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Fisseha Mengstie <sup>α</sup>, Hailemariam Gizaw <sup>σ</sup> & Desiye Tesfaye <sup>ρ</sup>

**Abstract-** Cross-sectional study for determining the prevalence of Ovine pasturellosis, in-vivo evaluation of the level of protective antibody titer before and after vaccination against the disease and proofing the farmers and animal health experts complaint of its cause on small ruminant production despite annual vaccination program against the disease in Adiyo district of Boka-Shuta and Buta Kebelle, Kaffa Zone. The study was conducted from July 2012 to June 2013 and the study kebelles were purposively selected based on sheep production potential, the disease's report and farmers' complaint on the vaccine's inefficacy for protecting the sheep against the disease. For these, the study was designed to answer the above stated objectives with two consecutive phases, viz. prevalence study followed by in-vivo antibody titer evaluation. For prevalence study, 192 blood samples were needed and calculated, but for more accuracy 200 samples were collected randomly from previously unvaccinated sheep population (in less than 1 year) against ovine pasturellosis disease. For in-vivo evaluation, 52 blood samples were randomly collected from selected sheep population after they were vaccinated against ovine pasturellosis disease with *P. multocida* biotype A-vaccine by grouping them based on history of vaccination status (not vaccinated in less than 1 year against ovine pasturellosis disease) and age group (greater than 6 months of age). Sample collection, preservation and transportation were performed according to the recommended standard procedures. Laboratory analysis, Indirect haemagglutination Inhibition Test was employed at National Veterinary Institute (NVI), Ethiopia for both studies. Thus, out of 200 serum samples, 175 (87.5%) were positive. However, there were no statistical significant difference ( $p \geq 0.05$ ) between study areas, age and sex of the animals. Regarding in-vivo evaluation of the level of protective antibody titer, it was found 87.5% before vaccination and 98.1% after vaccination. However, there was no statistically significant difference in between the study areas. In conclusion, Ovine pasturellosis was the major diseases of sheep in the study areas and the monovalent killed *P. multocida* biotype A-vaccine applied against ovine pasturellosis in the field was found effective in developing protective antibody in the vaccinated population. However, the complaint of the farmers and animal health

experts on the inefficacy of the applied vaccine despite annual vaccination program could be due to the presence of *M. haemolytica* serotypes which could not be cross-protected. And also, there were no research work on the serotypes present in the study areas. Therefore, comprehensive serological identification of involved serotypes for causing ovine pasturellosis should be performed, which could give the opportunity to know the exact antigenic structure present and indicate the use of multivalent vaccines combination against the disease in the study areas.

**Keywords:** antibody titer, in-vivo evaluation, ovine pasturellosis, prevalence, sheep, vaccination.

## I. INTRODUCTION

Ethiopia lies within the tropical latitude of Africa and has an extremely diverse topography, a wide range of climatic features and a multitude of agro-ecological zone which makes the country suitable for different agricultural production system. This contributed to the existence of a large diversity of farm animal genetic resource in the country (Anon, 2004). Sheep constitute the second major component of livestock in Ethiopia and they play a significant role in the nation's economy. Meat and milk are major sources of protein, and hides, live animals, and carcasses account for a significant proportion of exports. The increased demand for sheep meat, cash income and food security has increased their importance in the country (Alemu and Merkel, 2008). Despite the large livestock population of Ethiopia the economic benefits remain marginal due to prevailing diseases, poor nutrition, poor animal production systems, reproductive inefficiency, management constraint and general lack of Veterinary core (Anon, 1992).

In Ethiopia, Sheep and goats contribute 25% of the meat domestically consumed with a production surplus mainly being exported as live animals (Alemayehu and Fletcher, 1991; Tibbo, 2006). Both species also contribute 50% of the domestic needs in wool, about 40% of skins and 92% of the value of hides and skin exported (ILCA, 1993). The total income share of small ruminants tends to be inversely related to size of land-holding, suggesting that small ruminants are of particular importance for landless people. In some

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settings where, agriculture (crop production) provides only seasonal employment, rearing small ruminants would provide employment and income as a subsidiary occupation (Coppock *et al.*, 2006).

Indigenous small ruminants constitute greater percentage of ruminant population in Africa (Lebbie *et al.*, 1994). These flocks of animals are commonly found in the rural areas where they are owned and managed under extensive system (Otchere, 1986). Small ruminants play an important role in the lives of most people especially rural farmers whose livelihood entirely depend on them. They provide source of animal protein through their meat and milk (Fajemisin, 1991). Notwithstanding, they fetch a source of income when sold to meet some other family needs as well as play a vital social roles during ceremonies and festivals.

The importance of small ruminants (ie sheep and goats) to the socio-economic well being of people in developing countries in the tropics in terms of nutrition, income and intangible benefits (eg savings, insurance against emergencies, cultural and ceremonial purposes) cannot be overemphasized (Kosgey, 2004). Sheep and goats are important livestock species in developing countries because of their ability to convert forages, and crop and household residues into meat, fibre, skin and milk.

For an improved animal protein intake, there is need for improvement in the production of meat and other protein sources from the livestock industry. Sheep and goats offer a great potential in this respect due to their relative ease of breeding, management, ability to subsist on forages, hardiness, adaptation to a wide range of ecological zones and distribution among others. In recent times, sheep and goats production is becoming popular even among urban dwellers as result of the aforementioned merits (Umunna *et al.*, 2014).

Small ruminant management is seriously hindered by diseases in the tropics. Diseases are very important to farmers and affect the production of small ruminants in several ways. It increases cost of production, lowers production level, reduces the quality and quantity of animal products and generally causes great loss to the farmer (Abdullahi *et al.*, 2013). Respiratory diseases caused by concurrent infections have been identified as the leading health problem of small ruminants which accounts for up to 54% of the overall mortality of sheep in Ethiopian central highlands (Mukasa-Mugerwa *et al.*, 2000). *Mannheimia haemolytica* (formerly called *Pasteurella haemolytica*) and *Pasteurella multocida* are known to be the most prominent pathogens causing great economic losses in the domestic animal industry (Highlander, 2001; Confer, 1993). Pasteurellosis is a complex disease that develops when the immune system of the animal is compromised by stress factors mainly environmental stresses including inclement weather, feed shortage usually

assisted by inadequate management and husbandry practices (Bekele *et al.*, 1992).

As Kaffa Zones, pasteurellosis is considered to be the major sheep health problem and there have been a report on high rates of mortality and morbidity associated with the disease. And also, despite annual vaccination programs against pasteurellosis with the only commercially available Killed *P. multocida* biotype A containing vaccine (National Veterinary Institute, Ovine pasteurella vaccine) in the study areas, high mortality and morbidity continued to be observed and complaint by farmers and veterinarians. So far in the study area, it was the only vaccine type given to the farmer's sheep and was available in the market during the time of the research study. Therefore the present study was designed with the objectives of determining the prevalence of Ovine pasteurellosis, In-vivo evaluation of the level of protective antibody titer before and after vaccination with commercially available ovine pasteurellosis vaccine and proofing the farmers and animal health experts complaint of its cause on small ruminant production despite annual vaccination program against the disease in Adiyo district of Boka-Shuta and Buta Kebelle, Kaffa Zone.

## II. MATERIALS AND METHODS

### a) Study area, Study Design and Study Population

Cross-sectional study for determining the prevalence of Ovine pasteurellosis, In-vivo evaluation of the level of protective antibody titer before and after vaccination with commercially available ovine pasteurellosis vaccine and Proofing the farmers and animal health experts complaint of its cause on small ruminant production despite annual vaccination program against the disease in Adiyo district of Boka-Shuta and Buta kebele, Kaffa Zone, Southern Nations Nationalities and Peoples regional state was conducted from July 2012 to June 2013. The study kebelles were purposively selected based on sheep production potential, the disease's report and farmers' complaint on the vaccine's inefficacy for protecting the sheep against ovine pasteurellosis disease.

The study animals were Bonga sheep breed of both sex and all age group. Bonga sheep breed is geographically distributed and reared in Keffa, Sheka and Bench zones of Southern State and have physical feature and performance levels of Long fat tail with straight tapering end (98.4%); hair sheep; large size; predominantly plain brown (57.9%); both sexes are polled (Gizaw *et al.*, 2011).

### b) Sample Size determination

The sample size was calculated based on 2013 prevalence's reported by Maru *et al.*, (25%) in Haramaya district with 5% desired absolute precision at 95% confidence level using the formula recommended by Thrusfield (2005). Thus, 192 blood samples were

needed to calculate the prevalence rate of the population.

The study had two consecutive phases, i.e, prevalence determination and in-vivo antibody titer evaluation. For these, blood sample collection was performed prior to vaccination (to determine the prevalence of ovine pasturellosis) and post vaccination (to evaluate the effectiveness of ovine pasturellosis vaccine). The time interval for sampling of the study animals between before and after vaccination was 20 day.

#### c) Blood Sampling and Laboratory analysis

##### i. Prevalence Determination

A total of 200 blood samples were collected from sheep of previously unvaccinated against ovine pasturellosis disease (at least a history of less than 1 year) according to standard procedures from the animal's jugular vein using plain vacutainer tubes and sterile needles and allowed to clot for 1-2 h at room temperature, stored horizontally overnight at 4°C and finally, the serum was separated from the clot. The separated serum was labeled and transported to National Veterinary institute (NVI) laboratory using cold chain and it was kept under refrigeration (-20 °C) until tested to determine the level of Sero-positivity. The type of laboratory test employed was Indirect haemagglutination Inhibition Test.

##### ii. In-vivo Antibody Titer Evaluation

Among the sixteen Bonga sheep breed improvement Community sites (cooperatives), Boka-Shuta site was selected for the study. The type of vaccine used was Ovine pasteurolosis (*P. multocida* biotype A) which is currently produced by National Veterinary Institute, Ethiopia and marketed for field vaccination against ovine pasturellosis disease. The study animals, fifty two sheep of both sex were randomly selected by grouping them based on their history of vaccination status (not vaccinated in less than 1 year against ovine pasturellosis disease) and age group (greater than 6 months of age). The selected sheep were vaccinated with *P. multocida* biotype A vaccine. The vaccine was administered through sub-cutaneous (SC) route around lateral cervical vertebrae. Then after, blood samples were collected from the animal's jugular vein using plain vacutainer tubes and sterile needles and allowed to clot for 1-2 h at room temperature, stored horizontally overnight at 4°C and finally, the serum was separated from the clot. The separated serum was labeled and transported to National Veterinary institute (NVI) laboratory using cold chain to identify the level of specified antibody in their serum.

The type of laboratory test employed was indirect haemagglutination (IHA) test. IHA test was conducted according to the procedures of OIE (2004). The source of *Pasteurella multocida* serotypes of

biotype A was CIRAD-EMVT, France. A titer greater than or equal to 1:16 was taken as positive.

#### d) Data Management and Analysis

All data was first entered and managed using Microsoft Excel spread sheet and analyzed using STATA version 11. Descriptive statistics was employed to determine the prevalence while Chi-square ( $\chi^2$ ) test was used to measure the effect of predisposing factors. 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.

## II. RESULTS AND DISCUSSIONS

#### a) Prevalence of Ovine pasturellosis

Out of 200 serum samples, 175 (87.5%) was positive for ovine pasturellosis. The prevalence in between the study areas, age and sex had not statistical significant difference ( $p \geq 0.05$ ) (Table 1).

The present finding is higher than the report of 31.1% by Aschalew (1998) in Debre Birhan and 83% by Sisay and Zerihun (2003) in Wollo area. Ayelet et al (2004) reported higher prevalence of respiratory problems in July (64%) in central highlands of Ethiopia which had a positive correlation with rainfall pattern, suggesting that climatic conditions play a role.

The higher prevalence in this study could be related to various forms of stress factors as predisposing factors include environmental (heat, cold, wind, chill, crowding), managemental and/or infectious factors also reported by different Authors (Thompson *et al.*, 1977; Frank, 1989; Carroll and Forsberg 2007). Another finding by Mengstie (2014) indicated that, there were 81% prevalence of ovine pasturellosis and in Autmen and summer as explained by the farmers in the same study area predisposed to different stress factors.





**Table 1 :** Prevalence and Distribution of Ovine Paturellosis in Selected Community based Bonga Sheep Breed Improvement Site

Variables		Number of sample	Prevalence of ovine paturellosis	Sig.
Study Area	Boka-Shuta	100	87(87.0)	Ns*
	Buta	100	88(88.0)	
Age	Adult	112	97(86.6)	Ns
	Young	88	78(88.6)	
Sex	Female	148	130(87.8)	Ns
	Male	52	45(86.5)	
Total			175(87.5)	

\*Not significant

b) *In-vivo Antibody Titer Evaluation of Ovine Pasteurellosis Vaccine*

The level of protective antibody titer against ovine pasteurellosis before vaccination was 87.5 %, while after vaccination the antibody titer in response to *Pasteurella multocida* Bio-type A Vaccine was 98.1%. (Table 2 and Fig 1). There was no significant difference in level of antibody titer across sex and age ( $p > 0.05$ ). Similar observations were reported by Ferede et al (2013) when the level of protective antibody ( $> 1:16$ ) was increased from 32.5% (before vaccination) to 87.5% (after vaccination) which were vaccinated with *P. multocida* Bio-type A vaccine in northwest Ethiopian sheep and the author suggested that, 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.

In the present finding, however, despite annual vaccination programs against pasteurellosis using Killed *P. multocida* biotype A containing vaccine (National Veterinary Institute, Ovine pasteurella vaccine) in the study areas, high mortality and morbidity continued to be observed and complaint by farmers and animal health experts and these could be best explained by Ayelet et al (2004) studied in central highlands of Ethiopia, as incompleteness of the available vaccine for pasteurellosis which does not include all species and serotypes for *Pasteurella haemolytica* could not completely protect sheep from pasteurellosis.

**Table 2 :** Comparative Evaluation of Antibody Titer of Ovine Pasteurellosis Before and After Vaccination in response to *Pasteurella multocida* Bio-type A Vaccination

Variables	Study Area	Before vaccination (N= 200)		After vaccination (N= 52)		Sig.
		Positive ( $\geq 1:16$ ) (%)	Negative ( $\leq 1:16$ ) (%)	Positive ( $\geq 1:16$ ) (%)	Negative ( $\leq 1:16$ ) (%)	
Study area	Boka-shuta	87(87.0)	13(13.0)	25(49.0)	1(100)	0.313
	Buta	88(88.0)	12(12.0)	26(51.0)	0(0.0)	
Age	Adult	97(86.6)	15(13.4)	36(70.6)	1(100)	0.520
	Young	78(88.6)	10(11.6)	15(29.4)	0(0.0)	
Sex	Female	130(87.8)	18(12.2)	36(70.6)	1(100)	0.520
	Male	45(86.5)	12(13.5)	15(29.4)	0(0.0)	
Total		175(87.5)	25(12.5)	51(98.1)	1(1.9)	

\*Not significant

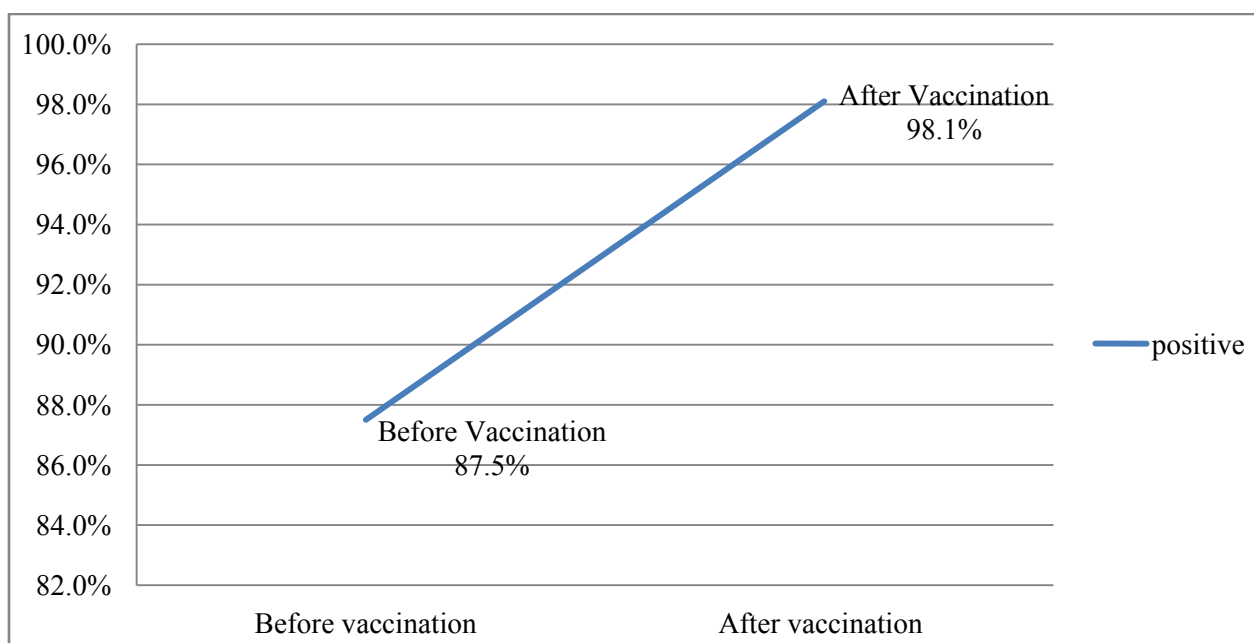


Figure 1 : Comparative Evaluation of Antibody Titer of Ovine Pasteurellosis Before and After Vaccination in response to *Pasteurella multocida* Bio-type A Vaccination

### III. CONCLUSION AND RECOMMENDATION

Ovine pasteurellosis was the major diseases of sheep in the study areas and the monovalent killed *P. multocida* biotype A-vaccine applied against ovine pasteurellosis in the field was found effective in developing protective antibody in the vaccinated population. However, the complaint of the farmers and animal health experts on the inefficacy of the applied vaccine despite annual vaccination program could be due to the presence of *M. haemolytica* serotypes which could not be cross-protected. And also, there were no research work on the serotypes present in the study areas. Therefore, comprehensive serological identification of involved serotypes for causing ovine pasteurellosis should be performed, which could give the opportunity to know the exact antigenic structure present and indicate the use of multivalent vaccines combination against the disease in the study areas.

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