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A Study on Ovine Pneumonic Pasteurellosis: Isolation and Identification of *Pasteurellae* and Their Antibiotic Susceptibility Test in Bishoftu District, Elfora Abattoir, Ethiopia

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Research Article

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ABSTRACT

Sheep constitute the second major component of livestock in Ethiopia and they play a significant role in the nation's economy. However, efficient utilization of this potential resource is hampered by combination of health problems, poor management and feed shortage. The study was conducted at ELFORA Debrezeit Abattoir in Eastern Showa Zone of Oromia regional state of Ethiopia with the objectives of isolation and identification of major *pasteurella* species (*pasteurella*, *multocida*, *Bibersteinia trehalosi* and *Mannheimia haemolytica*) from pneumonic sheep lung with culture and biochemical tests and to determine their antibiotic susceptibility test. From a total of 365 sheep lung swab samples, 130 *Pasteurella* species were successfully isolated and give the overall prevalence of 35.7%. Accordingly, 23 (6.3%) of the isolates were *P. multocida*, 40 (11%) were *B. trehalosi* and 67 (18.4%) were *M. haemolytica*. From positive isolates *M. haemolytica* account the highest percentages (51.5%) followed by *B. trehalosi* (30.8%) and *P. multocida* (17.7%). Origin, breed and age were significantly associated with *Pasteurella* isolates ($P<0.05$) whereas body condition were not statistical associated with *pasteurella* isolates ($p>0.05$). Despite diverse in the site of origins, the isolates exhibited uniformity in sensitivity to a majority of the antibacterial agents. The most effective drug was sulfamethoxazole (89.5%) followed by tetracycline (85%). The isolates were

susceptible to limited antimicrobial agents. All isolates were resistant to Streptomycin (92%) and Penicillin (85.6%) and completely resistant to vancomycin (100%). In conclusion, *Pasteurella* organism especially *M. haemolytica* is the most common cause of ovine pneumonic pasteurellosis in the study area. Good management and Chemoprophylaxis prior to transport reduce the disease burdens. Moreover, antimicrobial susceptibility test should be done before treatment except for critical cases.

INTRODUCTION

Small ruminants play an important role in income generation of people, source of nutrition like meat, milk and provide skin and wool around the world. Ethiopia lies within the tropical latitude of Africa and has an extremely diverse topography, a wide range of climatic features and a multitude of agroecological zone which makes the country suitable for different agricultural production system. This in turn has contributed to the existence of a large diversity of farm animal genetic resource in the country. Ethiopia is believed to have the largest livestock population in Africa. An estimate indicates that the country is a home for about 30.7 million sheep and 30.2 million goats. Despite the large livestock population of Ethiopia the economic benefits remain marginal due to prevailing diseases, poor nutrition, poor animal production systems, reproductive inefficiency, management constraint and general lack of Veterinary core. Among the respiratory disease, Pneumonia reported as the most important infections and frequently diagnosed in veterinary clinics and abattoirs in small ruminant in Ethiopia [1].

The term pasteurellosis or mannheimiosis or shipping fever is usually used for disease often respiratory caused by bacteria in the family Pasteurellaceae and genus *Pasteurella* which recently classified in the genera *Pasteurella*, *Mannheimia* and *Bibersteinia*. *Mannheimia haemolytica*, *Bibersteinia trehalosi* and *Pasteurella multocida* are the three most commonly isolated bacterial agents from ovine pneumonias that result in high rates of morbidity and mortality in sheep. Ovine pasteurellosis is one of the diseases that reduce sheep production and productivity and cause substantial economic losses not only in Ethiopia but also throughout the world.

Pasteurellaceae is small, Gram-negative *coccobacilli* or rods which may show bipolar staining. It is non-motile and non-spore forming, fermentative with few exceptions; ferment sugars like glucose, sucrose and maltose and most of them produce acid from common sugar but not hydrogen sulfide gas. It is aerobic or facultatively anaerobic with fastidious growth requirements. *Pasteurellaceae* is oxidase and catalase positive, reduces nitrate to nitrite and urease negative. Its growth on artificial media is enhanced by the addition of serum or blood on which they appear after 24 hours of incubation as round, smooth, greyish colonies of moderate size (1 mm-2 mm in diameter). The pathogenesis of ovine pneumonic pasteurellosis remains a subject of considerable speculation and controversy due to the complex nature of the disease and the lack of consistency in experimental results. However, the primary development of Ovine pasteurellosis highly mediated by complex interactions between the immunological status of the animal, the role of predisposing factors in the initiation of infection and the naturally existing causative organism in the upper respiratory tract. Both *Mannheimia* and *Pasteurella* are common commensal organisms of the tonsils and nasopharynx of healthy sheep. During certain factors such as stress, climatic change and other infectious diseases (virus, Bacteria and Lungworms) suppress the animal's immune system allowing opportunistic microorganisms (*Pasteurella* spp*)* to colonize the lung and cause ovine pasteurellosis.

The diagnosis of the disease is based on clinical signs, a variety of laboratory diagnostic techniques (including bacteriology, serology and molecular methods) and post mortem findings. Despite the application of advanced investigation and diagnostic techniques on both the organism and the affected animal species, pasteurellosis still continue to contribute to heavy losses in sheep production and remain a hazardous threat to human health worldwide. Since the disease is a complex multifactorial respiratory disorder in nature so good management practices which reduce stress factor, vaccination as well as early diagnosis and antibiotic treatment are the key methods of controlling disease [2].

Ethiopia recorded the highest number of outbreaks, cases and death in Africa according to AU-IBAR 2011 reports. Also some different studies conducted in Ethiopia indicated that pasteurellosis is a major threat to sheep production. Some of these studies were those in Amhara Regional State particularly and South Wollo Belay and Arsi Zone Mekonnen. Pasteurollosis is therefore a high-priority issue at the national level due to the significant economic losses it causes through morbidity, mortality and the high cost of treatment. However, even it is one of the most common respiratory problems in sheep and disease mostly diagnosed at clinic and abattoir in Ethiopia, very few studies have been conducted on identification and isolation of *Pasteurella* spp. in Ethiopia. Therefore, the objective of this study was to isolate and identify *Pasteurella* spp. from sheep slaughtered at ELFORA Debrezeit Abattoir with pneumonic lung lesions and to determine drug susceptibility pattern of these isolates [3].

MATERIALS AND METHODS

Study area

The study was conducted at Bishoftu district which is located in Eastern Showa Zone of Oromia regional state. The area is located at 9°N latitude and 40°E longitudes with an altitude of 1850 meter above sea level in the central highland of Ethiopia at 47 Km South East of Addis Ababa. The mean annual rainfall is 866 mm of which 84% is in the long rainy season (June to September). The dry season extends from October to February. The mean annual maximum and minimum temperatures are 26°C and 14°C respectively, with mean relatively humidity of 61.3%.

Study population

The study was conducted on apparently healthy sheep slaughtered at ELFORA Debrezeit abattoir with discrimination of their origin, breed, body condition and age. Currently the abattoir is one of the most facilitated modern export abattoirs in Ethiopia and is exporting meat of small ruminants to Saudi Arabia and UAE (Dubai). The animals slaughtered at the abattoir were purchased from different parts of the country particularly, Afar, Arsi and Borena and transported by vehicle. Therefore, animals were encountered different ecological areas and management conditions at their origin.

Study design

A cross-sectional study was undertaken from December 2017 to April 2018 to isolate and identify *Pasteurella* spp. from pneumonic lung lesions in sheep slaughtered at the abattoir.

Sample size determination

The sample size was calculated based on 2005 prevalence's reported by Mesele, in ELFORA Debrezeit Abattoir which was (38.9%) with 5% desired absolute precision at 95% confidence level using the formula recommended by Thrusfield. Thus, 365 lung swab samples were needed.

$$n = 1.96^2 \times P\exp(1 - P\exp)/d^2$$

Where,

n=required sample size, Pexp=expected prevalence, d=desired absolute precision, Using the above formula, the minimum sample size will be 365.

Sample collection and transportation

The abattoir was visited twice per week and on average 10 animals was included in the study per day using purposive sampling method. Up on ante-mortem examination, the age of animals was determined by dentition according to Getenby as young (<1½ years) and adult (>1½ years) and in to good, medium and poor body condition scores according to Thompson and Meyer. Also their origin and breed was recorded. Immediately after slaughter of apparently health animal, the pneumonic lungs of the slaughtered animal were visualized, palpated and incised and Lungs with consolidated inflamed area, deep red and sharply demarcated lesion with froth along the trachea, bronchi and cut surface of the lungs were considered as pneumonic lungs. After identifying this pneumonic lung with the above lesion on postmortem inspection, Lung swab was taken by incising using sterile scalpel blade and the inner surface of the incision was sampled with sterile swab. 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 National Veterinary Institute (NVI) laboratory.

Isolation and identification of *Pasteurella* species

The isolation and identification of *Pasteurella* were performed at the National veterinary institute laboratory using techniques recommended by Hardy Diagnostics, Santa Maria, CA, USA. The isolation and identification involves the following steps: first, the pre-enriched in tryptose Soya broth specimen was incubated for 24 hrs at 37°C. Secondly, after 24 hrs incubation, a loop full of the broth cultures were taken after homogenization and by using quadrant streaking method streaked over Tryptose Soya Agar (TSA) and immediately incubated aerobically at 37°C for 24 hours. Thirdly, from culture positive plates, typical colonies were subjected to gram's staining to study staining reactions and cellular morphology under light microscope at 100x magnification. Then mixed and gram-negative bacteria were further sub-cultured on both blood agar containing 7% sheep blood and MacConkey agar plates simultaneously by ignoring all gram positive and some gram negative bacteria which does not have characteristics of *pasteurella* species (such as *cocci* or *coccobacilli*, short rod). The growth of typical colonies on both blood and MacConkey agar was seen. On blood agar it was seen for the presence of haemolysis, the type of haemolysis, the general appearance of colonies (morphology, color, shape, size and consistency) and on MacConkey agar seen for the growth and ability to ferment lactose [4]. Fourthly, pure cultures of single colony type from both Blood and MacConkey agars were transferred onto nutrient agar-slants for a series of primary and secondary biochemical tests: Fifthly, primary biochemical tests such as catalase, oxidase, Motility and OF-test were done and the result is recorded. Lastly, the final identification of the bacteria to the species level was aided by using the secondary tests which include: indole, urease, nitrate reduction, MR-VP test were done and also gas production tests on TSI agar by metabolism of sugars such as glucose, sucrose and lactose following standard procedures [5].

Those biochemical tests which produce a narrow zone of beta haemolysis on Blood agar and grow on McConkey agar with lactose fermentation (pink colony), catalase positive, indole negative and fermentative with gas production forming a crack, positive to glucose, sucrose and lactose fermentation but negative to H_2S production on TSI media was interpreted as *M. haemolytica and* those biochemical tests which produce a narrow zone of beta haemolysis on Blood agar and grow on McConkey agar without lactose fermentation (no pink colony), catalase and

indole negative and fermentative with gas production forming a crack, positive to glucose, sucrose and lactose fermentation but negative to H_2S production on TSI media was interpreted as *B. trehalosi*. However, *P. multocida* was non-haemolytic on Blood agar and no growth McConkey agar, catalase and indole positive and fermentative with gas production forming a crack, positive to glucose and sucrose fermentation but no lactose and H_2S production on TSI media.

Antimicrobial susceptibility test

For this study, bacterial cultures that were already identified to their species level were used to check species which are resistant or susceptible to commonly used antibiotics and to identify a drug which is efficacious. In the laboratory, isolated colonies were suspended in nutrient broth and diluted to 0.5 McFarland turbidity and were swabbed onto Mueller-Hinton agar which was dispensed on a Petridish. Then antimicrobial discs (Sulfamethoxazole, Tetracycline, Ampicillin, Streptomycin, Vancomycin and Penicillin were fixed individually at different sites on the surface of inoculated agar plate evenly. Finally it was allowed to incubate at 37°C overnight and its zone of complete inhibition was measured to nearest millimeter using a ruler by holding on the back of inverted Petri dish and its zone of inhibition is compared to Zone Diameter Interpretive Standards (NCCLS) (Table 1).

Table 1. International zone interpretive chart for antimicrobials (inhibition zone diameter in mm).

Antimicrobial agent	Disc potency (µg)	Resistant (mm) (≤)	Intermediate (mm)	Suscepible (mm) (≥)
Sulfamethoxazole	25	10	Nov 15	16
Tetracycline	30	14	Nov 15-Nov 18	19
Ampicillin	10	13	Nov 14-Nov 16	17
Streptomycin	10	11	Dec-14	15
Vancomycin	30	14	Dec 15-Dec 16	17
Penicillin	10u	28	-	29

Data analysis

The data collected from abattoir was entered to Microsoft excel spread sheet 2013 program and the data was analyzed using SPSS version 20. Association of host risk factors with lung swab culture positives was calculated by descriptive statistics. A statistically significant association between variables was considered to exist if the computed p-value was less than 0.05. Origin, breed, body condition and age were considered as risk factors to see their association with the prevalence of pasteurellosis.

RESULTS

Colony characteristics and biochemical test result of the isolate

After 24 hr incubation at 37°C, cultures with colony characteristics of round (smooth) edge, greyish colour, small to moderate size (1 mm-2 mm in diameter) and mucoid consistency were observed on blood agar medium. Further sub-culturing and Gram staining of the isolates yielded gram negative *cocci* (*coccobacilli*) or short rods. Further, identification and discrimination tests were conducted with a series of the following confirmatory biochemical tests (Table 2).

Table 2. Results of biochemical test.

Biochemical tests	*P. multocida*	*B. trehalosi*	*M. haemolytica*
Blood agar (hemolysis)	-	+ (variable)	+
MacConkey (lactose fermentation)	- (No growth)	+ (without lactose fermentation)	+ (with lactose fermentation)
Gram stain	-	-	-
Colony colour	Gray	Gray	Gray
Colony consistency	Mucoid colony with round (smooth) adges	Mucoid colony with round (smooth) adges	Mucoid colony with round (smooth) adges
Shape/morphology	Pleomorphic *coccobacilli* or short rods	Pleomorphic *Coccobacilli* or short rod	Pleomorphic *Coccobacilli* or short rods
Colony size	Small to medium size (1 mm-2 mm in diameter)	Small to medium size (1 mm-2 mm in diameter)	Small to medium size (1 mm-2 mm in diameter)
Catalase test	+	-	+
Oxidase test	+	+	+
Motility test	-	-	-
O-F test	Facultative anaerobic (aerobic)	Facultative anaerobic (aerobic)	Facultative anaerobic (aerobic)
Indole test	+	-	-
Urease test	-	-	-
Nitrate reduction test	+	+	+
MR-test	-	-	-
VP-test	-	-	-
TSI test (sugar fermentation)	Glucose and sucrose positive	Glucose and sucrose positive	Glucose, sucrose and lactose positive

Note: +(positive result); -(negative result)

Bacterial isolation

Based on the above biochemical tests, 130 *Pasteurella* species were successfully isolated from 365 sheep lung swab samples which give an isolation rate of 35.7% of which 23 (6.3%) was *P. multocida*, 40 (11%) was *B. trehalosi* and 67 (18.4%) was *M. haemolytica*. Statistical association of different risk factor with total isolated positive and with each species of bacteria was described below.

Association of culture positive results with origin of animals

The association of culture positive with the origin of animals was studied. Accordingly, *P. multocida* 12 (7.8%) in afar, 3 (3.8%) in Arsi and 8 (8.4%) in Borena was isolated but the result of isolate with origin of animals has no statistically significant difference (P=0.498). The result of isolate for *B. trehalosi* indicate, 15 (9.7%) in Afar, 3 (3.8%) in Arsi and 22 (16.5%) in Borena and the result of study showed that there is statistical significant (P=

0.014). The isolate of *P. haemolytica* was 43 (27.9%) in Afar, 0 (0%) in Arsi and 24 (18%) in Borena with statistical difference (P=0.000). In present study, animals from afar origin was highly affected with a total positive isolates of 70 (45.4%) followed by Borena origin 54 (42.9%) and no isolation was observed in animals with Arsi origin. From this isolates *M. haemolytica* was the major isolated bacteria in relative to other isolate. The overall result of isolate within origin of animals indicate that there is positive association between origin of animals and bacteriological isolation (P=0.000) (Table 3).

Table 3. Association of culture positive results with origin of animals.

Origin	Afar	Arsi	Borena	Total	X^2	P-value	Overall x^2 (P-value)
P. multocida							
Negative	142 (92.2%)	75 (96.2%)	125 (90%)	342			
Positive	12 (7.8%)	3 (3.8%)	8 (8.4%)	23			
Total	154	78	133	365	1.39	0.498	
B. trehalosi							
Negative	139 (90.3%)	75 (96.2%)	111 (83.5%)	325			43.175 (0.000)
Positive	15 (9.7%)	3 (3.8%)	22 (16.5%)	40			
Total	154	78	133	365	8.52	0.014	
M.haemolytica							
Negative	111 (72.1%)	78 (100%)	109 (82%)	298			
Positive	43 (27.9%)	0 (0%)	24 (18%)	67			
Total	154	78	133	365	26.9	0	
Overall Positive within Origin	70 (45.4%)	6 (7.6%)	54 (42.9)	130			

Association of culture positive results with breed of animals

Breed of animals was also considered as risk factor for type and number bacterial isolates. The isolate of *P. multocida* was 0 (0%), 11 (9.2%) and 12 (9.7%) in Adal, Arsi and Black head Somali breeds respectively with statistical significant (p=0.002). None isolate was observed in Adal breed. The result of isolate for B. trehalosi was almost close to each other with 9.8%, 11.3% and 11.8% in Adal, Arsi and Black head Somali breeds respectively with no statistical significant (P=0.882). The result of isolate for *M.haemolytica* was 12 (21.3%), 14 (18.5%) and 14 (15.3%) in Adal, Arsi and Black head Somali breeds respectively and the result showed non-significant association (P=0.479). In current study Arsi and Black head Somali breed was highly affected with a total positive isolates of 47(39.5%) and 45 (36.3%) respectively while, Adal breed isolates were low. From these isolate *M. haemolytica* was the major isolated bacteria in relative to other isolate. The overall result of study indicate that there is significant association with breeds of animals and bacteriological isolation (P=0.034) (Table 4).

Table 4. Association of culture positive results with breed of animals.

Breed	Afar	Arsi	Black head somali	Total	X^2	P-value	Overall x^2 (P-value)
P. multocida							
Negative	122 (100%)	108 (90.8%)	112 (90.3%)	342			
Positive	0 (0%)	11 (9.2%)	12 (9.7%)	23			
Total	122	119	124	365	12.34	0.002	
B. trehalosi							
Negative	110 (90.2%)	105 (88.2%)	110 (88.7%)	325			
Positive	12 (9.8%)	14 (11.8%)	14 (11.3%)	40			13.65 (0.034)
Total	122	119	124	365	0.251	0.882	
M.haemolytica							
Negative	96 (78.7%)	97 (81.5%)	105 (84.7%)	298			
Positive	26 (21.3%)	22 (18.5%)	19 (15.3%)	67			
Total	122	119	124	365	1.474	0.479	
Overall Positive within Origin	38 (31.1%)	47 (39.5%)	45 (36.3%)	130			

Association of culture positive results with BCS of animals

In this study the isolate was compared within BCS of animals and the result of isolate shows 8 (10%), 4 (2.7%) and 11 (8%) for *P. multocida* in animals of poor, medium and good body condition score respectively but the result of study shows there is no statistical significant difference (P=0.055). The isolate of *B. trehalosi* was 11 (13.8%), 14 (9.5%), 15 (10.9%) in poor, medium and good BSC of animals respectively with no statistical significant variation (P=0.29). The result of isolate for *M.haemolytica* in animals with poor, medium and good BCS was 13 (16.2%), 30 (20.3%) and 24 (17.5%) respectively with no statistical significant variation (P=0.718). The major bacteriological isolate was higher in animals with medium and good body condition with 48 (32.5%) and 50 (36.4%) respectively but less isolate was observed in animals with poor body condition although the overall result of study showed no significant difference (P=0.291). *M. haemolytica* was the major isolated bacteria in relative to other isolate (Table 5).

Table 5. Association of culture positive results with Body Condition Score (BCS) of animals.

BCS	Poor	Medium	Good	Total	X^2	P-value	Overall x^2 (P-value)
P. multocida							
Negative	72 (90%)	144 (97.3%)	126 (92%)	342			
Positive	8 (10%)	4 (2.7%)	11(8%)	23			
Total	80	148	137	365	5.792	0.055	
B. trehalosi							
Negative	69 (86.2%)	134 (90.5%)	122 (89.1%)	325			7.33 (0.291)

Positive	11 (13.8%)	14 (9.5%)	15 (10.9%)	40			
Total	80	148	137	365	1.11	0.29	
M.haemolytica							
Negative	67 (83.8%)	118 (79.7%)	113 (82.5%)	298			
Positive	13 (16.2%)	30 (20.3%)	24 (17.5%)	67			
Total	80	148	137	365	0.663	0.718	
Overall Positive result within BCS	32 (40%)	48 (32.5%)	50 (36.4%)	130			

Association of culture positive results with age of animals

Isolate of *P. multocida* in young and adult animals was 17 (10.4%) and 6 (3%) respectively with statistical association (P=0.004). The result of isolate for *B. trehalosi* was 21 (12.9%) and 19 (9.4%) in young and adult animas respectively with no statistical significant difference (P=0.290). The result of isolate for *M. haemolytica* was 41 (25.2%) and 26 (12.9%) in young and adult aged animals with statistical association (P=0.003). It was a common bacteria isolated from young animals. In present study generally, young were highly affected with a total positive isolates of 79 (48.5%) than adult animals which was 51 (25.3%) with statistical association between isolate and age of animals (P=0.000) (Table 6).

Table 6. Association of culture positive results with age of animals.

Age	Young	Adult	Total	X^2	P-value	Overall x^2 (P-value)
P. multocida						
Negative	146 (89.6%)	196 (97%)	342			
Positive	17 (10.4%)	6 (3%)	23			
Total	163	202	365	8.5	0.004	
B. trehalosi						
Negative	142 (87.1%)	183 (90.6%)	325			23.93 (0.000)
Positive	21 (12.9%)	19 (9.4%)	40			
Total	163	202	365	1.118	0.29	
M.haemolytica						
Negative	122 (74.8%)	176 (87.1%)	298			
Positive	41 (25.2%)	26 (12.9%)	67			
Total	163	202	365	9.08	0.003	
Overall positive result within age	79 (48.5%)	51 (25.3%)	130			

Antimicrobial susceptibility test

In this study, the isolates were subjected to a panel of six antimicrobial discs. The antimicrobial susceptibility pattern of the isolates indicated that all isolates were sensitive to sulfamethoxazole (89.5%), tetracycline (85%) and Ampicillin (53%) with *B. trehalosi* and *P. multocida* shows a greater susceptibility and somewhat resistant respectively. Despite diverse in the site of origins, the isolates exhibited uniformity in sensitivity to a majority of the antibacterial agents. On the other hand all isolates were resistant to vancomycin (100%), Streptomycin (92%) and Penicillin (85.6%). All isolates were susceptible to sulfamethoxazole. Both *M. haemolytica* and *B. trehalosi* were susceptible to Tetracycline while *P. multocida* is somewhat resistant. From this result it can be concluded that sulfamethoxazole and tetracycline is a drug of choice while vancomycin, Streptomycin and Penicillin are drugs to which some isolates developed resistance while Ampicillin is intermediate drug.

DISCUSSION

The present study was conducted in ELFORA Debrezeit Abattoir. The animal's origin, breed, body condition and age were recorded on antemortem inspection. The sample was purposively taken from pneumonic sheep lung after detailed postmortem inspection. Pasteurellosis is a common respiratory disease of sheep caused by *Mannheimia* and *Pasteurella* species especially *M. haemolytica*, *P. trehalosi (B.trehalosi)* and *p. multocida* are the three most commonly isolated bacterial agents from ovine pneumonias that result in high rates of morbidity and mortality in sheep. Although, they found occasionally as normal inhabitant of the upper respiratory system, isolation of this organism from lower respiratory tract usually indicates a disease condition [6,7]. The bacteria can cause pneumonic pasteurellosis (pneumonic lesion development) and can also easily initiate infection in the body in man as well as in animals because of its toxigenicity that have deleterious effects on organs systems and immuno-responsiveness. In such cases there is chance of mixed infection occurrence (with other bacteria and viruses) which in turn makes *Pasteurella* organism a highly invasive, pathogenic and virulent. These bacteria posed health problem in most part of sheep breeding and rearing regions of Ethiopia and cause economic losses. In this study, an attempt was made to differentiate between *P. multocida*, *B. trehalosi* and *M. haemolytica* based on their colony appearance (color, consistency, shape and size), haemolytic pattern, growth on MacConkey agar and other biochemical tests and their antimicrobial susceptibility test for effective treatment [7,8].

From a total of 365 sheep lung swab samples, 130 Pasteurella species were successfully isolated and give the overall prevalence of 35.7% of which 23 (6.3%) was *p. multocida*, 40 (11%) was *B. trehalosi* and 67 (18.4%) was *M. haemolytica.* Of all isolates *M. haemolytica* was the predominant bacteria 51.5% followed by *B. trehalosi* 30.8% and *P. multocida* 17.7%. This indicates that, *M. haemolytica* was the major causative agent involved in ovine pneumonic pasteurellosis which reported an isolation rate of 63.8% and 67.6% respectively. This difference might be due to the type of sample taken and origin of animal that increase transportation stress plus stress overcrowded animals on the vehicle that can exacerbate the condition and even can lead to outbreaks if the origin is far from abattoir.

From positive isolates of *pasteurella* (130), *P. multocida* (6.3%), *B. trehalosi* (11%) and M. *haemolytica* (18.4%) were identified with significant difference among rates of isolation of the three species. Although the percentage isolation was relatively low 6.3%, the possible role of *P. multocida* in the etiology and pathogenesis of ovine pneumonia should not be under estimated. This is because *P. multocida* was rarely discovered from respiratory tract of sheep with very lower isolation rates from lung but it was discovered in nasal swab at the rate of 9.6%. The

isolation rate of *B. trehalosi* was in line with the finding reported 13.4%. In current study the overall isolation rate of *M. haemolytica* was found to be 18.4% this finding in line reported the isolation rate of 16.9%, 19% and 20% and 21.96% respectively, but higher isolation rate was reported by many researchers including with isolation rate of 25.2%, 40.8%, 52% respectively. These all difference might be due to the geographical variation of the origin of animals and time of sampling. Animals from different status of Veterinary service and vaccination program against pasteurollosis (*P. multocida* biotype A) and animals were transported long distance before being slaughtered [9-11].

The prevalence of ovine pneumonic pasteurellosis among geographical origins of the study animals was studied and the highest percentage 45.4% was found in sheep from Afar areas following by sheep from Borena areas 42.9% with significant difference (P=0.00). This may be due to transport stress (the origin was far from the abattoir, scarce of feed and water) and veterinary service of the origin. The isolation rate was compered among different breeds of animals and different prevalence rate was observed. The prevalence rate of Adal, Arsi and Black head Somali sheep was found to be 31.1%, 39.5% and 36.3% respectively but, the difference has no statistical significant (P=0.393) [11-14].

The prevalence rate of ovine pneumonic pasteurellosis of animals with medium and good body condition was 32.5% and 36.4% respectively but, there was statistically not significant differences (P=0.291). This might be due to differences in sample size. The prevalence is different in different age groups of animals with the highest in young animals with statistical difference (P=0.000). This result also elucidates pneumonic pasteurellosis occur in all ages of sheep, with the most susceptible in lambs during first life, and dams at lambing [15]. These is because the immune status of the animals being able to predispose to the bacterial infection and other pre-disposing etiological agents [16].

Antimicrobial susceptibility test of *Pasteurella* species was monitored to determine resistance development of the bacteria because antibiotic susceptibility studies should be renewed periodically. Increase in resistance against antibiotics in both *Mannheimia* and *Pasteurella* isolates. In this study, according to the antimicrobial susceptibility test results, sulfamethoxazole (89.5%) and tetracycline (85%) were the effective drugs whereas ampicillin (53%) was the only intermediate drug. Penicillin (14.4%) and streptomycin (8%) were inefficient drugs and vancomycin was totally inactive against all isolates [17].

One of the interesting findings of this study was the demonstration of the highest resistance of *Pasteurella* isolates against vancomycin (100%). However this study strengthens the statement vancomycin is active against most gram-positive bacteria but is not effective against gram-negative cells because of their large size and poor penetrability. In this study *P. multocida* showed resistance to penicillin, this result is in contrary to literature which indicates most strains of *P. multocida* are susceptible to penicillin. Susceptibility to sulfamethoxazole over 85% was very close to the rate reported. This might be due to difference in the strain of the isolate that may cause pasteurellosis in different species of animals or due to the existence of host factors that may affect the action of drug in sheep [18,19].

CONCLUSION

Sheep constitutes the second major component of livestock in Ethiopia. However, efficient utilization of this potential resource is hampered by combination of health problem, poor management and feed shortage. At the most end, Ovine pneumonic pasteurellosis was the major disease of sheep with economic importance in the study area in which *M. haemolytica* is the most common cause of infection followed by *B. trehalosi* whereas *P. multocida*

is rare. Young animals are mostly susceptible to the disease while adult also affected. It is highly complex multifactorial disease of sheep with endogenous or exogenous origin which could be associated with stress, immune compromization and adverse environmental condition. A combination of more definitive diagnostic methods, improved therapeutic agents and more rational management practices which reduce stress, good quality feed and Vaccine is suggestive for the control and prevention of the disease in the study area. In this line bacterial isolation and antibiotic susceptibility test should be conducted before treating with antibiotics except for critical ones. The three isolates were susceptible to limited antimicrobial agents such as sulfamethoxazole and tetracycline whereas vancomycin were totally inactive against the isolates.

RECOMMENDATIONS

Based on the results of this study the following points are recommended;

- Extension service should teach public that pasteurellosis is a disease which can be prevented by implementing good management strategy which can reduce /minimize stress.
- Extensive investigation should be taken for the determination of the magnitude and the distribution of the various species of *Mannheimia* and *Pasteurella* in different parts of the country to apply a cost effective and efficient prevention and control options is important.
- A continuous monitoring and evaluation of drug usage and drug resistance as well as vaccination programs which contain polyvalent *M. haemolytica serotypes* is needed to be implemented in the study area.

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