

Epidemiologic and Serologic Investigation of Multifactorial Respiratory Disease of Sheep in the Central Highland of Ethiopia

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ABSTRACT

The prevalence of respiratory disease syndromes was investigated in 859 sheep in North Shoa, in the central highland of Ethiopia. Pasteurellosis, known locally as “Engib,” has become a disease of major concern in the study area. During the 1-year study period, the highest respiratory morbidity rate (64%) was observed in July; the lowest occurred in December (5%). Sera from 160 sheep were examined for 10 serotypes of *Pasteurella haemolytica* biotype A and *P multocida* biotype A using indirect hemagglutination testing. Positive results were seen for the presence of specific antibodies to all serotypes. The most prevalent serotype was A2 (36%); the least prevalent was A9 (2%). The remainder of serotypes (A2 to A9) varied in frequency. Ten percent of the sera were positive for *P multocida* A. *Dictyocaulus filaria*,

Muellerius capillaries, *Protostrongylus rufescens*, *Mycoplasma ovipneumoniae*, and *M agalactiae* were also detected during the study period.

INTRODUCTION

The highland of Ethiopia comprises approximately 35% of the country and is known for sheep production, an important part of the national economy.¹ Approximately 75% of the nation’s 24 million sheep are found in the Ethiopian highland region. Although sheep represent a great resource for Ethiopia, the rate of productivity per animal is low. Sheep disease and poor animal management is largely responsible for this reduced productivity.²

Respiratory diseases are a great economic concern for sheep producers in the central highlands of Ethiopia. In North Shoa, outbreaks of respiratory diseases occur frequently, killing significant numbers of sheep. Potential respiratory diseases of sheep include pasteurellosis, maedi-visna,

pulmonary adenomatosis, and verminous pneumonia.^{3,4}

Pasteurellosis is a complex disease that develops when the immune system of the animal is compromised by stress factors such as crowding, transportation, draught, and inclement weather. Concurrent respiratory infestations by *Chlamydia psittacci*, 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.^{3,5}

P 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.^{5,6}

In North Shoa, 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. Pasteurellosis is therefore a high-priority issue at the national level due to the significant economic losses it causes through mortality, morbidity, and the high cost of treatment.

This study was conducted with the objective of determining the prevalence of different serotypes of *Pasteurella* species, assessing the environmental and managerial factors that enable the disease to proliferate, and detecting concurrent parasitic and mycoplasma infections that aggravate respiratory problems in sheep in this region of Ethiopia.

MATERIAL AND METHODS

Study Area

The study was conducted in Debre Berhan,

North Shoa, 130 km northeast of Addis Ababa, the capital of Ethiopia. Located at 9°36" N and 9°36" E, Debre Berhan is a plateau in central Ethiopia that lies at an altitude of 2780 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%.

Cross-Sectional Study

A total of 188 farmers were selected randomly for interviews (2 farmers per 2-km distance) by following the roads from Debre Berhan to Addis Ababa, Ankober, Debre Sina, and Mendida. 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, farming constraints, and the area of each individual's plot of land. The second part of the questionnaire inquired about sheep production—number, breed, sex, utilization, feeding system, diseases in chronological order of occurrence, vaccination history, type of barn, and use of medications.

Longitudinal Study

A total of 952 sheep owned by 32 farmers in four different peasant associations were selected for a 1-year longitudinal study. The sheep were clinically monitored and weighed monthly. All clinical findings, particularly respiratory syndromes, were recorded and serum and fecal samples were collected in 2-month intervals.

Laboratory Analysis

Fecal samples collected during the survey were examined with a modified Baerman technique to detect the presence of lungworm larvae. The test was conducted by collecting fecal samples, grinding them with mortar and pestle, wrapping the samples with gauze, and using water and a funnel to collect sediment overnight. The sediment in the jar was examined for the presence of lungworm larvae. A total of 157 and 96

fecal samples were examined in October and December, respectively.

Sera from 160 sheep were collected in March and examined for serotype-specific antibodies by indirect hemagglutination test for 10 *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.

Indirect enzyme-linked immunosorbent assay (ELISA) was used to detect the presence of *Mycoplasma ovipneumoniae*- and *M agalactiae*-specific antibodies in the sera collected during the study. Sera collected from 176 sheep were tested using microplates, positive and negative control sera, and antigen supplied by Centre National d'Etudes Veterinaires et Alimentaires (CNEVA-LPPR/France).

RESULTS

Answers to the questionnaire indicated that the average family size in the area is 6 to 7

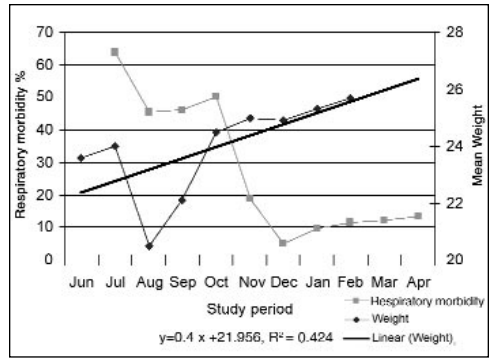


Figure 1. Respiratory morbidity and mean weight of 859 sheep in Central Highland, Ethiopia, during 1-year study period.^a

^aData were not collected for mean weight in March, April, and May or for respiratory morbidity in May and June.

and each farmer averages 17 sheep, 6 cattle, 4 chickens, 2 donkeys, 1 horse, and 1 goat. The dominant breeds of sheep were Menz (95%), Awassi-Menz cross (4%), and Horo and Adal breeds (1%). Most farmers (74.5%) keep their sheep in fully enclosed barns, 8.5% have animals in partially enclosed barns, and 17% have animals in open barns. Fifty-three percent of the interviewed farmers stated that their major source of income was the sale of small ruminants, mainly sheep, and the remainder of income came from the sale of animal by-products, firewood, poultry, and daily labor. A great majority of farmers (93.3%) used antihelmintics to treat their sheep and 46% 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, nasal discharge, and dyspnea. Respiratory morbidity was highest in July, when 549 of 859 sheep (64%) exhibited different respiratory syndromes (Figure 1). In December only 5% of the sheep exhibited respiratory syndromes.

An increase in mean body weight was observed during the study period with the exception of August. Mean body weight was lowest in August (20.5 kg) and highest in February (25.7 kg) (Figure 1).

Mortality associated with respiratory problems during the study period was low (< 0.1%): 25 sheep died from different causes, including parasitism.

Pasteurella Serotypes

The prevalence rates of *P haemolytica* A2 and A8 serotypes were the highest (36% and 35%, respectively), while serotype A9 was the least prevalent (2%) (Table 1). Ten percent of the sera were positive for *P multocida* A.

Respiratory Parasites

Of fecal samples examined for the presence of lungworm larvae in October, 55% were positive, while in December only 30% were positive for the parasite. Three species of ruminant lungworms (*D filaria*, *M capillaries*, and *P rufuscens*) were recovered; the most prevalent was *D filaria*.

Mycoplasma Species

Of 176 samples tested with indirect ELISA, 92% were positive for *M agalactiae* and 63.5% were positive for *M ovipneumoniae*.

DISCUSSION

Pneumonic pasteurellosis, known locally as “Engib,” is a disease of sheep 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.

During the 1-year study period the highest respiratory morbidity was observed in July (64%) and the least was in December (5%), exhibiting a positive correlation with rainfall pattern, suggesting that climatic con-

ditions play a role in respiratory problems in the area. The mortality associated with respiratory problems during the study period was low (< 0.1%), with 25 of the study sheep dying from different causes like parasitism, hyena bite, and poisoning with pesticides.

The animals’ mean body weight was lowest in August (20.5 kg) and highest in February (25.7 kg). The reduction of body weight in August might be attributed to the observed high respiratory morbidity in July and consequent loss of body condition. The results show that respiratory diseases affect sheep production through disease-related mortality as well as loss of body weight or reduction in body weight gain.

A multitude of *P haemolytica* biotype A serotypes were detected, with significant variation in prevalence among serotypes ($P < 0.001$), but due to lack of reference strains test were not conducted against serotype A12 and A16. The most prevalent serotypes recorded were A2 (36%) and A8 (35%), while A9 was the least with a prevalence of 2%.

The findings of this study are in partial agreement with reports that *P haemolytica* biotype A serotypes A2 and A1 are the most prevalent in Ethiopia, while A14, A11, and A9 were the least prevalent.⁷ In Sudan serotypes A2, A6, and A12 have been shown to be the most prevalent and A5, A7, and A11 were the least.⁸ In the United Kingdom serotype A2 is the most prevalent and A5, A8, A13, and A14 were the least.⁵

The high prevalence of *M ovipneumoniae* and *M agalactiae* found suggest that the circulation of these species in sheep of the area contribute to outbreaks of severe respiratory diseases. The role of mycoplasmosis in the development of ovine respiratory disease has also been reported.⁹

The prevalence of lungworm larvae recovered in the study was 55% in October and 30% in December. This finding was in close agreement with reports of a 73% prevalence rate of lungworm larvae in Debre Berhan,¹⁰ 58.6% in Assella,¹¹ and 84.6% in and around Addis Ababa.¹²

Table 1. The prevalence of *Pasteurella haemolytica* serotypes in sheep, Central Highland, Ethiopia, at a different dilution rate

	<i>P haemolytica</i> serotype										
	A1	A2	A5	A6	A7	A8	A9	A11	A13	A14	
1/20	38	15	39	9	25	19	36	3	5	35	
1/40	14	25	7	10	15	40	3	20	20	19	
1/80	7	32	0	13	14	16	0	26	34	11	
Number tested	160	160	160	160	160	160	160	160	160	160	
Number positive	59	72	46	32	51	75	39	49	67	65	
%P 20 ^a	37	45	29	20	32	47	24	31	42	41	
%P 40 ^b	13	36	4	14	16	35	2	29	33	19	
Geometric mean for positive 1/20 ^c	28	47	22	44	33	39	21	55	30	31	
Geometric mean for positive 1/40 ^d	50	59	40	59	54	49	40	59	61	52	

($\chi^2 = 29.4$, $P < 0.001$).

^aPercent positive at a dilution of 1/20.

^bPercent positive at a dilution of 1/40.

^cLevel of antibody titer at 1/20 dilution.

^dLevel of antibody titer at 1/40 dilution.

Keeping sheep in poorly ventilated and/or partially roofed and open barns also has a role in aggravating respiratory problems.

Researchers have suggested a synergistic effect of *Pasteurella* with *M ovipneumoniae*,¹³ bovine respiratory syncytial virus,¹⁴ parainfluenza-3,¹⁵ and environmental stress factors⁵ on the development of severe respiratory disease. 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 in the Central Highland of Ethiopia.

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