Pasteurellosis in Small Ruminants: biochemical Isolation, Characterization and Prevalence Determination in Relation to Associated Risk Factors in Fogera Woreda, North-West Ethiopia

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Abstract: The study was conducted from September 2014 to August 2015 in Fogera Woreda, Amhara Regional State, North-West of Ethiopia with the objectives of isolation and characterization of *pasteurella* species with culture and biochemical tests, and to determine its prevalence in relation to associated risk factors in apparently pneumonic sheep and goats. Out of 988 nasal swabs and blood samples from small ruminants (696 sheep and 292 goats) examined, 322 were found positive to pasteurellosis with overall prevalence 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*. Species, age, sex, management system and seasons of the year were found risk factors and significantly associated with *pasteurella* infection in small ruminants (P<0.05). The prevalence in sheep (37.1%), female (36.42%), young (< 2 years) (52.97%) and extensively managed animals (38.15%) were found higher than the prevalence in goats (21.9%), male (25.29%), adults (= 2 years) (21.26%) and sheep and goats managed under semi-intensive production system (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 clearly indicates 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: Prevalence - Pasteurellosis - Isolation - *M. haemolytica* - *P. multocida* - 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 [1]. In Ethiopia, like many developing countries, livestock may play multiple roles. Despite the huge number of cattle, sheep and goats and economic importance, the productivity is low due to the constraints of diseases, nutrition, poor management and poor performance of the indigenous breeds. These constraints result in poor reproductive performance of these animals [2]. On the basis of statistics acquired from different sources, livestock provides 16% of the total GDP (equivalent to 30% of agricultural GDP) and generates 14% of the country’s foreign exchange earnings [3].

Disease constraints like respiratory diseases contribute to the great financial losses and the socio-economic development of poor farmers in the area. These diseases cause a huge mortality and morbidity [4]. The disease occurs in food animals due to complex factors that often interact to produce disease. Various conditions such as climate, weather, weaning, transportation, poorly ventilated housing and nutritional deficiencies are 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 subsequent infection of the lungs is well documented [5].

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 [6].

The term pasteurellosis was broadly used to designate a number of infections in domestic animals caused by Gram-negative, non-motile, facultative anaerobic rods or cocccobacilli formerly grouped under the genus Pasteurella (after Louis Pasteur). It is worth mentioning that M. haemolytica and P. 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 [7].

Pasteurellamultocida is associated with hemorrhagic septicemia and enzootic pneumatic complex in sheep, goats and cattle and buffaloes [8]. Concurrent infections of the respiratory tract by viruses, bacteria and lungworms have been described and such disease conditions are commonly known as respiratory disease complex (RDC), indicating the difficulty to attribute to only one etiology [7, 9]. In the cool central highlands of Ethiopia, respiratory disease complex has been identified as leading. Irregular and insufficient vaccination program for diseases such as pasteurellosis and Pest des Petites Ruminants (PPR), lack of strategic mass drenching against lungworms and occurrence of viral infections may play significant roles in the persistence of respiratory disease complex in Ethiopia.

Even though pasteurellosis is one of the most economically important infectious diseases of sheep and goat in Ethiopia [10], there was lack of information on this regards in South Gondar Zone, especially in the study area, Fogera Woreda. Hence, the study was designed to isolate and identify pasteurella species that invade the respiratory tract of sheep and goats causing pneumatic and septicemic pasteurellosis, to determine the prevalence and to assess some of the determinant and risk factors associated with it.

MATERIALS AND METHODS

Study Area and Population

Study Area: The study was conducted on small ruminant pasteurellosis in Fogera Woreda, South Gondar Zone of Amhara Regional State; Ethiopia. Fogera is 625 kms farfrom Addis Ababa. Woreta is the capital of Fogeraand it is located at 55 kms fromBahir Dar (the capital city of Amhara Regional State) in the direction of Gondar. Fogerahas an altitude range of1,774 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. It has a minimum and maximum mean annual rain fall of 1,103 to 1,336 mm. The daily temperature can reach 12.0°C to 27.08°C with relative humidity of 22% to 80% [11]. The weather condition of Fogera is predominantly classified as ‘Woinadega’. The district has a total land area of 117,414 hectare, out of which 9,602.36 hectare 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 season similar to the rest part of the country. Mixed crop-livestock is the farming system in the area. Small ruminant production of the area is an integral component of the traditional farming system. In this area, extensive management system is dominant. Semi-intensive system of production is practiced to a lesser extent [12]. According to Fogera Woreda Office of Agriculture [13], the total number of livestock population in the study area were cattle 157,128, goats 27,867, sheep 7,607, chickens 246,496, bee hives 21,883, donkeys 13,189, mules 339 and horses 8. In addition, Fogera woreda has Exotic breeds which include: 22 heifers, 10 young bulls, 22 cows, 3 calves and 19 improved bee hives. Fogera woreda is the home of the “Fogera Breed” cattle, which is highly productive indigenous animal in the country known for its milk and meat production as well as traction power.

Study Population: The study animals were apparently pneumatic sheep and goats at Woreta city Veterinary Clinic and at the field areas of Fogera kept by individual farmers for subsistence and reserved as a means of capital for family members. These animals were managed almost under extensive farming system mixed with other species. The total number of small ruminant population in the study area were 35,474 from which 7,607 sheep and 27,867 goats. All of the study animals were clinically sick and showed pneumatic signs and were in all age groups. According to Gatby [14] (as cited in Tewodros and Dawit [15]), the study population was grouped into two age groups as young those which are < 2 years and adult those which are= 2 years.

Study Design: A cross-sectional study design was conducted to determine the prevalence of small ruminant pasteurellosisin sheep and goats showing pneumatic signs. Nasal swabs and blood samples were taken for
bacteriological culture and biochemical characterization of *Pasteurella* species beginning from September 2014 to August 2015 in Fogera Woreda.

**Sample Size:** 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 [16] was unable to use. This is because there is no any previous study conducted in this area on pasteurellosis of ruminants. As a result, a total of 988 small ruminants (696 sheep and 292 goats inclusive of all Kebeles of the Woreda) with pneumonic signs were randomly selected from the clinic as well as the field examined.

**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 [17]. 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 Bahir Dar Regional Veterinary Laboratory [17].

**Blood Sample:** Blood samples (for direct culturing and biochemical tests) were taken from the jugular vein of sheep and goats aseptically. After collection, samples were kept in an ice box for transportation to the laboratory.

**Isolation and Identification of Pasteurella Species:** The isolation and identification of *Pasteurella* were performed at the Microbiology Department of Bahir Dar Regional 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 hrs 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 hours [18]. 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 [18] 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 [19]. 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 [20, 21]. 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* [22, 30].

**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); pasteurellosis was isolated from 322 samples giving an overall prevalence of 32.6% (322/988) in the population studied. Out of 322 positives for pasteurellosis, 180 (55.9%) were from nasal swabs and 142 (44.1%) were from blood samples (Table 1). The isolated species of pasteurellosis were *M. haemolytica* and *P. multocida* with prevalence of 79.5% and 20.5%, respectively (Table 3).

There was a significance difference on the prevalence of pasteurellosis infection between sheep and goats ($\chi^2$ = 2.187, *p* < 0.05). The infection rate was higher in sheep (37.1%) compared to goats (21.9%) (Table 2).

Among the 988 small ruminants, higher prevalence (29.5%) was observed in young animals of less than two years of age while lower prevalence (13.9%) was observed in animals of greater than two years. The difference between the prevalence was statistically significant ($\chi^2$ = 6.360, *p* < 0.05) (Table 2).
Table 1: The isolation of pasteurellosis in nasal swabs and blood samples of sheep and goats:

<table>
<thead>
<tr>
<th>Species</th>
<th>No of Positives for Total sample</th>
<th>Total Isolates</th>
<th>Nasal swabs</th>
<th>Blood</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sheep</td>
<td>149</td>
<td>109</td>
<td>696</td>
<td>258(37.1%)</td>
</tr>
<tr>
<td>Goats</td>
<td>31</td>
<td>33</td>
<td>292</td>
<td>64 (21.9%)</td>
</tr>
<tr>
<td>Prevalence</td>
<td>55.9%</td>
<td>44.1%</td>
<td>988</td>
<td>322(32.6%)</td>
</tr>
</tbody>
</table>

Table 2: Prevalence of Pasteurellosis infection in small ruminants in relation to species, age, sex, management system and seasons of the year:

<table>
<thead>
<tr>
<th>Variable Tested</th>
<th>Prevalence (%)</th>
<th>Chi-square ($\chi^2$)</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sheep</td>
<td>696</td>
<td>258</td>
<td>37.1</td>
</tr>
<tr>
<td>Goats</td>
<td>292</td>
<td>64</td>
<td>21.9</td>
</tr>
<tr>
<td>Age Group</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;2 years</td>
<td>353</td>
<td>187</td>
<td>52.97</td>
</tr>
<tr>
<td>≥ 2 years</td>
<td>635</td>
<td>135</td>
<td>21.26</td>
</tr>
<tr>
<td>Sex of Animals</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>340</td>
<td>86</td>
<td>25.29</td>
</tr>
<tr>
<td>Female</td>
<td>648</td>
<td>236</td>
<td>36.42</td>
</tr>
<tr>
<td>Management System</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Extensive</td>
<td>726</td>
<td>277</td>
<td>38.15</td>
</tr>
<tr>
<td>Semi-intensive</td>
<td>262</td>
<td>45</td>
<td>17.18</td>
</tr>
<tr>
<td>Season of the Year</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Autumn</td>
<td>248</td>
<td>59</td>
<td>23.79</td>
</tr>
<tr>
<td>Winter</td>
<td>280</td>
<td>136</td>
<td>48.57</td>
</tr>
<tr>
<td>Spring</td>
<td>277</td>
<td>91</td>
<td>32.85</td>
</tr>
<tr>
<td>Summer</td>
<td>183</td>
<td>36</td>
<td>19.67</td>
</tr>
<tr>
<td>Total</td>
<td>988</td>
<td>322</td>
<td>32.6%</td>
</tr>
</tbody>
</table>

Table 3: Species of Pasteurellacae isolated by nasal swabs and blood cultures and their prevalence in small ruminants

<table>
<thead>
<tr>
<th>Species of Pasteurellacae</th>
<th>Type of Sample</th>
<th>Blood</th>
<th>Total positives</th>
<th>Prevalence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. haemolytica</td>
<td>143 Nasal swab</td>
<td>113</td>
<td>256</td>
<td>79.50</td>
</tr>
<tr>
<td>P. multocida</td>
<td>37 Nasal swab</td>
<td>29</td>
<td>66</td>
<td>20.50</td>
</tr>
<tr>
<td>Total</td>
<td>988 Nasal swab</td>
<td>322</td>
<td>32.6</td>
<td></td>
</tr>
</tbody>
</table>

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

Mannheimia haemolytica had a higher infection rate (79.50%) compared to that of P. multocida (20.50%). The difference in the prevalence of pasteurella species in sheep and goats was statistically significant ($\chi^2 = 11.370$, $p < 0.05$) (Table 3).

An assessment was made on pasteurella infection in relation to sex of small ruminants and estimated for a prevalence of 25.3% in males and 36.4% in females. There was statistically significant difference ($\chi^2 = 13.728$, $p < 0.05$) between male and female animals in susceptibility to pasteurellosis infection (Table 2).

To see the effect of management on the prevalence of pasteurellosis infection, 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 2).

The prevalence of pasteurellosis was observed among the different 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%), (Table 2).
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 [23] 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. [24], Aschalew [25] and Tesfaye [26] who reported 50.2%, 63.8% and 67.6%, respectively. On the other hand, the current result was slightly higher than that of Tilaye [27] 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 [24].

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. [24], Aschalew [25], Daniel et al. [28], Eshetu [29], Maru et al. [30], Mohammed [31], Tesfaye [26] and Asefa et al. [32]. Mannheimia haemolytica, which is a normal flora of the upper respiratory tract, may play a secondary role after the primary initiating agent suppressed the host defense mechanism and favors the multiplication of pasteurella species leading to bronchopneumonia in purely pneumonic animals [1]. 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 under estimated [30].

Concerning to the rate of pasteurellaspecies isolation 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 rate in sheep than that of goats (p<0.05). These results; however, are not in line with that obtained by Rasha et al. [32] 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 [33].

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. [30]. This might be due to the immune status of the animals being able to predispose to the bacterial infection and other pre-disposing etiological agents [24]. Similarly, this result is also in agreement with the findings of Gilmour and Gilmour [34], 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 ovine and caprine irrespective of pasteurella species was observed. A prevalence of 25.29% and 36.42% was found in male and female small ruminants, respectively. This result; however, contradicts to the findings of Maru et al. [30] 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 [35]. 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, receive more attention by small ruminant producers. Crop leftovers and remnants after human consumption, for instance, are provided primarily for males [36].

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 [15].

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. [32]. This is due to the high rain fall of the area in winter and spring which exposes the animals to cold stress. In addition, during spring and winter there is prevailing of most parasites. These parasites predominantly affect the gastrointestinal tract and respiratory system of sheep and goats causing immunocompromise. This in turn favors the multiplication and expansion of the normal inhabitant bacteria, *pasteurella*, leading to infection of the host (sheep and goats).

**CONCLUSIONS**

At the most end, pasteurellosis was the major disease of sheep and goat in the study area in which *M. haemolytica* is the most common cause of infection. Females, young animals and extensively managed animals were the risk factors of the disease. It also demonstrated that pneumonic pasteurellosis is a highly complex multifactorial disease particularly in sheep and goats which could be associated with stress, immune compromise, adverse environmental condition, previous illness or co-infection and misuse of traditional medicines. All these and other factors made the control and prevention of pasteurellosis more difficult. Measures such as, regular vaccination, rearing of sheep and goats separately and improving management practices by providing optimal sanitation and air quality in housing, minimizing transportation stress, providing good quality hay and water and supplement feeds should be taken into account to reduce the risk of the disease in animals.

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**REFERENCES**


33. Wilsmore, T., 2006. Diseases of small ruminants in Ethiopia, the veterinary epidemiology and economics research unit school of agriculture’s policy and development the university of read, UK., pp: 67-72.


