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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

Effect of infectious bursal disease virus on pathogenicity of avian influenza virus subtype H9N2 in broiler chicks

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In this experiment, pathogenesis of H9N2 avian influenza virus (AIV), experimentally infected with infectious bursal disease virus (IBDV) in broiler chicks was examined. Three groups of twenty were randomly selected. Day old chickens in group 1, were infected by 10^3 CID50 of IBDV intrabursaly, and in thirty days of age groups 1 and 2 were challenged with 10^6 EID50 H9N2, intranasaly-intraocular. Chickens in group 3 remained as control (uninfected with neither IBDV or AIV). Tracheal and cloacal swabs, and tissue samples, were collected at 3, 7, and 11 days postinoculation (PI). Serum samples examined for antibodies against avian influenza virus (AIV) by hemagglutination inhibition test (HI). IBD caused lower H9N2 antibody level. IBDV infected chickens (g1) shed AI virus for a longer period than AIV infected birds (g2), from both trachea and cloac. IBDV was related with AIV in brain and liver. Isolation of AIV from trachea, conjunctiva, bursa and lung in IBDV infected group (1), prolonged till 11 days PI. Our study provides evidence that a previous history of IBDV infection in chickens may cause them to be more susceptible to H9N2 low pathogenic avian influenza (LPAI) virus infection and may alter its tissue tropism.

Key words: Infectious bursal disease, avian influenza, virus shedding, broiler chicks.

INTRODUCTION

Avian influenza (AI) is a highly contagious disease caused by type A influenza virus, a genus of the family *Orthomyxoviridae.* Avian influenza viruses are divided into subtypes on the basis of two surface glycoproteins: hemagglutinin (HA) and neuraminidase (NA) (Swayne and Halvorson, 2008). Seventeen HA (H1 to H17) and ten NA subtypes (N1 to N10) have been identified (Tong et al., 2012, Zhu et al., 2012). Avian influenza (AI) has been reported in many countries from the Middle East region and Asia (Alexander, 2002). In 1998, an H9N2 subtype influenza A virus of low pathogenicity has been reported in the industrial poultry populations of Iran (Vasfi Marandi et al., 1999) and thereafter it has caused outbreaks in commercial broiler chickens in Iran (Nili et al., 2002). Outbreaks of H9N2 subtype also occurred in poultry in Pakistan (Bano et al., 2003). Considerable economic loss due to decreased production, increased mortality and the cost of vaccination have occurred following H9N2 infection in Iranian poultry industry (Vasfi Marandi and Bozorgmehrifard, 1999; Nili and Asasi, 2003). H9N2 influenza viruses are also considered to be one of the potential candidates for the next human pandemic (Butt and Smith, 2005). Therefore, it is imperative to understand the pathogenesis and properties of these viruses.

Infectious bursal disease (IBD), initially reported as

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Gumboro disease, is an acute, highly contagious virus infection of young chickens first described by Cosgrove (1962), who found B lymphocytes to be the primary target cells (Kauffer and Weiss, 1980). IBDV is important because it causes clinical disease and mortality in chickens 3 weeks of age or older and prolonged immunosuppression of chickens infected early in life leading to other infections and vaccination failures (Lukert, 1997). Its immunosuppressive effects were reported by others (Allan et al., 1972). Infection with IBDV reduces antibody response to other vaccinations (Faragher, 1974; Giambrone, 1976; Rosenberger, 1977; Muller, 2003, Westbury 2008), but the response against IBDV itself is normal (Skeeles and Lukkert, 1979). The present study was consequently undertaken to evaluate the effects of experimental IBDV infection in chickens by assessing the humoral responses of chickens to influenza virus subtype H9N2 in addition of its effects on H9N2 AIV pathogenicity for broilers.

MATERIALS AND METHODS

Challenge virus

A very virulent strain of Gumboro virus and avian influenza A virus subtype H9N2 were obtained from Razi Vaccine and Serum Research Institute (Iran), influenza virus was passaged in 9 to 11 days old emberyonated chicken eggs and used as a challenge virus in this study. The embryo infective dose (EID50) of infected allantoic fluid was calculated according to the Reed and Muench formula (1938). The virus was diluted 10 fold in sterile phosphate buffered saline (PBS) solution to obtain concentration of 10^6 EID50 in 1 ml. Ten fold serial dilutions of Gumboro virus was inoculated to 10 groups of five 21-day old chickens for evaluation of chicken infectious dose (CID50) by Reed and Muench method and 10^3 CID50 of diluted virus in sterile PBS was used to the trial.

Experimental design

Sixty one-day-old commercial broiler chicks were divided randomly into three groups, twenty chicks per group. All animal experiments were kept in separated cages in an isolated room and all biosecurity aspects were considered. Feed and water were available *ad libitum*. Day old chicks in group 1 were inoculated with 10^3 CID50 of infectious bursal disease virus intrabursaly. At the age of 30 days, groups 1 and 2 were challenged with 10^6 EID50/0/1 ml of H9N2 virus intraocculary-intranasaly. Birds in group 3 were not infected with neither IBDV or AIV (Table 1).

Table 1. Program of chickens infection in various groups.

Day of age	1	30
Challenge virus	IBDV	AIV
group1	+	+
group 2	-	+
group 3	-	-

Serology

At the days of 8, 29 and 42 days of age serum samples were collected from 10 birds per group and were tested for evaluation of H9N2 antibody titers by hemagglutination inhibition (HI) test. HI tests were performed following World Health Organization (WHO) recommendations (Webster et al., 2002).

Statistics

The mean titre of chickens antibody response was evaluated by 1-way analysis of variance (ANOVA) followed by $Danet_c$ and Tukey test, allowing for statistical comparisons among the different groups.

Duration of viral shedding

Tracheal and cloacal swabs were collected from three chickens per group on days 3,7 and 11 postinocculation with avian influenza H9N2 and stored at -70°C in sterile microtubes containing 1 ml buffered glycerol medium (50% sterile glycerol, 50% PBS) containing antibiotic-antimycotic.

Isolation of influenza virus from various organs

For studying effect of IBD virus on spread of AI virus in tissues samples, a comprehensive group of organs including trachea, lungs, conjunctiva, brain, liver, pancreas, bursa, thymus and kidney were collected from 3 birds per groups at 3, 7 and 11 dpi and samples from each group were pooled. Tissues were homogenized and 10% suspension was prepared by BHI medium. Suspensions were centrifuged at 1500 × g 10 min in 4°C then the supernatant was collected, and antibiotic (1000 IU/ml and streptomycin 2 mg/ml) and amphotericin B (0.02 mg/ml) (Dennis and Senne, 2008) were added. Suspensions of bursa were filtered before adding antibiotic.

Virus isolation

The influenza virus from various organs and swab samples was investigated by virus isolation method in 10-day-old embryonated chicken eggs. Five eggs were used for each tissue or swab sample and 200 µl/egg was inoculated to 10-day-old embryonated chicken eggs. Infected eggs were incubated for 48 h and then chilled at 4°C for no more than 24 h. Allantoic fluid was collected and a hemagglutination (HA) assay was performed. Samples showing agglutination of fresh chicken red blood cells were scored as positive.

RESULTS

Clinical observations

Results of daily monitoring of all groups showed that all chicks were clinically normal and did not show any abnormality prior to inoculation with influenza virus. From day two post-challenge, birds infected AIV started to show clinical signs such as depression, ruffled feathers, respiratory distress (coughing, sneezing and dyspnea), swelling of the periorbital tissues and sinuses, conjunctivitis, nasal and ocular discharge until day six post-inoculation (PI) that sings reduced. Mortality in IBD + AIV group was 33.3%.

Table 2. Mean titer of serum antibody levels against avian influenzavirus subtype H9N2(log2).

Group/day of age	8	29[a]	42[b]
IBD+AIV	5.8	1.15	8.3
AIV	5.8	1.4	10.14
Control	5.8	1.5	1

a = before inoculation of influenza, b=11 days after inoculation of influenza. Serum sampls were exmined by hemaglutination inhibition test to study the effect of gumboro disease on antibody production in chickens against avian influenza virus subtype H9N2. This test was conducted by WHO manual (webester, 2002).

Serology

HI test

There was no evidence of any change in specific antibodies against AIV or IBDV pre and post inoculation of control chickens. Mean antibody titers against influenza virus on the basis of log2 are shown in Table 2. 11 days after inoculation with influenza virus (42th days of age), significant differences were seen between IBD + AIV inoculated birds and AIVs. Results shows that infectious bursal disease can cause significant decrease of antibodys against H9N2 AI virus.

Duration of viral shedding

Chickens co-infected with AIV + IBD (group1) shed H9N2 AIV from day 3 to days 11 PI, while chickens in group 2 (AIV inoculated) shed the virus from day 3 to 7 PI in cloacal and tracheal swabs. Chickens of control group did not shed the virus (Table 3). In addition, chickens in group 1 had more positive sample/total in each time of sampling.

Isolation of influenza virus from various organs

The presence of the virus in various organs obtained from the inoculated and control birds at different days PI was determined by inoculation of 10% tissue suspensions in alantoic fluid of 9 to 11 days emberyonated chicken eggs. The results of the virus detection are shown in Table 4. The results show that most positive samples were detected on days 3 PI. The virus was isolated from the trachea, conjunctiva, lungs, pancreas, bursa, thymus and kidney of all experiment groups at 3 dpi. But in IBD + AIV group brain and liver samples were also positive. All samples except trachea and conjunctiva from other groups were negative at 7 dpi. Trachea, conjunctiva, bursa and lungs samples in IBD + AIV group were also positive till 11 dpi. 11 days PI all samples from AIV group were negative.

Table 3. Results of virus isolation in emberyonated chicken eggs (Tracheal and Cloacal swabs).

Group/day PI	Cloacal swabs			Tracheal swabs		
	3	7	11	3	7	11
1	3/3*	2/3	1/3	3/3	3/3	2/3
2	2/3	2/3	0/3	3/3	2/3	0/3
3	0/3	0/3	0/3	0/3	0/3	0/3

*Number of positive samples/total samples taken.group1 = IBD, g2 = AIV, g3 = Control. On 3, 7 and 11 days after inoculation of avian influenza subtype H9N2, tracheal , cloacal swab were collected using Dacron swabs. Each swab placed in a steriled and antimicotics were added.after 1 hour incubation in environment 200µml of swab medium was inoculated to 9 to 11 days old emberyonated chicken eggs via alantoic sac. Five eggs were used for each swab collected to determine the presence of virus. Infected eggs were incubated for 48 h and then chilled at 4°C for no more than 24 h. Allantoic fluid was collected and a hemagglutination (HA) assay was performed. This table shows positive sample/total sample. According to this table gumboro disease could increase period of virus shedding from trachea and cloaca.

DISCUSSION

In the last decade, frequent incidences of H9N2 AIV outbreaks have caused high mortality in broiler chicken farms in Iran and some other Asian countries, resulting in great economic losses (Nili and Asasi, 2002, 2003). However, the causative virus has not characterized as low pathogenic avian influenza (LPAI) viruses. So far, there has not been any clear explanation for such a definitive differences in mortality and severity of clinical manifestation between affected fields. One of the possible explanations for such a high mortality could be that it is due to mixed infection of the virus (H9N2) with other pathogens. Likewise, It has been declared that the factors such as management, concurrent bacterial or viral diseases, immunosuppression agents, age and strain of chicken, are the main reasons of the pathogenicity variation of H9N2 isolates (Aamir et al., 2007; Capua and Alexander, 2004; Guo et al., 2000; Toroghi and Momayez, 2006; Subler et al., 2008).

Bano et al. (2003) indicated that H9N2 subtype of AIV as a nonpathogenic virus can cause a severe infection in field condition in presence of opportunist secondary pathogens. They also showed that in chemically bursectomised chickens, H9N2 subtype can cause high mortality. Banani et al. (2002) and Nili and Asasi (2003) suggested that concurrent infections with infectious bronchitis and secondary bacterial infection such as ornithobacterium rhinotracheal, *Escherichia coli* and *Mycoplasma gallisepticum* may be important enhancers of the signs than the other factors in H9N2 infection in chickens. Ramirez et al. (2010) reported that previous infection of IBDV in chickens may render them more susceptible to avian influenza virus (AIV) infection, allowing for the potential introduction of AIVs in an otherwise resistant population. Since various strains of infectious bursal disease viruses and H9N2 AI viruses

Table 4. The results of virus detection from various organs of chickens at different days post inoculation with H9N2 AI virus.

Day PI	Group	Tr	Lu	P	Br	Li	C	K	Bu	Th
	1	+	+	+	+	+	+	+	+	+
3	2	+	+	+	-	-	+	+	+	+
	3	-	-	-	-	-	-	-	-	-
	1	+	+	-	-	-	+	-	+	-
7	2	+	-	-	-	-	+	-	-	-
	3	-	-	-	-	-	-	-	-	-
	1	+	+	-	-	-	+	-	+	-
11	2	-	-	-	-	-	-	-	-	-
	3	-	-	-	-	-	-	-	-	-

Tr= Trachea, Lu= Lung, Th= Thymus, Bu= Bursa of Fabricius, P= Pancreas, K= Kidney, Br= Brain, C=Conjunctiva, Li=Liver. group1=IBD+AIV, g2=AIV, g3= Control suspension. 10% of tissue samples were prepared by adding enough content of BHI(Brain Heart Infusion) to the tissues. After centrifuging at 1500g supernatant was collected and antibiotic-antimycotics were added. after 1 hour incubation in environment 200µml of supernatant was inoculated to 9-11 days old emberyonated chicken eggs via alantoic sac. five eggs were used for each tissue samples collected. eggs were incubated for 48 hr and then chilled at 4° C for no more than 24 hr. Allantoic fluid was collected and a hemagglutination (HA) assay was performed.this table shows that IBDV infection could prolonged presence and isolation period of avian influenza subtype h9n2 from tissues and caused precence of live virus in un common tissues(brain and liver).

commonly circulate in poultry farms in Iran, we carried out experimental coinfection of H9N2 AIV with IBD virus to investigate role of IBDV on some H9N2 pathogenisity factors.

In this experiment, IBDV caused lower AIV antibody levels significantly. Although antibody levels in IBD-infected birds were not severly affected, an observation indicating possible relative resistance, which might be consequence of the age that H9 exposure happened and/or the time between IBDV infection and exposure to H9N2. Otim et al. (2005) reported that Newcastle disease antibody levels after IBDV infection in chickens were lower than those of the control group, but they were still above log mean $2^{5.2}$, the 100% protective titer.

Inoculation of chicks with IBDV prolonged AI virus excretion from cloac and trachea comparing with AIV group, suggesting that this immunosuppressive agent may have also interfered with immune mechanisms that could have prevented virus replication (Otim et al., 2005).

Ramirez (2010) reported that previous history of IBDV infection in chickens may alter host range, tissue tropism or virulence. Results of tissue isolations indicated that prior infection with IBDV prolonged caused altered tissue tropism of H9N2 consequently, isolating the AI virus from liver and brain. There is a question that in which way, AI virus introduced in liver and brain of IBDV infected chickens, from localized infection or by viremia? IBDV might induce prolonged viremia, in AIV infection. Coinfection of IBD promoted the propagation of AIV and increased the pathogenicity and extended the period of H9N2 AIV shedding in broiler chickens and caused mortality under the present experimental conditions.

Conclusions

The results of this study indicated that:

1. Previous infection with infectious bursal disease virus promoted the propagation of H9N2 avian influenza virus and extended the period of its shedding from trachea and cloaca in broiler chickens.
2. It prologed isolation of H9N2 avian influenza virus from tissues and altered tissue tropism of it.
3. It increased the pathogenicity of H9N2 AIV and caused most mortality.

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ABBREVIATION

IBDV, Infectious bursal disease virus; **AIV,** avian influenza virus; **LPAI,** low pathogenic avian influenza; **CID50,** 50% chicken infective dose; **EID50,** 50% emberyo infective dose; **HI,** hemagglutination inhibition.

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Dairy cows mastitis survey in Adama Town, Ethiopia

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A total of 102 smallholder dairy farms and 303 cross bred milking cows were examined to determine the overall prevalence of mastitis, to identify risk factor, to isolate and identify bacterial pathogens and to conduct *in vitro* antimicrobial susceptibility test in Adama Town, Ethiopia using pre-tested questionnaires, California mastitis test (CMT), microbial isolation and identification and *in vitro* antibiotic susceptibility test. The overall mastitis prevalence was 73.4% (at herd), 39.5% (at cow) and 23.7% (at quarter) level of which (15.6 and 57.8%) at herd, (5.9 and 33.6%) at cow and (2.9 and 20.8%) at quarter level were clinical and sub-clinical mastitis respectively. The major isolates of subclinical mastitis cases were *Staphylococcus aureus* (26.8%), *Staphylococcus intermedius* (2.5%), Coagulase Negative *Staphylococcus* (CNS, 18.7%), *Streptococcus agalactiae* (5.8%), *Streptococcus dysgalactiae* (2.0%), *Streptococcus uberis* (4.5%) and *Escherichia coli* (6.5%) and from clinical mastitis were *S. aureus* (2.5%) and *S. agalactiae* (3.8%). Among the risk factors stage of lactation, parity and presence of teat lesions have been shown statistically significant ($p<0.05$) difference in the prevalence of mastitis. In the present study, out of the nine *in vitro* antimicrobials used, Gentamycine (3.1%), Chloramphinicol (4.2%), Vancomycin (17.6%), Streptomycin (25.4%), Kanamaycin (26.4%), Polymyxin B (48.6%), Penicillin (48.9%), Amoxicillin (68.7%) and Bacitracin (74.9%) showed resistance to mastitic pathogens. Gentamycine and Chloramphinicol were found to be more effective antibiotic among all the tested antibiotics. The main reasons for the occurrence of a high number of resistant strains in this study were the use of sub-therapeutic level of antibiotics and/or short treatment episodes and the long-term presence of infected cows in herds. Finally, due to the high resistance levels detected in the present study, it was believed that it is necessary to set up antimicrobial resistance (AMR) surveillance programs in the country.

Key words: Mastitis, bacteria, isolates, antibiotic, susceptibility, prevalence, risk factors.

INTRODUCTION

Ethiopia holds large potential for dairy development. The country currently manages the largest livestock population in Africa, estimated to be about 52.13 million head of cattle, 24.2 million sheep, 22.6 million goat, 8.73 million equine, 0.99 million of camel and 44.89 million poultry (CSA, 2012). Even though Ethiopia has huge number of livestock, the productivity has always been sub-optimal due to low genetic potential of the animals, poor nutrition and prevailing diseases. Mastitis is one of the most important economically devastating diseases of dairy cattle particularly for the backyard farmers in developing world, with different levels of economic losses (Hogeveen et al., 2011; Atyabi et al., 2006). Mastitis (Mast: breast, itis: inflammation) is one of the prevailing diseases characterized by inflammation of the mammary gland (udder) that causes physical and chemical changes in milk and leads to pathological condition of the glandular tissue, which may result due to microbial,

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thermal, chemical or physical causes (Fox, 2005). Although it may be caused by thermal or chemical or physical agents, the causes are almost entirely infectious and mostly bacterial. It is generally associated with poor hygienic and husbandry practices. The infection rate of mastitis in cows with pendulous udder is higher than those having non-pendulous udder (Hundera et al., 2005). In recent years, acquired antimicrobial resistance in bacteria is an increasing threat in human as well as in veterinary medicine. Hence, monitoring antimicrobial susceptibility in pathogenic as well as in commensal bacteria in animals is recommended by World Organisation for Animal Health (OIE) (Acar and Rostel, 2001). Such monitoring generates data of importance for therapeutic decisions and provides information on trends in resistance that might be cause for interventions regarding antimicrobial use.

In Ethiopia even if some studies have been conducted so far on the prevalence and the major cause of bovine mastitis in the country by Workineh et al. (2002), Biffa et al. (2005), Hunidera et al. (2005), Getahun et al. (2008), Mekibib et al. (2010), Mekonen et al. (2012), Bedane et al. (2012), Bekele et al. (2012), Alemu et al. (2013) and Zeryehun et a. (2013), it is insufficiently investigated and information related to magnitude of the disease, risk factor and antimicrobial susceptibility are scanty. Such information is important to envisage when designing appropriate strategies that would help to reduce its prevalence and effects. Therefore, the objectives of this study were to determine the prevalence of mastitis, to isolate and identify major mastitis pathogens, to perform *in vitro* antimicrobial susceptibility test and to assess risk factors.

MATERIALS AND METHODS

Study design

Study type

Cross sectional type of study was carried out from September 2008 to April 2009 in and around Adama town of Oromia regional state, Ethiopia. The prevalence rate of sub-clinical and clinical mastitis at cow level was calculated using the formula described by Wasserstein (1995).

Sampling

From in and around Adama town, 102 smallholder dairy farms (those had Holestein-Frisian zebu cross breed cows) were randomly selected for this study. Simple random sampling was carried out to select 303 lactating crossbreed cows from the total of smallholder's dairy farms in the study areas. The consideration during sample size determination includes 95% confidence interval, 5% precision and 60% prevalence from the previous studies in similar study areas (Workneh et al., 2002). Sample size was calculated using the formula described by Thrusfield (2005). Milk samples were taken in sterile universal bottles and closed with screw caps. The universal bottles were marked with a permanent marker, so that the markings were easy to read when the universal bottles were placed in a rack. The universal bottles were marked before sampling. The surface of the teat ends were cleaned by wiping with clean cotton dipped in 70% alcohol. Scrubbing with alcohol pads falls way short of sterilizing teat skin. An insulated cool box was used for transporting samples (Quinn et al., 2004).

Risk factor analysis

A questionnaire was developed, pre tested and administered to the smallholders' dairy owners of the animals. Data on each cow was collected in a format designed for this purpose. The animal level factors considered were parity numbers, herd size, stage of lactation and presence of teat lesion. The farm level factors were housing, farm hygiene, milking hygiene and milking sequence.

Clinical and subclinical analyses

California mastitis test (CMT)

CMT was carried out to screen sub-clinical mastitis and for selection of samples for bacterial culture. A small amount of milk from each quarter is squired into shallow cups in the CMT paddle, an equal amount of 3% CMT reagent was added to each cup and mixed well. A gentle circular motion was applied to a mixture in a horizontal plane for 15 s. Finally, the reactions were graded as negative, trace, 1+, 2+, and 3+, as described by Quinn et al. (2004). Cows and herds were considered positive for subclinical mastitis, when at least one quarter of a cow and one cow from the herd became positive for CMT, respectively. Definition of quarter was: one teat together with the part of cow's udder that it drains.

Clinical observation

Gross abnormalities indicated the clinical form of the disease was detected by physical examination of the udder for the presence of swelling, pain, hotness, disproportional symmetry, fibrosis, visible injury, tick infestation, atrophy and teat blindness. It was also recognized based on abnormalities in milk including flakes, clots and watery secretion.

Analysis of pathogens and their antimicrobial susceptibility

Microbial investigation was performed according to Quinn et al. (2004). The isolates were exposed to antimicrobial sensitivity using Kanamayacin (K 30), Streptomycin (S 10), Penicillin (P 10), Amoxicillin (Aml 2), Gentamycine (CN10), Chloramphenicol (C 30), Polymyxin (PB 300), Bacitracin (B 10) and Vancomycin (VA 30) discs *in vitro* disc diffusion (Kirby-Baur test method) was done based on Clinical and Laboratory Standards Institute (CLSI, 2010) at Microbiology Laboratory of Faculty of Veterinary Medicine of Addis Ababa University.

Statistical analysis

The data collected during the study periods were entered into MS-Excel spread sheet and analyzed using STATA software (STATA 2001). The effect of risk factors with possible association of the disease was analyzed using Chi-square. The associations between dependent and independent variables were tested by logistic regression model. For all the analysis performed, $p<0.05$ was taken as statistically significant (Snedecor and Cochran, 1989). Prevalence of bovine mastitis related to specific risk factors was determined as the proportion of affected cows out of the total

Table 1. Prevalence of clinical and subclinical mastitis at herd, cow and quarter levels based on clinical observation and culture.

Observation level	N	Prevalence of clinical mastitis		Prevalence of subclinical mastitis	
		Clinical observation in % (N)	Culture in % (N)	CMT in % (N)	Culture in % (N)
Herd level	102	15.6 (16)	100 (16)	57.8 (59/102)	98.3 (58)
Cow level	303	5.9 (18)	100 (18)	33.6 (102/303)	90.19 (92)
Quarter level	1172	2.98 (35)	100 (35)	20.8 (244/1172)	93.85 (229)

N: Number of observation; n: number of positive.

examined (Thrusfield, 2005). The prevalence of clinical and subclinical mastitis at herd, cow, and quarter level as defined by CMT score and bacteriological result was dependent variables. The independent variable at herd level included farm hygiene, barn floor status, milking hygiene and milking sequence. Stage of lactation was classified into three in such a way that the beginning of lactation referred to the first two months of lactation period, middle of lactation referred to the next five months period and end of lactation referred to the last weeks of lactation. A farm was considered to have good barn floor status, if the floor is made of concrete and bad if the floor is muddy. A farm was regarded as having good milking hygiene, if it practiced any one of the specific practices considered during the analysis.

RESULTS

Prevalence at quarter level

The results of this study showed that out of 2012 quarters, 47 (2.34%) were blinded. The overall quarter level prevalence of clinical and subclinical mastitis was 2.39% (n=47) and 88.01% (n=345), respectively. The individual quarter level prevalence of subclinical mastitis was 20.48% (n=103), 22.00% (n=110), 16.39% (n=78) and 20.78% (n=101) for the front right, front left hind right and hind left quarters, respectively. The results of univariate logistic regression revealed that quarter level prevalence of subclinical mastitis was not significantly different between the hind (21.44%) and front (26.03%) quarters and also the right (25.63%) and left quarters (21.96%).

Prevalence of clinical and subclinical mastitis

On the bases of clinical observation, 15.6% (n=16) herds, 5.9% (n = 18) cows and 35 (2.98%) quarters had clinical mastitis based on clinical observations and all the clinically mastitic positive herds, cow and quarter were 100% positive on bacterial culture. The prevalence of sub clinical mastitis was determined by CMT and microbiological cultures as presented in Table 1. From a total of 102 herds, 59 (57.8%) were positive based on CMT test and 98.3% of them were bacteriologically, culture positive. From the total 303 dairy cows, 102 (33.6%) of them were CMT positive and among this 90.19% were culture positive. From the total 1172 quarters, 244 (20.8%) quarters were CMT positive, 93.5% of them were culture positive (Table 1).

Risk factors affecting the prevalence of subclinical mastitis at cow level

The results of a univariate logistic regression revealed that the cow level prevalence of sub-clinical mastitis in the study area was significantly affected by stage of lactation and parity ($p<0.05$). All the cows (n=8) with teat lesion had subclinical mastitis. The prevalence of subclinical mastitis was significantly higher in cows at the end of lactation (78.82%) and in those with high parity number (65.69%). When the factors with p-value less than 0.25 were fitted in the multivariate model, only stage of lactation had significant effect on cow level prevalence ($p<0.05$). In the case of farm (herd) level prevalence of subclinical mastitis, only the practice of milking mastitic cow last had significant effect on the prevalence of subclinical mastitis ($p<0.05$). The prevalence was significantly higher (86.42%) in those which were not milking mastitic cows last (Table 2). Risk factors with p-value less than 0.25 were fitted in a multivariate model and only the practices of milking mastitic cow last had significant effect on the prevalence of subclinical mastitis ($p<0.05$). Risk factors with p-value less than 0.25 were fitted in a multivariate model and only the practices of milking mastitic cow last had significant effect on herd level prevalence of subclinical mastitis.

Bacterial isolates

From the total 118 lactating cows, 279 quarters of them were positive for mastitis either clinically or CMT tests. A total of 173 bacterial isolates were found, as presented in Table 3. Generally, the most important pathogens isolated from clinical cases were *Streptococcus agalactiae* (31.8%), *Staphylococcus aureus* (22.7%) and Coagulase Negative *Staphylococcus* (CNS, 13.6%). In case of subclinical mastitis, *S. aureus* (33.5%), CNS (24.2%), *S. agalactiae* (7.5%) and *Escherichia coli* (8.1%) were the most frequently isolated pathogens (Table 3).

From the total isolates, *S. aureus* (32.2%) and *S. agalactiae* (10.4%) were the major contagious pathogens and *E. coli* (8.7%), *Klebsiella* species (3.3%), *Enterobacter* species (2.2%), *Streptococcus uberis* (6.0%),

Table 2. Risk factors affecting the prevalence of subclinical mastitis at cow and herd level.

Factor	Categories	N	n (%)	P value	OR	95% CI of OR
At cow level						
Stage of lactation	Beginning	40	6 (15.00)	-	-	-
	Middle	178	43 (24.16)	0.237	1.80	0.68-4.81
	End	85	67 (78.82)	0.000	21.09	7.00-63.66
Parity	1-3	201	49 (24.38)	0.000	5.93	3.41-10.32
	> 3	102	67 (65.69)			
Teat lesions	-	8	8 (100%)	-	-	-
At herd level						
Herd size	1-5	94	66 (70.21)	0.776	1.27	0.24-6.70
	>5	8	6 (75.00)			
Udder washing before milking	Yes	62	39 (62.90)	0.38	0.36	0.14-0.94
	No	40	33 (82.50)			
Milking mastitic cow last	Yes	21	2 (9.52)	0.000	0.02	0.00-0.08
	No	81	70 (86.42)			
Hand washing before milking	Yes	6	4 (66.67)	0.828	0.82	0.14-4.76
	No	96	68 (70.83)			
Drainage structure	Good	60	41 (68.33)	0.551	1.30	0.54-3.14
	Bad	42	31 (73.81)			
Barn floor status	Good	63	41 (65.08)	0.125	2.08	0.82-5.29
	Bad	39	31 (79.48)			

N: Number of observation; n: number of positives; OR: odd ratio; CI: confidence interval.

Streptococcus dysgalactiae (3.3%) and *Arcanobacterium pyogenes* (1.6%) were the major environmental pathogens isolated. Other minor pathogens isolated included were CNS (23.0%), *Staphylococcus intermedius* (3.0%) and *Micrococcus* (2.5%) as shown in Table 3.

In vitro antimicrobial susceptibility test result

Antimicrobial sensitivity test was done for all isolates and the results of antimicrobial sensitivity tests are presented in Table 4. *S. aureus* was sensitive to Gentamycine (100%), Chloramphenicol (92%), Kanamayacin (90%), Vancomycin (80%) and Streptomycin (54%) and was resistant to Amoxicillin (62%), Penicillin (65.3%), Polymyxin B (89%) and Bacitracin (100%). In this study, Gentamicine, Chloramphenicol, Kanamayacin and Vancomycin were the most effective on *S. aureus* isolates. *S. intermedius* were sensitive to almost all antimicrobial disks applied. CNS was sensitive to Chloramphenicol (100%), Streptomycin (93%), Gentamycine (92%) and Vancomycin (70.2%) and was resistant to Penicillin (50%), Kanamayacin (72%), Amoxicillin (72%), Polymyxin B (80%) and Bacitracin (85%). *S. agalactiae* was sensitive to Gentamycine (100%), Chloramphenicol (100%) Vancomycin (84.2%), Penicillin (80%), and Streptomycin (52%) and was resistant to amoxicillin (100%) and Polymyxin B (92%) and *S. dysgalactiae* was sensitive to Polymyxin B (91%) and Amoxicillin (80%), but resistant to many of the other antimicrobial disks. *S. uberis* was sensitive to all other antimicrobial disks applied except to Bacitracin (70%) and Amoxicillin (65%) which were resistant. *E. coli* was sensitive to all antimicrobial discs except Bacitracin (80%), Penicillin (79%), Amoxicillin (75%) and Polymyxin B (65%) which were resistant. *Klebsiella* spp. Was sensitive to all antimicrobial discs except Bacitracin (75%), Polymyxin B (75%) and Amoxicillin (65%) which were resistant. *Enterobacter* spp. was sensitive to all antimicrobial discs except Bacitracin (80% resistance). *Micrococcus* species was sensitive to all antimicrobial discs applied except Streptomycin (62%), Polymyxin B (65%), Amoxicillin (75%) and Penicillin (75%), which were resistant.

Table 3. Bacteria species isolated from dairy cows clinical and subclinical mastitis.

Species of Bacteria Identified	Clinical		Subclinical		Total	
	N	%	N	%	N	%
S. aureus	5	22.7	54	33.5	59	32.2
CNS	3	13.6	39	24.2	42	23
S. intermedius	1	4.5	5	3.1	6	3.3
S. agalactaie	7	31.8	12	7.5	19	10.4
S. dysgalactaie	2	9.1	4	2.5	6	3.3
S. uberis	2	9.1	9	5.6	11	6
E. fesalis	1	4.5	5	3.1	6	3.3
E. coli	-	-	13	8.1	13	8.7
Enterobacter spp.	-	-	4	2.5	4	2.2
Klebssela spp.	-	-	6	3.7	6	3.3
Micrococcus	-	-	5	3.1	5	2.7
C. bovis	1	4.5	2	1.2	3	1.6
A. pyogens	-	-	3	1.9	3	1.6
Total	22	100	161	100	183	100

DISCUSSION

Prevalence and associated risk factors

This study showed that the overall prevalence of mastitis in crossbred cows in and around Adama was 73.4% at herd level, 39.4% at cow level and 23.7% at quarter levels of which 15.6 and 57.8% at herd level, 5.9 and 33.6% at cow level and 2.9 and 20.8% at quarter level were clinical and subclinical, respectively. The present overall cow level mastitis prevalence result (39.5%) is in close agreement with previous studies by Melesse et al. (2011), Bifa et al. (2005) and Bekele et al. (2012) who reported prevalence of 37.1, 34.9 and 34.3%, respectively. However, overall mastitis prevalence reported in the present study is relatively lower than the previous studies by Mekibib et al. (2010), Zeryehun et al. (2013), Bedane et al. (2012), Nibret et al. (2011) and Mekonnen et al. (2012) who reported prevalence of 71, 74.3, 59.1, 60.9 and 62.9%, respectively, but higher than the previous studies by Getahun et al. (2008) who reported 24.1%. The variability in the prevalence of bovine mastitis between reports could be attributed to difference in management of the farms. In this study, the clinical mastitis prevalence accounted for 5.9% whereas the subclinical mastitis was 33.6% of the share. The clinical prevalence of 5.9% in this study was comparable with that of Nibret et al. (2011), Melesse et al. (2011) and Benta and Habtamu (2011) who reported prevalence of 4.9, 8.5 and 5.3%, respectively. The present findings were lower than the findings of Mekibib et al. (2010), Zeryehun et al. (2013), Bedane et al. (2012), and Bifa et al. (2005) who reported prevalence of 22.4, 19.6, 21.1, 15.1 and 16.11%, respectively and higher than the findings of Mekonnen et al. (2012), Getahun et al. (2008) and Bekele et al. (2012) who reported prevalence of 3.9, 1.8 and 3.3%, respectively. The present subclinical bovine mastitis finding (33.6%) is in close agreement of Bekele et al. (2012), Bedane et al. (2012), Melesse et al. (2011) and Hundera et al. (2005) who reported prevalence of 31, 38, 28.6 and 34.6%, respectively. The present findings were lower than the findings of Alemu et al. (2013), Mekibib et al. (2010), Zeryehun et al. (2013), Mekonnen et al. (2012), Nibret et al. (2011) and Kerro and Tareke (2003) who reported prevalence of 41.2, 48.6, 55.1, 54.4, 56 and 62.9%, respectively and higher than the findings of Bifa et al. (2005) and Getahun et al. (2008) who reported prevalence of 23 and 22.3%, respectively. In this study similar to previous studies by Mekibib et al. (2010), Bedane et al. (2012), Zeryehun et al. (2013), Bekele et al. (2012), Getahun et al. (2008), Nibret et al. (2011), Mekonnen et al. (2012), Melesse et al. (2011), Bifa et al. (2005), kerro and tareke (2003), Workeneh et al. (2002), and Hussein (1999), the overall prevalence of clinical mastitis is lower than subclinical mastitis.

In Ethiopia, the subclinical form of mastitis (account high economic loss) was neglected and efforts have been concentrated on the treatment of clinical cases (Kerro and Tareke, 2003). According to Radostits et al. (2000), an affected quarter suffers on average 30% of reduction in productivity and an affected cow is estimated to lose 15% of its production for the lactation. As usual, the owners of smallholder dairy farms in the study areas were not well informed about the invisible loss from subclinical mastitis since dairy farming is mostly a sideline business in them.

In the present study, parity number 3 and above, late lactation stage and teat lesions were also found to increase occurrence of mastitis significantly ($p<0.05$). According to Erskine (2001), primiparous cows have more effective defense mechanism than multiparous

Table 4. Antibiotic sensitivity test.

Bacteria isolate	No. tested	K30%			S10%			P10%			Am12%			CN10%			C30%			PB300%			B10%			VA30%		
		R	I	S	R	I	S	R	I	S	R	I	S	R	I	S	R	I	S	R	I	S	R	I	S	R	I	S
S. aureus	59	-	10	90	46	-	54	65	6	29	62	16	22	-	-	100	7.9	-	92.1	8	-	92	100	-	-	16	4	80
S. intermedius	5	-	4	96	3	-	97	-	-	100	8	-	92	-	-	100	-	-	100	8	10	82	17	-	83	-	-	100
CNS	46	72	8	20	-	7	93	50	-	50	72	-	28	4	4	92	-	-	100	80	3	17	85	15	-	29.2	-	70.8
S. agalactiae	19	28	-	72	40	8	52	20	-	80	100	-	-	-	-	100	-	-	100	92	-	8	31	-	69	15.8	-	84.2
S. dysgalactiae	6	21	-	79	-	-	100	-	-	100	80	-	20	-	-	100	11	-	89	91	-	9	24	-	76	7	-	93
S. uberis	10	24	8	68	7	-	93	35	-	65	65	-	35	11	-	89	7	-	93	12	-	88	70	-	30	-	-	100
E. coli	13	25	-	75	35	-	65	79	-	21	75	-	25	8	-	92	-	-	100	65	-	35	80	-	20	5	5	90
Klebsiella spp.	6	4	-	96	11	-	98	25	-	75	65	-	35	-	-	100	-	-	100	75	-	25	75	-	25	20	-	80
Enterobacter	4	-	-	100	-	-	100	10	-	90	25	-	75	-	-	100	-	-	100	25	-	75	80	-	20	25	-	75
Micrococcus	5	-	-	100	62	7	31	75	-	25	75	-	25	28	-	72	25	-	75	65	-	35	24	-	76	25	-	75

S: Susceptible, I: intermediate, R: resistance, K30%: Kanamayacin, S10%: Streptomycin, P10%: Pencilline, AML12%: Amoxicillin, CN10%: Gentamycine, C30%: Chloramphenicol, PB300%: Polymyxin B, B10%: Bacitracin, VA30%: Vancomycin.

cows. The prevalence of subclinical infection increases as the stage of lactation progresses. In the case of farm (herd) level prevalence of subclinical mastitis, only the practice of milking mastitic cow last had significant effect on the prevalence of subclinical mastitis ($p<0.05$). The prevalence was significantly higher (86.42%) in those, which were not milking mastitic cows last (Table 4).

Bacterial isolation and identification

Klebsiella (3.3%), *Enterobacter* spp. (2.2%), *S. uberis* (6.0%), *S. dysgalactiae* (3.3%) and *A. pyogenes* (1.6%) were the major environmental pathogens isolated. Other minor pathogens isolated included were CNS (23.0%), *S. intermedius* (3.0%) and *Micrococcus* (2.5%) as shown in Table 3.

In the present study, *S. aureus* was the predominant pathogen (32.2%) of the area and this finding was comparable with the reports of Zingeser et al. (1991) (27%) and Barbuddhe et al. (2001) (23.2%). However, it was higher than the reports made by Hussein (1999) (10.6%). The reports of Kerro and Tareke (2003) (40.5%) and Hunderra et al. (2005) (44.4%) were higher than the present finding. The relative high prevalence of *S. aureus* in this study could be associated with lack of effective udder and hand washing before milking, use of separate clothes for drying, post milking teat dipping and disinfection of milking areas. The result of CNS (23.0%) in the current study is much lower than the finding of Hussein (1999) (42%). However, this result was much higher than the result of Miline et al. (2002), which was reported as 10%. CNS is a minor pathogen and normally considered as normal inhabitants of bovine udder (Gentilini et al., 2002). *S. agalactiae* prevalence (10.4%) in this study was lower than the finding of Kerro and Tareke (2001) (13.1%) and Bishi (1998) (27%). The 6.0% isolation result of *S. uberis* was comparable with Kerro and Tareke (2003) finding which was 5.1% and much lower than that of Miltenburg et al. (1996), that is 12.1%. Isolates of *S. dysgalactiae* (3.3%) were lower than the report of Kerro and Tareke (2003), which was 5.6%, *E. coli* (6.46%) was the predominant environmental pathogen isolated in the present study. The prevalence of environmental *E. coli* may be associated with poor farm hygiene and poor of stable areas. In this study, environmental pathogens were isolated, however a common understanding with increasing herd size, manure disposal and sanitation problem high to build up to bacterial population (coliform and environmental streptococcus) in the cows immediate environment.

Antibiotics sensitivity test

The antimicrobial sensitivity test results of this study are closer to the previous authors (Edward et al., 2002; Gentilini, 2002; Nesru, 1998; Kang, 2007; Sanmartin et al., 2007; Shakuntala et al., 2003).

The results of sensitivity tests of the organisms

isolated to antibiotics (Table 4) show that 3.1% of the strains were resistant to Gentamycin, 4.2% to Chloramphinicol, 17.6% to Vancomycin, 25.4% to Streptomycin, 26.4% to Kanamaycin, 48.6% to Polymyxin B, 48.9% to Penicillin, 68.7% to Amoxicillin and 74.9% to Bacitracin. Gentamycine and chloramphinicol were found to be more effective antibiotic among all the tested antibiotics. The main reasons for the occurrence of a high number of resistant strains in this study are the use of sub-therapeutic level of antibiotics and/or short treatment episodes and the long-term presence of infected cows in herds. Finally, due to the high resistance levels detected in the present study, it is believed that it is necessary to set up permanent resistance surveillance programs in the country.

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Full Length Research Paper

Preliminary study on prevalence of bovine tuberculosis in cattle owned by tuberculosis positive and negative farmers and assessment of zoonotic awareness in Ambo and Toke Kutaye districts, Ethiopia

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A cross sectional study was conducted from April to September, 2012 on cattle owned by tuberculosis positive and negative farmers and households to assess prevalence of bovine tuberculosis and zoonotic awareness of the households in Ambo and Guder districts, Ethiopia. A total of 398 cattle were tested using single comparative intradermal tuberculin test. The result was interpreted at >4 and >2 mm. An overall 1 and 4.02% prevalence of bovine tuberculosis at individual cattle and 7.02 and 24.56% at herd level were recorded at cut off >4 and >2 mm, respectively. Bovine tuberculosis was more prevalent in cattle owned by tuberculosis positive farmers (1.36 and 5% at individual cattle, 12 and 36% herd level) than in cattle owned by tuberculosis negative farmers (0.56 and 2.81% at individual cattle, 3.13 and 15.63% at herd level) at >4 and >2 mm cut off, respectively. Lack of awareness of the community about the zoonotic importance of the disease was observed. In conclusion, the present study indicated more prevalence of the disease in cattle owned by tuberculosis positive farmers than tuberculosis negative farmers and lack of zoonotic awareness of the households. Therefore, further study, collaboration between physician and veterinarians, and creation of awareness about zoonotic diseases were recommended.

Key words: Bovine tuberculosis, cattle, prevalence, tuberculosis positive and negative farmers.

INTRODUCTION

Tuberculosis (TB) is recognized as one of the most important threats to human and animal health causing mortality, morbidity and economic losses (Smith et al., 2006). It is communicable mycobacterial disease caused by members of *Mycobacterium tuberculosis* complex (MTBC) (CDC, 2008). MTBC include *M. tuberculosis*, *Mycobacterium bovis*, *Mycobacterium africanum, Mycobacterium microti, Mycobacterium canetti, Mycobacterium caprae* and *Mycobacterium pinnipedii.* Many of the species and subspecies of MTBC show specific host association. *M. tuberculosis* is specifically adapted to humans while *M. bovis* is most frequently isolated from domesticated cattle (Smith et al., 2006), although recent studies indicated that *M. tuberculosis* has been isolated from cattle and *M. bovis* from humans infected with bovine tuberculosis (BTB) and TB, respectively. In spite of variation in host specificity, the members of MTBC are characterized by 99.9% or greater similarity at nucleotide level, and are virtually identical at 16s rRNA sequence (Brosch et al., 2002).

Bovine tuberculosis is a chronic bacterial disease characterized by progressive development of tubercles in any tissue/organ of the body (Clarke, 1998). Infected cattle are the main source of infection for other cattle.

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Organisms are excreted in the exhaled air, in sputum, feces (from both intestinal lesions and swallowed sputum from pulmonary lesions), milk, urine, vaginal and uterine discharges, and discharges from open peripheral lymph nodes (Radostits et al., 2007).

Bovine TB has been widely distributed throughout the world and its enzootic occurrences have been reported in developing countries like African countries (Cosivi et al., 1998). The economic loss caused by this disease is enormous and great in animal production. Infected animal loses 10 to 25% of their productive efficiency. Direct losses due to the infection become evident by decrease in 10 to 18% milk and 15% reduction in meat production (Radostits and Blood, 1994). Apart from effects on animal production, it has also a significant public health importance (O'Reilly and Daborn, 1995). Currently, the disease in humans is becoming increasingly important in developing countries, as humans and animals are sharing the same micro-environment and dwelling premises, especially in rural areas, and susceptibility of AIDS patients to tuberculosis (Shitaye et al., 2007). Agricultural workers may acquire the disease by inhaling cough spray from infected cattle and develop typical pulmonary TB (Cosivi et al., 1998). It is estimated that *M. bovis* causes 10 to 15% human cases of tuberculosis in countries where pasteurization of milk is rare and bovine tuberculosis is common (Ashford et al., 2001).

In developing countries like Ethiopia, the socio-economic situation and low standard living area for both animals and humans are more contributing in TB transmission between human to human and human to cattle or vice versa. When the number of infected human individual increases, the possibility of transmission to cattle could also increase. Ethiopia is one of the African countries where tuberculosis is wide spread in both humans and cattle and the endemic nature of tuberculosis in humans and cattle has long been documented (Shitaye et al., 2007). Even though information about the disease is available in Ethiopia, there is no information in West Shewa zone generally and in Ambo and Guder districts specifically, where large populations of local zebu breed cattle are reared. Therefore, the main objectives of this study were to assess prevalence of BTB in cattle owned by TB positive and negative farmers and zoonotic awareness of the households in Ambo and Guder districts.

MATERIALS AND METHODS

Study area

This study was conducted from April to September, 2012 in four sites of Ambo (Meti site) and Toke Kutaye districts (Guder, Mutulu and Mugno sites), West Shewa Zone of the Oromia Regional State, Ethiopia. Ambo is the administrative center of the Zone and Ambo district located at a latitude and longitude of 8° 59′N, 37° 51′E and an elevation of 2101 m and 114 km West of Addis Ababa. Guder is the administrative town of Toke Kutaye district and geographically located at 8° 58'N, 37° 46'E and altitude of 1946 m above sea level. The area receives a mean annual rainfall of 900 mm (800 to 1000 mm) and annual temperature ranging from 15 to 29°C with average temperature of 22°C. West Shewa zone is generally a highland whose topography gave the area a characteristic climate that is conducive for the cattle husbandry.

Ambo Hospital, which is used as a catchment point, is the only referral hospital of West Shewa zone where TB patients from district health centers are referred for diagnosis. Based upon the information of TB prevalence from the zone and accessibility, Meti, Guder, Gunter and Mugno Health Centers were selected. Additionally, the areas were selected due to the fact that people are living in close association with their cattle in a homogenous condition, thus the dynamics of mycobacterial disease transmission between cattle and their owners can be clearly investigated.

Study type and design

A cross-sectional study was conducted on purposively selected 32 TB positive patients belonging to 25 households and 32 TB negative farmers and 57 cattle herds consisting of 398 cattle (25 herds of TB positive farmers and 32 herds of TB negative farmers) in the four sites. The study herd size varied from 2 to 20 cattle. 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 were considered as a herd to calculate the herd prevalence (Mamo et al., 2013).

Study subjects

This study was conducted on TB positive and negative farmers and their households, cattle owned by these two groups of units. Patients having cattle were identified at their respective clinic and traced back to their home being guided by health workers in health centers of the sites. A human TB case was defined as a TB positive patient diagnosed and confirmed at Ambo Hospital or other hospitals. TB negative farmers were purposively selected from the villages where these TB patients live, within 2 km radius, on the basis of absence of any member of the family that has shown any clinical sign suggestive or diagnosed as TB positive for the last five years and whether their cattle have not a communal grazing land with those TB positive farmer's cattle. All cattle above six months old in herds owned by TB patient and selected TB negative farmer were tested by using comparative intradermal tuberculin test. Both groups of farmers were interviewed to assess their awareness about zoonotic importance of bovine tuberculosis.

All the cattle included in the study were local zebu breed kept under extensive management system. Study animal related information on each tested cattle (such as sex, age, body condition score (BCS)) were collected and recorded at the time of the test. Age of the cattle was obtained from the owners. The body condition of each of the study animals was scored using guideline 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 (score 3) or fat (score 4 to 5).

Single comparative intradermal tuberculin test (SCITT)

SCITT was carried out by injecting bovine purified protein derivative (PPD-B) (Observe™ bovine tuberculin, AssureQuality Limited, National Center for Biosecurity and Infectious Diseases-Wallaceville, 66 Ward St, Upper Hutt, New Zealand) and avian purified protein derivative (PPD-A) (Observe™ avian tuberculin, AssureQuality Limited, National Center for Biosecurity and Infectious Diseases-Wallaceville, 66 Ward St, Upper Hutt, New

Table 1. Prevalence of BTB at individual cattle and herd level.

Site	No. of cattle examined	No. of positive		Prevalence at individual cattle level (%)		No. of examined herds	No. of positive herds		Prevalence at herd level (%)	
		>4	>2	>4	>2		>4	>2	>4	>2
Meti	98	2	7	2.04	7.14	12	2	5	16.67	41.67
Guder	40	0	1	0	2.5	6	0	1	0	16.67
Mutulu	118	1	5	0.85	4.24	19	1	5	5.26	26.32
Mugno	142	1	3	0.7	2.11	20	1	3	5	15
Total	398	4	16	1	4.02	57	4	14	7.02	24.56

Zealand) into two sites on the right side of the mid-neck. After 72 h, the skin thickness at the injection sites was measured. The 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 PPD-B was greater than the increase in skin thickness at the injection site for avian 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 >2 mm, the animal was considered as positive. A herd was considered as positive for BTB if it had at least one tuberculin reactor animal.

Data management and analysis

The collected data was coded and entered into Microsoft Excel spread sheet. Statistical analyses were performed using statistical package for social sciences (SPSS), version 15 software packages. A percentage was used to calculate the prevalence of TB in both groups at herd and individual cattle level. The presence of statistical significance difference of different risk factors in prevalence of bovine tuberculosis was analyzed using Chi-square test or Fishers' exact test when at least one of the cells had less than 5 value or count. Information generated through questionnaire was analyzed using percentage. In all cases, 95% confidence interval (CI) and $p<0.05$ was considered for statistically significant difference.

RESULTS

Prevalence study

An overall 1 (4 from 398 cattle) and 4.02% (16 from 398) prevalence of BTB at individual cattle level were recorded at cut-off >4 and >2 mm, respectively. At herd level, 7.02 (4 from 57 herds) and 24.56% (14 from 57 herds) overall prevalence of BTB were obtained at cut-off >4 and >2 mm, respectively (Table 1). There was no statistically significant difference between/among all the assessed risk factors (site, sex, age and BSC) in the prevalence of BTB at both cut-off ($p>0.05$) (Table 2).

On the basis of comparison of SCITT result between cattle owned by TB positive and negative farmers, higher prevalence of BTB at individual cattle level was observed in cattle owned by TB positive farmers (1.38 and 5.04% from 220 cattle) than in cattle owned by TB negative farmers (0.56 and 2.78% from 178 cattle) at >4 and >2 mm cut-off, respectively. From the examined cattle herds of TB positive households, 12 (3/25) and 36% (9/25) herds were positive at cut-off >4 and >2 mm, respectively, whereas 3.13 (1/32) and 15.63% (5/32) herds from TB negative households were recorded at the same cut-off, respectively (Table 3). There was no statistically significant difference among site, age and BSC, and between sexes in the prevalence of BTB at both cut-off ($p>0.05$) in cattle owned by TB positive farmers. There was statistically significance difference between sex groups in prevalence of BTB in cattle owned by TB negative farmers at cut-off >2 mm (Fishers' exact=5.503, $p<0.05$) but not at cut-off >4 mm ($p>0.05$). There was no statistically significant difference among site, age and BCS groups at both cut-off ($p>0.05$) in cattle owned by TB negative farmers as shown in Table 2. The association between reports of human cases of tuberculosis in the households and reactor cattle in the household's herd was not statistically significant (Fishers' exact=1.606, $p=0.314$ and $\chi^2=2.914$, $p=0.088$ at >4 and >2 mm cut-off, respectively).

Generally, the disease is more prevalent in female cattle than male and cattle having medium BCS (Table 2). Young adult cattle (≥2<5 age) were found more susceptible followed by adult (≥5≤9 age).

Public health awareness

Results of interview conducted on 57 households (25 TB positive farmers and 32 TB negative households) revealed lack of awareness of the community about the zoonosis impact of the disease, but the survey showed 80.7% (46/57) of them were aware of BTB (Table 4). Both TB positive and negative farmers had less awareness of zoonotic importance of the disease with only 31.5 (18/57) and 42.1% (24/57) respondents had awareness on the transmission of TB from human to cattle and BTB from cattle to human, respectively. From feeding habit, 87.9% (51/57) of the respondents consume raw or non-treated soured milk. Higher proportion TB positive farmers who chew and spit tobacco (52% (13/25)) to their cattle were found to be more vulnerable

Table 2. Prevalence of BTB and association of different risk factors to skin test positivity at >4 and >2 mm cut-off point for bovine tuberculosis in the study areas.

Variable	Category	No. of examined	No. of positive (%)		χ^2/Fishers' exact		p-value	
			>4	>2	>4	>2	>4	>2
Overall								
Site	Meti	98	2 (2.04)	7 (7.14)	1.621	4.070	0.7943	0.254
	Guder	40	0 (0)	1 (2.5)				
	Mutulu	118	1 (0.85)	5 (4.24)				
	Mugno	142	1 (0.7)	3 (2.11)				
Sex	M	200	1 (0.5)	5 (2.5)	1.031	2.408	0.371	0.134
	F	198	3 (1.52)	11 (5.56)				
Age	<2	59	0 (0)	2 (3.39)	2.155	4.999	0.541	0.172
	≥2<5	115	1 (0.89)	8 (6.96)				
	≥5≤9	170	3 (1.76)	6 (3.53)				
	>9	54	0 (0)	0 (0)				
BCS	Lean	109	0 (0)	1 (0.92)	3.328	4.442	0.189	0.109
	Medium	216	2 (0.93)	10 (4.63)				
	Fat	73	2 (2.74)	5 (6.85)				
Cattle owned by TB positive farmers								
Site	Meti	46	1 (2.17)	5 (10.87)	0.678	5.222	0.878	0.156
	Guder	20	0 (0)	0 (0)				
	Mutulu	55	1 (1.82)	3 (5.45)				
	Mugno	99	1 (1.01)	3 (3.03)				
Sex	M	108	1 (0.93)	5 (4.63)	0.302	0.61	1.000	1.000
	F	112	2 (1.79)	6 (5.36)				
Age	<2	34	0 (0)	2 (5.88)	1.142	1.723	0.767	0.632
	≥2<5	60	1 (1.67)	4 (6.67)				
	≥5≤9	101	2 (1.99)	5 (4.95)				
	>9	25	0 (0)	0 (0)				
BCS	Lean	55	0 (0)	1 (1.82)	1.146	1.702	0.564	0.427
	Medium	123	2 (1.63)	7 (5.69)				
	Fat	42	1 (2.38)	3 (7.14)				
Cattle owned by TB negative farmers								
Site	Meti	52	1 (1.92)	2 (3.85)	2.437	1.830	0.487	0.608
	Guder	20	0 (0)	1 (5)				
	Mutulu	63	0 (0)	2 (3.17)				
	Mugno	43	0 (0)	0 (0)				
Sex	M	92	0 (0)	0 (0)	1.076	5.503	0.483	0.025
	F	86	1 (1.16)	5 (5.81)				
Age	<2	25	0 (0)	0 (0)	1.589	6.042	0.662	0.110
	≥2<5	55	0 (0)	4 (7.27)				
	≥5≤9	69	1 (1.45)	1 (1.45)				
	>9	29	0 (0)	0 (0)				
BCS	Lean	54	0 (0)	0 (0)	4.769	3.127	0.092	0.209
	Medium	93	0 (0)	3 (3.23)				
	Fat	31	1 (3.23)	2 (6.45)				

Table 3. Prevalence of BTB in cattle owned by TB positive and negative farmers from the sites.

Site	No. of cattle examined	No. of positive (prevalence in %) at individual cattle level		No. of examined herds	No. of positive herds (prevalence in %) at herd level	
		>4	>2		>4	>2
Owned by TB positive						
Meti	46	1 (2.17)	5 (10.87)	5	1 (20)	3 (60)
Guder	20	0 (0)	0 (0)	3	0 (0)	0 (0)
Mutulu	55	1 (1.82)	3 (5.45)	6	1 (16.67)	3 (50)
Mugno	99	1 (1.01)	3 (3.03)	11	1 (9.09)	3 (27.27)
Sub total	220	3 (1.36)	11 (5)	25	3 (12)	9 (36)
Owned by TB negative						
Meti	52	1 (1.92)	2 (3.85)	7	1 (14.29)	2 (28.57)
Guder	20	0	1 (5)	3	0	1 (33.33)
Mutulu	63	0	2 (3.17)	13	0	2 (15.38)
Mugno	43	0	0	9	0	0
Sub total	178	1 (0.56)	5 (2.81)	32	1 (3.13)	5 (15.63)

Table 4. Public health awareness about zoonotic importance of bovine tuberculosis.

Variable	Overall	TB positive farmers	TB negative farmers
Heard about BTB before	80.7 (46/57)	80 (20/25)	81.25 (26/32)
Know transmission of BTB from cattle to human	42.1 (24/57)	48 (12/25)	37.5 (12/32)
Consumption of unpasteurized or raw milk	89.47 (51/57)	84 (21/25)	93.75 (30/32)
Chew and spit tobacco to cattle	36.84 (21/57)	52 (13/25)	25 (8/32)
Herd graze with other herds	82.46 (47/57)	80 (20/25)	84.38 (27/32)

Where values are in percentage

to the disease than TB negative farmers (25% (8/32)).

DISCUSSION

Prevalence study

Both cut-off point (>4 and >2 mm) were utilized in the current study to indentify positive cattle. According to the OIE (2009) recommendations, the cut-off point for positivity of the Comparative Intradermal Tuberculin Test (CIDT), calculated as the difference between skin thicknesses after bovine tuberculin (B) and avian tuberculin (A) injections (B-A), is >4 mm. This cut-off point is used worldwide although it is likely that local conditions influence test performance. In Ethiopia a cut-off >2 mm, with CIDT test sensitivity of 69% was recommended for local zebu breeds (*Bos indicus*) (Ameni et al., 2008). The corner stone of TB control in cattle is the accurate detection and removal of infected cattle (Adams, 2001).

The overall 1 (4 from 398 cattle) and 4.02% (16 from 398) prevalence of BTB at individual cattle level were recorded at cut-off >4 and >2, respectively. The results of the current study are in line of agreement with reports from different areas. In Ethiopia, 0.8% from Hamer, 1.3 and 6% prevalence from Uganda were reported by Tschopp et al. (2010), Inangolet et al. (2008) and Bernard et al. (2005), respectively at >4 mm cut-off. Higher prevalence was also reported from different parts of Ethiopia. According to Mamo et al. (2013), the individual animal prevalence of BTB in cattle of Afar pastoralists was 11% at ≥4 mm cut-off and 18% at ≥2 mm cut-off. Ameni et al. (2008) also reported higher prevalence of 13.5 and 16% at a cut-off of >4 and >2 mm, respectively. The difference in prevalence of BTB might be related to influence by breed of cattle and type of farming (intensive, semi-intensive, extensive), housing and gathering of animals at grazing and watering areas. The fact that zebu cattle are relatively resistant to BTB than European breed (Radostits et al., 2007) and practice of mixed farming system in which cattle included in this study were under extensive farming system during dry and wet seasons (Oromiya Livelihood Zone Reports, 2008) agrees with the lower prevalence recorded in the present study as TB is more of disease intensification (Shitaye et al., 2007).

Slight high herd level prevalence of BTB, 7.02 (4 from 57 herds) and 24.56% (14 from 57 herds), were obtained

at cut-off >4 and >2, respectively. The current result is lower than herd prevalence report from other parts of Ethiopia. The herd prevalence of 44 and 56% at ≥4 and ≥2 mm cut-off, respectively was reported from Afar (Mamo et al., 2013). At cut-off >4 mm, the herd prevalence of 19% (5/27) was reported from Boji district (Laval and Ameni, 2004). The higher dairy herd level prevalence (51.4%) at >4 mm cut-off was also reported from Jimma (Tigre et al., 2011). The difference might be related to the epidemiological factors that favor the transmission of BTB, which include herd sizes, communal grazing and watering of diverse species of animals (Mamo et al., 2013).

Higher prevalence of the disease was observed in cattle owned by TB positive farmers than TB negative farmers both at herd and individual cattle level. In Northwest Ethiopia, out of the total 77 households examined, 11 TB cases were found. Of this, 36.4% (4/11) had reactor herds (Nega et al., 2012). 62.5% (5/8) of the households that had TB patients in their family owned reactor cattle in their dairy herd in study conducted in Jimma, Ethiopia (Tigre et al., 2011). At individual cattle level, 1.36 and 5% prevalence of BTB at >4 and >2 mm cut-off in cattle owned by TB positive farmers were observed in the current study, which is higher than prevalence of the disease in cattle owned by TB negative farmers (0.56 and 2.81% at the same cut-off). This agrees with the work of Fetene et al. (2011) who reported significantly higher prevalence of TB in cattle owned by TB patients than in cattle owned by non-tuberculosis owners and isolated *M. tuberculosis* and *M. bovis* from sputum and fine needle aspiration specimens of TB patient cattle owners. *M. tuberculosis* was also identified in grazing cattle in Central Ethiopia (Ameni et al., 2011). In Nigeria, slight higher prevalence of 11.8% was reported in cattle owned TB positive herd man after tracing back (Danbirni et al., 2012). The trend of high prevalence of TB among human patients in Nigeria is similar to the trend observed among cattle populations; thus indicating a relationship between the disease in human and infection in cattle (Abubakar, 2007). The presence of higher TB reactor cattle in cattle owned by TB positive farmers than TB negative farmers could suggest that either of them could be a source of infection for the other as the disease may be cyclical (cow-to man and man-to cow) (Cosivi et al., 1998).

Generally, the disease is more prevalent in female cattle than male and cattle having medium BCS. Young adult cattle (≥2<5 age) were found more susceptible followed by adult (≥5≤9 age). This is in line of agreement with the work of Lackech et al. (2012) in which the disease is found to be more prevalent in young adult and medium body conditioned cattle. According to Nega et al. (2012), analysis for the effect of risk factors revealed that the animal level of prevalence of BTB increased with age up to the age of 7 years, and was then observed to decrease slightly. This could be because as the age increases the probability of acquiring TB infection also increases (Barwinnek and Taylor, 1996). On the other hand, the decrease in prevalence is associated with immune status of the animal (Buddle et al., 2003).

In this study, there was no statistically significant difference between/among all the assessed risk factors (site, sex, age, BSC) in the overall prevalence of BTB and BTB in cattle owned by TB positive farmers at both cut-off. There was statistically significant difference between sex groups in prevalence of BTB at cut-off >2 mm but not at cut-off >4 mm. Absence of significant difference among age groups is in line with report from Akaki, Ethiopia (Lakech et al., 2012). Similar to other studies conducted in different parts of Ethiopia at cut-off >4 mm (Ameni et al., 2007; Tschopp et al., 2010; Gumi et al., 2011; Biffa et al., 2011; Mamo et al., 2013), there was no association between body condition score and tuberculin skin test positivity at both cut-off points. Absence of association in this study might be related to the number of sample size.

In the present study, the association between reports of human cases of tuberculosis in the households and reactor cattle in the household's herd was not statistically significant. This agrees with the works of Ameni et al. (2003). However, it is different from previous reports by Ameni et al. (2001) and Tigre et al. (2011) who reported statistically significant associations between human tuberculosis cases and reactor herds.

Public health awareness

Questionnaire survey of households showed that 80.7% of them were aware of BTB with low level knowledge about zoonosis of the disease. This agrees with report from Cameroon, which indicated 81.9% of cattle handlers know BTB, however with 67.9% of them knew as BTB is zoonotic (Ndukum et al., 2010). Assessment of the knowledge of cattle owners about BTB in Wuchale Jida district, Ethiopia showed that 38.3% (36 of 94) of the respondents knew that cattle can have tuberculosis, and 30.8% (29 of 94) recognized that BTB is zoonotic (Ameni et al., 2003).

The proportion of which BTB contributes to total tuberculosis cases in humans depends on the prevalence of the disease in cattle, consumer habits, socio-economic conditions, level of food hygiene (Ashford et al., 2001) and medical prophylaxis measures in practice (Tigre et al., 2011). According to the result of this study, 89.47% consume unpasteurized or raw milk. Similarly, studies conducted in different parts of Ethiopia indicated the habits of raw milk consumption. The current result is slightly higher than 85.7% report from Jimma town, Ethiopia (Tigre et al., 2011). Study conducted in Wuchale Jida district indicated 52.1% (49 of 94) households' has habit of consuming raw milk (Ameni et al., 2003), which is lower when compared with the current result. From TB

positive farmers' family, about 84% (21/25) consume raw milk. This is in close agreement with 81.8% (9/11 of TB positive households) raw milk consumption in Northwest Ethiopia (Nega et al., 2012). Consumption of unpasteurized fresh and soured milk potentially infected with *M. bovis* was found to cause milk-borne infection with BTB, which can result in non-pulmonary TB (Lee and Mills, 2000).

Only little proportion of the respondents was found to be aware about the transmission of the disease from cattle to human and vice versa. In line with the current result, Ameni et al. (2003) reported that 30.8% (29 of 94) of the respondents know that BTB is zoonotic.

The higher proportion TB positive farmers who chew and spit tobacco to their cattle could be due to transmission of *M. bovis* to humans through inhalation of the cough spray from infected animals during spitting and results in pulmonary TB or transmission of *M .tuberculosis* from human to cattle as the organism can spread to the animal when the person with TB spits or the sputum of TB positive person can contaminate the tobacco when chewed. The source of *M. tuberculosis* to animal is most frequently considered to be a human being with active tuberculosis expelling the causal agent through sputum, less often through urine or feaces. These could easily contaminate animals' feed and water (Radostits et al., 2007). This agrees with the finding of Ameni et al. (2011) in Central Ethiopia.

Keeping cattle in close proximity to their house and calves in their house is a common practice of households in the study area. This indeed can facilitate zoonosis impact of the disease. According to Bogale (1999), conditions such as customs of consuming raw milk, keeping cattle in close proximity to the owner house and using cow dung for plastering wall or floor and as source of energy for cooking do exacerbate the chance of spread of tuberculosis as zoonosis in Ethiopia.

In conclusion, this study indicated more prevalence of BTB in cattle owned by tuberculosis positive farmers than tuberculosis negative farmers and lack of zoonotic awareness of the households. Although this study could not established the source of the infection whether it was from the human to cattle or vice versa, further study, establishment of collaboration between physician and veterinarians to trace back positive patient to get profile of their cattle and creation of awareness about zoonotic importance of the disease in the community were recommended.

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Full Length Research Paper

Epidemiology of gastrointestinal nematodes of Horro sheep in Western Oromiya, Ethiopia

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This study was conducted during May 2011 to December 2012 in Western Oromiya to determine the prevalence of gastrointestinal nematodes in naturally infected Horro sheep and associated risk factors. A total of 1680 faecal samples were examined using flotation and modified McMaster methods. Identification of all isolated nematodes was performed on larvae recovered from pooled faecal cultures and worms collected from slaughtered animals. The overall prevalence was observed to be 24.7% (95% confidence interval: 22.6 to 26.8) and majority of the infected animals (88.9%) had low faecal egg counts per gram (50 to 800). Season, grazing management, age, agro-ecology and body condition scores showed significant association ($p < 0.001$) with prevalence and mean nematode faecal egg counts recorded. Results revealed that *Haemonchus contortus* was the most prevalent parasite detected followed by *Trichostrongylus* species. The Horro sheep were infected with diversified gastrointestinal nematodes that can seriously affect the health and productivity of the animals. Many animals were sub-clinically infected without attracting awareness of farmers to undertake control measures. Therefore, to improve the production potential of this indigenous breed of sheep and the livelihood of the farmers, control strategies based on the epidemiology of the parasites and production systems should be implemented.

Key words: Horro sheep, gastrointestinal nematodes, epidemiology, prevalence, Oromiya, Ethiopia.

INTRODUCTION

Ethiopia possesses highly diversified indigenous sheep breeds parallel to its diverse agro-ecology and production systems (Galal, 1983). The country is the home to a large population of sheep estimated to be 25.9 million (Central Statistical Agency (CSA), 2010). Horro sheep are one of the prominent indigenous breeds mainly distributed in Western Oromiya Region of Ethiopia. They belong to the long fat tailed breed group. They are uniform in colour having creamy white, dark tan or spotted short smooth hair. It is a large framed local breed and the fat tail is triangular in shape hanging straight down (Kassahun, 2000). They have an estimated population of 3.4 million and wide distribution from highland to lowland in their natural habitat. The breed is a valuable genetic resource usually characterized to have good reproductive performance, fast growth rate and large mature size compared to some of the traditional breeds (Abegaz et al., 2000).

Gastrointestinal (GI) nematode parasites are a major cause of mortality and sub-optimal productivity in grazing livestock in pastoral systems worldwide (Hoglund et al., 2009). As a consequence, the control methods mainly rely on the use of curative or preventive treatment with anthelmintics which, on many farms, lead to an ever increasing anthelmintic resistance problem.

On the other hand in many countries in sub-Saharan Africa including Ethiopia, helminth control options are

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limited. This is mainly an outcome of restricted access to anthelmintics by smallholder farmers, because of expense or shortage and low practices of alternative control methods like grazing management. Under such circumstances exploiting genetic variations in host resistance to gastrointestinal nematode parasites is a relevant option both to control parasites and improve the productivity of sheep industry. Some indigenous breeds reported in Africa including the Nigerian West African Dwarf sheep (Idika et al., 2012), the Red Maasai sheep in Kenya (Baker et al., 1998) and the Djallonkè sheep of West Africa (Goossens et al., 1997) showed superior genetic variations in host resistance to nematode parasites than some other local breeds. However, in Ethiopia on-station comparative studies conducted on two indigenous breeds of Menz and Horro sheep did not show consistent breed difference in nematode faecal egg counts (Tembely et al., 1998; Rege et al., 2002). Hence, further study to generate baseline epidemiological data on nematode infection status of Horro sheep is required to manage the parasites.

In Western Oromiya, sheep are important component of the farming system and reared traditionally by smallholder farmers. Natural pasture is the major feed resource and grazing animals are continuously exposed to nematode parasites which contribute to loss of production and unthriftiness. The humid and warm climatic conditions are virtually favorable for the widespread occurrence of parasitic diseases in the region. However, most of the studies hitherto conducted were directed to other parts of the country. Studies carried out in central highlands (Assefa, 1997), in southern (Asegede, 1990; Amenu, 2005) and eastern parts of the country (Sissay et al., 2007; Dereje, 2008) have generated data on epidemiology and production losses caused by GI nematode parasites in small ruminants but not in Horro sheep. As a result, data available on nematode parasites of Horro sheep in their native habitat are scanty. The objectives of the present study were therefore, to determine the prevalence of gastrointestinal nematodes in Horro sheep and investigate associated risk factors that may facilitate the development and implementation of control strategies relevant to the production systems.

MATERIALS AND METHODS

Study areas

This study was conducted in 6 selected districts of Western Oromiya with different agro-ecological locations. Highland represented by Horro and Jimma Arjo located about 325 and 385 km West of Addis Ababa, respectively. Mid altitude included Guto Gidda, Sasiga and Bedele situated 335, 353 and 480 km West of Addis Ababa in that order. The lowland involved resettlement sites in Jimma Arjo, Bedele, Dabo Hana and Guto Gidda in upper Didessa and Uke/Anger valleys. The elevations are 2000 to 2500, 1500 to2000 and below 1500 m above sea level in highland, mid altitude and lowland, respectively (Ministry of Agriculture (MOA), 1998; Production Estimates and Crop Assessment Division (PECAD), 2013). The mean annual temperature ranges recorded are 10 to 15, 15 to 20 and 20 to 25°C for highland, mid altitude and lowland, respectively. The average relative humidity is above 60.0%. All areas have two distinct seasons with a unimodal rainfall distribution.

The rainy season extends from May to September with the rainfall peak occurring from July to August and dry season from October to April. The areas receive a total annual rainfall of 1200 to 2000 mm (NMA, 2011) with small variations between areas. Vegetation that constitutes natural pasture of the highland area is mainly grass family along with other *Trifolium* species. The common grasses include species of *Andropogon*, *Cynodon* and *Pennisetum.* In the mid altitude natural pasture and crop residues comprise major feed resources and the commonest grasses of the area include *Chloris pychynothrix*, *Cenchrus ciliaris* and *Hyparrhenia* species. Natural pastures provide more than 90% of the livestock feed in lowlands with wide range of grasses, legumes and other herbs (Alemayehu, 2003).

Study animals

Horro sheep of all age and sex groups kept by smallholder farmers were included in the study. Majority of the families possessed on average 5 to 7 animals in a flock. Mating is predominantly uncontrolled and they are year-round breeders. Average age at first service of 7.8 months and age at first lambing of 13.3 months are reported for the female. The lambing interval of 7.8 months and average twinning rate of 40.0% were recorded for the breed (Edea et al., 2012).

Natural pastures from communal grazing lands were the principal sources of feed for sheep and other livestock during rainy season and crop residues were the major supplements available after harvest. Mostly, a large number of different livestock including sheep are grazed together on communal grazing pasture. Some farmers used tethering for sheep in the home vicinity. In some places, sheep grazing on native pasture at 20 animals per hectare all year round was reported (Tesfaye and Diriba, 2006). All sheep are grazed together during daytime and housed at night.

Study design and sample size

A cross-sectional study was carried out to determine the prevalence of GI nematodes. A cluster sampling was used to select the samples (Bennett et al., 1991; Toma et al., 1999) and the required sample size was calculated using a formula (Thrusfield, 2005). Initially the number of clusters equivalent to the number of flocks to be sampled in one agro-ecology was determined. The desired absolute precision was set to be 0.05 and an expected prevalence of 30% was considered (Fekadu B, Regional Veterinary Diagnostic Laboratory, Bedele, personal communication). Based on the relative population size in each agro-ecology, the cluster number was proportionally reallocated for each agro-ecology and 1680 sheep (240 flocks) were sampled for the study.

Method of sampling

In each study area, clusters of sheep or flocks possessed by households were considered during sampling. First, the list of household heads was obtained from the local Development Agents (DA). A lottery system was used to randomly pick a household head and subsequently the flock directly owned by the family was included in the sample. Then all the sheep in the flock were sampled for the study.

Parasitological study

All faecal samples were collected directly from the rectum of the animals (Hendrix, 1998). For individual samples, an average of 5 g of faeces was collected in a screw-capped universal bottle. The samples were clearly labeled corresponding to detailed information recorded and transported to the Regional Veterinary Diagnostic Laboratory in Bedele for analysis. When delay was expected in transport, samples were preserved in 10% formalin to prevent larvae from hatching (Hansen and Perry, 1994). Faeces for pooled culture of 10 to 15 animals were collected separately based on 3 g from each animal and were mixed thoroughly in the laboratory.

Faecal egg counts per gram (EPG) were determined for each sample following the modified McMaster technique described by Ministry of Agriculture, Fisheries and Food (MAFF, 1986) using saturated sodium chloride (specific gravity of 1.2) as flotation fluid. The degree of faecal egg output per gram was determined as described by Hansen and Perry (1994) in mixed infection with different GI nematode species. Six pooled faecal cultures (2 from each agro-ecology) were prepared following the method described by MAFF (1986) and larvae (L_3) were recovered by means of Baermann technique. The larvae were examined under a magnification of 250x and at least 200 identified from each culture using the keys and morphological characteristics described by MAFF (1986).

A total of 12 complete abomasa and intestines were obtained from sheep brought from our study areas and slaughtered in restaurants. All animals were adult males above one year old and 6 of them were slaughtered during rainy season and the other 6 in dry season. Collection of adult nematodes and their developmental stages were done according to method described by Hansen and Perry (1994). Identification was performed in the laboratory based on morphological keys provided by MAFF (1986) and Urquhart et al. (1996).

Questionnaire and body condition scoring

A semi-structured questionnaire was administered to sheep owners to collect some demographic data and grazing management used for the animals. In addition, the parasite control practices exercised in the areas were assessed and recorded based on interviews (Bartley et al., 2003). Body condition scoring (BCS) was done according to Ethiopia Sheep and Goat Productivity Improvement Program (ESGPIP, 2009) recommendation by careful visual observation and palpation of muscle mass and fat cover over the lumbar region. Animals of poor condition (BCS ≤2.0) and good condition scores (BCS 3.0 to 5.0) were identified and sampled (Behnke et al., 2011).

Data analysis

All data collected were stored in Microsoft Excel spreadsheet. Percentage was used to measure prevalence of infection and nematode species identified as shown in Tables 1,2,3,4 and 7. The EPG was logarithm transformed as $\log_{10}$ (EPG + 1) to minimize a skewed distribution and used in all procedures of analysis. Chi-square (χ^2) test statistic of SPSS for Windows, Version 16.0 (2007) and IBM SPSS Statistics for Windows, Version 20.0 (2011) were used to compare nematode species distribution and to test the association between nematode infection and each risk factor. The independent-samples t-test was used to compare EPG means within each age group and the General Linear Model (GLM) procedure of the Statistical Analysis System (SAS, 2002), version 9.00 was used to analyze the effects of main factors on least squares means (LSM) nematode faecal egg count. In all analysis, statistical significance was considered at 0.05 or less probability level.

RESULTS

Questionnaire survey and body condition score

According to grazing system, 81.4, 3.8 and 14.8% of sheep sampled were kept under open grazing, tethering and mixed grazing combining both methods, respectively. In the study areas, parasite control practices largely relied upon the use of anthelmintics. Out of 150 farmers interviewed; 143 (95.30%), 1 (0.70%) and 3 (2.0%) responded they use entirely anthelmintics, traditional medicine and both methods to treat their animals against parasite, respectively. The remaining 3 (2.0%) replied that they did not use any method. Among the respondents, 20 (13.30%), 58 (38.70%) and 52 (34.70%) replied they use anthelmintic treatments once, twice and three times a year in that order only targeting the sick or animals in poor conditions. Body condition score assessment revealed that 78.0% of sheep sampled had relatively good condition scores (BCS, 3.0 to 5.0), while 22.0% of the animals scored thin or classified to have poor body condition scores (BCS ≤2.0).

Prevalence of gastrointestinal nematode infections

The overall prevalence of gastrointestinal nematode infections of Horro sheep based on faecal egg count was 24.7% (95% CI: 22.6 to 26.8). The prevalence were higher in the lowland (30.7% [95% CI: 26.2 to 35.2]) and mid altitude (30.7% [95% CI: 26.9 to 34.5%]) which were significantly different ($p < 0.001$) from prevalence recorded in highland (15.7% [95% CI: 12.9 to 18.5%]). Majority of the infected sheep (88.9%) had low nematode faecal egg counts per gram (50 to 800). Only 4.1 and 7.0% of the animals showed moderate (800 to 1200) and heavy (>1200) EPG counts, respectively (Table 1).

The nematode prevalence showed great seasonal variation based on wet and dry period of the year and the difference was significant ($p < 0.001$). As a result, a high prevalence of 41.9% (95% CI: 38.7 to 45.1) was observed during wet season (Table 2). In this study, no significant difference ($p > 0.05$) was seen in nematode infection between male (25.0% [95% CI: 22.5 to 27.5]) and female animals (24.0% [95% CI: 20.0 to 28.0]). The prevalence of 27.0% (95% CI: 23.0 to 31.0), 24.7 (95% CI: 18.1 to 31.3) and 23.7% (95% CI: 21.1 to 26.3) were recorded in lambs, yearlings and adult sheep, respectively. The effect of age was significant ($p < 0.05$) for lambs and adult sheep. But it was not significant ($p > 0.05$) for lambs and yearlings as well as yearling and adult age groups. A prevalence of 50.8% (95% CI: 45.7 to 55.9) was observed in sheep of poor condition score (BCS ≤2.0) compared to prevalence recorded for animals in good condition (BCS 3.0 to 5.0) and the difference was significant ($p < 0.001$) for both populations (Table 3). The grazing system used for the animals had a significant effect ($p < 0.001$) on nematode infection. Sheep grazed

Table 1. Prevalence of gastrointestinal nematode infections and faecal egg counts in Horro sheep based on agro-ecologies.

Agro-ecology	Sample	Prevalence (%)	95% CI (%)	EPG category (%)		
				Light	Moderate	Heavy
Highland	674	106 (15.7)[a]	12.9–18.5	91 (85.8)	6 (5.6)	9 (8.5)
Mid altitude	599	184 (30.7)[b]	26.9–34.5	168 (91.3)	5 (2.7)	11 (6.0)
Lowland	407	125 (30.7)[c]	26.2–35.2	110 (88.0)	6 (4.8)	9 (7.2)
Total	1680	415 (24.7)	22.6–26.8	369 (88.9)	17 (4.1)	29 (7.0)

Values within a column followed by letters a and b: χ^2 (1df, n = 1273) = 40.40, p < 0.001; a and c: χ^2 (1df, n = 1081) = 33.80, p < 0.001 (significantly different).

Table 2. Prevalence of gastrointestinal nematode infections and faecal egg counts in Horro sheep based on seasons.

Season	Sample	Prevalence (%)	95% CI	EPG category (%)		
				Light	Moderate	Heavy
Rainy	955	400 (41.9)[a]	38.7–45.1	354 (88.5)	17 (4.2)	29 (7.3)
Dry	725	15 (2.0)[b]	1.0–3.0	15 (100.0)	0	0
Total	1680	415 (24.7)	22.6–26.8	369 (88.9)	17 (4.1)	29 (7.0)

Values within a column followed by letters a and b: χ^2 (1df, n = 1680) = 351.30, p < 0.001 (significantly different).

Table 3. Prevalence of gastrointestinal nematode infections and faecal egg counts in Horro sheep based on body condition**.**

Body condition score (BCS)	Sample	Prevalence (%)	95% CI	EPG category (%)		
				Light	Moderate	Heavy
Good (BCS 3.0-5.0)	1310	227 (17.3)[a]	15.3 – 19.3	224 (98.7)	0	3 (1.3)
Poor (BCS ≤2.0)	370	188 (50.8)[b]	45.7 – 55.9	145 (77.1)	17 (9.0)	26 (13.9)
Total	1680	415 (24.7)	22.6 – 26.8	369 (88.9)	17 (4.1)	29 (7.0)

Values within a column followed by letters a and b: χ^2 (1df, n = 1680) = 173.90, p < 0.001 (significantly different).

under tethering were more affected than animals kept under open grazing. Similarly, animals reared under mixed grazing of both methods had more infection than free grazers. However, no significant difference was noted between tethered and mixed grazers (Table 4).

Nematode faecal egg counts

In the present study, in all group of animals, the nematode faecal egg output increased during rainy season compared to dry season. The degree of faecal egg count was generally low for both sexes throughout the study period. In male animals, there was virtually higher faecal egg count than female counterparts. However, the differences were not significant between male and female in each age group (Table 5).

The effects of agro-ecology, season, age, grazing system and animal condition were significant for least squares means ± standard error (LSM ± SE) of logarithm transformed faecal egg counts. However, the effect of sex was not significant. There were significant differences between lowland and mid altitude as well as between lowland and highland for LSM ± SE of faecal egg counts ($p < 0.001$). But, no significant variation was seen between mid altitude and highland. Similarly, differences were significant for seasons, lambs and adult sheep, free grazers and tethered animals, animal in good condition and poor condition (Table 6).

Prevalence of nematode species

Identification of third stage larvae (L_3) from coprocultures resulted in a nematode composition presented in Table 7. Among the prevalent worms, *Haemonchus contortus* was the most dominant parasite (31.8%) with significant difference in distribution between lowland and highland as well as between mid altitude and highland. But the distribution was not significantly different between

Table 4. Prevalence of gastrointestinal nematode infections and faecal egg counts in Horro sheep based on grazing management.

Grazing system	Sample	Prevalence (%)	95% CI	EPG category (%)		
				Light	Moderate	Heavy
Open grazing	1369	273 (19.9)[a]	17.8 – 22.0	249 (91.2)	8 (2.9)	16 (5.9)
Tethering	63	33 (52.4)[b]	39.9 – 64.9	30 (90.9)	1 (3.0)	2 (6.0)
Mixed	248	109 (43.9)[c]	37.6 – 50.2	90 (82.6)	8 (7.3)	11 (10.1)
Total	1680	415 (24.7)	22.6 – 26.8	369 (88.9)	17 (4.1)	29 (7.0)

Values within a column followed by letters a and b: χ^2 (1df, n = 1432) = 37.50, $p < 0.001$; b and c: χ^2 (1df, n = 311) = 1.43, $p > 0.05$; a and c: χ^2 (1df, n = 1617) = 67.04, $p < 0.001$.

Table 5. Mean comparison of nematode faecal egg counts between female and male sheep within age group.

Age group	Sex	Sample	No. infected	Means ± SE	t-value	df	p-value
Under 6 months	F	286	77	2.28 ± 0.05	1.43	122	>0.05
	M	173	47	2.41 ± 0.08			
6-12 months	F	112	28	2.14 ± 0.09	0.17	39	>0.05
	M	54	13	2.16 ± 0.13			
Above 1 year	F	828	201	2.21 ± 0.03	0.44	248	>0.05
	M	227	49	2.25 ± 0.07			
Total	-	1680	415	-	-	-	-

Means ± SE for Logarithm transformed faecal egg count (Log_{10} [EPG + 1]), Standard error of the mean (SE).

Table 6. Effects of factors on Least squares means of nematode faecal egg counts of Horro sheep.

Factor		LSM ± SE of Log_{10} (EPG + 1)	F-value	p level
Agro-ecology	Lowland	0.40 ± 0.08[a]	16.73	***
	Mid altitude	0.75 ± 0.06[b]		
	Highland	0.65 ± 0.06[bc]		
Season	Wet	1.09 ± 0.05[a]	213.86	***
	Dry	0.11 ± 0.07[b]		
Sex	Female	0.60 ± 0.05	0.07	NS
	Male	0.61 ± 0.06		
Age	Lamb	0.68 ± 0.06[a]	5.50	**
	Yearling	0.61 ± 0.08[ab]		
	Adult	0.51 ± 0.05[bc]		
Grazing management	Open grazing	0.47 ± 0.03[a]	3.84	*
	Tethering	0.79 ± 0.12[b]		
	Mixed	0.54 ± 0.09[ab]		
Body condition score	Good (BCS 3.0-5.0)	2.05 ± 0.03[a]	98.65	***
	Poor (BCS of ≤ 2.0)	2.49 ± 0.03[b]		

Values within a column followed by different letters (a, b, c) within each factor category are significantly different; Not significant (NS, $p > 0.05$); *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

lowland and mid altitude. The *Trichostrongylus axei* and *Trichostrongylus colubriformis* occurred widely distributing in all agro-ecologies and the difference was not significant between each zone. The *Ostertagia/*

Table 7. Prevalence of nematode species based on coprocultures and distribution in different agro-ecologies**.**

Nematode species	Distribution of nematode species (%)			Mean (%)	χ^2	df	p level
	Lowland	Mid altitude	Highland				
Haemonchus contortus	42.0[a]	37.0[a]	16.5[b]	31.8	33.7	2	0.001
Trichostrongylus axei	10.0[a]	9.0[a]	6.0[a]	8.3	2.3	2	0.32
Ostertagia/Teladorsagia circumcincta	0.0[a]	3.0[b]	25.5[c]	9.5	90.4	2	0.001
Bunostomum trigonocephalum	13.0[a]	8.0[ab]	6.0[b]	9.0	6.3	2	0.04
Cooperia curticei	0.0[a]	8.0[b]	1.5[c]	3.2	23.6	2	0.001
Nematodirus filicollis	0.0[a]	6.0[b]	19.5[c]	8.5	51.3	2	0.001
Strongyloides papillosus	3.0[a]	4.0[a]	0.0[b]	2.3	7.6	2	0.02
Trichostrongylus colubriformis	12.0[a]	11.0[a]	10.0[a]	11.0	0.4	2	0.81
Chabertia ovina	5.0[a]	2.0[a]	3.0[a]	3.3	2.90	2	0.23
Oesophagostomum columbianum	15.0[a]	12.0[a]	12.0[a]	13.0	1.1	2	0.58

Values within rows followed by different letters (a, b, c) are significantly different

Teladorsagia circumcincta and *Nematodirus filicollis* were more prevalent in the highland areas.

From abomasa and intestines collected from 12 slaughtered sheep, gastrointestinal nematodes were recovered only from 10 animals. The worm recovery was high and all animals harboured nematodes during the rainy season compared to low or no counts detected in dry season. Mixed infections with two or three nematode species were common and the overall mean worm burden was observed to be 104.50 ± 47.50 (Mean ± SE). The mean worm burden of 165.20 ± 66.30 and 13.50 ± 5.70 were recorded in rainy and dry seasons, respectively which were significantly different ($p < 0.05$). Totally, 7 species of nematodes namely *H. contortus* (60.0%), *Oesophagostomum columbianum* (40.0%), *T. colubriformis* (20.0%), *T. axei* (10.0%), *Bunostomum trigonocephalum* (20.0%), *Trichuris ovis* (20.0%) and *Strongyloides papillosus* (10.0%) were isolated from slaughtered sheep.

DISCUSSION

Prevalence of nematode infections

In this study, 11 species of gastrointestinal nematodes representing 10 genera were detected. *H. contortus* was the predominant parasite of Horro sheep occurring in all agro-ecologies with decreasing prevalence from lowland to highland areas. The *T. colubriformis* and *T. axei* constituted the next most prevalent nematode species followed by *O. columbianum, B. trigonocephalum* and *O./T. circumcincta* in decreasing order. Other species including *N. filicollis*, *Chabertia ovina*, *Cooperia curticei*, *S. papillosus* and *T. ovis* were the nematode species recorded in the study areas. Similarly, majority of the species were reported from indigenous sheep breeds in different parts of the country (Asegede, 1990; Assefa, 1997; Abebe and Esayas, 2001; Dereje, 2008).

Even though few slaughtered animals were studied to generate information about prevalence of nematode species and worm burden in general, 6 similar species of nematodes detected from coprocultures and additionally *T. ovis* were recovered during necropsy examination. The *H. contortus* was the most dominant worm in prevalence occurring in 60.0% of the slaughtered sheep followed by *Trichostrongylus* species which accounted for 40.0%. Other species occurred in less percentage and the species prevalence was in agreement with coproculture results. In this study, a mean nematode burden of 104.50 ± 47.50 was recorded which was lower compared to the mean burden of 1371.60 ± 263.40 reported for indigenous sheep breed in Southern Ethiopia (Amenu, 2005) and a mean burden of 1124.60 ± 669.60 recorded in Afar sheep from Eastern Ethiopia (Dereje, 2008). The mean worm burden of 165.20 ± 66.30 and 13.50 ± 5.70 recorded in wet and dry seasons, respectively complied with the level of prevalence and mean faecal egg count observed in respective season.

Many studies showed several gastrointestinal nematodes of the family Trichostrongylidae parasitize sheep around the world. Particularly, *H. contortus* is the major and economically the most important nematode parasite of small ruminants in the tropical and subtropical regions of the world (Achi et al., 2003; Fontenot et al., 2003; Terrill et al*.,* 2004). These prevalent species, namely *H. contortus* and *O. columbianum* which have intrinsically high biotic potential (Hansen and Perry, 1994) were expected to have considerably contributed to pasture contamination. Furthermore, *H. contortus* with its short generation interval in sheep host might be expected to significantly influence the epidemiology of GI nematodes in the study population. The occurrence of these species was largely influenced by the seasonal variation in rainfall pattern and larval recovery from coproculture was high in rainy season and negligible in dry season parallel to the prevalence recorded. Similar trend of prevalence was reported in Pakistan (Lateef et

al., 2005) and in Ethiopia (Sissay et al., 2007).

The overall prevalence of gastrointestinal nematode infection in Horro sheep was found to be 24.7% (95% CI: 22.6 to 26.8) and much of the infection seemed to be sub-clinical and could indirectly cause production losses without apparent clinical signs. Other contrasting findings were also reported in different parts of the country including 16.4% in Central Ethiopia (Bekele et al., 1992), 98.9% in Southern Ethiopia (Amenu, 2005) and 55.0% in sheep and 22.5% in goat flocks in Afar region (Dereje, 2008). These results are compliant with the consensus that prevalence varies greatly from region to region, corresponding to ecological and climatic diversity as well as the existing host ranges (Njau et al., 1990). Yet, a relatively low prevalence recorded in this study should not be overlooked to receive due attention to institute control measures, because, many studies indicated that gastrointestinal nematodes are the leading causes of productivity losses in small ruminant production in Ethiopia (Demelash et al., 2006).

Risk factors

In the present study, some factors influencing the epidemiology of gastrointestinal nematode parasites in Horro sheep have been investigated. The agro-ecology based study revealed a relatively high prevalence in lowland and mid altitude areas as compared to prevalence recorded in highland. Meanwhile, analysis of variance of LSM of logarithm transformed EPG for effects of factors showed significant difference between the agro-ecological zones. The LSM for nematode egg counts were higher in mid altitude and highland zones than in lowland and the difference was significant. But no significant variation was observed between mid altitude and highland. This might be largely the influence of variation in geographic and climatic conditions existing between each zone. The result was also consistent with other reports from Ethiopia (Demelash et al., 2006) and Australia (Waller et al., 1995).

Season was a factor seen critically influencing the epidemiology of gastrointestinal nematodes of sheep in the study areas. Both prevalence and the LSM of EPG were affected by season with significant rise in wet season which declined to a negligible low level in the middle of dry season. Likewise, seasonal fluctuations in nematode faecal egg counts which followed seasonal rainfall pattern were reported from different studies in the country (Fikru et al., 2006; Sissay et al., 2007). Similar to the present result, seasonal influences on worm faecal egg counts were reported in areas with distinct rainy and dry seasons in Kenya (Nginyi et al., 2001) and Tanzania (Keyyu et al., 2005).

The effect of sex on nematode prevalence of sheep was investigated. In this study, no significant variation was observed between male and female hosts despite slightly higher infection noticed in male sheep. Similar finding was reported in grazing ruminants in Western Oromiya (Fikru et al., 2006). However, the result was inconsistent with a finding reported in Pakistan where higher prevalence was observed in female sheep (Lateef et al., 2005). In this finding, the mean EPG was not significantly different between female and male animals. Also, within each age group (lambs, yearlings and adults), there was no significant variation in mean EPG between female and male animals. On the other hand, the mean EPG between lambs and adult sheep varied significantly irrespective of sex. These results are in agreement with the finding reported in Eastern Ethiopia (Sissay et al., 2007). In this study, the mean EPG count for adult females in breeding age including lactating ewes did not show any significant increase over male counterparts during the study periods. This result did not coincide with a view that breeding ewes become more susceptible to helminth infections (Huntley et al., 2004; Houdijk et al., 2006; Al-Shaibani et al., 2008). One possible explanation is that farmers give more attention to animals in production and increase the treatment regimen against endoparasites using anthelmintics which could reduce nematode infection in such group of animals.

The results also showed that, even if the differences were not significant, male animals had more nematode fecal egg counts than females in all age groups. In a study conducted at central highland of Ethiopia, male lambs had higher mean EPG than female lambs (Rege et al., 2002). The reason was not clear, but some evidence from literature supports that entire male animals are more susceptible than females to some helminth infections as a result of androgen activity (Urquhart et al., 1996). The prevalence and LSM of EPG were seen to decrease with increasing age of sheep. Nematode infection and the LSM of EPG were high in lambs with significant difference from adult age group. This complies with the result reported by Hansen and Perry (1994). In contrast, no significant differences were observed between lambs and yearlings as well as between yearlings and adult age group.

The prevalence and LSM of EPG were high for poor body condition scored sheep and significantly different from good body condition scored animals. This result concurs with other reports (Keyyu et al., 2003; Van Wyk et al., 2006). The higher prevalence recorded in the former group supported the local tradition experienced by farmers to select their animals for treatment. Farmers used the loss of conditions in their animals as a marker to identify and present for treatment mainly to minimize the treatment expenses. Possibly this practice deserves further field study perhaps to optimize for use in targeted selective treatment to manage nematode parasites of sheep. Similar field survey based on live weight gain was advocated by Jackson et al. (2009) as one possible tool to identify animals for targeted selective treatment with anthelmintics in an attempt to control anthelmintic resistance problems as a result of exploiting refugia.

The influence of grazing method used for the animals was significant for prevalence of gastrointestinal nematode infections and LSM of EPG count. The study conducted in sheep kept under different grazing management showed high prevalence in tethered animals followed by those maintained under alternate use of tethering and open grazing. On the other hand, low prevalence was recorded in sheep managed under open grazing. The differences between open grazing and tethering as well as between open grazing and mixed grazing were significant. Similarly, the variation was significant for LSM of EPG count between open grazers and tethered population. Farmers used tethering mostly during rainy season when pasture production was relatively surplus. In the study areas, this coincided with the time when pasture contamination with nematode eggs was more likely to occur from infected animals parallel to high prevalence recorded. In tethering of sheep and goats, outbreaks of parasitic gastro-enteritis have been reported in Tanzania, Nigeria, Kenya and Cameroon (Kambarage and Kusiluka, 1996) which was consistent with the present finding. In open grazing system, the low prevalence observed could indicate that animals freely grazed in the extensive grazing field had less exposure to infective larvae on the pasture or may have better resistance to the worm challenge than tethered animals.

In this study, despite the variations observed in prevalence and nematode faecal egg counts as influenced by different risk factors, 88.9% of the sheep sampled had low EPG counts (50 to 800). Only 4.1 and 7.0% of the animals showed moderate (800 to 1200) and heavy (>1200) EPG counts, respectively. Similar observation was also previously reported in the area (Fikru et al., 2006) and in many countries in sub-Saharan Africa (Bekele, 1991). Perhaps this result could be a preliminary indication of the genetic potential of Horro sheep breed that are well adapted and thrive to produce under nematode challenges in their natural habitats.

Alternative treatment of animals with high nematode infections may be considered under the existing production system in the study areas. Farmers developed a tradition of taking their animals to veterinary clinics for treatment against endoparasites when they detect clinically sick, emaciated, diarrheic or animal exhibiting loss of production. This is a kind of targeted selective treatment which could minimize the selection pressure for anthelmintic resistant gastrointestinal nematode populations on farms and treatment expenses for smallholder farmers.

CONCLUSION AND RECOMMENDATION

Conclusively, the results of this study showed that Horro sheep are infected with diversified gastrointestinal nematodes that can seriously affect the health and productivity of the animals. These parasites affected all age and sex groups and their prevalence varied from place to place based on agro-ecology, husbandry practices and seasonal rainfall pattern. Many animals were sub-clinically infected without attracting awareness of farmers to undertake control measures. Therefore, to improve the production potential of this indigenous breed of sheep and the livelihood of the farmers, control strategies based on the epidemiology of the parasites and production systems should be implemented. Improvement of grazing management for the animals particularly where tethering is in practice could minimize the risk of infection for susceptible animals.

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Short Communication

Preliminary studies on synchronization of estrus with double injection of prostenol in dwarf does *(Capra hircus)* and role of macro minerals in estrus

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A study was conducted to examine the efficacy of prostenol for synchronizing estrus in dwarf goats *(Capra hircus)* and role of concentration of macro minerals during estrus phase. A lot of goats (n=8) were selected from the flock maintained at Nuclear Institute for Agriculture and Biology (NIAB) Farm, Faisalabad on the basis of their post-partum period >2 months. Goats (n=5) were given 2 prostenol (an analogue of prostaglandin F2α (PGF2α) injections of 125 µg/animal), 12 days apart while 3 goats were kept as control. The does responded double injection at an interval of 15 days, because of immaturity of follicles. A teaser buck was introduced in the herd for estrus detection and visual symptoms were also observed. Blood sampling was carried out during estrus phase for sodium, potassium and phosphorus determination. Estrus was observed after 48 to 96 h with a mean time of onset of estrus at 72 ± 33 PGF2α injection. Estrus was observed in all treated goats (100% response) while no estrus was exhibited by any animal of the control group, because they were not injected by injection (PGF2α). Regarding macro-minerals, potassium in estrus was found to be higher (10.83 ± 1.89 ppm) in treated animals as compared to that of the control animals, while sodium and phosphorus levels were found to be the same in treated as well as control groups. It was concluded that double injection of prostenol (125 µg/animal) was efficient in synchronizing estrus in goats and potassium might have some important role during estrus phase in goats.

Key words: Estrus synchronization, goats, prostaglandins, macro-minerals.

INTRODUCTION

Prostaglandins F2α (PGF2α) and their analogues have been used successfully to synchronize estrus in buffaloes, goats and sheep. The double injection regimen aims at achieving higher rates of estrus synchronization at the 2nd PGF2α injection without the need to detect the estrus status of the animals before the first injection. Prostenol which is the cheaper analogue of PGF2α, works well in goats and gives best results in goats.

Minerals are very important in animal feed and are classified as micro and macro elements (Abdelhameed, 2000). Phosphorus deficiency is associated with decreased reproductive performance in dairy cows. Inactive ovaries (anestrus, delayed sexual maturity and low conception rates) have been reported when phosphorus intakes are low (Smith et al., 1979). Sabir (2005) mentioned that, deficiency of potassium leads to infertility, weak muscles and bones and hormonal defects which appeared in extra secretion of adrenal gland, loss

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of appetite, botulism. Akinsoyino (1986) reported that hypokalemia and hyperkalemia lead to delayed growth, poor production, and respiratory arrest, heart failure, kidney failure and finally death occur (Siribaddana, 2011) respectively.

Deficiencies, excesses or malabsorption of minerals contribute to several diseases of maternal, fetal, hormonal dysfunction and exert negative effect on the reproductive efficiency and it was reported that a mineral deficiency can cause infertility, abortion and still birth (Apgar et al., 1992).

There is no single study describing the role of macro-minerals at estrus phase of estrus cycle after synchronizing goats with PGF2α. Therefore, the present study was designed to investigate the role of different concentrations of sodium, potassium and phosphorus in estrus to observe the efficiency of double injection regimens of prostenol with an interval of 12 days for estrus synchronization in the goats.

MATERIALS AND METHODS

A herd of eight female goats (Teddy × Beetal), weighing 30 to 35 kg reared at Livestock Farm, Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad, Pakistan were selected on the basis of their post-partum period >2 months. Does were divided into 2 groups. Group 1 does (n=5) were (n=5) given two injections (i/m) of 250 µg (PGF2α)/animal (Selmore, Pharmaceutical Industries, Lahore) with twelve days apart and group 2 (control group, n=3) were given no treatment. The blood sampling was carried out on days 0 and 12th when PGF2α were given and on estrus day. Estrus and duration of the estrus were determined by continuous observation. The teaser buck was introduced into the herd for estrus detection. Blood samples were analyzed for sodium, potassium and phosphorus by following procedures.

Samples collection

Blood samples (5 ml) were collected from the jugular vein with a syringe and blood was immediately transferred to the tube. The blood samples were allowed to clot for 4 h at room temperature and then centrifuged at 2500 rpm for 20 min, and then serum was frozen at -20°C. Hemolytic free serum samples were harvested into clean polypropylene vessels and were frozen at -20°C for serum mineral analysis.

Sample preparation

Two milliliters serum sample was placed in a 50 ml centrifuge tube, and 1 ml of 1 N HCL was added. They were mixed and allowed to stand for 10 min. 8 ml of 10% trichloroacetic acid (TCA) was also added. The mixture was mixed very well and allowed to stand for 30 min. At 2000 rpm centrifugation, supernatant was collected and the precipitate was washed with 3 ml TCA. It was then centrifuged again and the supernatant fluids were combined.

Sample analysis

The sodium and potassium in digested samples were read on Flame photometer (FP, Jenway, PFP-7, England). Phosphorus determination was done by Spectrophotometer (Cecil, CE-1021, England). Foslam et al., (1975) respectively.

Table 1. Estrus synchronization rate in dwarf does with double injection of PGF2α (Prostenol).

Parameter	Control (n=3)	Treated (n=5)
Synchronization rate (%)	0 (0/3)	100% (5/5)
Time of onset of estrus	-	72 ± 33
Estrus duration	-	37.3 ± 2.33

Statistical analysis

The mean (±standard error (SE)) values of sodium, potassium and phosphorus of treated and control groups were compared.

RESULTS AND DISCUSSION

Estrus synchronization

The results of estrus synchronization are presented in Table 1. All the treated does presented in estrus. The animals showed vaginal discharge, bleating, redness of vagina, flehman posture, stands to be mounted and finally copulation. The animals were observed in estrus for 30 to 40 h with a mean duration of 37.3±2.33 h. Overall, 100% estrus response was found in all treated does. No estrus behavior was found in control group, because they were not injected with injection of PGF2α.

These results are comparable with the results reported by Nuti et al. (1992). The authors reported that mean time from injection (PGF2α) to behavioral estrus was 46 to 48 h with 95 to 100% does estrus response. Beck et al. (1993) reported that estrus response and timing of estrus in goats treated with prostenol (125 µg) on days 6 and 12 of the estrus cycle was 100%.

Sodium, potassium and phosphorus

A mean (±SE) value for sodium on days 0, 12 and at estrus is presented in Table 2. It was found out that there is no difference of sodium and phosphorus concentration levels in treated and control groups on 0, 12th and on estrus days. Mean (±SE) value for potassium on days 0, 12 and estrus are presented in Table 2. Higher values of potassium in treated does than that of the control group were found on estrus day.

Sodium and chloride are critical in the electrolyte balance. In addition, sodium affects the absorption of sugar and proteins from the digestive tract. Salt deficiencies can affect the efficiency of digestion and indirectly the reproductive performance of cows. Potassium functions in acid-base balance, osmotic pressure and the amount of water retained in the body. High levels of

Table 2. Serum concentrations of sodium, potassium and phosphorous in dwarf does affected by PGF2α injection.

Mineral	Sodium			Potassium			Phosphorus		
Day	0	12th	Estrus	0	12th	Estrus	0	12th	Estrus
Treated (n=5)	42.4 ± 4.33	45.8 ± 10.82	46.2 ± 1.29	8.4 ± 1.29	8.15± 1.61	11 ± 1.83	11.89 ± 135.34	580 ± 154.82	525 ± 22.93
Control (n=3)	35.33 ±12.50	42.33 ± 7.76	45.33 ± 7.37*	7.08 ± 2.80	8.15± 1.61	8.66 ± 2.08*	243 ± 92.26	293 ± 52.43	525.33 ± 14.18*

Values (Mean ± SD) with difference in the columns. Concentration showed in ppm. *Animals showed no estrus response.

potassium may inhibit magnesium absorption and cause metabolic problems, especially in grazing systems. Other studies also report lower fertility in cows fed high levels of potassium or diets in which the potassium-sodium ratio was too wide (Rivera, 2011).

Mean (±SE) value of phosphorus is presented in Table 2. No difference in phosphorus levels was observed on days 0, 12 and estrus day.

Phosphorus deficiency leads to decreased growth, unthriftness, decreased milk production, poor conception, lower fertility and calving percentage (Bredon and Dugmore, 2005). Phosphorus is commonly referred to as the “fertility” mineral. Inactive ovaries, delayed sexual maturity and low creatinine have been attributed to low phosphorus intake (Lopez et al., 2004). In ruminants, majority of phosphorus is excreted through the feces (69% of the total) with approximately 30% being excreted through the milk and only about 1% being excreted through the urine (Phillips, 2000).

Conclusions

Prostenol, a synthetic cheaper product of PGF2α is the best hormone for estrus synchronization in goats and had given 100% estrus response. Role of macro-minerals, especially potassium, might have some role in behavioral estrus. Further study should be conducted to identify the exact role of potassium during estrus phase of the estrus cycle.

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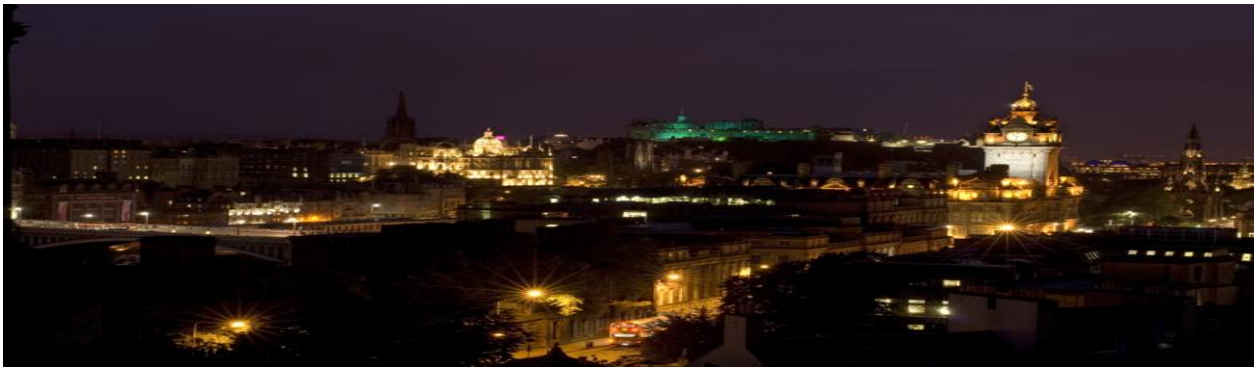

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