International Journal of Pharma Medicine and Biological Sciences

ISSN 2278 – 5221 www.ijpmbs.com
Vol. 2, No. 4, October 2013

Research Paper

SERO-TYPING AND EVALUATION OF THE LEVEL OF PROTECTIVE ANTIBODY TITER IN NORTHWEST ETHIOPIAN SHEEP BEFORE AND AFTER OVINE PASTEUROLLOSIS VACCINATION

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Investigation on Serotypes of Ovine Pasteurellosis and Evaluation the level of protective antibody titter before and after ovine pasteurollosis vaccination was conducted from May 2010 to April 2012 in model sheep breeding villages of Farta and Lay Gaint districts of Amhara Regional State, Northwest Ethiopia. The objectives of the study were to investigate the prevailing field serotypes of ovine pasteurollosis and to evaluate the level of antibody titer of ovine Pasteurellois after vaccination in model sheep breeding villages. Accordingly, blood from 71 sheep were collected aseptically using non-heparinized blood collecting tubes and examined for serotyping of Mannhaimia. haemolytica and *P. multocida* using indirect haemagglutination (IHA) test at National Veterinary Institute, Debre Zeit, Ethiopia. Accordingly, *M. haemolytica* serotypes were found with prevalence of A1 (33.1%), A7 (31.8%) and A2 (28.5%) respectively. The least prevalent sero-type was Pasteurella Multocida biotype-A (6.6%). In addition, sera from 40 sheep were aseptically collected and examined to evaluate level of protective antibody titer before and after *P. multocida* Bio-type A vaccination. The overall population protective antibody titer ($\geq$1:16) before and after vaccination was 32.5% and 87.5% respectively. There was no significant difference ($p > 0.05$) in antibody titer detected between study location, breed and age. However, there was a significant difference ($C^2=25.2$; df=1, $p=0.000$) in level of protective antibody titer before and after vaccination. In conclusion, *Manhaemia haemolytica* serotypes were highly prevalent in the study areas. Although *Pasteurella multocida* Bio-type A serotype was the least prevalent, the vaccine applied against it in the field was found effective in developing protective antibody in the vaccinated population. However, the monovalent killed *P. multocida* biotype A-vaccine may not protect the different *Manhaemia haemolytica* serotypes isolated in the study areas. Therefore, the development of a multivalent vaccine using the most prevalent Pasteurella serotypes will help to effectively prevent ovine pasteurellosis.

Keywords: Antibody titer, Serotypes, Ovine Pasteurellosis, Vaccination, Sheep

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INTRODUCTION

In the Ethiopian highlands, sheep and their products provide direct cash income through the sale of live sheep, wool and hides. They are living banks for many farmers, and are closely linked to the social and cultural lives of millions of resource poor farmers. According to Agyemang *et al.* (1985), 40% of total income of small holder farmers comes from the sale of sheep and used to purchase agricultural inputs and household goods. Although sheep represent a great resource for Ethiopia, the rate of productivity per animal is low (Ayelet *et al.,* 2004). Sheep disease and poor animal management is largely responsible for this reduced productivity (Ademosun, 1992). Ovine pasteurellosis is one of the most economically important infectious diseases of sheep with a wide prevalence throughout the continents (Mohamed and Abdelsalam, 2008). Both *Mannheimia* and *Pasteurella* species are commensally resident in the respiratory tract of healthy ruminants and are capable of causing infection in animals with compromised pulmonary defense system. The disease is essentially triggered by physical or physiological stress created by adverse environmental and climatic conditions such as extremely bad weather, poor management, overcrowding, transportation or previous infection with respiratory viruses, mycoplasma or some other pathogenic organisms (Mohamed and Abdelsalam, 2008).

Mannheimia haemolytica, the cause of ovine pasteurellosis, exists in two biotypes, A and T. These biotypes further divide into serotypes based on their surface antigen. Type A comprises A1, A2, A5, A6, A7, A8, A9, A10, A11, A12, A13, A14, and A16; type T comprises T3, T4, T10, and T15. Biotype A is particularly associated with pneumonic pasteurellosis in sheep, whereas biotype T causes systematic pasteurellosis in lambs (Gilmour *et al.*, 1983; Gilmour and Gilmour,1989). All serotypes can be involved in pneumonic pasteurellosis in sheep, but serotype A2 is the most commonly isolated serotype from cases of ovine pneumonic pasteurellosis (Barbour *et al.*, 1997; Davies *et al.*, 1997)

As Farta and Lay gaint districts are characterized by drought, adverse environmental and climatic conditions such as, critical feed shortage and poor management practices, ovine pasteurellosis is considered to be the major sheep health problem in the study areas. Despite biannual vaccination against ovine pasteurellosis with a monovalent vaccine (inactivated *P. multocida* biotype A), there have been a report on high rates of mortality and morbidity associated with ovine pasteurellosis in the study areas. However, no studies have been conducted on identification of the prevailing field serotypes of ovine pasteurellosis and its vaccine effectiveness at the field. Thus, the motive behind to conduct this study was due to the scarcity of the above information in the study areas.

This study was therefore, conducted with the objective of identifying the prevailing field serotypes of ovine pasteurellosis disease and to evaluate the effectiveness of its vaccine applied in model sheep breeding villages of Amhara Region.

MATERIALS AND METHODS

Study Area

This study was conducted in model sheep breeding villages of Farta and Lay Gayint districts of South Gonder Zone, Northwest Ethiopia. Farta district is situated at 11°402 N latitude and 38° E longitude and located at about 100 km north-east

of Bahir Dar, capital city of the Amhara Region, Ethiopia. It lies within an altitude range of 1920-4135 m above sea level. The district receives an average annual rain fall of 900-1099 mm and a mean-range temperature of 9-25°C. The rainy season ranges from May to September. Abebaw and Melaku (2009); Alemtsehay and Girma (2006) as cited by Shigdaf Mekuriaw (2011) the district's major socioeconomic problem is food insecurity.

Lay Gayint district is located 175 km from Bahir Dar and lies between altitude ranges of 1300-3500 m above seas level. It receives an annual average rain fall of 600-1100 mm and mean minimum and mean maximum temperatures of 9 and 19°C respectively. It is characterized by drought, sever soil erosion, poor soil fertility, frost and shortage of arable land, crop disease and pest, landslide and feed shortage (South Gonder Zone BOA, 2008)

Study Animal Description

The study animals were farta, washera and cross (Farta X Washera) sheep of both sex and all age found in farta and Lay gaint model sheep breeding villages. Farta sheep is said to be small size and slow growing. Where as, Washera sheep breed is recognized as one of the promising indigenous sheep breed. As Sisay (2009) reported that the Washera sheep breed has an important genetic potential for growth and adaptation to a wide range of agro-climatic conditions and generally, high growth rate and twinning rates are among the desirable traits identified for this breed (Solomon *et al.,* 2008a; Mengistie *et al.,* 2009a). The dominant sheep breed reared in Lay Gaint and Farta districts is local Farta sheep. How ever, over five years, washera breed was introduced to the present study areas by the Amhara region Bureau of Agriculture and Rural Development (BoARD) in collaboration with Andassa Livestock Research Center aimed at improving the production and productivity of Farta sheep by crossbreeding.

Study Design, Sampling and Data Recording

Cross-sectional study was employed for serum collection for sero-typing of ovine pasteurella and evaluation of the effectiveness of ovine pasteurellosis disease vaccine. Study areas were sampled purposively based on sheep ownership. While for serum collection, sheep were sampled randomly.

Vaccination of Animals

Two model sheep villages namely Adis alem (Farta) and Damot (Lay gaint) were taken as study area. The type of vaccine used was Ovine pasteurollosis (*P.multocida* biotype A) which is currently produced by National Veterinary Institute, Ethiopia. The vaccine was administered through sub-cutaneous (SC) route around lateral cervical vertebrae. All sheep above six month of age found in the study village was vaccinated.

Specimen Collection and Laboratory Analysis

A total of 71 blood samples were collected for the analysis of pasteurella serotypes and 84 blood samples (42 before vaccination; 42 after vaccination) were collected to evaluate the effectiveness of ovine pasteurollosis vaccine applied in the study model sheep villages. The time interval between before and after vaccination was 20 day.

About 10 ml of blood was collected from the jugular vein of each sheep by using plain vacutainer tubes and needles. The blood was allowed to clot for 1-2 h at room temperature, stored horizontally overnight at 4°C and then the serum was separated from the clot by

centrifugation at 3000 rpm for 15 min. Then the separated serum was labeled and kept under refrigeration (–20°C) until tested.

Test Procedure for Sero-typing and Antibody Titer Evaluation of Ovine Pasteurellosis Vaccine

All serum samples were sent to National Veterinary instituite (NVI) for laboratory sero-typing as well as antibody titer evaluation. The type of laboratory test employed was indirect haem-agglutination (IHA) test. IHA test was conducted according to the procedures of OIE (2004). The source of *Pasteurella haemolytica* and multocida serotypes of biotype A was CIRAD-EMVT, France. A titer greater than or equal to 1:16 was taken as positive and an agglutination rate of >50% was taken as positive during sero-type investigation.

Data Management and Analysis

All data was first entered and managed using Microsoft Excel spread sheet and analysis of data was made through STATA version 11. Descriptive statistics was employed to determine the prevalence of sero-types of ovine pasteurellosis and level of protective antibody titer while Chi-square (X^2) test was also used to measure the effect of risk factors (study location, breed, age and sex) on the distribution of Pasteurella serotypes and level of protective antibody development before and after vaccination. A significance level ($p<0.05$) and confidence level (95%) was set to determine the presence or absence of statistically significant difference between the given parameters.

RESULT

Investigation of Sero-types of Ovine Pasteurellosis

In this study a relatively higher prevalence of *M. haemolytica* A1 (33.1%), A2 (28.5%) and A7 (31.8 %) serotypes were and lower prevalence of *P. multocida* biotype A (6.6%) were recorded in the study areas (Table 1).

Relatively higher prevalence of *M. haemolytica* serotype A1 (33.1%) followed by A7 (31.8%) and A2 (28.5%) were recorded. While *P. multocida* biotype A (6.6%) was found the least prevalent in the study areas. The investigated sero-types and their prevalence were computed across study location, breed, age and sex. How ever, no significant association ($P>0.05$) was found among computed parameters (Table 2).

Antibody Titer Evaluation of Ovine Pasteurellosis Vaccine

The level of protective antibody titer against ovine pasteurollosis before vaccination was 32.5 %, while after vaccination the antibody titer in response to *Pasteurela multocida* Bio-type A Vaccine was 87.5% (Table 3 and Figure 1). There was no significant difference in level of antibody titer across study location, breed and age ($p > 0.05$). But There was significant ($X^2=25.2$; $P=0.000$) difference in level of protective antibody titer before and after vaccination (Table 2).

In the present study causes of ovine pasteurolosis, *M. haemolytica* and *P multocida* biotype A were identified. The identified *Mannheimia haemolytica* serotype were A1, A2 and A7 with prevalence of 33.1%, 28.5% and 31.8 % respectively. The prevalence of *Pasteurella multocida* Bio-type A was found lower (6.6%). The present finding is found compatible with previous reports which have shown been that *Mannheimia haemolytica* A1, A2 and A6 are dominant in United Kingdom (Gilmour *et al.,* 1979), *M. haemolytica* A1 is dominant in Denmark (Angen *et al.,* 2002)

Table 1: Distribution of *Pasteurella* Sero-types in Model Sheep Villages of Farta and Lai Gaint Districts

Variables	Mannheimia haemolytica Serotypes			Pasteurella multocida serotype	
	A1	A2	A7	A	Sig.
Location	N (%)	N (%)	N (%)	N (%)	Ns
Farta	26(17.2)	20(13.2)	27(17.9)	4(2.6)	
Lai-Gaint	24(15.9)	23(15.2)	21(13.9)	6(4.0)	
Total	50(33.1)	43(28.5)	48 (31.8)	10(6.6)	
Sex					Ns
Male	4(2.6)	2(1.3)	4(2.6)	0	
Female	34(22.5)	32(21.2)	31(20.5)	10(6.6)	
Total	38(25.1)	34(22.5)	35(23.2)	10(6.6)	
Age					Ns
Young	0	1(0.7)	1(0.7)	0	
Adult	38(25.2)	33(21.9)	34(22.5	8(5.3)	
Total	38(25.2)	34(22.5)	35(23.2)	8(5.3)	
Breed					Ns
Washera	14(9.3)	15(9.9)	15(9.9)	4(2.6)	
Farta	18(11.9)	13(8.6)	13(8.6)	3(2.0)	
Cross	6(4.0)	6(4.0)	7(4.6)	0	
Total	38(25.2)	34(22.5)	35(23.2)	7(4.6)	

Note: Sig.=Significance level; Ns=Not significant.

Table 2: Overall Prevalence of Ovine Pasteurellosis in the Study Model Sheep Villages

Sero-types of Ovine Pasteurellosis	Prevalence (%)
M. haemolytica **A1**	33.1
M. haemolytica **A7**	31.8
M. haemolytica **A2**	28.5
P. multocida **A**	6.6

and *M. haemolytica* A1, A2 are dominant in Hungary (Fodor, 1984). The current result is also almost compatible with previous studies

Figure 1: Comparative Evaluation of Antibody Titer in response to Pasteurella multocida Bio-type A Vaccination

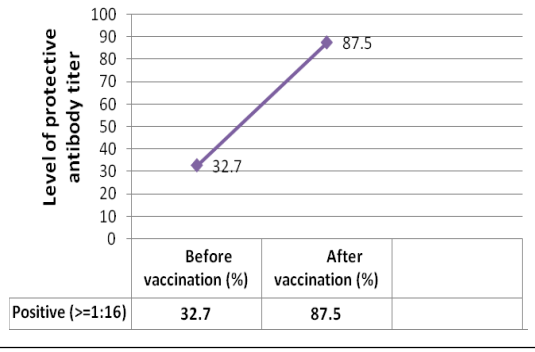

Table 3: Comparative Evaluation of Antibody Titer of Ovine Pasteurollosis Before and After Vaccination

	Before Vaccination		After Vaccination	
	Positive (≥1:16)	Negative (<1:16)	Positive (≥1:16)	Negative (< 1:16)
Model sheep village	N (%)	N (%)	N (%)	N (%)
Farta (Adisalem)	9(22.5)	10(25.0)	16(40)	3 (7.5)
Gaint (Damot)	4(10.0)	17(42.5)	19(47.5)	2(5.0)
Total	13(32.5)	27(67.5)	35 (87.5)	5(12.5)

conducted in North shoa, Ethiopia which shown that the prevalence rates of *M. haemolytica* A2 (36%), A8 (35%), serotype A9 (2%) and *P. multocida* A (10%) (Ayelet *et al, 2004).* As stated by Pegram *et al.* (1980), *M. haemolytica* serotypes A2 and A1 are the most prevalent in Ethiopia, while A14, A11, and A9 were the least prevalent.

On the other hand, the present findings are not comparable with other findings reported from New Zealand which showed *M. haemolytica* A2 is dominant (Prince *et al*, 1985) and in Sudan serotypes A2, A6, and A12 have been shown to be the most prevalent and A5, A7, and A11 were the least (Hussein and Elsawi Mohamed, 1984). The discrepancy might be attributed due to differences in laboratory test (in the present study all ovine pasteurella serotypes were not investigated due to limited availability of Ag) and other possible variations associated with the epidemiology *M. haemolytica* serotypes between study areas.

The overall protective antibody titer (>1:16) before vaccination and after vaccination was 32.5% and 87.5% respectively. The higher protective antibody titer recorded in the vaccinated population could be due to the result of *P. multocida Bio-type A* vaccine, which induced higher level of invivo antibody production. This finding is consistent with the epidemic theory which suggests that if 70% of the population is immune, the disease outbreak is unlikely to occur because there are not enough susceptible to propagate an epidemic (Thrusfield, 1995)

Thus it has been expected that the mortality and morbidity reports associated with ovine pasteurolosis in the study areas was due to multiple *M. haemolytica* serotypes which could not be cross-protected by *P. multocida* bio-type A vaccine. And this finding also supported by (Ayelet *et al*., 2004) who reported that incompleteness of the available vaccine for pasteurellosis which does not include all species and serotypes for *Pasteurella haemolytica* could not completely protect sheep from pasteurollosis.

CONCLUSION AND RECOMMENDATION

Investigation on serotypes of ovine pasteurellosis revealed that *Manhaemia haemolytica* serotype A1, A2 and A7 were investigated in the study areas. Although *Pasteurella multocida* Bio-type A serotype was the least prevalent, the vaccine applied against it in the field was found effective in developing protective antibody in the vaccinated population. However, the monovalent killed *P.*

multocida biotype A-vaccine may not protect the different *Manhaemia haemolytica* serotypes isolated in the study areas. Therefore, vaccinate sheep flock with multivalent vaccine containing the most prevalent Pasteurella serotypes will help to effectively prevent ovine pasteurellosis in the study areas. Moreover, it is recommended that comprehensive evaluation of sero-typing of ovine Pasteurella organism would give the opportunity to find the exact antigenic structure and to prepare the most effective vaccine in the future.

ACKNOWLEDGMENT

We would like to extend our gratitude to Amhara Regional Agricultural Research Institute, for funding of this study. Our gratitude is also extended to animal health technical assistances Mehari Ayalew and Samuel Taye, who paid invaluable contributions in the successful completion of this work

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