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ARTICLE

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Full Length Research Paper

Sheep and goats pasteurellosis: Isolation, identification, biochemical characterization and prevalence determination in Fogera Woreda, Ethiopia

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A cross sectional study was carried out on pasteurellosis of small ruminants in Fogera Woreda, Ethiopia. The objectives of this research were isolation and characterization of the bacterial species by culture and biochemical testes, and determining the prevalence in apparently pneumonic small ruminants. Out of the total 988 samples examined, 322 were detected positive for *Pasteurella* with an overall infection rate of 32.6%. Of which, 180 (55.9%) were from nasal swabs and 142 (44.1%) were from blood samples. Accordingly, 79.5% of the isolates were *Mannheimia haemolytica* and 20.5% were *Pasteurella multocida*. Significantly ($p < 0.05$) higher prevalence was detected in sheep (37.1%), females (36.42%), young (52.97%), and extensively managed ruminants (38.15%) than those in goats (21.9%), males (25.29%), adults (21.26%) and semi-intensive production systems (17.18%), respectively. Similarly, the frequency of infection was significantly ($p < 0.05$) higher in winter (48.6%) and spring (32.85%) as compared to autumn (23.79%) and summer (19.67%). In conclusion, this finding showed that the disease is highly prevalent in the study area. Thus, an integrated application of vaccination and overall management measures should be implemented to prevent and control the disease in animals.

Key words: Pasteurellosis, Isolation, *Mannheimia haemolytica*, *Pasteurella multocida*, Sheep and Goats, Identification, Fogera, Ethiopia.

INTRODUCTION

Ethiopia has diverse animal resources and its relatively large livestock population (approximately 100 million) is well adapted to and distributed among diverse ecological conditions and management systems (Aiello and May, 1998). Like other developing countries, livestock may

give a wide variety of importance in Ethiopia. Although the presence of large animal populations and its economically significance, the occurrence of diseases, malnutrition, poor-management systems and poor-performances of the local breed makes the productivity of animals to be

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very low. The above most mentioned problems lead to poor reproductive performance of sheep and goats (Lobago et al., 2006). As reported by many researchers in different areas of the country, livestock provides 16% of the total GDP (= 30% of agriculture GDPs) and generates 14% of the country's foreign exchange earnings (CSA, 2009).

Diseases such as respiratory tract infections leads for the huge financial loses and the socio economic developments of low income animal producers in the area. Such infections results in high morbidity and mortality of animals (Zewdie, 2004). The disease occurs in food animals due to complex factors that often interact to produce disease. A wide variety of factors such as weathers, climates, transportations, weaning, poorly-ventilated houses, and malnutrition were well known to play a pre-disposing role as the animal's immunity weakness. In such conditions, occurrence of the normal flora of the upper respiratory tract and subsequence infection of the lungs is well documented (Radostits et al., 2000; Baundreaux, 2004).

Respiratory tract infections are of common occurrence in various species of domestic animals. However, pneumonic pasteurellosis, also known as respiratory mannheimiosis, is most common example with a wide prevalence in ruminants. The disease in its typical clinical form, is highly infectious, often fatal and with very serious economic mortality in many animals in which the disease accounts for approximately 30% of the total cattle deaths worldwide (Baundreaux, 2004).

It is important to mention that *Mannheimia haemolytica*, and *Pasteurella multocida* constitute the most important members of the family Pasteurellaceae that pose serious hazards in livestock industry. These species are commensally resident in the animal body as normal constituent of the naso-pharyngeal microflora and are all capable of causing infection when the body defense mechanisms are impaired. Their presence is mainly confined to ruminants with most adequately characterized strains originating from cattle, sheep and goats (Biberstin and Hirsh, 1999).

Pasteurella multocida is associated with haemorrhagic septicemia and enzootic pneumonic complex in sheep, goats, and cattle and buffaloes (Jones et al., 1997). Respiratory tract disease complexes such as bacteria, lungworms and viruses had been contributed for the spread and multiplication of pasteurellosis in ruminants (Biberstin and Hirsh, 1997; Quninn et al., 2002). In the cool central highlands of Ethiopia, respiratory disease complex has been found as leading. Uncoordinated vaccination schedules for diseases such as pasteurellosis and Pest des Petites Ruminants (PPR), lack of strategic mass drenching against lungworms and existence of viral diseases may attribute significant roles in the persistence of respiratory disease complex in Ethiopia.

Although pasteurellosis is among the most economically significant infectious diseases of sheep and goats in Ethiopia (Mohamed and Abdelsalam, 2008), there was no any previous research conducted in Fogera Woreda. Hence, this research was proposed to isolate and characterize *pasteurella* species that affect the respiratory tract of sheep and goats which cause pneumonic and septicemic pasteurellosis, to determine the prevalence, and to estimate related factors which have direct and indirect association with the bacteria.

MATERIALS AND METHODS

Study area

The study was undertaken in Fogera Woreda, South Gondar zone of Amhara Region, Ethiopia. Fogera is located 625 km away from the capital city, Addis Ababa. Woreta is the capital of Fogera and it is located at 55 km from Bahir Dar (the capital city of Amhara Regional State) in the direction of Gondar. Fogera has an altitude range of 1,774 to 2,410 meter at sea level and a latitude and longitude of 11° 46' to 11° 59' E and 37° 33' to 37° 52' N, respectively. The minimum and maximum mean annual rain fall of the area ranges from 1,103 to 1,336 mm. The area receives a temperature range of 12.0 to 27.08°C daily. Similarly, the humidity of the area is relatively 22% up to 80% (CSA, 2008). The weather condition of Fogera is predominantly classified as 'Woinadega'. The district has a total land area of 117,414 ha, out of which 9,602.36 ha is grazing land. 76% of the zone is Flat land, 11% mountain and hills and the rest 13% is valley bottom. It has bi-modal rainfall. The short rainy season being from March 15 to May and long rainy seasons are predominantly seen in June, July and August. Crop and livestock production practices are common to the study area. Sheep and goat farming system of the localities are an integral part to the traditional rearing ways. In this area, extensive management system is dominant. Semi-intensive system of production is performed to a lesser extent (ARSBARD, 2006). As stated by Fogera Woreda Office of Agriculture and Rural Development (2004), the animal populations are estimated to be: 157,128 cattle, 27,867 goats, 7,607 sheep, 246,496 chickens, 21,883 beehives, 13,189 donkeys, 339 mules, and 8 horses. In addition, Fogera woreda has exotic breeds which include: 22 heifers, 10 young bulls, 22 cows, 3 calves and 19 improved beehives. Fogera woreda is the origin to the Fogera cattle breed's that are extremely productive indigenous cattle in Ethiopia famous to their meat as well as milk productions and traction power.

Study population

The study animals were apparently pneumonic small ruminants at Woreta city Veterinary Clinic and at the field of Fogera reared by small ruminant producers for subsistence and reserve as a means of income for their families. Small ruminants are commonly reared under extensive system of production and are kept with other animal species. The total sheep and goat populations of the study area are estimated to be 35,474 from which 7,607 sheep and 27,867 goats. All small ruminants under study were clinically sick and showed pneumonic signs and were in all age groups. As stated by Gatenby (1999) (as cited in Tewodros and Dawit, 2015), the study animals were grouped into two age categories as young (< 2 years), and adults (≥2 years).

Study design

Cross sectional study design was used to estimate the infection rate of sheep and goats pasteurellosis that showing pneumonic signs. Swab and blood specimens were submitted for bacteriological isolation and biochemical characterization of *pasteurella* species.

Sample size determination

In this study, the sample size determination in random sampling for infinite population using expected prevalence of small ruminant pasteurellosis at 5% desired absolute precision and 95% confidence interval according to Thrusfield (2005) was used. Since there is no any previous study conducted in this area on pasteurellosis of ruminants, 50% expected prevalence was used. As a result, a total of 988 small ruminants (696 sheep and 292 goats) with pneumonic signs were randomly selected from the clinic and the field.

$$n = \frac{1.96^2 P_{exp} (1 - P_{exp})}{d^2}$$

Where, n = required sample size; P_{exp} = expected prevalence; d = desired absolute precision.

Sampling procedures

Nasal swab

Each animal was individually identified and restrained by an assistant and kept fixed. After disinfection of external part of the nose with 70% alcohol, a sterile cotton-tipped swab was inserted into the nostril and rotated against the wall of the nasal cavity (Carter, 1984a). The swab was placed in labeled sterile test tube that contains 3 ml of tryptose soya broth, and then kept in an ice box for transport to the Laboratory (Carter, 1984a).

Blood samples

Aseptic procedures were followed while taking blood samples from the Jugular veins of small ruminants. The blood samples were subjected to different biochemical tests and direct culturing. Samples were transported to the required laboratory through an ice box jar after its collection in the field.

Isolation and Identification of *Pasteurella* species

The isolation and identification of *Pasteurella* were performed at the Veterinary Laboratory using techniques recommended by Hardy Diagnostics, Santa Maria, CA, USA. The isolation and identification involves the following steps: First, the specimen was incubated for 24 h at 37°C. A loop full of the broth cultures were taken and streaked over an identified Petri-plate containing blood agar base supplemented with 7% sheep blood and immediately incubated aerobically at 37°C for 24 h (Quinn et al., 1994). Secondly, from culture positive plates, typical colonies were subjected to gram's staining to study staining reactions and cellular morphology. Mixed and gram-negative bacteria were further sub-cultured on both Blood and MacConkey agar plates (Quinn et al., 1994) for further analysis. The growth of typical colonies on both Blood and MacConkey agar was characterized using Blood agar for the presence of haemolysis,

the type of haemolysis, the general appearance of colonies and the ability to ferment lactose (Sharma and Adlakha, 1996). Thirdly, pure cultures of single colony type from both Blood and MacConkey agars were transferred onto nutrient agar-slants for a series of primary biochemical tests. Final identification of the bacteria to the species level was aided by secondary biochemical tests (Carter, 1984b; Quinn et al., 1994). If the organism is able to produce a narrow zone of haemolysis on Blood agar and grow on MacConkey agar, but unable to produce Indole, interpreted as *M. haemolytica*. If the organism unable to produce haemolysis on Blood agar and cannot grow on MacConkey but able to produce Indole, is interpreted as *P. multocida* (MacFadinn, 2000; Marru et al., 2013).

Data analysis

Data were entered and managed in MS Excel work sheet. The analysis was conducted using SPSS 16.0. Prevalence of pasteurellosis at animal was expressed as percentage with 95% confidence interval (CI) by dividing the total number of animals positive to pasteurellosis to the total number of animals examined. An animal was considered as positive for pasteurellosis if it was positive either through nasal swab or blood culture. The significance of differences between the prevalence of pasteurellosis was determined using Fisher's exact test when the numbers within the categories were too small for Chi-square test. Age, sex, species, management system and seasons of the year were considered as risk factors to see their association with the prevalence of pasteurellosis.

RESULTS

Overall prevalence

Out of the total 988 nasal swabs and blood samples collected and cultured (696 from sheep and 292 from goats); *pasteurella* isolates were detected from 322 specimens giving an overall infection rate of 32.6% in the population studied. Of the 322 positives for *Pasteurella*, 180 (55.9%) were isolated from nasal swabs and 142 (44.1%) were from blood specimens (Table 1 and Figure 1).

P. multocida and *M. haemolytica* were the two major species of *pasteurella* isolated. Significantly ($\chi^2 = 11.370$, $p < 0.05$) higher prevalence was recorded in *M. haemolytica* (79.50%) than in *P. multocida* (20.50%) (Table 2 and Figure 2).

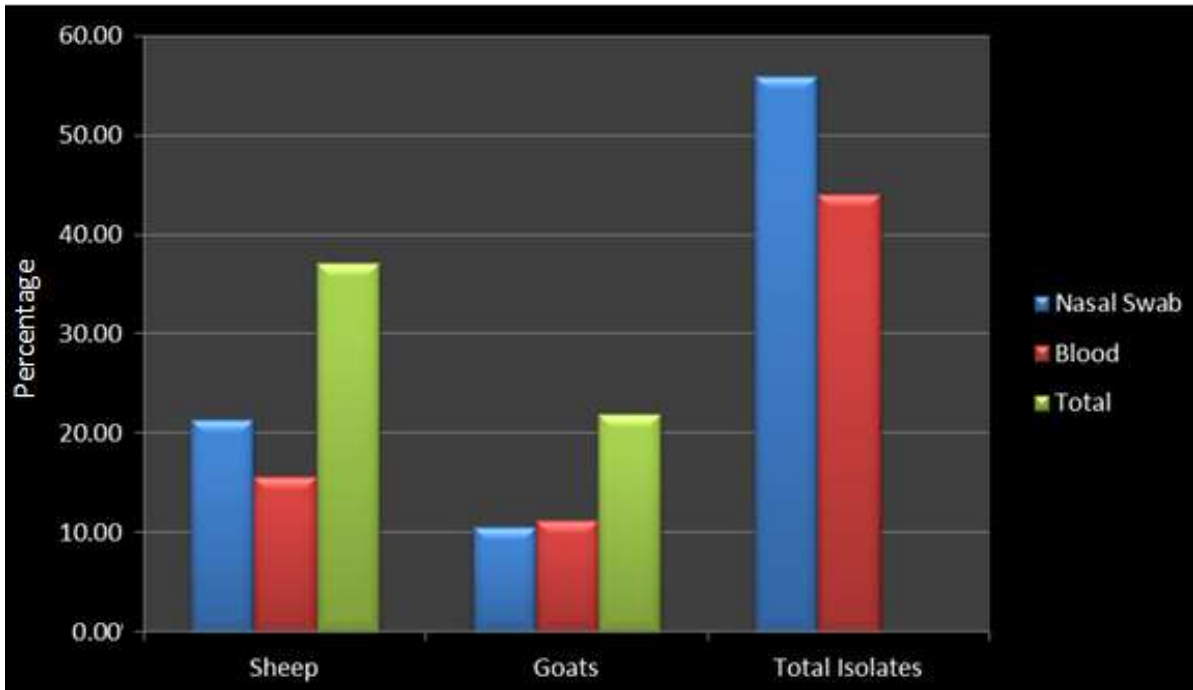
Comparing the infection rate of pasteurellosis in species of small ruminants, significantly ($\chi^2 = 2.187$, $p < 0.05$) higher prevalence was detected in sheep (37.1%) than that of goats (21.9%) (Table 3).

Out of the 988 small ruminants examined, only 135 (21.26%) adults (≥ 2 years) were found infected as compared to young animals (< 2 years) (52.97%; 187 out of 353). The difference was statistically significant ($\chi^2 = 6.360$, $p < 0.05$) (Table 3).

Based on our examinations on *pasteurella* infection in relation to sex of small ruminants, it was a significant ($\chi^2 = 13.728$, $p < 0.05$) prevalence of 36.4 and 25.3% was estimated in female and male animals, respectively

Table 1. Isolation of *Pasteurella* in sheep and goats from nasal swabs and blood specimens.

Species	No. of positives		Total sample	Total isolates
	Nasal swab	Blood		
Sheep	149	109	696	258 (37.1%)
Goats	31	33	292	64 (21.9%)
Prevalence	55.9%	44.1%	988	322 (32.6%)

**Figure 1.** Isolation of *Pasteurella* in sheep and goats from nasal swabs and blood specimens.**Table 2.** Isolation of *Pasteurella* species with nasal swabs and blood culture.

Species of <i>Pasteurellaceae</i> Isolated	Type of sample		Total positives	Prevalence (%)
	Nasal swab	Blood		
<i>M. haemolytica</i>	143	113	256	79.50
<i>P. multocida</i>	37	29	66	20.50
Total		988	322	32.6%

M. haemolytica: *Mannheimia haemolytica*; *P. multocida*: *Pasteurella multocida*.

(Table 3).

To see the effect of management on the prevalence of pasteurellosis, sheep and goats were categorized under extensive and semi-intensive management systems. A prevalence of 38.2% was observed in animals kept under extensive management system while 17.18% in animals reared under semi-intensive system. The difference

between the prevalence was statistically significant (Fisher's exact = 0.021, $p < 0.05$) (Table 3).

The infection rate of pasteurellosis was assessed among the four seasons of the study period. The frequency of infection was significantly ($\chi^2 = 0.428$, $p < 0.05$) higher in winter (48.6%) and spring (32.85%) as compared to autumn (23.79%) and summer (19.67%)

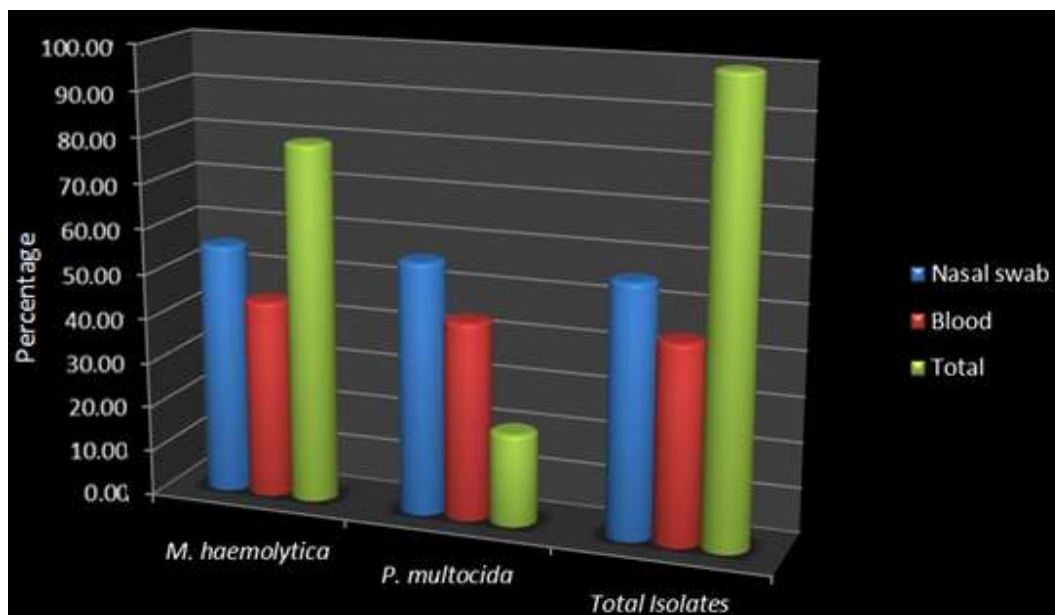


Figure 2. Isolation of *Pasteurella* species with nasal swabs and blood culture in small ruminants.

(Table 3).

Cultural characteristics of isolated *Pasteurella* species

P. multocida isolates were round, smooth (mucoid) and non-hemolytic on blood agar. All *P. multocida* were gram-negative, coccobacillary and did not grow on MacConkey agar. Whereas; *M. haemolytica* was able to grow on MacConkey agar and showed hemolysis on blood agar. It also grew as pin point red colonies.

Biochemical characteristics of isolated *Pasteurella* species

The biochemical examination result indicated that both the species were positive to catalase, oxidase, and able to ferment glucose as well as sucrose. Similarly, both the isolates of *Pasteurella* species were negative to urease. *M. haemolytica* isolates were unable to produce Indole while *P. multocida* isolates were characteristically produced Indole (Table 4).

DISCUSSION

In the present study, the overall prevalence of small ruminant pasteurellosis was found to be 32.6% in which 37.1 and 21.9% were recorded in sheep and goats,

respectively. This finding coincides with Yeshwas et al. (2013) who reported 33.1% in Farta and Lay Gaint Districts of Amhara Regional State, North-West Ethiopia. However, the current finding was lower than that of Abera et al. (2014), Aschalew (1998), and Tesfaye (1997) who reported 50.2, 63.8 and 67.6%, respectively. On the other hand, the current result was slightly higher than that of Tilaye (2010) who reported 28.4%. This might be due to the different ways of taking samples from purely pneumonic sheep and goats, improved health facilities, laboratory facilities, ecology of the study areas and predisposing factors (Abera et al., 2014).

Comparing the two *Pasteurella* species, *M. haemolytica* constituted 79.5% of the total positives indicated that *M. haemolytica* was the major causative agent involved in small ruminant pneumonic pasteurellosis. Although the infection rate varies, this finding is consistent with previous reports of Abera et al. (2014), Aschalew (1998), Daniel et al. (2006), Eshetu (1991), Maru et al. (2013), Mohammed (1999), Tesfaye (1997), and Asefa et al. (2001). *M. haemolytica*, which is a normal flora of the upper respiratory tract, may play a secondary role after the primary initiating agent that suppressed the host defense mechanism, and favors the multiplication of *Pasteurella* species leading to bronchopneumonia in purely pneumonic animals (Aiello and May, 1998). Although the percentage of isolation was relatively low (20.5%), the possible role of *P. multocida* in the etiology and pathogenesis of sheep and goat pneumonia should not be underestimated (Maru et al., 2013).

Concerning to the rate of *Pasteurella* species isolation

Table 3. The infection rate of *Pasteurella* in sheep and goats in relation with different variables.

Variable	Animal		Infection rate (%)	(χ ²)	P. value
	Tested	Positive			
Species					
Sheep	696	258	37.1	2.187	p < 0.05
Goats	292	64	21.9		
Age					
< 2 years	353	187	52.97	6.360	p < 0.05
≥ 2 years	635	135	21.26		
Sex					
Male	340	86	25.29	13.728	p < 0.05
Female	648	236	36.42		
Management system					
Extensive	726	277	38.15	F. exact = 0.021	p < 0.05
Semi-intensive	262	45	17.18		
Season					
Autumn	248	59	23.79	0.428	p < 0.05
Winter	280	136	48.57		
Spring	277	91	32.85		
Summer	183	36	19.67		
Total	988	322	32.6		

Table 4. Cultural and Biochemical characteristics of *pasteurella* species isolated from sheep and goats:

Biochemical Test	Species of <i>Pasteurella</i> isolated	
	<i>M. haemolytica</i>	<i>P. multocida</i>
Catalase	+	+
Oxidase	+	+
Urease	-	-
Glucose	+	+
Sucrose	+	+
Lactose	+	-
Indole	-	+
Hemolysis	+	-
Growth on MacConkey agar	+	-

in sheep and goats, it was 32.6% (322 out of 988), 258 positive isolates with recovery rate of 37.1% in sheep while it was 64 positive isolates with recovery rate of 21.9% in goats.

In comparison between sheep and goats, the isolation percentage of pasteurellosis was significantly higher in sheep than that of goats ($p < 0.05$). These results; however, are not in line with that obtained by Rasha et al. (2014) who recovered *pasteurella* species from sheep and goats with recovery rate of 56 and 44%, respectively. This difference might be due to the difference in sampling

procedures and sample taking from apparently healthy and purely pneumonic sheep and goats. The other possible difference might be the epidemiological and ecological differences of the study areas. Similarly, the difference in the prevalence of the two species might be due to the difference in grazing behavior of these species of ruminants. Sheep predominantly deep graze; pick up more bacteria so have higher exposure than goats which mostly consume browse. Goats with their browsing behavior consume uncontaminated matter with bacteria, so being less exposed to infection and therefore, have

lower prevalence than sheep (Wilsmore, 2006).

In this study, higher rate of infection was associated with young age groups (52.97%) of sheep and goats as compared to adults (21.26%) ($p < 0.05$). This finding coincides with Maru et al. (2013). This might be due to the immune status of the animals being able to predispose to the bacterial infection and other predisposing etiological agents (Abera et al., 2014). Similarly, this result is also in agreement with the findings of Gilmour and Gilmour (1989) that elucidates pneumonic pasteurellosis occur in all ages of sheep and goats, with the most susceptible in lambs and kids during first life, and dams at lambing.

In the current study, a significant association between pneumonic pasteurellosis and sex of apparently sick sheep and goats irrespective of *Pasteurella* species was observed. A prevalence of 25.29 and 36.42% was recorded in male and female small ruminants, respectively. This result; however, contradicts to the findings of Maru et al. (2013) in Haramaya District who reported that sex has no any association in *Pasteurella* infection in sheep. This difference in prevalence between female and male animals is probably due to the fact that resistance to infection is abrogated at the time of parturition and during early lactation. This pre-parturient relaxation of resistance results in the females' inability to resist the inhabitant bacteria which cause higher level of infection (Craig, 1994). The way that males and females treated in terms of nutrition may also attribute for such differences. Males are kept for fattening to be sold later, except some which are kept for breeding, receives more attention by small ruminant producers. Crop leftovers and remnants after human consumption, for instance, are provided primarily for males (Alemu et al., 2006).

In the present study, the level of prevalence was compared between animals kept under extensive and semi-intensive management systems. The prevalence was higher (38.15%) in small ruminants kept under extensive system of production, and the difference between the prevalence was statistically significant ($p < 0.05$). The difference in the prevalence rate that were found between the management systems might be due to the proportion of number of animals tested for semi-intensive system was relatively small as compared to extensive system. An extensive management system (free grazing) which allows unrestricted contact between animals might also have contribution to the spread of pasteurellosis in animals in the extensive system. In case of semi-intensive management system it could be associated with better management practices like introducing sheep and goats after being vaccinated for pasteurellosis and the routine cleaning of the house may decrease the establishment of infection in the semi-intensive production system and this makes small ruminants not being exposed more by pasteurellosis (Tewodros and Dawit, 2015).

In the current study, the seasonal variation of the

isolates of *Pasteurella* was also determined. The frequency of infection was significantly ($p < 0.05$) higher in winter (48.6%) and spring (32.85%) as compared to autumn (23.79%) and summer (19.67%) which is in agreement with Rasha et al. (2014). This is due to the high rain fall of the area in winter and spring which exposes the animals to cold stress. Moreover; almost all major disease causing parasites are prevailing in these seasons (spring and winter) of the study area. It is well known that these parasites predominantly affect the gastrointestinal and respiratory tracts of sheep and goats causing immune-compromization. This in turn, favors the multiplication and invasion of the normal inhabitant bacteria, *Pasteurella*, leading to infection of the host (sheep and goats).

Conclusions

In general, small ruminants were highly affected by pasteurellosis in the areas where this research was conducted. Species, sex, age, management system and seasons of the year were found the risk factors and significantly associated with the distribution and dissemination of the disease in sheep and goats. In conclusion, avoiding small ruminants from stress causing conditions and providing adequate feed and water as well as regular vaccination is highly advisable to minimize the influence of this disease.

Conflicts of Interests

The authors have not declared any conflict of interests.

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