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Prevalence, histopathological findings and aerobic bacteria flora associated with pneumopathies in goats slaughtered at the Nsukka abattoir

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Abstract

This study was conducted to determine the prevalence, histopathological findings and aerobic bacteria flora associated with pneumopathies in goats slaughtered for human consumption at the Nsukka abattoir. The lungs of 342 goats were examined for gross lesions and samples were collected for histopathological and bacteriological examinations. Microscopic findings of this study showed that a total of 136 (39.8%) of the samples had various types of pneumopathies including bronchopneumonia, interstitial pneumonia, hyperemia, haemorrhages, oedema, etc. Bronchopneumonia was observed in 54 (39.70%) of the 136 lungs with pneumopathies while interstitial pneumonia and non-pneumonic pneumopathies were recorded in 44 (32.35%) and 38 (27.94%) respectively. Seventy-nine (58.11%) out of the examined 136, were recorded during the wet season and 57 (41.9%) in the dry season. Ninety-six (70.6%) of the cases were found in Kano brown goats, 39(28.67%) in West African dwarf goats and 1(0.73%) in Sahel goats. More of the females examined had pneumopathies. Aerobic bacteria isolated from the lungs with pneumopathies were *E. coli*, *Klebsiella pneumoniae, Mannheimia haemolytica, Streptococcus pyogenes, Staphylococcus aureus*, *Bacillus subtilis*, *Proteus vulgaris* and *Pasteurella multocida*. *E. coli* with a prevalence rate of 73.5% was the most predominant isolate. There was no significant association between the lung lesions observed and the associated aerobic bacterial isolates, seasons, sexes and breeds.

Keywords: Aerobic bacteria isolates, Bronchopneumonia, Interstitial pneumonia, Pneumopathies, prevalence

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Introduction

Food animal production has become a strategic and greatly important subsector for diversification of income and the fight against malnutrition and poverty in the urban and rural areas of the world (Bala *et al.*, 2011). Chakraborty *et al*. (2014), pointed out that the great Indian leader and freedom fighter M. K. Gandhi "father of the Indian nation" designated goats as "poor man's cow," emphasizing the importance of small ruminants in poor countries.

Goat is assuming a great importance in Nigeria as source of high quality meat in addition to milk and skin (Emikpe *et al.*, 2011). In Nigeria, one of the major limiting factors to the successful rearing of goats is disease (Ugochukwu, 2008). Pneumonia in goats is one of the most important infections of caprines frequently diagnosed in veterinary clinics, veterinary teaching hospitals and abattoirs (Elsheikh & Hassan, 2012). Factors such as crowding, dust, damp humid weather or stress can increase the incidence of the disease (Weiser *et al*., 2003). Pneumonia has been associated with heavy economic losses to farmers including mortality, emaciation, poor weight gain, poor meat quality and condemnation of the affected lungs during meat inspection (Tijjani *et al*., 2012a). In our study area, to the best of our knowledge, there has not been a comprehensive research work done on this topic, hence the need for the study. The objectives of this study were to determine the prevalence, histopathological findings and identify the aerobic bacteria flora associated with pneumopathies in goats slaughtered at the Nsukka abattoir and to determine the effect of seasons, species, breed and sex on the prevalence of pneumopathies in the goats slaughtered at the Nsukka abattoir.

Materials and Methods

Study area

This study was conducted in Nsukka, a town in Nsukka Local Government Area of Enugu state, Nigeria. It situated at latitude 6°51'24"N and longitude 7° 23'45"E, and has an estimated population of 1,377,001. Nsukka is (NPC, 2006). The climate here is tropical. The average temperature in Nsukka is 24.9 °C. The average annual rainfall is 1579 mm (Inyang, 1978).

Study design, sample collection and study duration

The study was conducted over a 6 - month period, 3 months in the dry season (Dec, 2013-Feb, 2014) and 3 months in the rainy season (April, 2014-June, 2014). The caprine lung samples with gross pneumopathies were collected in duplicates from goats looking sick and some looking apparently normal slaughtered at the Nsukka abattoir, placed in sterile sample bottles and appropriately labelled. One portion was used for histopathological tissue processing and studies while the other portion was used for bacteriological studies.

Gross and histopathological studies

The lungs of each slaughtered goat were examined and the nature and distribution of the pneumopathic lesions were properly recorded as described by Thomson (1981).

Sections of the lungs observed grossly to have lesions were fixed in 10% buffered formalin in appropriately labelled sample bottles and sent to Histopathology Laboratory, Department of Veterinary Pathology and Microbiology, University of Nigeria, Nsukka. After fixation, the tissue was dehydrated in graded alcohol (70, 80, 90 and 100%), cleared in xylene, embedded in paraffin wax and sectioned at 5µm thickness, using a microtome. The tissue sections were then stained with haematoxylin and eosin using procedures described by Bacha & Bacha (2012). The Slides were examined under light microscope at appropriate objectives and photomicrographs of representative lesions were taken using Motic camera.

Isolation and Identification of aerobic bacteria

Tissue sections from pneumopathic lung samples were aseptically collected, put into appropriately labelled sterile sample bottles and transported in ice packs to the Diagnostic Microbiology Laboratory, Department of Veterinary Pathology and Microbiology, University of Nigeria, Nsukka. These tissue materials were streaked on blood and MacConkey agar plates and incubated at 37°C for 24 hours. Representative morphological colony types were picked and purified on blood, nutrient and MacConkey agar. Colonial characteristics on blood, nutrient and MacConkey agar were noted. Gram positive Cocci were subjected to Mannitol salt agar, catalase, CAMP and coagulase tests adhering to standard protocols as described by Sharma (2009). Gram negative rods and coccobacilli isolates were cultured on Eosin methylene blue agar, Giemsa staining was performed, the following biochemical tests were also performed, namely; indole, methyl red, Voges proskauer, citrate utilization, nitrate reduction and urease tests as well as reaction on triple sugar iron agar were performed using standard procedures as outlined by Harley & Prescott (2002). Bacterial isolates were identified by colonial, microscopic and biochemical characteristics according to standard procedures as described by Hubbard (2010).

Statistical analysis

Descriptive statistics involving frequencies and percentages of occurrence of pneumopathies in goats and the associated aerobic bacterial isolates were used. Chi-square statistics was used to determine the level of association between the seasons, breeds, sex and the occurrence of pneumopathies. Significance was accepted at $P < 0.05$.

Results

Out of the 342 caprine lungs examined, 136 (39.8%) were observed microscopically to have pneumopathies included bronchopneumonia, interstitial pneumonia, hyperemia, haemorrhages and oedema. Of the 136 lungs with pneumopathies, 98 (72.05%) were pneumonic, which included bronchopneumonia and interstitial pneumonia, while the other observed pneumopathies were 38 (27.94%) with hyperemia representing 8 (5.88%), haemorrhages 18 (13.23%) and oedema 10 (7.35%). Figure 1 shows the prevalence of the individual observed pneumopathies as percentage ratio of total number of pneumopathies observed. Majority of the pneumonic pneumopathies were bronchopneumonia. The gross lesions observed were mainly those of frothy exudates in the trachea and oozing of fluid from the cut surface of the lungs indicating pulmonary oedema with a frequency of 7.35%, pulmonary haemorrhage with an occurrence frequency of 13.23%, hyperemia (5.88%), purulent exudative pneumonia, congestion and consolidation in the cases of suppurative and fibrinous bronchopneumonia. There was also fibrinous exudation into the pleura and parenchyma of the affected lungs in cases of Fibrinous bronchopneumonia while in cases of

interstitial pneumonia, the lung lesions appeared grossly as reddish and meaty in consistency.

Histopathological examination revealed the two types of bronchopneumonia; fibrinous pneumonia and suppurative bronchopneumonia were observed in 11.02% (*n* = 15/136) and 28.67% (*n*= 39/136) respectively, while interstitial pneumonia were observed in 32.35% (*n*=44/136). The lungs observed microscopically with bronchopneumonia were characterised by neutrophilic infiltrations in the alveolar spaces and lumens of the bronchioles and bronchi, and in 3 of the cases, there was a mixture of various amounts of cell debris and macrophages observed in these areas (Plate I). The lungs with interstitial pneumonia were characterised by interalveolar space infiltrated with predominantly lymphocytes, macrophages and a few neutrophils (Plate II). Histopathological findings in the cases of hyperaemia, included active hyperemia in which blood vessels were engorged with blood while in cases of pulmonary oedema, there was serous fluid accumulation in the alveoli and bronchioles. In some cases, the pleura was distended but this was not due to pleuritis but due to hydrothorax and in cases of haemorrhage, there was the presence of red blood cells in the interalveolar spaces in an ecchymotic pattern in most cases. There were several cases of backpooling of blood (Plate III), observed at the Nsukka abattoir.

Eight aerobic bacteria species were isolated from the lungs with pneumopathies in the following descending order of prevalence, *E. coli* 100 (73.5%), *Klebsiella pneumoniae* 32 (23.52%), *Mannheimia haemolytica* 23 (17%), *Staphylococcus aureus*

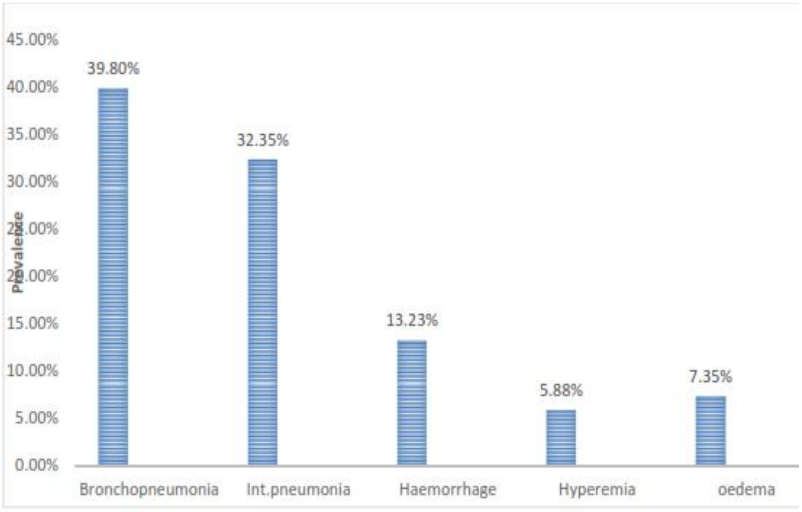

Figure 1: Prevalence of pneumopathies as percentage ratio of total number of pneumopathies observed in goats slaughtered at the Nsukka abattoir

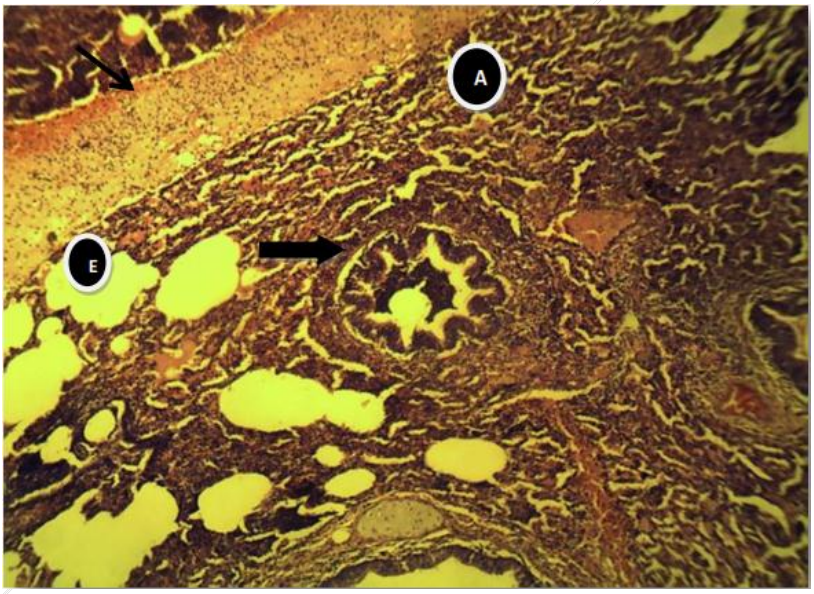

Plate I: Catarrhal bronchopneumonia in the lung of a Kano Brown goat
Note –Neutrophilic exudates in bronchiolar lumen (Thick arrow), thinner arrow pointing at distended interlobular space, infiltrated with polymorphonuclear cells, multifocal areas of alveoli distension (E) and alveoli collapse (A). X400 H&E

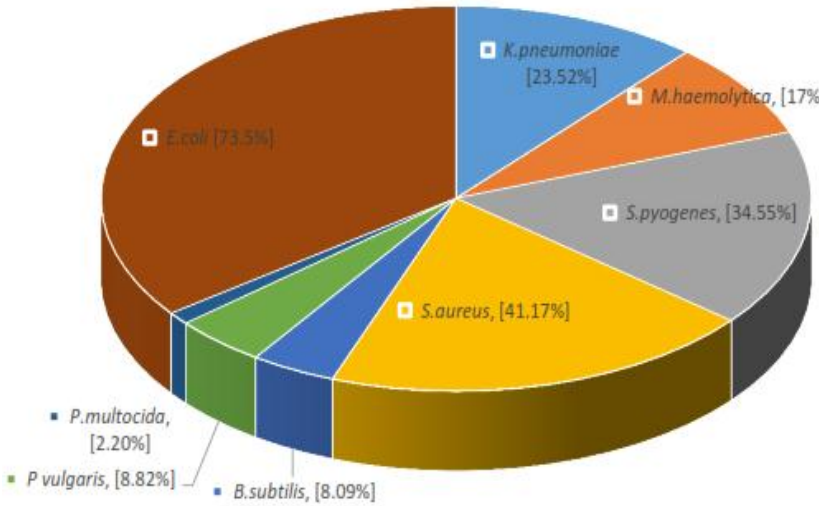

Figure 2: Percentage of aerobic bacteria isolates from lungs with pneumopathies in goats slaughtered in the Nsukka abattoir

56 (41.17%), *Streptococcus pyogenes* 47 (34.55%), *Proteus vulgaris* 12 (8.82%), *Bacillus subtilis* 11 (8.09%) and *Pasteurella multocida* 3 (2.20%), respectively (Figure 2).

Sex distribution of the cases of pneumopathies showed that more of the females than males were observed to have pneumopathies and more pneumopathies were recorded in the wet season than in the dry season (Figure 3). Of the 136 goats with pneumopathies 79 (58.11%) were recorded during the wet season and 57 (41.9%) in the dry season. 96 (70.6%) of the cases were found in Kano brown goats, 39(28.67%) in West African dwarf goats and 1(0.73%) in Sahel goats (Table 1). The chi-square analysis results (Pearson's Chi-square = 5.948, p-value = 0.546) showed that there is no significant association ($P<0.05$) between the histopathological findings observed in the goats examined and the Kano brown breed of goats, similarly, the chi-square analysis results (Pearson's Chi-square = 9.220, p-value = 0.237) showed that there is no significant association ($P<0.05$) between the histopathological findings observed in the goats examined and the WAD breed of goats. Therefore, was no significant association ($p>0.05$) between the lung lesions observed and the breeds with pneumopathies and seasons.

Discussion

Different types of pneumopathies both pneumonic and non-pneumonic were observed during histopathological examination. The types of pneumonia observed in this study are in agreement with the most common types of

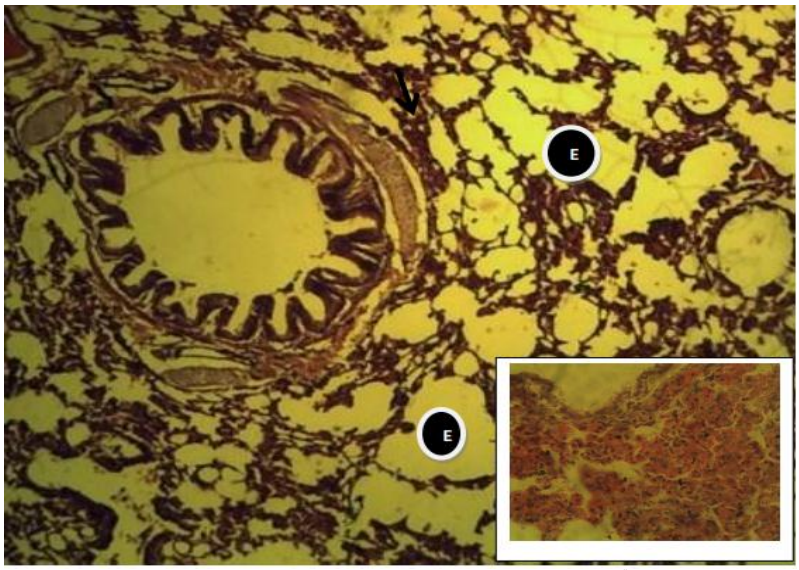

Plate II: Chronic interstitial pneumonia in the lung of a Kano Brown goat X 100 H&E. Note - (Arrow) interalveolar space infiltrated with inflammatory cells and distended alveoli (E). Inset showing infiltrating mononuclear cells (mainly lymphocytes, macrophages and few neutrophils) X1000 H&E

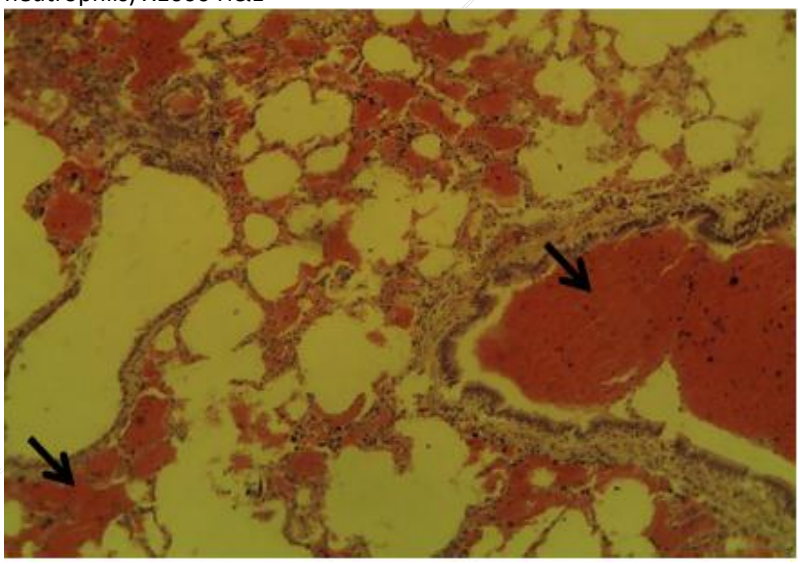

Plate III:- Active hyperemia (arrows) of the interalveolar space X 400 H&E in the lung of a West African dwarf goat

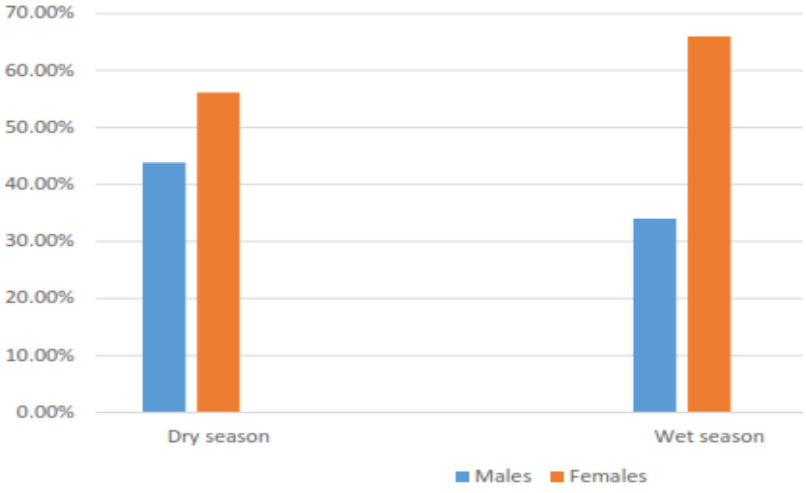

Figure 3: Sex distribution of pneumopathies observed in goats slaughtered at the Nsukka abattoir

Table 1: Breed distribution of Goats with pneumopathies slaughtered at Nsukka abattoir

Season	Breed			
	Kano Brown	West African Dwarf (WAD)	Sahel	Total
Dry season	38	18	1	57
Rainy season	58	21	0	79
Total	96	39	1	136

pneumonia encountered in previous studies. Yesuf *et al*. (2012) and Ashraf *et al.* (1986) reported such findings in small ruminants in Ethiopia and Pakistan. Upadhyaya & Rahman, (1993) also reported them in goats in their study conducted in Assam, India.

In this study, 28.67% of the pneumonic lungs amongst goats with pneumopathies showed the characteristics of catarrhal bronchopneumonia and lower than the 58%, 51.22% and 45.24% reported by Ashraf *et al.,* (1986), Yesuf *et al.* (2012) and Azizi *et al*. (2013) respectively in small ruminants. Also, fibrinous bronchopneumonia observed in this study 11.02% amongst goats with pneumonia was lower than the results 26.72%, 39.02% reported by Oruç (2006) and Yesuf *et al.* (2012) respectively but higher than 9% and 7.14% reported by Ashraf *et al*. (1986) and Azizi *et al.* (2013) respectively. The variation in the results could be attributed to differences in the predisposing factors such as production systems, transport stress, climatic changes, unhygienic conditions and a low level of herd health status which these animals were exposed to. The 32.35% prevalence rate of interstitial pneumonia recorded in this study is higher than 19%, 18.3%, 20.9% and 23.61% recorded by Upandayaya & Rahman, (1993), Oruç (2006), Azizi *et al.* (2013) and Yesuf *et al. (*2012) in small ruminants, respectively. The prevalence in this present study is comparable to that of Tijjani *et al*. (2012b), who reported a prevalence of (32%) in small ruminants but lower than the 41% recorded by Ashraf *et al.* (1986) in small ruminants. This is because the presence of interstitial pneumonia indicates other causes such as allergen and viruses are involved in the development of pneumonia (Azizi *et al.*, 2013). There were several cases of backpooling of blood, that were attributed to the slaughtering method of severing the jugular vein used at the Nsukka abattoir.

The *Escherichia coli, Mannheimia haemolytica and Pasteurella multocida* found in this study as the dominant aerobic bacterial organism isolated from fibrinous types of bronchopneumonia have been reported previously (Upadhyaya & Rahman, 1993; Oruç, 2006; Yesuf *et al.*, 2012; Azizi *et al.*, 2013). *Staphylococcus spp*, *Streptococcus spp* and *E. coli* were the predominant aerobic bacteria isolated from catarrhal bronchopneumonia. This is similar to the findings of Yesuf *et al.* (2012). However, *E. coli* was also isolated from some cases of catarrhal bronchopneumonia in this study.

In this study, *Staphylococcus aureus, Streptococcus pyogenes and E. coli* were the most dominant aerobic bacteria isolated. *Staphylococcus aureus* observed in this study is one of the predominant aerobic bacteria isolates from cases of interstitial pneumonia, which was also reported by Azizi *et al.* (2013). The aerobic bacteria isolated from the cases of interstitial pneumonia in this study could be due to the fact that geographic location, nutrition and climate are determining factors on the type of micro organism causing pneumonia (Azizi *et al*., 2013). The type of aerobic bacteria isolated in this study are similar to the isolates reported in small ruminants in Maiduguri, Northeast Nigeria (Tijjani *et al.,* 2012b) and Zaria, North central Nigeria (Raji *et al.,* 2000).

The variation in the prevalence rates of different types of pneumopathies in goats in different seasons may be attributed to the variation in nutrition, breed and the effect of stress factors involved (transportation, crowding and heat). Transport stress, overcrowding and nutritional shortage could possibly play a constructive role in predisposing the goats to different types of pneumonia (Yesuf *et al.,* 2012).

In this study, more of the Kano brown with a total number of 96 than the WAD (39) and Sahel (1) goats examined had pneumopathies. This difference was mainly due to the proportion of breeds examined because Kano brown goats was the most common breed slaughtered at the Nsukka abattoir and could also occur due to genetic variation of the breed which influences their immune response and host receptor interaction with colonizing bacteria of the selected species (Yesuf *et al.,* 2012).

Majority of the cases of pneumopathies were observed in females than in males for the dry and wet seasons. The results confirm findings by Raji *et al*. (2000) that rainstorms, heavy rainfall and dry harmattan are important stress factors in the pathogenesis of pneumonia of sheep and goats. In addition the Kano brown and Sahel goats were transported over long distances about 820km from the northern part of the country to reach the market, in Southeast Nigeria. They were also crowded and mixed at market places with the West African dwarf goats, during transportation and lairage.

The study period coincides with low environmental temperatures, representing a recognizable stressful condition. This is in agreement with the role of these commensal organisms could be enormous (Yimer & Asseged, 2007).

In conclusion, based on the results obtained in this study, the prevalence of most of the observed pneumopathies were higher in the rainy season than in the dry season, it was also higher in females examined and it was also concluded that several bacterial species inhabit the respiratory tract of apparently normal goats but stressful conditions could turn these known harmless commensals into disease causing bacteria.

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