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Epidemiology of Ovine Pasteurollosis in Lume District, East Shewa Zone of Oromiya Region, Ethiopia

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Abstract
The study of ovine pasteurellosis was conducted in Lume districts, East Shoa Zone of Oromia region, Ethiopia to determine the prevalence of M*annheimia haemolytica*, *Pasteurella trehalosi* and *Pasteurella multocida* from nasal swabs (384), abattoir specimens (145*),* and the serotype diversity among the species from sheep sera (150). A total of (115) isolates of *M. haemolytica*, *P. trehalosi* and *P. multocida* were isolated from nasal swabs of apparently health and clinically sick sheep and from pneumonic lungs. The *M. haemolytica, P. trehalosi and Pasteurella multocida* was isolated from the nasal swabs (11.2%), (7.6%) and (2.1), whereas *M. haemolytica* isolates from pneumonic lungs (11.7 %)*, P. trehalosi (*10.3*)* whereas *Pasteurella multocida* was the lowest among species isolated (2.1 %). The overall isolation rate of *M. haemolytica* and *P. trehalosi* and *Pasteurella multocida* was 15.7%, 11.5% and 2.9 respectively. From 145 lung samples collected and cultured, Pasteurella was isolated successfully in 35 (24.1%) sheep.Out of 35, the percentage recovery rate of *M.haemolytica* 17(11.7%), *P.trehalose* 15(10.3%) and *P.multocida* 3(2.1) % from the lung lesion sample respectively. On the basis of these results *M. haemolytica* and *P.trehalose* was the most common cause of pasteurellosis in sheep at Lume district. A total of 150 sheep sera were examined for serotype specific antibodies using indirect haemagglutination test for *M. haemolytica, P.trehalose* and *P.multocida* serotypes. Variation in prevalence among the different serotypes was observed (P< 0.001). The IHA test revealed that serotype A1, A2,A7,T3,T10,and T15 were the dominant serotypes with 23.3%, 42.6%,32,51.3,29.3 and 30% positive by IHA whereas serotypes PAs (*P.multocida* biotype A) and T4 were the least positive with 14.6% and16% respectively. Generally Both bacterial and serological results of this study showed that the causal agents of pasteurellosis are prevalent in the area, and serotypes A1,A2, A7, T3,T10 and T15 were dominant over the other serotypes.
Keywords*:* *Mannheimia and pasteurella* spp, nasal swabs *Pasteurella*,isolation, serotypes,IndirectHaemagglutination(IHA),Lume(EastShewa).

INTRODUCTION

Ovine pasteurellosis is disease mainly caused by *Mannhaemia haemolytica(Pastuerella hemolytica)*, *Pastuerella multocida* and *Pastuerella trehalosi (Bibersteinia trehalosi) are* the three most commonly isolated bacterial agents from pneumonias that result in high rates of morbidity and mortality in sheep. *Pasteurella multocida* and *Mannheimia haemolytica* are causative agents of several economically significant veterinary diseases. Serious infectious diseases as fowl cholera, bovine hemorrhagic septicemia, and porcine atrophic rhinitis are caused by *P. multocida* whereas *M. haemolytica* is the causative agent of shipping fever or pneumonic pasteurellosis in sheep (Michael, 2008).

Pasteurella multocida was first found in 1878 in fowl cholera-infected birds. However, it was not isolated until 1880, by Louis Pasteur - the man whom *Pasteurella* is named in his honor. Now strains of *P. multocida* are grouped serologically into 5 capsular types (A, B, D, E and F) and 16 somatic lipopolysaccharide-types (1–16). *P. multocida* strains have also been characterized by outer membrane protein (OMP)-type and 16S rRNA-type. 16S rRNA-typing revealed that the majority of clinical isolates belong to a single lineage containing seven 16S-types. However, a range of capsular types, OMP-types and host species were represented, indicating significant heterogeneity between closely related strains (Richard, 2009).

Mannheimia haemolytica has been the subject of extensive reclassification in the past: first called *Bacterium bipolare multocidum* by Theodore Kitt in 1885, it was renamed *Pasteurella haemolytica* in 1932 and classified into two biotypes (A and T) based on its ability to ferment the sugars arabinose and trehalose, respectively. These biotypes were further subdivided into 13 A serotypes (A1, A2, A5, A6, A7, A8, A9, A11, A12, A13, A14, A16 and A17) and 4T capsular serotypes (serotypes 3, 4,10 and 15), based on results from an indirect haemagglutination test (Biberstein and Gills, 2002). After years *pasteurella haemolytica* biotype A was allocated to a new genus and renamed *Mannheimia haemolytica* while the 4T serotypes named *Bibersteinia trehalosi.* Recently because of serotypes A11 differently classified as *M. glucosida, M. haemolytica* is considered having twelve serotypes (A1, A2, A5-A9, A12-14, A16 and A17) based on capsular antigen typing (Sarah, 2011). The genus *Mannheimia* now contains several species including *M. haemolytica, M. granulomatis, M. glucosida, M. ruminalis* and *M. varigena* (Mohamed and Abdelsalam, 2008).

In Ethiopia, a number of attempts have been made so far to identify the different serotypes of both *Mannheimia* and *Pasteurella* spp. in small ruminants. Recent work on pasteurellosis of small ruminants has

demonstrated different species and serotypes of, *M. haemolytica* and *P. trehalosi* such as A1, A2, A5, A6, A7, A8, A9, A11, A12, A13, A14 and T3, T4, T10 and T15. Of which A1, A2, A8, A7, T3 and T4 are the dominant serotypes (Gelagay, 1996; Tesfaye, 1997; Aschalew, 1998; Mekonnen, 2000; Mesele, 2005).

Small ruminant production in the study districts is playing an important role in generating cash income, manure, social value, meat, skin. The predominant practices are mixed management system, traditional housing and grazing of natural pasture. Several investigations have been conducted in different countries and regions on the species and its serotypes that cause Pasteurellosis in sheep. The 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 Debre Birhan (Gelagay *et al.,* 2004) and South Wollo (Belay, 2007) and Oromia Regional State particularly selected sites of Arsi Zone (Mekonnen, 2000) as well as those in Debre Birhan, Harshin and Jijiga (Deressa *et al*.) but there is no available study in East Shoa Zone of Oromia region. In Lume district, despite annual vaccination against pneumonic pasteurellosis with a monovalent vaccine (inactivated *P. multocida* biotype A), there are high mortality and morbidity following respiratory distress (OBOARDAR, 2012/2013). Pasteurollosis is therefore a high-priority issue at the national level due to the significant economic losses it causes through mortality, morbidity, and the high cost of treatment. The main problem is absence of an extensive study on epidemiology of this disease as well as absence of a cost effective prevention and control methods which suits best for different phenotypes and serotypes of the agent.

Therefore, the objectives of this study were to isolate and characterize most prevalent *Pasteurella* and *Mannheimia* species in sheep and to determine the possible major serotypes of *Pasteurella* involved in respiratory symptoms/infections of sheep in Lume districts of East Shoa zone of the Oromia region.

MATERIALS AND METHODS

The study area

The study was conducted in Oromia Region; East shewa Zone at Lume district. The area is located at 74km from Addis Ababa and 27 km from Bishoftu, at Longitude between 38º56'E-39 º17'E and Latitude 8º34'N - 8º34'N. The average Elevation of the area is 1780m above sea level. The area have average annual rain fall 969.35mm, and mean annual temperature of 20.4 ºC. Management system of the animals is extensive production system. The main farming system is mixed farming and sheep are the predominant animal species kept in the area. Traditional housing and grazing of natural pasture are the predominant husbandry practices (OBoARDAR, 2012/2013). The district was selected due to having high population of small ruminants, high reports of the pasteurollosis disease challenge and it also the schematic area of the sponsorship.

Study animals

For the determination of contributing factors to epidemiology of ovine Pasteurellosis, indigenous sheep breeds belonging all age, sex and health status kept under extensive management system were sampled.The study animals were clinically healthy and sick sheep from five veterinary clinics for nasal swab and serum sampling and apparently healthy and slaughtered sheep from hotels were visited during sample collection. Accordingly 384 nasal swabs, 145 lung lesions and 150 serum samples were collected from the study area.

Study design

The type of the study was cross-sectional, with simple random sampling technique which was conducted from october 20014- March 20015 to establish the prevalence of *Pasteurella trehalosi*, *Pasteurella multocida* and *Mannheimia haemolytica* isolated from nasal swab of apparently healthy and sick sheep ,lung lesion from privately owned hotels in Lume district using isolation rates on culture and biochemical tests to identify their distribution among different age groups, sex, and health status and to determine the serotype prevalence of *Mannheimia haemolytica P.trehalosi and P.multocida* from serum samples of sheep collected in the study district. The study PAs were selected by purposive sampling technique based on sheep population within PAs. The number of sheep to be sampled from each PAs of the districts was determined simply by equally dividing the share of the PAs and finally individual sheep sample was selected purposely from each PAs and then bacterial samples were collected from each sample individual at time of visit. Since the prevalence of ovine pasteurellosis in the selected district were not known, the sample size were determined using the formula given by Thrusfield (2005) based on maximum expected prevalence of 50%. For this study 95% level of significance was considered.

Sample collection and Laboratory analysis

Nasal Swab collection

Nasal swabs were collected from randomly selected sheep. Before collecting the swabs, the nostrils of the animals were well cleaned with cotton wool soaked in 70 % ethyl alcohol. Then sterile cotton-tipped swabs in screw-capped test tube moistened with tryptose soya broth (Oxoid, Hampshire, England) were inserted into the

nostrils of each sheep, and the mucosa surface rubbed by rotating the swabs. The swabs were then placed back into 3 ml of sterile tryptose soya broth (Oxoid, Hampshire, England) in universal tubes and transported packed in ice to the laboratory after collection.

Tissue sample collection

Lung lesion was collected from randomly selected slaughtered sheep at Lume privately owned hotels during the study period. Piece of affected part of lung of the corresponding animals were taken after close inspection and put into separate sterile containers and transported to the laboratory in cool box (Sisay and Zerihun, 2003).

Blood sample

Blood samples for serum extraction were collected directly from jugular vein using sterile needles and plain vacuntainer tubes from randomly selected sheep flock. Up to 5-8 ml of blood was withdrawn and the tubes left to stand in inclined position over night at an ambient temperature to allow clotting, and the sera were collected using sterile Pasture pipette and transferred to sterile testes tubes, labeled and stored at -20 ^{o}C in deep freezer until they were.

Bacteriological Examination

In the laboratory nasal swabs were incubated immediately at 37 ^{o}C for 24 hours. Whereas lung samples were disinfected with 70 % alcohol and dried, and the samples were cut into pieces with sterile scissors assisted by tongue forceps and put into 3 ml of tryptose Soya broth (Oxoid, Hampshire, England) in universal tubes. The universal tubes were loose capped and incubated at 37 ^{0}C for 24 hours. After 24 hours incubation of the nasal swabs, lung tissues samples in tryptose Soya broth (Oxoid, Hampshire, England), a loop full of culture was transferred onto blood agar (Titan Biotech Limited, Bhiwadi) containing 7% difibrinated sheep blood and MacConkeys agar (Titan Biotech Limited, Bhiwadi), and then streaked by inoculating loop. The plates were incubated at 37 ^{0}C for 24 hours, and after 24 hours incubation, blood agar plates examined for the presence and type or absence of haemolysis and general appearance of the colonies including colour, shape, size and contour. The colonies suggestive of *Pasteurella* and *Mannheimia* were selected and smears were stained with Gram's staining and microscopically examined under oil immersion. Gram-negative, coccobacilli, short rods with or without bipolar staining were subjected for oxidase, urease, catalase, indole and H_2S production tests. Colonies, which were oxidase positive, urease negative, indole negative/positive, catalase positive/negative and yellow (acid) slant, yellow (acid) butt. , H_2S negative in TSI agar were subjected for further biochemical tests. The growth on MacConkeys agar (Titan Biotech Limited, Bhiwadi) was examined for the presence or absence of growth and lactose fermentation as indicated by pink coloured colonies and non-lactose fermentation by absence of pink colour and general appearance of colonies. Accordingly colonies were grouped as lactose fermenters (LF) and non-lactose fermenters (NLF). Plates with *Pasteurella* and *Mannheimia* like colonies were kept for further biochemical testing whereas mixed colonies were further sub-cultured.

Biochemical characteristics

After primary characterization and oxidase, urease, indole, catalase and H_2S test of the isolates, biochemical characterization of the organisms was preformed based on Glucose, lactose, maltose, salicin, sucrose, trehalose and xylose and nitrate reduction tests. *Mannheimia haemolytica* isolates were selected on the basis of xylose and lactose fermentation and lack of fermentation of trehalose and salicin and were catalase positive. Whereas, isolates of *Pasteurella trehalosi* utilised only trehalose and salicin and were catalase negative. *Pasteurella multocida* were selected on the basis of indole production, nitrate reduction, characteristics sweetish odour and absence of growth and haemolysis on MacConkey and blood agar, respectively.

Serotyping

Sera were serotyped for *M. haemolytica* using the indirect haemagglutination (IHA) test introduces by Biberstein (1978) for serotyping *Mannheimia haemolytica.* Serotping was conducted using capsular extract antigen. Briefly, Capsular antigen was extracted from a 24 hr culture of bacteria of known serotypes in tryptose Soya broth, which was inactivated in a water bath at 60 0C for 30 minutes, and centrifuged at 3000 rpm for 30 minutes; the clear supernatant was collected into sterile test tubes to be used as capsular extract antigen. Fresh sheep blood was collected in Alsever‟s solution at proportion of 3:5. The suspension was centrifuged at 2500 rpm for 5 minutes, washed twice with phosphate buffer saline solution (PBSS), and again centrifuged at 2500 rpm for 5 minutes. For sensitisation of the sheep red blood cells (RBC), 50µl of packed RBC were added to 5 ml of capsular extract antigen, and then 50 µl of glutaraldehyde was added and homogenized with gentle shaking, incubated for 1 hr at 37 OC. After incubation the suspension was centrifuged and washed twice with PBSS. Finally, the pellet was adjusted with PBSS to give a1% suspension of RBC. In v bottomed micro-plates 50 µl of PBSS were added to all wells and 50µl of test sera to the first column and serially diluted by pipetting 50µl up to column 12. Fifty

microliters of sensitised RBC were added to each well and incubated for one hour at 37 OC. Results were recorded based on complete or more than 50% agglutination seen in each well. The titre showing 1/20 dilution and above were taken as positive.

Data analysis

For interpretation of the results, after entry of the collected data into the Microsoft Excel sheet, it was summarized by descriptive statistics and then displayed by tables and graphs to illustrate the relationships between the dependent variables (each *Pasteurella* species and their total) and independent variables (PAs, age, sex and health status). Chi-square ($X2$) tests for repeated measures were used to test relationship between dependent variable (*Pasteurella* species distribution) and different independent host and environmental factors. For these analyses SPSS statistics 20 and Epi Info were used. Prevalence of *Pasteurella* and *Mannheimia* isolates was analysed using percentages and serotype distribution compared using percentages and mean percentages.

RESULTS

Characteristics of the isolate

Pasteurella and *Mannheimia* isolates were revealed different morphological features on smear made from fresh isolate cultures. The isolates were Gram-negative, short ovoid rods with an occasional tendency to bipolar staining. Cells arranged in chain were also observed. The *Mannheimia haemolytica* and *pasteurella trehalosi* revealed moist, smooth greyish, odourless and haemolytic colonies on blood agar, while *P. multocida* revealed round, greyish, non-haemolytic occasionally mucoid colonies. All the isolates of *Mannheimia haemolytica* and *pasteurella trehalosi* were grown on MacConkey‟s agar and showed pink to red small pinpoint colonies. None of *P. multocida* isolates showed growth on MacConkey agar. A total of 80 isolates from nasal swabs and 35 isolates pneumonic lungs were identified using biochemical tests. All the isolates were positive for oxidase and negative for urease and H_2S production but the *P. multocida* isolates were positive for indole production. All the isolates were able to utilize glucose fermentative. Bacteriological and biochemical test results are shown in table 6.

Table 6. Summary of biochemical tests for M. *haemolytica*, *P trehalosi* and *P multocida.*

Type of test	Type of species			Total positive
	M.Haemolytica	*P.Trehalosi*	*P.Multocida*	
Haemolysis on blood agar*	60	44	-	104
Growth on MacConkey agar	60	44	-	104
Distinct odour	-	-	11	11
Oxidase	60	44	11	115
Catalase activity	60	-	11	71
Indole production	-	-	11	11
Urease activity	-	-	-	-
H2S production in TSI Slant	-	-	-	-
Nitrate reduction	60	44	11	115
Glucose	60	44	11	115
Sucrose	60	44	11	115
Maltose	60	44	11	115
Lactose	60	-	-	60
Salicin	-	44	-	44
Xylose	60	-	-	60
Trehalose	-	44	-	44

Bacteriological findings

From 529 samples (384 nasal swabs and 145 lung lesion) collected and cultured, Pasteurella was isolated successfully in 115 sheep. Out of 115, 80 (21.1%) were from nasal cavities and 35 (24.1%) from lungs. The prevalence of *M.haemolytica* 43(11.2%), *P.trehalose* 29(7.6%) and *P.multocida* 8(2.1%) from the nasal swab sample respectively. Whereas in lung lesion, of the samples which were culture positive, 17(11.7%) of the isolate was *M. haemolytica* and 15(10.3%) of the isolate was *P.trehalose* and 3(2.1%) of the isolate was *P.multocida* . On the basis of these results *M. haemolytica* and *P.trehalose* was the most common cause of pasteurellosis in sheep at Lume district regardless of the health status, age, sex and other attributes .

Table 7. Prevalence of M. *haemolytica*, *P. trehalosi* and *P. multocida* isolates of sheep

Type-of sample	No-of sample processed	Total positive	Species identified		
			M.haemolytica	*P.multocida*	*P.trehalosi*
Nasal swab	384	80(21%)	43(11.2%)	8(2.1%)	29(7.6%)
Lung lesion sample	145	35(24.1%)	17(11.7%)	3(2.1%)	15(10.3%)
Total	529	115(21.7%)	60(11.3%)	11(2.1%)	44(8.3%)

From the total isolates *Mannheimia haemolytica, P. trehalosi* and *P. multocida* 43(11.2%), 29(7.6%) and 8 (2.1%) positive in nasal swabs respectively. Out of these isolates, 21(9.3 %) and 60 (38.2%) were isolated from apparently healthy and clinically sick sheep with respiratory syndrome, respectively. There was high significant difference (P<0.001) between isolates from nasal swabs of healthy and clinically sick sheep with respiratory syndrome.

Table 8. The prevalence of *M. haemolytica*, *P. trehalosi* and *P. multocida* from apparently healthy and clinically sick sheep.

Status of sheep	No (%)	Isolated species (%)		
		M.haemolytica	*P.trehalosi*	*P.multocida*
Healthy sheep	227(21)	11(4.8)	8(3.5)	2(0.8)
Diseased sheep	157(59)	32(20.3)	21(13.3)	6(3.8)
Total	384(80)	43(11.2)	29(7.5)	8(2.08)

Regardless of species of the isolates and PAs the prevalence of the isolates in Qoka was 27.2%, Sheran dibandiba 14.2%, Jido 19.4%, Biyo 23.3%and in Mojo 21.1%.

These show there were variations in distribution of the agent among the five study PAs but the variation is not statistically significant because p=0.788.

Table 9. Distribution of total positivity nasal swab among different epidemiological risk factors

Risk factors	Level	No examined	No positive	Prevalence in %	X^2	P-value
PAs	Qoka	77	21	27.2	1.17175	0.788
	Sh/dibandiba	77	11	14.2		
	Jido	77	15	19.4		
	Mojo	76	16	21.1		
	Biyo	77	18	23.3		
Age	< 3mth	65	15	23.1	10.24	0.001
	4-6mth	57	16	22.8		
	7-9mth	63	14	20.3		
	10-12mth	54	11	17.3		
	1-3yrs	53	9	17.1		
	4-6yrs	47	8	17.1		
	7-9yrs	44	11	25		
Sex	Female	201	47	23.4	0.0296	0.863
	Male	183	34	18.6		
Health status	Healthy	227	21	10.2	8.858	0.0001
	Sick	157	59	38.2		

Age distribution of the total positivity shows that the isolation rate of the agent varies significantly among different age groups (P=0.001).The isolation rate of the agent increases from age categories 1-3mth (15) to 4-6mth (16), from7-9mth (14) to 10-12mth (11) ,1-3yrs (9)and from4-6year (8) to 7-9yrs (11). Whereas Sex and distribution of agent has no statistical significant variation even if there were variations observed. When the distribution of the agent by their species was seen among PAs,there were variations observed in distribution of a single species of the isolate among PAs. Even if variation is observed among PAs distribution of isolates was independent of PAs which means they have no statistically significant association between the isolates and PAs. Likewise age and sex distribution of isolates were examined for similarity in distribution of isolates among different age groups and sex of sheep. A Pearson chi-square test computed for existence of dependency between isolates and age of sheep shows that there was dependency between age of sheep and distribution of the disease agents which implies that at 95% confidence level distribution of isolates is dependent on age of sheep (P-

value<0.05). That is statistically there is significant association between distribution of isolates and age of sheep. Sex isolate cross tabulation shows that there was no significant variation in distribution of isolates both within female and male sheep and also between female and male sheep. That is distribution of isolates was independent of sex of sheep; sex of sheep does not limits distribution of isolates. This implies that sex (host factor) is not a determinant factor of the disease pasteurellosis in sheep.

Table 10. Distribution of *Pasteurella spps* among different epidemiological risk factors

Risk factor	**Level**	**No examined**	**No Positive**	***M.haemolytica* No= (%)**	***P.multocida* No= (%)**	***P.trehalos* No= (%)**	**X2**	**P-value**
PAs	Qoka	77	21	10(47.6)	3(14.3)	8(38.1)	8.197	0.224
	Sh/dibandib	77	11	5(45.5)	1(9.1)	5(45.5)		
	Jido	77	15	9(60)	1(6.7)	5(33.3)		
	Mojo	76	16	9(56.3)	1(6.3)	6(37.5)		
	Biyo	77	18	11(61.1)	2(11.1)	5(27.8)		
Age	< 3mth	65	15	7(46.7)	2(13.3)	6(40)	2.926	0.232
	4-6mth	57	13	5(38.5)	1(7)	7(53.9)		
	7-9mth	63	14	8(57.1)	1(7)	5(35.8)		
	10-12mth	54	11	7(63.7)	1(9)	3(27.3)		
	1-3yrs	53	9	4(44.4)	1(11.1)	4(44.4)		
	4-6yrs	47	8	5(62.5)	1(12.5)	2(25)		
	7-9yrs	44	11	7(63.7)	1(9)	4(36.4)		
Sex	Female	201	47	27(57.4)	3(6.4)	17(36.1)	2.462	0.292
	Male	183	34	16(47.1)	5(14.8)	8(23.6)		
Health status	Healthy	227	21	11(33.3)	2(9)	8(23.9)	4.398	0.111
	Sick	157	59	32(60)	6(10)	21(40)		

Isolates and health status of sheep shows that there were dependencies between isolates and health status of sheep, indicating that distribution of isolates were dependent of health status of sheep.

For the purpose of *Mannheimia* and *Pasteurella* isolation, lung lesion of sheep were examined from slaughtered sheep at voluntary hotels in which highest number of sheep slaughtered on daily basis were selected for three months, November, December and January during the study period. Sampling of the specimen was performed at a time.

From 145 lung samples collected and cultured, Pasteurella was isolated successfully in 35 (23.4%) sheep. Out of 35, the prevalence of *M.haemolytica* 17(11.7%), *P.trehalose* 15(10.3%) and *P.multocida* 3(2.1) % from the lung lesion sample respectively. On the basis of these results *M. haemolytica* and *P.trehalose* was the most common cause of pasteurellosis in sheep at Lume district. However, the observed prevalence of isolates from lung in sex and age group has no impact on the isolation of pasteurella species.

Table11. Distribution of total positivity lung lesion among different epidemiological risk factors

Risk factor	**Level**	**No sample**	**No positive**	**Prevalence**	X^2	**P-value**
Age	young	35	16	45.7	2.7560	0.097
	Adult	110	23	20.9		
Sex	Female	31	8	25.8	1.1348	0.567
	Male	114	31	27.1		

Sero typing

Sheep Sera collected from Lume districts East shoa Zone of Oromia region, namely Qoka, Sheran dibandiba , Jido, Biyo and Mojo were serotyped using the indirect haemoagglutination (IHA) test as per Biberstein (1978) for serotyping of *M. haemolytica,P.multocida and P.trehalosi*. A total of 150 serum samples, thirty sera from each PAs were examined for specific antibodies. The *M. haemolytica.P.multocida and P.trehalosi* serotype positives have been computed for each PAs.

Table 12. Prevalence distribution of *Pasteurella* serotypes in sheep sera in different PAs of Lume districts

	Seropositive (%) at dilution ≥1/20					
Mannhaemia/ Pasteurella serotypes	Jido No=30	Qoka No=30	Sh/dibandiba No=30	Mojo No=30	Biyo No=30	Total No (%)
A1	7(23.3)	3(10)	14(46.6)	9(30)	2(6.6)	35(23.3)
A2	17(56.6)	15(50)	11(36.6)	9(30)	13(43.3)	65(43.3)
A7	13(43.3)	7(23.3)	8(26.6)	11(36.6)	9(30)	48(32)
PA	5(16.6)	4(13.3)	4(13.3)	6(20)	3(10)	22(14.6)
T3	18(40)	17(56.6)	15(50)	16(53.3)	11(36.6)	77(51.3)
T4	12(40)	6(20)	9(30)	7(23.3)	8(26.6)	42(28)
T10	8(26.6)	11(36.6)	7(23.3)	9(30)	9(30)	44(29.3)
T15	13(43.3)	8(26.6)	9(30)	7(13.3)	8(26.6)	45(30)

Variation in prevalence among the different serotypes was observed,in mojo district, serotype A1 (30%) followed by A2 (30%) and A7 (36.6%),PA(20%),T3(53.3%),T4(23.3%),T10(30%) and T15(23.3%). in Qoqaa A1 (10%), A2(50) and A7 (23.3 %) PA(13.3%),T3(56.6%),T4(20%),T10(36.6%) and T15(26.6%)., each, in Sheran Dibandiba A1 (46.6%), A2 (36.6 %) , A7 (26.6%), PA(13.3%),T3(50%),T4(30%),T10(23.3%) and T15(30%). In Jidoo A1 (23.3%), A2 (56.6%) and A7 (43.3%), PA(16.6%),T3(40%),T4(40%),T10(26.6%) and T15(43.3%). and in Biyo, A1 (6.6%), A2 (40%) , A7 (30%) , PA(10%),T3(36.6%),T4(26.6%),T10(30%) and T15(26.6%). were the dominant serotypes detected. In present study results there was higher prevalence of *M. haemolytica* A1 (23.3%), A2 (42.6%), A7 (32%), T3 (51.3) ,T10(29.3) and T15(30%) serotypes were lower prevalence of *P.multocida* biotype A (14.6%) and T4 (16%) were recorded in the study areas.

Table 13. Overall Prevalence of Ovine Pasteurellosis in the Study Districts

Sero-types of ovine pasteurollosis	**Total**	**Prevalence %**
M.hemolytica A1	35	23.3%
M.hemolytica A2	64	42.6%
M.hemolytica A7	48	32%
P.multocida biotype A	22	14.6%
P.trehalosi T3	77	51.3%
P.trehalosi T4	24	16%
P.trehalosi T10	44	29.3%
P.trehalosiT15	45	30%

DISCUSSION

Pasteurellosis in sheep caused by *M. haemolytica* and *P. trehalosi* have posed health problem in most part of sheep breeding and rearing regions of Ethiopia due to the significant economic losses they causes through mortality, morbidity, and the high cost of treatment Gelagay, et al., (2004). In this study, an attempt was made to differentiate between *M. haemolytica*, *P. trehalosi* and *P. multocida* based on their growth on MacConkey agar, haemolytic pattern, Oxidase and Catalase activity, Indole and H2S production, colony morphology and fermentation of different sugars with acid production Quinn et al., *(*2002) .

The aetiological agent of pasteurellosis in sheep was found to be wide spreading in the study area. Though there were little variation in biochemical characteristics, the results of morphological, staining, colony, cultural and biochemical activities were in total agreement with those documented by Merchant and packer (1983), Carter and Chengappa (1991), Carter (1984) and Quinn et al., (2002). The haemolytic activity of *M haemolytica* and *P. trehalosi* isolates were lost after subsequent subcultures and were in agreement as reported by Carter and Chengappa (1991), Quinn et al., (2002). Two distinct colony types were observed on MacConkey agar, lactose fermenter (*M. haemolytica*) with pink and non-lactose fermenter *(P. trehalosi*) other than pink colour. Whereas the *P. multocida* isolates neither grew on MacConkey agar nor haemolysed sheep red blood cells as observed by Carter and Chengappa (1991) and Quinn et al., (2002). The isolates of *M. haemolytica, P. trehalosi* and *P. multocida* were positive for oxidase test and negative for urease, HS2 production in TSI agar slant, and Indole test. Further they utilized glucose fermentatively, but the *P. multocida* isolates were indole positive which coincided with those described by Quinn et al., (2002*)*. The *Mannheimia haemolytica* isolates fermented xylose and lactose, but failed to ferment trehalose and salicin, and were catalase positive, whereas isolates of *P. trehalosi* were fermented. trehalose and salicin, and were catalase negative the results are in agreement with Carter (1984) and Quinn et al., (2002).

In the present study, *M. haemolytica and P. trehalosi* were isolated at the rate of 11.2% and 7.6% from nasal swabs, 11.7 % and 10.3% from pneumonic lungs, respectively. Isolation of *M. haemolytica* and *P.*

trehalosi from nasal swab and pneumonic lungs may indicate that these species are important species in the induction of pneumonic pasteurellosis in the study area.

The low percent of (2.1%) both from nasal swab and lung lesion P. *multocida* isolates might indicate the occasional involvement of this species in the pneumonic pasteurellosis and similar reports have been made by Merchant and Packer (1983) Carter and Chengappa (1991), Aschalew (1998), Mekonnen (2000), Sisay and Zerihun (2003) and Belay (2007). In contrary to our observation Mesele (2005) reported high incidence rates of 15.4% and 25% of P. *multocida* from sheep slaughtered at Jijiga and ELFORA abattoir respectively. This might be due to the time of sampling and geographical variation, where sampling was conducted in June and sampled sheep were from Somali lowlands where very poor Veterinary infrastructure and vaccination against pasteurollosis (*P. multocida* biotype A) was not conducted for considerable number of years, and animals were transported long distance before being slaughtered at both abattoir, whereas, our study was conducted , where regular vaccination was done in sheep with monovalent *P. multocida* vaccine which is attributed to the low incidence of infection. From total isolates of nasal swabs of clinically sick sheep with respiratory syndrome, *M. haemolytica* and *P. trehalosi* constituted 20.3 % and 13.3% in their proportion, respectively. This result is in agreement with the findings of Tesfaye (1997) and Aschalew (1998) in highlands of central Ethiopia, where they reported 28.8 % and 35 % for *M. haemolytica* and 42 % and 58.3 % for *P. trehalosi*, respectively from nasal swabs of sheep with pneumonic symptoms. The high proportion of *Mannheimia* and *Pasteurella* species in nasal swabs of clinically sick sheep could be due to these organisms are normal inhabitants of the upper respiratory tract of sheep and invasion to the lower tissue (lung) of clinically sick sheep when the immune system of the animals is compromised by different factors (Lopez, 1995) and pneumonia associated with these bacteria species recorded as primary diseases in sheep elsewhere (Gilmour, 1993).

Accordingly, there was a significant difference existing among isolation rates at different time in different areas even if there is no significant difference in isolation rates among the three species in all of the studies at different time and areas. This implies that there must be a continuous survey to be held in different areas to know a recent rate for each of the species implicated for ovine pasteurellosis and to design a cost effective and efficient prevention and control strategies suited for each area.

Age distribution of the three species associated with ovine pneumonic pasteurellosis disclose that there was an association in distribution of the species among different age groups of sheep and at the same time distribution of the species was also associated with age groups of sheep. According to the present study different age group distribution of the isolates looked like as follows: within age group 1-3mth it was 23.1%, 4-6mth (22.8%), 7-9mth (22.2%), 10-12mth (20.3%), 1-3year (17.1%), 4-6year (17.1%) and 7-9 year it was (25%). This result ease also in agreement with findings of Gilmour and Gilmour [26], 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 but not agreement with Zuber (2009) in Iraq with slight fluctuation in age grouping which was in 1-3mth age category it was 2.1%, 4-8mth (7.7%) and 9-12mth (7.1%).

Sex shows that there was no significant variation in distribution of the species between female and male sex and that the distribution was independent of sex of sheep. In the present study prevalence of the agent in female sheep was 23.4% while it was18.6% in male. This result was in agreement with Behailu (2012) where female 29.2% and male 24.6%, and also with Belay (2007) and Kuod (2013).

The prevalence of *M. haemolytica*, *P.trehalosi* and *P.multocida* was almost similar in nasal swabs(11.2%),(7.6%) and (2.1%) and in lung sample (11.7%),(10.3%) and (2.1%) respectively. In this study *M. haemolytica* and *P. trehalosi* in pneumonic lungs may indicate that these species are important species in the induction of pneumonic pasteurellosis in the study area. The low percent of (2.1%) P. *multocida* from lung lesion isolates might indicate the occasional involvement of this species in the pneumonic pasteurellosis. No associations were observed between the risk factors and pneumonic pasteurellosis at lung lesion sample ($P > 0.05$).This study result is in agreement with the reports of Kaoud (2010) prevalence of 52% for *Mannheimia* and 42% for *P. trehalosi.*

The serological results showed that the predominance distribution pattern of *M. haemolytica and P.trehalosi* serotypes between the PAs of Lume districts were almost similar. This might be due to similar agro climatic and animal management systems. The results of IHA test were tabulated to know the overall prevalence and distribution of *M, haemolytica* serotypes in East shoa zone of Lume districts. The prevalence of each serotype was varied. Of 150 sheep sera tested, A1 (60 %), A2 (42.6%), A7 (32%) and PA (14.6%) ,T3(51.3%),T4(16%),T10(29.3) andT15 (30%) were detected. A mong A1 (60 %), A2 (42.6%), A7 (32%) and PA (14.6%),T3(51.3%), T10(29.3) and T15 (30%) were the dominant serotypes and *P.multocida* biotype A were the least detected serotypes. The result of this study is in agreement with the reports of Yeshwas Ferede (2013) P.*haemolytica* A1 (33.1%), A2 (28.5%) and A7 (31.8 %) serotypes where as a lower prevalence of *P. multocida* biotype A (6.6%) were recorded in South Gonder Zone Farta and Lay Gayint districts, the result this study was also in agreement with that of Sisay and Zerihun (2003) who reported that 29 % and 16.9 % prevalence for A1, in East and Northeast Ethiopia, respectively, which was the most prevalent serotype. Hussein

and Mohammed (1984) in Sudan identified the predominant A2 and A6 serotypes and A13 and A14 were among the lowest identified serotypes. Prince *et al* (1985) and Gilmour and Gilmour (1989) in New Zealand identified A2 as the most prevalent serotype. Gelagay (1998) also identifies in central highland of Ethiopia A2 and A6 were the most dominant ones, while Lhan and Keles (2007) in Turkey identified A2 and A6 as most prevalent and A8 was the least identified respectively, which was the most prevalent serotype. This deference in dominance of serotype might be due to geographical location, the time of sampling, serotypes involved in pasteurellosis which varied from year to year, area to area and flock to flock (Gilmour and Gilmour, 1989).

Conclusion

In East shewa zone Oromia region in Lume districts, sheep are the most kept animal than other species and plays an important role in the economy and livelihood of farmers. These small ruminants serve as a principal source of cash income for household expense as well as domestic consumption. However, efficient utilization of this resource is impaired by different factors, such as health problem, poor management and shortage of feed. Knowing epidemiology of the disease and contributing factors to the disease has a paramount importance in designing a cost effective prevention and control method. For that matter knowing prevalence of the disease to strain level helps one to design a best intervention method using vaccine or antibiotics. Management practices directed at reducing stress are important in preventing *Pasteurella*/*Mannheimia* associated disease in sheep. Both bacterial and serological results of this study showed that the causative agents of pasteurellosis are prevalent in the area, and serotypes A1,A2, A7, T3,T10 and T15 were dominant over the other serotypes.The current pasteurellosis vaccine, which is used for the control and prevention of pneumonic pasteurellosis, does not include more prevalent agents in the field apart from the strains of *M. haemolytica* which are incriminated in causing pasteurelosis. On the basis of the results obtained the following are recommendations are proposed.

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