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Research Article

Isolation, Identification and Molecular Characterization of *Pasteurella multocida* from Cases of Cattle from Selected Areas

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ABSTRACT

The current research work was conducted from November 2019 to August 2020 to isolate, identify and molecularly characterizing *Pasteurella multocida* from cattle diseased with Hemorrhagic Septicemia (HS) in Bambasi and Assosa districts in Benishangul Gumuz Regional State with the objective of the existing vaccine improvement against Bovine pasturellois diseases in Ethiopia. For this purpose, a total of 30 samples were collected from clinical cases following outbreaks of HS in cattle. The samples were transported to National Veterinary Institute of Ethiopia using transport medium and then processed for isolation of *P. multocida*. There were 4 samples isolated as *P. multocida*. The isolates were identified by cultural and morphological features, biochemical features and pathogenicity tests. With the aim of additional confirmation by means of Pathogenicity study and inocucity estimation, the 4 isolates were inoculated on rabbits and the 4 (100%) isolates were found to be confirmed as Pasteurella up on post-mortem examination. The study of PCR assay revealed the presence of *P. multocida* serotype B2, and absence of *P. multocida* serotype E2. In conclusions *P. multocida* serotype B2 in the designated area is considered as possible pathogens in resulting HS in cattle. However, further study supported by DNA sequencing is essential to finalize these conclusions. At the end based on the conclusions made, the recommendations were forwarded.

Keywords: Asosa; Bambasi; Cattle; Haemorrhagic; septicemia; *Pasteurella multocida*; Polymerase chain reaction

Abbreviations: ALFD: Assosa District Office of Livestock and Fishery Development; BLFD: Bambasi District office of Livestock and Fishery Development; CLSI: Clinical and lessons Standard Institute; CSA: Central Statistical Agency; CSY: Casein Sucrose Yeast; EA: Environmental Agency; FMD: Foot and Mouth Disease; HS: Hemorrhagic Septicemia; NMSA: National Meteorology Service Agency; NVI: National Veterinary Institute; OMA: Outer Membrane Protein; SDS: Sodium Dodecyl Sulfate; TEM: Transport Enrichment Medium.

INTRODUCTION

Even though Ethiopia is one of the largest countries with cattle population in Africa the productivity from the livestock sector has been insignificant due to the existence of infectious diseases like Pasteurellosis which affect the productivity of the sector [1,2].

There are two forms of pasteurellosis namely; Pneumonic and Septicemic pasteurellosis. Septicemic pasteurellosis is caused by *P. multocida*. It is supposed that Louis Pasteur was a person who identified for the first time the bacterium *P. multocida* as the causative agent of fowl cholera before 125 years ago. During his essential experiment he also showed though many passage this bacterium loses its virulence activity, but still inoculation the attenuated strain can enhance the protective immune response [3].

The species name "Multocida"in Latin means killing several which was invented viewing predominantly considerable mortality related as a consequence infection by this bacterium [4]. Among the family of pasturelliace *P. multocida* consists of a varied group of gram negative bacteria which are liable for wide range of economically important infections of animals, including cattle and buffalo [5].

Morphologically, *P. multocida* comprises a small, gram-negative, non-spore-forming, none-motile and penicillin sensitive coccobacillus with bipolar staining characters. The bacteria characteristically seem as a single bacillus on Grams' stain: though, pairs and short chains can also be seen [6]. Based on serological classification the strains of *P. multocida* are currently grouped in to five groups namely; A, B D E, and F according to capsular configurations. Similarly, based on somatic serotype classification it is grouped in to 16 somatic serovars (1-16).

Based on serological classification the strains of *P. multocida* are

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currently grouped in to five groups namely; A, B, D, E, and F capsular sero groups. Similarly, based on somatic serotype classification it is grouped in to 16 somatic serovars (1-16). It can also exist assembled based on their outer membrane protein type 16S rRNA-type. The ribosomal RNA (r RNA) of gram negative bacteria consists of two structures namely; 16 rRNA and 23 rRNA. Thus based on the 16 rRNA-classifications there are seven 16S type which belongs to a single family of the majority of clinical [7].

P. multocida frequently occurs as a commensal in the upper respiratory tracts of several live stocks, poultry and domestic pet's species [8]. However, it is kown to cause a wide range of economically important diseases like Haemorrhagic Septicemia (HS) in hooved animals specifically in cattle and buffalo in many Asian and African countries resulting in to high death and morbidity [9-11].The stains of *P. multocida* causing HS consists of serotype B:2, B: 2, 5 and B;5 in Asian countries and E;2 in African countries [12]. Moreover, in addition to causing HS it is known to result I atrophic rhinitis in Swine, fowl cholera in poultry [4,13]. Serogroup A and D are identified to infect calves, pigs,' sheep, goats and rabbits in which they cause different infections [14]. Moreover, serogroup F has been identified as a causal agent of fowl cholera but [15], in latest period, it has similarly been recognized in some mammalian species in different biosphere parts [16].

HS is a very acute, lethal form of disease of animals particularly that of cattle. It is one of the most communal economically significant infections of water buffalo and cattle in tropical areas of Asia, Africa and the Middle East. In Ethiopia also it is one of devastating diseases resulting in economic loss [17]. The disease alone does not result in high morbidity and mortality rate, but concurrent infection due to stressing factors like undernourishment, viral and bacterial attack of immune system, inclement weather, dehydration, nasopharyngeal settlement and shipment [18]. Tentative diagnosis can be made in many ways but, confirmation can be established by isolation of the causative organisms and their conventional and molecular characterization [19].

In Ethiopia the animal quarantine issue is underrated and is very poor due lack of policy regarding controlling system of the restriction of animal movement within the country and to the noubouring countries. Due to these cattle move with in the country without any restriction for marketing grazing and water. Likewise, cattle always come in the country from borders liker South Sudan where *P. multocidasero* type E2 and B2 causing HS are endemic. However, due to limited study, in Ethiopia particularly serotype E was not reported even though the suspection is very high. This is due to lack of detailed molecular based information on the prevailing serotypes at present. National Veterinary Institute (NVI) is producing only vaccine against Multocida type B2, and there is some complainton current available vaccine from some areas in Ethiopia, and this might be due to emerging of new strains which might be serotype E. Therefore, the molecular identification of the serotypes would improve our understanding of the epidemiology of strains involved to the disease as well as provide information on the most appropriate serotypes to be used for successful vaccination against bovine HS.

Thus, the general objective of the study is to determine the occurrences and molecularly characterize *P. multocida* strains causing HS for the purpose of vaccine improvement.

The specific objectives are:

- Isolation and Identification of Strains *P. multocida* from cattle in selected areas using conventional method.
- Molecular characterization of identified strains (Serotyping) for vaccine improvement.

LITERATURE REVIEW

Overview on is caused by *P.Multocida*

HS is a very acute, highly lethal form of disease of animals specifically buffalo, cattle, and bison. It is one of the most communal economically important infections of water buffalo and cattle in tropical areas of Asia, Africa and the Middle East, with erratic eruptions happening in southern Europe. The Disease is most devastating to smallholder farmers where husbandry and preventive practices are deprived and free-range management is common. Usual disease happens occasionally in sheep, pigs, and goats and has been conveyed in donkeys, camels, elephants, camels, yaks, and several species of deer and other wild ruminants [20].

Etiology

The Office International des-Epizoot (OIE) defined the conventional HS based on Carter and Heddleston classification system as the diseases caused by *P. multocida* serotypes B:2 and E:2. Currently, serotype B:2 has been known in most areas where the disease is widespread. However, serotype E:2 has been found only in Africa [12].

Transmission

The common transmission route of HS from one animal to another is *via* inhalation or ingestion in the course of direct contact of adulterated feed and water. The causative bacteria are assumed to feast mostly in respiratory excretions, but they can also be establishing in other secretions and excretions, comprising feces and urine. Some animals remain carriers of the organisms in lymphatic tissues like in the tonsils, and shedding it periodically in nasal secretion during stress conditions [21].

Epidemiology

Three aspects affect the world wide circulation of HS: climatic circumstances, husbandry practices and the species of animal [22]. Later on disease eruption alive animals may come to be latent, asymptomatic carries of contagion. Herd immunity is high and so no new cases happen. Conversely, succeeding the contact of naïve animals, for instance, after animal travels, or the birth of new naïve animals, further disease outbreaks may occur. The infection was nearly non-existent where there was a prevalence of peaks. Intermittent sporadic eruptions occurred in areas with landscape, climate and animals that were between these dissipations [23].

Pathology and pathogenesis

On necropsy, the best evident gross lesion is subcutaneous oedema in the submandibular and brisket areas [24]. Petechial hemorrhages are originating subcutaneously and in the thoracic cavity. Furthermore, congestion and numerous degrees of consolidation of the lung might happen [24]. The death will happen contained by 24-36 hours, have only few petechial hemorrhages on the heart and wide spread blood accumulation of the lung, while in animals that die after 72 hours [24].

Geographic distribution

HS is an important infection of cattle and water buffalo in Asia, Africa and the Middle East. The primary incident is in Southeast

Asia. The disease has likewise been transferred sporadically in Europe, where this disease is thought to occur frequently in southern Europe but has similarly continued observed rarely in further nations, counting those with humid weathers like Estonia, Latvia, Georgia and Ukraine. Numerous eruptions have remained conveyed as 2010 in Central Europe like Germany, Hungary and Serbia, signifying that the causal bacteria might have remained presented to this area after seemingly being lacking for eras [24]. HS-inducing serotypes of *P. multocida* are not supposed to flow in North America, Australia or New Zealand. They remained similarly supposed to be lacking from South and Central America; nonetheless, this disease was conveyed to the World Organization for Animal Health (OIE) from Colombia in 2007, Venezuela in 2015 and Ecuador in 2018. There appears to be little or no published material around these eruptions [25,26].

Diagnosis

Clinical signs: HS diseased cattle develop peracute or an acute course of infection with signs of septicemia comprisingnasal discharge, malaise, recumbency, fever, dyspnoea, oedema and hemorrhages resulting in high mortality rates (OIE, 2012).

Postmortem lesions: HS is rapid in its course, therefore, the infected animals' remains in good condition after death. Gross pathological lesions are mutable and might be understated and comprise circulatory and degenerative disorders. Liver looks yellowish-brown in pigment, hard and degenerated, with a clear lobular configuration. The tracheal mucosa is hyperemic, comprising plentiful frothy fluid, and the lungs are edematous and bloody. The spleen is enflamed, with smoothed boundaries and splenomegaly. The occurrence of coagulated blood in blood containers is due to dispersed intravascular coagulation [26].

Bacteriological method: Bacteriological medium like Blood agar, Nutrient agar, Casein-sucrose yeast (CSY) broth, Dextrose-starch agar, CSY agar CSY-blood agar, Enriched tryptophan agar and MacConkey agar (Oxoid, England), is used as selective medium because *P. multocida* cannot grow on MacConkey. The colony character of *P. multocida* like color, shape, size, consistency and a gram staining of culture media is considered. Conditions like growth reticence, motility; hemolytic property of growing bacteria was also seen [27]. The grams staining result under microscope reveals gram negative, small size rod shape of coccobacilli was being appreciated.

Serological technique: Serotyping using serological assays used comprise an agglutination test for somatic typing, rapid slide agglutination or indirect hemagglutination assays for capsular typing, agar gel immune diffusion for both capsular and somatic typing, and counter-immunoelectrophoresis for the rapid identification of capsular types B and E. Some sequesters can be hard to type with these traditional approaches [27].

Molecular identification: Molecular acid based diagnostic test has been establishing to be subtler and trustworthy than the conservative method of bacterial identification. The main benefit of nucleic acid based tests is that they decrease the time consumption and permit the discovery of the organism's genome even if it is in tiny quantities, thus increasing the sensitivity and specificity of the test [28]. Polymerase chain reaction is one such test that can be used for the identification of organisms at any level, like strain, species, genus or all members of a domain, just by using explicit primer sequence [29].

Treatment

Antibiotics are the only effective drugs for the treatment of HS. However, they are only effective only if they are taken place immediately after the start of clinical signs. Monitoring the animals for febrile condition and treating them on the onset of the disease is advisable during the outbreak of the infection IV antimicrobials drugs like Oxytetracycline, pen-strep and various sulfonamides [30].

Control and prevention

Extended and unselective use of antibiotics against *P. multocida* can lead to Multi Drug Resistant (MDR) [31]. Consequently, an effective control of disease can merely is attained by vaccination. Formalin-inactivated, alum-precipitated vaccine of *P. multocida* currently the most broadly applied vaccine for preventing HS. Though, immunity persists for 4 to 5 months and protecting efficacy is only 60% [32].

MATERIALS AND METHODS

Research area

The research was accompanied from December 2019 to August 2020 at two designated districts in Assosa Zone of Benishanigul Gumuz Regional State, namely Bambasi and Assosa. Assosa town is the clericalfocus of the Benishanigul Gumuz Regional State, and located 676 km West of Addis Ababa. Assosa zone is one of the 3 clerical zones of Benishanigul Gumuz Regional State. It is found in the Southwest part of the region. Assosa area is composed of 70 clerical farmworker associations and 4 Assosa town "kebeles" [33] which is located at 9.60° and 10.45° N and 34.20° and 34.58°E longitude. The elevation of Assosa arrays from 580 to over 1544 meter above sea level [34]. The entire zone of the district is 2317 Km2 of which area is categorized by low land flat agro-ecology rendering to National Meteorology Service Agency [34]. Bambasi district is situated in Benishanigul Regional State Southern west of the Assosa zone and 616 km west of Addis Ababa at 9.45-9.75°N and 34.35-34.88°E with a least and supremeelevation of 1350 m and 1770 m overhead sea level. Bambasi Woreda has the livestock populace 38964 cattle, 11,990 goats, 3452 sheep, 1995 donkeys, and 38442 poultry [35]. These regions were designated on the foundation of antiquity for occurrence of cattle HS, cattle drive to market, foraging land, drenching points and extent of cattle populace by the district office of livestock and fishery development.

Research design

The research design was a cross sectional study and it was intended for isolation of *P. multocida* type B2 and E2. The study area was selected purposively based on epidemiological geographical location as Benishangul Gumuz regional state is the neighboring region to South Sudan where *P. multocida* serotype E is endemic. Three visits were made to the study area purposively based on outbreak information. The choice of the animals was established on distinctive clinical signs of HS, and sampling of swabs and blood were completed from clinic and resident areas where eruption happened for isolation and identification purpose.

Research methods

Target population: The study population was local breed cattle infected with HS from the study area. The target populations were determined based on outbreak information of HS for the Isolation and identification of *P. multocida* with the prominence of HS Causing Serotypes namely, B2 and E2 from indigenous cattle

breeds.

Sampling technique: The technique used for sample collection from infected animals were purposive sampling and each animal was selected based on prominent specific clinical sings of HS from the study area where outbreak occurs and from those HS infected cattle brought to veterinary clinic in the study area. Two types of samples were collected namely, blood and swab samples. Swab was collected from nasal cavity of sick animals by introducing nasal swab about 5 cm deep in to the nasal cavity. Similarly, from those animals with high body temperature the blood was collected from jaguar vein after disinfecting the area with 70% alcohol.

Sample size: The sample size was determined based on the number of infected animals that were considered as an outbreak of HS in the study area. Thus, the purposive sampling method was used, therefore, the number of cattle sampled from Asosa, Bambasi and districts were 30 during the total three visits to the study area.

Data analysis method: For interpretation of the results, the whole data was entered into the Microsoft Excel sheet Data management and Analysist, and then it was analyzed using SPSS version 20. Regarding the molecular detection, the banding patterns of individuals' strains were scored based on the presence or absence of the bands with the appropriate base pairs.

Isolation and identification of bacterial isolates

Cultural methods: All samples were plated onto nutrient agar (Oxoid, England) and incubated at 37°C overnight [36]. Single specific colony was taken and subculture on 10% sheep blood agar, then incubated at 37°C for 24 hours to observe the hemolytic effect of the organism. At the same time another single colony was taken to check the grams' stains result under microscope. Sub culturing on selective medium called MacConkey agar (Oxoid, England) was done as *P. multocida* do not grow on this media [37].

Biochemical methods: Succeeding biochemical tests were done for identification of *P. multocida*. These tests include Oxidase, catalase, indole, H_2S, motility and fermentation of glucose, mannitol, sucrose, Dextrose, maltose, lactose, using methods described by Cowan and Steel with modification of using CSY agar (Oxoid, England) [23,38,39]. The result found were recorded and compared for verification 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).

A filter paper was soaked with the substrate Kovac's oxidase reagent moisten the paper with a sterile distilled water. Then s single colony to be tested was placed on the filter paper. Finally Observation around inoculated area of paper for a color change to deep blue or purple within 10-30 second was detected. A negative reaction is indicated by absence of color. The Swab method was also done by sinking swab into reagent and then traces an isolated suspect colony and was detected for color change within 5-10 seconds and its result was similar with wet filter paper. Catalase test was done by putting a single colony of growth from culture onto a clean microscope slide. A few drips of H_2O_2 were added onto the smear. It was assorted with a tooth pick. A positive outcome was revealed the fast occurrence of O_2 as showed by tangent foam formation [40].

Pathogenicity test: 24 hours' growth from Tryphosa agar slant was suspended in 1 ml sterile physiological saline solution as labeled by Bain et al. [39]. The mean suspension density of each sample having 1 × 109 CFU/ml was evaluated at 540 nm. To evaluate the Pathogenicity of samples, rabbit was chosen and 0.5 ml growth suspension was injected by ear vein. Postmortem examination of the lifeless animal was done and re-isolation of the bacterium from the heart blood were cultured on Tryptose agar and incubated at 37°C for 24 hours.

Molecular identification: The pure growth of the bacteria was processed for molecular characterization through Polymerase Chain Reaction (PCR) using universal and capsular serotype specific primers of *P. multocida* (Table 1) following [37].

RESULTS

Isolation and identification

Cultural characteristics of *P. multocida*: Up on culturing of the total collected 30 blood and swab samples, it was revealed that only 4 samples out of 30 samples (13.3%) were found to show the cultural characteristics of *p. multocida* (Small, glistening, mucoid no hemolytic on blood agar and didn't grow on MacConkey agar). All four isolates of Pasteurella are short coccobacilli with rounded ends, stained negative by Gram staining. Bipolar coloration of cells can be seen when smears of the studied culture are stained by Romanovsky-Giemsa method (Figures 1-4) [40].

Pathogenicity test activity of *P. multocida*: With the aim of further confirmation like Pathogenicity study and acuity estimation, the 4 isolates were injected to Rabbit. All of the samples caused hemorrhages with in the heart and mortality in the animals. Replication of all of these tests on samples of died rabbit confirmed the isolates were Pasteurella (Figure 5).

Biochemical characteristics of *P. multocida*: All the four isolates of biochemical characterization revealed that they belonged to Pasteurella genus. All strains isolated from Asosa and Bambasi districts were oxidase, Indole and catalase positive, did not procedure hydrogen sulfide, did not have hemolytic effect and were negative in Voges-Proskauer reaction. All isolates fermented glucose, Dextrose mannitol, sucrose, and were negative for maltose andlactose (Figures 6-10) [14].

Table 1: Primers used for characterization of *P. Multocida*.

Type of primer	Direction	Name of primers	Sequences	Mole. weight
Universal primer	Forward primer	KMT1 KMT1T7	5'ATCCGCATTTACCCCAGTGG3'	460 bp
	Reverse primer	KMT1SP6	5'-ATTC-3'	
Serotype	Forward primer	CAP E	5'TCCGCAGAAAATTAATTGACTC3'	511 bp
E specific	Reverse primer	CAP E	5'GCTTGCTGCTTGATTTTGTC-3'	
Serotype	Forward primer	KTT72	5'AGGCTCGTTTGGATTATGAAG3'	620 bp
B specific	Reverse primer	KTSP61	5'ATTCCGCTAACACACTCTC3'	

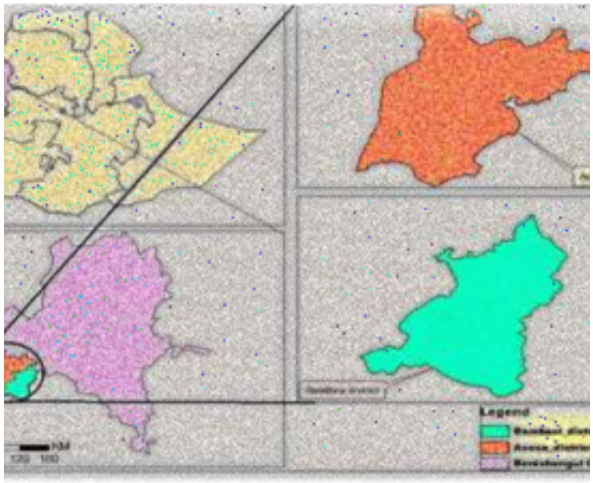

Figure 1: Study area created by Arch GIS.

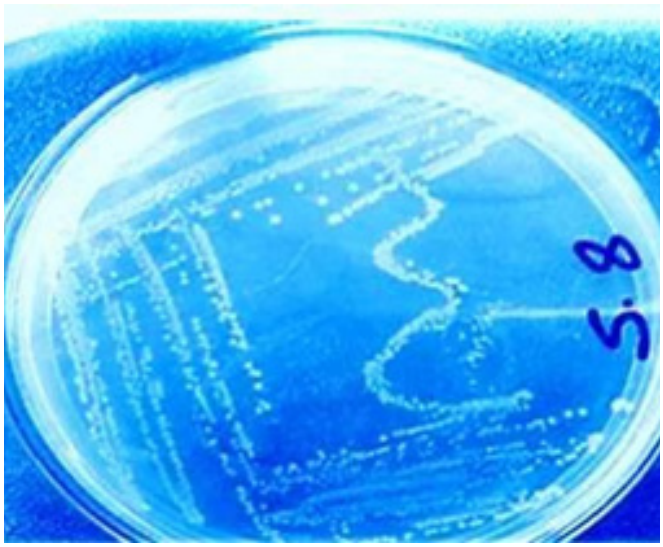

Figure 2: Growth colony of *P. multocida* on nutrient agar.

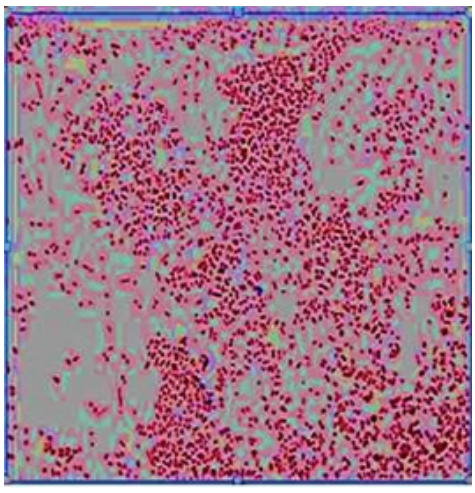

Figure 3: Grams' stain isolated *P. multocida*.

Figure 4: *P. Multocida* non hemolytic colony on blood agar.

Figure 5: Re-isolation of *P. multocida* from rabbit showing hemorrhage in the heart.

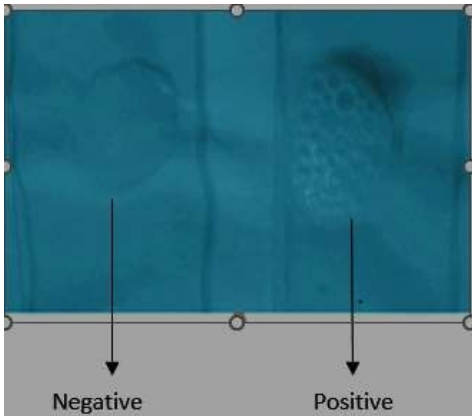

Figure 6: Catalase test of *P. multocida*.

Figure 7: Oxidase test of *P. multocida*.

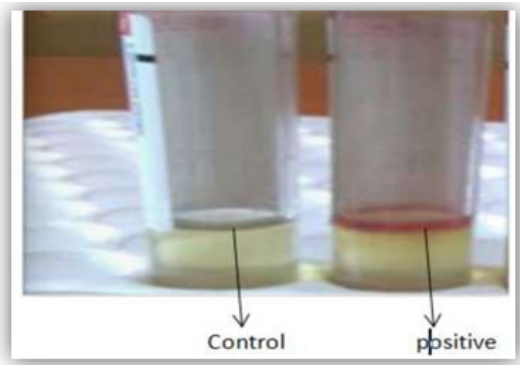

Figure 8: Indole test of *P. multocida*.

Figure 9: H2S and motility test of *P. multocida*.

Figure 10: Conventional PCR using different primers for *P. multocida* characterization. Note: Lane M: DNA marker, lanes 1-4: extracts from the isolates amplified using universal primer (primer KTT1T7 and primer KMT1SP6), lane 5 -8: the same isolate amplified using serotype B6 specific primer (primer KIT72 and primer KTSP61), lane 9-12: the same sample amplified using serotype E specific primer (primer CAPE forward and reverse). Lane 13 and 14 represented Negative controls whereas; lane 15 and 16 represents serotype Band E specific primers respectively which are positive controls for universal conventional PCR. Lastly, lane 17 exemplifies positive control for serotype B6 *P. multocida*.

DISCUSSION

Pasteurella multocida is an important pathogen causing a number of diseases in various domestic animals. The most important disease is HS resulting in heavy economic losses. This study was under taken at Benishangul regional state namely Asosa and Bambasi district aimed to isolate and identify *P. multocida* serotypes resulting to HS by conventional and molecular methods. Up on culturing the organisms shows small, glistening, mucoid no hemolytic on blood agar and didn't grow on MacConkey agar. Morphology by garm's stain showed that it was gram negative coccobacilli under microscope. The biochemical Characteristics were oxidase, Indole and catalase positive, did not procedure hydrogen sulphide. All isolates fermented glucose, Dextrose mannitol, sucrose, and were negative for maltose and lactose. All these specific charactestics are similar to those described by Chesbrogh [41,42]. Similarly, Choudhury et al., [43] and Rahman [44] also stated the morphological features of *p. multocida* with grams' negative and coccobacilli, with no haemolytic effect on blood agar which is similar to this study. Study by Cowan and Steel [38] also similar to this finding regarding the biochemical futures of *P. multocida* like positive for Oxidase, catalase, Indole tests and negative for H2S. Shiva chandra [13] also reported similar biochemical characteristics for *P. multocida* type B characteristics colonies of *P. multocida*, as described by Choudhury et al., [43] and Yami et al., [17].With the aid of further confirmation like Pathogenicity study and acuity estimation on rabbit the selected organisms showed death and haemorrhage in the heart upon post mortem examination which is typical characteristics of pathogenic effect of *P. multocida* as described in Bain et al. [39].

The overall isolation percentage of *P. multocida* from the total number of samples examined was 3.39%. The various characteristics of *P. multocida* isolated during the present investigation are in conflict with the findings of Francis and Carter who reported the isolation of 17.5% *P. multocida* from calves in Zambia [31]. This might be due to the small number of animals diagnosed due to the disease in this specific study. For confirmation of *P. multocida* serotypes B:2 (6:B) and E:2 (6:E) as the principal causes of HS the PCR test was done based on Townsend et al. by using universal and specific primers [37].

Although serotype B: has been mainly reported in Asian countries and E:2 in African countries based on Townsend [37],both serotypes have been recovered from the disease in some African countries as Townsend [37]. Likely, in this study PCR remains only 100% specific for isolate of *P. multocida* type B cultures with the predominant somatic antigen being either type 2 were identified by the amplification of a 620 bp fragment. However, the isolates in study area were not detected by conventional PCR using the amplification of an approximately 511 bp fragments with the CAPE forward and reverse serotype E primers which disagree with the study by Carteret., aland De Alwis [45], which says serotype E is the African serotype causing HS. Even though it is too early to exclude the effect of the emergence of new stains for ineffectiveness of the vaccines against Haemorrhagic Septicemia, lack of maintaining cold chain during transportation and storage of vaccines, inappropriate dose and route of administration and lack of strategic vaccination might be the cause of vaccine ineffectiveness [46,47]. However, still it is very important to cover a wide further epidemiological study area to conclude these suggestions.

CONCLUSION AND RECOMMENDATIONS

In conclusion, HS is the most important disease of cattle in Africa caused by *P. multocida* serotypes. The disease has been an extended standing delinquent with eruptions of the disease occurring mostly in the yearly basis. This is the preliminary study in Ethiopia, which comprises about the spread of both HS causing strains namely Serotype B and E at the same time in cattle in the field. Though, it was concluded that districts of study area were accepted as HS positive with serotype B specific and negative with serotype E specific. From the total sample collected from infected animals with HS 13% of samples were found to be positive for serotype B specific. However, no samples were found to be positive for serotype E specific. Isolates were highly pathogenic to rabbit. This study constitutes a crucial and initial step allowing in designing or improving the effectiveness and efficiency of the existing vaccine to support pasteurellosis disease controls in the country. Therefore, based on above conclusions the following recommendations were forwarded:

- The molecular investigation should be supported by the like DNA sequencing as to confirm *P. multocida* strains that will ultimately helpful in designing an effective and efficient vaccine

- Too early to still recommend that *P. multocida* serotype E is not circulating in Ethiopia, therefore, wide area coverage needed for further study in the area.

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