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# Isolation And Identification Of Cattle Pasteurellosis In Asossa And Bambasi Districts, Western, Ethiopia

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**Abstract**: The study was conducted from Nov 2021 to May 2021 in Bambasi and Assosa districts, western Ethiopia. The objective was to isolate *mannhemia* and *pasteurella* species, and to assess the associated risk factors from cattle , and antibiotic susceptibility profile of the isolates. The bacteriological examination revealed, 134/384 (34.89%) ov erall isolates of *Pasteurellosis.* 21.6% *M. haemolytica*, 9.63% *P. multocida* and 3.6% *B. trehalosi* of pastuerella spec ies were isolated by culturing and biochemical tests. The higher isolation rate of *M. haemolytica*, indicated as major cause in the study area. Age was found to be potential risk factors in which young animals were highly affected. The antimicrobial susceptibility profile of the isolates were carried out using disc diffusion method used. The isolates w ere susceptible to most of the antibiotic disks used such as amoxicillin, chloramphenicol, cephalexin, polymyxin-B a nd kanamycin and florifenicol. However, resistance was observed to Tetracycline, Erythromycin, and Penicillin G. T hus, an integrated application of overall management and vaccination should be implemented as prevention and cont rol measures. Therefore, strict measures like proper vaccination and antibiogram test to select effective drugs should be regularly implemented.

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**Key words**: *Asossa, Bambasi, Cattle,* M. *haemolytica, P. multocida, B. trehalosi*

## 1. INTRODUCTION

Classical hemorrhagic septicemia is a particular form of pasteurellosis caused by *Pasteurella multocida* and manifested by an acute and highly fatal septicemia mainly in susceptible cattle and water buffaloes (Blackall *et al.,* 2007). Pneumonic pasteurellosis is one of the priority diseases that deserve control. However, control of pneumonic pasteurellosis is difficult task that requires integration of various techniques. Developed and developing countries practice various control mechanisms for primary diseases. While developing countries including Ethiopia could not apply the strategies used by developed countries because of economic reason. Hemorrhagic septicemia (HS) is an acute, fatal, septicemia disease of cattle and buffaloes caused by specific serotypes of the bacterium family *Pasteurellaceae* and genus *Pasteurella* which recently classified in the genera *Pasteurella*, *Mannheimia*, and *Bibersteinia* (Blackall *et al.,* 2007). Bacteria of the family *Pasteurellaceae* are involved in a variety of economically important diseases in food-producing animals and pasteurellosis is a multi-factorial respiratory disorder (Catry, 2005).

Among these genera Pasteurella is the causative agent of hemorrhagic septicemia. *P. 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 (Katherine, 2008). 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). From these serotypes B2 and E2 are only the causative agents of HS in cattle mainly in Asia and Africa (Garner, 2003).

*P. multocida* is isolated from upper respiratory tract and blood sample that result in high rates of morbidity and mortality in cattle. *P. multocida* is 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* (Michael, 2008).

P. *multocida* is common inhabitants of the tonsils and nasopharynx of a variety of healthy cattle. In cattle, *P. multocida* are believed to be opportunistic bacteria that colonize the lung and other organ after some predisposing risk factors. The initiating risk

factors can be from stresses by mildly pathogenic agents such as Foot and mouth disease, parasitic infections, as well as from mechanical dust, heavy rain, and transportation.

In most instances, these insults alone do not result in significant epidemics with high morbidity or mortality; however, when these and other stressors are compounded by infection with *Pasteurellaceae*, the result can be increased morbidity and death (Michael, 2008). Generally infection results when an animal is compromised by any of the variety of stress factors such as inclement weather, transportation, malnutrition, bacterial invasion of host defense, viral infections, nasopharyngeal colonization and dehydration (Hawari *et al*., 2008).

The diagnosis of the disease is based on the clinical signs, gross pathological lesions, morbidity and mortality patterns, and confirmation by isolation of the pathogens and their conventional and molecular characterization (Ragy, 2005).

Even though pneumonic pastuerellosis is one of the most economically important infectious diseases of cattle in Ethiopia (Mohamed and Abedel Salam, 2008), there was lack of information on this regards in Benishangul Gumuz, western Ethiopia especially in Asossa and Bambasi Districts of study area. Hence, the study was designed to isolate and identify Mannheimia and Pastuerella 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 cattle in Assosa and Bambasi districts. Therefore, the study was conducted with the objectives of isolation and identification of *Pastuerella species* from cattle in the study area, antimicrobial resistance profile *and* assessment of the potential risk factors.

## 2. MATERIALS AND METHODS

### 2.1. Study Area

Assosa town is the administrative centre of the Benishagul Gumuz Regional state, and located 676 km west of Addis Ababa. The study was conducted in Asossa and Bambasi districts, from November 2021 to May 2021. Within districts it was studied in five peasant association here after called namely: Asossa kebeles namely: Megele38, Megele30, Megele31 and Gambla, Bambasi peasant association of household. Asossa zone has 214 peasant associations, stretching over an area of 18,340.55 kilometer square, with human population of 270,980. The region is found in the north west of the country between latitude of $9^0$ and $11^0$ N and longitude of $34^0$ and $35^0$E and its altitude is from 700-1560 meter above sea level. Annual rain fall is between 900-1500 mm with uni modal type of rainfall that extends from April to October with peak rainy periods from June to August, and annual temperature ranges between 25- $35^0$c (NMSA, 2014; CSA, 2015). Asossa zone, the livelihood of the society largely depends on mixed livestock and crop production. It has 35.6% of the livestock population of the region constituting 81,939 cattle, 167,281 goats, 10,231 sheep, 14,089 donkeys, 40,3153 poultry, 29 horses and 59,695 beehives (CSA, 2005; CSA, 2016).

Asossa district has 74 kebeles covering an area of 2317 $km^2$ with human population of 47,666. And also it is located between $8^0$30" and $4^0$27" N and 34°21" and 39°1" E. It has an altitude range of 1000-1570 meter above sea level and its annual temperature ranges between $16^0$c- $34^0$c. Besides this, Bambasi district has 38 kebeles stretches over an area of 2210.16 square kilo meter with human population of 62,693 and annual temperature ranges between $21^0$c - $35^0$c (CSA, 2015).

### 2.2 Study Animals

The study population constitute indigenous zebu cattle found in selected districts. Cattles vary in sex, age groups, body condition scores, and managed under small holder mixed crop- livestock farming system which are kept under traditional extensive husbandry system with communal grazing and watering points. For the Isolation and Identification of *Pasteuerellosis* from indigenous cattle breeds, different risk factors were considered. These potential variables/factors include origin, sex, age, body condition scores, herd management system and vaccination history.

### 2.3 Study Design

A cross sectional study was carried out from November 2021 to May 2021 for isolation, identification and assesses antimicrobial resistance profile of pasteurella isolates from small household farms.

### 2.4 Sample size Determination

Total sample size for cattle nasal swab sample collection, isolation and enumeration of pasteurella species were assigned according to Thrustfield (2007). A 5 % absolute precision (5% sampling error) at 95% confidence interval was used during estimation of the sample size. Since there is no similar work done in the Asossa and Bambasi district, the expected prevalence was taken as 50%. Therefore, the total sample size for the study were calculated using the following formula for each sampling units.

$$n = \frac{(1.96)^2 x\ P\ (1-P)}{d^2}$$

Where: n=the total sample size, p=expected prevalence (50%), d=desired absolute precision / marginal error between the sample and population / (5%), (0.05) at 95% CI,

Zα/2 = the standard normal deviation corresponding 95% of confidence level = 1.96
n = (1.96) x (1.96) x (0.5) x (1-0.5)/ (0.05) x (0.05) = 384; accordingly, from a total of 384 cattle swab; 261 were sampled from Asossa and 123 swab sampled from Bambasi districts.

**2.5 Study methods**

**2.5.1 Sample Collection, transportation**

Each study animal was individually identified and restrained by an assistant and kept fixed before the sampling procedures. A total of 384 nasal swabs were collected aseptically from apparently healthy animals as well as clinically pneumonic cattle showed respiratory sign such as an irregular, breathing pattern and grunting on expiration, coughing, a serious nasal discharge, and apparently healthy ones. Risk factors such as sex, age, breed, body condition scores, management system, and vaccination history of the sampled animals were recorded. Swab sample collection was done, after disinfecting the skin around sampling site or around the noses by 70% alcohol. The sample was taken deep in to nasal cavity by 5cm long swab after disinfecting. Samples were collected from clinical sick cattle which were selected purposely from the two study districts in test tube having, nutrient broth for swab which were labeled and coded immediately. The samples were transport to Assosa, Regional Veterinary Laboratory for culturing and identification using bacteriological methods. In the laboratory nasal swabs were incubated immediately at $37^0$C for 24 hours. Besides this, methylene blue staining was done for bipolar pasteurella observation (Ashraf *et al*., 2011). All samples were processed bacteriologically.

**2.5.2 Isolation and Identification of Isolates**

Up on pure culture of bacterial colonies on blood agar and mac conkey agar, bacterial colonies were characterized. So, each bacterial colony, identification was made using series of primary and secondary biochemical tests following standard procedures (Quinn *et al*., 2002).

Bacterial cultures, were firstly characterized based on primary identification, such as the cellular morphology of the bacteria; gram stain, oxidase, catalase and oxidation-fermentation tests to determine the genus of the bacterial isolates. Pure colonies were streaked to nutrient agar. Species of the bacterial isolates were determined using variety of secondary biochemical tests, such as indole production and motility tested in SIM medium, hydrogen supplied production was tested on triple iron sugar agar. Fermentation reaction for sugars (glucose, lactose, maltose, mannitol, trehalose and arabinose) were conducted according to the procedure distributed by Quinn *et al.,* 2002.

All activities from streaking primary media to secondary biochemical tests conducted for final identification of bacterial isolates to the species level. *P. multocida* on blood agar had colonies non-haemolytic round, smooth or mucoid, all the isolates failed to grow on Mac conkey on the basis of gram staining, the isolates were found to be gram negative, coccobacillary rod with or with out bipolar staining on primary identification *M. haemolytic* and *B. trehalosi* were forms round, smooth, translucent, grayish with beta- distinct zone of haemolysis on blood agar, grows on mac conkey agar. Therefore, the isolates were identified to the species level based on the serious of results obtained from the primary identification to secondary biochemical tests.

**2.5.3 Biochemical tests**

For further identification of the culture and Gram stain bacteria which were suggestive of *pasteurella/ Mannheimia/ Bibersteinia* different biochemical tests were conducted by selecting those which aid in differentiation among *pasteurella/ Mannheimia/ Bibersteinia, trehalosi* genera and species. These included Indole and Catalase test fermentation of maltose, lactose, glucose, sucrose, trehalose and Oxidase, Sorbitol, and motility tests. The data obtain were recorded and compared for confirmation of the isolates to which species they were belong. The Oxidase test was used to determine those organisms which possessed the cytochrome oxidase enzyme. The test was used as an aid for the differentiation of Neisseria, Moraxella, Campylobacter and Pasteurella species (oxidase positive).

Wet filter paper was also used. A strip of filter paper was soaked with a little freshly made 1% solution of the reagent. A speck of culture was rubbed on it with a platinum loop. A positive reaction was indicated by an intense deep-purple color, which appeared within 5-10 seconds, a “delayed positive” reaction by coloration in 10-60 seconds, and a negative reaction by absence of coloration or by coloration later than 60 seconds.

Direct Plate Method was used by adding 2-3 drop of reagent directly to suspect colonies on an agar plate. The Kovac’s oxidase reagent was used and the result was seen with in 5 to 10 minutes dark purple color change as positive reaction. The Swab method was also done by dipping swab into reagent and then touches an isolated suspect colony and was observed for color change within 5-10 seconds and it’s result was similar with wet filter paper.

Catalase test was used by placing a small amount of growth from culture onto a clean microscope slide. To avoid a false positive result it was not used

metal loop. A few drops of $H_2O_2$ were added onto the smear. It was mixed with a tooth pick. Since the metal loop gives false positive result when we use with $H_2O_2$, it was avoided metal loop or needle use with $H_2O_2$ in this test. A positive result was shown the rapid evolution of $O_2$ as evidenced by bubbling. A negative result was no bubbled or only a few scattered bubbles. Biohazard glasses were disposed in disposal container.

Indole test was used on a sterilized test tube containing 4 ml of SIM broth culture. Inoculate the tube aseptically by taking the growth from 18 to 24 hrs culture. Incubate the tube at 37°C for 24-28 hours. 0.5 ml of Kovac's reagent was added to the SIM broth culture. The result was read for the presence or absence of red ring at the top of broth. So among 384 samples, on 37 *P. multocida* were indole positive. *83 M. haemolytica* were Indole negative (HPA, 2010).

**2.5.4. In vitro Antimicrobial Sensitivity Tests**

Antimicrobial susceptibility profile of the isolates were determined using standard disc diffusion methods described by Kirby- Bauer on Mueller- Hinton agar. The antimicrobial sensitivity tests of pastuerella species from pure culture was carried out by standard disk diffusion methods and interpreted according to the National Committee for Clinical Laboratory Standard guidelines (NCCLS, 1999).

The following common antimicrobials applied in the country were assayed; chloramphenicol (CAF30g), erythromycin, florifenicol (FFC-30Ng), penicillin G (10IU ), Tetracycline (TTC-30 Ng), Polymixin B (30 g), Amoxicillin ( Amox-20Ng), kanamycin ( 30g), and Cephalexin(30g).

About 4-5 similar colonies from the blood agar were suspended in a propagating medium of nutrient broth and incubated for 2-8 hr duration of incubation .Then, the bacterium culture was taken and re suspended into saline water. It was adjusted until similar to 0.5McFarland standards (NCCLS, 1999). Using sterile cotton swab with applicator stick the suspended bacteria from the broth was uniformly distributed on the surface of Mueller- Hinton agar plate. Antibiotic discs were applied on the plate using sterile forceps ensuring complete contact with the agar surface. 4-5 Antibiotic disks regularly placed in one plate 3cm apart and 1.5cm from the edge antibiotic impregnated paper discs and the plates were incubated at 37 $^0$c for 8-12hr. Finally, it was allowed growing at 37$^0$C overnight, the diameter of the zone of inhibition was measured using a transparent ruler by holding on the back of inverted Petridish, to the nearest millimeter, based on the zone of inhibition, the isolates were categorized as Resistance, intermediate and susceptible. The result was compared to the standards (Zone Diameter Interpretive Standards and equivalent Minimum Inhibitory Concentration Break Points of the NCCLS Performance Standards for Antimicrobial Susceptibility Testing) and the species of the isolates were related with a drug effective against them or with a drug to which they are resistant (Kehrenberg, 2001).

**2.6 Data Analysis**

Processing of data was done by computer software. Data was coded and entered to MS Excel spreadsheet and checked for accuracy, coded, analyzed using Stata software version 10. The association between the dependent variables and independent variables such as age, sex, body condition, and vaccination status and management practices was analyzed. Chi-square ($x^2$) tests for repeat measure was used to test relationship between dependent variable (*Pastuerella* species) and different independent host and environmental factors. For all analysis, 95% CI and $P<0.05$ was set for statistical significance of an estimate.

**3. RESULTS**

**3.1 Bacteriological Isolation**

In the present study, 384 nasal swab samples were collected from Asossa and Bambasi districts.

From 384 animals examined, 34.89% of overall isolates were found to positive to the pastuerellosis during the study period. So that, 83/384(21.6%) of *M. haemolytica*, 37/384 (9.63%) *of P. multocida* and 14/384(3.64%) of *B. trehalosi* were isolated with bacteriological method. The collected nasal swab samples were processed microbiologically for isolation and identification of *pasteurellosis*. Variations in sex, age, management, animals' body condition (availability of improved feed and water supply) and vaccination status were used as risk factors for assessing the occurrence of hemorrhagic septicemia in cattle.

**3.1. Culture Characteristic of isolates**

On blood agar, the colonies of *P. multocida* isolates were non-hemolytic, round, smooth or mucoid. All *P. multocida* isolates were gram negatives, coccobacillary and did not grow on mac conkey agar, whereas, *B. trehalosi* and *M. haemolytica* were able to grow on macconkey and showed Beta- hemolysis on blood agar also grow as pin point red colonies. 37 isolate of *P. multocida* were grown on blood agar, nutrient agar and the grown isolate were subjected to subculturing for pure colony appreciation and bacterial colonies were characterization as rod coccobacilli, smooth and rough colony, gram stain was shown gram negative, the rest, *B. trehalosi* and *M. haemolytica* isolates were not grown on MacConkey, on blood agar hemolysis were detected on *M. haemolytica*. After each bacterial colony was characterized, identification of bacteria through primary and secondary biochemical

tests was conducted. Moreover, *Pastuerella species* were identified based on methylene blue staining showing bipolar staining.

### 3.2. Biochemical activities 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, and 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 1).

**Table 1. Result of biochemical characteristics of bacterial species isolated cattle**

| **Tests** | ***Species*** | | |
|---|---|---|---|
| | *M. haemolytica* | *P. multocida* | *B. trehalosi* |
| Hemolysis | + | - | + |
| Growth on Mac conkey | + | - | + |
| Catalase | + | + | + |
| Oxidase | + | + | + |
| Indole | - | + | - |
| Urease | - | - | - |
| Lactose | + | - | + |
| Sucrose | + | + | + |
| Glucose | + | + | + |
| Maltose | + | - | + |
| Mannose | + | + | + |
| Arabinose | + | - | - |
| Mannitol | + | + | + |
| Trehalose | - | - | + |

### 3.3 Questionnaire Survey

A structural questionnaire with the primary objective of eliciting the multifactorial predisposing factors of pasteurellosis was conducted. The information was collected with those pertinent questions about; risks associated respiratory problems of cattle in the study area. Data on animal transportation, management, feed type, weather condition, and stress causing factors was assessed. Accordingly, the questionnaire survey was conducted to determine various aspects of bovine pasteurellosis in study areas. The result showed respiratory disease as the most important disease
problems, in addition, the associated, risk factors with bovine pasteurellosis were identified by respondents.

Among the assessed risk factors, age was one of the most predisposing factors to pastuerellosis in which young animals were found to be more susceptible than adults, according to the respondents as indicated in Table 2. The main stress as causing factors were indicated by the respondents such as climate change (9.89%), previous sickness (64 %), feed shortage (36.72%), drought (23.69%) and community dry season (53.64%) as indicated in Table 3.

### 3.3 Antimicrobial susceptibility profile of the bacterial isolates

From a total of 134 isolates of *Pastuerella species* obtained from the study, antimicrobial susceptibility tests were performed on 13 isolates. Due to the relatively small size, no separate analysis was undertaken for isolates and were tested for antimicrobial sensitivity for 6 different types of antibiotics. So that, the antibiotic susceptibility tests of bacteria species isolated during the study period from nasal swab carried out by disc diffusion method showed that almost *M. haemolytica* (n=13) 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 antibiotic sensitivity tests conducted for p. multocida also indicated that florifenicol, Chloramphenicol, Kanamycin, Cephalxin, Polymyxin B, Amoxicillin were the most effective antibiotic. Similarly, antibiogram profile of *B. trehalosi* revealed that all isolates were susceptible to most of the drugs tested except against penicillin, erythromycin and tetracycline. The antimicrobial sensitivity test result indicated that the bacterial isolates were more susceptible six antibiotic discs used even if the degrees of susceptibility vary in table 4. In this study, there was statistical significant difference between age and the occurrence of the disks.

**Table 2: Cattle *Pasteurellosis* in selected "woreda" association with risk factors**

| Factor | Level | No. of examined | Prevalence (%) | $X^2$ | P-value |
|---|---|---|---|---|---|
| District | Assosa | 261 | 46 (17.62%) | 12.97 | 0.01* |
| | Bambasi | 123 | 37 (30.08%) | | |
| Age | >= 2yr | 133 | 32(24.06%) | 1.36 | 0.7 |
| | 3-7yr | 162 | 32 (19.75%) | | |
| | > 7yr | 88 | 19 (21.59%) | | |
| Sex | Male | 165 | 35 (21.2%) | 0.036 | 0.85 |
| | Female | 218 | 48 (22.01%) | | |
| Management | Good | 152 | 47(30.92%) | 12.7 | 0.000** |
| | Poor | 231 | 36 ( 15.6%) | | |
| Body condition | Poor | 106 | 20 (18.86%) | 0.69 | 0.71 |
| | Medium | 116 | 26 (22.41%) | | |
| | Good | 161 | 37 (22.98%) | | |
| Vaccination status | Non-vaccinated | 121 | 31(25.6%) | 1.62 | 0 .20 |
| | Vaccinated | 262 | 52(19.84%) | | |

**Table 3. Result of questionnaire survey**

| Variable | | Frequency | Percentage (%) |
|---|---|---|---|
| Previous history of sickness | Yes | 247 | 64 % |
| | No | 136 | 35.4 % |
| Predisposing factors | Weaning | 50 | 13.02% |
| | Shipping | 60 | 15.63% |
| | Shortage of feed | 141 | 36.72% |
| | Drought and crowding | 91 | 23.69% |
| | Unknown | 42 | 10.94% |
| Major Stress | Transportation | 39 | 10.15% |
| | Climatic change | 38 | 9.89% |
| | Housing system | 145 | 37.76% |
| | Un known | 162 | 42.18% |
| Season | Dry season | 206 | 53.64% |
| | Rainy season | 178 | 46.35% |
| Drenching of drugs | Yes | 264 | 68.75% |
| | No | 120 | 31.25% |
| Frequency of out break | Frequency | 148 | 38.5% |
| | Not | 236 | 61.5% |
| Seriousness of the disease | very serious | 286 | 74.47% |
| | moderate | 93 | 24.22% |
| | Not as such serious | 5 | 1.30% |
| Control method applied | Antibiotic | 132 | 35% |
| | Vaccination | 139 | 36% |
| | Improving management | 113 | 29% |

**Table 4. Antimicrobial sensitivity test result**

| Bacterial isolates | PG | E | K | TTE | 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) | 9S) | 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) |

*S= number of bacterial isolate susceptible to the corresponding antibiotic disc used, CAF= Chloramphenicol, TTE= tetracycline, E= erythromycin, K= kanamycin, A=amoxicillin, PG= penicillin G, PB= polymyxin B, F= florifenicol, CP= cephalexin*

## 4. DISCUSSION

In the present study, overall bovine pasturellosis prevalence was (34.89%). 83/384 (21.6%)

*M. haemolytica*, 37/384(9.63%) *P. multocida*, and 14/384(3.64%) *B. trehalosi* were investigated on bacteriological analysis. Comparing the bovine pasteurellosis species identified, *P. mannhimia* was major causative agent involved in bovine pneumonic pasteurellosis. This finding was consistent with the previous reports of (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 favours the multiplication of pastuerellosis species leading to bronchopneumonic in purely pneumonia animal (Aiello and May, 1998). Although, the percentage isolation of *B. trehalosi* was relatively low (3.64%), it plays great role in the etiology and pathogenesis of bovine pneumonia should not be under estimated.

In the present study, 134/384 (34.89%) overall, bovine pasteurellosis prevalence was identified in Assossa and Bambasi districts. The present findings were comparable to Tilaye (2010), who reported 28.4 % of bovine pasteurellosis, in Debre zeit /Bishoftu/, Ethiopia. This finding was low as compared to Dereje A *et al.* (2014) who reported 50.2% of overall prevalence of bovine pneumonic pasteurellosis, 39.3% *P. multocida* and 46.4% *M. hemolytica*, in Bedelle districts, western Ethiopia. Besides this, Aschalew (1998) and Tesfaye (1997) who reported high prevalence of bovine pasteurellosis of 63.8 % and 67.6% respectively. This might be due to the different ways of taking sample from purely pneumonic cattle, improved health facilities, laboratory facilities and predisposing factors. However, comparably, Yami B.(2017) reported 3.4 % bovine pastuerellosis in Asossa and Bambasi Districts, which was low as compared to the current findings.

In this study, 9.6% *P. multocida prevalence* was isolated and identified from suspected cases or out breaks of hemorrhagic septicemia of cattle in Assosa and Bambasi districts by conventional bacteriological method. On blood agar media, the isolated bacteria produced small, round, grayish colonies with no hemolysis. Gram's staining revealed presence of Gram negative small rod shaped bacteria. The isolated organisms fermented glucose, sucrose and but not maltose and lactose. These fermented sugars produced acid without gas. The organisms also gave positive indole test and negative methyl red (MR). All these findings are similar to those reported by Cheesbrough (2006) as specific for *Pasteurella* spp. The isolated organisms were also found Gram negative and morphologically they were coccobacillary in shape. On Blood agar the isolated organisms produced grayish, opaque, circular, translucent colonies and with no hemolysis that resembles the characteristics colonies of *P. multocida*, as described by Choudhury *et al*. (1985) and Rahman *et al*. (2016). Biochemically the isolated organisms were found positive for oxidase, catalase, indole tests, negative from MR.

On the other hand, both *M. haemolytica* and *B. trehalosi* were showed hemolysis on blood agar, growth on Macconkey agar, indole negative, lactose positive, and maltose positive. *P. haemolytica* was arabinose positive and trehalose negative. While *B. trehalosi* was arabinose negative and trehalose positive (Choudhury *et al*., 1985; Rahman *et al*., 2016).

The organisms were found positive for sucrose, dextrose, mannitol and negative for lactose and maltose. Results of these biochemical tests suggested that the isolated organisms could be considered as *P. multocida.* Shivachandra *et al*. (2011) also reported similar biochemical characteristics for *P. multocida* type B. characteristics colonies of *P. multocida*, as described by Choudhury *et al*. (1985).

With regard to pasturellosis associated risk factors, higher rate of infection was associated with young age groups as compared to adults. This is because of immune status of animal being able to bacterial infection and other predisposing agents. Age distribution of the total positivity showed that, the isolation rate of the agents were not significant among different age groups (P>0.05). The status of the isolation rate of the agent was decreased when age of cattle increase so older animals are relatively resistant to pastuerellosis than younger. On the same ways age distribution of the total positivity shows that the isolation rate of the agent is not statistically significant among different age groups (P>0.05). But the status of the isolation rate of the agent decreased when age of cattle increase so older animals are relatively resistant to pastuerellosis than younger. Younger cattle less than or equals to 2 year old were 24.06% and adult cattle between 3 year and 7 years old were 19.75% positive for isolated agents , older cattle greater than 7 years were 21.6%. On this study anybody can conclude that young cattle were twice more susceptible to the isolates than the adult cattle. This study shows agreement with young age susceptibility report by (De Alwis *et al.,* 1976).

Sex distributions of the agent show no statistically significant variation even if there were variations observed. Females are 22.01 % positive while male are 21.2%. It is possible to say in study area females was more susceptible than male cattle. Even though there were not previous result discussed sex risk factors for occurrence of hemorrhagic septicemia, the sex difference might have seen in female during lactation, pregnancy and heat period/oestrus/ due to compromised natural immunity leads to stress on female cattle than male. This is similar with the study conducted to isolate pneumonic *M. hemolytica and P.*

*multocida* by Dereje *et al*., (2013) in Bedele district from apparently health cattle.

Vaccination practices have shown statistically significant which were similar P=0.02 for pastuerellosis occurrence. 25.6% of non-vaccinated and 19.84% of the vaccinated animals was susceptible to isolates. This study result was related with reported of veterinary immunology (Dowling *et al.,* 2004). It is concluded that HS can be prevented/minimized its occurrence by proper and seasonal vaccination program.

Cattle with good management practices were less susceptible to the isolated agent of HS than the poorly managed cattle. Cattle with poor body condition 30.9% were also affected by the isolates *Pasturellosis*. Whereas cattle with good body condition 15.6% were infected by isolated agents. This study is related to the positivity report of disposing factor of Sheikh *et al*. (1996) in India. Out of 30.9% positive animals to pastuerellosis those with poor body condition, poor management practices and non-vaccinated were about 25.6%. This study is in agreement with the report of De Alwis and Sumanadasa (1982).

The quality of questionnaire is an important tool in individual cases detection. The predisposing factor, in the initiation of pneumonic pasteurellosis (Jones *et al*., 1997) which might be due to reduced immune status of animal was surveyed based on the questionnaire. Accordingly, the main stresses causing factors were indicated by the respondents such as climate change (9.89%), previous sickness (64%), feed shortage (36.72%), drought (23.69%), and common in dry season (53.64%). This was in agreement with the previous findings Bruere *et al*., 2002.

In this study, the seasonal variation of the isolates was not determined due to shortage of time, moreover, even, if identification of serotype is so important. It was not conducted due to lack of laboratory facilities.

Antibiotics are important remedies in modern farm animal production. The use of these chemical agents should be based on an accurate diagnosis since there is an increasing incidence of bacterial resistance to antibiotics in humans. This phenomenon was attributed to the use of anti-microbial drugs in food-producing animals. Also, there is a concern about possible residues in animal products.

Antimicrobial sensitivity pattern of the isolates were determined in this study. All the isolates *P.multocida, M. haemolytica* and *B. Trehalosi* were susceptible 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 *p. multocida* isolates showed resistance against pencilline G. Also some of *M. hemolytica*, *p.multocida* and about half of *B. trehalosi*, did not show susceptible to erythromycin. As result, obtained indicated, the therapeutic success could be achieved using most of antibiotic in the study area. The findings was in line with the previous report (Disassa *et al*., 2013).

## 5. CONCLUSION

Pasturellosis was the major disease of cattle identified in the study area. *M. haemolytica, P. multocida and B. trehalosi* were the prominent cause of bovine disease in the present study. Origin and management system were risk factors for the disease. Young animals, dry season, shortage of feed, drought, crowding and drenching of drugs are factors which were exposed to the pastuerellosis. It also demonstrated that pasteurellosis is a highly complex multifactorial disease particularly in cattle which could be associated with stress, compromised immunity, adverse environmental condition, previous illness and co-infection made the control of pasturellosis in question. The antibiotic sensitivity tests of the isolates indicated them to be susceptible to most of the drug; however, the isolates showed resistance to some of the antibiotic disks. Moreover, an integrated management system, vaccination, controlling of the predisposing factors and use of broad spectrum antimicrobials prophylactic and treating of sick animals is suggestive. Finally, public awareness against pastuerellosis and continuous monitoring and evaluation of drug should be implemented, so hemorrhagic septicemia and/or pneumonic pastuerellosis is a disease which can be prevented by implementing a management strategy which can avoid stress; in designing an effective and efficient prevention and control options.

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