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Full Length Research Paper

Isolation of diverse bacterial species associated with maedi-visna infection of sheep in Ethiopia

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Bacterial species associated with maedi-visna (MV) infection and occurrence of respiratory disease complex (RDC) in sheep in the cool central highlands of Ethiopia was investigated. Of the 80 sheep examined, 61.25% (n= 49) were found to be MV seropositive and 38.75% (n= 31) were MV seronegative. At post-mortem examination, out of the 49 MV seropositive sheep, 75.51% (n= 37) showed pneumonic lesions in the lungs and a further 23 (74.19%) seronegative sheep were also found pneumonic. Overall, 87.5% (n= 70) of the lungs were culture positive and no bacteria were isolated from 12.5% (n= 10) of the lung samples. The majority of the bacterial species were isolated from grossly pneumonic lungs (75.71%, n= 94) and MV seropositive sheep (62.3%, n= 71). Of the total 114 bacteria isolated, 63 were gram-positive and 51 were gram-negative. Almost all gram-negative isolates (96.08%, n= 49) were recovered from pneumonic lungs. The bacterial isolates were classified into 18 genera and 23 species, some of which are known pathogens and some are opportunists. Involvement of diverse microbial groups in the development of RDC in the study area is discussed.

Key words: Diverse bacteria, Ethiopia, maedi-visna, pneumonia, sheep.

INTRODUCTION

Respiratory diseases in food animals are due to complex factors that often interact to produce disease. Various conditions such as inclement weather, weaning, transportation, poorly ventilated housing and nutritional deficiencies are known to play a predisposing role as the animal's immunity weakens sequel to the stressful conditions. In such conditions, flare up of the normal flora of the upper respiratory tract and subsequent infection of the lungs is well documented (Radostits et al., 2000). In Ethiopia, concurrent infections of the respiratory tract by viruses, bacteria and lung worms have been described and such disease conditions are commonly known as respiratory disease complex (RDC), indicating the difficulty to attribute to only one etiology (Teferi, 2000; Tibbo et al., 2001; Woldemeskel et al., 2004).

In the cool central highlands of Ethiopia, respiratory disease complex has been identified as the leading health problem of small ruminants for the last three decades which reportedly accounts for up to 54% of overall sheep mortality in the region (Mukasa-Mugerwa et al., 2007). The unusually chilly weather in the region which at times goes down to -8.5°C is an aggravating factor and the involvement of bacterial, viral and parasitic pathogens in sheep RDC have been documented by earlier epidemiological studies (Ayelet et al., 2001; Obasi et al., 2001; Tsegaw, 2004). Higher prevalence of the disease in adult and older sheep in the region showing progressive emaciation, dry coughing, occasional nasal discharge, increased respiratory rate, mouth breathing followed by paresis of hind quarter and recumbence have also been reported (Mackie et al., 1995; Tibbo et al., 2004).

Recent investigations on maedi-visna (MV) infection of sheep in the study area showed that the disease has already established itself among sheep breeds in the central highlands and controlling further dissemination to other unaffected regions has become a national agenda (Ayelet et al., 2001; Tibbo et al., 2001; Tsegaw, 2004;

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Woldemeskel et al., 2004). Irregular and insufficient vaccination program for diseases such as pastuerellosis and Peste des petitis ruminant (PPR), lack of strategic mass drenching against lung worms and occurrence of viral infections such as MV have been stipulated to play significant roles in the persistence of respiratory disease complex in this part of Ethiopia which accounts for about 75% of the county's 24 million sheep.

The present study was therefore initiated to further deepen our understanding of bacterial infections associated with MV and the role of such concurrent infections in the occurrence of the respiratory disease complex.

MATERIALS AND METHODS

Study area

The study was conducted from October 2005 to June 2006 at the Debre-Berhan Agricultural Research Center (DBARC). DBARC is located at 9°36 N, 39°38 E and 2780 m above sea level on the cool central highlands of Ethiopia. The climate has a bimodal rainfall pattern consisting of a long rainy season (June - September), short rainy season (March - May) and dry season (October - January); average annual rainfall of 954 mm, and a mean minimum and maximum temperatures 1.3 and 16.3°C respectively. *Andropogon, Festuca* and *Pennisetum* grasses mixed with *Trifolium Semenese* dominate the research center vegetation cover (Tibbo et al., 2004).

Study animals and management

The study was started with 1,083 sheep at the centre with Awassi, local sheep breeds and their crosses which were one year old and above of both sexes. The study population considered were sheep that died due to various causes and those apparently healthy ones slaughtered for culling purposes once they finished experimental trials at the centre. Totally, 80 sheep (36 males and 44 females) were sampled from which 34 were in a moribund state due to progressive pneumonia and 46 were apparently healthy slaughtered ones. The age of the sheep ranged from 1 to 7 years as determined by their dentition. The sheep usually graze on natural pasture and they had free access to hay during the dry season. All sheep were drenched with albendazole in September and April (after rainy seasons) and trichlabendazole in November. They were also vaccinated against ovine pasteurellosis and PPR in June and sheep pox in October.

Study design

A longitudinal study was undertaken to establish the diversity of bacteria infecting the lungs of dying and slaughtered sheep in association with MV infection and occurrence of pneumonia. A total of 80 sheep (34 from moribund animals and 46 from sacrificed) were included in the study based on systematic random sampling. Clinical, serological, gross pathological and bacteriological investigations were carried out.

Sample collection and analysis

Serology

A total of 80 sera were collected immediately before slaughter from apparently healthy sacrificed sheep (n= 46) and from those at the terminal stage of the progressive pneumonic illness or other diseases (n= 34). Blood was collected and recorded directly from the jugular vein in sterile plain vacutainer-tubes (Belliver Industrial Estate, Plymouth, UK) and individual animal data (age, sex, breed, body condition, and disease history) were also collected. The tubes were then kept at 37°C so as to allow clotting and sera separation. Sera were transferred into 5 ml sterile test tubes and preserved at -20°C until assayed. Agar gel immunodiffusion (AGID) test (Institut Pourquier, Montpllier-France) was used to determine the presence of specific antibodies against MV virus and to estimate sero-prevalence in the study population. The test is also used to distinguish MV infected from non-infected.

Gross pathology

Post-mortem examination was conducted either immediately after death on those clinically sick sheep with progressive pneumonia (n= 34) and apparently healthy slaughtered sheep (n= 46). During autopsy, special emphasis was given to respiratory organs.

Cultural and biochemical assays

Lung samples, irrespective of pathological status were collected from all (n= 80) the study sheep for bacteriological assays. From pathologic lungs, tissues were collected from the periphery of the lesion containing normal and pathological parts. All samples were transported to the laboratory for further processing. Standard aseptic procedures were followed for bacterial isolation; the surface of the lungs were cleaned with denatured alcohol and flamed immediately following the procedures of Carter (1984) followed by searing with heated spatula before the inner tissues were chopped and inoculated into sterile screw capped test tubes containing 5 ml of brain heart infusion (BHI) broth. The inoculated tubes were incubated, slightly loose capped aerobically at 37°C for 24 h. Similar procedures were followed for grossly non-pathologic lung specimens.

After 24 h, a loop full broth culture was plated onto a sheep blood agar media (BBL^{R}, Becton Dickinson, USA) by quadrant streaking method and incubated aerobically at 37°C for 24 h (Quinn et al., 2002). After 24 h incubation, the plates were observed for presence of bacterial colony. Colonies were then further sub-cultured by half plating on blood and MacConkey agars (Oxoid, Basingstock, England) side by side and incubated at 37°C for 24 h aerobically. After 24 h, presence or absence of growth and lactose fermentation on MacConkey agar were evaluated. On the blood agar, morphological characteristics and size of the colony, presence or absence of haemolysis and pigment production were observed and noted. The type of haemolysis was also examined and recorded. Thereafter single isolated colonies were aerobically sub cultured on BHI agar slant and preserved at 4°C for downstream biochemical and fermentative identification tests. Bacterial identification was done according to Barrow and Feltham (1995). Colony characteristics on blood agar, growth on MacConkey agar, catalase and oxidase tests, hydrogen sulfide (H_2S) production, indole, methyl red (MR), Voges-Proskauer (VP), citrate utilization, urease, oxidation-fermentation, motility and different carbohydrate fermentation tests were applied for colony and gram stain bacteria.

Clinical case study

Flock visits were done every day and records on feeding, alertness, specific clinical symptoms and treatment given. In addition, vaccination and drenching with antihelmenthics, body condition check-ups were documented. Animals found sick were separated and kept in patient pens with due care.

Table 1. Maedi-visna detection by age group.

MV status	Young	Adult	Total
Positive	6 (28.57)*	43 (72.89)	49 (61.25)
Negative	15 (71.43)	16 (27.12)	31 (38.75)
Total	21	59	80

Young < 1 year old; adult ≥ 1 year old; *numbers in brackets indicate percentages computed along columns.

Table 2. Overall maedi-visna infection and occurrence of gross pneumonic lesions.

	Lung pneumonic status		
Maedi-visna	**Pneumonic**	**Non-pneumonic**	**Total**
Negative	23(28.75)*	8(10.00)	31
Positive	37 (46.25)	12(15.00)	49
Total	60 (75.00)	20 (25.00)	80

X^2 = 19.06 p>0.05; *numbers in parenthesis indicate percentages of the total.

Data analysis

Data generated from clinical, serological, gross pathological, and bacteriological studies were analyzed with the statistical analysis systems of (SAS) and Stata (2003) versions.

RESULT

Clinical findings

Of the 34 sheep that died during the study period, 47.06% (n= 16) had been sick with respiratory disease manifested by overt symptoms like nasal discharge, coughing, difficulty in breathing with abdominal lift, fever and progressive emaciation. All sick sheep received a cocktail of antimicrobial treatment. None of the 46 sacrificed sheep showed signs of respiratory illness.

Serological findings

The agar gel immunodiffusion test detected the presence of antibodies against MV virus in 61.25% (n= 49) of sera tested (Table 1). Adults were found to be more affected [72.89 %, n= 43] by maedi-visna than the younger ones (28.57 %, n= 6).

Postmortem findings

Post mortem examination revealed the presence of gross pneumonic lesions in 75.51% (n= 37) of the 49 MV sero positive sheep and in 74.19% (n= 23) of the 31 MV sero negative sheep. Gross pneumonic lesions did not significantly differ (P > 0.05) with the MV status of sheep. Overall, of the total 80 sheep lung samples examined, 60 (75.0%) were found to be grossly pneumonic (Table 2).

Bacteriological findings

87.5% (n= 70) bacteria was isolated, of which 75.71% (n= 53) and 24.29% (n= 17) were found from pneumonic and non-pneumonic lungs respectively. 10 lung samples were found culture negative. Of those culture positive pneumonic lungs, 62.26% (n= 33) were from MV seropositive sheep while the rest 37.73% (n= 20) were from MV seronegative sheep. From culture positive non-pneumonic lungs (n= 17), 58.82% (n= 10) were from MV seropositive sheep and the remaining 41.18% (n= 7) were from MV seronegative sheep. Of those lungs which were culture negative for bacteria (n= 10), 7 were pneumonic and 3 were non-pneumonic.

Concerning mixed bacterial infections, 21 (35.0%) of the 60 pneumonic lung samples yielded only one type of bacterial species while two types of species were cultured from 30 (50.0%) of the lung specimens including lung pathogens of *M. haemolytica*. Furthermore, 2 (3.33%) lungs yielded 3 bacterial species and the remaining 7 (11.67%) specimens were culture negative whereas, *Staphylococcus* and *Streptococcus* groups were dominantly (55.0%, n= 11) isolated from the 20 non-pneumonic lung samples. In addition, two bacterial species were isolated from 5 lungs (25.0%) while one sample gave 3 types of bacterial species and the remaining 3 lungs (15%) yielded no bacteria (Table 3). Overall, 114 bacterial species were identified from 70 lung samples which were classified into 18 genera, and 23 of the isolates were identified to species level as shown in Table 3.

The classical lung pathogens, *Mannheimia haemolytica* and *Pasteurella multocida,* were isolated at a relatively higher rate (7.89%, n= 9 and; 7.02%, n= 8, respectively) than the other isolated bacterial species. However, other opportunist microbes were also isolated at higher percentages including *Staphylococcus epidermidis* (13.16%, n=15), *E. coli* (11.41%, n=13), *Staphylococcus aureus* (9.65%, n=1) and *Citrobacter* spp. (7.89%, n=9).

Overall, 51 (44.74%) isolates were gram negative and 63 (55.26%) were gram positive. Isolation of bacteria from pneumonic lungs did not significantly differ from non-pneumonic lungs (P > 0.05). Regarding mixed bacterial infection, however, pneumonic lungs were found to be infected with diverse bacterial species than non-pneumonic lungs (P < 0.05) (Table 3).

DISCUSSION

Respiratory tract infections in sheep, especially pneumonia continues to be an important cause of economic

Table 3. Mixed bacterial isolation from pneumonic and non-pneumonic lungs.

Lung status	Culture Negative	Single species	Two species	Three species	Total
Pneumonic	7(8.75)*	21 (26.25)	30 (37.5)	2 (2.5)	60 (75.00)
Non-pneumonic	3(3.75)	11 (13.75)	5 (6.25)	1 (1.25)	20 (25.00)
Total	10 (12.5)	32 (40.0)	35 (43.75)	3 (3.75)	80 (100)

X^2= 19.086 P<0.001; *numbers in parenthesis indicate percentages of the total .

loss (Tibbo et al., 2001). Progress in understanding its pathogenesis has been slow because of its complex etiology and varied epidemiology. Among respiratory tract infections, bacterial diseases have drawn attention due to their varied clinical manifestations, disease severity and emergence of strains resistant to a number of chemotherapeutic agents (Woldemeskel et al., 2002). Hence, there is a need to analyze the extent of involvement of different pathogenic bacterial agents in respiratory tract diseases of sheep so as to implement appropriate control measures. In the present study, investigation on bacterial species associated with maedi-visna (MV) infection was undertaken to further our understanding of the microbial consortia involved in the occurrence of respiratory disease complex (RDC) in sheep in the cool central highlands of Ethiopia.

The overall maedi-visna sero prevalence of 61.3% found in our study differs from previous reports of similar studies in the region as Ayelet et al., (2001) and Tsegaw (2004) observed lower seroprevalence of 5.4% (11/203) and 6.04% (9/149) respectively. Woldemeskel et al. (2002) and Seyoum (2005) reported sero-positivity of 74% (78/105) and 70.4% (176/250) respectively; these findings are in agreement with our results. Such differences might be ascribed to sample size and sampling strategy. Woldemeskel et al. (2002) tested only clinically pneumonic sheep while Ayelet et al. (2001) and Tsegaw (2004) made a general survey of the population and the present study was purposive where moribund animals irrespective of the sickness were included.

This study also confirmed the general trend that maedi-visna is detectable at higher rates in adults than young animals; particularly when less sensitive tests are carried out. Our data showed that only 6 (12.25%) young animals were found sero-positive for maedi-visna and the remaining 43 (87.75%) were adults; this finding is in line with the result of Woldemeskel et al. (2002), Ayelet et al. (2001) and Tsegaw (2004). An antibody against the maedi-visna virus accumulates over time and this makes detection of long time infected animals easier than those at the very early stage of the disease (Radostits et al., 2000). Concerning sex difference, from maedi-visna sero-positives, 17 (34.69%) were males and 32 (65.31%) were females.

Records at the research centre (DBARC) indicated that pneumonia is the most important cause of culling. A study by Zeleke (1998) indicated that 60% (94/155) of sheep mortality at the centre over a period of nine months was attributed to pneumonia. Our data also confirmed the higher prevalence of pneumonia in the region as 75.0% (n= 60) of the lungs examined were found pneumonic irrespective of overt clinical symptoms (Table 2). The post mortem data further showed that 75.5% (n= 37) of the 49 MV sero positive sheep had various levels of pathologic lungs while 74.2% (n= 23) of the 31 MV seronegative sheep had similar pathologic lung conditions. Differences between MV seropositivity and development of pneumonia were not statistically significant (P > 0.05) which contradicts the remarks of Ayelet et al., (2001), Concha-Bermejillo (1997), Saman (1999) and Seyoum (2004) who showed correlation between MV infection and development of pneumonia. Involvement of diverse groups of bacteria (Table 3) and the possibility of infection with other viruses, other than maedi-visna in pneumopathies of sheep is well documented elsewhere (Radostits et al., 2000).

Cultural and biochemical assays identified 114 bacterial strains of which the classical lung pathogens *M. haemolytica and P. multocida* were well represented (7.89%, n= 9 and 7.02%, n= 8, respectively), though two species of *Staphylococcus* were the single largest group (22.8%, n= 26) isolated in the present study followed by *E. coli* (11.4%, n= 13). *Streptococcus* species including *Streptococcus pneumoniae* (5.3%, n= 6) and three species; *Streptococcus uberis, Streptococcus pyogenes and Streptococcus bovis* (2.6%, n= 3) were also among the frequently isolated bacteria in this study. Ozkara (1998), Raji et al. (1999), Rajivkumar et al. (2000), Zeleke (1998), Wakwaya (1999) and Teferi (2000) also reported isolation of diverse bacterial species from sheep sick of respiratory diseases. Even though numerically more gram positives (n= 63) were isolated from the overall lung tissues, gram negative bacteria were dominantly isolated from pneumonic lungs (96.08%, n= 51). A strong correlation was observed between the presence of gram-negative bacteria and development of pneumonia (P <0.001), which significantly vary from the presence of gram-positive bacteria (Table 4). Isolation of diverse groups of bacterial strains in the present study will further enlighten our understanding of the respiratory disease complex in the study area.

Overall, more diverse groups of bacteria were isolated from MV sero-positive sheep (62.3%, n= 71) than sero-negatives [37.7%, n= 43]. The classical bacterial lung

Table 4. Frequency of bacterial isolates from pneumonic and non-pneumonic lungs.

Type of bacteria	Lung status		
	Pneumonic	Non Pneumonic	Total
Gram negative			
P. multocida	6	2	8
M. haemolytica	9	0	9
E. coli	13	0	13
Citrobacter spp	9	0	9
Acinetobacter spp	3	0	3
Neisseria spp	1	0	1
Enterobacter spp	3	0	3
Proteus spp	1	0	1
P. aeruginosa	1	0	1
K. pneumoniae	2	0	2
P. trehalosi	1	0	1
Gram positive			
S. pneumoniae	5	1	6
r. uberis	3	0	3
S. epidermidis	10	5	15
Bacillus spp	4	2	6
S. bovis	2	1	3
C. pseudotubercullosis	2	2	4
Micrococcus spp	3	1	4
Lactobacillus spp	0	1	1
S. pyogenes	2	1	3
S. areus	8	3	11
E. faecalis	2	0	2
R. equi	4	1	5
Total	**94**	**20**	**114**

pathogens in sheep pneumopathies such as *M. hemolytica* and *P. multocida* were isolated twice more frequent in MV seropositive sheep than MV seronegative sheep (Table 5). The immuno-suppressive nature of MV infection might partially explain the presence and higher frequencies of diverse groups of bacterial strains in MV seropositive sheep.

Our data indicated the potential role of the *Staphylococcus* species in the occurrence of respiratory disease complex as two species namely *S. aureus* (13.2%, n=15) and *S. epidermidis* (9.6%, n=11) were dominant among the gram positive isolates. Gillespie and Timony (1981) discussed that though species like *Staphylococcus* are not the usual pathogens of the lungs, they are indeed known to be opportunistic pathogens found everywhere including on the upper part of the respiratory tract. Such opportunist microbes are capable of invading and localizing in any part of the body under suitable conditions. Thus, the S*taphylococcus* species isolated at higher rates in the present study might have invaded the lungs as the animals' immune system got compromised due to extreme weather conditions of the study area and the concomitant MV or infection by other pathogens not covered in the current investigation. Similar bacterial isolations of *Staphylococcus* groups from the lungs of sheep were reported elsewhere by Seyid (1997) in Ethiopia, Barbour et al. (1997) and Rajivkumar et al. (2000). The specific roles of opportunist pathogens in the initiation of infection by producing a variety of enzymes and toxins were further discussed by Gillespie and Timony (1981).

Likewise, the isolation of majority (80%) of the 15 *Streptococcus* species namely *S. pneumoniae* (5.3%, n=6), *S. uberis*, *S. bovis* and *S. pyogenes* at similar proportions (2.6%, n= 3) from pneumonic lungs probably tell their association with RDC. *Streptococcus* species can also cause pneumonia in combination with other causative agents like *Mycoplasma* species or viruses (Sasani et al., 1998). Several workers also isolated these organisms from respiratory tract infections of sheep (Al-Sultan, 1995; Ozkara, 1998; Raji et al., 1999; Rajivkumar et al., 2000). *Bacillus* species were also isolated at a higher rate (5.3%, n=6) from pneumonic lungs (Table 4). *Bacillus* species has been described as ubiquitous microbes found in nature as normal micro-flora thus their role in the pathogenesis of respiratory infections is be-

lieved to be insignificant (Carter et al., 1995). Our findings agree with the studies of Rajivkumar et al. (2000), Savitha (2003) and Tekleselassie (2004) who frequently isolated *Bacillus* species from the lungs of sheep.

The involvement of *Rhodococcus equi* in ovine pneumonia has not been well described in literature and one of the few reports by Tekleselassie (2004) indicated that this bacterium was isolated from the respiratory tract of sheep in Ethiopia. The bacterium, however, is known as a normal inhabitant of soil and intestinal tract of animals and studies showed that the bacteria can replicate at warm temperatures in soils enriched with feces of herbivores (Quinn et al., 2002).

Isolation of *Corynebacterium pseudotuberculosis* from both pneumonic (n= 2) and non-pneumonic (n= 2) lung tissues in the present study is in line with various reports. The bacterium is known to associate with sporadic cases of pneumonia in sheep (Radostits et al., 2000). Although *C. pseudotuberculosis* is frequently isolated from *Caseous lymphadenitis* (Quinn et al., 2002), numerous reports maintain isolation of this bacterium from pneumonic lungs (Elyas, 1993; Ozkara, 1998; Raji et al., 1999; Tekleselassie, 2004) as well as non-pneumonic lung samples of sheep (Barbour et al., 1997; Soni et al., 1990) as in the present study. Other bacterial species like *Micrococcus* and *Enterococcus faecalis* species isolated in this study have a secondary pathogen role. *Micrococcus* species are known as normal inhabitants of the respiratory tract but could contribute to infection pathogenesis as a secondary invader together with other primary pathogens. *Micrococcus* species were isolated by several workers from mixed respiratory tract infections of sheep (Barbour et al., 1997; Rajivkumar et al., 2000; Tekleselassie, 2004).

Finding the classical lung pathogens *P. multocida* (15.6%, n= 8), *M. haemolytica* (17.6%, n= 9) and the common bacteria *Escherichia coli* (25.5%, n= 13) as the dominant isolates of the total gram-negative organisms (n= 51) in the present study particularly from pneumonic lungs, once more confirm their role as respiratory tract pathogens. The *Pasteurella* and *Mannheimia* species together account for 33.3% of the gram negative isolates and interestingly all the *E. coli* isolates were from pneumonic lung samples. It is well known that *E. coli* plays an important role as an etiological agent for various disease conditions encountered in man and animals Sasani et al. (1998) accounted that *E. coli* might produce lymphoproliferative interstitial pneumonia in ovines. Soni and Sharma (1990), Elyas (1993), El-Sukhon (1995), and Rajivkumar et al. (2000) also reported similar isolation of *E. coli* from pneumonic cases.

Pasteurella species are normal inhabitants of the upper respiratory tract of sheep and can cause pneumonia either alone or in conjunction with other organisms during stressful conditions. Acute fatal respiratory disease caused by *Pasteurella* species is a regular feature in disease surveillance reports and these species are the most common causative agents of clinical acute pneumonia in sheep in the study region (Deressa, 2002; Zeleke, 1998). The recently renamed *P. haemolytica* as *M. haemolytica* and its role as a cause of pneumonic pasteurellosis is well-documented (Gilmour et al., 1983; Mishra et al., 2000; Radostits et al., 2000; Watt, 1996). All the nine isolates of *M. haemolytica* recovered in this study were from pneumonic lung samples alone or in combination with the other types of bacteria. Our findings are in accordance with several workers who illustrate *M. haemolytica* as the most frequent pathogen isolated from enzootic pneumonia as well as apparently healthy sheep (Concha-Bermejillo, 1997; Dwivedi et al., 1990; Joshi et al., 1990; Mackie et al., 1995; Salgam et al., 1998; Sheikh et al, 1995; Sisay et al., 2003; Ward et al., 1997).

Nine of the gram negative lung isolates were identified as *Citrobacter* species (Table 5). Similar isolations of *Citrobacter* species were reported by Rajivkumar et al., (2000) who described these species as normal inhabitants of the upper respiratory tract of man and animals and stipulated that along with *Enterobacter* species may aggravate respiratory infections. The three *Acinetobacter* species were exclusively isolated from pneumonic lungs of sheep. The clinical significance of *Acinetobacter* species in the pathogenesis of pneumonia is yet to be documented. Likewise, three isolates of *Enterobacter* species in the present study were entirely recovered from pneumonic lungs. To our knowledge, there is no published report indicating the isolation of *Enterobacter* species from the respiratory tract of sheep. Furthermore, a highly virulent pathogen in domestic animals, *Klebsiella pneumoniae,* which is often associated with cases of pneumonia, was among the unique isolates in this study recovered exclusively from pneumonic lungs. Elyas (1993), Al-Sultan (1995) and Savitha (2003) captured this pathogen from pneumonic cases.

Together with other *Pasteurella* species, *Pasteurella trehalosi* is also known as a normal inhabitant of the upper respiratory tract particularly in the tonsils and it has been incriminated for its role in the pathogenesis of pneumonia in sheep (Sisay et al., 2003). In this study, one isolate of *P. trehalosi* was recovered from pneumonic sheep lung simultaneously with *M. haemolytica*. Our study once more confirmed the presence of mixed infections by *Pasteurella* species in pneumonic cases of sheep in the study area. Such mixed infections of the lungs were repeatedly reported (Al-Sultan, 1995; Concha-Bermejillo, 1997; Soni et al., 1990). Typing *Mannhemia* isolates which could have given details of the serotype diversity was not done in the present study. Nevertheless, worthy of mention are the works of Sisay and Zerihun (2003); Ayelet (2001); Wakwaya (1996); Zeleke (1998) who serotyped *M. haemolytica* and *P. multocida* isolates and showed the presence of diverse serotypes in various localities of the cool central highlands of Ethiopia. *M. haemolytica* serotypes A1, A2 and A8 were found to be the dominant ones. *P. aeruginosa, Proteus* and *Neisseria* species were each isolated only once. Such species in most cases proliferate and cause

Table 5. Number of bacterial isolates from sheep lungs and their Maedi-visna serological status

Bacterial species	Maedi-visna serological status		
	seronegative	seropositive	Total
P. multocida	3	5	8
M. haemolytica	3	6	9
Str. pneumoniae	2	4	6
Str. uberis	1	2	3
E. coli	5	8	13
Staph. epidermidis	7	8	15
Bacillus spp	3	3	6
Str. bovis	0	3	3
C. pseudotuberculosis	2	2	4
Micrococcus spp	2	2	4
Lactobacillus spp	0	1	1
Str. pyogenes	0	3	3
Staph. aureus	4	7	11
E. faecalis	0	2	2
Citrobacter spp	4	5	9
R. equi	3	2	5
Acinetobacter spp	1	2	3
Neisseria spp	1	0	1
Enterobacter spp	1	2	3
Proteus spp	1	0	1
P. aeruginosa	0	1	1
K. pneumoniae	0	2	2
P. trehalosi	0	1	1
Total	**43**	**71**	**114**

disease as a sequel to immunosuppressive conditions and tissue debilitations. Related isolations of these opportunistic pathogens from the respiratory tracts of sheep and their role in the development of pneumonia have been extensively described (Al-Sultan, 1995; Elyas, 1993; Ozkara, 1998). In most cases, such opportunists were captured in mixed respiratory tract infections in association with *M. haemolytica* (Mishra N., 2000).

In conclusion, respiratory diseases have been identified as the leading health problem of sheep in the cool central highlands of Ethiopia and the etiological agents involved are yet to be fully described. The present study illustrated the involvement of diverse bacterial species in respiratory tract infections of sheep. Isolation of more diverse bacterial species from MV sero-positive sheep in the present study further enlightened the role of primary viral attack as a potential predisposing factor for subsequent bacterial infections of the lung and development of bacterial pneumonia. The study further elucidated the overall dominance of gram-negative bacteria in pneumonic cases of sheep in the study area. The real benefits of using killed vaccine preparations from *P. multocida* biotype A in the prevention of ovine pneumonia in the region should be revised. Further studies on the involvement of other viral pathogens in the development of respiratory disease complex and design of polyvalent vaccines and vaccination strategies are recommended for the successful control of the disease in the region.

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