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Bronchoalveolar Lavage Fluid Cellular and Haematological Changes in Different Types of Caprine Pneumonia

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Summary: Goats in the tropics are often reared under the traditional extensive and semi-intensive management systems. These and other factors influence the pattern of pneumonia complex in goats. We investigated the bronchoalveolar lavage fluid (BALf) cellular changes and haematological response in different types of caprine pneumonia in Nigeria. Haematological indices and BALf cells were analysed from 300 goats randomly selected from 700 goats comprising different breed, age and body scores. The pneumonia status was well characterised using standard pathological tools. Data is summarized as Mean ± SEM and compared using non-parametric statistics at 5% significance. There was leukocytosis in the pneumonic animals. The overall lavage recovery rate was 55.5%. The differences in Haemoglobin concentration, and Lymphocyte-Neutrophil ratio were significant ($p<0.05$). BALf changes in the neutrophil, macrophage and eosinophil counts were significantly different ($p<0.05$). The diagnostic features including increased percentage neutrophils, Macrophage-Neutrophil ratio and eosinophils observed in BAL were reliable and also correlated positively to the pathological findings. BAL should be considered a component of the diagnostic approach to caprine pneumonia complex, as it may accurately aid diagnosis and identification of the causal organisms.

Keywords: Bronchoalveolar lavage, Haematology, Pneumonia, Comparative, Caprine

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INTRODUCTION

Goats constitute 42.3% of Nigerian ruminant livestock population and contributing about 12.7% of the total agricultural gross domestic product (Lawal-Adebowale 2012). Small ruminant production have been limited by myriads of infectious diseases of which respiratory infections are of paramount importance (Emikpe *et al.*, 2013).

Bronchoalveolar lavage (BAL) is a tool in diagnosis of respiratory diseases (Reynolds 2000, Ezeasor *et al*., 2012 and Lee *et al.*, 2015). The cellular changes support clinical diagnosis in the absence of biopsy (Rottoli and Bargagli, 2004, Lee *et al.*, 2015). However, the correlations between BAL fluid (BALf) analysis and corresponding morphological features of the lungs have not been well defined in caprine pneumonia complex.

Previous studies in our laboratory has shown that caprine pneumonia in Nigeria is more of bronchopneumonia with fibrinous (30%) and suppurative (10%) types, others observed were interstitial pneumonia (15%), broncho-interstitial pneumonia with giant cells (40%) and atelectasis (5%) (Emikpe *et al.*, 2013, Jarikre *et al.*, 2016) Furthermore, the cellular response in the respiratory tract after vaccination and safety of non-conventional route of vaccine administration was well assessed using bronchoalveolar lavage celluar changes and haematology respectively in goat (Ezeasor *et al*., 2012; Tenuche *et al*., 2013, Ezeasor et al., 2014) however the use of BAL in evaluating normal and diseased lungs in goats is still scanty in literature especially in pneumonia of ruminants (Mohammad *et al*., 2007), horses (McKane, 2010), companion animals and man (Rottoli and Bargagli, 2004). In addition, due to non-specificity of the clinical signs in respiratory diseases and auscultation, examining the cytological characteristics of the respiratory secretions do provide both aetiology and an indication of treatment response (McKane, 2010), hence in tropical environment where management systems encourages transportation stress (Minka *et al.*, 2009) and other stressors, the health of those animals need to be evaluated routinely using these techniques. With the current focus on caprine respiratory diseases internationally, and the fact that BAL has been explored to determine the cellular and humoral responses to infectious agents' in cattle (Blodörn *et al.*, 2015) and experimentally in goats (Ezeasor *et al*., 2012; Tenuche *et al*., 2013), emphasis should then be on the cellular changes in naturally occurring caprine pneumonia which have not been previously investigated.

This study investigates the BALf cellular and haematological changes in the different pattern of caprine pneumonia in a tropical setting.

MATERIALS AND METHODS

The source of animals and pattern of pneumonia have been previously described (Jarikre *et al.*, 2016). Blood and bronchoalveolar lavage samples from 300 goats out of 700 were randomly collected for haematology and BALf analysis.

Haematology

Packed cell volume (PCV) and haemoglobin (Hb) concentration were determined by microhaematocrit and cyanmethemoglobin methods, respectively (Schalm *et al*. 1975). Red blood cells (RBC), White blood cells (WBC) and Platelets were counted using haemocytometric methods and light microscope (Schalm *et al*. 1975). Differential leucocyte counts were estimated from Romanowsky-stained smear at high dry (40X) and oil magnification (100X) counting 200 cells and classifying by type. Mean corpuscular volume (MCV) and Mean corpuscular haemoglobin concentration (MCHC) were calculated. Plasma protein was determined using the refractometer.

Bronchoalveolar lavage fluid

Lavage was as described by Khin (2009) and modified by Ezeasor *et al* (2012). Briefly, following slaughter, the trachea together with the lungs were resected and lavaged by introducing 40 ml of warm sterile phosphate buffered saline (PBS), pH 6.8 into the lungs. This was followed by gentle massage of the lungs before the fluid was re-collected into a measuring beaker. The colour and consistency of the BALf was noted before centrifuged at 2000 rpm for 15 minutes and the supernatant decanted. The sediment was smeared on clean glass slides, fixed with methanol and stained with Giemsa for cytological details. The BALf cellular differential count was as described by Dawson *et al.* (2005), 400 cells were counted on each of the stained slides with the reader blinded as to the identity of each goat.

Statistics Analysis

Data was presented as Mean ± SEM, and compared using non-parametric statistics at 5% significance.

RESULTS

Distribution of pneumonia

Of the 300 goats, 224 had varying pneumonic lesions bronchopneumonia (148), broncho-interstitial pneumonia (49), interstitial pneumonia (37), granulomatous pneumonia (3) and verminous pneumonia (2) while 61 were normal.

Haematological changes

The haematologies of pneumonic and non-pneumonic goats are presented in table 1. Generally, in pneumonic goats, there were increases in the haemoglobin concentration (HB), white blood cells, platelet, lymphocytes, and neutrophils. And a slight decrease in plasma protein. The leukocytosis, neutrophilia and hypoproteineamia were significant ($p<0.05$). The haematological changes for the different types of pneumonia are shown in Table 2.

There was significant decrease in HB of goats having broncho-interstitial, interstitial and granulomatous pneumonia; leucocytosis in goats with bronchopneumonia and leucopaenia in goats with interstitial and verminous pneumonia; thrombocytosis in goats with granulomatous pneumonia; lymphocytosis and neutrophilia in goats with bronchopneumonia; and increased Lymphocyte-Neutrophil ratio in goats with granulomatous pneumonia ($p<0.05$).

Bronchoalveolar lavage fluid (BALf) cytology

The colour of the lavage fluid recovered was clear to turbid with a top foamy layer, the volume recovered ranged from 15 ml to 25.3 ml, while the overall mean volume recovered was 22.2 ± 3.5 ml. The overall lavage recovery rate was 55.5% of the 40 ml of saline solution instilled into the lungs. Most of the stained smears showed a pinkish background with strands of mucus and fibrin. The BALf differential cell counts are shown in Table 1 and 2.

Generally, BALf changes in the pneumonic goats included significant increases in neutrophils, alveolar macrophages, Macrophage-Neutrophil ratio, lymphocytes and eosinophil counts ($p<0.05$). The cellularity was low to high constituting alveolar

Table 1. Haematological and BALf cellular count changes in normal and pneumonic goats

	Normal (61)	Pneumonic (239)	p-value
Haematology			
PCV %	28.3±0.7	28.1±0.4	0.80
HB g/dl	8.7±0.3	8.5±0.1	0.59
RBC $x10^3/\mu L$	6.1±0.4	8.3±2.1	0.60
WBC $x10^3/\mu L$*	$10.3±0.4^a$	$12.8±0.1^b$	0.03
PLT $x10^3/\mu L$	147±14.6	135±32.7	0.24
LYM $x10^3/\mu L$*	$8.8±0.9^a$	$11.0±2.3^b$	0.04
NEUT $x10^3/\mu L$	0.8±0.1	1.1±0.2	0.64
L:N	12.3±0.4	12.7±0.3	0.44
MON $x10^3/\mu L$	0.3±0.0	0.3±0.1	0.62
EOS $x10^3$ /µL	0.1±0.0	0.1±0.0	0.86
PP (g/dl)	7.8±0.1	7.4±0.3	0.62
MCV fl	56.6±2.9	55.9±1.6	0.85
MCHC g/dl	30.8±0.6	30.6±0.7	0.800
BALf			
NEUT cells $x10^2$/Ml	$7.7±1.5^a$	$127±8.4^b$	0.00
MQ cells $x10^2$/Ml	$47.8±6.4^a$	$175±5.3^b$	0.00
MQ:N	$3.1±0.5^a$	$5.9±0.6^b$	0.00
LYM cells $x10^2$/Ml	$32.0±3.4^a$	$73.2±3.7^b$	0.02
PC cells $x10^2$/Ml	$6.2±2.0^a$	$11.3±1.1^b$	0.08
EOS cells $x10^2$/mL	$0.1±0.1^a$	$7.5±1.8^b$	0.01
MAST cells $x10^2$/Ml	0.2±0.1	0.2±0.1	0.92

MON- monocytes, Neut- neutrophils, LC- lymphocytes/plasma cells, L:N- lymphocyte-neutrophil ratio, MQ- macrophage, MQ-N ratio macrophage:neutrophil ratio, EOS- eosinophil, MAST- Mast cells. Values with different superscript are significantly different across row at 5%

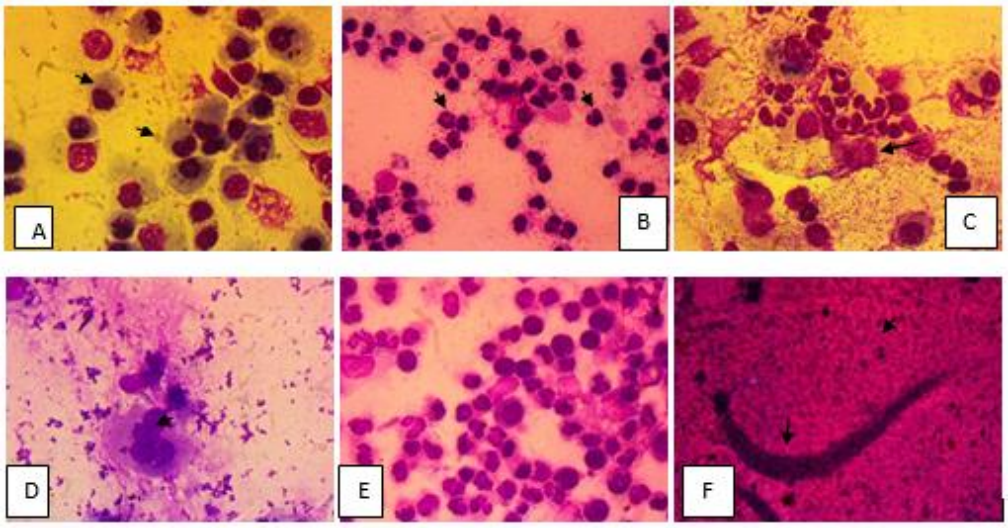
Table 2. Haematological and BALf cellular count changes in the different pattern of pneumonia in caprine goats

	Normal (61)	Broncho Pneumonia (148)	Broncho-interstitial (49)	Interstitial pneumonia (37)	Granulomatous pneumonia (3)	Verminous pneumonia (2)	p-value
Haematology							
PCV	28.4±0.7	28.2±0.5	28.6±1.0	27.1±1.0	24.0±5.7	27.0±0.0	0.72
HB*	8.7±0.3	8.7±0.2^{a}	8.1±0.3^{b}	8.2±0.4^{b}	7.5±2.1^{b}	9.2±0.2	0.04
RBC	6.1±0.5	9.9±3.4	6.1±0.5	5.4±0.5	6.5±2.6	4.5±0.0	0.93
WBC x10^{3*}	9.4±0.4^{a}	14.9±0.2^{b}	9.5±0.4	7.1±0.4^{c}	10.0±2.0	7.4±2.3^{c}	0.03
PLT x10^{3*}	133±6.3^{a}	142±6.5	136±8.3	123±8.7	205±81^{b}	145±0.7	0.05
LYM x10^{3*}	8.4±0.8^{a}	13.2±3.8^{b}	8.5±1.0	6.3±0.6	9.1±1.5	6.5±3.2	0.01
NEUT x10^{3*}	0.8±0.1^{a}	1.3±0.4^{b}	0.8±0.1	0.6±0.1	0.7±0.3	0.6±0.3	0.03
L:N*	12.3±0.4	12.3±0.3^{a}	13.3±0.6	13.3±0.7^{b}	15.7±4.4	11.1±0.3	0.02
MON x10^{3}	0.2±0.1	0.4±0.1	0.2±0.1	0.2±0.0	0.2±0.1	0.2±0.0	0.83
EOS	0.01±0.0	0.01±0.0	0.01±0.0	0.01±0.0	0.0±0.0	0.1±0.0	0.95
PP	7.8±0.9	7.3±0.4	6.9±0.1	8.3±1.5	6.4±0.2	6.3±0.3	0.88
MCV	56.6±2.9	53.2±1.9	60.6±4.4	61.0±4.4	48.4±3.4	60.1±0.1	0.36
MCHC	30.8±0.6	31.2±0.5	29.0±1.0	30.2±0.8	30.6±2.2	33.3±0.7	0.30
BALf cells x10^{2}/Ml							
NEUT*	7.1±8.1^{a}	165±10.1^{b}	72.3±13.0	57.3±15.2^{b}	89.3±85.4	64.0±16.0	0.00
MQ*	45.2±6.0^{a}	155±6.7^{b}	205±10.2	208±10.7^{b}	249±55	192±32.0	0.00
MQ:N	3.1±0.5	4.4±0.6	10.1±1.6	5.9±1.4	11.5±11.2	3.3±1.3	0.00
LYM	30.0±2.9	60.3±4.3	90.5±8.4	109±10.2	42.7±3.5	56.0±16.0	0.00
BPC	5.5±1.8	9.5±1.2	17.1±2.7	11.7±3.2	8.0±8.0	20.0±20.0	0.09
EOS*	0.0±0.0^{a}	8.4±2.4^{a}	5.1±2.5	3.9±3.3^{a}	0.0±0.0^{a}	68.0±52.0^{b}	0.01
MAST	0.2±0.1	0.02±0.02	0.3±0.3	0.9±0.5	0.0±0.0	0.0±0.0	0.06

MON- monocytes, Neut- neutrophils, LC- lymphocytes/plasma cells, L:N- lymphocyte-neutrophil ratio, MQ- macrophage, MQ-N ratio macrophage:neutrophil ratio, EOS- eosinophil, MAST- Mast cells. Values with different superscript are significantly different across row at 5%.

Table 3: Percentage BALf differential counts observed in the different types of pneumonia

Pneumonia type	Neutrophil	Macrophage	Lymphocyte	Plasma cell	Eosinophil	Mast cell	Total
Normal	6.1%	56.5%	32.1%	5.0%	0%	0.3%	100%
Bronchopneumonia	41.1%	39.2%	15.1%	2.4%	2.1%	0%	100%
Broncho-interstitial	18.2%	52.9%	23.2%	4.4%	1.3%	0.1%	100%
Interstitial	14.6%	53.3%	27.9%	3.0%	1.0%	0.2%	100%
Granulomatous	20.5%	66.2%	11.3%	2.0%	0%	0%	100%
Verminous	16.0%	48.0%	14.0%	5.0%	17.0%	0%	100%



Figures 1 A-F: Photomicrographs showing different BAL cellular changes: A-Alveolar macrophages (arrow). B-Neutrophils. C- Degenerate neutrophils (arrow). D- Giant cell (arrow). E- Mixed cellular population. F- parasitic larva (arrow). Giemsa X1000

macrophages (fig A) and other inflammatory cells, with a few phagocytosed bacteria, fungi and larva stages of helminthes present. Inflammatory cells include neutrophils (fig B), macrophages, lymphocytes (figures E), plasma cells, and eosinophils.

Morphologically, some of the neutrophils were degenerate (fig C) in the BALf (76), with numerous

intracellular bacteria rods in phagocytic cells (21). The macrophage-neutrophil (M:N) ratio varied greatly with pattern of pneumonia ($p<0.05$). The percentage BALf differential counts observed in the different types of pneumonia are shown in Table 3. There was significant changes in the percentage neutrophils, macrophages, lymphocytes and eosinophil counts ($p<0.05$).

In the pneumonic goats, 80 of the goats had neutrophil counts within range (<10%), 152 severe neutrophilia (>50%) and 68 moderate neutrophilia (in between). Concurrently, 127 had lymphocyte counts less than 15% (normal) and 173 with lymphocytosis (>15%). Low level of oesinophils (<1-2%), free erythrocytes and erythrophagocytosis (haemorrhage), giant cells (fig D), intracellular bacteria and Curschmann's spirals were also observed in a few of the BALf.

In bronchopneumonia, there was increased cellularity, clear to pinkish background and with a remarkable increase in neutrophils, macrophages and eosinophils ($p<0.05$). Numerous bacteria rods are free in smear and intra-cellular in neutrophils and macrophages. A few of the neutrophils are degranulated and degenerate. In broncho-interstitial pneumonia, there was increase in macrophages, macrophage-neutrophil ratio (M:N), lymphocytes and neutrophils ($p<0.05$). A few syncytial giant cells were present.

Interstitial pneumonia also showed remarkable increases in macrophages and lymphocytes ($p<0.05$). Similar pattern was observed in granulomatous pneumonia with increases in macrophages and M:N ($p<0.05$), while verminous pneumonia showed increases in eosinophils ($p<0.05$).

DISCUSSION

This study has been able to show the haematological and BALf cellular changes in the caprine pneumonia complex. These changes could serve as predictors in the diagnosis of caprine pneumonia in a tropical setting. Possible diagnostic indices to predict pneumonic lesions in these goats includes (macrophage-neutrophil ratio from BALf and blood leucocyte counts as erythrogram indices could be non-specific.

The prevalence, patterns and type of pneumonia in the examined goats has been previously described (Jarikre et al., 2016). Meanwhile, 300 goats with correlating signalment, body condition, haematological, BALf and morphological findings were reported.

The values for the haematological parameters observed were similar to those previously reported in goats by Daramola et al (2005) and Ezeasor *et al* (2015). The leukocytosis and neutrophilia observed are suggestive of tissue injury and or inflammation. Most of the other haematological parameters were within range of this specie. However, the leucogram changes are indicative of stress.

BALf differential cell count has been shown to be an accurate predictor of the cellular changes occurring in the lungs (Lee *et al.,* 2015). In this study, the differential cell count was used to ascertain the presence or absence of inflammatory response in the lungs. It was observed that there was a strong correlation between morphological changes and macrophage-neutrophil ratio (M:N). This was also reported in cattle and experimentally in goats (Tenuche *et al*., 2013; Ezeasor *et al*., 2015).

The amount of mucus in lower airways increases with pulmonary irritation. Specific causes of increased mucus or mucopurulent exudate includes bacterial, fungal or parasitic pneumonia, and chronic bronchitis. However, the significance of mild increases in the amounts of mucus in the airways remains unresolved and the point at which increased mucus is significant has not been defined. This was observed in this as also reported in horses with increased amounts of mucus, but no, or mild increases in the number of neutrophils and many activated macrophages (McKane, 2010). Mild elevations in the proportions of inflammatory cells, often accompanied by a mild increases in mucus, probably represent a normal response to noxious stimuli and in all probability do not contribute to decreased respiratory function (Lee *et al.*, 2015).

Quantification of the total number of cells/ml of sample retrieved may also help to indicate overall cellularity as well as assisting interpretation of relative numbers of individual types of inflammatory cells in BALf however defining cut-off values for normal percentages of inflammatory cells is difficult due to considerable variation between studies. It has been considered that BALf should have < 5% neutrophils, < 2% mast cells and <0.5% eosinophils. Wider ranges in the proportions of lymphocytes (30-60%) and macrophages (40-70%) have been reported (Taniuchi *et al*., 2009). However, from our observation neutrophil counts above 10% was suggestive of pneumonia. The presence of macrophages in all types including normal underscores the importance of alveolar macrophages in pulmonary clearance.

The high neutrophilia and lymphocytosis observed suggests tissue reactions to stress, bacterial and or viral injuries in the lungs. A number of factors may influence the accuracy of these counts, including variable dilution by infused saline and large amounts of mucus which can trap cells. Nonetheless, increase in the number of epithelial cells in samples was relatively rare. On the other hand, a few epithelial cells with degenerative changes were observed. Moreover, viruses and bacterial toxins cause direct damage to the

airway epithelium in cases of respiratory infections (Reynolds, 2000; Emikpe and Akpavie, 2011, 2012).

Pulmonary alveolar macrophages (PAMs) are the most abundant inflammatory cell in the BALf even from normal lung; this was very much evident in our findings. Therefore, increased proportions of these cells are difficult to detect. Occasionally increased amounts of mucus, increased total nucleated cell count (TNCC) and increased numbers of activated macrophages were quite useful. Lymphocytes also occur in higher proportions in BALf. Neutrophils respond to a variety of stimuli, and their numbers may fluctuate rapidly. This, however, reflects the innate response of the pulmonary system. Also, the effectiveness of the defense mechanisms in face of injury or irritants in the airways and gaseous exchange compartments in goat.

The usefulness of BAL as compared to other forms of washings cannot be overemphasized. BAL gives a more accurate representation of lung pathology than upper respiratory tract aspirates, except when lesions are localised to the cranioventral lung lobes (Espinasse, 1991). The volume of saline instilled is quite adequate and volume recovered is in range of those reported from 60–500 ml in large animals and 20- 50ml in small ruminants. The volume of fluid instilled may directly influence recovery rate and some studies have suggested that differential cell counts may be biased by small volume lavages (McKane 2010).

The Indications for BAL are generally not vague including signs of lower respiratory tract (LRT) disease, fever of unknown origin, poor performance, the presence of mucopurulent material in the trachea and possibly dyspnoea (Dawson *et al*. 2005). The colours of the BALf observed from the goats were also important. Normal BAL are clear or mildly turbid, whereas increased turbidity and presence of flocculent material reflects increased mucus, cells and cellular debris suggested pneumonia as was reported by Dawson *et al.*, (2005), while BALf differential cell counts were useful in determining the stage of the inflammatory process. In the initial stages of the caprine pnumonia complex, multinucleated syncytial giant cells were frequently observed. This is the case in broncho-interstitial pneumonia; neutrophils were few and alveolar- macrophages predominated (Allen, 1992, Blodörn *et al.*, 2015). In well advanced stages, neutrophils predominated. Cytological examination can therefore be used to assess stages of the pneumonic process. Occasionally, as when lungworm is involved, findings were diagnostic (eosinophilia). However, the low incidence of verminous pneumonia in this study may not be unconnected to the widespread use of broad spectrum anthelmintic.

The surface area has been the most widely used method for assessing severity of pulmonary consolidation in both experimental and naturally occurring cases of pneumonia. This method also has its shortfalls, especially not picking onset of pneumonic lesions, technicalities and experience involved. Nonetheless, its clinical usefulness cannot be overemphasized. Thus, the combination of gross consolidation, morphological changes and BALf analysis may to a large extent accurately detect pneumonia.

In conclusion, BAL should be considered a component of the diagnostic approach to caprine pneumonia, as it may accurately aid diagnosis and identification of the causal organisms. In Nigeria, where respiratory disease is a perennial problem in goats, it is worthwhile to ensure that investigation is carried out early enough in an outbreak, for effective diagnosis. Given the recognized losses and treatment costs associated with respiratory disease in ruminants, the relative expense of this procedure and laboratory techniques should not preclude its use in routine investigations.

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