (13.28%) were trypanosome positive and there was a significant difference ($P < 0.05$) among the age groups.

Prevalence of trypanosome infection in cattle was analyzed according to the agro ecological zone: lowland (< 1500 masl) and midland (1500 to 1800 masl). Accordingly, the prevalence in animals sampled from lowland was 40 (13.28%) and in animals from midland was 34 (13.07%). The difference between two ecological zones were statistically not significant ($P > 0.05$).

Haematological findings

The mean PCV (%) values during the study period were 22.96 ± 2.61 in parasitaemic and 25.78 ± 4.06 in aparasitaemic animals. Statistical analysis was made using t-tests to compare mean PCV value of parasitaemic and aparasitaemic animals. When the results were compared, parasitaemic animals had lower mean PCV than aparasitaemic animals, and there is statistically significant difference ($P < 0.05$) between the two variables.

DISCUSSION

The results on tsetse fly survey in this study revealed the presence of a single *Glossina* species, known as *G. pallidipes* and identified as the major vector of

Table 1. Summary of the results of entomological survey in Amaro Special District.

PA	Alt	Lat	Long	Traps	Days	*Glossina* spp found		Other biting flies caught				
						G.pallidipes	F/T/D	Sto	Tab	Hea	Total	F/T/D
Jelo	1300	5°47	37°58	15	2	200	6.6	783	58	841	1682	56.06
Dorbade	1324	5°51	37°55	20	2	64	1.6	365	138	503	1006	25.15
Shero	1153	5°45	37°54	20	2	46	1.15	307	54	361	722	18.05
Goble	1331	5°45	37°55	20	2	48	1.2	363	29	412	804	20.1
Dano	1100	5°44	37°45	7	2	12	0.85	35	30	65	130	9.28
Total	-	-	-	82	-	370	2.25	1853	309	2182	4344	26.48

PA = Peasant association, Alt = altitude, Long = longitude, F/T/D= flies/trap/days, Sto= stomyxs, Tab = tabanus, Hea= haematopota.

Table 2. The number of flies dissected and infection rate of *Glossina pallidipes* based on sex and age.

Sex	No. dissected	Age	No. of flies infected by trypanosome species (%)			Overall infection rate (%)
			T. congolense	*T. vivax*	*T. brucei*	
Male	30	19	0	1 (3.33)	0	1 (3.33)
Female	172	31	3 (1.74)	9 (5.23)	1 (0.58)	13 (7.55)
Total	202	-	3 (1.48)	10 (4.95)	1 (0.49)	14 (6.93)

$\chi^2 = 99.82$, $P = 0.00$.

Table 3. Prevalence of trypanosome infections and species of trypanosomes identified in cattle in the study area.

PA	N	+ve	*Trypanosome species* (%)				Overall infection (%)
			T. congolense	*T. vivax*	*T. brucei*	Mixed	
Jelo	98	11	10	1	0	0	11.2
Dorbade	108	22	15	4	0	3	20.37
Shero	92	10	5	2	0	3	10.86
Goble	95	13	10	2	1	0	13. 68
Dano	111	14	14	0	0	0	12. 61
Kore biko	57	4	4	0	0	0	7.01
Total	561	74	58	9	1	6	13.19

$\chi^2 = 304$; $P = 0.00$.

trypanosomosis in Amaro special district, southern Ethiopia. Other biting flies including *Stomoxys*, haematopota, and *Tabanus* that transmit the parasites mechanically were also found in the study area. The overall apparent density of tsetse and other biting flies were 2.25 and 26.48 flies/trap/day (F/T/D), respectively. There was significant difference ($P < 0.05$) in tsetse flies density between surveyed peasant associations; Jelo and Dano of Amaro Special District ranging from 6.6 to 1.15. This might be attributed to the altitude and vegetation type and coverage of the two sites. The trypanosome infection rate in a population of tsetse may vary with sex, age and the sampling method (Jordan, 1974). Sex ratio and age composition of the flies were assessed in this study and higher numbers of female and adult flies were recorded. The presence of high number females might result in high population density which is indicative for future high infection rate. Similar results have been reported by Msangi (1999), Mohammed-Ahemed and Dairri (1987) and Leak (1999) which showed that in unbiased sample, female would comprise between 70 to 80% of the mean population.

A total of 202 *G. pallidipes* were dissected, and an overall of 6.93% of *G. pallidipes* in Amaro Special District of Southern Ethiopia harbors *T. vivax, T. congolense and T. brucei. T. vivax* is the most prevalent species identified in the tsetse fly. According to Adams et al. (2010), *T. vivax* is considered to be one of the most important of the salivarian trypanosomes because of its pathogenicity to cattle and its relatively high infection rates in tsetse. Similar

Table 4. Prevalence of trypanosome infection in relation to sex, age and altitude categories.

Risk factor	N	+ve	Species detected				Prevalence (%)	d.f	χ^2-value	*P*-value
			T. congolense	*T. vivax*	*T. brucei*	Mixed				
Sex										
Male	292	39	31	5	0	3	13.35	1	1.299	0.254
Female	269	35	27	4	1	3	13.01	-	-	-
Age (years)										
2-4	95	12	10	1	0	1	12.63	2	117.348	0.000
5-7	165	22	17	3	0	2	13.33	-	-	-
>7	301	40	31	5	1	3	13.28	-	-	-
Altitude										
Mid-land	260	34	27	4	0	3	13.07	1	2.995	0.083
Low-land	301	40	32	5	0	3	13.28	-	-	-
Total	561	74	58	9	1	6	13.19	-	-	-

findings in other *Glossina* species were reported. An overall infection rate of 5·1% of *Glossina morsitans submorsitans* by the three species of trypanosomes was reported in Radom National Park of Bahr El Arab (Mohammed-Ahmed et al., 1989).

More trypanosome infections were observed in female tsetse with an infection rate of 6.43% amongst the female flies while 0.49% infection rate was found in male flies. There was significant difference in the proportion of tsetse infected with trypanosomes between male and female flies (χ^2 = 2.01; *P* = 0.00). The reason for a higher infection rate in females might be due to their better life expectancy as suggested by Jordan (1974). The lower infection rate found in male flies can be explained by the low average age of traped male flies (20 days or less). The overall trypanosome prevalence (13.19%) found in the present study is relatively high when compared with the apparent density of *G. pallidipes* (2.25%) but it was well compromised with trypanosome infection rate of *G. pallidipes* (6.93%). The relatively higher fly infection rate and trypanosome prevalence as compared to low tsetse challenge can be explained by the higher fly- animal contact.

T. congolense in cattle was the most prevalent trypanosome species in the study area that accounts for the overall percentage of about 78.37% (58/74). Similar studies indicated that the most prevalent trypanosome species in tsetse-infested areas of Ethiopia are *T. congolense* and *T. vivax.* Rowlands et al. (1993) reported a prevalence of 37% for *T. congolense* in southwest Ethiopia. Abebe and Jobre (1996) reported an infection rate of 58.5% for *T. congolense,* 31.2% for *T. vivax* and 3.5% for *T. brucei* in southwest Ethiopia. In this research work, age was found to be a risk factor; higher infection rates were observed in adult animals in both altitude levels. This is logically associated to the fact that young animals are also naturally protected to some extent by maternal antibodies (Fimmen et al., 1982) but adult animals travel and cross-different vegetation types for grazing, watering, as well as for draught and harvesting crops to tsetse high challenged areas. *T. congolense* infection is a chronic disease that increase infection rates with age. *T. congolense* infection is usually higher in adult animals than younger ones (McDermott and Coelman, 1999).

In the present study, a relatively lower mean PCV values were observed in parasitaemic animals, but the difference is statically significant among aparasitaemic and parasitemic animals. The result of this study was in accordance with Rowlands et al. (2001) who observed in an increase in PCV value, the proportions of positivity decreases and hence mean PCV was a good indicator for the health status of animals in an endemic area. The lower mean PCV value in parasitaemic animals than the aparasitaemic animals is reported by several authors (Leak, 1987;

Afewerk, 1998; Muturi, 1999; Tewelde, 2001). The development of anaemia is one of the most typical signs of trypanosomosis cased by *T. congolense* in the susceptible cattle breeds (Murray and Dexter, 1988). The level of anaemia or the PCV usually gives a reliable indication of the disease status and productive performance of an infected animal (Trail et al., 1991).

Conclusion

This study presents findings on the trypanosome infection rate of *G. pallidipes* and prevalence of cattle trypanosomes in Amaro Special District of southern Ethiopia. The study indicated that *G. pallidipes* was the only *Glossina* species with the apparent density of 2.25%. The trypanosome infection in vector and host animals were highly prevalent than tsetse population density in the study area. This result could be due to fly-cattle contact relationship which increases the prevalence of trypanosome in both vector and host animals. Therefore, vector controlling and treating infected cattle with prophylactic or chemotherapeutic measures should be given to mitigate the problem in the study area.

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Full Length Research Paper

Bovine tuberculosis and its associated risk factors in pastoral and agro-pastoral cattle herds of Afar Region, Northeast Ethiopia

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Bovine tuberculosis (BTB) has a potential public health risk and economic impact in pastoralist community whose livelihood depends on their livestock. A cross-sectional study was carried out from September, 2008 to June, 2011 on 1087 cattle under pastoral and agro-pastoral production system in four districts of Afar Pastoral Region of Ethiopia using comparative intradermal tuberculin skin test to estimate the prevalence of BTB and assess the associated risk factors for infection. The individual animal prevalence of BTB in cattle of Afar pastoralists was 11% (95% confidence interval (CI): 9 to 13%) with ≥ 4 mm cut-off and 18% (95% CI: 16% to 21%) with ≥2 mm cut-off. The herd prevalence was 44% (95% CI: 36 to 51%) and 56% (95% CI: 48 to 63%) at ≥4 and ≥2 mm cut-off points, respectively. In bivariate analysis, the prevalence was significantly associated with study districts, herd size, sex and age, and in multivariable logistic regression analysis, the statistical significance was maintained with study district, age and herd size of the cattle. In conclusion, the present study revealed a moderately high prevalence of BTB in Afar Pastoral Region of Ethiopia and further investigation is recommended to assess the zoonotic significance of the disease to the pastoralist communities of the region.

Key words: Bovine tuberculosis, prevalence, risk factors, comparative intradermal tuberculin test, Afar pastoral region, Ethiopia.

INTRODUCTION

Bovine tuberculosis (BTB) is a chronic, granulomatous mycobacterial infectious disease caused mainly by *Mycobacterium bovis*, which is a member of *Mycobacterium tuberculosis* complex. BTB is a zoonotic disease with a potential health risk to human and has economic significance to livestock sub-sector (Ayele et al., 2004). Though BTB is controlled in developed countries through test-and-slaughter method, the disease poses a significant problem to the economy of the livestock sub-sector and remains a potential public health

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threat in developing countries where controlling programs are lacking. In Africa, approximately 85% of cattle and 82% of human population lives in areas where BTB is partly or not controlled at all in animals (Cosivi et al., 1998) and consumption of raw animal product is a common practice in rural and pastoralist communities of the continent, which creates a potential risk for zoonotic transmission of *M. bovis* (Daborn et al., 1996).

Ethiopia possesses the largest cattle population in Africa with the total of about 51 million of cattle (CSA, 2010). The livestock sub-sector in general contributes about 45% to the gross domestic product of the country's agriculture based-economy (Behnke, 2010). In addition, the sector plays a crucial role in livelihood of the pastoralist communities, who own 42% of the country's livestock in the lowland arid and semi arid regions. In Eastern Africa, Ethiopia has the largest pastoralist population (7 to 8 million) which depends on livestock for their livelihood (Markakis, 2004). The main feature of pastoralist's way of life is that they move from place to place in search of water and pasture for their livestock.

In Ethiopia, BTB is known to be endemic with prevalence ranging from 3.4 to 50% depending on husbandry method, with extensive rural setting showing low prevalence as compared to intensive dairy farms (Ameni et al., 2007; Berg et al., 2009). In spite of large population of livestock, very few studies were carried out in pastoral area of Ethiopia in which prevalence of 0.8% BTB in cattle of Hamer pastoral district (Tschoop et al., 2010) and prevalence of 5.5% of BTB in cattle of Borena pastoral area (Gumi et al., 2011) were reported. However, so far there is no report of BTB in cattle of Afar Pastoral Region of Northeast Ethiopia.

The Afar pastoral communities of Ethiopia are characterized by owing large numbers of livestock with diversity of species of animal. The consumption of raw animal products such as milk and very close physical contact creates a significant risk for transmission of zoonotic diseases like BTB. In addition, the existing epidemiological setting in Afar Pastoral Region is characterized by the presence of large herds of cattle, interspecies mixing of herds of animals at watering point, grazing area, at night in the village and the existence of climatic stress factors in the pastoral regions could suggest the existence of a potential risk factors for infection and transmission of diseases such as BTB in the livestock and pastoral communities of the region. Despite the large livestock population and existence of potential risk factors in the pastoral region, the epidemiology of BTB in the herds of cattle owned by pastoralist has not been well investigated so far.

The present study, therefore, was designed to investigate the epidemiology of BTB and assess the associated risk factors in the Afar Pastoral Region of Ethiopia.

MATERIALS AND METHODS

Study area

The study was conducted from September, 2008 to June, 2011 in four districts namely (Amibara, Dubti, Afambo and Chifra districts) of Afar Pastoral Region. The Afar Pastoral Region is located in northeast of Ethiopia between 39° 34' to 42° 28'E longitude and 8° 49' to 14° 30' N latitude (Figure 1). The region shares common international boundaries with Eritrea in the north-east and Djibouti in the east, and it is characterized by an arid and semi-arid climate with low and erratic rainfall. Rainfall is bi-modal throughout the region, with a mean annual rainfall below 500 mm in the semi-arid western escarpments and decreasing to 150 mm in the arid zones to the east. The altitude of the Region ranges from 120 m below sea level in Danakil depression to 1500 m above sea level. Temperatures vary from 20°C in higher elevations to 48°C in lower elevations. The human population of Afar region is 1.5 million in which the majority are pastoralists who largely depend on livestock production for their livelihood (Afar National Regional State (ANRS), 2010).

There are about 1.9 million Afar breed cattle in Afar Region, of which 90% of the cattle are managed under pastoral production system and the rest 10% in agro-pastoral production system (ANRS, 2010). The four districts were selected based on the cattle population, accessibility of their sub districts and presence of potential risk factors. Because of the presence of large pasture land and rivers in the districts, animals from different districts migrate to river banks and vast pasture lands where intermixing of different species (cattle, camel, goat and sheep) and herds of livestock occur. In Amibara and Dubti districts, there are large state-owned and private cotton farms, which after harvesting, become grazing sites where large number of herds of different species of livestock (cattle, camel and small ruminants) from various districts congregate to graze on the leftovers of the harvest, creating a potential risk factor for interspecies (cattle, camel, goat and sheep) and interherd disease transmission. Majority of the grazing land and watering points in Amibara district are shared by wild animals (including oryx, warthog, gazelle and zebra) from the Awash National Park. It was very common to observe cattle grazing in close proximity with wild animals in the pasture land of Amibara district. The sites selected in Amibara, Chifra and Dubti districts were pastoral and that of Afambo were mainly agro-pastoralist in their production system.

Study design

A cross sectional study was conducted in the four districts of Afar Pastoral Region and a total of 17 sub districts were included in the study based on the inclusion criteria (accessibility, security, and willingness of the pastoralists to participate in the research). All settlements (villages) in each sub district were included after obtaining the elder's consent to participate in the study. In our study, cattle owned by one owner and/or his close relatives, in which the animals shared common grazing sites, watering points, kept at night in common site and move together during migration, were considered as a herd to calculate the herd prevalence. In settlements which had super-herd, larger herd composed 500 to 600 animals, herd selection was made proportionally to represent each cluster in the super-herd. A total of 180 herds were tested and the final analysis was carried out on 171 herds, the rest 9 (5%) were drop-outs in which they were not available for reading after 72 h of tuberculin injection. In each herd, individual cattle were selected randomly after recording all the animals in the herd.

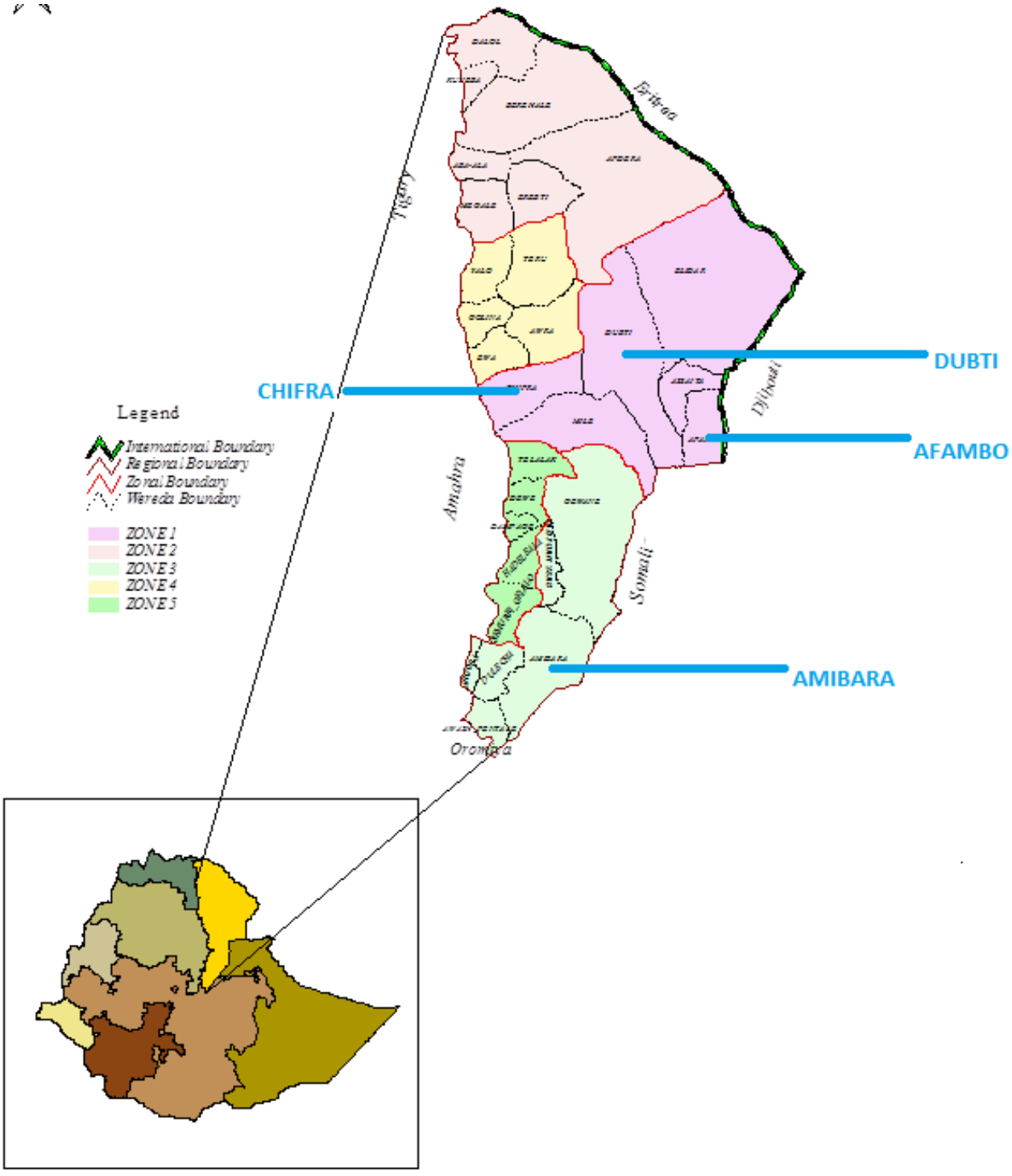

Figure 1. Map of Afar Pastoral Region indicating the study districts.

Study animals

For the Comparative intradermal tuberculin skin test (CIDT), cattle above the age of six months having no clinical symptom of any disease were included. Study animal related information on each tested cattle (such as sex, age, body condition score, lactation and reproductive status, parity number (number of calving)) were collected and recorded at the time of the test. All the cattle in this study were Afar (Danakil) breed of cattle which are categorized under Sanga breed group. Each animal was de-wormed with antihelmintic drug after testing and collecting fecal sample. Sample

size was determined according to Thrusfield (1995) considering the recommendation for sample size estimation involving three or more cluster stages (Thrusfield, 1995). Based on this estimation, the estimated sample size was 1,152. Hence, a total of 1,147 cattle were tested, although 5% were not available for the reading after 72 h of tuberculin injection, and hence were considered as drop-outs. Thus, the final analysis of the data was based on the results of 1087 cattle tested.

Comparative intradermal tuberculin skin test (CIDT)

CIDT was carried out by injecting both bovine purified protein derivative (PPD) and avian PPD (Observe™ bovine and avian tuberculin, AsureQuality Company, Mt. Wellington, Auckland, New Zealand). Two sites on the skin of the mid-neck of the cattle, 12 cm apart, were shaved, and skin thickness was measured with a caliper. One site was injected with an aliquot of 0.1 ml of 2,500 IU/ml bovine PPD into the dermis, and the other was similarly injected with 0.1ml of 2,500 IU/ml avian PPD. After 72 h, the skin thickness at the injection sites was measured and recorded. Results were interpreted according to the recommendations of the Office International des Epizooties (OIE, 2009) at ≥ 4 mm cut-off and also at ≥ 2 mm cut-off (Ameni et al., 2008). Thus, at cut-off ≥ 4 mm, if the increase in skin thickness at the injection site for bovine PPD (PPD-B) was greater than the increase in skin thickness at the injection site for avian PPD (PPD-A) and PPD-B minus PPD-A was less than 2 mm, between 2 and 4 mm, or 4 mm and above, the animal was classified as negative, doubtful, or positive for BTB, respectively. At cut-off ≥ 2 mm, if the difference between B and A was greater or equal to 2mm, the animal was considered as positive, while if the difference is less than 2 mm, the animal was considered as negative. When the change in skin thickness was greater at PPD-A injection site, the animal was considered positive for mycobacterial species other than *Mycobacterium tuberculosis* complex. A herd was considered as positive if it had at least one tuberculin reactor animal.

Body condition scoring

The body condition of each of the study animal was scored using the guidelines established by Nicholson and Butterworth (1986). Accordingly, on the basis of observation of anatomical parts such as vertebral column, ribs, and spines, the study animals were classified as lean (score, 1 to 2), medium (3 to 4), or fat (greater than 5).

Fecal sample examination

Fecal samples from CIDT tested cattle were collected during tuberculin injection directly from the rectum of each animal using sterile glove and placed in labeled vials containing 10% formalin solution and then transported to the laboratory for microscopic examination using floatation technique (Soulsby, 1982), and eggs of the parasites were classified based on their morphology and size.

Data management and analysis

Data were classified, filtered, coded using Epidata software and Microsoft Excel sheet, and was transferred and analyzed using STATA version 11 (Stata Corp., Collage station, TX). Pearson chi-square was used to evaluate the statistical significance of the associations of different categorical variables with skin test results and McNemar's chi-square was used to assess the association of PPD-A and PPD-B results. Bivariate and multivariable logistic regression analyses were performed to quantify crude and adjusted effects of pre-specified risk factors on tuberculin reactivity. P-value less than 5% was considered statistically significant. In cases of estimating the effect of different risk factors in terms of odds ratio (OR) with corresponding 95% confidence interval, statistical significance was assumed if the confidence interval did not include one among its values.

RESULTS

Individual animal prevalence

On the basis of CIDT, the animal prevalence of BTB was 11% (119/1087) with 4 mm cut-off point and 18.4% (200/1087) with 2 mm cut-off point. At 4 mm cut-off point, there were statistically significant differences in proportions of bovine positive reactor animals between the four districts (χ^2 = 21.7, P = 0.000), herd size category (χ^2 = 8.72, P = 0.013), sex (χ^2 = 6.96, P = 0.008), age category (χ^2 = 21.12, P = 0.000) (Table 1). At 2 mm cut-off point, in addition to the factors indicted above, there was a statistically significant difference in proportion of bovine positive reactors between the pastoral and agro-pastoral production system (χ^2 = 3.8, P = 0.05) where a higher proportion of positive reactors in cattle under pastoral production system than those in agro-pastoral production system. Multivariable logistic regression analysis (Table 2) showed that older cattle (9 years and above) had 2.66 times the odds of being tuberculin reactors compared with those cattle less than 2 years old (adjusted OR = 2.66; CI =1.21-5.84). Cattle found in Amibara district had also the higher odds of being tuberculin positivity in relative to those cattle in Chifra district (adjusted OR = 6.56; CI = 1.63 to 28.73). At both cut-off points, there was no statistical significance difference in the proportion of bovine tuberculin positivity between groups in relation to body condition score, breed, gastrointestinal parasite infestation status, lactation status, reproductive status, and number of parity. The gastrointestinal parasite infestation status in general was low both in tuberculin nonreactors and reactor cattle. In majority of the tested animals eggs of *Trichostronglus* species were the most common parasite eggs identified in this study.

Herd prevalence

The herd prevalence was 44% (95% CI = 36 to 51%) and 56% (95% CI = 48 to 63%) at ≥ 4 mm and ≥ 2 mm cut-off points, respectively. In multivariable logistic regression analysis, herds found in Amibara district had the higher

Table 1. Association of different risk factors to skin test positivity at 4 mm cut-off point for bovine tuberculosis in Afar Pastoral Region of Ethiopia.

Variable	Number of cattle examined	Number of positive (%)	χ^2	p-value
Districts				
Chifra	106	2 (1.9)	21.768	0.000
Dubti	151	10 (6.6)		
Afambo	137	9 (6.6)		
Amibara	693	98 (14.1)		
Herd size				
<11	330	50 (15.2)	8.720	0.013
11≤X<31	533	50 (9.4)		
≥31	224	19 (8.5)		
Sex				
Male	112	4(3.6)	6.968	0.008
Female	975	115(11.8)		
Age*				
<2	183	9(4.9)	21.123	0.000
2-5	220	13(5.9)		
5-9	419	55(13.1)		
>9	265	42(15.9)		
BCS				
Poor	298	31(10.4)	0.140	0.932
Good	596	66(11.1)		
Fat	193	22(11.4)		
Production system				
Pastoral	937	109 (11.6)	3.271	0.071
Agro-pastoral	150	12 (6.7)		
GIT Parasite				
Absence	246	16(6.5)	2.811	0.094
Present	131	15(11.5)		
Lactation status				
Lactating	274	23(8.4)	2.453	0.117
Non-lactating l	277	14(5.1)		
Reproductive status				
Pregnant	174	12(6.9)	0.280	0.597
Non-pregnant	299	17(5.7)		
Parity (Calving) number				
<2	83	3(3.6)	3.776	0.151
2≤X<5	87	7(8.1)		
X≥5	47	6(12.8)		

*A given age range includes its lower bound and excludes its upper bound. BCS: Body condition score; GIT: gastrointestinal tract.

Table 2. Multivariable logistic regression analysis of tuberculin reactors with various host-related risk factors at 4 mm cut-off point.

Variable	Number of cattle examined	Number of positive in CIDT	Crude odds ratio (95% CI)	Adjusted odds ratio (95% CI)
Districts				
Chifra	106	2	1	1
Dubti	151	10	3.68 (0.79-17.18)	2.79 (0.58-13.45)
Afambo	137	9	3.65 (0.77-17.29)	2.49 (0.16-37.49)
Amibara	693	98	8.56 (2.07-35.27)	6.84 (1.63-28.73)
Herd size				
<11	330	50	1	1
11≤X<31	533	50	0.57 (0.38-0.88)	0.54 (0.35-0.85)
≥31	224	19	0.51 (0.29-0.90)	0.42 (0.23-0.77)
Sex				
Male	112	4	1	1
Female	975	115	3.61 (1.30-9.98)	1.66 (0.55-4.98)
Age*				
<2	183	9	1	1
2-5	220	13	1.21 (0.50-2.90)	1.05 (0.42-2.56)
5-9	419	55	2.92 (1.41-6.04)	2.11 (0.98-4.56)
>9	265	42	3.64 (1.72-7.68)	2.66 (1.21-5.84)
BCS				
Poor	298	31	1	1
Good	596	66	1.07 (0.68-1.68)	1.06 (0.66-1.70)
Fat	193	22	1.10 (0.62-1.97)	1.17 (0.62-2.18)
Production system				
Pastoral	937	109	1	1
Agro-pastoral	150	12	0.54 (0.27-1.06)	1.45 (0.15-13.41)

CI: Confidence interval; BCS: body condition scoring. *A given age range includes its lower bound and excludes its upper bound.

odds of showing tuberculin positivity in relation to those cattle in Chifra district (adjusted OR = 8.15; 95% CI = 1.77 to 37.59), and no significant association was found between herd positivity, herd size and production system (Table 3).

Association of tuberculin reaction to bovine and avian PPD

Comparative result of skin reaction to PPD-A and PPD-B is summarized in Table 4. Based on the ≥ 4 mm cut-off point, a statistically significant association was observed between the skin reaction to PPD-A (avian) and PPD-B (bovine) (χ^2= 75.98; p-value = 0.000). As indicated in Table 4, 0.5% of the tested cattle responded positively to both PPD-A and PPD-B. On the other hand, 10.5% of them reacted only to PPD-B, while 1.8% reacted only to PPD-A.

DISCUSSION

BTB is known to be endemic in Ethiopia (Hailemariam, 1975), and in spite of a good deal of studies carried out in Ethiopia in the last decade, very few addressed the

Table 3. Multivariable logistic regression analysis of herd positivity with selected herd risk factors at 4 mm cut-off point.

Variable	Number of herds examined	Number of positive herds (%)	Crude odds ratio (95% CI)	Adjusted odds ratio (95% CI)
District				
Chifra	17	2 (11.8)	1	1
Dubti	32	8 (25)	2.5 (0.47-13.39)	2.39 (0.44-12.93)
Afambo	13	6 (46.2)	6.42 (1.03-40.26)	5.71 (0.89-36.31)
Amibara	109	59 (54.1)	8.85 (1.93-40.58)	8.15 (1.77-37.59)
Herd size				
<11	99	39 (39.4)	1	1
11≤X<31	57	26 (45.6)	1.29 (0.67-2.49)	1.23 (0.61-2.48)
≥31	15	10 (66.7)	3.08 (0.98-9.69)	2.44 (0.75-7.94)
Production system				
Pastoral	158	87 (55.1)	1	1
Agro-pastoral	13	8 (61.5)	1.11 (0.36-3.44)	-

CI: Confidence interval; BCS: body condition scoring.

Table 4. Response of PPD-A and PPD-B* at 4 mm cut-off point.

PPD A result	Number (%) of animals with PPD-B result		Total number (%)
	Positive	Negative	
Positive	5 (0.46)	15 (1.4)	20 (1.84)
Negative	114 (10.48)	953 (87.67)	1067 (98.16)
Total	119 (10.94)	968 (89.05)	1087 (100)

*Positive and negative reactions were according to OIE guideline with skin indurations ≥4 mm and <4 mm, respectively, McNemar's chi-square=75.98; p-value=0.000.

epidemiology of BTB in pastoral cattle of the country, which owns 42% of the country's cattle population and occupied 61% of the landmass of the country (PFE et al., 2010). In the present study, a moderately high animal prevalence was recorded at ≥ 4 mm cut-off point. The highest was reported in Amibara (14.1%) and lowest in Chifra district (1.9%), indicating a variation in prevalence within the region. The overall prevalence obtained, in general, was higher than the previous reports from other pastoral area of Ethiopia and Uganda. Thus, in Ethiopia, from Hamer and Borna, 0.8 and 5.5% prevalence were reported by Tschopp et al. (2010) and Gumi et al. (2011), respectively, while in Uganda a prevalence of 1.3% was reported by Inangolet et al. (2008). It was also higher than the 4.1% prevalence in cattle under traditional extensive grazing system of Boji [western Ethiopia] which were reported by Laval and Ameni (2004). The difference might be related to the epidemiological factors that favors the transmission of BTB in the Afar Region, which include large herd sizes, communal grazing and watering of diverse species of animals including camel, cattle, goat and sheep, and an extensive seasonal mobility within and outside the districts, which creates favorable condition for wide range of interspecies contact (cattle, camel, goat, sheep and some wild animals such as Oryx, antelope and warthog).

In addition, the herds owned by individual pastoralist congregate together, forming a larger herd of the village and this larger herd composed of 500 to 600 of cattle and moves together to grazing and watering site. Such type of herd structure was observed particularly in Amibara district where conflict between Afar and Issa Somali tribes has been common and this intermixing of the herds might have increased the chance of contracting the infection, as it is demonstrated by higher prevalence in the district (14.1%) as compared to the others.

Moreover, during months of November to February, large number of livestock congregate in the cotton irrigation farms (2 to 4 weeks) to graze on the leftover of the cotton farm and different species of livestock (camel, cattle, and small ruminants) coming from neighboring zones and districts interact at the specific point, which create a favorable condition for close contact between animals and potential risk for transmission of diseases such as BTB among the animals. Such epidemiologically conducive conditions could lead to higher prevalence of BTB in the Afar Region as compared to the prevalence in other pastoral regions in Ethiopia. In addition, because of the presence of extensive range land in the districts, particularly in Amibara district, wild animals including oryx, gazelle, warthog and zebras (in and around Awash National Park) were observed grazing in close proximity with cattle which suggests a possible exposure for potential risk of disease transmission either way. The possibility of transmission of *M. bovis* between wildlife and cattle has been reported from other part of Africa and Europe (Woodford, 1982; Phillips et al., 2003; Cleaveland et al., 2005).

On the other hand, the result of the present study was much lower than the higher prevalence of BTB reported in urban intensive dairy farms of Ethiopia, where Holstein and crossbreeds cattle predominantly form the composition of the farms under intensive management system (Ameni et al., 2003, 2007). This difference might be mainly related to the intensive husbandry system practiced and the breed susceptibility (Ameni et al., 2007; Tsegaye et al., 2010). In our study, the animals tested were zebu Afar breed of cattle managed under extensive pastoral husbandry system which might be one reason for the differences in result, as the zebu breeds are known to be relatively resistant to BTB as compared to Holstein and other cross breeds managed under intensive system (Ameni et al., 2007,Cadmus et al., 2010).

The prevalence of BTB showed an increase with age and this finding was in agreement with previous reports by others (Kazwala et al., 2001; Oloya et al., 2006; Ameni et al., 2007; Inangolet et al., 2008; Regassa et al., 2010, Cadmus et al., 2010; Biffa et al., 2011). As indicated by these authors, the possible reasons could be the fact that older animals had longer and repeated chance of exposure to mycobacterial infection during their life time.

Furthermore, it has been observed that cows were more positive reactor than bulls, which is in agreement with other studies (Inangolet et al., 2008; Cadmus et al., 2010). In the Afar pastoral system, the majority (90%) of their herds is composed of cows kept exclusively for milk production and kept for longer time than the bulls, which form less than 10% of the herd, as bulls are sold or slaughtered in their early age. This condition might be the reason for higher tuberculin positivity in cows than bulls. As milk is consumed raw in Afar communities, the high prevalence in cows might create a potential risk for public health in the pastoralist community and need further investigation to identify its zoonotic significance and further design a control strategy in the region.

Similar to other studies in Ethiopia (Ameni et al., 2007; Tschopp et al., 2010; Gumi et al., 2011; Biffa et al., 2011), there was no association between body condition score and tuberculin skin test positivity. In addition, no statistical significant association was observed between tuberculin positivity and gastrointestinal parasite infestation which was different from previous finding by Ameni and Medihn (2000), which could be due to the difference in geographic locations and climatic condition which determines the existence and load of parasite in the area. In the present study with arid and semi arid climatic condition, the parasite infestation load was low, and immune compromising parasite such as *Fasciola hepatica* (Flynn et al., 2007, 2009) were not abundant as that of the highland area and hence might not have affected the overall tuberculin reactivity of the animals, while Ameni and Medhin (2000) did their study in highland where fasciolosis was highly prevailing.

Finally, because of the poor infrastructure facilities such as road accessibility and insecurity to the remote sites in the pastoral setting, part of the study was carried out with some level of convenient sampling method which can be taken as the limitation of this study.

Conclusion

To the best of our knowledge, this is the first BTB study done in Afar Pastoral Region of Ethiopia. The study revealed a moderately high prevalence of bovine tuberculosis in cattle and the presence of epidemiological risk factors for infection and transmission among cattle of the Region. Considering the fact that the Afar pastoral communities have very close contact with their animals and depend entirely on their livestock for subsistence through consumption of raw milk and other animal products, the findings of this study emphasizes the need for further investigation on isolation of the specific *Mycobacterium* species causing BTB in livestock, and their zoonotic significance in the Afar pastoral community, in order to design control options of the disease both in livestock and humans living in pastoral setting.

Abbreviations

BTB, Bovine tuberculosis; **CIDT,** comparative intradermal tuberculin skin test; **PPD,** purified protein derivative.

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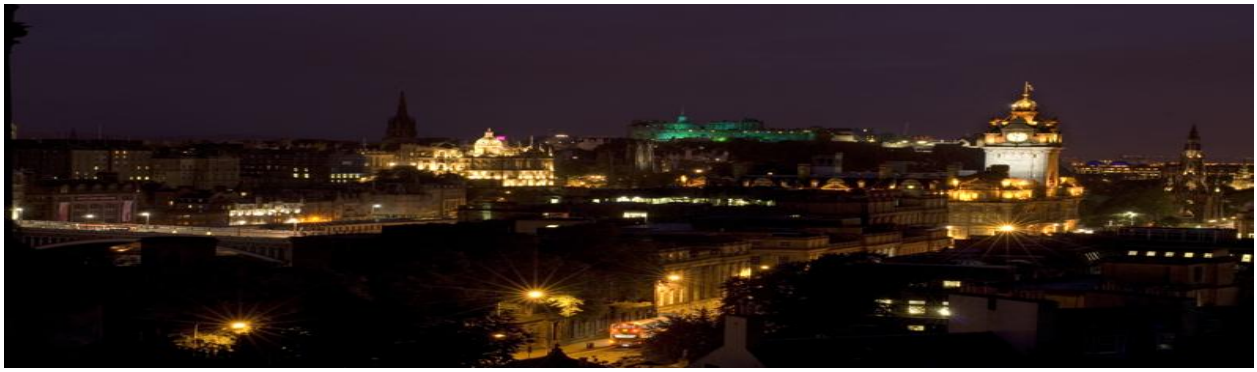

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