INTERNATIONAL JOURNAL OF CURRENT RESEARCH IN BIOLOGY AND MEDICINE
ISSN: 2455-944X
www.darshanpublishers.com
DOI:10.22192/ijcrbm Volume 2, Issue 3 - 2017

Original Research Article

DOI: http://dx.doi.org/10.22192/ijcrbm.2017.02.03.002

# Study on the Epidemiology and Strain Identification of Small Ruminant Pneumonic Pasteurellosis for Vaccine Production in Selected Districts of Wolaita Zone, SNNPR

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**Abstract**

This study was carried out from December 2015 to July 2016 in Wolaita Zone of the SNNPR, Ethiopia to know the dominant serotypes of *Maheinemia hemolytica* and *Pasteurella multocida* biotype A circulating in small ruminants in the region for new vaccine strain production and to investigate the role of potential risk factors associated with the occurrence of the disease. Pasteurellosis, known locally as "Oshencha," has become a disease of major concern in the study area. Sera from 384 sheep and goats were examined for three serotypes of *Pasteurella haemolytica* A and *P multocida* biotype A using indirect hemagglutination testing. Positive results were seen in 354 samples for the presence of specific antibodies to all four serotypes examined. The prevalence rates of *P haemolytica* A7 and A2 serotypes were the highest (82.6% and 81.8%, respectively), followed by serotype A1 (76.8%). Six percent of the sera were positive for *P multocida* A. The possible causes for the occurrence of the disease are discussed and the results are compared with works done on small ruminants in Ethiopia and abroad. Finally based on the results obtained, recommendations are forwarded for further study and for the control and prevention of small ruminant pneumonic pasteurellosis.

**Keywords:** Respiratorry syndrome, pasteurellosis, small ruminant, Wolaita, Ethiopia

## 1. Introduction

Small ruminant (sheep and goats) production is an important activity for smallholders, particularly for resource poor farmers in many parts of the country. They provide vast range of products and services such as immediate cash income, meat, milk, skin, manure, risk spreading/ management and social functions (Adane and Girma, 2008). They are also main sources of foreign currency (Berhanu *et al*., 2006). Small ruminants with their higher reproductive capacity and growth rates are ideally suited to production by resource-poor smallholders (Tibbo, 2006). According to a recent report by Central Statistics Authority of Ethiopia, there are about 26.1 million and 21.7 million sheep and goat population heads in Ethiopia respectively and about five million sheep and goats are slaughtered every year in the country which indicates their potential for meat production (CSA, 2008).

Though small ruminant production is playing an important role in the improvement of income for poor farmers, poverty and hunger alleviation and can contribute a major role in the country developmental plan; its production and productivity and producers' benefits are far below expectations due health constraints, feed shortage both in quality and quantity, low genetic potential and management problems (Zinash *et al*., 2001).

Among others infectious diseases are the major constraints to small ruminant production in Ethiopia (Zinash *et al*., 2001; Sisay, 2006; Tibbo, 2006; Tsedeke, 2007; Getahun, 2008; Gizaw *et al.*, 2010). Of all diseases those affecting the respiratory system are generally the leading causes of morbidity and mortality in large domestic animals and are a major source of economic loss to farmers. Furthermore, these diseases predispose animals to other infections (Zinash *et al*., 2001).

Pneumonic pasteurellosis in small ruminants is one of the main respiratory disease complexes and generally is thought to result from invasion of the lung by *Pasteurellaceae* (Alley *et al.*, 1999; Lopez, 2001; Donachie, 2007; Mohamed and Abdulselam, 2008). *Pasteurella multocida*, *Mannheimia haemolytica*, and *Bibersteinia trehalosi* (all formerly in the genus *Pasteurella*) are the three most commonly isolated bacterial agents from pneumonic pasteurellosis that result in high rates of illness, morbidity, and mortality in domestic animals (Gilmour and Gilmour, 1989; Brogden *et al.*, 1998; Alley *et al.*, 1999; Donachie, 2007). According to the taxonomical studies based on genotypic and phenotypic analysis there are about 17 serotypes of these organisms (Younan and Fodor, 1995; Biberstein and Hirsh, 1999).

In Wolaita zone of SNNPR, despite annual vaccination against pneumonic pasteurellosis with a monovalent vaccine (inactivated *P. multocida* biotype A), there are high rates of mortality and morbidity following respiratory distress. This is accompanied with strong compliant from the field veterinarians and farmers in the region for the failure of vaccination against this disease and this could probably be due to the difference in the vaccine strain used and the serotype of the organisms prevalent in the region.

In Wolaita zone of SNNPR, outbreaks of pneumonic pasteurellosis occur frequently, killing significant numbers of sheep and goats. Pneumonic pasteurellosis is therefore a high-priority issue at the national and regional level due to the significant economic losses it causes through mortality, morbidity, and the high cost of treatment. Based on the background information and the need for identifying the causes of pneumonic pasteurellosis of small ruminants in the region the present study was conducted with the objectives: to establish the prevalence of different serotypes of *Maheinemia hemolytica and Pasteurella multocida*, to know the dominant serotypes of *Maheinemia hemolytica and Pasteurella multocida* in the region for new vaccine strain production and to investigate the role of potential risk factors associated with the occurrence of the disease.

## 2. Materials and Methods

This work was a collaborative endeavor among various stakeholders having direct or indirect concern in the small ruminant production and farmers communities.

### 2.1 Study area

The study was conducted in Wolaita Sodo, SNNPR, 130 km northeast of Addis Ababa, the capital of Ethiopia. Located at 9°36'' N and 9°36'' E, Wolaita Sodo is a plateau in southern Ethiopia that lies at an altitude of 2300 m above sea level with a bimodal rainfall pattern consisting of a long rainy season from June to September and a short rainy season in February and March. The area has mean annual temperature of 12.6°C, an average annual rainfall of 956 mm, and a mean relative humidity of 59.6%.

### 2.2 Study population

The study was conducted on 384 local breeds of sheep and goats in two different peasant associations without discrimination of their age, sex, body condition and coat color. They were in different age group and body condition. Most of them were apparently healthy during clinical examination.

### 2.3 Study methodology

#### 2.3.1. Study type

The study was a cros-sectional study undertaken to establish the prevalence of different serotypes of *Pasteurella hemolytica* and *Pasteurella multocida biotype*A; assessing the environmental and managerial factors that enable the disease to proliferate and detecting concurrent infections that aggravate pneumonic pasteurellosis in sheep and goats in this region of Ethiopia.

#### 2.3.2 Sample size

The sample size for the present study was determined according to the formula given by Thrusfield (2005) for random sampling method. A 5% absolute precision and 95% confidence interval was used for determining sample size. An expected prevalence of 50% was used to determine the maximum sample size. Accordingly,

about 384 sheep and goats from each woredas was included in the study.

$$N = \frac{1.96^2 x Pexp (1-Pexp)}{d^2}$$

Where N = the total sample size, Pexp= expected prevalence, d= absolute precision

### 2.4 Data collection and sampling procedure

#### 2.4.1 Clinical examination

Before sampling, general physical examination was conducted on each animal. Data regarding current clinical manifestations of disease were recorded with special attention to the respiratory system. All these information were preceded by age, sex, body condition and other related information. The age and body condition of sheep and goats were determined according to Gatenby (1991) by observing the dentition of the sheep and goats Aitken (1995) respectively.

#### 2.4.2 Questionnaire Survey

Pre-tested structured questionnaire was distributed to veterinary personnel and farmers in the respective districts to determine management and husbandry risk factors, which are known or thought to influence the spread and maintenance of pneumonic pasteurellosis. Farmers were given a two-part questionnaire; the first part asked about general farming systems including the number of families, types and number of domestic animals and farming constraints. The second part of the questionnaire inquired about sheep and goat production– number, breed, sex, utilization, feeding system, diseases in chronological order of occurrence, vaccination history, type of barn, and use of medications.

### 2.5 Specimen collection and transportation

Blood samples were collected from jugular vein of each animal with no history of vaccination for pneumonic pasteurellosis using plain vaccutainer tubes and needles. The blood samples were identified by each individual animal and allowed to clot at room temperature. Then serum was separated from clotted blood by centrifugation. The separated sera were stored at –20 $c^o$ until tested by indirect hemagglutination test which is a confirmatory test.

### 2.6 Serological examination

Sera from 384 sheep and goats were collected in April and examined for serotype-specific Anti-bodies by indirect hemagglutination test for four pathogenically important *P haemolytica* A serotypes and *P multocida* A. Known *Pasteurella* serotypes were cultured in tryptose broth at 37°C overnight, inactivated in a 60°C water bath for 30 minutes, and centrifuged at 3000 rpm for 30 minutes; the clear supernatant antigen was retained. Five milliliters of sheep blood, treated with Alsever's solution (18.66 g dextrose/glucose, 4.18 g sodium chloride, 8 g sodium citrate, and 1 L distilled water) was centrifuged at 2500 rpm for 5 minutes, washed twice with phosphate buffered saline (PBS), and again centrifuged at 2500 rpm for 5 minutes, and mixed with 50 µL of packed red blood cells (RBC) with 5 mL of antigen. Then 50µL gluteraldehyde was added and homogenized with gentle shaking, incubated for 1 hour at 37°C, centrifuged and washed two times, and finally suspended with 5 mL of PBS. In V-bottomed microplates 50µL of PBS were dispensed to all wells; 50 µL of test sera were added to the first column and serially diluted by pipetting 50 µL up to column 12. Fifty microliters of sensitized RBC were added to each well and incubated for 1 hour at 37°C. An agglutination rate of 50% or more was taken as a positive result, and those showing a hemagglutination reaction in 1/40 dilution and above were taken as a positive sample.

### 2.7 Data management and analysis

The data was entered in to Microsoft excel spread sheet and coded appropriately. For data analysis SPSS version 20, was used. In this data analysis, descriptive statistic was used to determine the proportion of the different serotypes. Chi-square was used to test the association among risk factors and the identified serotype.

## 3. Results

### 3.1 *Pasteurella* Serotypes

The prevalence rates of *P haemolytica* A7 and A2 serotypes were the highest (82.6% and 81.8%, respectively), followed by serotype A1 (76.8%) (Table 1). Six percent of the sera were positive for *P multocida* A.

**Table 1.** The prevalence of *Pasteurella* serotypes in sheep and goats, Wolaita Zone, Ethiopia.

| Serotype | Frequency | Prevalence |
|---|---|---|
| P hemolyticaA7 | 317 | 82.6 |
| P hemolyticaA2 | 314 | 81.8 |
| P hemolyticaA1 | 295 | 76.8 |
| P multocidaA | 23 | 6 |

### 3.2 Questionnaire data

Answers to the questionnaire indicated that the average family size in the area is 6 to 7 and each farmer averages 11 sheep, 6 cattle, 4 chickens, 2 donkeys, 1 horse, and 4 goats. Most farmers (74.5%) keep their sheep in fully enclosed barns, 8.5% have animals is partially enclosed barns, and 17% have animals in open barns. A great majority of farmers (93.3%) used antihelmintics to treat their sheep and 26% of them vaccinated their sheep against pasteurellosis. Nineteen percent of farmers surveyed complained about animal production constraints. Diseases, a shortage of feed, inadequate veterinary service, and a lack of access to improved breeds were listed as factors contributing to animal production problems. An outbreak of respiratory disease was not observed during the study period except for minor syndromes such as cough and nasal discharge.

## 4. Discussion

Pneumonic pasteurellosis, known locally as "Oshencha," is a disease of sheep and goat that is of prime concern in Ethiopia. Despite annual vaccination programs against pasteurellosis using killed *P multocida* biotype A-containing vaccine (National Veterinary Institute, Ovine pasteurella vaccine), high mortality and morbidity continue to be observed by farmers and veterinarians. A pasteurellosis outbreak was not observed during the study period, which might be because animals developed a lasting immunity from the previous outbreak.

Out of the total 384 serum samples subjected for serological investigation, 354 (92.2%) samples show seropositivity to *Pasteurella* antibodies but the rest 30 (7.8%) were negative. This prevalence rate is higher than the works of Gelagay *et al.,* (2004), Hussein and Elsawi (1984) and Pegram *et al.,* (1980) who identify the various serotypes of *Pasteurella Haemolytica* at a relatively lower prevalence rate. This variation in the isolation rate could be due to variation in sample size, sample collection and preservation or it could be also due to variation in the severity of risk factors and geographical areas. In general, three *P haemolytica* biotype A Serotypes (A1, A2 and A7) and *P multocida* A were detected, with significant variation in prevalence between the two species ($P < 0.001$).

*P haemolytica* biotype A serotypes A7 was one of the commonly identified serotype in this study. It was detected at a rate of 82.6%. This is in agreement with Pegram *et al.,* (1980) who identified it at a prevalence of 74.5% from sheep and goats and is relatively higher than the results of Gelagay *et al.,* (2004) and Hussein and Elsawi (1984) who establish 33% and11% prevalence rate of P *haemolytica* biotype A serotypes A7 respectively. Seropositivity to *P haemolytica* biotype A serotypes A2 antibodies was found to be 81.8%. This result is higher than the result of Gelagay *et al.,* (2004) and Hussein and Elsawi (1984) who identified it at a rate of 45% and 53.3% from sheep serum respectively, but comparable with the results of Gilmour (1989) who recovered at a prevalence of 76.2% from sheep serum.

*P haemolytica* biotype A serotypes A1 was identified at a prevalence of 76.8%. There is no previous report indicating high prevalence of this serotype from small ruminants except Gelagay (2004) who establishes a prevalence rate of 37%. Even though this serotype is considered as non-pathogenic in sheep and goat (Biberstein, 1978), its significant rate of identification from serum samples by various workers, such as Gelagay, (2004) in sheep and Tekleselasse, (2005) in goat and in this study could indicated that this serotype has a role in the development pneumonic pasteurellosis outbreaks.

Six percent of the sera were positive for *P multocida* A. This result is in agreement with most of previous workers such as Gelagay *et al.,* (2004), Gilmour (1989) and Hussein and Elsawi (1984) who detected at a rate of 10%, 5.33%, and 8.4% from sera of sheep and goats respectively. Of the various serotypes of *pasteurell* species, *M. haemolytica* serotype A2 and A1 are the major pathogen responsible for diseases in sheep, causing a fibrinous, necrotic pneumonia. But *P multocida* A serotype is not as well-characterized as *M. haemolytica* serotype A2 and A1 serotypes, which mainly causes pneumonia in cattle (Biberstein, 1978). Researchers have suggested a synergistic effect of *Pasteurella* with environmental stress factors on the development of severe respiratory disease (Pegram *et al,* 1980). Considering the existence of multiple pathogenic serotypes of *pasteurella* as encountered in this study, weather changes and other stressful conditions the importance of pneumonic pasteurellosis

will be higher and need due attention and detail investigation.

Our findings support the need for the development of a multivalent vaccine using the most prevalent *P haemolytica* serotypes as well as strategic deworming, and improved housing conditions for sheep and goats in Wolaita zone of Ethiopia. This can contribute towards improving the survival and productivity of sheep and goats which in turn increases their vital role in the food security of communities living in rural Wolaita where there is inadequate percapita production of food.

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How to cite this article:
Ymisrach Yonas, Amenu Goa and Berhanu Butako. (2017). Study on the Epidemiology and Strain Identification of Small Ruminant Pneumonic Pasteurellosis for Vaccine Production in Selected Districts of Wolaita Zone, SNNPR. Int. J. Curr. Res. Biol. Med. 2(3): 8-13.
DOI: http://dx.doi.org/10.22192/ijcrbm.2017.02.03.002