Assessment Of Mannheimia Haemolytica Serotypes Affecting Ovine Species In Assela And Surrounding Areas, Arsi Zone Of Oromia Regional State, Ethiopia

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Abstract: This study was conducted from December, 2006 to March, 2007 to assess *Mannheim haemolytica* serotypes effecting ovine species from pneumonic lungs of apparently healthy sheep slaughtered at the backyard in Assela, Arsi zone, Oromia regional sate of Ethiopia. From the total 300 sheep examined during the slaughter, 150 (50%) lungs were observed to be pneumonic out of which 130 (87%) lungs were sampled and processed for isolation and identification of *M. haemolytica*. The detailed bacteriological analysis made on those pneumonic lungs indicated that there was no *M. hemolytica* organism in the affected lungs sampled for this study. As this study was based on apparently healthy sheep, the organized study which includes the epidemiology, bacteriological and serological investigation at the time of occurrence of the disease should be devised and applied to suggest the possible *M. haemolytica* serotypes for future vaccinal development in the country.
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1. Introduction

Ethiopia is endowed with large livestock resource, with small ruminant assuming a great share in the socio-economic activities of about 85% of the population (FAO, 1993). The country's holding is estimated 24 million sheep and 18 million goats. This constitutes about 15% of small ruminant population in Africa (ILCA, 1993).

In the East African highlands, the Ethiopian especially the central one with altitude of above 1500 m.a.s.l is known for sheep production. They sustain 92% of the human, 75% of sheep and 28% of goat population of the country, and account for 20% of the total sheep population of tropical Africa (ILCA, 1980, Alemayehu, 1991)

Unlike cattle, sheep and goats are capable of a remarkable adaptability to diverse environmental conditions, are amenable to case of management and serve as a source of income and cash security, particularly in the transitional small holder farmers of the developing countries. In Ethiopia, they cover more than 30% of all domestic meat consumption and generate cash income from exports of meat, live animal and hide (Fletcher and Zelalem, 1991). In high altitude farms, sheep represent only 9% of the total capital value of livestock but produce 63% of the cash income derived from livestock and provide 23% of the subsistence value of food livestock production could contribute to the attainment of food self-sufficiency in the country, particularly in protein requirements for the growing human population as well as to increase export earnings.

However, even though sheep represent a substantial portion of Ethiopian livestock resource, the productivity per animal is very low. Factors such as inadequate nutrition, poor state of health, traditional production system, low genetic potential and the lack of inputs provided for improvement are presumed to be the causes for low productivity (Ademosum, 1992).

Infectious and parasitic diseases are among the major problems affecting sheep production in Ethiopian highlands. Among infectious diseases of small ruminants, respiratory problems are the leading constraints (Teklaye *et al*., 1992). Infectious and parasitic pneumonia in sheep is serious and account for 38.2% of the total cases generally observed and 80% of the over all mortality rates in the highlands of Ethiopia (Taklaye *et al*, 1992). It has been also observed that 18.6% of the morbidity rate and 10.6% of mortality were due to bronchopneumonia mainly caused by pasteurella organism (ILCA Annual Report, 1988).

Pasterurellosis has been known to be one of the most common bacterial diseases of sheep and goats and causes great economic losses in the most sheep rearing countries of the world (Gilmour, 1993). Pasteurellosis in sheep and goats has been known to be caused mainly by *M. heaemolytica* which causes pneumonia in sheep and septicemia in young lambs (De Alwis, 1993).

In Ethiopia, pneumonic pasteurellosis was reported from different regions on a tentative basis as a considerable small ruminant health problem. *Mannheimia haemolytica* has been isolated from

sheep in some parts of the country, and studies on the prevalence have revealed a frequent occurrence in the highlands with high morbidity and mortality (Pegram *et al*, 1979; Aschalew,1998) Losses due to death were also noticed in sheep kept confined at stations and detained in quarantine for export (Pegram, 1980).

Environmental and management factors that may stress animals are part of the normal flock problem and little can be done to remove them. Thus, Control could best be achieved by immunization. There has been a considerable activity in the development of vaccines for the control of pasteurellosis in sheep. But an effective vaccine has proved elusive, due to multiple serotype distribution and poor immunogenicity of the most prevalent serotype A_2. However, the development of multivalent vaccines containing all serotypes of *M. haemolytca and* the use of adjuvant vaccines has improved efficiency and they appeared to be effective in many flocks although their use has not prevented the occurrence of disease in all situations (Radositits *et al*, 1994; Black *et al*, 1997).

An inactivated monovalent *Pasteurella multocida* type A vaccine (NVI product, Ethiopia) has been used routinely for the control and prevention of pneumonic pasteurellosis in Ethiopia, though its efficiency is questionable as most of the *M. heamoytica* serotypes so far known were isolated and identified (Pegram *et al*, 1979; Bekele, 1996; Tesfaye, 1997; Aschalew, 1998). However, this antigenic diversity was not given due attention in immunizing animal in the country, and protection against *M. haemolytica* strains was not provided. Hence, developing a polyvalent vaccine that incorporates antigens of the most predominant serotypes and important immunogens is of paramount importance particularly in countries like Ethiopia where it is very difficult if not impossible, to remove viral diseases and other environmental risk factors that predispose animals to the disease, pasteurellosis.

On account of the foregoing, therefore, knowledge of the overall disease prevalence and serotype distribution in the country is a prerequisite for launching a sound control program besides some sero-prevalence and isolation and identification activities done on *M. heamolytica* in the central highlands of the country up to now. The highlands in Arsi region bearing similar agro economical feature to those of the central highlands with a possibility of being prone to the effects of the disease, however, it has not been investigated for pasteurellosis in small ruminants. Information on the distribution and significance of these serotypes of *M. haemolytica* is scant and not well documented in different agro-ecologies and production systems of Ethiopia. Further more, the currently used vaccinal strain does not include *M. haemolytica* serotypes. Hence the objectives of the current study were:

- to determine the most dominant serotypes of *M. haemolytica* in apparently healthy sheep slaughtered at the back yard of Assela restaurants.
- to recommend the possible *M.haemolytica* serotypes for future vaccinal development in the country.

2- Materials And Methods

2.1 Study Area

The study was conducted from December, 2006 to March, 2007 in Assela, capital of Arsi Zone, in the Oromia Regional State, Ethiopia. Assela is situated 175 km southeast of Addis Ababa, at the base of mount Chilallo. Arsi zone is one of the administrative zones in the Oromia Regional State known for its wheat production. It is located 6^o 59' N latitudes and 38^o 41' to 40^o 44' E longitude southeast of the country (Annex l). Topographically, the altitude of the zone ranges from 500 to 4130 m.a.s.l, where a central plateau (2000-2500m.as.l) predominates with a narrow lowland area in the rift valley. Three climatic zones, including an arid, tropical highland and temperate forms are known to exist. An average annual temperature of 20^oc -25^o_c and rainfall of 200mm in the lowlands and 10^o_c – 15^o_c with a rainfall of 400mm in the highlands are recorded.

As in many other parts of the country, agriculture contributing 75.67% of the total GDP is the leading economic activity of the area. A mixed farming system covering 90% of the total agricultural activities, with crop production accounting for 45.33% and livestock for 30.34% of the GDP is typical of the area. Sheep stand next to cattle as the dominant livestock asset in the zone (Table 1).

Table 1: Livestock resource data of Arsi zone, 1997 census

Zone	Cattle	Sheep	Goats	Horses	Donkeys	Mules	Camels	Poultry	Total
Arsi	2,249,479	928,603	467,221	197,365	154,701	36,016	11,716	1,189,497	5,234,598

Source: Arsi Plan and Economic Development Office (APEDO, 1999) and Arsi zone Agricultural Regulatory Office.

Five restaurants in Assela were selected for sampling pneumonic lungs of apparently healthy sheep slaughtered at back yard, since small ruminants were not slaughtered at the Assela abattoir.

2.2 Study Animals

The study was conducted on any apparently healthy sheep slaughtered at back yard of restaurants at Assela town. The origins of the animals were from the surrounding 'areas of Assela (Sagure, Agura and Iteya) and Assela town.

2.3. Sampling Method and Sample Size

Purposive sampling of pneumonic lungs from apparently healthy sheep was used in this study. Assuming expected prevalence of the disease to be 20%, desired absolute precision of 0.05 and 95% confidence limit, about 300 lungs from sheep slaughtered at restaurants were examined, out of which 150 lungs were found pneumonic and only 130 lung samples taken for bacteriological study.

2.4. Case definition

The pneumonic lungs sampled were those lungs with irregular consolidations which vary from dark red to grey pink and grey. *Mannheimia hemolytica* is the new nomenclature for the formerly known *Pasteurella haemolytica* biotype A.

2.5. Study Design

2.5.1 Sample Collection

The surface of a pneumonic lung was rubbed with cotton soaked in 70% ethanol to minimize surface contaminants and a piece of lung was cut using a sterile pairs of scissors holding with tissue forceps, then put in to a sterile screw-capped universal bottle containing 3ml of Tryptose soya broth. Sample bottles were identified by labeling and transported to the laboratory in an icebox with in six hours.

2.5.1 Media Preparation

Various types of media were prepared and used for culturing the sample and to carry out subsequent procedures that aid in species identification. These include: Blood agar, Nutrient agar, Tryptose broth, Mac Conkey agar, Oxidation- Fermentation (O-F) medium, Christensen's urea medium, Triple Sugar iron (TSI) agar, SIM medium. Their composition and step of preparation was given in Annex 2.

2.5.2 Sample processing

Pneumonic lung tissues were bacteriologically examined for the isolation and identification of pasterurella organisms. This was carried out in Assela Regional Veterinary laboratory and National Veterinary Institute (NVI).

Isolation

Once the samples were taken to the Assela Veterinary laboratory, primary isolation was made using, Tryptose soya broth, Blood agar, Mac Conkey agar, Gram stain, oxidase, catalase, and

O-F using standard procedures (Merchant and Packer1983; Carter, 1984; Quinn *et al*., 1994). The lung tissue was cut with disinfected scissors. The scissors and tissue forceps were heated over the flame between each sample processing. Then, the cut surface was streaked over one half of a Petridish containing blood agar base supplemented with 7% sheep blood with an inoculating loop. The plates were then be incubated at 37^{O}_{C} aerobically for 24 hours.

The growth on the primary culture plates was characterized on the next day and *M.haemolytica* like colonies which were smooth, round, white or grey and more or less hemolytic were subsequently streaked on to another blood agar and MacConkey agar for further examination. Smears were made and the organisms were characterized by using light binocular compound microscope at 100 x magnification under oil immersion objective. Hence, colonies which gave gram negative coccobacilli or short rods with or with out bipolar staining on smears and which showed growth on MacConkey were transferred to nutrient agar and further biochemical tests were done at the National Veterinary Institute (NVI).

Identification of Isolates

A 24 hours pure Mannheimia *hemolytica* suspected cultures (isolates) were subjected to the following biochemical tests for identification following the standard recommended procedures (Carter, 1984; Quinn *et al*., 1994).

- Catalase test using 3% hydrogen peroxide.
- Oxidase test using N,N,N', N',-tetramethyl-p- phenylenedtamine dihydrochloride.
- Indole test using Kovac's reagent.
- Hydrogen sulfide production test on Triple Sugar Iron (TSI) agar.
- Urease test using urea medium.
- Oxidative –Fermentative (O-F) test using base medium.
- Motility test using SIM medium.
- Carbohydrate fermentation tests using 1% sugar solutions of lactose, arabinose and trehalose.

Accordingly, out of the total 130 pneumonic lungs examined or cultured, only 22(17%) showed *M. haemolytica* like characteristics on primary isolation. Thus detailed biochemical tests were carried out on these isolates (Annex 3) and *M. haemolytica* organism was identified based on its ability to produce oxidase and catalase and ability to grow on MacConkey and its inability to produce urease and indole, lack of motility, exhibit yellow reaction on slant and butt of TSI but no gas and H_2S and their ability to ferment lactose, Arabinose and Trehalose (Annex 4).

3- Results

Several visits between December, 2006 and March, 2007 were made to five restaurants in Assela and a total of 300 lungs of apparently healthy sheep slaughtered at back yard were inspected. From the total lungs examined, 150 (50%) were found to be pneumonic, of which 130(87%) were sampled for bacteriological analysis and brought to the department

of bacteriology of Assela Regional Veterinary Laboratory (Table 2).

Table 2: Number of lungs examined, sampled and processed during the study period.

Months	No slaughtered	No of pneumonic lugs	No. sampled	No.of Mannheimia like isolates
December	35	25 (71%)	20 (80%)	2 (10%)
January	120	60 (50%)	55 (92%)	6 (10%)
February	85	48 (57%)	41 (85%)	9 (22%)
March	60	17 (28%)	14 (82%)	5 (36%)
Total	300	150 (50%)	130 (87%)	22 (17%)

The detailed biochemical tests (indole, urease, motility tests, TSI agar and carbohydrate fermentation tests like lactose, trehalose and arabinose) indicated that the 22 isolates which resembled *M. haemolytica* on primary isolation were no more *M. haemolytica* organism.

Discussion

It has been known that *M. haemolytica* is normally found inhabiting the nasopharynx and tonsils of healthy sheep (Davis, 1987; Shreeve and Thomson, 1970). However, the organism has been known not to constitute the normal lung flora in healthy sheep (Ewers *et al*., 2004, Brogden *et al*., 1998, Radositis *et al*., 1994).

Following stressing environmental factors or shipping, the number of *M. haemolytica* found in the nasal cavity have been known to increase (Gilmour and Gilmour, 1989) and this rise in number of the organisms results in their entry in to the lungs and cause development of pneumonia (Gray and Thomson, 1971; Whitly *et al*., 1992; Bruere, 2003): the later has been known to be assisted by impairment of pulmonary clearance in situations of stress, environmental or climatic change and concurrent viral or bacterial infections of the lungs (Davies *et al*., 1983; Brogden *et al*., 1998; Bruere *et al*., 2002)

M. haemolytica pneumonia in sheep has been known by acute bronchopneumonia with plural and pericardial exudates and consolidation of the lung (Gilmour and Gilmour, 1989; Radositis et al., 1994) at necropsy.

The present study was made on the assumption that the *M. haemolytica* organisms isolated from the pneumonic lungs of apparently healthy sheep slaughtered was the cause of pneumonia. It has been known that in acute pneumonia where the animals show over clinical signs of fever, depression, weight loss, isolation from flock, muco-purulent nasal discharge, increased respiratory rates and rales of anterior thorax (Gilmour and Angus, 1983) and chronic non-progressive pneumonia (sub-clinical pneumonia) where the animals (sheep) does not show visible clinical signs (Alley, 1987b; Bruere et al., 2002), *M. haemolytica* has been known to be the main bacterial agent responsible for the lung damage (Alley, 2002), though the frequency of recovery of this organism has been known to be higher in acute pneumonia (Jubb *et al*., 1985; Daniel *et al*., 2006).

The results of the bacteriological study made on 130 pneumonic lung samples obtained from apparently healthy sheep indicated no isolation of *M. haemolytica* from these pneumonic lungs. This result agrees with reports of Tasfaye (1997), who reported that *M. haemolytica* was isolated (in some cases) only from tonsils but not from pneumonic lugs at Dessie abattoir.

In the study made on the New Zealand lambs, Goodwin-Ray, (2006) reported the presence of cases of no isolation rate of *M. haemolytica* organism from pneumonic lungs. This could happen as a result of:

- the infection with M. haemolytica which caused the pneumonia in the lungs sampled was no longer active but the pathology remained.
- despite the necessary possible precautions taken in this study to carefully transport the sample to the laboratory due to the delicate nature of the organism, they may have died.
- the pneumonia observed in the sampled lungs might have been caused by other pathogens; other bacteria, viral, fungal, parasitic agents. Oruc (2006) in his study on lambs with out breaks of pneumona (and from cases which died from pneumonia) in Turkey reported *E.coli*, *P.multocida*, *P.aeuroginosa*, *Staphylococcus*, *Actynomyces*, *Streptococcus* and *Mycoplasma* organisms from pneumonic lungs besides *M. haemolytica*. Similar reports were made by Goodwin-Ray (2006). On the other hand, information obtained from the study area indicated that there was no occurrence of ovine pneumonic pasteurellosis during the study period which might add to probable cause for lack of M. *haemolytica* isolates. Therefore, further Organized study which comprise the sick, contact and apparently healthy subjects with different organs sampled be carried out to father confirm the current study.

Conclusion And Recommendations

In Arsi region, as in many other parts of Ethiopia, livestock production plays a pivotal role in sustaining agricultural productivity. In this regard,

small ruminants assume a great share in the live stock sector of the region. The central high land plateau of Arsi is the major sheep rearing area, where most of sheep populations of the region are concentrated. In these areas sheep are important Assets that secure cash income and covers part of domestic expenses of local farmers.

Pneumonic pasteurellosis, caused by *Mannheimia haemolytica*, and important pathogen of ruminants, is a major bacterial disease in the area that lead to loss of a substantial number of sheep all year round during outbreak times through mortality. Even through in the present study the *Mannheimia haemolytica* was not isolated from apparently healthy slaughtered sheep and no out break was reported this year, the presence of number of strains and presence of several contributing factors to the disease demonstrate the continuing economic importance of *Mannheimia haemolytica* infection.

Based on this facts and related factors of the predisposing sheep to infection the following points are recommended.

- All cases of pneumonia were not only due to *Mannhemia haemolytica* therefore, a study should be undertaken to investigate the over all epidemiology of respiratory disease complex and/or in association with pneumonic pasteurellosis in small ruminants of the area.
- The present study did not include, diseased and in contact sheep and different specimens from them. Further more, the obtained isolates were not identified due to the fact that our focus was on *Mannhemia haemolytica.* There fore, the organized study which includes both bacteriological and serological investigation at the time of out breaks of the disease (ovine pneumonic pasteurellosis) should be devised to apply control strategy that reduce the major constraints of sheep production in the area so that the farmers in the area in particular, and the country in general will benefit from the large sheep population of the area.
- Pneumonic pasteurellosis accompanies stress factors including environmental or management, infectious and non-infectious and thus these factors in the area should be investigated in relation to pasteurellosis in the area.

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