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Article

Serotyping and identification of predisposing factors for the occurrence and distribution of ovine pasteurellosis in Bonga sheep breed

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Abstract: Cross-sectional study for investigating the prevailing field serotypes and predisposing factors for the occurrence and distribution of ovine pasteurellosis in Bonga sheep breed of different agro-ecology of Adiyo (highland), Gawata (lowland) and Debub Bench (Midland) of Kaffa and Benchi Maji in southern Ethiopia was conducted during July 2012 to June 2013. For this purpose, a questioner survey to identify predisposing factors and laboratory analysis on samples to investigate the prevailing field serotypes were performed. In these regard, a total of 210 farmers were interviewed and 70 blood samples from each agro-ecology (total of 210) was randomly collected following standard procedures. Laboratory analysis was performed by indirect haemagglutination (IHA) technique at National Veterinary Institute (NVI), Debre Zeit, Ethiopia. The finding showed that, 34.8% of the farmer's household main sources of income were dependent on sheep production in which ovine pasteurellosis had 81% of occurrence. Management of sheep like housing and sanitation of the barn were predisposing factors. Irrespective of agro-ecology, *M. haemolytica* A2 (52.4%) followed by A7 (42.4%) and A1 (31%) were detected where as *P. multocida* serotype was not detected. Among identified serotypes, highest prevalence of *M. haemolytica* A1 (37.1%) were detected in lowland where as A2 (62.9%) in Midland and lowland with equal prevalence and A7 (85.7%) in midland. Therefore, ovine pasteurellosis was the major disease of sheep identified with multiple losses in the production and *M. haemolytica* is the most common cause identified. Measures such as, improving management practices by providing optimal sanitation in housing, providing good quality supplement feeding during rainy season and minimizing stress factors should be taken into account to reduce the prevalence. In addition, the currently use vaccine (*P. multocida* biotype A) not incorporate the identified serotypes so that may not protect the disease. As a result, use of multivalent vaccine by incorporating those identified serotypes is necessary to effectively prevent the disease.

Keywords: agro-ecology; ovine pasteurellosis; predisposing factors; prevalence; serotypes; Sheep

1. 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).

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). Of the various *M. haemolytica* strains, *M. haemolytica* serotype A2 is the major pathogen responsible for diseases in sheep, causing a fibrinous, necrotic pneumonia, also called "shipping fever" (Ackermann and Brogden, 2000). *P. multocida* was found present in lung lesions of sheep affected by pneumonia as well (Rudolph *et al*., 2007; Daniel *et al*., 2006). The disease, in its typical clinical form, is highly infectious, often fatal and with very serious economic impact in animal industry (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).

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). Concurrent respiratory infestations by *Chlamydia psittaci*, viruses (parainfluenza-3, reovirus, adenovirus, and respiratory syncytial virus), *Mycoplasma* species (*M. ovipneumoniae*, *M. arginini*, *M. agalactiae,* and others) and lungworms (particularly *Dictyocaulus filaria)* can also suppress the animal's immune system, allowing opportunistic microorganisms (*Pasteurella haemolytica* and rarely *P. multocida,* A and D) to colonize the lung and cause pasteurellosis (Blood and Radostits , 1994; Gilmour and Gilmour , 1989). Physiological response to stressors includes suppression of the immune system; consequently, prolonged stress may increase susceptibility to pathogens and to morbidity and mortality (Brogden *et al*., 1998; Carroll and Forsberg, 2007; Knowles *et al*., 1995).

As Kaffa and Bench Maji Zones, pasteurellosis is considered to be the major sheep health problem. Despite 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 the disease in the study areas. However, no studies have been conducted on identification of the prevailing field serotypes of it. 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 investigating the prevailing field serotypes of ovine pasteurellosis and predisposing factors for its occurrence and distribution in Bonga sheep breed of South Nations and Nationalities Region.

2. Materials and Methods

2.1. Study area, study design and study population

A cross-sectional study for investigating the prevailing field serotypes of ovine pasteurellosis in Bonga sheep breed of SNNPR state of Ethiopia (Kaffa and Benchi Maji Zone) was conducted from July 2012 to June 2013. From kaffa and Benchi Maji zone, Adiyo (highland), Gawata (lowland) and Debub Bench (Midland) were randomly selected. From each selected agro-ecology, representative sampling sites (Boka/Shuta, Qonda Zuria and Faniqa) were selected. Using simple random sampling technique, blood samples from individual study animals were collected.

The study animals were Bonga sheep breed of both sex and all age. 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). Questioner survey was also conducted using structured format by interviewing the owners to know the management of sheep and to identify predisposing factors for the occurrence of ovine pasteurellosis.

2.2. Sample size, sampling procedures and laboratory analysis

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). Accordingly, a total of 210 blood samples from the jugular vein of each sheep by using plain vacutainer tubes and needles were collected. Then, The blood was allowed to clot for 1-2 h at room temperature, stored horizontally overnight at 4^{O}C and finally, the serum was separated from the clot by centrifugation at 3000 rpm for 15 min. The separated serum was labeled and transported to National Veterinary institute (NVI) laboratory for sero-typing using cold chain. It was kept under refrigeration (–20 ^{O}C) until tested. The type of laboratory test employed was indirect haemagglutination (IHA) test according to the procedures of OIE (2004).

2.3. 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 of sero-types while Chi-square (X^2) test was used to measure the effect of predisposing factors on the distribution of Pasteurella sero-types. 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.

3. Results

3.1. Questioner survey result

The questioner survey result on the occurrence of diseases and management of small ruminants revealed that, ovine pasteurellosis was the most prevalent (85% in Debub Bench followed by 78.6 % in each of Adiyo and Gawata) with great losses in the production. 83.2% of interviewed farmers responded that, there were sudden deaths in their flock associated with symptoms of ovine pasteurellosis. Regarding livestock importance for the life of the farmers, out of 210 respondents, 73 (34.8%) household's main sources of income were dependent on sheep production (Table 1).

Different factors were identified by the farmers as a source/predisposing factors for the occurrence and distribution of ovine pasteurellosis in the study areas. Among these, sheep without housing at night had 100% of occurrence while those have housing at night; sanitation and frequency of cleaning during wet and dry season have great contribution for its occurrence (Table 2).

3.2. *M. haemolytica* serotypes and prevalence

Overall prevalence of *M. haemolytica* A2 110/210 (52.4%), A7 89/210 (42.4%) and A1 65/210 (31%) serotypes was detected irrespective of agro-ecology. The prevalence of the different *M. haemolytica* serotypes in computed parameters as indicated in Table 3, highest prevalence was detected: A1 in Lowland (37.1%); A2 in midland and lowland (62.9% in each); and A7 in midland (85.7%). Regarding age groups, adults had higher level of isolation with A2 (70.2%) followed by A7 (62.3%) and A1 (39.5%). Due to lack of reference strains, other *Mannheimia haemolytica* serotype test was not conducted.

M. haemolytica A2 and A7 serotypes had significantly higher isolation in midland and lowland than highland agro ecology ($p=0.000$) and also, adult age group had significantly higher rate of isolation ($p=0.000$) (Table 3). *P. multocida* serotype was not detected in all the collected serum samples.

4. Discussion

In the present finding, 34.8% of the farmer's household income was dependent on sheep production and one of the most prevalent identified sheep diseases was ovine pasteurellosis as 81% of the interviewed farmers ranked it first with multiple devastating effects on the production by high mortality and morbidity. This could be due to poor ventilated and/or partially roofed and open barns and poor sanitation of the barn had a role in aggravating respiratory problems as reported by Ayelet *et al*. (2004) which is in agreement with the present finding by out of 28 farmers they keep their sheep flock in open barn, all (100%) reported signs of ovine pasteurellosis. Various forms of stress factors have been incriminated as predisposing factors include environmental (heat, cold, wind, chill, crowding), managemental and/or infectious factors also reported by different Authors (Thompson *et at*., 1977; Frank, 1989; Carroll and Forsberg 2007). During Autmen and summer, there were highest occurrences and mortality of sheep by ovine pasteurellosis which was explained by the farmers as sheep allowed staying long time in the barn without feed rather than grazing out in response of rain.

Table 1. Questioner result on the importance and distribution of small ruminant diseases.

Distribution of ovine pasteurellosis and importance of livestock		No.	No. of respondents	Prevalence (%)
Occurrence of ovine pasteurellosis in the Study Locations	Adiyo (Boka/Shuta)	70	55	78.6
	Debub Bench (Faniqa)	70	60	85.7
	Gawata (Qonda Zuria)	70	55	78.6
Sudden death in the sheep flock associated with signs of ovine pasteurellosis	No	55	41	74.5
	Yes	155	129	83.2
House hold main source of income	Crop production	210	62	29.5
	Sheep production		73	34.8
	Other livestock and their product		35	16.7
Total		**210**	**170**	**81**

Table 2. Different variables associated with the occurrence and distribution of ovine pasteurellosis identified by the farmers.

Variables		No.	No. of respondents	Prevalence (%)
Sheep sheltered at night	No	28	28	100
	Yes	182	142	78
Total		210	170	81
Barn cleaning frequency during dry season	Every day	161	130	80.7
	Every two day	21	20	95.2
Total		182	150	82.4
Barn cleaning frequency during wet season	Every day	31	13	41.9
	Every two days	120	100	83.3
	Every three days	31	27	87.0
Total		182	140	76.9
Season with high number of cases/occurrence of ovine pasteurellosis	Autmen	170	91	53.5
	Spring		4	2.4
	Summer		40	23.5
	Winter		13	7.6
	Year round		22	12.9
Total		170	170	100
Season with high mortality with ovine pasteurellosis	Autmen	170	82	48.2
	Summer		46	27.1
	Spring		8	4.7
	Winter		30	17.6
	Year round		4	2.4
Possible reasons for occurrence of ovine pasteurellosis in different seasons	Feed shortage, hot weather condition and dust particles	170	37	21.8
	Rain, cold weather condition and insufficient time of grazing		124	72.9
	hot weather condition, rain and feed shortage		9	5.3
Affected age and production groups by ovine pasteurellosis	Adults		11	6.5
	All age group		8	4.7
	Ewe		67	39.4
	Ewe and lamb		67	39.4
	Lamb		17	10.0
Total		**170**	**170**	**100**

Table 3. Distribution of *M. haemolytica* sero-types in Bonga sheep breed.

Variables		*M. haemolytica* Serotypes	No.	Positive	Prevalence (%)	Value	X^2
Agro ecology	Highland	A1	70	20	28.6	19.16	0.384
	Lowland		70	26	37.1		
	Midland		70	19	27.1		
	Total		210	65	31.0		
	Highland	A2	70	22	31.4	18.48	0.000
	Lowland		70	44	62.9		
	Midland		70	44	62.9		
	Total		210	110	52.4		
	Highland	A7	70	5	7.1	91.301	0.000
	Lowland		70	24	34.3		
	Midland		70	60	85.7		
	Total		210	89	42.4		
Age	Adult	A1	114	45	39.5	8.473	0.004
	Young		96	20	20.8		
	Total		210	65	31.0		
	Adult	A2	114	80	70.2	31.657	0.000
	Young		96	30	31.3		
	Total		210	110	52.4		
	Adult	A7	114	71	62.3	40.44	0.000
	Young		96	18	18.8		
	Total		210	89	42.4		
Sex	Female	A1	115	36	31.3	0.015	0.903
	Male		95	29	30.5		
	Total		210	65	31.0		
	Female	A2	115	67	58.3	3.524	0.061
	Male		95	43	45.3		
	Total		210	110	52.4		
	Female	A7	115	63	54.8	16.011	0.000
	Male		95	26	27.4		
	Total		**210**	**89**	**42.4**		

Despite annual vaccination programs against pasteurellosis using killed *P multocida* biotype A-containing vaccine, high mortality and morbidity was observed by farmers as these vaccine may not protect the different *Manhaemia haemolytica* serotypes isolated in the present study could be also another justification. 39.4% of farmers explained that, ewes and lambs are mostly affected by ovine pasteurellosis. Predisposing factors, such as lack of sufficient energy or protein, inadequate colostrum consumption, specific vitamins or certain minerals, also may compromise immunity (Carroll and Forsberg 2007).

Comparing the two *Pasteurella* spp., the only identified *M. haemolytica* serotypes was the major causative agent involved in ovine pasteurellosis in the study areas. The identified *Mannheimia haemolytica* serotype were A2, A7 and A1 with prevalences of 52.4%, 42.4% and 31%, respectively. The findings of this study are in agreement with *P haemolytica* biotype A serotypes A2 and A1 are the most prevalent in Ethiopia (Pegram *et al.*, 1980) and 36% of *Mannheimia haemolytica* A2 reported by Ayelet *et al.*, 2004 which is also the most prevalent In the United Kingdom reported by Gilmour and Gilmour, 1989. In Sudan *Mannheimia haemolytica* A2 is among the most prevalent serotypes while *Mannheimia haemolytica* A7 is the least (Hussein and Mohamed, 1984).

5. Conclusions

Ovine pasteurellosis was the major disease of sheep in the area identified with multiple losses in the production and *M. haemolytica* is the most common cause. Though correlation of the disease with identified possible factors by the farmers were not done during the present study, the poor management of sheep could be predisposing factor for ovine pasteurellosis in the production. Measures such as, improving management

practices by providing optimal sanitation in housing, providing good quality supplement feeding during rainy season and minimizing other stress factors should be taken into account to reduce the prevalence. In addition, the present findings also support the need for the development of a multivalent vaccine using the most prevalent *P haemolytica* serotypes as the monovalent killed *P. multocida* biotype A-vaccine which is currently provide to the farmers of the study area may not protect the different *Manhaemia haemolytica* serotypes isolated.

Conflict of interest

None to declare.

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