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Full Length Research Paper

Isolation of *Mannheimia* and *Pasteurella* species and the associated risk factors from pneumonic and seemingly healthy cattle as well as the antibiotic susceptibility profiles of the isolates

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The study was conducted from October 2012 to April 2013 at Bedelle district, Western Ethiopia. The aim was to isolate *Mannheimia* and *Pasteurella* species, and to assess the associated risk factors from pneumonic and apparently healthy cattle, and antibiotic susceptibility profiles of the isolates. Out of 329 samples from animals (185 nasal swabs from clinic and 144 lung tissues abattoir) examined, 50.2% was found to be positive to the disease. The bacteriological examination revealed 28 (8.51%) overall isolates of *Mannheimia haemolytica* (46.4%), *Pasteurella multocida* (39.3%) and *Bibersteina trehalosi* (14.3%) were recovered respectively in which 17 (9.19%) and 11 (7.63%) bacterial isolates were obtained from nasal swabs and pneumonic lungs, respectively. The higher isolation rate of *M. haemolytica* indicated it as the major cause in the study area. Age was found to be the potential risk factor in which young animals were highly affected. The antimicrobial susceptibility profiles of the isolates were carried out using disc diffusion method. The isolates were susceptible to most of the antibiotic disks used: amoxicillin, chloramphenicol, cephalexin, polymyxin-B, kanamycin and florifenicol. However, moderate resistance was observed to Tetracycline, Erythromycin and Penicillin-G. Thus, an integrated application of overall management and vaccination should be implemented as prevention and control measures.

Key words: Bedelle, cattle, *Mannheimia, Pasteurella*, pneumonic pasteurellosis, antimicrobial susceptibility test.

INTRODUCTION

Ethiopia has diverse animal genetic resource and its relatively large livestock population (approximately 100 million) is well adapted to and distributed among diverse ecological condition and management system (Aiello and May, 1998). In Ethiopia, like many developing countries, livestock play multiple roles. Despite the huge number of cattle and economic importance, the productivity is low due to the constraints of disease, nutrition, poor manage-

ment and poor performance of the indigenous breeds. These constraints result in poor reproductive performance of these animals (Lobago et al., 2006). 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 (Central Statistical Agency, 2009).

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 (Zewdie, 2004). The disease occurs in food animals due to complex factors that often interact to produce disease. Various conditions such as climate, weather, weaning, transpor-tation, poorly ventilated housing and nutritional deficiencies are known to play a predisposing role as the animal's immunity weakens. In such conditions, occurrence of the normal flora of the upper respiratory tract and subsequent infection of the lungs is well documented (Radostitis et al., 2000).

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

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 coccobacilli formerly grouped under the genus *Pasteurella* (after Louis Pasteur). For several decades, the genus *Pasteurella* was believed to be only one single genus with numerous species infection of farm animals, particularly in ruminants (Angen et al., 1999b).

However, with more recent advancements in molecular biology involving DNA hybridization studies and 16s rRNA sequencing, most of the formerly recognized species were found to share a number of common features and become the subject of intensive revision and reclassification. In this respect, *Pasteurella haemolytica*, biotype A was allocated to a new genus and renamed *Mannheimia*. This new genus contains several species including *Mannheimia heamolytica, Mannheimia granulomatis*, *Mannheimia glucosida, Mannheimia ruminals* and *Mannheimia varigena*. The name *Mannheimia* was given in tribute to the Germen Scientist Welter Mannheimia for his significant contributions in the recent taxonomy of the family Pasturellacae. On the other hand, *Pasteurella haemolytica* biotype T was first reclassified as *Pasteurella trehalosi*. In addition avian *Pasteurella* species including *Pasteurella gallinarum, Pasteurella paragallinarum* and *Pasteurella volantinium* were similarly removed to new separate genus named as Avibacterium (Blackall et al., 2005).

Before the establishment of this newly revised classification, *P. haemolytica* was known to comprise two bio-types A and T, based on fermentation of arabinose and trehalose respectively. Within these two biotypes, 17 serotypes were further identified on the basis of soluble or extractable surface antigen by passive heamaglutination test (Carter and Cole, 1990). Serotypes 1, 2, 5, 6, 7, 8, 9, 11, 12, 13, 14, 16 and 17 belong to biotype A which was reclassified as *M. haemolytica*. However, serotype 11 was later reclassified as *M. glucosida* . The rest of serotypes (3, 4, 10 and 15) which belong to biotype T finally moved to separate new genus as mentioned. It is worth mentioning that *M. haemolytica, P. multocida* and *P. trehalosi (Bibersteina)* constitute the most important members of the family *Pasturellacae* that pose serious hazards in livestock industry. These species are commensally resident in the animal body as normal constituents of the nasopharyngeal micro flora and are all capable of causing infection when the body defense mechanisms are impaired. Their presence is mainly confined to ruminants with most adequately characterized strains originating from cattle, sheep and goats (Biberstin and Hirsh, 1999).

P. multocida, is associated with hemorrhagic septicemia in cattle and buffaloes and enzootic pneumonia complex in young ruminants (Jones et al., 1997). Concurrent infections of the respiratory tract by viruses, bacteria and lung worms have been described and such disease conditions are commonly known as respiratory disease complex (RDC), indicating the difficulty to attribute to only one etiology (Biberstin and Hirsh, 1999; Quninn et al., 2002). In the cool central highlands of Ethiopia, respiratory disease complex has been identified as leading. Irregular and insufficient vaccination program for diseases such as pastuerellosis and Pest des Petites Ruminant (PPR), lack of strategic mass drenching against lung worms and occurrence of viral infections may play significant roles in the persistence of respiratory disease complex in Ethiopia.

Even though pneumonic pasteurellosis is one of the most economically important infectious diseases of cattle in Ethiopia (Mohamed and Abdelsalam, 2008), there was lack of information on this regards in Southern Ethiopia especially in study area. Hence, the study was designed to isolate and identify Mannheimia and Pasteurella species that invade the lower respiratory tract of cattle causing pneumonic pasteurellosis, to assess potential associated risk factors and determine their antibiogram

susceptibility pattern from pneumonic and apparently healthy cattle and in Bedelle District, Western Ethiopia.

MATERIALS AND METHODS

Study area

The study was conducted from October 2013 to April 2014 in the selected, Illubabor Zone, in Bedelle District of Oromia regional state, Western Ethiopia. The area is located 480 km from the capital city, Addis Ababa, to the West, along the way to Metu. The area is geographically positioned at an altitude of 1900-2000 m above sea-level (m.a.s) and 8-9° N latitude and 36-37°E longitude. The mean annual rain fall in the area is 1500 mm. The mean seasonal temperature varies from 20-25°C from October to January and decline to a level of 15-25°C during the rest of the months (Central Statistical Agency, 2009).

The area is within an agro-ecological zone and is prone to high rainfall and multi-species animal is preferred and most practiced system of livestock production. The area is among the known coffee-growing in the country. Agriculture is the main livelihood in the area in which cattle and sheep are kept as the major livestock which are highly important for the livelihood of the local population. The rearing system of cattle in study sites depends on natural grass and crop residues that are kept in traditional management system.

Study animals

The study population constitutes indigenous zebu cattle of varies sex, age groups, body conditions scores and managed under smallholder mixed crop-livestock farming system which are kept under traditional extensive husbandry system with communal grazing and watering points.

This study included all cattle with respiratory signs presented to the Bedelle Veterinary Clinic and/or site of veterinary clinics found in the District. For comparative study, live animals without pneumonic signs and non-pneumonic lungs from slaughter house were also included in the study. The study was conducted on local breed cattle selected from the district and 329 study animals were sampled. The origin, sex, age and body condition scores of the animals were the potential variables used to associate with the prevalence rate.

Sample collection

Each study animal was individually identified and restrained by an assistant and kept fixed before the sampling procedures. A total of 185 nasal swabs were collected aseptically out of which clinically pneumonic cattle (n=96) from animals showed respiratory signs such as an irregular breathing pattern and grunting on expiration, coughing, a serous nasal discharge, apparently healthy ones (n=89) for comparison purpose. All samples were processed bacteriologically.

The methods followed were based on the procedures of Sisay and Zerihun (2003). Sterile cotton-tipped, 20-25 cm long nasal swabs, moistened in sterile tryptose soya broth, were directed via the ventral nasal meatus in to nasopharynx, that is, the swabs were carefully inserted into nostril; rolled gently; put back to the test tube containing broth; and the tubes were capped.

The swabs were then kept in an ice box. The specimens collected were transported to Bedelle Regional Laboratory for bacteriological analysis. At the time of sample collection aseptic procedures were implemented to avoid getting contamination from the external nares; thus, the external nares were decontaminated with cotton soaked with 70% ethanol. A total of 144 lung tissue samples from both non-pneumonic (n=75) and pneumonic (n=69) from slaughtered animals were collected immediately after slaughter. The samples were placed in separate sterile universal bottles, labeled and kept cooled in the ice box and transported to the laboratory. Sampling method used was purposive sampling and comparative.

In addition, data were gathered from owners by using structured questionnaire survey to assess the risk factors for pneumonia in the study area. Finally, data gathered were sorted and analyzed using standard methods.

Sample processing and culturing

Nasal swab samples were streaked on sheep blood agar plates and incubated at 37°C for 24 to 72 h. The same samples were parallely streaked on MacConkey agar for primary differentiation of the pathogen following standard procedures. Colonies were characterized and those giving Gram-negative coccobacilli or short rods with or without bipolar staining on smears were subcultured for identification. A 24 h pure *Pasteurella* suspected culture (isolate) was subjected to biochemical tests using standard procedures.

A section of lung samples were aseptically taken from the edge of the lesion in the case of pneumonic cases and the samples were processed before inoculation into appropriate media as follows: the surface of the lung tissue passed through the Bunsen burner several times to avoid surface contamination and transferred to Petri dish. Then the surface was heated with spatula and incised and minced with sterile forceps, scalpel blade. Then the tissue specimens were placed i to screw capped test tubes containing TSB and was incubated for 2 h at 37°C and then streaked on to both blood agar plate (BAP, 5% blood in blood agar base) and MacConkey agar plates. The plates were incubated at 37°C for 24 h. The grown colonies were characterized and subcultured on nutrient agar plate to get pure culture for further biochemical tests following standard protocol (Quninn et al., 2002).

Isolation and identification of bacterial isolates

To obtain pure cultures of a single bacterial colonies type from blood agar and MacConkey agar only, bacterial colonies characteristics of rod, coccobacilli, rough or smooth, Gram negative, with or with no growth on MacConkey, presence or absence heamolysis on blood agar were subjected to sub culturing. Then, each bacterial colony was characterized and further bacterial identification was made using series of primary and secondary biochemical tests following standard procedures (Quninn et al., 2002).

Bacterial cultures were firstly characterized based on primary identification characteristics such as the cellular morphology of the bacteria; Gram staining, oxidase, catalase and oxidation fermentation tests to determine the genus of the bacterial isolates. After, pure colonies were obtained, transferred to nutrient agar media.

Furthermore, species of the bacterial isolates were determined using a variety of secondary biochemical tests. Indole production and motility was tested in SIM medium, hydrogen supplied production was tested on triple iron sugar agar and SIM medium. Fermentation reactions for sugars (glucose, lactose, maltose, mannitol, trehalose and arabinose) were conducted according to the procedure described by Quninn et al. (2002). All activities from streaking primary media to secondary biochemical tests were conducted for final identification of bacterial isolates to the species level. *P. multocida* on blood agar had colonies non-heamolytic round, smooth or muccoid, all the isolates failed to grow on MacConkey on the basis of Gram staining, the isolates were found to be Gram negative, coccobacillary rods with or without bipolar

Table 1. The overall prevalence of *Pasteurellacae* in Bedelle District, Western Ethiopia.

Total animal examined	+ve animals	Prevalence
Pneumonic (nasal swab)	96	58.2%
Pneumonic (lung tissues)	69	41.8%
Total (329)	165	50.2%

staining on primary identification. *M. haemolytica* and *B. trehalosi* were forms round, smooth, translucent, greyish with β- distinct zone of heamolysis on blood agar, grows on MacConkey agar. Therefore, the isolates were identified to the species level based on the series of results obtained from the primary identification to secondary biochemical test.

In vitro antimicrobial sensitivity test

Antimicrobial susceptibility profiles of the isolates were determined using standard disc diffusion methods as described by Kirby -Bauer on Mueller-Hinton agar supplemented with defibrinated 5% sheep blood. The antibiotic susceptibility tests of *Pasteurella* species from pure culture was carried out by standard disk diffusion methods and interpreted according to the National Committee for Clinical Laboratory Standards (NCCLS) guidelines (NCCLS, 1999). The following common antimicrobials applied in the country were assayed: chloramphenicol (CAF 30 g), erythromycin, florifenicol (FFC-30Ng), penicillin- G (10 IU), tetracycline (TTC-30 Ng), polymixin-B (30 g), amoxicillin (Amox-20 Ng), kanamycin (30 g), and cephalexin (30 g).

About 4-5 similar colonies from the blood agar were suspended in a propagating medium of nutrient broth and incubated for 2-8 h duration of incubation then the bacterium culture was taken and resuspended into saline water then it was adjusted until similar to 0.5 McFarland standards according to the guide line (NCCLS, 1999). Using sterile cotton swab with applicator stick, the suspended bacteria from the broth was uniformly distributed on the surface of Muller Hinton agar plate, containing 5% of sheep blood, at three different planes.

The plates were dried for some minutes by putting upside down. Antibiotic discs were applied on the plate using sterile forceps ensuring complete contact with the agar surface and 4-5 discs regularly placed in one plate 3 cm apart and 1.5 cm from the edge antibiotic impregnated paper discs and the plates were incubated at 37°C for 8-12 h. Finally, the diameters of the zone of inhibition was measured using a transparent ruler to the nearest mm. Based on the zone of inhibition, the isolates were categorized descriptively as resistant, intermediate and susceptible.

Questionnaire survey

A structured questionnaire with the primary objective of eliciting the multifactorial predisposing factors of pneumonic pasteurellosis was conducted. The information was collected with those pertinent questions about: risks associated respiratory problems of cattle in the study area.

In this study, structured questionnaires was prepared and administered to the livestock owners and animal health practitioners. The questionnaire was pre-tested on some farmers to assess the response rate and consistency of replies as well as to monitor any source of bias in the course of interview. Data on animal transportation, management, feed type, weather condition and stress causing factors was collected.

Data management and analysis

The collected data was coded and entered into Microsoft Excel, 2007 spread sheet. The data were analyzed using SAS software (SAS Version 9.2). Descriptive statistics called person Chi-square test was used to determine the statistical significance for categorical data. The data were also summarized using comparison of frequency, percentages (EpInfo, 6.04), drawing graphs. In addition, other statistical associations were also to summarize generated data on the rate of isolation, identification and sensitivity patterns difference.

The effect of age, adverse environmental condition, vaccination, nutritional and immune status observed through the questionnaire survey were interpreted and summarized. For data analyzed statistically, confidence level was held at 95% and $P<0.05$ was set for significance.

RESULTS

Out of 329 animals examined, 50.2% were found to be positive to the disease during the study period. The overall isolates were 28 (8.51%) with *M. haemolytica* (46.4%), *P. multocida* (39.3%) and *B. trehalosi* (14.3%) recovered on bacteriological analysis respectively. The culture results of samples from nasopharyngeal swabs (185) of pneumonic and non-pneumonic cattle and lung tissue (144) were taken from apparently healthy slaughtered cattle (Table 1 and 2).

Bacteriological isolation

From 185 nasal swab samples examined from respiratory tracts of cattle, 8 (4.32%), 6 (3.24%) and 3 (1.62%) of *P. multocida, M. haemolytica and B. trehalosi* were isolated respectively. While 144 lung tissue samples were collected from Bedelle Municipal Abattoir, 3 (2.08%), 7 (4.86%) and 1 (0.69%) of *P. multocida, M. haemolytica and B. trehalosi* were isolated. The total culture results of isolates from both the nasal swabs and lung tissues were 8.51% that comprises three different bacterial species (Table 3).

Among 96 nasal swabs collected from cattle showing respiratory signs, 15 (15.6%) were positive for *Pasteurella* and *Mannheimia* species. Whereas out of 89 nasal swabs collected from apparently healthy cattle, only2 (2.25%) were positive. From the total of 17 (9.2%) bacterial species isolated, nasal swabs originated from both pneumonic and healthy animals.

Of 144 lung tissues sampled from apparently healthy slaughtered cattle, 75 of the lung tissues were non-pneumonic and neither *Pasteurella* nor *Mannheimia* species were isolated. Although, the remaining 69 lung tissues were pneumonic and yield 11 (15.9%) isolates was recorded. The recovery rate of the bacterial isolates from animals showing signs of respiratory tract and non-pneumonic cattle of nasal swabs and lung tissues originated samples showed variation with higher rate of isolates from nasal swab as shown in Table 4.

Table 2. Summary of total isolate with corresponding health status and total sample processed.

Health status	Type of samples		Total sample	Total Isolate
	Nasal swabs	Lung tissues		
Pneumonic	96 (51.9%)	69 (47.9%)	165(50.2%)	26 (92.9%)
Non-pneumonic	89 (48.1%)	75(52.1%)	164 (49.8%)	2 (7.14%)
Total	185	144	329	28

Table 3. Distribution of *Pasteurellacae* species isolated from nasal swabs and lung tissues of cattle in and around Bedelle.

Types of sample	***Pasteurellacae* species isolated**			Total
	P. multocida	***M. heamolytica***	***B. trehalosi***	
Nasal Swab	8 (4.32%)	6 (3.24%)	3 (1.62%)	17 (9.19%)
Lung tissues	3 (2.08%)	7 (4.86%)	1 (0.69%)	11 (7.63%)
Total	11 (3.34%)	13 (3.95%)	4 (1.22%)	28 (8.51%)

Table 4. Comparison on the recovery rate of bacterial isolates with respiratory signs and apparently healthy cattle.

Health status of animal	Type of samples	No. of samples	No. of +ve	(%) of +ve
Pneumonic cattle	Nasal swabs	96	15	15.6
Non-pneumonic	Nasal swabs	89	2	2.25
Apparently healthy	Lung tissue	75	0	0.00
Pneumonic	Lung tissue	69	11	15.9
Total		329	28	8.51

Cultural characteristics of isolates

On blood agar, the colonies of *P. multocida* isolates were non-hemolytic, round, smooth or muccoid. All *P. multocida* isolates were Gram negative, coccobacillary and did not grow on MacConkey agar. Whereas, *B. trehalosi* and M. *haemolytica* were able to grow on MacConkey agar and showed *β*-heamolysis on blood agar also grow as pin point red colonies.

Biochemical characteristics of the isolates

The biochemical test carried out showed that all the isolates were positive for oxidase, catalase and able to ferment glucose, mannitol, sucrose mannose. All *Mannheima* species ferment mannitol, glucose, maltose, arabinose and sucrose except trehalose.

M. haemolytica isolates were positive for catalase and oxidase. *B. trehalosi* unlike others can ferment trehalose but not arabinose and lactose. It was found to be catalase; indole and urease negative (Table 5).

The comparison result of isolated bacterial species between pneumonic and non-pneumonic lung tissues showed 3 (2.08), 7 (4.86) and 1 (0.69) of *P. multocida, M. haemolytica and B. trehalosi*, respectively with none of them isolated from of non-pneumonic lung tissues. There were no bacteria isolates revealed from samples of non-pneumonic lung tissues. However, 11 (7.63%) of the isolates were characterized from pneumonic lung (Table 6).

Antimicrobial susceptibility profiles of the bacterial isolates

The antibiotic susceptibility tests of bacterial species isolated during the study period from nasal swabs and lung tissues as carried out by disc diffusion method showed that almost *M. heamolytica* (n=13) were found to be sensitive to most of the drugs tested such as: florifenicol, amoxicillin, polymyxin B, kanamycin, cephalexin and chloramphenicol. However, moderate resistance was noted against erythromycin, penicillin and tetracycline.

The antimicrobial susceptibility tests conducted for *P.*

Table 5. Results of biochemical characteristics of bacterial species isolated from cattle.

Characteristics (tests)	Species		
	M. haemolytica	*P. multocida*	*B. trehalosi*
Heamolysis	+	-	+
Growth on MacConkey	+	-	+
Catalase	+	+	+
Oxidase	+	+	+
Indole	-	+	-
Urease	-	-	-
Lactose	+	-	+
Sucrose	+	+	+
Glucose	+	+	+
Maltose	+	-	+
Mannose	+	+	+
Arabinose	+	-	-
Mannitol	+	+	+
Trehalose	-	-	+

Table 6. The distribution of bacterial isolates from the lung tissue samples.

Bacterial isolate	Pneumonic lungs		Non pneumonic lungs	
	No. of isolate	Isolate (%)	No. of isolate	Isolate (%)
P. multocida	3	2.08	0	0
M. haemolytica	7	4.86	0	0
B. trehalosi	1	0.69	0	0

multocida also indicated that florifenicol, chloramphenicol, kanamycin, cephalexin, polymyxin and amoxicillin were the most effective antibiotics. Simililarily, antibiogram profile of *B. trehalosi* revealed that all isolates were susceptible to most of the drugs tested except against penicillin-G, erythromycin and tetracycline.

The antimicrobial susceptibility test result indicated that the bacterial isolates were more susceptible to six antibiotic discs used even if the degrees of susceptibility vary (Table 7). In this study, there was statistical significant difference between age and the occurrence of the disease.

The isolated bacteria indicate young cattle, 13 (92.9%) were more susceptible to pneumonic pasteurellosis than adult cattle, 2 (66.7%) (Table 8).

Questionnaire survey

The questionnaire survey was conducted to determine various aspects of bovine pneumonic pasteurellosis in the study areas. The result shows respiratory disease as the most important disease problem. In addition, the associated risk factors with bovine pneumonic pasteurellosis were identified by the respondents.

Among the assessed risk factors, age was one of the most predisposing factors to pneumonic pasteurellosis in which young were found to be more susceptible than adults according to the respondents. The main stresses causing factors were indicated by the respondents such as climatic change (80.0%), previous sickness (64.3%), feed shortage (37.0%), drought (23.7%) and commonly, dry season (53.5%) (Table 9).

DISCUSSION

In the present study, the overall prevalence of bovine pneumonic pasteurellosis was found to be 50.2% in which 58.2 and 41.8% were recorded at clinic and abattoir, respectively. The finding was slightly lower than that of Aschalew (1998) and Tesfaye (1997) who reported 63.8 and 67.6%, respectively. However, the result was higher than that of Tilaye (2010) who reported 28.4%. This might be due to the different ways of taking sample from purely pneumonic cattle, improved health

Table 7. Summary of antimicrobial sensitivity test result descriptively.

Bacterial isolate	PG	E	K	TTC	CAF	CP	PB	F	A
P. multocida	6(S)	7 (S)	11(S)	6(S)	11(S)	11 (S)	11 (S)	11 (S)	11(S)
M. haemolytica	7(S)	9 (S)	13(S)	7(S)	13 (S)	13(S)	13 (S)	13(S)	13(S)
B .trehalosi	2(S)	2 (S)	4 (S)	3 (S)	4 (S)	4(S)	4(S)	4 (S)	4 (S)

Where (S) is = Number of bacterial isolate susceptible to the corresponding antibiotic discs used. CAF = chloramphenicol, TTc = tetracycline, E = erythromycin, K = kanamycin, A = amoxicillin, PG = penicillin, PB = polymyxin-B, F = florifenicol, CP = cephalexin.

Table 8. Summary of isolation rate with age group.

Age group	Health status	Types of Isolates (%)			Total
		M. haemolytica	*P. multocida*	*B.trehalosi*	
Young	Healthy	-	1 (7.14%)	-	1 (7.14)
	Pneumonic	5 (35.7)	5 (35.7%)	3 (21.5%)	13 (92.9)
Adult	Healthy	-	1 (33.3)	-	1 (33.3)
	Pneumonic	1 (33.3)	1 (33.3)	-	2 (66.7)

X^2=8.59 Where, X^2 = Chi- square; P-value =0.04.

facilities, laboratory facilities and predisposing factors.

The prevalence of *M. haemolytica* was almost similar both at abattoir (4.86%) and clinic (3.24%). *P. multiocida* was discovered from nasal swabs and lung tissues. Pneumonic pasteurellosis was only positively associated with the age of suspected bovine particularly *P. multiocida*.

This result is lower than the findings of Eshetu (1991), Nurhusein (2005), Mohammed (1999) and Radostitis et al. (1994) that were reported as 13, 8.7, 40.8 and 56% respectively in pneumonic lungs. This difference might be due to the type of sample taken from purely pneumonic lung tissues and improved health facilities in the current study area.

B. trehalosi was also isolated both from nasal swab (1.62%) and lung tissues (0.69%). This was in agreement with the report of Tesfaye (1997) and Tilaye (2010) in which the species contributes in the occurrence of RDC. The pasteurella and Mannhemia species were isolated from nasal swabs as well as lung tissues, however, the isolation rate varies.

In the present study, the overall isolates were 28 (8.51%) with *M. haemolytica* (46.4%), *P. multocida* (39.3%) and *B. trehalosi* (14.3%) were recovered on bacteriological analysis respectively. Comparing the three *Pasteurella* species, *M. haemolytica* (46.4%) was the major causative agent involved in bovine pneumonic pasteurellosis. This was consistent with previous reports (Tesfaye, 1997; Tilaye, 2010; Eshetu, 1991; Mohammed, 1999).

M. 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 animal (Aiello and May, 1998). Although the percentage isolation of *B. trehalosi* was relatively low (14.3%), it plays great role in the etiology and pathogenesis of bovine pneumonia should not be under estimated.

According to studies carried out by Sisay and Zerihun (2003) the prevalence of *M. haemolytica* and *P. trehalosi*, 75% from nasal swabs of apparently sheep, in the high lands of Wollo from local abattoir 205 lungs were investigated (34%) which showed pneumonia, *Mannheima* and *Pasteurella* were isolated from 59% of these pneumonic lungs and 69% of the respective tonsils. However, the present study shows lower rate of isolation of these species of bacteria. This lower rate of isolation of *Mannheimia* species and *Pasteurella* species may be due to the difference in epidemiological area and as well the anatomical structure of study sampling in addition to species study variation. Also, this higher isolation rate by the previous studies might be attributed also to the type of sample taken which were purely pneumonic, variations in the animal species in different study areas.

Comparing the three bacterial species, *M. haemolytica* constituted higher percentage of the total isolates, this implies that *M. haemolytica* is the major causative agent involved in bovine pasturellosis. This finding was consistent with other previous reports (Aschalew, 1998; Tesfaye, 1997; Mohammed, 1999; Daniel et al., 2006) that indicated *Mannheima* and *Pasteurella* species are the causative agent of pneumonic pasteurellosis in most animal species of all climatic zones in which ruminants are usually asymptomatic carriers (Thomson, 1998). *M.*

Table 9. Summary of the results of questionnaire survey.

Variable		Frequency	Percentage (%)
Management system	Extensive	151	81.6
	Semi intensive	34	18.4
	Intensive	0	0.00
Previous History Sickness	Yes	119	64.3
	No	66	35.7
Predisposing factors	Weaning	24	13.0
	Shipping	29	15.6
	Shortage of feed	68	37.0
	Drought and Crowding	44	23.7
	Unknown	20	10.7
Drenching of drugs	Yes	72	38.9
	No	113	61.1
History of stress before became sick	Yes	127	68.7
	No	58	31.3
Major stress causing factor	Transportation	19	10.3
	Housing system	18	9.70
	Climatic change	70	80.0
	Unknown	78	42.2
Season	Dry season	99	53.5
	Rainy season	86	46.5
Frequency of out break	Frequently	71	38.4
	Not Frequently	114	61.6
Seriousness of the disease	Very serious	138	74.6
	Moderate	45	24.3
	Not as such serious	2	1.10
Control method applied	Antibiotic	64	34.6
	Vaccination	67	36.2
	Improving management	54	29.2

haemolytica and *P. multocida are* considered to be normal inhabitants of nasopharyngeal mucosa (Ames et al., 2002) but not of the lung and considered to be opportunistic pathogens . *M. haemolytica* is considered to be the major cause of BRD but it is rarely isolated on nasal swabs in healthy animals. Similarly, the present study result also revealed neither *Pasteurella* nor *Mannheima* species were isolated from the non-pneumonic lung but only isolates of *P. multocida* were obtained from nasal swabs of nonclinical cases. *M. haemolytica* has been known to be the main bacterial agent responsible for the lung infection and has been known to be higher in acute pneumonia (Daniel et al., 2006).

In this study, higher rate of infection was associated with young age groups as compared to adults ($p<0.05$). This might be due to the immune status of the animal being able to predispose to the bacterial infection and other predisposing etiological agents. Furthermore, antimicro-bial sensitivity patterns of the isolates were determined in this study. All the isolates (*P. multocida, M. haemolytica* and *B. trehalosi*) were susceptible to most of the antibiotics

discs used. Bacterial resistance to antibiotic is becoming a subject of interest and attention
of these days. However, moderate resistance against penicillin-G, erythromycin and tetracycline was noted. About half of *M. haemolytica* and of *P. multocida* isolates showed resistance against penicillin-G. Also some of *M. haemolytica, P. multocida* and about half of *B. trehalosi* did not show susceptibility to Erythromycin. As the result obtained indicates, the therapeutic success could be achieved using most of antibiotics in the study area. The finding was in line with the previous report (Disassa et al., 2013).

The quality of questionnaire is an important tool in individual cases detection. In the present study, the predisposing factors in the initiation of pneumonic pasteurellosis (Jones et al., 1997) which might be due to reduced immune status of the animal was surveyed based on the questionnaire. Accordingly, the main stresses causing factors were indicated by the respondents such as climatic change (80.0 %), previous sickness (64.3%), feed shortage (37.0%), drought (23.7%) and common in dry season (53.5%). This was in agreement with the previous findings (Bruere et al., 2002).

In the present study, the seasonal variation of the isolates was not determined due to shortage of time. Moreover, even if identification of serotypes is so important, it was not conducted due to lack of laboratory facilities.

However, the study has the following strengths: the sample was collected from both abattoir and clinic which shows the reliability of the data, the isolates were obtained from pneumonic bovine that made us compare the clinical case with ethological agents, all laboratory works were followed standard procedures and quality control was exhibited in each step of the work.

Conclusion

Generally, pneumonic pasteurellosis was the major disease of cattle in the study area and *M. haemolytica* is the most common cause. Young animals were the risk factor for the disease. It also demonstrated that pneumonic pasteurollosis is a highly complex multifactorial disease particularly in cattle which could be associated with stress, compromised immunity, adverse environmental condition, previous illness (co-infection), misuse of traditional medicine and this made the control of pasteurellosis more difficult.

Furthermore, the antibiotic sensitivity tests of the isolates showed them to be susceptible to most of the drugs. However, the isolates showed resistance to some of the antibiotic disks. Moreover, there must be an integrated management system, vaccination, controlling of the predisposing factors and use of broad spectrum antimicrobials as a prophylactic, and treating of sick animals is suggested.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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