



Original Article

Isolation and Identification of *Pasteurella* Species from Lung Lesion of Caprine Slaughtered at Helimex, Bishoftu, Ethiopia

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Abstract

A cross-sectional study was conducted from October, to 2013 to April 2014 with the aim of isolating and identifying *pasteurella species* in lung lesion (pneumonic lung) of apparently healthy goat slaughtered at Hasin Nuru Export Abattoir. All goats were originated from Bale and Harar area. Samples were collected aseptically from the lung lesion of 384 investigated Goat and standard microbiological techniques were used for isolation and identification of *pasteurella species*. From the 384 specimen collected for bacteriological examination, 301 were positive for *pasteurella species*. A total 301 *pasteurella species* recovered in which 274 and 27 of them were *Manehemia hemolytica* and *Pasteurella multocida*, respectively. The isolation rates of *Manehemia hemolytica* was 74.4% and *Pasteurella multocida* was 7%. Therefore, the major *pasteurella species* isolated from lung lesion of caprine in this study was *Manehemia hemolytica* and, *Pasteutella multocida* was the minor isolates. In conclusion *pasteurella* organism especially *Manemia hemolytica* is one of the most cause of pneumonic pasteurellosis in caprine and rarely *Pasteurela multocida* so Chemoprophylaxis need to be given to small ruminants prior to transportation or other stresses.

Keywords: Caprine, Pneumonic lung, *Manehemia haemolytica*, *Pasteurella multocida*.

Introduction

Africa has a population of 205 million sheep and 174 million goats representing approximately 17% and 31% of the world total, respectively (FAO, 1993). Within Africa, the distribution of small ruminants varies widely, with a higher concentration found in dry areas than in humid areas. Sheep and goats produce only about 16% of the world's meat, despite their higher contribution to the total world livestock population (CTA, 2003).

Ethiopia has a large livestock population in Africa, which is estimated to be around 34-40 million TLU .out of which 17% and 12% of cattle and small ruminants, respectively, are found in Ethiopia (FAO, 1994).

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Small ruminants play an important role in nutrition and income of people around the world. They serve primarily as source of meat also provide milk, skin, and wool (Mbilu, 2007). Goats are playing a major role in the livelihood of many people in the world particular in tropic and subtropic zones. Pneumonia in goats is one of the most important infections that are frequently diagnosed in veterinary clinics and abattoirs of the country. The bacterial agents that probably causing the disease were either rarely investigated or out of the scope of studies. This is due to the growing interest in studying contagious caprine pleuropneumonia, which is more common in goats and causes heavy losses (OIE, 2008)

Small ruminant production in the country however is still constrained by various factors. The major constraints facing sheep and goats production include disease, inadequate nutrition, poor genetic potentials of the local stock, marketing, social factors, structural constraints and shortage of high level of trained man power (Awgichew *et al.*, 1995). Of these, multifactorial infectious diseases of small ruminants cause substantial loss through morbidity and mortality (Teklye *et al.*, 1995). Thomson 1998 stated that all diseases, those affecting the respiratory system are generally the most important in every species of domestic animals.

Owing to their remarkable adaptability to adverse environments, goats assume important position in Ethiopian livestock economy. In combination with sheep, they supply more than 30% of all domestic meat consumption, and generate income from exports of live animal, meat and skin (Alemayehu and Fletcher, 1991). Moreover, due to less opportunity for alternative land uses, livestock (mainly goats) production is the only economic activity supporting the livelihood of the communities in the arid areas of the country (Coppock, 1994). Hence increase in goat production is needed both to maintain food self-sufficiency and to increase export earnings.

Bacterial infection of the respiratory tract may be primary, occurring in healthy individuals or secondary to a large number of conditions which depress resistance. Secondary bacterial infection occurs especially when the bacterial growing in the nose and throat extends downwards, usually giving a mixed infection (Megra *et al.*, 2006). Pasteurellosis broadly refers to any of the disease conditions caused by species of the genus *Pasteurella* (Dziva and Mohan, 2000; Davies *et al.*, 2003). *Pneumonic pasteurellosis* is an acute infectious disease that causes widespread financial losses because of death, reduced live weight, delayed marketing, treatment costs and unthriftiness among survivors (Aiello *et al.*, 1998; Davies *et al.*, 2001; Ozbey *et al.*, 2004).

Mannheimia haemolytica, formerly known as *Pasteurella haemolytica*, (Tefera and Smola, 2002; Christensen *et al.*, 2003; Sisay and Zerihun, 2003) is the bacterium most frequently isolated from shipping fever, which affects sheep and goats of all ages worldwide (Falade, 2002; Ozbey *et al.*, 2004). *Mannheimia* species naturally inhabit the upper respiratory system (tonsils and nasopharynx) of healthy sheep and goats and other wild and domestic animals (Chen *et al.*, 2002).

Infections result when an animal is compromised by any of a variety of stress factors as inclement weather, shipping (transportation), malnutrition, bacterial invasion of host defense, viral infections, nasopharyngeal colonization and dehydration, (Radostits *et al.*, 1994; Baron, 1996; Aiello *et al.*, 1998). Various *M. haemolytica* virulence factors influence the outcome of bacterial-host interactions (Davies *et al.*, 1997).

Bacterial species included within the genera *pasteurella* and *mannheimia* have been classified on the basis of their phylogenetic characteristics (Tefera and Smola, 2002). Capsular serotyping provides the primary basis for the classification of strains and epidemiological typing of *M. haemolytica* (Peterson *et al.*, 2001). Furthermore the purified organism is subsequently classified according to phenotypic traits such as morphology, carbohydrates fermentation patterns and serological properties. However, culture conditions can influence the expression of these attributes thus diminishing the stability and reliability for phenotypic

methods for strain identification (Matsumoto and Strain, 1993). So, genotyping techniques have been used extensively to differentiate epidemiologically significant strains of *P. multocida* (Lainson *et al.*, 2002).

Based on background information and the need for identifying the causes of diseases of caprine the present study was conducted with the following objectives;

- To determine prevalence of *Pastereulla species* encountered in lesion of caprine lungs.
- To isolate and identify *Pastereulla species* involved in pneumonic lesion of caprine.

MATERIALS AND METHODS

Study site

The study was conducted in Bishoftu town, East Showa zone of Oromia regional state. The area is located at 9⁰N and 40⁰E with an altitude of 1880 meter above sea level in the central highland of Ethiopia at 47Km South East of Addis Ababa. It has annual average rain fall of 1152mm of which 84% fall down during the long rainy season that extends from June to September. The mean annual maximum and minimum temperatures are 30.7^c and 8.5^c respectively and the mean relative humidity is 61.3% (NMSA, 2006).

Study Population

Study was conducted on caprine slaughtered at Hashim Nuru Export Abattoir with discrimination of their origin, body condition and age.

Study Design

Cross-sectional study was under taken to establish the prevalence of *pastereulla species* from lung lesions involved in pneumonia of caprine slaughtered at Hashim Nuru Export Abattoir.

Sample Size Determination

The sample size required for this study was determined on the formula given by Thrusfield (2005). There is no previous study on *pasteurella species* in caprine lung lesion in the study area recorded. $N = \frac{1.96^2 \times P \exp (1-P_{\exp})}{d^2}$. Where N = required sample size, Pexp = expected prevalence, d = desired absolute precision (usually 0.05). Therefore, to calculate the total sample size, the following parameters was used: 95% level of confidence (CL), 5% desired level of precision and with the assumption of 50% expected prevalence of *Pasteurella species* in caprine lung lesion by using the above formula the required sample size becomes 384 caprine lung lesions.

Data Collection and Sampling Procedure

Clinical data

Before slaughter, general physical examination will have conducted on each animal. Data regarding current clinical manifestation of disease will recorded with special attention to the respiratory system. All this information was proceed by origin, age and other related information.

Pathological Data

Post mortem examination was made by visual examination and through palpation of air ways, lung and the corresponding bronchial lymph nodes for the presence of any lesion. The gross appearance, location and size of the lesions will be recorded. An incision was made in to the lesions for further observation. Details of the lesions were recorded.

Sample Collection and Transportation

Immediately after slaughter, lung tissue sample with a size of greater than 10x10cm, sometimes the whole lung showing pneumonic lesions were collected using sterile forceps and scalpel blade. The lung specimen was then placed separately in sterile plastic bags kept in ice box and transported to National Veterinary Institute (NVI) for bacteriological examination. Sampling was done according to Carter (1984), Quinn *et al.* (1999) and Fekadu (2005).

Sample processing

In the laboratory pneumonic lung sample were processed for isolation of bacteria. To avoid surface contaminants, the surface of the lung tissues will seared with a hot spatula (Carter, 1984). Exudates will collected from the interior portion using sterile pasture pipette through the seared surface. In case when there was no exudates small pieces of lung tissue sample will collected from the sterilized surface area with the help of sterile forceps and scalpel blade and inoculated in to sterile screw capped test tube with 5ml of brain heart infusion (BHI) broth or blood agar base(BBL[®]). The inoculated broth tube was incubated lose capped aerobically at 37°C for 24 hours. After 24 hours of incubation, a loopful broth culture was plated on to the sheep blood agar (BBL[®], Becton Dickison, USA) by quadrant streaking method and incubated aerobically at 37°C for 24 hours (Sisay and Zerhun, 2003).

After 24 hours of incubation, the plates were observed for the growth of the bacterial colonies. The size and morphology the colony, pigment production, presence of haemolysis and the type of haemolysis was observed and noted. Then the isolated colonies will sub-cultured by half plating on blood agar and MacConkey agar (Oxoid, Basingstroke, England) and incubated at 37°C for 24 to 48 hours. Then a single colony will sub-cultured on BHI agar or Blood agar and incubated for 24 hours at 37°C. after obtaining pure colonies primary and secondary identification tests was conducted according to the standard technique recommended by Fekadu (2004);Quinn *et al.*, (1999) and Carter (1984).(Annex 2)

Data Analysis

The data was entered to Microsoft excel spread sheet and coded appropriately. For data analysis SPSS version 16 was used. Descriptive statistics was used to summarize the data collected. The prevalence of *Pasteurella spp* was calculated as the number of positive isolates divided by the total number of samples examined. Chi square was used to test the association among risk factors and the lesions.

RESULTS

During the study period, 384 pneumonic caprine lungs (198 Bale and 186 Harar) were examined for presence of *Pasteurela species* in lung lesion of apparently healthy goat slaughtered at hashim nur export abattoir. The overall prevalence of pastereulla species (*Manehemia haemolytica* and *Pastereulla multocida*) from caprine pneumonic lung collected and cultured was 78.4 % (301/384).

Out of 301, 274(71.4%) of isolates was *Manehemia haemolytica* and 27(7)% of isolates was *Pasteurella multocida*. on the basis of these results *Manehemia hemolytica* was the most common cause of pneumonic pasteurelosis in goat in both origin (Bale and Harar).

Higher pastereulosis prevalence 41.7 % (160/384) was observed in goat from bale than the prevalence of goat from harar 36.7% (141/384). The difference in prevalence of pastereullosis between the two origin was found to be statically not significant ($\chi^2=1.4$; $p>0.05$).

On the other hand, age specific study revealed higher pasteurellosis prevalence in young 83.7% (164/384) than in adult 72.9%(137/384). The difference among prevalence in age status was found to be statically significant ($\chi^2=6.4$; $p<0.05$).

Table 1: Prevalence of pasteurellosis in pneumonic lung of goat in Helimex, Bishoftu.

Factors		No of sample	Test +ve	Percentage (%)
Age	Young	196	164	83.7
	Adult	188	137	72.9
$X^2= 6.4$ $df= 1$ $\alpha= .011$				
Origin	Bale	198	160	80.8
	Harar	186	141	75.8
$X^2= 1.4$ $df= 1$ $\alpha= .234$				
Over all		384	301	78.4

DISCUSSIONS

In the present study, *Pasteurela species* were isolated in 78.4% of the caprine pneumonic lung (lung lesion). Of which *Manehemia hemolytica* was accounted for 71.4% and *Pasteurella multocida* was discovered only 7%. These bacteria have been reported by different workers in different anatomical sites and animals. However, their loads vary from site to site and/or animal to animal. Several workers (Okolo,1985; Almeida *et al.*, 1986; Mohammed,1999; Tesfaye,2004) reported this bacteria from caprine pneumonic lung; while others (Richard *et al.*,1986; Alsutan,1995; Barbour *et al.*, 1997; Mohamed, 1999) were isolated it from ovine pneumonic lungs. In addition, Shemsedin (2002) reported it from camels. The invariable isolation of these organisms from pneumonic lungs of various animal species might indicate their significance in different respiratory syndromes.

The more pathogenic species aerobic *Manehemia hemolytica* was isolated in higher proportion from pneumonic lung (71.4%) as opposed erlier report of Mohammed (1999) and Tesfaye (2004) lung infection rate. The higher isolation rate, in present study, might be attributed to the size of lung samples, which are large and incomparable.

According to Baker (1998), stress factor with or without viral infections interact to suppress the muco-ciliary clearance mechanism, which allow the proliferation of commensal bacteria in the respiratory tract. In spite of the low percentage frequency (7%) of isolation of *Pasteurella multocida* in this study attention is drawn to the pathogenic potential of this organism for goats. Different workers (Mohammed, 1999, Tesfaye, 2004) also isolated *Pasteurella multocida* from respiratory tract of sheep and goats. In agreement with previous reports (Mohammed, 1999, Tesfaye, 2004) in terms of infection intensity. *Manehemia haemolytica* assumes greater prominence in caprine pasteurellosis.

Pasteurella multocida is a potential pathogenic bacterial organism, which has been incriminated, in both human and animal infections where it causes of ten times severe respiratory abnormalities that can terminate in death (Dritz *et al.*, 1996). The successful isolation of *Pasteurella multocida* in this investigation is interesting not only because of its traditional role as a disease causing aerobic bacteria but also because its toxigenicity (Hall *et al* 1996,). Its toxin has been reported to have deleterious effect on organ system and immune responsiveness (Hall *et al* 1987; Cheville and Rimler, 1989).

Although the percentage isolation was relatively low (7%), the possible role of *Pasteurella multocida* in the etiology and pathogenesis of caprine pneumonia should not be under estimated. Although, it may be found occasionally as normal inhabitat of the respiratory system, experimentally, evidence has shown that under certain condition associated with debilitation, nutrition and climatic factors, this organism may singly or in concert with other

organism, flare-up to cause severe infection with high morbidity and mortality (Ugochukwu, 2008)

Comparing the two pasteurilla species, *Manehemia hemolytica* constitute 71.4% of the total, indicated that *Manemia hemolytica* was the major causative agent involved in caprine pneumonic lung. This is consistent with previous reports of Aschalew (1998), Eshetu(1991), Mohammed(1999) and Tesfaye(1997) of ovine pneumonic pasteurellosis. *Manemia hemolytica*, which is a normal flora of upper respiratory tract, host defense mechanism, and favors the multiplication of pasteurilla species, Leading to bronchopneumonia in purely pneumonic animals (Aielo, 1998).

In current study a significant association between pneumonic pasteurellosis and age of goat was observed. In agreement with findings of Gilmour (1989) that elucidate pneumonic pasteurellosis occur in all ages of sheep and goat with, the most susceptible in lambs and kids during first life, and dam at lambing.

Conclusions and Recommendations

Goat constitutes the second major component of live stock in Ethiopia. However, efficient utilization of this potential resource is hampered by combination of health problem, poor management and feed shortage.

Pneumonic pasteurellosis is the major disease of goat in the area and entails substantial loss in the number of goat all the years' round. Despite annual vaccination program against the disease high mortality and morbidity do occur.

In the present study quite a good number of pasteurilla organism are isolated from caprine with pneumonic lung. This indicates pasteurilla organism especially *Manemia hemolytica* is one of the most cause of pneumonic pasteurellosis in caprine and rarely *Pasteurella multocida*. In one way or another, this piece of work can serve as a clue for other researcher who wants to study the subject matter.

Based on the result of present study the following recommendations are forwarded:-

- Vaccine development from number of serotype of *M. hemolytica* that have been isolated from cases of ovine pasteurellosis should be encouraged.
- Identification of serotype of *M. hemolytica* from various part of Ethiopia should be continued.
- There has to be epidemiological investigation in order to identify the most common predisposing risk factor and management factors which lead to pneumonic pasteurellosis in small ruminant should be reduced.
- Chemoprophylaxis need to be given to small ruminants prior to transportation or other stresses.
- Immunization of goat against respiratory disease complex should be based on the identification of the under lying cause. For instance, *M. hemolytica* is more prevalent than *P. multocida* in the present study. However, inactivated monovalent *P. multocida* type A vaccine is being used for control and prevention of caprine pasteurellosis in the area.

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