CHAPTER ONE

GENERAL INTRODUCTION

1.1 Background

The small ruminants have integrated into our everyday livelihood the worldover. They contributes to economy and provide products for human consumption. In Africa alone, there are several millions of sheep and goats at the bottom of the food chain. These animals are kept by every household especially in the suburbs where they are managed semi-intensively (Ademosun, 1985). Small ruminant alone contribute sixteen percent of meat and protein needs of humans, and an average of thirteen percent to the gross domestic product from agricultural sector (Thornton, 2010). In Nigeria, over eighty percent of the sheep and goat are owned by rural households, in the different regions of the country (Ademosun, 1988).

The production of sheep and goat is attractive because of the low initial investments, however, the system of management influence the spread and outcome of respiratory diseases (Diallo *et al.* 2007; Emikpe *et al.* 2013). Over the years, pneumonia in small ruminants has caused enoumours production losses. Wosu (1994) estimated 71%, and Ameh *et al.* (2000) reported over 90% mortality among goats. This lingering problem of pneumonia may rub off on food and protein supply in the face of human looming population explosion (OIE, 2008). Most respiratory infections are fatal due to aerosolisation of the causative agents which include viruses and bacteria, and gaseous exchange requirements of the respiratory system (Hagglund *et al.,* 2006). However, the different mechanisms put the milieu of pathogens under check, but an overwhelming primary infection may compromise the host pulmonary defenses and enhancing the severity of respiratory infection (Hodgson *et al.* 2005)..

Emphasis of researches had also been on the description of pneumonia commonly observed in sheep and goats with very little knowledge on the pathogens that may be currently involved in our environment. Morphological studies of more than three decades underscored the

importance of giant cell pneumonia in goat (Ikede, 1977), and purulent pneumonia in sheep (Ikede, 1978). The causative agents investigated by different workers over the years included Peste des petits ruminant virus (Taylor and Abedunde, 1979; Obi *et al.* 1985), Mycoplasma, *Pasteurella spp*, (Ojo, 1975) and helminth parasites. Nonetheless, pneumonia remains a major limitation to goat production due to the ineffectiveness of chemotherapy and vaccination. Serological evidence of Parainfluenza-3 was reported in goats by Obi and Ibu (1990). Bacterial complications of primary viral infection have also been suggested as the reason for severity and inability to control caprine pneumonia (Emikpe *et al.* 2011). These studies opened the scope but more needs to be investigated on pneumonia in small ruminants for effective control of caprine pneumonia which is still a major problem in our environment.The knowledge on the dynamics and variations in pneumonia requires an update especially as it relates to the morphological change in goat so as to elucidate the role of different pathogens and or factors predisposing the animals to pneumonia.

Generally, this is need to evaluate the influence of different factors that contribute to pneumonia complex of goats in Nigeria. This study is designed to therefore evaluate the associated factors, aetiology, pathology, and markers of infection towards improving therapeutic and vaccination strategies for caprine pneumonia in Nigeria.

1.2 STATEMENT OF PROBLEM

Pneumonia remains a major limitation to goat production in Africa because of the ineffectiveness of chemotherapy and vaccination currently used for its control. In Nigeria, there is dearth of information on bacteria-viruses interaction involved in caprine pneumonia.

1.3 JUSTIFICATION FOR THIS STUDY

Small ruminant contribute immensely to the economy and livelihood of human population in African and Southeast Asian countries as well as the Mediterranean. These animals are valuable by virture of the meat, milk, and wool products. More so, the ever rising human population pressure, coupled with the soaring cost of dairy and beef production have increasingly drawn attention to improving productivity of goats.

Goat production in Nigeria is of immernse economic benefit. Despite the importance, two principal constraints impede the optimal performance of this animal, diseases and inadequate food supply. The most serious single cause of production losses ascribable to disease among all small ruminants in West Africa is Pneumonia. In general, pneumonia account for over one million USD loss yearly in Nigeria (Hamdy *et al.,* 1976), 1.5 million USD in Iran, 39 million USD in India (Bandyopadhyay, 2002) and upto fifteen million USD in East Africa (Thombare and Sinha, 2009). Irrespective of these obvious challenge in Africa and middle East, the global economic impact of pneumonia in small ruminant is still far elusive.

More so, goats are fundamental to the welfare and economic livelihood of peasant farmers, women and youths in Africa. The present global concern on poverty alleviation (sustainable development goal one) and improving the welfare of the rural poor can be built on the improvement of goat production. Further more, harnessing the mucosal immune response for prompt vaccination and control of pneumonia in goats may be important in reducing losses in goat production through optimisation of effective immune response. The research in caprine pneumonia may also allow for innovation and development in vaccinology.

1.4 RESEARCH QUESTIONS

1. What is the distribution and pattern of pneumonia in Nigerian goats?
2. What factors are involved in pneumonia in goats in Nigeria?
3. What are the markers and pathogens involved in caprine pneumona?
4. Which are the pathogens involved in caprine pneumona in our environment?
5. What is the role of the pathogens and oxidative stress in the pathology of pneumonia in the goats?
6. How can the use of vaccines be optimized for the control of caprine pneumonia?
7. What is the effect of combined intranasal vaccination on control of caprine pneumonia?

1.5 AIM OF STUDY

This study is designed to evaluate the associated factors, aetiology, pathology, and markers of infection towards improving therapeutic and vaccination strategies for caprine pneumonia in Nigeria.

1.6 OBJECTIVES OF STUDY

1. To study the patern and predisposing factors involved in caprine pneumonia.
2. To determine the haematological, and bronchoalveolar lavage cellular changes in caprine pneumonia.
3. To evaluate and quantify antioxiadant and prooxidant levels in the bronchoalveolar lavage fluid from pneumonic and normal caprine lungs.
4. To culture and isolate bacteria organisms involved in caprine pneumonia.
5. To immunohistochemically detect some viral and bacterial agents involved in caprine pneumonia.
6. To evaluate the effect of combined vaccinations and intranasal routes in control of caprine pneumonia.

CHAPTER TWO

LITERATURE REVIEW

2.1 Small ruminants

Small ruminant meet the day to day exchange needs of farmers and rural households. They constitute a reliable investment for capital and production return.The goat was designated as "poor man's cow," by M. K. Gandhi in India, emphasizing its contribution to the economy of the rural poor (Chakraborty *et al.* 2014).

Goats have played multiple roles in the support of mankind for over 7000 years. While goats were originally domesticated in Southwest Asia they quickly moved into Africa and now found in every environment on the continent (Peacock, 1996). Goats are embedded in human culture, kept in a wide range of agro-ecological zones and management systems in Africa (Peacock, 2005). The goat has a rich historical perspective even in human history, myths and beliefs.

2.2 Historical perspectives on goats

Goat (*Capra hircus*) was the first farm animal to be domesticated (8000 BC) in Ganj Darech, which is today known as Iran (Boyazoglu *et al.* 2005). The goat is present in religion, economy, nutrition, customs, and culture. Even the skin of the goat (aegis) was said to have miraculous qualities. Goats played an important role in the rural economies of Egypt, Palestine, Crete and Mycenae. The goat is recognised as the mother of the world (*Pzakriti*), represented by three colours; Red, White and Black, the three *gunas* or primordial qualities of life (Encyclopedia Mythica, 2004).

Goat's milk and meat have been consumed throughout the world for many years. The skin is used for parchment, clothes and liquid container for travellers (Boyazoglu *et al.* 2005). The value of the goat in the survival of the desolate masses of Western Europe during the Middle

ages in periods of great famine is also worth mentioning. Goats were ferried on ships by the early explorers for Cooking, and fresh milk. Angora goat was common in the Middle East since the time of Moses (13th century BC), it was introduced to Europe (1554) by King Charles V. From the three basic breeds around the world; Asian European and African, various other types were identified.

A paradigm shift in production methods was recoreded at the turn of the 19th century. Specialised system replaced conventional plant and animal husbandry, and intense production was designed to feed the urban populations that developed around fast growing cities. Milk-producing cow units were established and goats were relegated to more marginal and poor areas, where their role was limited to providing rural communities with milk and manure for fertiliser. However, the drop in the price of wool in early 19^{th} century encouraged a move towards goat farming. Nonetheless, goat farmers were considered as being on the lowest step of the social ladder, where they remained for a long time. The worst depression in the history of the wool industry began in the late 1920s, this again contributed to a revival of the goat sector. An important reduction in the European goat population took place in the middle of the 20th century, due essentially to the enforcement of extremely stringent laws against goats, which were considered a threat to the environment (Mavrogenis and Sinapis, 2003).

With the exception of Greece, goat populations declined significantly over the past 100 years. The reduction in goat numbers was particularly significant following the two World Wars. Food shortages encouraged an intensified approach to agriculture, which, among other things, promote the development of the dairy cow. Furthermore, the cultivation of grass in the form of intensive prairies was not favourable to the goat and the wave of mortalities due to gastroenteritis and pneumonia had been alarming. The 20th century was the turning point in goat production. It created different conditions, prospects and orientations that heralded a more dynamic phase of management for goat farming. High productivity in agriculture and even certain scientific advances were contested due to the growing sensitivity for the environment. The goat symbolised ecological agriculture and diversification. The conditions that prevail today can be described as being multi-faceted and relatively complex. The problems and prospects of goat production vary depending on where in the world the farming activity takes place as this involves different cultural, financial or geopolitical issues in each

case. Therefore, in addition to examining the population figures, products and characteristics (advantages and shortcomings) on a global level, the problem should also be approached with respect to the economy of specific developed and developing countries (Iniguez, 2004). Most countries in Africa have witnessed growing populations in recent years. Milk production has increased worldwide by a meagre 8.7%, while meat production by an impressive 40%.

2.2.1 Importance of goats in developing countries

Goat farming is important for food production, employment and economic values. Asia and Africa hold up to 96% of the world's goat population (Boyazoglu *et al.*. 2005). The goat is adapted to the different climates, and widely acceptable by the people. The continuous and rapid increase in goat populations and products shows that it is important for the continuous increase of human population (Iniguez, 2004). Goat is also considered sacred for religious, traditional or cultural ceremonies (Alexandre *et al.* 2002). However, the goat sector suffers from poor funding.

Rural households mostly train and own goats, and these households are characterised by poverty and low agricultural input (Lebbie, 2004). There is no sufficient infrastructure, marketing research and extension services. Goats have not been accorded adequate attention in Nigeria. Most importantly in areas of its management and health conditions, bacause mortalities are recorded annual. Pneumoenteritis, pneumonia, helminthiasis, and mange are the prevalent diseases encountered by farmers.

2.2.2 Importance of Goats in developed countries

After a century of being perceived in a negative light, goat farming is now viewed by developed countries more positively. The change has different origins, reflecting different dynamics, and raises other problems (Boyazoglu et al. 2005). The positive development pertains to the resilience the goat demonstrated in comparison to livestock. The goat enjoys a new profile, and gradual acceptance of its superior breeding and product quality. Goat milk from goats is reputed and recommended for consumption. The lack of production restrictions has played a significant role in the maintenance and increases in the credibility of goat breeding. Furthermore, the healthy and ecological image of goat products is associated with

agrotourism (Lebbie *et al.* 2004). Finally, goats are now used more frequently to actively and positively modify vegetation cover (Hart, 2001).

Nevertheless, many factors impede the growth of goat farming in both developing and developed countries. The goat is considered a culprit in deforestation and desertification. A few of the population are not familiar with or do not like the taste of goat milk, and product. More so, the principal factor that limits goat production is the sociological or societal outlook.

Goat breeds fall into overlapping, general categories. They are distributed broadly as dairy, fiber, meat, skins, and or as companion breeds. The dairy goats are some of the oldest defined animal breeds for which breed standards and production records have been kept. The breeds include; Pygmy, Cashmere, Mohair, Angora, Anglo-Nubian, Alpine, Abaza, Boer, Bengal etc. Over 200 domestic goat breeds are known all over the world.

2.3 Common breeds of goat in Nigeria

Some of the common breed of goats in Nigeria include; the Red Sokoto, West African Dwarf, Sahelian (plate 2.1), Kano brown and other mixed breeds.

2.3.1 Red sokoto goat: The Red Sokoto goat (or Red Maradi) is the predominant breed of goat found mainly in the Sudan and Sahel savanna and or Sokoto province, North-West Nigeria (plate 2.1a). The Northern region of Nigeria supply more than 60% of the goat population (Obua *et al.*, 2012). However, it is characterized by dry season which often causes serious shortage of feed for the ruminants.

2.3.2 West African Dwarf (WAD) Goat: The West African Dwarf goat (WADG) is common in Southern Nigeria (plate 2.1b). It is indigenous to the 18 countries of West and Central Africa. The Dwarf goats are linked to pygmy goat, which is one of the ten primary goat breeds believed to have originated from the wild Benzoar goat, indigenous to the

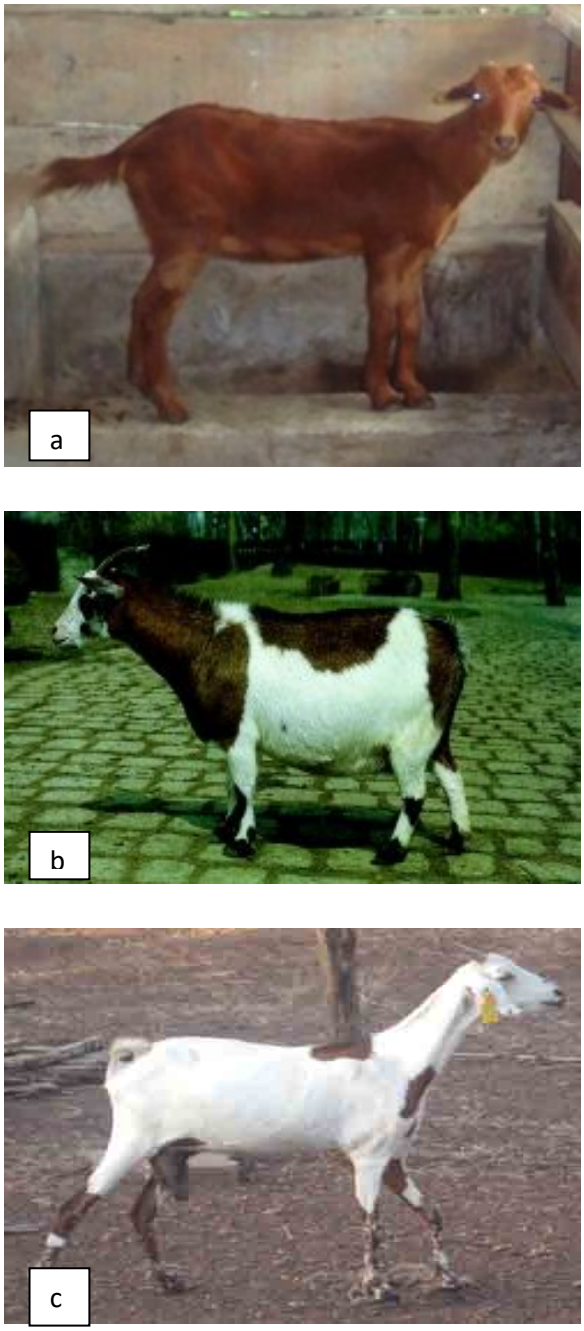


Plate 2.1. Breeds of Goat in Nigeria. a) Red Sokoto (maradi) having a red colour coat and lean body mass. b) West African dwarf with short limbs and variable body coat. c) Sahelian with long limbs and floppy ears.

middle East (*Capra aegagrus*). WADG is generally used, but the breed is synonymous to Cameroonian, Nigerian, Guinean and Fouta Djallon goat. The WADG industry constitute 30% of livestock in Africa, with 17% and 12% meat and milk products respectively (Lebbie, 2004). The goat census record indicate over 150 million goats in Sub-Saharan Africa comprising, and more than eighty breeds in the different agro-ecological zones (Oseni and Ajayi, 2014).

There are two distinct types of the West African Dwarf Goat, and Nigeria hosts the largest WADG population. The **Humid** WADG differ from the and the **Savanna** WADG phenotypically, in body weight (Lawal-Adebowale, 2012). WADG have unique ability to adapt well to its native habitat, including surviving in trypanosome-endemic zones (trypanotolerant). They also have high fertility and prolificacy, which largely compensate for its small size and low body weight in comparison to commercial breeds. The socio-economic importance of WADG is reflected in the typical agrarian settings and the expression‘bank on the hoof’. However, the morbidity and mortality attributed to parasitic infection and pneumonia is quite alarming.

2.3.3 Sahelian goat (Borno White): The Sahelian goat belongs is most suited to desert or semidesert environments and intolerant to high humidity. The breed is widely distributed in the arid and semiarid zones of the Sahel, north of 12°N from central Chad in the east to the Atlantic coast in the west and well into the southern Sahara. This is the most suitable place, where most of the world’s Sahelian goats are found, although some numbers of this goat breed can also be found elsewhere in the world, especially in such areas where suitable environments or conditions are available, such as Australia. The Sahelian goat is not a trypano-tolerant and usually do not survive for long in forest and dense savanna. They are well-adapted to nomadic life.

Respiratory disease is still a major problem in goat despite the research resources and control measures The interaction and complication of multiple pathogens leads to severe damage of the respiratory organs, morbidity and loss of goats. The uniqueness of the respiratory system in ruminants allow for precipitation of aerogenous pathogens and also spread of lesions within the lung. The nature of the immune response and other host responses will also determine susceptibility of these animals to pneumonia.

2.4 Respiratory System- Anatomy and Histology

Generally, the respiratory system allow for exchange of gases between the blood and the atmosphere and improve the quality of the inspired air with flow regulate. The respiratory system begins at the nostrils, through which the air enters the nasal cavities, and is continued by the nasopharynx, larynx, trachea, bronchi, bronchioles and to the lungs (Plate 2.2).

A astratified squamous epithelium lines the nostrils and nasal vestibule, the epithelium transits caudally to an intermediate epithelium, stratified cuboidal and pseudostratified ciliated columnar epithelium towards the alar region (Kahwa and Purton, 1996). The epithelium is specialized for sensing along the olfactory region. There are surface mucus/goblet cells and submucosal glands in the epithelia lining the nasal conchae and septum (Bloom and Fawcett, 1976; Mariassay and Plopper, 1983). Lymphoepithelium and lymphoid aggregates are present in the nasal caity.

There are slight variations on the epithelial lining of the larynx; stratified squamous epithelium to pseudostratified ciliated columnar epithelium. Submucosal glands are numerous producing acid rich to mixed mucosubstances. The trachea and bronchi are lined by simple columnar epithelium. There is an increase of mucus cells as the airway diameter decrease, while submucosal glands decrease as airway diameter decrase (Kahwa and Purton, 1996).

Pseudostratified ciliated columnar epithelium, simple columnar ciliated and nonciliated epithelium (Clara cells) and goblet cells line the bronchioles. Clara cells stains negative to Acian Blue or Periodic Acidic Schiff stain (AB/PAS). The distal bronchioles and respiratory bronchioles (interrupted by alveoli) are lined simple cuboidal epithelium.The epithelial lining of alveoli is simple cuboidal to squamous epithelium comprising type 1 and type 2 pneumocytes (Kahwa 1992).

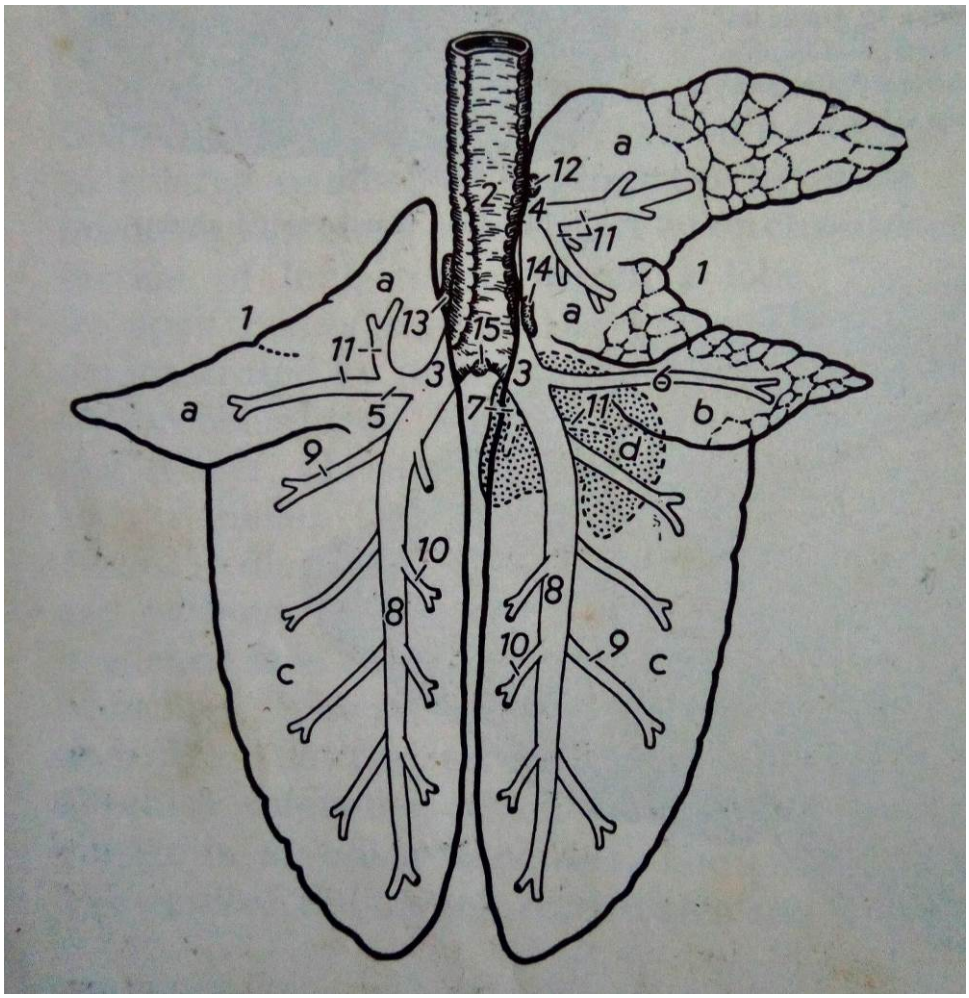


Plate 2.2: Schematic drawing of the lobation, bronchial tree and lymph nodes of the lungs in the goat (dorsal aspect)

a Apical lobe; b Cardiac lobe; c Diaphragmatic lobe; d Accessory lobe (stippled).
1 Cardiac notch; 2 Trachea; 3 Principal bronchus; 4-8 Lobar bronchi; 4 Tracheal bronchus; 5 Cranial bronchus; 6 Middle bronchus; 7 Accessory bronchus; 8 Caudal bronchus; 9 First (left) and third (right) ventral segmental bronchi of the caudal lobe; 10 Second dorsal segmental bronchus of caudal lobe; 11 Further segmental bronchi; 12 Cranial tracheobronchial lymph node(s); 13 Left tracheobronchial lymph node(s); 14 Right tracheobronchial lymph node(s); 15 Middle tracheobronchial lymph node(s). (Nickel *et al.*, 1973)

The morphology of the tracheobronchial tree in the goat is not too different from other mammals. There are mucuos cells in the upper respiratory epithelium, decreasing inversely with the airway diameter (Kahwa and Purton, 1996). Presence of submucosal glands as in sheep (Mariassay and Plopper, 1983; Plopper et al. 1983) and cat, differing in mouse (Pack et al. I981), guineapig (Nadel et al. 1979), and rabbit (Plopper et al. 1984). Acid rich and mixed mucus are produced in equal proportion, same as in ox, sheep (Mariassay and Plopper, 1988), Rhesus monkey (Plopper et al. 1989), and man (Spicer et al. 1983), but differing to pig (Jones *et al.*, 1975) with neutral mucosubstances. The bronchiolar epithelium is same as in pig, dog (Majid, 1986), horse (Pirie, 1990) and man (Ten Have-Opbroek et al. 1991). Although, Getty (1975) showed the absence of respiratory bronchioles in ruminant unlike in rabbit (Plopper et al. 1983), rat (Massaro et al. 1984) and horse (Pirie, 1990). Also, the bronchiolar bronchiolar epithelium is devoid of submucosal glands in goat (Plopper et al. 1989). The alveoli is devoid of mucous cells (Atwal et al. 1979). However, the respiratory bronchiolar epithelium in the goat are well developed and prominent. The mucosal surface of the bronchiolar airway and alveoli are suppose to be sterile while the pulmonary clearance mechanisms are optimised for innate immune response (fig 2.3).

2.5 Immune Response in the Respiratory Tract

Mucosal surfaces of the body are unique due to the continual assault by pathogens from the environment. The pathogens gain entrance through contact, attachemt before replicating and induction of lesion (Kaul and Ogra, 1998). The respiratory, gastrointestinal, and urogenital surfaces have unique mucosal immunity. The lungs and respiratory mucosae are exposed to continual assaults from aerolised microbes. Maximal mucosal response is reauired at the mucosal surface for physiologic and immunity at respiratory surface.

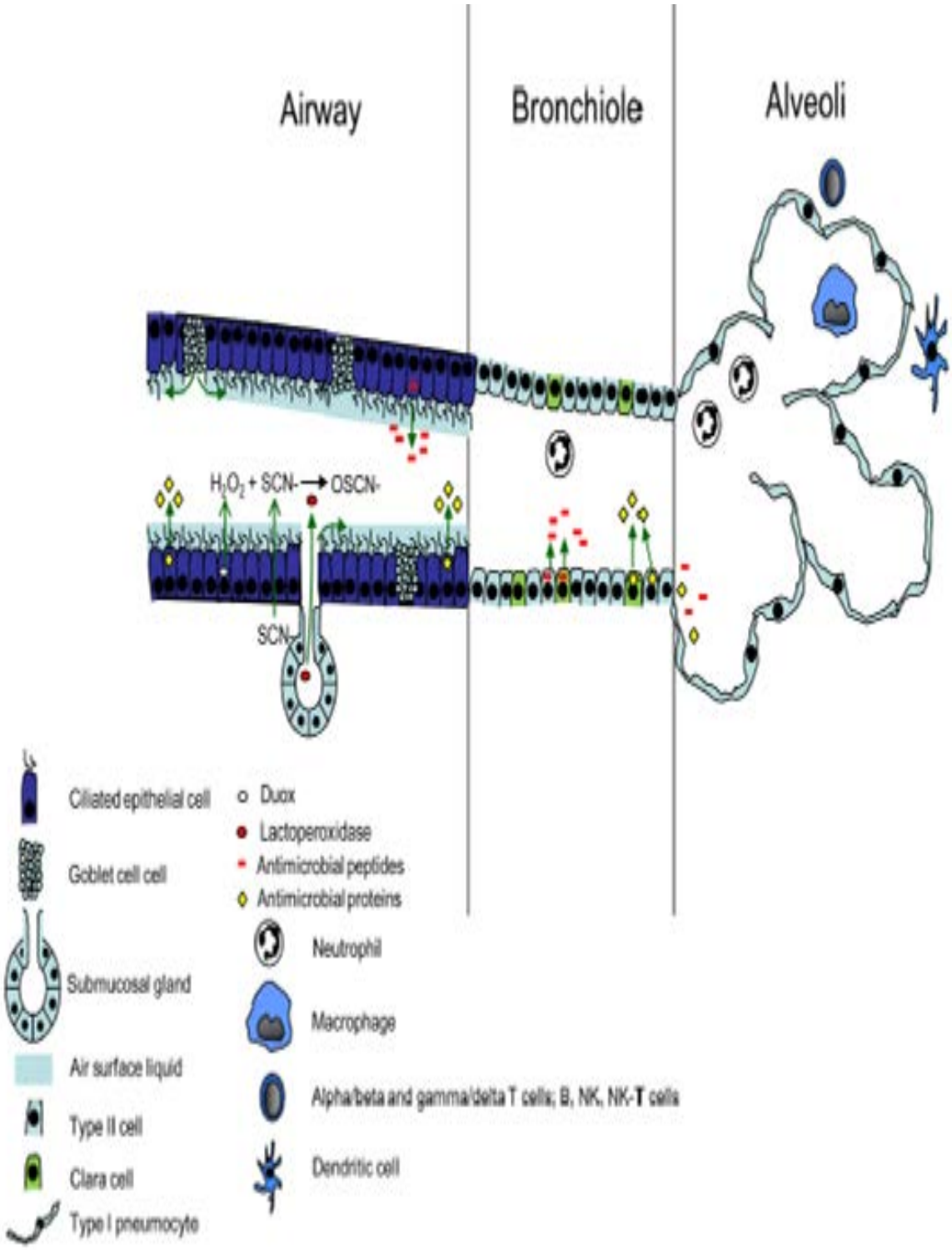


Figure. 2.3. Scheme on the mucosae and pulmonary defense at the airway and alveoli. (Source: Ackermann et al. 2010)

The pulmonary defense and clearance comprise physical, cellular and antimicrobial defense systems. The physical barriers include the epithelial cells, surfaces projections and junctional complexes, prevent pathogens and other matters from direct attachment and entery into the lungs. The epithelium secretes mucus containing soluble chemical mediators, lysozyme, lactoferrin, collectin and defensins which have antimicrobial properties. These soluble mediators also recruit phagocytic cells and opsonisation of pathogens and matter. The phagocytic and inflammatory are quick to response due to nonspecificty of their receptors.

The immunity at the mucosal surface has an induction part comprising mucosal associated lymphoid tissue (MALT) which process and present antigens and effector part responsible for long lasting immunity (memory-effector) (Kiyono and Fukuyama, 2004). The memory cells move to the MALT, lymph nodes and circulation for eventual specificity at the mucosal surface (Fig 2.4), under the influence of cell surface adhesion molecules (mucosal addressin cell adhesion molecule-1 MAdCAM-1) and chemokines.

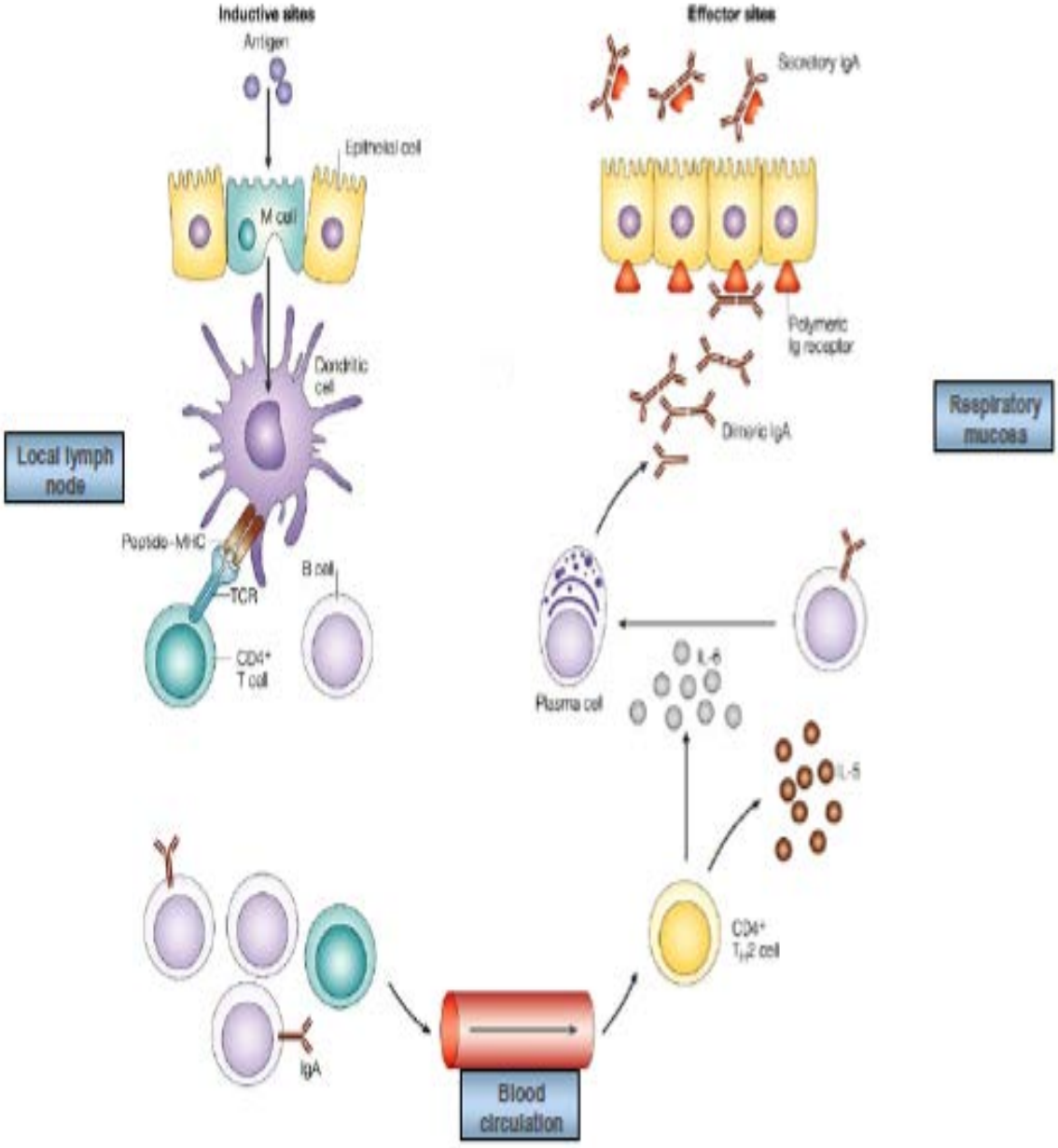


Figure 2.4. The Respiratory mucosal immune system depicting differentiation of lymphoid cells and effector arm of immune response (Modified from Nature Reviews 2004).

2.5.1 Specific innate immune responses in the ruminant

The combinatinations of physical barriers, resident microflora, expression of chemokines from the respiratory epithelium, mucosal and histiocytic cells serve in the innate immune response of the respiratory tract. These may be considered as follow;

2.5.1.1 Commensial microflora: The upper respiratory tract is colonized by a variety of bacterial pathogens that are inhaled, replicated in the tonsillar crypts and nasal/sinus mucin. Colonization of these organisms within regions of the upper respiratory tract mucosa may occupy micronutrients and receptor sites resulting in reduced colonization by pathogenic bacteria. *Bacillus sp., Streptococcus sp. Streptomyces sp. Micrococcus sp*., and *Pseudomonas sp* are some commensal bacteria isolated from the upper respiratory tract. *Mannheima haemolytica, Pasteurella multocida, Mycoplasma sp, Histophilus somni, Streptomycetes sp., Neisseria spp. and Bacillus spp*. have been demonstrated in nasopharyngeal swabs of healthy calves (Allen *et al.* 1991). The deeper lung remains relatively sterile in healthy animal; however, these organisms may cause lesion in the lung as it was demonstrated in bronchoalveolar lavage fluid in cattle (Angen *et al.* 2009).

2.5.1.2 Pathogenic microflora: Myriad of microbial agents have been reported to be infectious to the respiratory tract (Respiratory disease complex RDC). These include viruses that have a tendency to infect immunocompromised lung: Herpesvirus; Peste des Petits Ruminant virus, Respiratory Syncytial virus, Coronavirus, Parainfluenza virus, Adenoviruses, and Bovine viral diarrhea virus in ruminants. *Mycoplasma spp.* inhibits function and activity of ciliated respiratory epithelial cells. Initial viral infections, toxins such as 3-methyl indole, or other immunosuppressive conditions allow increased replication of other bacterial pathogens: *Mannheimia, Pasteurella, Histophilus, Archanobacterium* and *Chlamydiaceae. Mannheimia, Pasteurella* and *Histophilus* may also colonize the tonsil and mucous of the nasal meatus and sinuses.With stress, their replication increases and the area of colonization spreads in the respiratory tract (Agarwal and Marshall, 2001).

2.5.1.3 Respiratory airways: Ruminants have a relatively long tracheobronchial tree which increases the amount of dead space volume in comparison to dogs, pigs and horses (Kahwa and Purton 1996; Kirschvink, 2008). The increased dead space affects the amount of fresh oxygen that can be delivered to lung and increases the risk of alveolar hypoventilation with partial obstruction. The increased dead space may not affect respiratory tract immunity per se; however, it may allow increased surface area for particulate deposition and increased transit time of inhaled vapors, gases and particulate matter (Ackermann *et al.* 2010).

Hairs along the external nares provide a physical barrier to inhalation of large particulate matter. Squamous cells that line the anterior nares form a layer of stratified squamous epithelium which is more resistant to microbial adhesion compared to pseudostratified epithelium.

2.5.1.4 Mucociliary apparatus: The air-surface liquid (ASL) lining the upper respiratory tract and pulmonary airways is generated largely from submucosal glands and goblet cells. It provides a layer of protection against inhaled particulate matter, aerosols, vapors, and microbial pathogens (Fig. 2.3). The ASL has two layers, a periciliary sol layer close to the apical cell surface and a gel/mucus layer that is toward the airway lumen. Ciliary beat occurs principally within the sol layer which is less viscous than the gel layer. Dehydration may increase the viscosity of the ASL, likewise aggregates of DNA and filamentous actin that accumulate from degraded neutrophils, leukocytes, and necrotic epithelial cells along with bacterial biofilms (Ackermann *et al.* 2010). Sneezing and coughing help to expel particulates and ASL/mucin aggregates and also induces some dilation of the airway lumens. The antimicrobial activity of the ASL is becoming increasingly understood.

Air surface liquid was **o**nce considered simply a lubricant for airway function, the ASL has a very active role in innate defense. Water accumulates within the periciliary sol layer after secretion of chloride ions by epithelial cells, submucosal glands and serous cells and water is resorbed as sodium is removed from the layer by epithelial Na^+ channels (ENaC). In addition to providing a microenvironment for ciliary activity, the periciliary sol layer maintains a proper pH (slightly more acidic than blood). The gel layer of the ASL is composed of mucin glycoproteins and proteoglycans secreted by goblet cells and submucosal glands. The amount

of this layer can increase with chronic inflammatory conditions, allergic conditions, and cholinergic stimulation. The mucin glycoproteins are tethered to the apical membrane of the subjacent epithelial cell or secreted. The protein backbone of the mucin glycoproteins is encoded by *MUC* genes, which contain repeating serine and threonine amino acids that form O-linkages with oligosaccharides (Ackerman *et al.* 2010).

The ASL contains numerous molecules that mediate antimicrobial as well as pro- and anti-inflammatory activity, immunomodulation and wound healing (Grubor *et al.,* 2006). The osmotic gradient is maintained by sodium and chloride level regulated by sodium and chloride transport pumps and channels which allows proper protein, enzyme, and peptide activity. The presence and activity of lactoferrin and lysozyme have been known for many years. Lactoferrin binds and sequesters iron from microbial agents, and lysozyme disrupts membranes of bacteria. Additional components of the ASL have been indentified in cattle and other species and increasingly appreciated for the immune and immunomodulatory role. These include antimicrobial peptides such as defensins, cathelicidins, and larger proteins. Three major subclassifications of defensins but two; alpha (α) and beta (β) defensins are expressed in ruminants.

The beta-defensins include tracheal, lingual and enteric antimicrobial peptides (TAP, LAP & EAP), produced by respiratory epithelia, with TAP having the highest level of expression. TAP was the first mammalian defensin identified and cloned (Diamond *et al.*, 1991). These defensins form pores in the membranes of bacteria, enveloped viruses, and other microbial agents resulting in their rapid lysis. Beta defensins have numerous other activities that range from exacerbating the acute inflammatory reaction; triggering histamine release from mast cells, to triggering the adaptive immune response through their chemotactic properties to dendritic cells, to inducing wound healing via their mitogenic function in epithelial cells (Boudreux *et al.,* 2005). Ruminants neutrophils have numerous alpha defensins as well; however, these are produced in the ASL only after neutrophils recruitment and degranulation. Cathelicidin antimicrobial peptides are also produced in neutrophils and can be released by lung epithelia; however, epithelial expression is apparently low and limited. Airway epithelial cells also produce RNAase, an RNA helicase with antimicrobial properties; however, the expression levels of RNAase in cattle and goat is not known. Neutrophils express 13 alpha

defensins that are released upon degranulation, facilitating microbial killing and acute inflammatory response (Ackermann *et al.* 2010). Binding of defensins and other antimicrobial peptides to host membranes is limited presumeably due to the higher cholesterol content of mammalian cells and thus, autotoxicity by these peptides is not a feature of their activity.

In the alveoli, alveolar macrophages engulf particulate matter and pathogens that manage to escape aforementioned physical barriers and structures into the deep lung. Once activated, the macrophages release cytokines, chemokines, and other soluble mediators that stimulate an inflammatory and/or immune response (Dyer *et al.* 1989). Alveolar lining cells (type I pneumocytes) are covered by a layer of surfactant (surface active agents), composed of phosphatidylcholine and other phospholipids which prevents alveolar collapse by its effect on apical membrane surface tension. Surfactant is approximately 80% phospholipids, 10% neutral lipids and 10% surfactanct proteins (SP) A-D secreted by cuboidal pneumocytes and specialized respiratory bronchiolar epithelial (Clara) cells (Mcgowan, 2014). Intracellularly, surfactant proteins B and C are associated with surfactant and maintain surfactant folding/structure until release. SP A and SP D are also associated with surfactant and released by psuedostratified ciliated cells, type II pneumocytes and Clara cells into the airway and/or alveolar lumen and have very potent antimicrobial and immunomodulatory roles. Surfactant protein A (SP-A) and D (SP-D) bind and inactivate microbial agents. Both of these proteins have a carbohydrate recognition domain (CRD) that binds to mannose residues of microbial pathogens. Once bound, the pathogen and surfactant protein complex aggregate and taken up by alveolar macrophages. This has been well documented for respiratory syncytial virus (RSV) in humans (Grubor *et al.* 2006), individuals with altered surfactant proteins A and/or D due to nucleotide polymorphisms have increased numbers of RSV infection and severity. SP-A activate macrophages, enhancing uptake and killing of microbial pathogens. In addition, SP-A present within the alveolar lumen liquid is drained by the pulmonary lymphatics into the blood (Ackermann *et al.* 2010). Increased levels of SP-A in blood is associated with pneumonia and a useful biomarker of pneumonia severity.

2.5.1.5 Pulmonary Epithelia: The airway and alveolar epithelial cells produce the air-surface liquid along the respiratory tract. In between the pseudostratified ciliated epithelium are goblet

cells and also submucosal glands that release mucin products. The respiratory bronchiole and alveolar mucosa (Clara cells and Pneumocytes II) produce surfactant and surfactant proteins to line lung aveoli. Clara cells produce cytochrome p450 isoenzymes (mixed function oxidases) for detoxification of inhaled and haematogenous toxins. However, this contributes to the mechanism of some toxins like 3-methyl indole (3- MI), a toxic metabolite, from 3-methylindololamine, that cause injury and necrosis of mucosa epithelial cell. Leading to loss of the epithelial barrier and the overlying air-surface liquid containing antimicrobial factors. Clara cell secretory protein 10 (CC10) is another potent immunomodulator in the lung involved in synthesis and expression of phospholipase A2, interferon gamma, and immunoglobulin A (Caswell and Williams, 2007). Generally, it is an immunosuppressive and antiflammatory protein that is important in limiting the collateral damage caused by inflammatory cells.

2.5.1.6 Microbial pattern recognition molecules: The epithelial cells are important to sensing, signaling and effector response of respiratory systems to the external environment. Most particulate matter and microbial agents are removed from the inhaled air in the nares, nasal conchae and trachea leaving the deeper lung sterile and relatively free from particulate material.

These matter, along with mists, vapours, and gases may escape through the ASL and bind the lung epithelia to trigger activation, cell injury, metaplasia, or cell death in the respiratory tract. Microbial agents produce a number of conserved molecular patterns that include substances such as teichoic acid from Gram-positive bacteria, lipopolysaccharide (LPS) from Gram-negative bacteria, cytokine-phosphate-guanine (CpG), DNA, single-and double-stranded RNA (dsRNA), flaggelin, fungal zymosan, and lipopeptides. Most respiratory pathogens produce pattern associated molecular pattern that are recognized by epithelia, alveolar macrophages, and intravascular macrophages (Ackerman *et al.* 2010).

The intravascular macrophages; macrophages within the small capillaries of alveolar walls, attached to the underlying endothelial cells are very active in metabolic generation of inflammatory mediators such as prostaglandins and leukotrienes. Neutrophils from acute pneumonia recognize PAMPs, while alveolar macrophages, dendritic cells, NK cells, NK T cells, T and B lymphocytes also respond to microbial PAMPs (Ackermann *et al.* 2010).

The lung expresses a variety of extracellular, cell surface, endosomal and cytoplasmic receptors (pattern recognition receptors PRR) that recognize microbial PAMPs. Extracellular PRR include lipopolysaccharide (LPS) binding protein, mannan-binding lectins (ficolin and collectins), and C-reactive protein and serum amyloid protein. Single nucleotide polymorphisms (SNP) in these mannan-binding lectin C are involved in increased pneumonia in pigs (Lillie *et al.,* 2007). LPS, a gram negative bacterial component binds LPS binding protein, soluble CD14 and the toll like receptors TLR to induce inflammation in the lung. TLR 4 was also activated by the envelope protein of paramyxo viruses (Ackermann, 2005).

The respiratory viruses infect lung epithelial cells to induce formation of non-capped, 5′triphosphated RNA, long ds RNA, ssRNA, viral DNA, along with their capsids, matrix proteins, and non-structural proteins. They signal through TLR 3, 7, 8 and 9, and cytosolic viral pathogen recognition receptors. Other cytosol receptors include the leucine-rich repeat nucleotide-binding oligomerization domain-like receptors (NOD)-like receptors (NLRs) which can detect viral, bacterial and other pathogens that enter the cytoplasm. The NLRs stimulate cell activation and formation of inflammasome. The inflammasomes induce activity by triggering caspase 1 activation that results in cleavage of inert interleukin 1β to active IL-1β and active IL-18. IL-1 beta and IL-18 bind their respective receptors on leukocytes and other cells to triggers pro-inflammatory responses. These products are also known as danger-associated molecular patterns (DAMPs) or alarmins (Ackermann *et al.* 2010).

2.5.1.7 Oxidative killing by respiratory epithelia: An oxidative defense system (ODS) has been identified in human and ovine respiratory tract. This may also be present and active in goat. The ODS requires activity of epithelial enzymes dual oxidases1 and 2 (Duox1 and Duox2) which are members of the NADPH oxidase family, generate hydrogen peroxide onto the ASL. In the presence of thiocyante (SCN-), the weakly microbicidal hydrogen peroxide is converted to hypothiocyanite (OSCN-) which is short-lived but highly microbicidal (Romero *et al.* 1995).

2.5.1.8 Cytokines, chemokines and interferons: Cytokines invoke acute inflammation through activation of endothelial cells, epithelial cells, and acute inflammatory cells into the site of injury. Lymphocytes migrate to the lung through different set of chemokines.

Viral infections activate the receptors to stimulate production of type I interferons (IFN alpha and beta) from numerous cell types (Fishaut *et al*. 1980). The interferons bind Jak/Stat receptors to induce expression of anti-viral substances which bind and trap the infecting viruses, inhibiting initiation, translation and replication (Fink and Cookson, 2005). Other innate effector cells contribute to the pulmonary defence system in addition to respiratory epithelia (Fishaut *et al.* 1980). They participate in vascular and cellular phases of acute inflammation, synthesis of chemical mediators, cytotoxic and oxygen dependent killing of pathogens.

2.5.2 Factors that limits the respiratory mucosal immunity

The anatomical features of the ruminant lung predispose it to infections (Kahwa and Purton, 1996). There is an increased dead space area, and a trachea-bronchus branching early to the right, interlobular septae with limited connections, increased resistance and decreased compliance (Kirschvink, 2008). Collateral ventilation is reduced due to a lack of; a) broncho-alveolar communication (*channels of Lambert*), b) alveolar pores (*pores of Kohn*) and c) interbronchiolar connections (*channels of Martin*). Atelectasis occurs readily and areas of the lung remain consolidated with reduced functional gaseous exchange. Thus, these anatomic and physiologic conditions increase the demands for protective immune responses.

Several environmental factors and managerial issues (fig 2.5) also lower the respiratory tracts immunity; transportation, weaning, overcrowding, changes in social structure, precipitation, fluctuations in temperature, humidity, air exchange, lighting, sounds, changes in feedstuffs, feedlot floor conditions, and other microbial agents (Mitchell et al. 2007; Minka et al. 2009). Corticosteroids, catecholamines, acetylcholine, and substance P may affect basal level of TAP and LAP expression. Dexamethasone exposure reduced TAP and LAP expression upon exposure to LPS (Zamri et al. 1999). Viral infection reduced LPS-induced expression of TAP (and lactoferrin) in cattle (Al-Haddawi et al. 2007). Other conditions that impair immune responses may include single nucleotide polymorphisms within genes encoding innate and adaptive immune responses of the lung. However, no large scale studies have been completed in ruminant to date.

The innate immune response was viewed as the basal, most basic immune response to pathogen but it is difficult to enhance therapeutically (Ackermann et al. 2010). There is redundancy and overlap in the function of innate immune products that protect the respiratory tract. However, loss of some innate factors is clearly associated with increased incidence and/or severity of respiratory disease. Dehydration, genetic changes and expression of innate immune factors, stress, and primary infection (fig 2.5) contribute to poor respone of innate immunity. Reducing the effects and duration of these factors are a first step in enhancing innate immune activity. Therapies aimed toward enhancing activity of some innate immune responses may become a viable option and adjunct to management, vaccination, and antimicrobial agents in the nearest future.

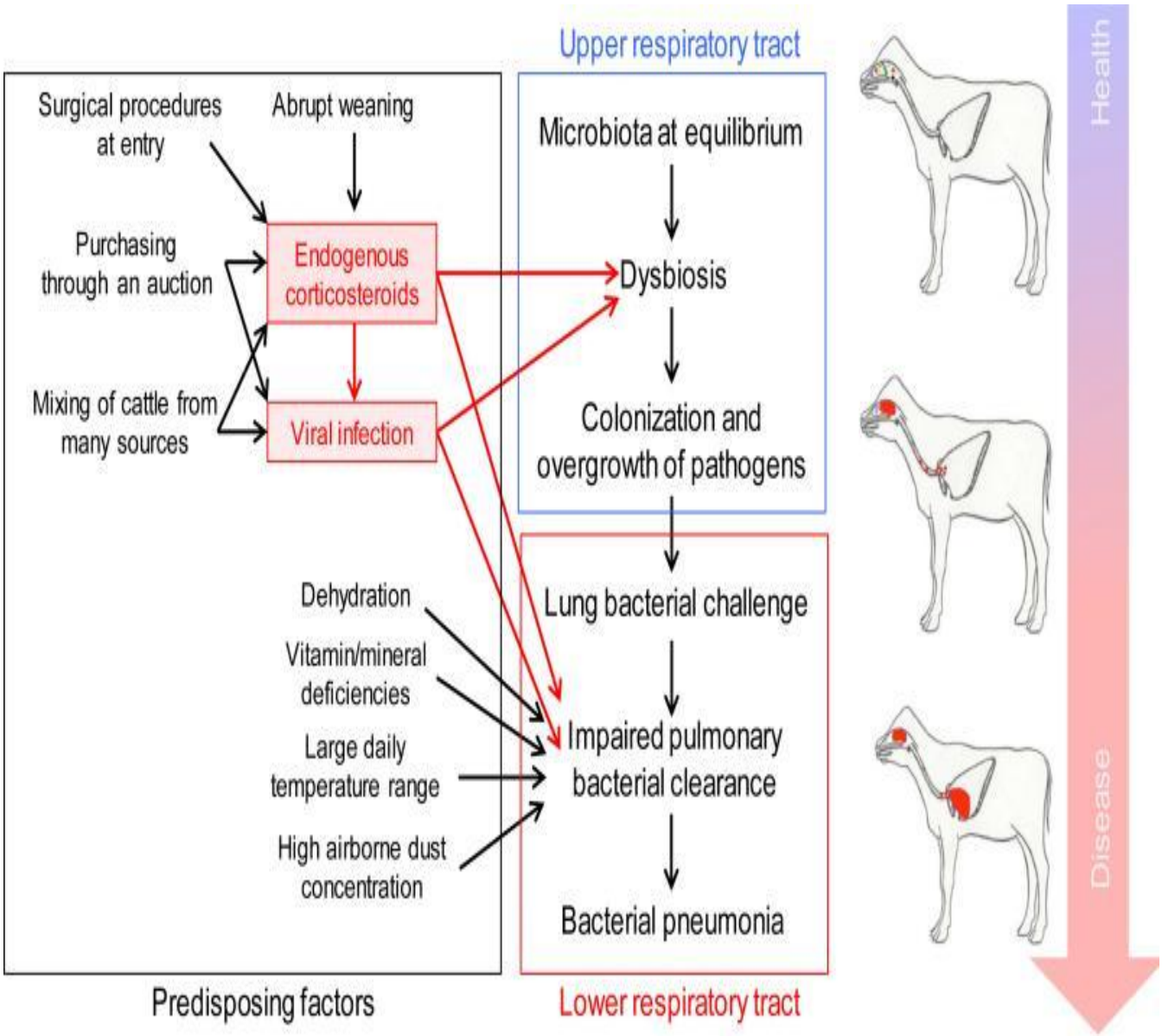


Figure 2.5. Factors that can reduce pulmonary defense system and contribute to respiratory distress in livestock.

2.6 Caprine Pneumonia

Respiratory distress in the lambs and kids are major cause of morbidity, and pneumonia being a common problem in ruminant production (Ramirez- Romero and Brogden, 2000). Caprine pneumonia is as a result of complex infectious and managerial processes that allow for interaction of several microbial pathogens. Pnuemonic studies in cattle (Akpavie *et al.* 1991;Welsh *et al.* 2004), sheep (Ikede, 1978), goats (Ikede, 1977; Mellado *et al.* 1991; Al-Qudah *et al.,* 2008) have been considered with less emphasis on causative agents other than PPR and Manheimia in goats in our environment. Thus, there is a knowledge gap on the pattern and pathology of caprine pneumonia in our environment. And inspite of the enoumnous resources invested in its control over the years, pneumonia in goats still remain a major limitation.

Pneumonia have been observed more in young goats, however, stress andor secondary bacterial infection contribute to severity in adult goats (Emikpe *et al.,* 2013). Male goats were reported to be at higher risk of infection due to the reproductive instinct of sniffing the females during estrus and possibility of faecal shedding of infective virions, and shedding of infectious agents in the faeces. An eleven-year study carried out on small ruminants showed that pneumonia remains an important condition with 69.8% incidence (Emikpe *et al.* 2013).

Shoo (1989), Gonzalez and Maheswaran (1993), Brogden et al (1998) hypothesised pneumonic pasteurellosis involvement on the severity of viral induced pneumonia. *Mannheimia haemolytica (Mh)* in PPRV infection in goats was studied experimentally by Emikpe (2009). However, the interaction of viruses and bacteria in natural caprine pneumonia needs to be investigated. Nonetheless, the control of pneumonia in goats is still far from being achieved. The possible roles of other respiratory viruses and bacteria is not well elucidated in our environment. One or a few studies have reported antibodies to Parainflunza-3 virus (Obi and Ibu, 1990), mycoplasma and other pathogens. This knowledge gap may have resulted in the poor vaccinal response, control and persistence of caprine pneumonia in our environment. The markers for early detection of pneumonia are needed for effect give control of pneumonia in goats.

2.7 Diagnostic Biomarkers of Pneumonia

From the review thus far, some innate immune molecules can be assessed for respiratory tract function/activity or stage/severity of clinical pneumonia. The Surfactant protein A (SP-A) production by lung epithelia increases markedly in fetal lung near term, and it delineates the level of lung maturation at birth. There is increase SP-A production in viral pneumonia, because it is drained by the pulmonary lymphatic vessels through the thoracic duct to the systemic circulation (Arckermann et al. 2010). Thus, serum SP-A assay has potential to be a marker of respiratory tract maturation and pneumonia outcome.

Aich *et al.* (2009) showed acute phase proteins are good clinical markers of pneumonia severity. Serum associated amyloid (SAA), haptoglobin, alpha 1-acid glycoprotein produced by liver in response to IL-1 and TNF alpha are produced in pneumonic condition. SAA and haptoglobin may be used to differentiate acute and chronic pneumonia, while alterations in haptoglobin and apolipoprotein A1 are associated with viral infections. Metabolic and elemental compounds (glucose, low density lipoprotein LDL, valine, phosphorus, and iron) are biomarkers of viral infection and disease outcome (lactate, glucose, iron). Other markers may include haematologic indices, bronchoalveolar lavage and oxidative stress parameters.

2.7.1 Oxidative stress

Oxidative stress is an emerging concept in medicine, which monitors the metabolic status and requirements of the body. It arises due to excess Reactive Oxygen Species (ROS) and imbalance of antioxidants. The shift in prooxidants and antioxidants result in biochemical and physiological changes.Oxidative stree is manifested as lesions of different sort in the organ-system in the body (Blier, 2014). Evaluation of oxidative stress serve as pointers to organ impairment, clinical signs and diagnosis of disease conditions. Different oxidative stress markers are measurable. Hitherto, ROS was considered for their deleterious effects of on tissue damage (Almroth, 2008). However, they are now recognized in homeostasis, inter- and intracellular signaling and different pathophysiologies (Halliwell and Gutteridge, 1999). Poor enzyme activity, DNA assaults, protein and lipid oxidation are evidence of oxidative stress (Hensley and Floyd, 2002).

Important antioxidant enzymes include cytochrome P450 (CYP1A) involved in generation of oxygen radical ($O^{2\bullet-}$), superoxide dismutase (SOD), catalase (CAT), Glutathione peroxidase (GPx) and Glucose-6-phosphate dehydrogenase (G6PDH) (fig 2.6). Ascorbic and Retinoic acids and tocopherol are also important dietary antioxidant (Dalton *et al.,* 1999). Glutathione (GSH) is a cytoplasmic antioxidant involved in direct detoxification of ROS. (Stephensen *et al.* 2002).

Oxidative stress and antioxidant status have been repeated in animal models and in humans, but there is a considerable paucity of studies validating the use of antioxidants as biomarkers to assess oxidative stress of caprine species. Ideally, biomarkers of oxidative stress would be measurable in specimens that can be collected relatively easily, and the analysis procedure should be applicable to stored specimens.

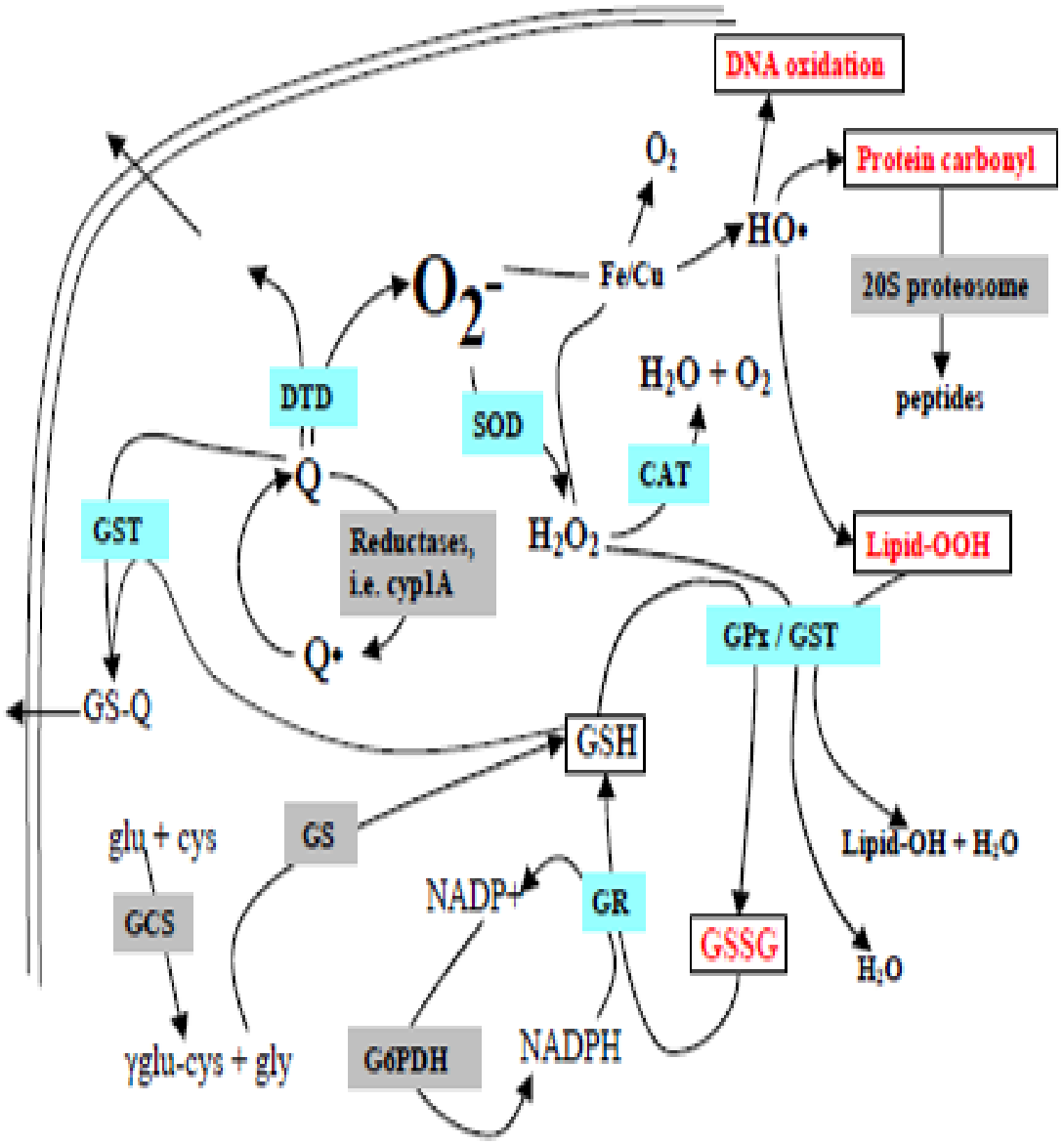


Figure 2.6. Schematic diagram showing antioxidant enzymes, oxidized molecules, and products in oxidative stress.

GPx is involved in lipid peroxidation and formation of Malondialdehye (MDA) from polyunsaturated fatty acids (PUFAs), and this is generally quantified by the thiobarbituic acid reactive substances (TBARS), and 4-hydroxynonenol (4-HNE) formations (Lovell et al. 1997; Lu, 1999; Grune et al. 2004).

The ODS requires activity of epithelial enzymes dual oxidases 1and 2 (Duox1and Duox2) which are members of the NADPH oxidase family to generate H_2O_2. The Dual-Functioning Oxidase/Lipid peroxidase LPO Defense contributes to airway sterility. Ackermann *et al.* (2010) have shown that the oxidative system kills bacteria by producing hypothiocyanite ($OSCN^-$) in an LPO-catalyzed reaction.The mechanism(s) by which $OSCN^-$ eliminates microbes is not known, but $OSCN^-$ can oxidize thiol groups in surface proteins. LPO has high affinity for SCN^- and also iodide (I^-). LPO can catalyze the oxidation of I^- to hypoiodite (OI^-) in the presence of H_2O_2 (Grune et al. 2004). Although I^- is not a physiologic component of the airway surface liquid (ASL), when present, I^- allows hypoiodous acid (HOI) generation by the LPO/Duox enzymes. There is some evidence that $OSCN^-$ and OI^- have slightly different spectrums of antimicrobial activity. However, $OSCN^-$ lacks activity against RSV in vitro, OI^- has anti-RSV activity in vitro and in vivo (Ackermann, 2005). The lung also has other oxidative systems with antimicrobial activity; the phagocytic nicotinamide adenine dinucleotide phosphate oxidase, myeloperoxidase MPO, and nitric oxide synthase. Of these, MPO convert potassium iodide (KI) and sodium thiocyanate (NaSCN) to a halide in the presence of H_2O_2, and neutrophils expressing MPO in viral infection. The amount of MPO in ASL and the extent to which MPO in ASL contributes to halide formation in ASL are not known.

The system is potent against multiple respiratory pathogens in vitro. Potential antiviral activity of this system needs large animal models of viral infections because mouse airways lack sufficient LPO and rat airways contain only a few LPO secreting cells. The ovine respiratory tract is similar to that of humans in terms of LPO and Duox expression and other physiologic and immunologic features. After in vitro studies and pilot work in lambs, Ackermann *et al.* (2010) reported that the antimicrobial activity of Duox/LPO may be optimized in vivo through the supplementation of KI or NaSCN a in number of respiratory infections. Amelioration of

respiratory viral infections by enhanced oxidative responses is significant because the therapy is relatively inexpensive, available, and easily distributed and administered.

2.7.2 Bronchoalveolar lavage

Bronchoalveolar lavage (BAL) is an alternative sampling method that overcomes the limitations of biopsy in diagnostic laboratories for the detection of respiratory pathogens. Fresh cellular material is collected from the lower airways and alveoli free of the degenerate cells and mucus which characterise samples collected from the nasopharynx. BAL fluid is also the sample of choice for the isolation of respiratory viruses (Kimman *et al.,* 1986). Cytological examination of the fluid provides an indication of the stage and type of inflammatory process taking place in the lung. In theory, bacteriological examination of the collected fluid may yield more useful information than culturing from the upper respiratory tract where potential respiratory pathogens may be found as commensals.

Three main BAL techniques have been described: nasal tube sampling; transtracheal sampling (Espinasse *et al.,* 1991); and collection by endoscope (Allen *et al.,* 1992). BAL assay the gaseous exchange area of the lungs for ongoing or established pulmonary lesions (Hodgson, 2006). Surfactant (top foamy layer) on the BAL fluid (BALf) is characteristic and an an indication that the alveoli have been sampled.

Enumerating the cellular content of BALf (cells/ml) suggests an inflammatory process in the lung, support diagnosis and prognosis of pulmonary disease (Hodgson, 2006). BAL has been widely applied as a routine tool in diagnosis of respiratory disease (Reynolds, 2000). The BAL technique is safe and the cell type explains the observed clinical features (Rottoli and Bargagli, 2004). However, the application of BAL still remains controversial based on a variety of techniques or methods with considerable variations and implications. There are correlations between BALf cells and corresponding tissue cell extractions especially for lymphocyte infiltration in granulomatous lung disease (Reynolds, 2000; Taniuchi *et al*., 2009). Evaluation of BALf and enumeration of the cells have not been well defined and incorporated in relation to the pathological conditions of caprine respiratory disease complex.

2.7.3 Immunohistochemical staining

In 1903, Paul Ehrlich published the first comprehensive textbook describing histological and histochemical staining techniques ("Encyclopedia of Microscopical Technique," Enzyklopa¨die der Mikroskopischen Technik). His immunological studies begun in 1890 when he was an assistant at the Institute for Infectious Diseases under Robert Koch. Antigen-antibody interaction hypothesized a "lock-and-key" which triggers antibodies formation by Paul Ehrlich In 1897. Albert H. Coons attached fluorescent dye (fluorescein isocyanate) to antibody for localisation of antigen. The concept of putting a visible label on an antibody molecule appeared both bold and original. His initial results were described in two brief papers. Fluorescent antibody technique have also been applied in a wide variety of experimental settings. Presently, localization of pathogen antigens in tissues aid diagnosis of infectious conditions.

Fluorophore like colloidal gold or biotin have been used to demonstrate antigens (Harlow and Lane, 1999). Electron-dense labels using colloidal gold are characteristic at electron microscopy (Roth *et al.,* 1978). Biotin label is an integral part of avidin biotin complex ABC technique necessary for enhancement of signals. Digoxigenin (DIG) or dinitrophenol (DNP) are also, potent in enhancement of signals. For their visualization, enzyme or fluorophore-conjugated secondary antibodies are affordable. Enzyme labels are usually coupled to secondary antibodies or to (strept)avidin The latter is used for detection of biotinylated primary or secondary antibodies in ABC methods. Enzyme labels routinely used in immunohistochemistry are horseradish peroxidase and alkaline phosphatase (from calf intestinl). Glucose oxidase from *Aspergillus niger* and *E. coli* b-galactosidase are only rarely applied. The enzyme label are qualified using chromogens like diaminobenzidine (DAB).

2.8 Aetiology of Caprine Pneumonia

The infectious causes of pneumonia in goats are responsible for the mortalities and uncontrollable nature of caprine pneumonia. The range from viruses, mycoplasma, bacteria, fungi and parasites, but the widespread use of broadspectrum anthelminths have drastically reduced the menance of verminous pneumonia in our environment.

2.8.1 Peste des petits ruminants virus (PPRV)

2.8.1.1 History of PPRV

PPR is a viral disease of small ruminant first reported in the Cote d'Ivoire (Langannec, 1942) and confirmed in Dahomey (Benin) in 1944. Subsequently it was recognized as occurring in the small ruminant ("lagoon") breeds (dwarf breeds) in the southern regions of Nigeria, Togo and Benin where small ruminants were highly susceptible to the disease, and in Senegal in 1955. The disease has similar clinical features Rinderpest RP eventhough they are different antigenically (Pastoret, 2006). PPRV is a Morbillivirus in the family *Paramyxoviridae* and order *Mononegavirales* (Gibbs *et al.,* 1979). Other Morbilliviruses of veterinary and medical importance include canine distemper virus, feline Morbillivirus, cetacean morbillivirus, phocine distemper virus and Measles virus. The isolation of PPRV was first successful in sheep cell culture (Gilbert and Monnier, 1962).

PPR isa problem of goats across Africa, China and Middle East (Fentahun and Woldie, 2012). It was diagnosed in Indian subcontinent by Shaila *et al.* (1989). There is dearth of knowledge in PPR epidemiology (Felix, 2013). Buffaloes, Cattle and Camels are carriers involved in transmission of PPR in Africa and India (Govindarajan *et al.,* 1997; Roger *et al.,* 2000). The considerable importance of PPR as a factor limiting goat production in West Africa has been recognized (OIE, 2008). The acute disease is common in goats, but rare in Sahelian sheep. In goat population, the morbidity and mortality is high in kids and young goats**.**

2.8.1.2 Epidemiology and Economic Importance of PPR

PPR is notifiable since inclusion in the OIE list of animal diseases. The disease has spread as far as into Tibet, China. PPR may have been under diagnosed due to the non-specificity of the available diagnostic tests considering its clinically relatedness and antigenic similarity to other

morbilliviruses (Taylor, 1979). In last two decades, the disease was diagnosed in East and Northern Africa (Banyard *et al.* 2010; Munir *et al.* 2013).

Phylogenetic studies of PPRV fusion gene (F), grouped the strains into 4 distinct lineages (Shaila et al. 1996; Dhar et al. 2002). However, analysis of nucleoprotein gene (N) gave a better phylogeney (Kwiatek et al. 2007) (figure 2.7). Eventhough there was no much difference in the phylogeny the Nig/75 strain fell into lineage II from 1 on the N tree. The haemagglutininneuraminidase (HN) gene is much more reliable in PPRV transmission studies (Balamurugan et al. 2010). Lineages I and II are exclusive to West Africa. Lineage III is common in Middle East and East Africa, while lineage IV is common but not exclusive to Asia (Kwiatek et al. 2011; Munir et al. 2013). However, multiple lineages of the virus are present in same geographical area (Luka *et al*. 2012).

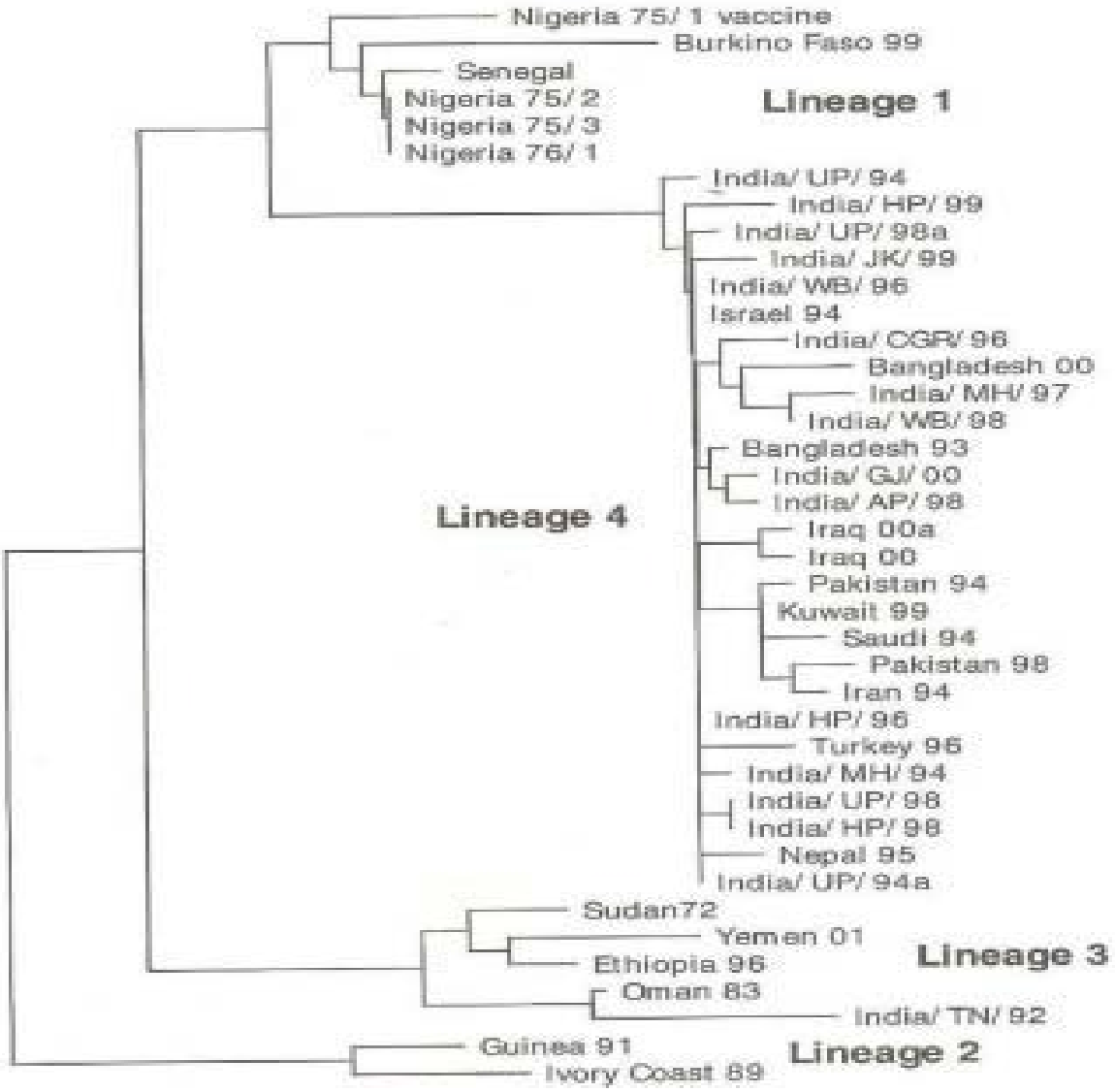


Figure 2.7. Phylogenetic analysis of PPRV isolates according to the N gene.

PPR has been considered a major constraint for small ruminant production however, impact of the disease has not been fully evaluated. The case fatality rate may be high as 100% (Ezeokoli et al. 1986; Rossiter and Taylor, 1994; Nanda et al. 1996). It is a concern to small rural households and farms who keep them as source of income.

2.8.1.3 Morphology, Lifecycle and Antigenicity of PPRV

PPRV is an enveloped virus with pleomorphic shape and size of between 150 to 700 nm (Durojaiye *et al*., 1984). The genome is negative-stranded RNA which contain 15,948 nucleotides and six genes that code eight different proteins (Kumar et al 2014); six structural proteins -nucleocapsid protein (N), phosphoprotein (P), fusion protein (F), matrix protein (M), haemagglutinin-neuraminidase protein (HN), large protein (L) and two non-structural proteins (C and V) in the sequence 3'-N-P/C/V-M-F-HN-L-5' (fig 2.8).

The attachment of PPRV is mediated by the *signaling lymphocyte activation molecule* (SLAM) or CD150 protein on lymphocyte, macrophages and dendritic cells and and Nectin-4 on epithelial cells (Adombi et al. 2011; Meng et al. 2011; Munir et al. 2013). These receptors contributes to the pantropic nature of Morbilliviruses. It takes about 6–8 h for the virus to grow in cell culture (Kumar et al. 2013). The stages of the infection commences whence the virus *attaches and delivers its nucleic acid into the* host cell for replication (fig 2.9).

Gilbert and Monnier (1962) cultured the virus in primary lamb kidney cells, but it was later grown on African green monkey kidney (Vero) cell line (Lefevre and Diallo, 1990), on marmoset lymphoblastoid (B95a) cell line (Meng et al. 2011) and on monkey CVI cells (Munir et al. 2013) due to the low turnout in the previous cultures. The monkey cell line (CHS-20) was used for growing wild strain of PPRV in clinical samples (Adombi et al. 2011). The expression of SLAM in the tissue was directly proportional to cell tropism of PPRV.

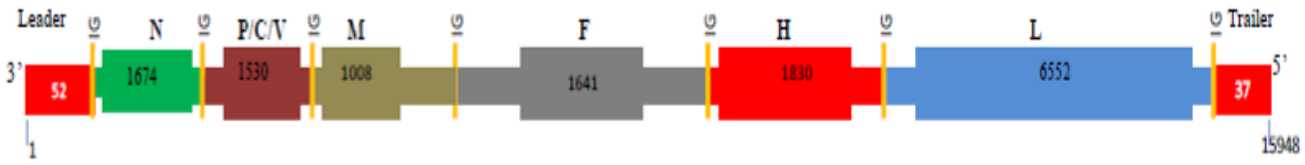


Figure 2.8. Structural organization of PPRV Genome in the sequence 3'-N-P/C/V-M-F-HN-L-5. (Source: Kumar et al. 2014**)**

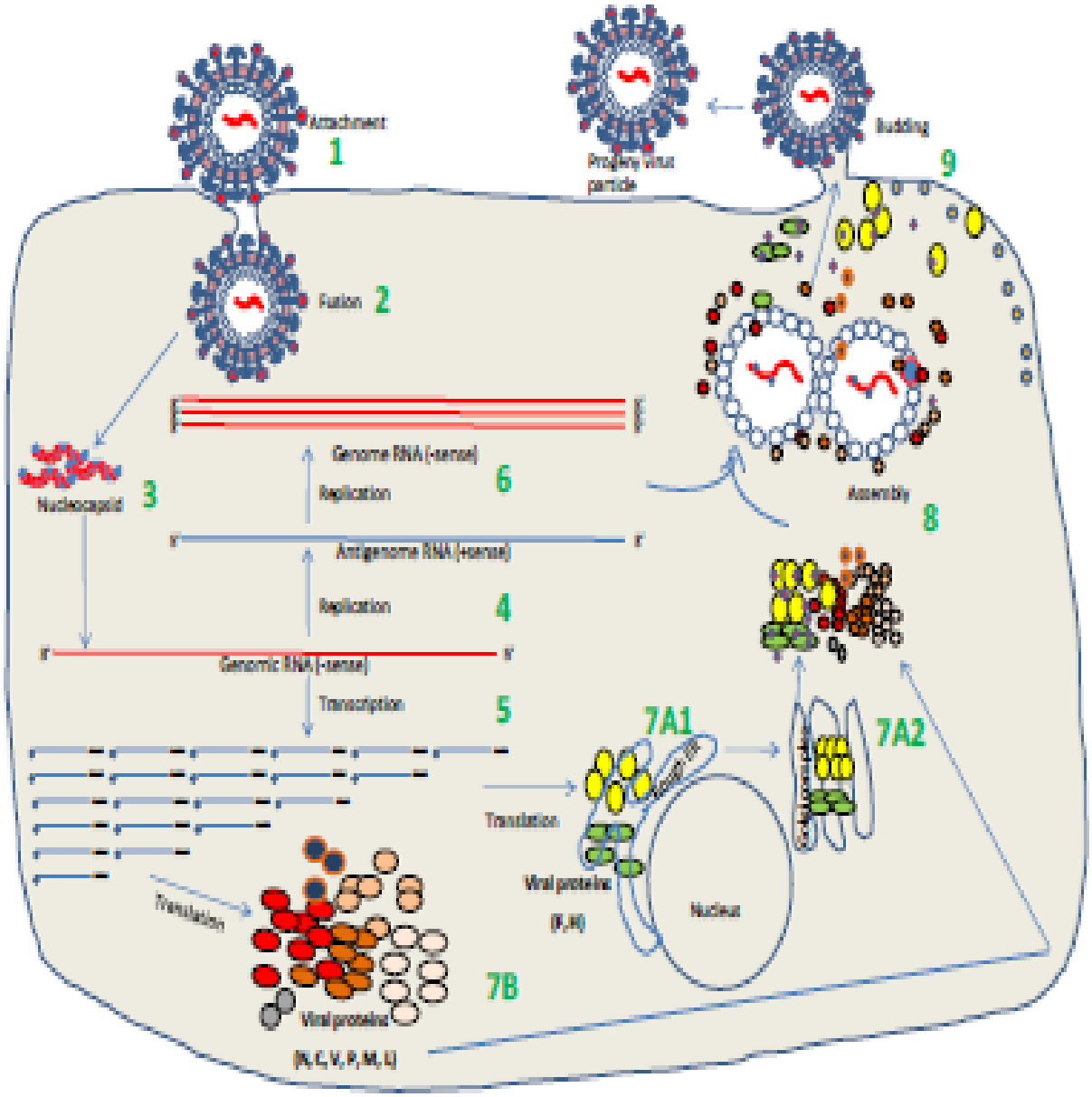


Figure 2.9. Scheme showing life cycle of PPRV. (1). Virion attachment (HN protein) to receptor on host cell epithelium (SLAM/Nectin-4). (2). Fusion of F and HN on virus to plasma membrane (3). Genome released from virus into cytoplasm of host cell. (4). Replication of RdRp genome encoded in virus (5). mRNA synthesis by the virus-encoded RdRp. (6). RNA synthesis. (7). Viral protein Synthesis (F & H on RER (7A1), translocation on Golgi apparatus (7A2), post-translational modifications. N, P, C, V, M & L synthesized on ribosomes (7B). (8). Progeny or new virions assembly. (9). Progeny virions budding on plasma membrane. Source; Kumar et al. 2014.

2.8.1.4 PPRV Virulence Determinants

Several factors contribute significantly to the dissemination of the virus and severity of PPR; PPRV have been detected in Asiatic lion, Buffalo, Camels, Cattle, and Pigs (Balamurugan *et al.* 2012a). Wild ungulates and small ruminants show clinical disease and act as reservoir for PPRV (Munir, 2013). Effect of age, breed, sex and seasonal influences have been associated with the severity of PPR in domestic sheep and goats (Amjad *et al.* 1996; Dhar *et al.* 2002; Munir *et al.,* 2009). Goats are apparently more susceptible to PPR than sheep probably due to the high PPRV neutralizing antibodies sheep (Munir *et al.,* 2009). More so, the rate of PPR recovery is lower in goats compared to sheep (Wosu, 1994), the infection may spread clinically in goats while sparing sheep in same flock (Animal Health Australia, 2009). However, commingling of sheep and goats was associated with the seropositivity in sheep (Al-Majali *et al.* 2008). Mortality in goats was also associated with low levels of neutralizing antibodies and weaning of maternal antibodies (Srinivas and Gopal, 1996; Shankar et al. 1998; Ahmed *et al.* 2005; Atta-ur-Rahman *et al.* 2004; Waret-Szkuta *et al.* 2008).

Mortality to PPR is high in African breeds than European breeds (Couacy-Hymann *et al.* 2007). And the dwarf breeds are the most susceptible within African breeds (Abubakar et al. 2012). PPRV is shed from body secretions and excretions of natural orifices 10 days after infection and in the pyrexic phase of infection. Formites and inanimate objects may aid transmission of the virions from sneezing, coughing and faecal material (Couacy-Hymann *et al.* 2007; Ezeibe *et al.* 2008).

2.8.1.5 Pathogenesis and Clinical Presentation of PPR

The mucosal surface of the respiratory and gastrointestinal system serves as route of entry to PPRV. The virus multiplies in the regional lymph node before spreading to circulation. The ensued viraemia disseminates the virus to the body sytems where it causes tissue damage, lesions and clinical signs. The severity of the lesions is influenced by the age, breed, innate immunity and viral virulence. Moreover, concurrent infections may complicate the clinical presentation of the disease; peracute, acute, subacute or subclinical (Kulkarni *et al.* 1996).

Peracute PPR occur in kids and lambs because of absence of maternal antibodies (Obi *et al.* 1983). There is pyrexia and the animal usually may not survive the pyrexic phase.

Acute PPR is characterized by an incubation of 3–4 days, pyrexia, oculo nasal discharge, sneezing, coughing, dyspnea, necrosis, erosion and ulceration of mucosal epithelium, diarrhea, dehydration, emaciation and death. Death of the animal occur 10–12 days post-pyrexia. The case fatality rate is high and recovery is rare if not managed supportively (Diallo, 2006).

Subacute PPR is characterized by an incubation of >6 days, low-grade pyrexia, and recovery 10–14 days after infection.

2.8.1.6 Gross and Histopathological Lesions of PPR in goats

The lesions of PPR are erosive to ulcerative necrotizing stomatitis and enteritis, linear congestion of caecal, colonic andor rectal mucosae ('zebra striping'), congestive and oedematous lymphadenomegally. Severe pulmonary congestion and consolidation, frothy exudate in the airway. Tracheitis, bronchitis, atelectasis and interstitial pneumonia, focal degenerative lesions are also rarely noticeable in the liver (Munir *et al.* 2013).

Histopathological changes of multifocal degeneration, defoliation and necrosis of the airway epithelial cells are followed by clara cell and cuboidal pneumocyte hyperplasia, multinucleated giant cells, intranuclear inclusion bodies, and ependymal cell necrosis (Aruni *et al.* 1998; Yener *et al.* 2004; Kul *et al.* 2007). There is knowledge gap on the neurotropism of PPRV (Kul *et al*, 2008). The antigens were demonstrated in neuronal body and processes in the temporal, frontal and olfactory lobes, and dendrites in the hippocampal pyramidal cells (Galbraith *et al.*, 1998 and 2002).

2.8.1.7 Responses Against PPRV

Cytokine responses-Neutralizing antibodies, complement system and cytokines are evoked in PPRV infected animals. IFN-γ inhibits viral replication (Atmaca and Kul, 2012). IFN-γ and interleukin-4 (IL-4) were inversely proportional in vaccinated and infected goats (Patel *et al.* 2012). Elevated level of IFN-γ and TNF-α were observed in intravascular monocytes, syncytial cells, and mononuclear cells (Opal and DePalo, 2000; Atmaca and Kul, 2012). Inducible nitric oxide synthetase (iNOS) was also expressed with TNF-α (Moussallem *et al.*, 2007; Svitek and von Messling, 2007). IL-4, IL-10 and IL-1 expressions have been compared

in PPRV infection. IL-10 down regulatesexpression of TNF-α and IL-1 (Atmaca and Kul, 2012).

Passive immunity- Passive or maternal antibodies protects kids against natural PPR. However, this protection only last 3–5 months (Orkpeh, 1989; Libeau *et al.*, 1992; Awa *et al.*, 2000; Bodjo *et al.*, 2006).

Cellular and humoral immunity- Little is known on the induction of adaptive immunity by PPRV. Humoral and cellular responses were developed against HN protein for neutralization of PPRV *in vitro* (Sinnathamby et al. 2001), while the F protein antibodies neutralizing and protective against clinical PPR. Lymphocyte epitopes were mapped on the viral envelope for recombinant vaccines; T lymphocyte epitope (N-terminus, amino acids 123–137 and C-terminus, amino acids 242–609) of HN protein (Mitra-Kaushik *et al.*, 2001; Sinnathamby *et al.*, 2001). Four and two B lymphocyte epitopes were mapped on the N and HN protein respectively (Renukaradhya *et al.*, 2002). The N protein induced strong $CD8^{+}$T-cell activity and splenic peri-arteriolar lymphoid hyerplasia (Sinnathamby *et al.*, 2004).

Immune suppression-Morbilliviruses cause immunosuppression, predisposing the host to secondary infections. The Izatnagar/94 strain induced leukopenia, lymphopenia and poor antibody response (Rajak *et al.*, 2005). The immunosuppressive effect arises as early as 4–10 days of clinical PPR (Raghavendra *et al.*, 1997; Kumar *et al.*, 2001; Rajak *et al.*, 2005). The Nigeria/75/1 (vaccinal) strain suppressed bovine and caprine lymphocytes *in vivo* (Heaney *et al.*, 2002). This suppression needs to be further investigated as the strain is currently being used as live attenuated vaccines. Steriod induced immune-suppressed animals showed severe clinical PPR (Jagtap *et al.*, 2012), with lesions present in liver, kidney and heart. Therefore, it shows that stress and the virus have interference on production of viral neutralizing antibodies.

The nonstructural proteins of paramyxoviruses control immune regulations. V protein impairs production of IFN-α/β, and nuclear factor kappaB (NF-kB) pathway (Munir et al. 2013) while C protein inhibits IFN induction (Boxer et al. 2009).

Haematological responses: There is regenerative anaemia due to haemorrhagic enteritis leukopenia, monocytopenia, lymphopenia and ((Olaleye, 1985; Aikhuomobhogbe and Orheruata, 2009; Sahinduran *et al.* 2012). The thrombocytopaenia is associated with increased activated partial thromboplastin time (APLTT) and prothrombin time (PT) due to decrease platelets, and consumptive coagulopathy. However, disseminated intravascular coagulation may result from liver damage, and hypoalbuminaemia (Yarim *et al.* 2006; Addass *et al.* 2010).

Biochemical responses: The replication of PPRV in the renal epithelial cells cause damage and increase in blood urea nitrogen and creatinine (Sahinduran *et al.* 2012). The liver enzymes; aspartate aminotransferase, alkaline phosphatase and gamma glutamyl-transferase increases in serum of infected animal (Yarim *et al.* 2006; Sahinduran *et al.* 2012). PPRV elevate levels of bilirubin, cholesterol and sialic acid in infected animals (Yarim *et al.* 2006). Cell-mediated immune responses increases the sialic acid content in serum.

2.8.1.8 Diagnosis of PPR

The diagnosis is based on history of direct contact, clinical signs, serology, postmortem findings, histopathology, immunohistochemical staining, viral isolation and or molecular detection of the virus (Singh *et al.* 2004; Chauhan *et al.* 2009; Fentahun and Woldie, 2012).

Agar gel immunodiffusion, virus neutralization, complement fixation, haemagglutination inhibition, and competitive ELISA assays have been used for detection of PPR antibodies, although with varying specificity (Osman *et al.*, 2008). Competitive ELISA showed high specificity (Fentahun and Woldie, 2012), likewise Immunocapture ELISA (Mahajan *et al.* 2013).

These serologic assays use monoclonal antibodies specific to N protein (Lefevre *et al.* 1991; Yunus and Shaila, 2012). The N protein is also easily targeted in other diagnostic tests due to its abundance (Diallo *et al.* 1994; Libeau *et al.* 1995). However, conservative nature of the HN protein makes it useful in diagnoses of morbilliviruses (Saliki *et al.* 1993; Anderson and McKal, 1994; Singh *et al.* 2004; Munir *et al.* 2012a, 2013).

The trends and throughput of molecular tools has improved diagnosis of PPR by nucleic acid detection of PPRV. PCR, quantitative real-time RT-PCR, RTPCR ELISA amplifies the

virions in clinical samples in many folds for viral detection (Saravanan et al. 2004; Balamurugan et al. 2012; Abera et al. 2014). Loop-mediated isothermal amplification (LAMP) aslo detected PPRV from clinical samples based on the "N" gene amplification (Dadas et al. 2012; Dhama et al. 2014).

Prompt diagnosis of PPR is crucial for effective control, containing of outbreaks and minimizing economic losses. Most PPR outbreaks were diagnosed based characteristic clinical lesions which are quite difficult to distinguish from other viral stomatitides. Thus, confirm of clinical diagnosis via laboratory testing, demonstration of antibodies and detection of the antigens in serum and tissues.

2.8.1.9 PPRV Vaccination and Vaccines

Post-infection or vaccination immunity are required for effective control strategies of PPR. **Serum immunization using** hyperimmune serum protected goats for short period of at least 10 days (Ihemelandu et al. 1985). However, the protection was increased to 9 months with concurrent administration of PPRV (Adu and Joannis, 1984). This method is limited by the cost of growing hyperimmune serum, availability of virulent PPRV and shelf life of blood (<10 days).

Heterologous vaccines- An attenuated Plowright's tissue culture RP vaccine (TCRPV) was administered to protect goats against PPRV due to their cross-protection (Bonniwell, 1980). It protected goats and produced maternal antibodies in kids (Adu and Nawathe, 1981). However, the global concern on RP eradication has halted the use of the TCRPV.

Homologous vaccines- Efforts of Gilbert and Monnier (1962), Diallo et al (1989) and Taylor and Abegunde (1979) on culture and isolation of PPRV from a goat which died of PPR clinically in 1975 culminated in the development of the vaccinal strain (Nig/75) which was adapted to Vero cells at 37°C for attenuation and vaccination of animals. The effective dose ranged from $10^{0.8}$ to 10^{3} $TCID_{50}$/animal. The Indian PPRV isolates (Sungri/96, Arasur/87 and Coimbatore/97) were also assessed against field strains of PPRV (Saravanan et al. 2010). The Egypt/87 strain is also attenuated and commercialized for vaccination purpose (Nahed et al.

2004). However, thermodegradation and instability of these vaccines are major limitation to control of PPR (Munir et al. 2013).

Recombinant marker vaccines- the poor thermostability of PPRV homologous vaccines has led to the incooprating viral epitopes into other carrirer organisms. F and HN proteins of PPRV were expressed in capripox virus which was later introduced to goats for antibody production and protection against PPRV (Jones et al. 1993; Romero et al. 1995).The antibody was protective against PPRV (Guinea- Bissau/89) and capripox (Yemen isolate). Baculoviruses (Ghosh et al. 2002), *Bombyx mori* (Rahman et al. 2003), peanut plants (*Arachis hypogea*) and canine adenovirus (Qin et al. 2012) have also been used for recombinant PPRV vaccine.

Subunit vaccines- subunit vaccines are developed using synthetic biology and reverse genetic system of PPRV glycoproteins for production of neutralizing antibodies against N, F & HN protein (Mahapatra et al. 2006; Parida et al 2007; Buczkowski et al. 2012).

Advances in vaccinology is shifting to production of multivalent vaccines because of the complexity of pneumonia in ruminants (Diallo et al. 2002). The use of antiviral drugs and RNA interference (RNAi) to inhibit PPRV relication have also been suggested. It is expected to tackle the menance of PPR, however, molecular genetic tools are needed to silence the expression of N protein through RNAi. However, large scale and *in vivo* studies are needed to validate the claims.

2.8.2 PARAINFLUENZA-3 (PI3)

Parainfluenza-3 virus (PI3V) infects the respiratory tract of sheep and goats, it belongs to the family *Paramyxoviridae* of order *Mononegaviralis*, RNA non-segmented negative single stranded genome (Gafer et al. 2009**).**The virus is spherical to pleomorphic in shape measuring 150 to 350nm in diameter. The genomic RNA is contained within a helical nucleocapsid that is enclosed within the lipid bilayer. Inserted into the envelope are glycoprotein pikes that extends approximately 8 to 12 nm from the surface of the membrane (Field et al. 1996). PI3Vwas first isolated frrom sheep (Hore, 1966), and serological surveys showed that it is widely spread in sheep population in many countries (Fenner et al. 1996). The infection showed signs of mild pyrexia, coughing and nasal discharge for several days and at necropsy there were areas of pulmonary consolidation, particularly in ventral parts of the cranial lobe. Gafer et al (2009) isolated thevirus in sheep than in goats. The lower recovery in goats was attributed to less number of goat samples collected and managemental conditions. There are three ovine PI3V strains or serotypes; G_2 strain, C_5L_6 strain and DH-1 strain.

Anita et al (2015) underscored the role of PI3V in caprine pneumonia. The virus causes different clinical syndromes in sheep and goat especially respiratory infections (Obi and Ibu, 1990). Serological surveys showed that the infection is widely spread among small ruminant population (Sendow et al. 2002; Yener, 2005). PI3V affects the function of alveolar macrophages and destroys the cilia on the bronchial mucosa. This affects the host's natural clearance mechanism for removing pathogenic organisms from the respiratory tract and renders them susceptible to secondary bacterial infection (Caswell and William, 2007). Much studies needs to be done on prevalence and characterization of PI-3 in our environment, considering its endemicity in developed climate, its exclusion in vaccines, and the inadequacies of diagnostic tools. PI3V is a neglected virus in animals and humans alike. The human strain (hPI3V) is an important pathogen in children (Karron and Collins 2007).

2.8.2.1 Nature and Epidemiology of PI3V

Aside studies of Obi et al. (1990), little is known on PI3V in Africa. Solis-Calderon et al. (2007) reported the Seroprevalence in Mexico. The dynamic is obviously obscured in livestock production. However, it was suggested that PI3V antibodies varies proportionally with age of animals in a flock (Frank and Marshall, 1973). The pattern of seroconversion, excretion of the virus and duration of immunity were observed in calves (Frank and Marshall 1971).

The effect of weather on infection and occurrence of parainfluenza pneumonia was established in temperate climates (Allen et al. 1992; Fulton et al. 2000). The virus is inhaled attaching to the mucosal surface in the respiratory tract. An infection dose of 10^6 to 10^7 $TCID_{50}$ was experimented by Frank and Marshall, (1973). There is cross species protection of neutralizing antibodies to PI3V. the bovine strain (bPI3V) reactive antibodies protected ungulates (Harwood et al. 2008). Thus, typing of the strains for confirmatory diagnosis may require sequencing of the isolates. Meanwhile, cross specie infection with PI3V have been reported for bPI3V in sheep (Rudolph et al. 2007), calves, goats (Yener et al. 2005), children (Karron and Collins, 2007), primates and pigs (Qia et al. 2010). Lyon et al. (1997) reported minor genetic differences between the bovine and ovine strains. The human strain was used to protect primates and humans against parainfluenza pneumonia (Karron and Collins 2007; Sato and Wright, 2008). However, questions surrounds the infective ability of hPI3V in cattle.

2.8.2.2 Pathoology of Parainflenza 3 virus

The most studied PI3V is the bovine strain (bPI3V). The infective virions attaches to the respiratory mucosal surface by N-acetylneuraminic (salic) acidand its HN glycoprotein. The attachement causes degradation of mucus and penetration of epithelial cells (Matrosovich *et al.*, 2004). The virus releases its nucleocapsid into the cytoplasm with formation of characteristic intracytoplasmic inclusion bodies in the respiratory epithelium through polymerase-mediated transcription of mRNAs and translation of proteins.

The exact molecular mechanisms involved in virus-host cell relation needs further investigation. The cellular injury is redundant but may follow different mechanisms in the

different host cells and species (Karron and Collins, 2007). The lesions are typified by loss of cilia, dwarfing of epithelial cells, intracytoplasmic inclusion bodies, degemeration and proliferation of epithelial cells with syncytium formation (Garcin et al. 1998; Fink and Cookson, 2005).

Zhang et al (2005) suggested that immune complex complicates the mechanism of parainfluenza infection, especially through hyper responsiveness and cellular responses in humans. PIVs induce hyper responsiveness in the airway. An histamine mediated airway responsiveness was reported in Rubulavirus (parainfluenza virus SV5) infection of dogs (Lemen et al. 1990). Ogunbiyi et al (1988) also reported an increased release of vasoactive amines (histamine) from mast cells in calf lungs infected by bPI3V.

The virus also grow tremendiously in pulmonary effector cells (Schrijver et al. 1995). It caused cytotoxicity of epithelia and pulmonary alveolar macrophages, frustrated phagocytosis, and poor metabolism of arachidonic acid and secretion of prostaglandins (Liggitt et al. 1985; Slauson et al. 1987; Laegreid et al. 1989). The accessory proteins of PI3V (C, V, and D) inhibits induction of interferon α/β (Komatsu et al. 2004). Attachment of the virus to the olfactory epithelium was observed in Sendai virus challenged mice (Mori, 1996). The giant cells appear late in infection with two and seven nuclei. The giant-cell pneumonia rarely leads to death except in immunosusceptible hosts. However, persistent parainfluenza infections induce morphologic and functional alterations.There is peribronchiolar aggregate of lymphocytes and bronchiolar epithelial necrosis, with formation of pulmonary oedema (Porter et al. 1991).

There is pyrexia of approximately 1 week duration, coughing and hacking of the dorsum mucopurulent oculonasal discharge, rhinitis, tachypneoa and inappetence (Allen et al. 1978). The lesions are usually complicated by other pathogens like *Mannheimia haemolytica* and *Mycoplasma spp* (Fulton et al. 2000, Gagea et al. 2006).

Grossly, the pneumonic lesions include atelectasis and consolidation, lymphadenomagally and interlobular emphysema are differentiating feature of BRSV (Ellis, 2010). Histopathologic changes include bronchitis/bronchiolitis and alveolitis (Kapil et al. 1997).

Intracytoplasmic eosinophilic inclusion bodies are found by 2 and 7 days after infection (Bryson *et al.* 1979) while formation of epithelial syncytia may be variable.

2.8.2.3 Immunity to PI-3

The presence of haemaglutinin inhibition immunoglobulins was observed in PI3V infection. The dynamics of mucosal IgA and IgM, IgG humoral antibodies was also detected in intranasal PI3V inoculated calves with out prior exposure (Morein, 1970). Virus neutralization antibodies persisted for a few months (5), while nasal secretory antibodies declined after few weeks. There was anamnaesia in serum and mucosal humoral responses after reexposure to PI3V. The changes in nasal and serum humoral response were correlated with length of virus shedding (Gates et al. 1970). Mucosal antibody levels were associated antigen specificity of the PI3V in cattle. The molecular weights of the viral proteins (HN, F, and N) correlated with the nature of mucosal response in calves (Toth and Frank, 1988). Response to HN are high in primary infection, while F-specific antibodies are more in re-exposure to the virus(van Wyke Coelingh et al. 1990).

The influence of maternal derived antibodies were observed in colostrum for calves. The calves shed the virus, and were clinically ill after aerosol exposure to the virus. However, calves having maternal antibodies had low concentration of virus specific neutralizing antibodies.

The knowledge of cell-mediated immune response to PI3V needs further investigation. However, delayed hypersensitivity, virus-specific lymphocyte proliferation, leukocyte migration inhibition,and cytotoxic activity of natural killer cells were observed in cattle (Bamford et al. 1995). CD81 CTL conferred protection against hPI3V in humans. The humoral and CTL however partly provided immunity in the lower respiratory tract (Ellis, 2010).

2.8.2.4 Diagnostic approaches to PI3V

The best clinical diagnosis of PI3V is through viral isolation in nasal secretions taken by polyester-tipped swabs into viral transport medium (West et al. 1999). PI3V grows in turbinate and renal epithelial cell line. Virus identification is through cytopathic effects on the culture cells, intracytoplasmic inclusion bodies, syncytium giant cells, and haemadsorption of guinea pig red blood cells (Minnich and Ray, 1981), or immunofluorescence (IF) staining (McFerran and McNulty, 1992) or immunohistochemical techniques (Haines et al. 1992). The virus should be handled with care as it's instability and false-negative results from clinical samples. IF staining can be done for rapid diagnosis of PI3V (McFerran and McNulty, 1992).

10-14 days acute and convalescent serum can be used for PI3V diagnosis. This diagnosis of PI3 was done using haemagglutination inhibition or viral neutralization, and ELISA which is reliable (Graham et al. 1998). Serology has its own limitation in PI3V and other viral diagnosis in ruminants. There is persistence of passive immunity against PI3V even without re-exposure. Gates et al (1970) and Thomas (1973) reported that titers of less than 1/20 HI. Serial sampling should be done in relation to onset of disease (Thomas, 1973; Allen et al. 1978). IgM-based ELISA differentiates recent and previous PI3 exposure.

The gross and histologic lesions are suggestive of PI3V. Haines (1992) differentiated these lesions from that caused by RSV by demonstrating the PI3V antigensusing immunohistochemical staining. The greatest limitation of virus detection is to know the appropriate time. Bryson et al. (1979) however isolated PI3V from the nose of cattle for up to 9 days after exposure to the virus.This timing may influence outcome of analysis (Fulton et al. 2000; Gages et al. 2006). Inspite of serological limitation, paired serum samples and antigen detection are the most readily available means for PI3V investigation. PI3V is characterized by focal purulent bronchopneumonia, pulmonary congestion and oedema grossly, and nacute, severe diffuse necrotizing and fibrinous or suppurative bronchopneumonia microscopically (Rosadio et al. 2011). Other tests like direct immunofluorescence , multiplex PCR and RT-PCR are good for demonstrating PI3V nucleic acid. Sequencing and restriction enzyme amplification of F gene differentiate the isolates (Maidana et al. 21012). A new nucleic acid sequence based amplification (NASBA) technique was developed for HN and P genes.

2.8.2.5 Control and Vaccination for PI3

The effect of environmental factors;housing and other stressors complicates the PI3 and other viral pneumonia. The develompent of PI3V vaccine commenced with its isolation (Gale 1968). Attenuated injectable vaccines to hPI3V were induced humoral and rarely mucosal immunity after two different injections in cattle (Probert et al. 1978). The humoral response decreased viral shedding after exposure to the virus. The introduction of modified live virus (MLV) PI3 vaccines through parenteral and intranasal delivery were recored by Salt et al (2007) and Vangeel et al (2009). The available PI3V vaccines are polyvalent containing temperature-sensitive mutants.

Intranasal PI3V vaccination enhanced chemotactic activity of pulmonary alveolar macrophages and cellular immunity in ruminant (Allen *et al.* 1978). However,the optimal dose and the maximal period of protection of the vaccine needs further investigation.

Evaluation of the PI3V vaccines has been difficult because of the complexity and multiple agents involved in pneumonia (Stilwell *et al.*, 2008). The whole or attenuated viral antigen is useful for eliciting viral antibody. Column chromatographic techniques purified and separated most of the viral structural proteins (Henrickson, 2003). Intranasal inoculation of vaccine in laboratory animals produced polyclonal antibodies for serologic tests. A repeat (booster) immunization was required for maximal response. More so, monoclonal antibodies against all serotypes of PIV are now commercially available for specific serological tests.

2.8.3 Respiratory syncitial virus (RSV)

RSV is a *Pneumovirus* from the family *Paramyxoviridae*. It causes respiratory infections and economic losses in human and livestock (Baker et al. 1985; Ceribasi et al. 2013). There are bovine, ovine and caprine isolates of RSV (ie BRSV, ORSV & CRSV) in ruminants (Eleraky et al. 2001). It lowers the mucosal immunity of the respiratory tract.

2.8.3.1 RSV Morphology and Pathogenesis

The virus has the characteristics of the paramyxovirus; RNA genome that is negatively stranded, non segmented and enveloped (Lamb and Parks, 2007). The outer coat contains different proteins; surface glycoprotein [G], surface fusion protein [F] and small hydrophobic protein [SH] (Figure 2.10). It has a helical nucleocapsid containing nucleoprotein (N), phosphoprotein (P), RNA-dependent polymerase protein (L), M protein and a transcriptional anti-termination factor (M2-1). The genomic RNA is ~15,000 nucleotides in length.

The virus was first observed in primates with respiratory distress, and therefore identified as chimpanzee coryza agent (Morris et al. 1956). It was later named **respiratory syncytial virus** due to its characteristic cytopathic effect in culture (Parrott et al.1973).

RSV infection is mediated by attachement of its G protein to glycosaminoglycan on host cell receptor. Fusion is by F protein to host cell plasmalemma and nucleocapsid release into the cell (Feldman et al. 1999). F or G viral neutralizing antibodies protect animals with in flock.

2.8.3.2 Epidemiology of RSV

RSV infection in ruminants is worldwide due to transboundary movement of animals (Ames, 1993). The infectivity rates are high among herds. Cattle are reservoir; however, sheep come down with infection (Masot et al. 2000). Transmission within herd is by aerosols, and directly by new infected animals, and indirectly by formites. Herd size and managemental farm practices contributes to risk of infection (Ohlson et al. 2010).

The influence of weather was observed in RSV outbreaks (Valarcher and Taylor, 2007). The morbidity is high up to 60% among dairy herds (Elvander, 1996). RSV affect all groups of animals irrespective of age, however, it is a function of exposure (Raaperi et al. 2012).

Climate change favours spread of RSV all year round (Valarcher and Taylor, 2007). The mechanism behind viral survival within population is not yet known. Nonetheless, Stott et al (1980) suggested influence of chronicity in transmission of RSV infection.

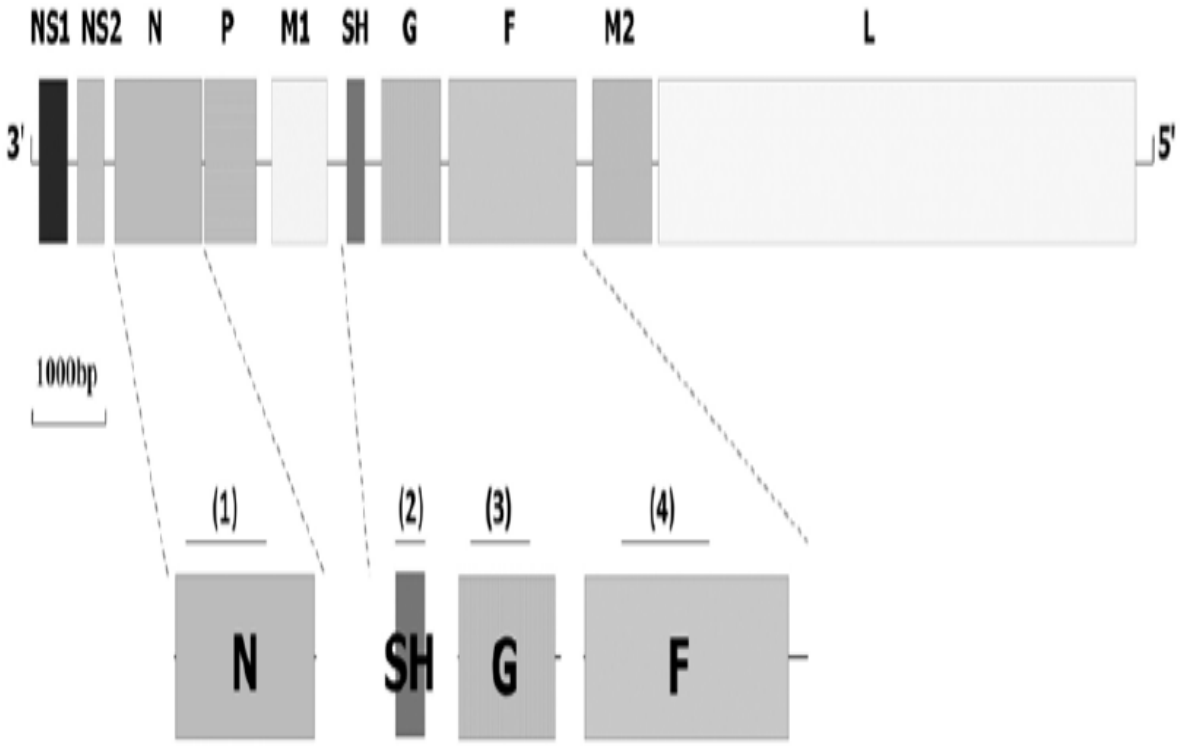


Figure 2.10. Scheme showing BRSVgenome. (1)- N region (nt 1294 to nt 1984); (2)- SH complete genome (nt 4268 to 4513); (3)- G region (nt 4864 to 5353); (3)- F region (nt 6071 to nt 6812). This analysis was given in reference to RSV NC_001989 strain. (Source; Sarmiento-Silva et al. 2012**)**

Seromonitoring of herds is required for establishing RSV infection. Molecular tools have also provided valuable knowledge on nature, transmission and biology of RSV (Valarcher et al. 2000; Spilki et al. 2006). Phylogentic analysis identified mutations on the G protein and strains. The antigenic variation of the strains was based on negative reactions with polyclonal antibodies (Lerch et al. 1989). Moree so, heterogenicity of the viruses could be estimated from the molecular sizes of the structural proteins (Mallipeddi and Samal, 1993). Many (4) antigenic variants (A, B, AB, untyped) are of similar antigenic group. Further phylogenetic analysis showed two main branches of RSV; Branch I with subgroups a & b, Branch II with three subgroups and all making up 5 lineages (Sarmiento-Silva et al. 2012).

However, structural antigenic connection was reported for all RSV, and for bovine and caprine isolates (Alansari and Potgieter 1994). The virus was isolated from goats that did not show clinical respiratory sign (Masot et al. 1993). RSV infection was demonstrated in goats through serological and pathological lesions (Jetteur et al. 1990; Yesilbag and Gungor, 2009). Clinically there was fever, weakness, nasal discharge, dyspneoa, cough, and anorexia in affected animals (Caswell and Williams, 2007). These signs may become intense with mixed infections or bacterial complication.

2.8.3.3 Pathology of RSV infection in the respiratory tract

Grosslly, there is mucopurulent exudate bronchi and bronchiolar lumen, irregular atalectasis in the cranioventral lobes, consolidation and emphysema in the lungs. Histopathologically, it is characterized by bronchitis, bronchiolitis, mononuclear cell infiltrations in alveolar septum, lymphoid hyperplasia, hyperplasia of epithelial cells, acidophilic inclusion bodies and syncytial cells (Gulbahar et al. 2002). The viral inclusion body and syncytial cell formations may be inconsistent due to virulence and infective dose of the virus, incubation period, host age and specie (Masot et al. 1993; Gulbahar et al. 2002). More so, the pathological changes may progress with complication from other opportunistic infections.

2.8.3.4 Diagnosis of RSV

The diagnosis can be made considering the gross, microscopic lesions and viral antigen demonsration (Gulbahar et al. 2002; Ceribasi et al. 2013). Serum neutralizing antibodies were reported in domestic sheep, goats, cattle and bighorn sheep ***(****Ovis cunudensis****)*** (Dunbar et al., 1985). The seroprevalence in free-ranging mountain goats (*Oreamnos americunus*) ***was*** 42% (Dunbar et al. 1986). Much is yet to be understood on the control of RSV in ruminants. Available vaccines against RSV are polyvalent containing different antigenic components.

2.8.4 Pneumonic pasteurellosis

Pneumonic pasteurellosis is characterized by severe fibrinous bronchopneumonia, and septicaemia (Mohamed and Abdelsalam, 2008). It is caused by Gram-negative non-motile facultative bacteria Pasteurella. Phylogenetic comparison of universal bacterial gene sequences (16S rDNA) has recently become a key character for bacterial classification. This has resulted in improved classifications of many groups and enabled re-evaluation of the selection of phenotypic characters used for identification.

2.8.4.1 Classification of Pasteurella species

Pasteurella is associated with devastating disease conditions in farm animals. The bacteria causes necrotizing to haemorrhagic lesions in domestic and wild life animals. DNA hybridization and 16S rRNA sequencingidentified the different subtypes (Christensen et al. 2003a).

The family consist of two genera *Pasteurella and Mannheimia* (Angen *et al.*, 1999a). Mannheimia was named after Walter Mannheim for his contributions to Pasteurellaceae. *M. haemolytica* is grouped as two biotypes; biotype A due to fermentation of L-arabinose and biotype T due to fermentation of trehalose. There are 1–17 *M. haemolytica* serovars (Miller, 2001). *Pastuerella multocida* has three subspecies; *Pastuerella multocida* subspecie *gallicida*, *Pastuerella multocida* subspecie *multocida* and *Pastuerella multocida* subspecie septic based on DNA–DNA hybridization **(**Blackall et al.1998). However, the three subtypes have similar

genotypic characteristics (Petersen et al. 2001)**.** There is about 99.9% homology between sheep (PM82) and Haemorrhagic septicaemia (HS vaccine P52) strains (Dey et al. 2007).

M. haemolytica causes enzootic pneumonia in cattle, mannheimiosis in cattle, sheep and goats, bacterial septicaemia and mastitis in sheep and non-specific inflammatory lesions in animals (Quinn et al. 2002). *P. multocida* causes haemorrhagic septicaemia, enzootic pneumonia, fowl cholera, snuffles or fatal septicaemia in animals. Pasteurella and Mannheimia were also considered primarily pathogens in human (Ashley et al. 2004). Both bacteria are however normal microflora in healthy animals(Shewen and Conlon, 1993; Al-Tarazi and Daghall, 1997). The mean colony count of *M. haemolytica* in the respiratory tract was high in sick animals (Thomson et al. 1975).

The *Mh* biotype "A" serotype 1 induced pneumonia in cattle while serotype 2 induces ovine and caprine pneumonia (Morck et al. 1989). Serotype 7 was isolated in acute ovine pneumonia (Odugbo *et al.*, 2004a), serotypes A6, A9 and A11 were also pathogenic in sheep (Odugbo *et al.*, 2004b) and goat (Emikpe, 2009). The clinical and pathological manifestations of mmannheimiosis in goats are not apparently different from sheep.

2.8.4.2 Clinical features of pneumonic pasteurellosis/mannheimiosis

The clinical signs develop within two weeks of infection or exposure to stressful factors (Radostits et al. 2006). There is dullness, depression, mucopurulent oculonasal discharge, cough, and (Mohamed and Abdelsalam, 2008).

Cattle, sheep and goats develop pyrexia, young animals are very much susceptible developing sudden clinical signs, depression and death.

2.8.4.3 Pathogenesis and virulence factors of *Pasteurella spp*

The sequential development of the lesions in the respiratory tract is mediated by complex interactions of the pathogen virulence factors, immunological status of the host and other confounding factors. Stress and/or viral infections impair the pulmonary clearance of bacteria from the nasopharynx thus allowing gravitational flow along the tracheal, bronchi, bronchioles and alveoli. Bacteria endotoxins cause extensive intravascular thrombosis, ischaemic necrosis and severe fibrinous inflammation (Mohamed and Abdelsalam, 2008; Lopez, 2012). Immune

complex formation contribute to the vascular response, leucocyte migration, phagocytosis and hydrolytic breakdown of tissues (Kim, 1977). The extent and degree of tissue damage depends bacteria growth, endotoxin, other virulence factors and host immunity (Hilwig et al. 1985).

The virulence factors promote adhesion, and colonization of the pathogen. The cell capsule fimbriae, endotoxin, leucotoxin, outermembrane protein and extracellular enzymes are established virulence determinants (Zecchinon et al. 2005). Others include siderophore-mediated iron uptake (Otto et al. 1992), transferrin-binding protein (Ogunnariwo and Schryvers, 1990), neuraminidase (Abdullah et al. 1992), and IgG1 specific protease (Lee and Shewen, 1996).

2.8.4.4 Pathology of pneumonic pasteurellosis

Grossly, there is fibrinous (lobar) bronchopneumonia, pleuritis and effusions. Interstitial oedema and congestion forms the marbled appearance of the lung (Lopez, 2012). The lesion is bilateral, cranioventral and horizontal tothe tracheal bifurcation. The apical and cardiac lobes are affected parts, and extensive to the diaphragmatic lobe. Foci of coagulative necrosis are in the affected lung, irregular and demarcated zone of inflammation. The thoracic lymph nodes (mediastinal and bronchial) are hyperaemic, haemorrhagic and or inflamed while froth is abundant in the upper respiratory tract.

Microscopically, there is diffuse vascular congestion, oedema, vascular thrombosis, alveolar epithelial necrosis and fibrinous exudate. The necrotic alveoli is swirled by a rim of elongated cells (swirling macrophages) or “oat cells” that are degenerated neutrophils and alveolar macrophages. Bacterial colonies are in the necrotic tissue.

The gross and histopathological lesions of *Mh* in cattle were similar to *P. multocida* induced lesions. However, the lack of focal necrosis and abundant neutrophils in *P. multocida* pneumonia may be characteristic (Ames et al. 1985). More distinguishing features include the fulminating fibrinous lobar pneumonia in *Mh* and fibrinopurulent bronchopneumonia in *P multocida* (Haritani et al. 1987).

The lesions of mannheimiosis are similar in ruminants (Brogden et al. 1998). It was acute haemorrhagic or fibrinonecrotic in sheep, goats and cattle (Zamri et al. 1991; Brogden et al. 1998), and presence of focal abscesses in the goat. The presence of thrombi in large vessels underscores the procoagulant effect of *Mh* endotoxin.

2.8.4.5 Diagnosis of Pneumonic pasteurellosis

Fresh tissue samples are requireed for *Pasteurella/Mannheimia* culture and cytologic staining for bipolar rods. The medium for bacterial isolation include blood agar, chocolate agar and the special medium (casein/sucrose/yeast agar) supplemented with fresh unclotted blood. Dextrose based agar, and trypticase soy agar are equally good for bacterial growth.Methylene blue or Leishman's stain demonstrate bipolar rods. Rapid slide agglutination test, indirect haemagglutination assay and agar gel precipitation test are serologic tests suitable in typing capsular and somatic antigen. The counterimmunoelectrophoresis, Dot immunobinding assay, and immunoblotting have been used for characterization of outer membrane proteins and rapid diagnosis (Minakshi et al. 2000). The comprehensive analysis of OMP profiles of *Pm* by immunoblotting showed that it is highly antigenic and immunodiagnostic.

PCR and specific primer pair was used to amplify the 353 base pair fragment of the 16s rRNA gene (Dhama et al. 2012). DNA finger printing was used for characterization of isolates (Brown, 2008; OIE, 2009). Southern hybridization confirmed bacteria sequence and virulence. Genotypic DNA fingerprinting, restriction endonuclease analysis, and repetitive extragenic palindromic REP-PCR were differentiated the the Pasteurella subtypes (Hopkins et al. 1998).

2.9 Control of Respiratory Disease

Vaccination is the most effective way of respiratory disease control in livestock. The use and administration of antibiotics and other therapeutics for prophylaxis and treatment of respiratory disease conditions have not been able to effectively control the menance of pneumonia in ruminants. It was been established that better and maximal immune response was achieved through inhalation/intransal route when compared to the conventional subcutaneous or intramuscular route of vaccination. Further more, the problems associated

with issues of cold chain and vaccine failures needs to be addressed by thinking of preferable vaccine delivery systems and adjuvants that may prolong the shelf life of the vaccine, and improve the immunogenicity of vaccines.

The complexity of caprine pneumonia also warrants research innovation in areas of vaccinology for production of multivalent vaccines. This can only be achieved by first updating and elucidating the causative agents in our environment and incorporating the antigens as vaccine against caprine pneumonia. This phenomenon needs to be harnessed and enhanced for optimal protection of goats and other livestock against the serious threat of pneumonia.

Vaccine efficacy relates largely to the vaccine material, route of vaccination and the mode of delivery which needs to be chosen carefully so as to induce the best possible response (Streatfield 2005).

2.9.1 Route of vaccination

The intramuscular injection is the most common route of administration. The thigh and deltoid muscles are the sites of choice, since injection into the gluteal region has been known to cause damage to the sciatic nerve and lameness in animals. Other routes of administration that can be used in animals, include epidermal, intradermal, intravenous, intranasal, and oral. The latter two routes are particularly attractive for two reasons. First, under the right circumstances, intranasal and oral regimens can induce both systemic and mucosal immune responses, enhancing protection at the interfaces where the vast majority of pathogens gain access to the body. Secondly, any method that obviates the need for sterile syringes and needles will be an immediate boon to the developing world. In the course of therapeutic injections as well as vaccinations, syringes and needles are commonly reused in poorer areas where sterilization is often inadequate (Mak and Saunders 2011).

Recent studies on the control and prevention of some respiratory diseases of ruminants have shown a strong immune response following mucosal immunization (Sabri et al. 2013, Ezeasor et al 2015). This is achievable by delivering the vaccine through the organism's natural route of entry into the body thereby creating a mucosal barrier against pathogens in the environment.

CHAPTER THREE

3.0 PATTERN AND ASSOCIATED FACTORS OF PNEUMONIA IN GOATS

3.1 INTRODUCTION

Goats are are of economic value to humans serving as a source income, protein, and hide. Goats (*Capra hircus*) are a descendant from the species *Capra oegragus,* and are the first ruminant to be domesticated (Kumar et al. 2003). The economic importance of goats cannot be overemphasized because they serve as source of income to the poor, comparably cheap to manage and are essential livestock in rural households (Emikpe et al. 2013). However, goats are on the receiving end of adverse environmental influences, stressful managemental practices and conditions which predispose to respiratory diseases.

Pneumonia no doubt is a major problem that needs to be controlled for improvement of sheep and goat production worldwide (OIE, 2008). It causes severe morbidity manifested physically as unthriftiness or weight loss (Jarikre et al. 2013) and large scale mortality in domestic and wild goats (Ostrowski et al. 2011). Pattern of pulmonary consolidation or pneumonia serves as a reliable clinical parameter in auscultation. This was in different experimental conditions; Emikpe and Akpavie (2011) reported a mean score of 6.83 in WAD goats, 3-80% in aerosol vaccinated calves (Jericho and Langford, 1982) and 32% in Markor goats (Ostrowski *et al.*, 2011), but little is known of the influence of age, sex, body score and breed on the pattern of caprine pneumonia in Nigerian goats.

The knowledge of the lobar and morphologic pattern of pulmonary consolidation especially with respect to age, sex, breed and body score in goats will provide insight as to the probable etiology, route of exposure to the causative agent, pathogenesis of the lesions, effect on pulmonary function, possible complications and sequelae of the pulmonary lesion. This study investigated the pattern of lung consolidation in natural infections and attempts to identify the

associated risk factors associated with caprine pneumonia in Nigeria, so as to elucidate and aid the understanding of caprine respiratory disease complex in Nigeria.

3.2 MATERIALS AND METHODS

3.2.1 Study location

This study was conducted at the slaughter post of the University of Ibadan Zoological garden, Ibadan, south west Nigeria for over 72 weeks where an average of 30 goats were slaughtered weekly. The sampling was between March 2014 and August 2015.

3.2.2 Study Animals

The goats were sourced mostly from the northern region in Nigeria and transported to the University of Ibadan where the animals were meant to feed the captive wild carnivores housed in the prestigious zoo. A total of 700 indigenous goats comprising West African Dwarf (WAD), Red Sokoto (RS) and Sahelian (S) breeds were examined.

3.2.3 Ante-mortem examination

The sex of the goats were determined and the age by the dentition as described by Lasisi *et al*. (2002). The general body conditions were evaluated and scored systemically as described by Battaglia (2001) on a scale of 1-5 (amount of fat and muscle at key anatomical points); 1= very thin (poor), 2= thin (fair), 3= normal (good), 4=fat (obese) and 5= (vey obese). However, the goats were either scored as good, fair or poor.

3.2.4 Post-mortem evaluation of pneumonia

Gross examination of the lungs for changes in consistency, texture, color and degree of distribution was as described by Lopez (2012). The distribution and nature of pulmonary consolidation and other lesions were determined grossly by observation and palpation. The degree of pulmonary consolidation and lobe involvement as a percentage of the total lung volume was stimated using morphometric evaluation (Jericho and Langford, 1982; Emikpe and Akpavie, 2011). Sections from all the lung lobes; apical, middle and caudal were taken

from both lungs into sample bottles with adequate volume of fixative for histological processing and examination.

3.2.5 Histopathologic evaluation

The stepwise protocol for histological processing of the tissues was done using the the automatic tissue processor and as described by William (2004). Briefly, the lung tissues were fixed in 10 times volume of neutral buffered formalin for at least 24 hours before the commencement of dehydration. Dehydration was done by automated means using the automated tissue processor (Shandon-Elliot^{R}) in graded concentration of alcohol as follows: 70%, 80%, 90%, 95%, 95%, 100% for one hour each. At the end of processing schedule the tissues were totally dehydrated and subsequently cleared in Xylene for two hours. Clearing prepares the tissue for infiltration in molten paraffin wax. Infiltration with molten paraffin wax was completed in a wax oven for four hours at a fairly high temperature before embedding. The tissues were carefully and consciously positioned in the orientation they will be sectioned (Embedding). The embedded tissues were left until the wax solidifies. Sectioning was done using the microtome (Leica) at a thickness of 4µ. The sections were floated out in a floating water bath. Satisfactory section(s) were picked up with frosted edge microscope glass slides. The slides carrying the sections were appropriately labeled with a pencil. They were arranged in a slide carrier ready for staining in an oven at 40^{0}c for 30 minites for dewaxing and allowing the sections stick to the slide. The slides were dewaxed in Xylene (twice) for 15 mins each, rehydrated in decreasing gradient of ethanol (100%, 100%, ,80%, 70%) for 30 seconds each. They were then rinsed in water for 3 mins before sttaining in haematoxylin for 15mins. The excess stain was washed off with water, differentiated in 1% acid alcohol for 3 – 5 secs, rinnsed in water for 3–5 mins, counterstained in 1% Eosin for 3 secs. The slides were further dehydrated with Ethanol dipping and remained in the xylene until they were mounted using DPX mountant (Atom Scientific UN19993, UK)

The slides were examined for pathological changes under the microscope (Olympus CX2), considering the vascular, interstial and parenchymal (bronchi, bronchiolar and alveolar) changes of the lung. Photomicrographs of appropriate lesions or morphological changes were taken by a digital camera (Amscope MU900) connected to the computer and binocular

microscope. Morphological diagnosis was made from each goat and the pulmonary lesions were grouped according to the morphological changes.

3.2.6 Microbial Culture (*Pasteurella species*)

Samples from the affected fresh lung were taken from the lesion (pneumonic) aseptically in universal bottles containing brain heart infusion broth. Those that yielded growth were streaked on blood agar plate (BAP, 5% blood in blood agar base). The same samples were parallel streaked on MacConkey agar for primary differentiation of the pathogen following standard procedures. The culture dishes were placed in the incubator at 37^0C for 24 hours. The grown bacterial colonies were characterized based on cellular morphology and Gram staining. The organisms that were negative to the stain, cocci or rod-like in shape, having or without staining at both ends (bipolar) on microscopic examination were further identified on nutrient agar plate to get pure culture for further biochemical tests following standard protocols (Quinn et al. 2002).

A 24 hour pure culture or colony of *Pasteurella/Mannheimia* was characterised biochemically. Indole production and motitlity was used in Sulfide Indole motility (SIM) medium, hydrogen sulfide production was tested on triple iron sugar agar and SIM medium. Fermentation reactions for sugars (glucose lactose, maltose, mannitol, trehalose and arabinose) were conducted (Abera et al. 2014).

3.2.7 Statistical analysis

The distributions of the animals according to age, sex, breed, body score, management and season were presented in frequencies and percentages.

The pneumonic status of the goats were analysed and compared using Chi square and Odd ratio. The pulmonary consolidation scores were presented in Mean±Standard error of mean (M±SEM), compared using Student T-test, ANOVA and Duncan Multiple Range Test at α=0.05 on Graph Pad Prism version 21 and SPSS version 20.

3.3 RESULTS

3.3.1 Distribution of the animals

Two hundred and twelve (30.3%) were WAD, 388 (55.4%) RS and 100 (14.3%) Sahelian (mixed) breeds. Twenty six (3.7%) of the animals were one year old, 215 (30.7%) two years old and 459 (65.6%) above 2 years old. Six hundred and eighty five (97.9%) male and 15 (2.1%) female.

3.3.2 Clinical examination

One hundred and sixty two (23.1%) of the animals were in in good body condition, 340 (48.6%) apparently fair body condition, and 198 (28.3%) in poor body condition (Table 3.1). Clinically, the signs observed are shown in Plate 3.1 and Table 3.1.The prevalence of pneumonia in the goats studied was 418 (59.7%) while 282 (40.3%) were normal.

3.3.3 Consolidation pattern

From the 418 (59.2%) pneumonic goats; different patterns of pulmonary consolidation were observed grossly (Plate 3.2) including cranio-ventral with marked consolidation of the apical to middle lobes 294, 56 caudo-dorsal consolidation affecting the diaphragmatic lobes, accentuated and markedly distended interlobular pattern with fibrinous deposits (marbling) 51 and multifocal 8, emphysematous 4 and haemorrhagic 5.

The right lung had higher consolidation and followed by the caudal lobes in the goats. The right cranial (apical) lobe had the highest occurrence of consolidation followed by right middle lobe, right cranial posterior lobe, left cranial lobe and caudal lobes. The assessory lobe was the least consolidated in the examined goats.

The RS breed had the highest incidence of pulmonary consolidation and the least was observed in the sahelian/mixed breeds. Furthermore, animals with fair and poor body conditions had highest occurrence of pulmonary consolidation compared to those in good body condition ($p<0.05$). And more male animals were observed with high incidence of pulmonary consolidation (figure 3.3).

It was also observed that the incidence of pneumonia occurred with increase in ages of the goats across breeds; however more pneumonic cases were observed from above 2 years old

and in RS breed. Similar pattern was observed in goats with fair and poor body condition scores, the incidence of pneumonia high in poor body condition goats (Table 3.2).

Morphometrically, the mean pulmonary consolidation score was 8.1±0.5 in the pneumonic goats, it was 7.8±0.5 in pneumonic male goats and 21.4±8.7 in the pneumonic female goats. The influence of age was quite remarkable in the susceptibility to pneumonia as higher consolidation score was observed in goats less than one year old. The RS breed had higher consolidation score than the WAD and less in the Sahelian breed. The goats with poor body condition score and in clinically debilitated states had higher consolidation scores. The specific mean consolidation score on the different pulmonary lobes are shown in figure 3.2.There was a significant correlation between lobar consolidation on each sides of the lung ($p<0.05$).

Plate 3.1: (a) Goats observed ante-mortem for signs of respiratory distress and body condition score.

Plate 3.1. (b) A goat with rough hair coat, bony prominence and mucopurulent nasal discharge (arrow).

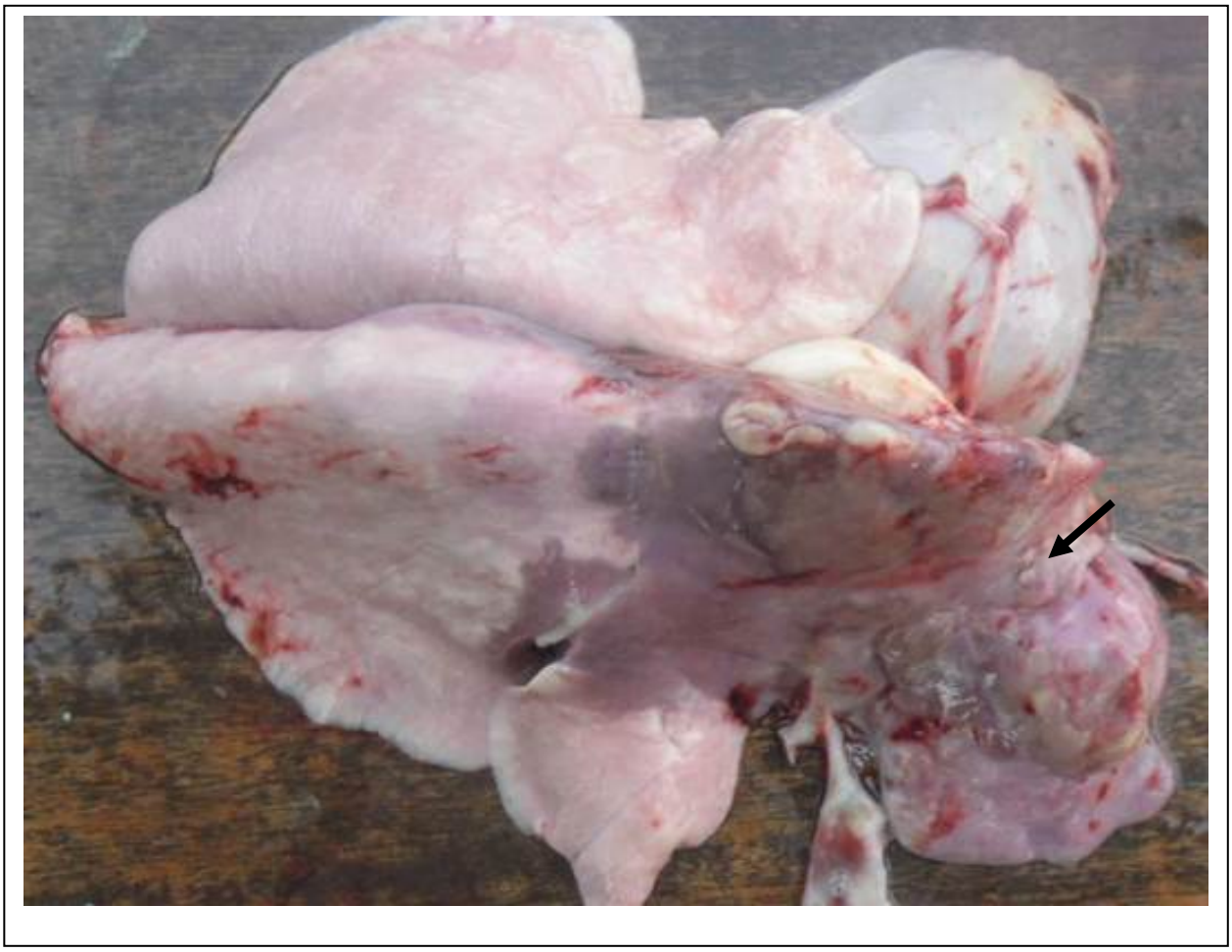


Plate 3.2. Lung from a goat with 92% consolidation (dark red and firm) of the right cranial lobes (arrow) and fibrinous exudate on the pleura.

Table 3.1. The frequency of clinical parameters and signs taken during ante-mortem examination of the goats.

Score	Number	Weight looss	Nasal discharge	Ocular discharge	Dyspnoea	Cough	Rough hair coat	Diarrheoa	Weaknes s	Pyrexia
Good	162	0	0	3	0	7	0	0	0	0
Fair	340	45	47	23	28	28	15	15	0	11
Poor	198	82	100	39	91	75	83	43	14	37
Total	700	127 (18%)	147 (21%)	65 (9%)	119 (17%)	110 (16%)	98 (14%)	58 (8%)	14 (2%)	48 (7%)

Table 3.2: Distribution of the goats based on gross diagnosis of pneumonia (n=700)

	Non-Pneumonic	Pneumonic	TOTAL	Odd ratio	p-value
Breed					
WAD	70 (33%)	142 (67%)	212 (100%)		
RS	166 (43%)	222 (57%)	388 (100%)		p=0.029
SH	46 (46%)	54 (54%)	100 (100%)		
TOTAL	282 (40%)	418 (60%)	700 (100%)		
Sex					
M	274 (40%)	411 (60%)	685 (100%)	0.583	p=0.045
F	8 (53%)	7 (47%)	15 (100%)		
TOTAL	282 (40%)	418 (60%)	700 (100%)		
Age					
≤1	14 (54%)	12 (46%)	26 (100%)		
2	83 (39%)	132 (61%)	215 (100%)		p=0.326
>2	185 (40%)	274 (60%)	459 (100%)		
TOTAL	282 (40%)	418 (60%)	700 (100%)		
Body condition score					
Good	81 (50%)	81 (50%)	162 (100%)		p=0.016
Fair	127 (37%)	213 (63%)	340 (100%)		
Poor	74 (37%)	124 (62%)	198 (100%)		
TOTAL	282 (40%)	418 (60%)	700 (100%)		

WAD-West African Dwarf, RS- Red Sokoto, SH- Sahelian. M- Male, F- Female,

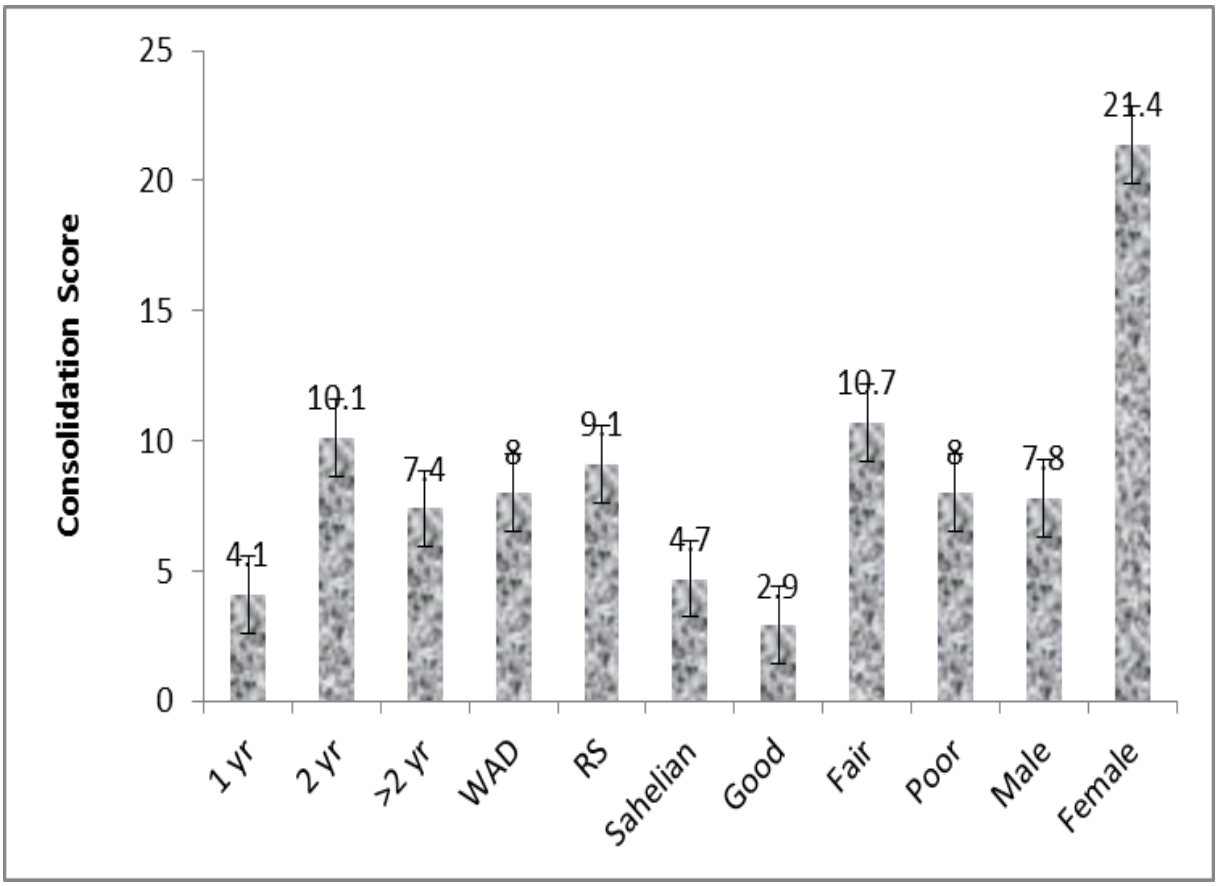


Figure 3.2. Distribution of the consolidation scores in examined goats across Nigeria

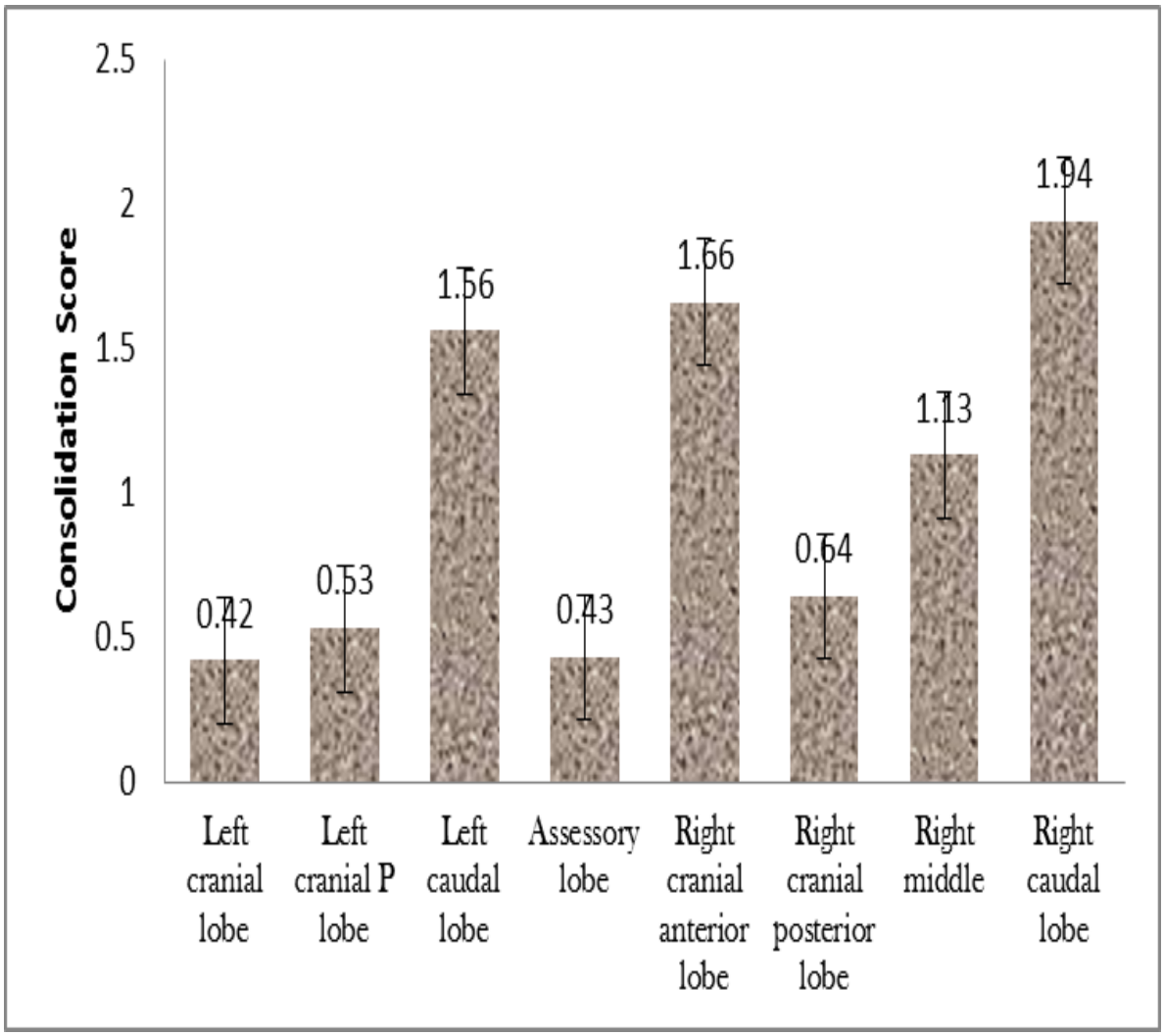


Figure 3.3. Lobal distribution of pulmonary consolidation scores from the examined goats.

3.3.4 Histopathology

The histological morphologies of the pneumonic goats include bronchopneumonia (41.6%) - fibrinous and suppurative (Plate 3.4); broncho-interstitial pneumonia (8.7%) with giant cell formation (Plate 3.5); interstitial pneumonia (7.9%); verminous pneumonia 0.3% (Plate 3.6) and granulomatous pneumonia (0.9%). There were also a few with congestion, edema, and atelectasis, and normal lung (40.7%). The distribution across breeds, sex, age and body scores of the animals is shown in Table 3.2.

The bronchiolar and alveolar epithelial cells were hyperplastic (Plate 3.7), degenerate to necrotic with cellular infiltrates and cellular debris in the lumen of the airway. Some of the degenerate epithelial cells had pale to oesinophilic intracytoplasmic inclusion bodies (Plate 3.8). The inflammatory cells varied from neutrophils, macrophages and lymphocytes.

3.3.4.1 Bronchopneumonia

There was hyperaemia. The bronchi, bronchioles and alveoli contained exudate and cellular debris. The exudate varied from oedema, fibrin, neutrophils, and abundant alveolar macrophages. Infilling of alveoli, bronchioles and small bronchi obliterated airspaces with attenuation of septae. The bronchial associated lymphoid tissue (BALT) was moderately hyperplastic, and pleura thickened with formation of a few condensed fibrin thrombi in the parenchyma.

3.3.4.2 Broncho-interstitial pneumonia

There was also hyperaemia, degeneration, necrosis and sloughing of bronchial and bronchiolar mucosa epithelium. Peribronchial mononuclear cellular infiltrates extending to the broncho-alveolar bundle. The alveolar cells were also necrotic with presence of macrophages, lymphocytes, a few neutrophils and syncytial giant cells in air spaces. Some of the degenerate epithelial cells contain eosinophilic to basophilic intranuclear inclusion bodies.

3.3.4.3 Interstitial pneumonia

There was thickening or accentuation of the alveolar septae due to congestion of of alveolar capillaries, haemorrhages and or infiltration of mononuclear inflammatory cells including

lymphocytes and macrophages. Disruption of alveolar lining and formation of hyaline membrane was also common.

3.3.4.4 Granulomatous pneumonia

There was diffuse expansion of the interstitium by infiltrating mononuclear cells. Alveoli were obliterated. There was presence of necrotic centre flanked by abundant macrophages, lymphocytes and plasma cells, and an outer rim of connective tissue.

3.3.4.5 Verminous pneumonia

This was characterized by presence of parasitic segments in airways (bronchi and bronchioles) with an associated peribronchial reaction and also identification of larva stages in the alveoli.

Comparatively, bronchopneumonia was common in all the three breeds as compared to interstitial in RS, verminous and granulomatous pneumonia in WAD ($p<0.05$). Similar pattern of the body score as seen in the consolidation was observed with histological type of pneumonia ($p<0.05$). However, these distribution was more in the male goats and those 2 years above ($p>0.05$).

3.3.5 Bacterial pathogens isolated

Gram negative, coccobacillary rods with or without bipolar staining were the most isolated bacteria from the pneumonic goats. The organisms were identified and differentiated biochemically;

P. multocida- colonies were non-haemolytic, round, smooth or mucoid, and did not grow on MacConkey agar.

M. haemolytica- colonies were round, smooth, transluscent, greyish with β-distinct zone of haemolysis on blood agar, and grew on MacConkey agar.

Other bacteria isolated include *Staphyloccocus aureus, Bacillus spp, Klebsiella spp, Pseudomonas spp* and *Escherichia coli* (Table 3.4).

Table 3.3: Histological classification of pneumonia in the examined goats

	Normal	Broncho-pneumonia	Broncho-interstitial	Interstitial pneumonia	Verminous pneumonia	Granulomatous pneumonia	Total	P
Breed								
WAD	73 (10.4%)	101 (14.4%)	19 (2.7%)	16 (2.3%)	1 (0.1%)	2 (0.3%)	212 (30.3%)	
RS	165 (23.6%)	153 (21.9%)	31 (4.4%)	35 (5.0%)	1 (0.1%)	3 (0.4%)	388 (55.4%)	0.422
SH	47 (6.7%)	37 (5.3%)	11 (1.6%)	4 (0.6%)	0	1 (0.1%)	100 (14.3%)	
TOTAL	285 (40.7%)	291 (41.6%)	61 (8.7%)	55 (7.9%)	2 (0.3%)	6 (0.9%)	700 (100%)	
Sex								
Male	277 (39.6%)	288 (41.1%)	59 (8.4%)	53 (7.6%)	2 (0.3%)	6 (0.9%)	685 (97.9%)	
Female	8 (1.1%)	3 (0.4%)	2 (0.3%)	2 (0.3%)	0 (0%)	0 (0%)	15 (2.1%)	0.631
TOTAL	285 (40.7%)	291 (41.6%)	61 (8.7%)	55 (7.9%)	2 (0.3%)	6 (0.9%)	700 (100%)	
Age								
≤1	14 (2.0%)	6 (0.9%)	5 (0.7%)	1 (0.1%)	0	0 (0%)	26 (3.7%)	
2	83 (11.9%)	94 (13.4%)	21 (3.0%)	14 (2.0%)	0	3 (0.4%)	215 (30.7%)	0.313
>2	188 (26.9%)	191 (27.3%)	35 (5.0%)	40 (5.7%)	2 (0.3%)	3 (0.4%)	459 (65.6%)	
TOTAL	285 (40.7%)	291 (41.6%)	61 (8.7%)	55 (7.9%)	2 (0.3%)	6 (0.9%)	700 (100%)	
Body score								
Good	79 (11.3%)	68 (9.7%)	4 (0.6%)	8 (1.1%)	1 (0.1%)	2 (0.3%)	162 (23.1%)	
Fair	130 (18.6%)	137 (19.6%)	35 (0.5%)	36 (5.1%)	0	2 (0.3%)	340 (48.6%)	0.05
Poor	76 (10.9%)	86 (12.3%)	22 (3.1%)	11 (1.6%)	1 (0.1%)	2 (0.3%)	198 (28.3%)	
TOTAL	285 (40.7%)	291 (41.6%)	61 (8.7%)	55 (7.9%)	2 (0.3%)	6 (0.9%)	700 (100%)	

WAD-West African Dwarf, RS- Red Sokoto, SH- Sahelian. M- Male, F- Female,

Table 3.4: Bacteria isolated from the Pneumonic lungs of the examined goats (n=70)

Isolate	Total No. sample examined	No. of samples positive	Bacterial isolated (%)
E. coli		20	29%
Klebsiella sp.		15	21%
Pasteurella sp.	70	31	44%
Streptococcus sp.		12	17%
Bacillus sp.		8	11%
Pseudomonas		5	7%
Mannheimia h.		61	87%

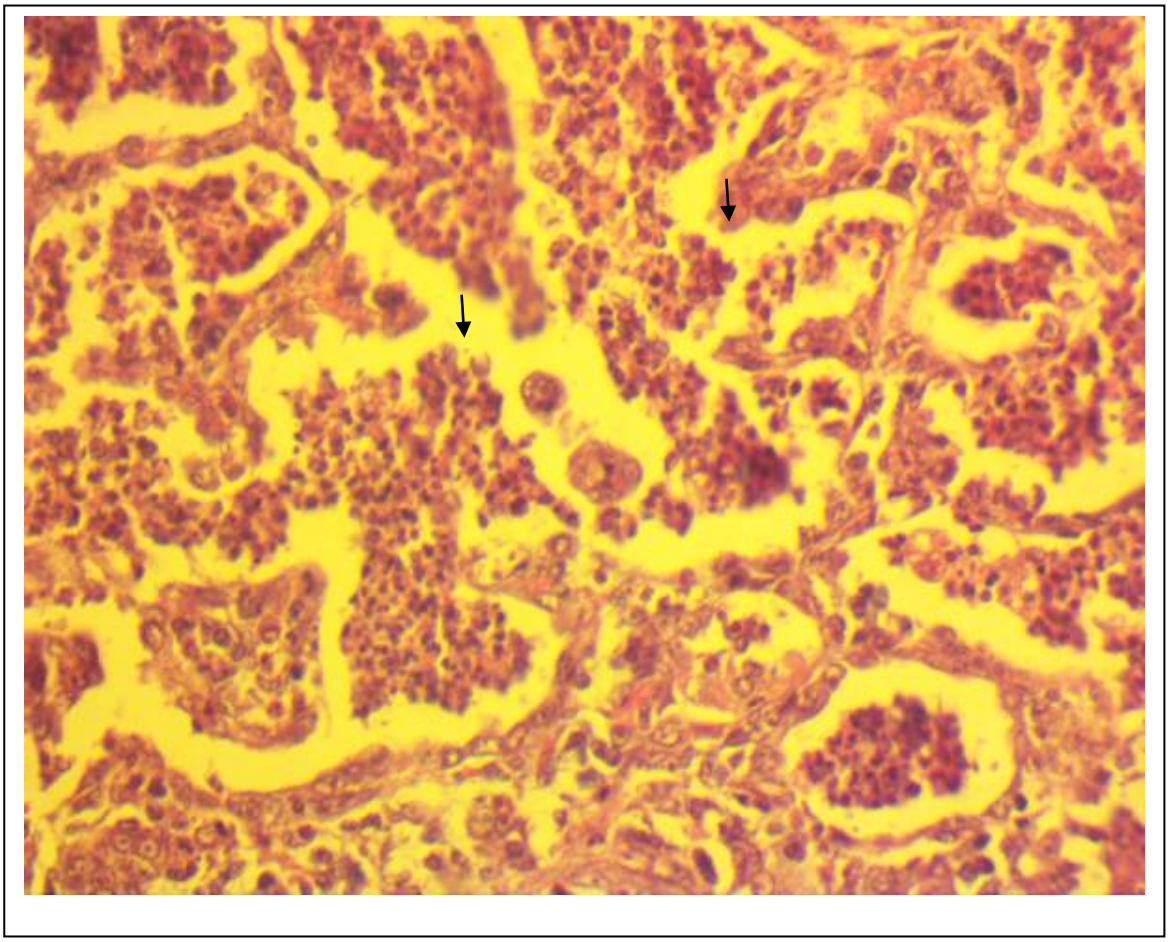


Plate 3.4. Photomicrograph of the lung section showing diffuse accumulation of inflammatory cells (neutrophils, and macrophages) in alveolar spaces with exudates (arrow). H and E stain x400

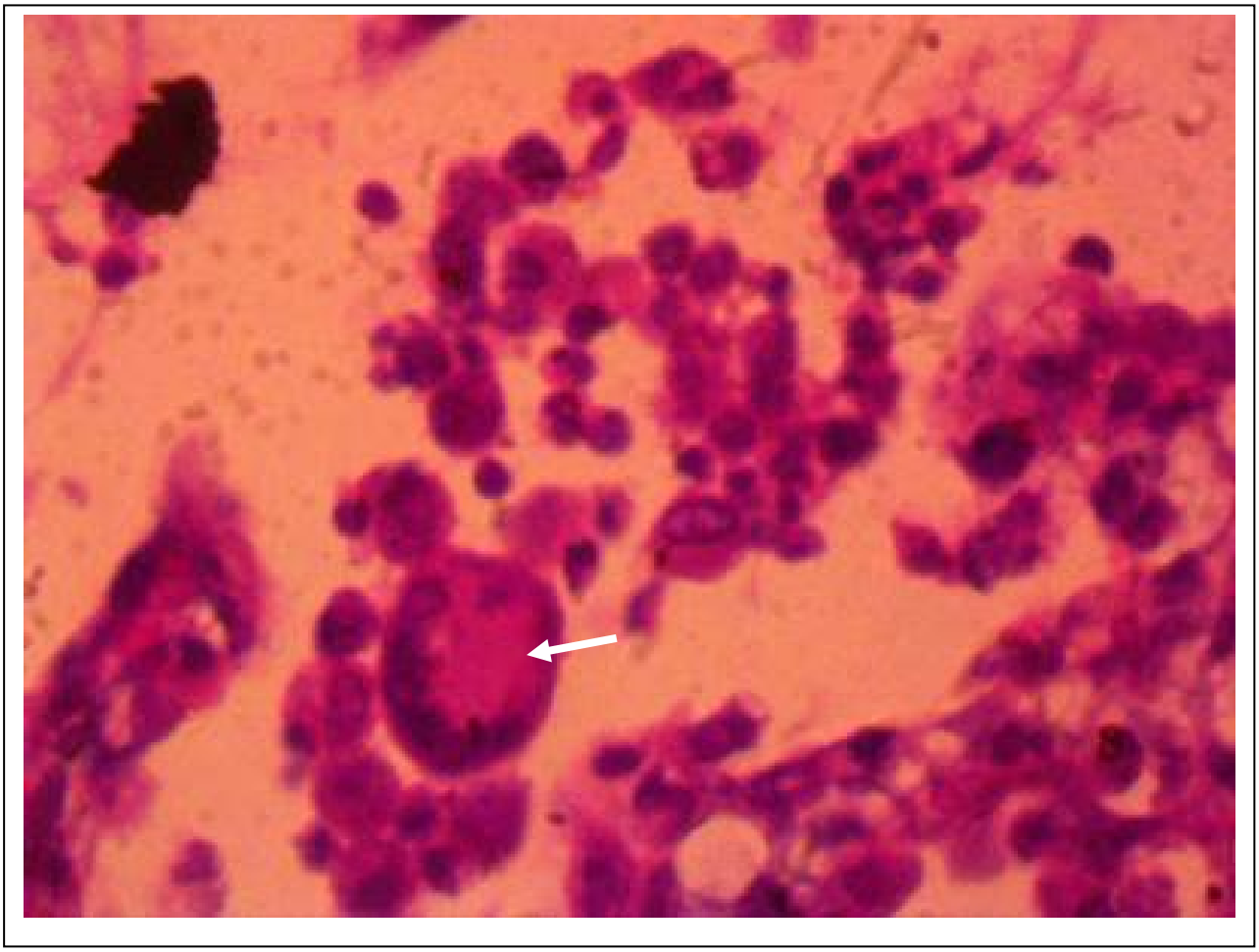


Plate 3.5. Photomicrograph of a section of the lung with broncho-interstitial pneumonia showing a giant cell (arrow) and other macrophages in air space. H and E. x1000

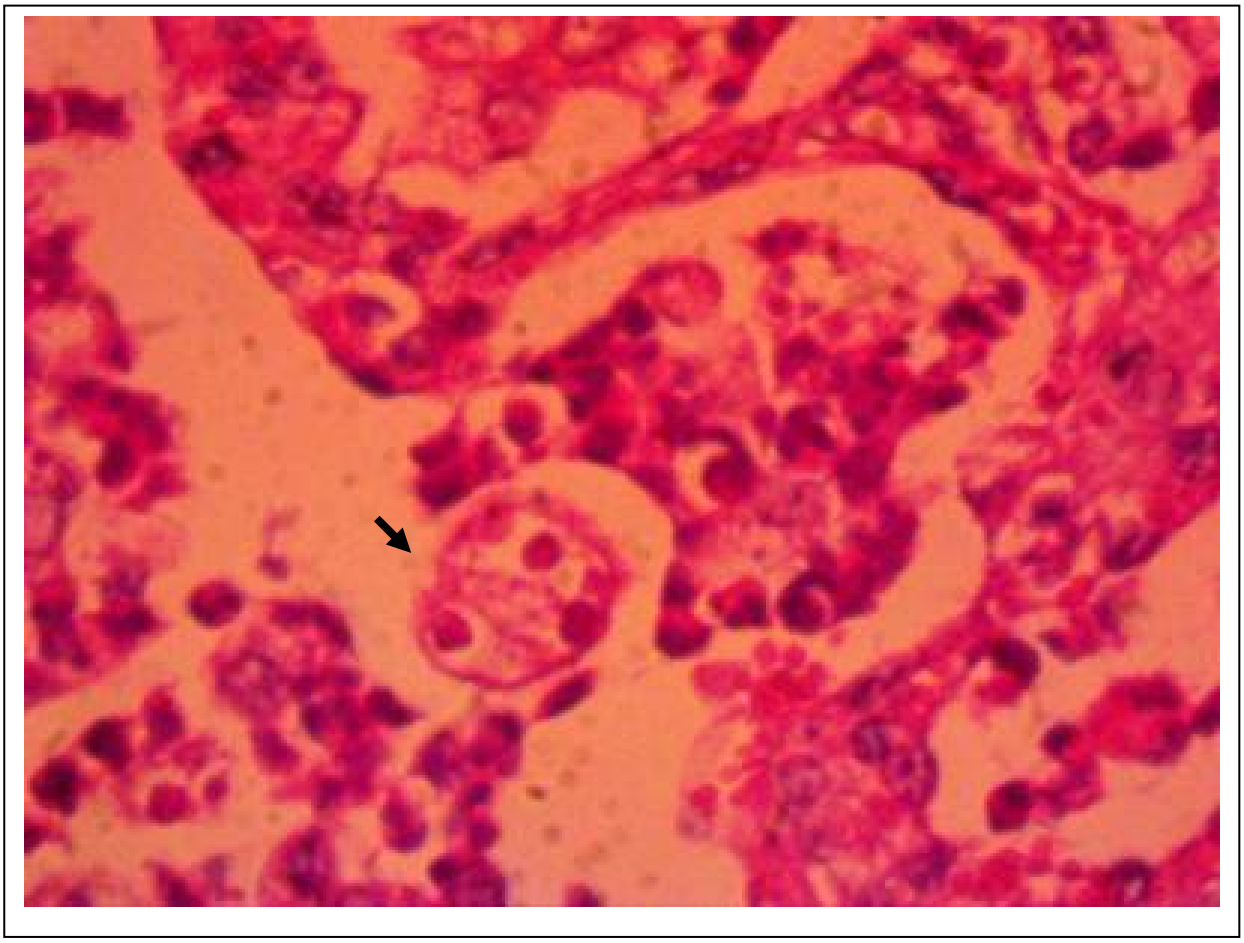


Plate 3.6: Photomicrograph showing a section of parasitc larva (arrow) and eosinophils in alveolar space from lung of a pneumonic goat. H and E x400.

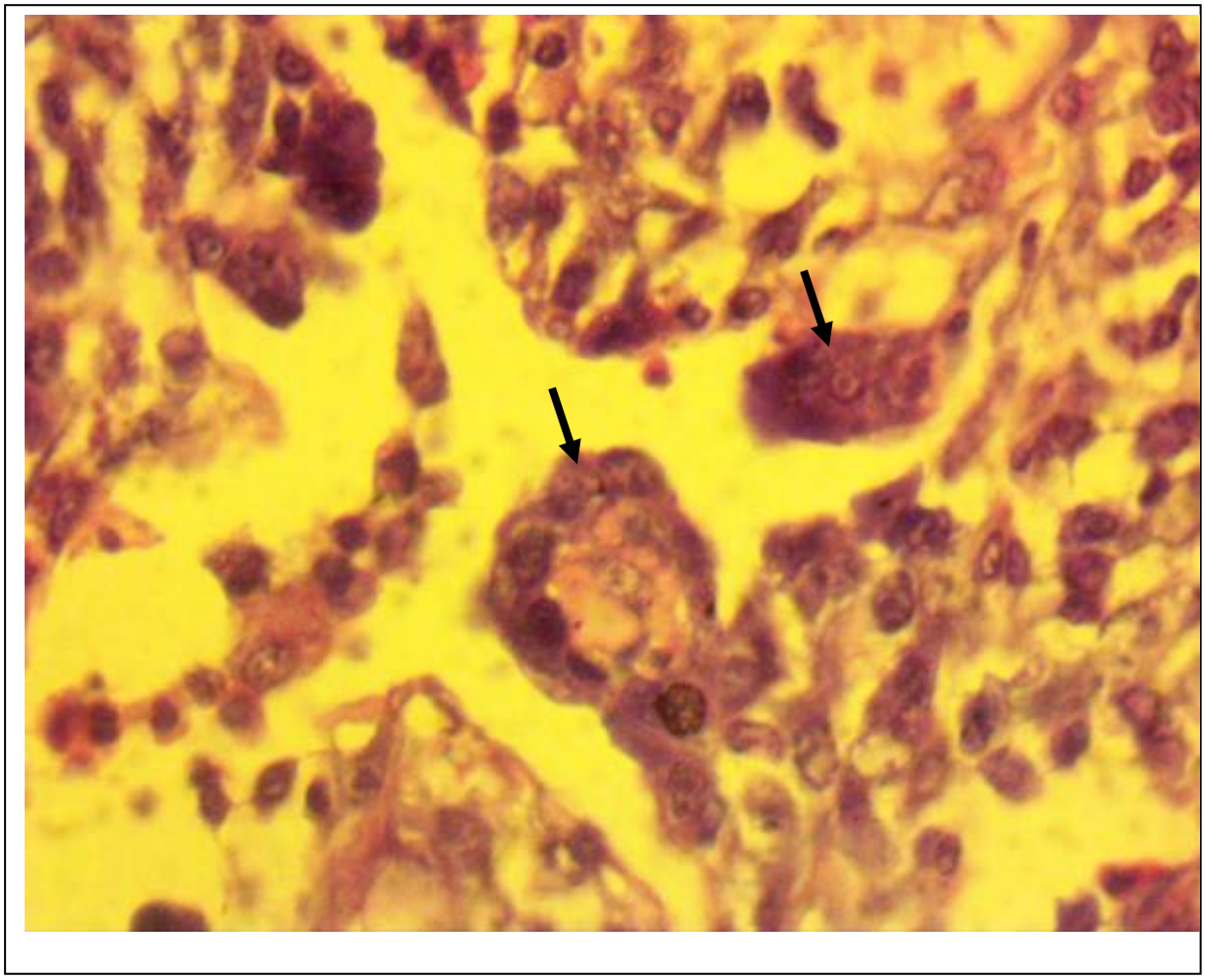
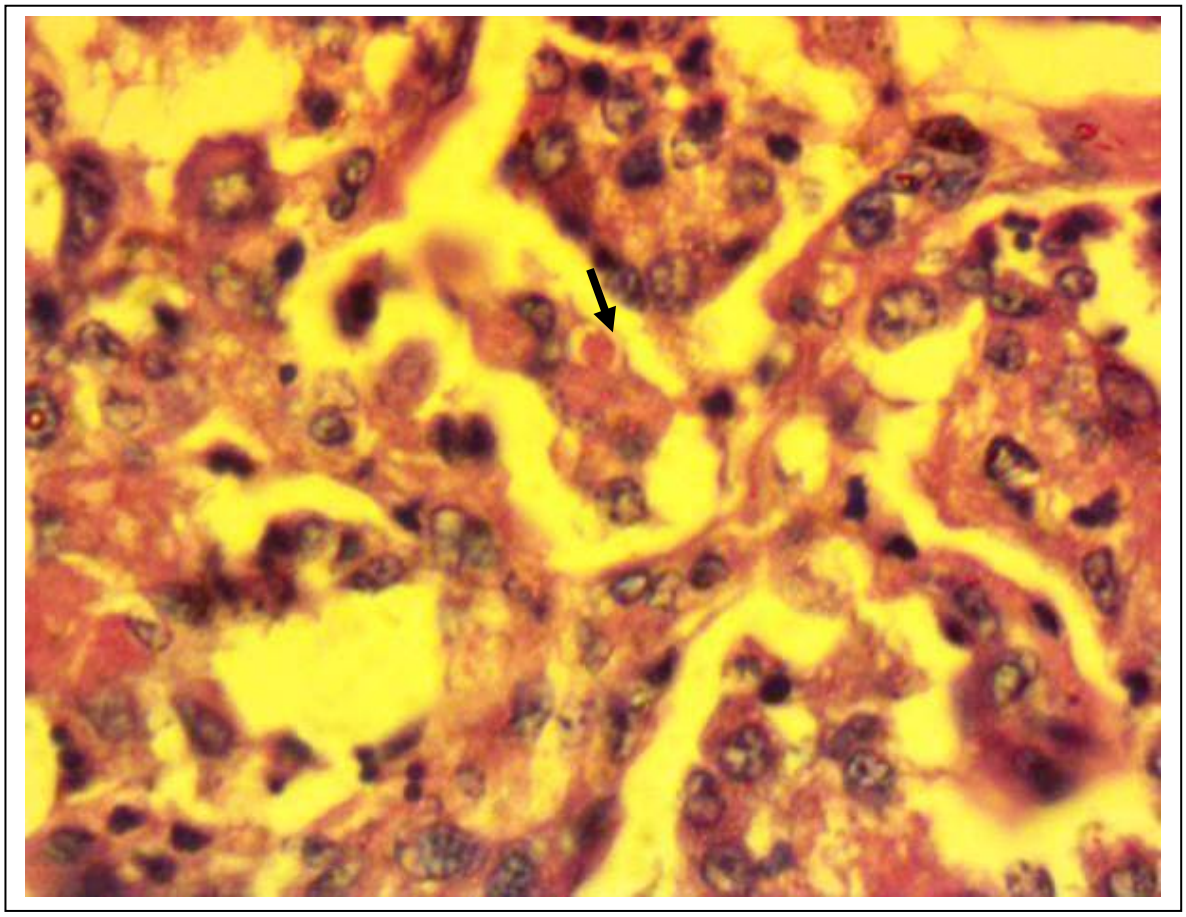


Figure 3.7: Photomicrograph of a section of a pneumonic caprine lung showing syncytial giant cells (arrows). .H and E x1000.



Plates 3.8: Photomicrograph shows syncytial giant cells in the alveoli with intracytoplasmic oesinophilic inclusion body (arrow).
H&E x1000

3.4 DISCUSSION

This study elucidated the risk and predisposing factors in pneumonia complex in Nigerian goats. This is part of a progressive study following our preliminary observations (Emikpe et al. 2013). Natural pneumonia in goats needs to diagnosed and characterized from field investigations to ascertain the true nature and assessment of the economic loss it causes in goats (Elsheikh and Hassan, 2012). This study gives a detailed pathological scoring of the lung lesions in natural cases of caprine pneumonia in Nigeria.

Consolidation is the hallmark of pneumonia which is best detected in the lungsby its firm texture on palpation. These lesions may be dark red, gray-pink, or gray depending on the age and nature of the process, but a color change without altered texture may simply represent patchy areas of congestion or hemorrhage (Akpavie, *pers comm*). In this study, the high consolidation observed on the lobes of the right lung suggests inhalation route involvement due to the abrupt and early branching of the bronchus. Moreover, the caudal lobe involvement often suggests hematogenous route and atypical pneumonia (Lopez, 2012).

The influence of age in this study was remarkable in the susceptibility to pneumonia as observed in a previous study where there was increased susceptibility of the young adult animals (Emikpe et al 2013) and has been attributed to increased immuno-susceptibility (Pope et al. 2013).

Our finding of 60.5% pulmonary consolidation is very much consistent with the findings of Bakke (1982) in a study from 209 sheep lungs in Norway. However, the mean consolidation was slightly higher than 6.83% reported by Emikpe and Akpavie (2011) in experimentally induced caprine pneumonia but within the range of 3-80% observed by Jericho and Langford (1982) in calves, these variations could be adduced to environmental influences.

The higher consolidation score observed in goats within two years of age corroborates the susceptibility of young adults to pneumonia which could be due to deficient pulmonary clearance. The RS breed had more consolidation score than the WAD breed and lesser in the mixed breed. This could be attributable to transport or other managemental stress associated with the transport of RS between far north to other regions of Nigeria. The impact of

transportation stress needs to monitored and evaluated for appropriate control of pneumonia in goats.

Animals with poor body conditions and clinically debilitated states had higher consolidation scores similar to the findings of Emikpe and Akpavie (2011) in WAD goats, in finished pigs (Ostanello *et al*.., 20007), and in cattle (Jarikre *et al*., 2013). It appears that those with fair body scores were in advanced stages of pulmonary consolidation. The high consolidation scores in male animals may be due to the managemental factors allowing free ranging of the animal and increased activity of the male goat. The pattern of bronchopneumonia further corroborates findings of Emikpe et al (2013) from archival lung samples of sheep and goats considering influence of season and stress. More so, this study underscored the importance of Mannheimiosis in this environment.

Nonetheless, better conditions of management and appropriate balance of other stressors may reductce respiratory problems in the goats because there may not be enabling conditions for infectious agents or commensals to disrupt pulmonary clearance mechanisms. More so, active surveillance for infectious pneumonic organisms will effectively reduce and control spread of these organisms in this specie which is experiencing ravaging mortality across Africa. Worthy of mention are mortalities from Pest des petits ruminants *PPR* virus (Kumar et al. 2014), *Mannheimiosis* (Emikpe and Akpavie, 2011; Odugbo et al. 2004), and *Mycoplasmosis* (Thiaucourt et al. 2011; Kılıc, 2013) which would also be elucidated. In this study, fibrinous bronchopneumonia was the commonest pattern of pneumonia. Sex and body score were significant associated factors in caprine pneumonia. Transport stress may be responsible for the high consolidation in RS.

The consolidation associated with the viral infections has been established to be mild due to the fact that viruses do produce patchy consolidation affecting a small part of the lungs and the pattern of the pneumonia is usually interstitial (Munir et al. 2013). A peak consolidation was associated with the secondary bacterial complication of *Mannheimia haemolytica*, and other pathogenic bacteria (Emikpe and Akpavie, 2011; Truong et al. 2014, Shaikh and Crowe, 2013). The degree of consolidation has been associated with bacteria pathogenicity and

immunosuppression of host from virus reduced efficiency of alveolar macrophages in pulmonary clearance (Shaikh and Crowe, 2013).

Obviously with these multifactorial influences on caprine pneumonia complex, multivalent vaccines had been proposed as a good solution to major upper and lower respiratory disease constraints in small ruminant production systems in lesser developed countries throughout the world (Herbert *et al.*,. 2014). And also the prospect of immunomodulation and the advantages of mucosal immunity raise hope in the face of these daunting respiratory challenges in small ruminant populations.

In conclusion, this study elucidated the consolidation pattern of natural pneumonia in goats in the tropics and influence of different factors. It will also help to piece together the pathogenesis and prognosis, whether or not lesions have common features irrespective of the inciting causal factors. This information will also help increase the body of knowledge on risk factors and possible causal agents involved in caprine pneumonia complex especially in Nigeria.

CHAPTER FOUR

4.0 HAEMOGRAM AND PULMONARY FLUID CHANGES IN PNEUMONIC AND NORMAL GOATS

4.1 INTRODUCTION

The livestock population in Nigeria comprise over 43% ruminants with sheep and goat contributing more than 13% through agricultural gross domestic product to the GDP of the economy(Lawal-Adebowale, 2012). However, these animals suffer from infectious diseases where pneumonia is of paramount importance (Emikpe et al. 2013). For this problem to be resolved appropriate pointers and markers of infection and pneumonia need to be identified for prompt and early diagnosis. These markers may include haematological indices and evaluation of fluid from the lower airway and gaseous exchange areas.

Haemtological and other clinicopathological examination of animals are very good indicator of illhealth. Bronchoalveolar lavage (BAL) samples the airsapces to evaluate the cellular dynamics and nature of changes in respiratory diseases (Reynolds 2000, Ezeasor et al. 2013 and Lee et al. 2015). The nature and dynamics of cells in the lower respiratory tract explains the clinical diagnosis in the patient (Rottoli and Bargagli, 2004, Lee et al. 2015). This diagnostic tool has not been well utilized in daprine pneumonia.

The cellular dynamics in bronchoalveolar lavage fluid (BALf) needs to determined and correlated with the different histologic types of pneumonia. The types include suppurative bronchopneumonia, interstitial pneumonia, and broncho-interstitial pneumonia (Emikpe et al. 2013) The cellular changes was compared in different vaccination routes against PPR in goat (Ezeasor et al. 2013; Tenuche et al. 2013, Ezeasor et al. 2015), however this needs to be done in a large goat population and natural/field conditions to establish a baseline data for BALf changes in the different pneumonia. Just as it was tried in bovine (Mohammadi et al. 2007; Blodörn et al. 2015), equine (McKane, 2010),and humans (Rottoli and Bargagli, 2004). The

findings will contribute significantly to clinical parameters of auscultation and respiratory rate evaluation (McKane, 2010), for prompt and accurate diagnosis of pneumonia in goats.

The challenges of biopsy from the lung for laboratory investigation is surmounted by BAL technique. Analysis of BALf may also indicate presence of acute phase proteins, immunoglobulins, surfactants (Blodörn et al. 2015), which may serve as good compliments to haemogram and biochemical changes in the goats having pneumonia.

This study was therefore set to investigate the haemogram, BALf and cellular dynamics as markers of pneumonia in Nigerian goats.

4.2 MATERIALS AND METHODS

4.2.1 Study Animals

Three hundred goats of different pneumonic status and normal were randomly sampled cross sectionally. The source of animals and pattern of pneumonia have been described. Blood and bronchoalveolar lavage fluid were collected from the 300 goats. Pneumonia was established in the goats using standard techniques.

4.2.2 Blood work

Two mls of blood was collected from the jugular vein of each goat into Heparinised universal bottles for haematological analysis in the Veterinary Clinical Pathology Laboratory, University of Ibadan. The different haematological parameters were determined following standard techniques.

Microhaematocrit method was used to determined the haematocrit value (%), while and cyanmethemoglobin method was used for haemoglobin concentration (g/dl) (Schalm *et al*. 1975). Red cell, leucocyte and thrombocyte counts were evaluated using haemocytometric methods. The leucocyte differentials were done on Romanowsky-stained smear under the microscope (Olympus C2X) by counting 400 cells from 10 fields per high power field. The other red cell indices of Mean corpuscular volume and Mean corpuscular haemoglobin concentration were calculated while plasma protein level (g/gl) was done by the refractometer.

4.2.2.1 Packed cell volume (PCV) determination: 3/4 of microhaematocrit (heparinised) capillary tube was filled with blood from appropriately labled whole blood in a heparine tube by capillary action one end of the tube was sealed with plastercine. The tube was spined at 1,200rpm in microhaematocrit centrifigue for 5 minutes (Jain, 1986). The PCV was read using microhaematocrit reader.

4.2.2.2 Haemoglobin concentration determination: The concentration of haemoglobin (Hb) was determined by cyanohaemoglobin method (Jain, 1986). Briefly 20µl blood was mixed with 4ml of modified drabkin's solution (potassium ferricyanide, 200mg, potassium cyanide, 50mg, potassium dihydrogen phosphate 140mg, diluted to 1L with water and at pH 7.0. This mixture was allowed to stand for 3 minutes before the haemoglobin concentration was read with Autospectrophotometer (spectrum lab 23A) at a wavelength of 540nm. The actual value of Hb concentration was extrapolated from a standard haemogblobin curve. For the solution to work perfectly well the drabkin's solution was kept in a dark cupboard after preparation before using.

4.2.2.3 Determination of total red blood count (RBC)**:** The red cell and leucocyte counts were done manually. Differential WBC counts were done manually (Jain, 2010). By the use of Neubauer haemocytometer and the number of cells were expressed as 10^{12} Rbc per litre of blood and 10^{9} white blood cells per litre of blood respectively.

Procedure: 1ml of RBC diluting fluid (Daciers fluid) was taken into test tube. 0.05µl of whole blood was added into the Daciers fluid (99ml of 3 percent aqueous solution of sodium citrate, 1ml of 40 percent formaldehyde) to keep and preserve the shape of the cells. This was gently mixed and allowed to stand and counted at X40 magnification under the microscope.

4.2.2.4 Determination of white blood cell counts (WBC): Procedure: 0.5ml of WBC diluting fluid (3 percent aqueous solution of acetic acid to which 1 percent gentian violet was added) was taken into a test tube. 25µl of whole blood was added into the fluid and gently mixed thoroughly and allowed to stand. It was counted at x40 magnification.

4.2.2.5 Differential Leucocyte Counts DLC: DLC was manually counted as described by Jain (2010). Freshly prepared Giemsa stain solution was used. Preparation: 66ml of glycerol was

measured into conical flask, 1g of giemsa powder added into it. The mixture was allowed to boil in the water bath for 90 -120 minutes and mixed at intervals for thorough boiling then 10% xylene was added. After it had attained the desired time allotted, it was removed and allowed to cool before methanol (Analar grade) 66ml was added into it. It was then mixed thoroughly, sieved and kept in a dark cupboard for a week before using. The stain was used at 10% with distilled water.

Thin blood smear was done using clean glass slide. Capillary tube was filled with whole blood. This was done by capillary action. A 0.04ml blood was dropped on the clean slide, and a spreader was used to evenly spread the blood at an angle 45^0 to make a very good thin blood smear. This was allowed to dry under room temperature, then fixed with methanol (Analar grade) and allowed to dry; it was then immersed in Giemsa stain solution for 45min. After staining, it was allowed to dry, then viewed under the microscope at x100 magnification using Leica Galen III Microscope to identify and count blood cells present. Thin blood smear stained with Giemsa as described by Kelly (1984) was used; 400 white blood cells were enumerated and differentiated.

4.2.3 BAL collection and analysis

The BAL was collected as described by Khin (2009) and Ezeasor *et al* (2013). Briefly, the entire pluck was removed after slaughter of each goat. The wind pipe (trachea) was transected at the level of its bifurcation before 40 ml of warm sterile phosphate buffered saline (PBS) of pH 6.8 was intotroduced into the lower respiratory tract and alveolar spaces. The lung was gently rocked for washing of the air spaces before pouring back fluid into a graduated collecting beaker. The BALf was noted for presence of foamy layer, colour and consistency. It was spinned in a macrocentrifuge at 3000 rpm for 15 minutes to for separation of supernatant and sediment cells.

The supernatant or fluid was aliquoted into cryotubes and stored at -20^{o}C while the cell sediment was spread on clean glass slides, fixed with methanol and stained with Romanousky-Giemsa for cytological examination of cellular details and differential.

A few of the spread cells were stained unfixed in 0.5% toluidine blue in 0.5 molar hydrochloric acid for 30 minutes (Cole *et al*., 1980, pp 223). The cellular details and differentials were taken and counted as described by Dawson et al (2005), where the total nucleated cells were counted in ten fields with a 40 fold magnification of the objective Lens (maximum of 400 cells) from the slides under identity to the reader. The mean cell number per field was calculated.

4.2.4 Statistical analysis

Data was summarised generically (percentages), presented descriptively as Mean±Standard error of mean (M±SEM), then compared using parametric statistics (T-test and ANOVA) at α=0.05.

4.3 RESULTS

4.3.1 Distribution and pattern of pneumonia in the goats

In the goats, there were those with pulmonary consolidation (224); out of which 148 were bronchopneumonia, 49 broncho-interstitial pneumonia, 37 interstitial pneumonia, 3 granulomatous pneumonia and 2 verminous pneumonia. The remaining 61 lungs from the goats had normal colour, consistency and shape.

4.3.2 Haemogram

The changes in the maemogram of the goats pneumonic and norma in table 4.1. There was leucocytosis, thrombocytosis, lymphocytosis, neutrophilia and hypoproteinaemia in the goats ith pneumonia (α=0.05). The haemogram values in the goats with different morphological type of pneumonia (table 4.2) also ahow the increases and or decreases are significant.

Moderate anaemia (decrease haemoglobin concentration) was observed in the from goats with broncho-interstitial, interstitial and granulomatous pneumonia. There was leucocytosis in goats having bronchopneumonia and leucopaenia was observed in goats having interstitial andor verminous pneumonia; thrombocytosis in goats having granulomatous pneumonia; lymphocytosis and neutrophilia in goats having bronchopneumonia; and decreased Neutrophi-Lymphocyte (NL) ratio in goats with granulomatous pneumonia (α=0.05).

4.3.3 BALf evaluation and cellular changes

The bronchoalveolar lavage fluid had a frothy layer on surface, and colour variation from clear and turgid (66%), mucoid (21%), slightly cloudy (8%) and haemorhagic (5%). The recovered BALf volume was between 15 ml to 25.3 ml, with an average volume of 22.2 ± 3.5 ml in the goats. There was an average of 56% recovery rate from every 40 ml of saline washing of the lungs.

On cytological examination, the Giemsa stained smears had a pinkish to bluish background indicating presence of fibrinous exudate and mucus. The BALf smear was of high cellularity showing resident and inflammatory cells. The cellular components of the BALf were alveolar macrophages (plate 4.1) neutrophils (plate 4.2), lymphocytes, plasma cells, oesinophils, mast cells and giant cells (plate 4.4). The cellular differentials from each BALf are shown in table 4.1 and 4.2. There was increase in neutrophils, alveolar macrophages, Macrophage-Neutrophil ratio (M:N), lymphocytes, mast cells (plate 4.5) and eosinophil counts in BALf from pneumonic goats (α=0.05). There were bacterial rods, a few phagocytosed bacteria (plate 4.3), fungi, parasitic larvae, red blood cells, erythrophagocytosis and Curschmann's spirals (mucus) in the different BALf smears.

BAlf smear from some of the goats with severe pulmonary consolidation showed abundant neutrophils (76), a few degenerate (plate 4.3), and coccobaccili organisms (21). The M:N ratio was directly proportional to the different types of pneumonia. The percentage leucocyte counts in the BALf are shown in table 4.3. 80 BALf had less than 10% neutrophil counts, 152 BALf had more than 50% neutrophil counts. 127 BALf had lymphocyte counts less than 15% and 173 BALf had more than 15% lymphocyte counts. The eosinophil counts were between 1-2%,

BALf from bronchopneumonic goats was characterised by remarkable cellularity, pink staining background, high neutrophil, degenerate neutrophils and bacterial rods.

BALf from broncho-interstitial pneumonic goats was characterized by increased macrophage, M:N, lymphocyte, neutrophil and a few giant cells.

BALf from interstitial pneumonic goats was characterized by presence of macrophages, lymphocytes and neutrophils .

BALf from goats with granulomatous pneumonia were characterized with high macrophages and M:N while those from goats with verminous pneumonia had remarkable increase in eosinophil counts.

Table 4.1: Haemogram and BALf dynamics in goats with normal and consolidated lungs

	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 α=0.05

Table 4.2: Haemogram and BALf dynamics in normal and pneumonic goats in Nigeria

	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 α=0.05

Table 4.3: Infflamatory cell proportions in BALf from normal and pneumonic goats

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%

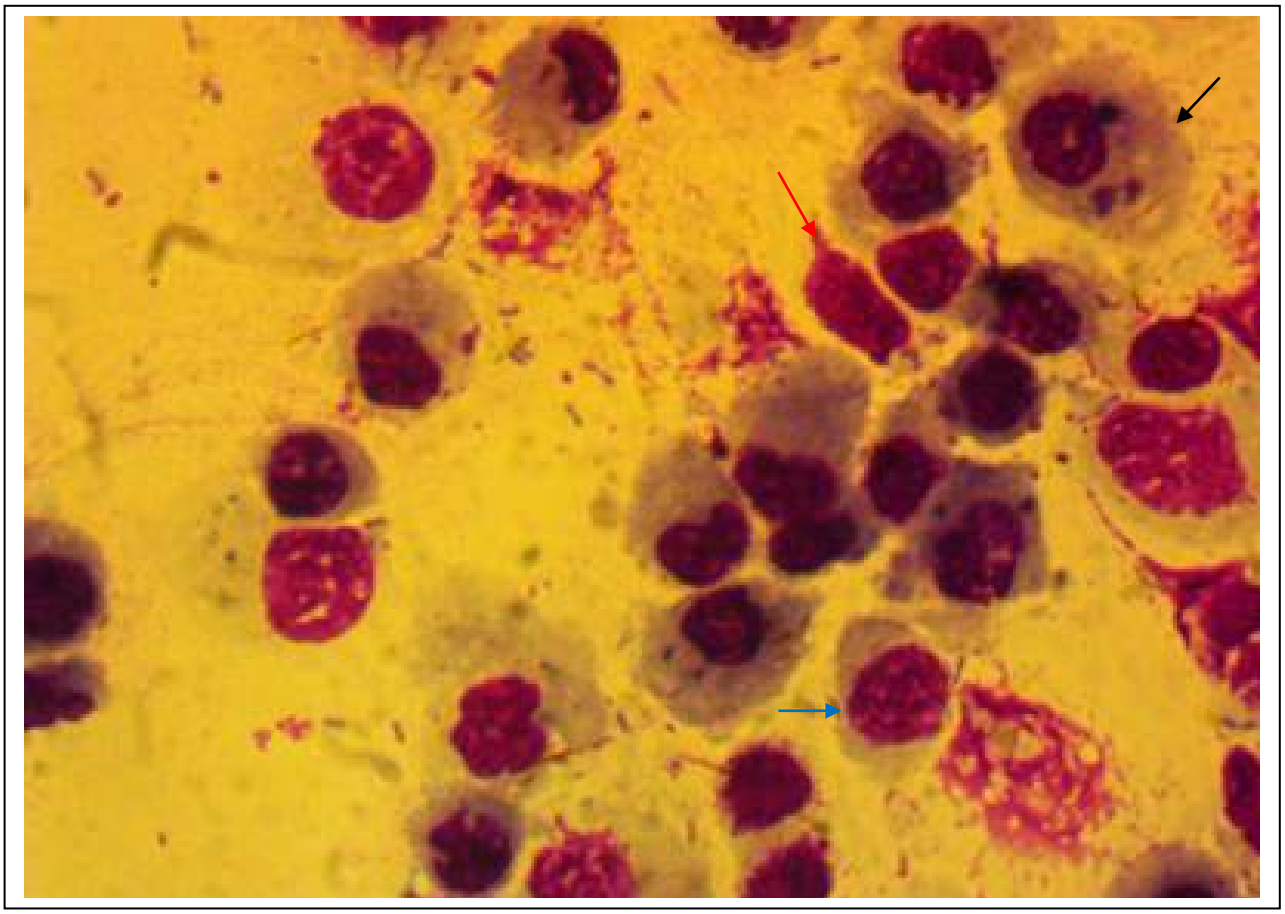


Plate 4.1. Photomicrograph from cytology of bronchoalveolar lavage fluid showing alveolar macrophages (black arrows), lymphocytes (blue arrow) and pneumocytes (red arrow). Giemsa stain (x1000)

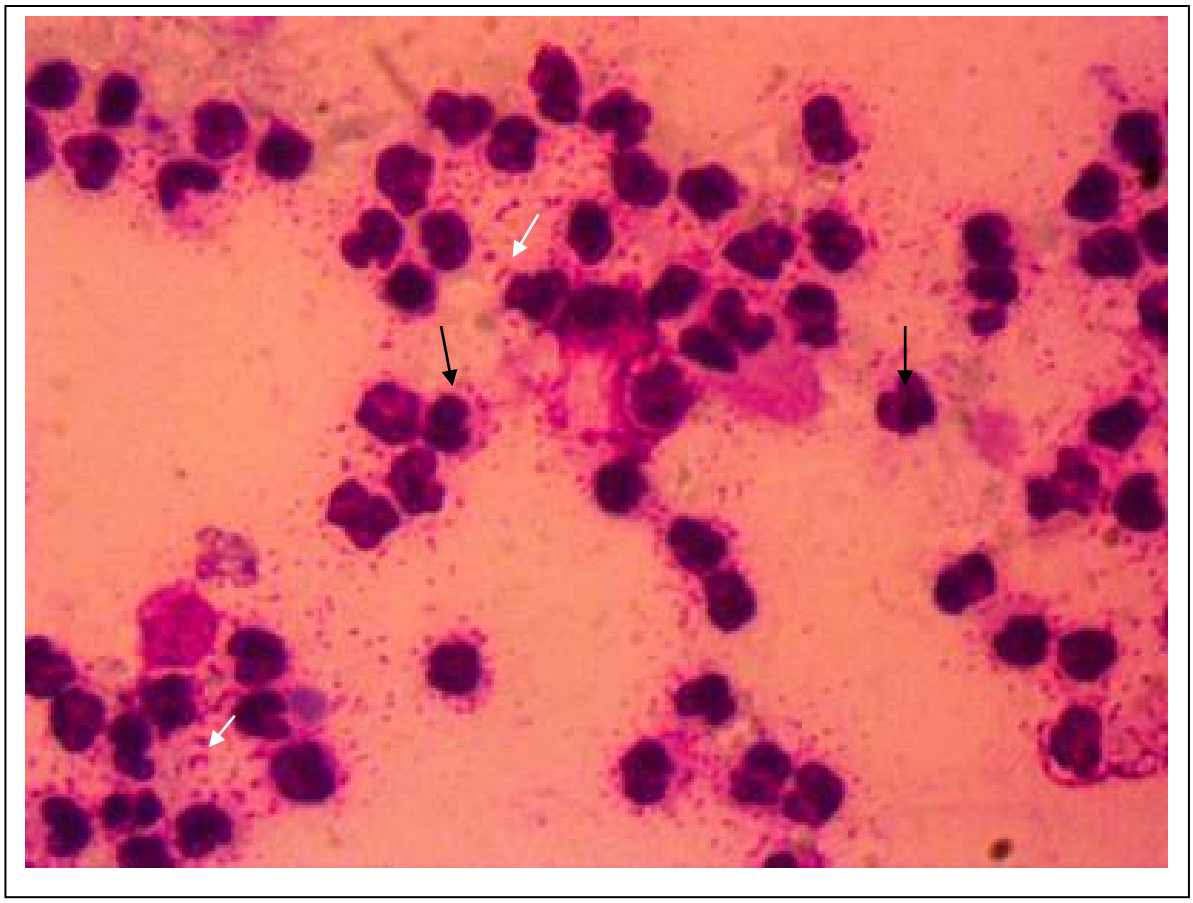


Plate 4.2. Photomicrograph from cytology of bronchoalveolar lavage fluid showing predominantly neutrophils (black arrows) and numerous bacteria rods (white arrows). Giemsa stain (x400).

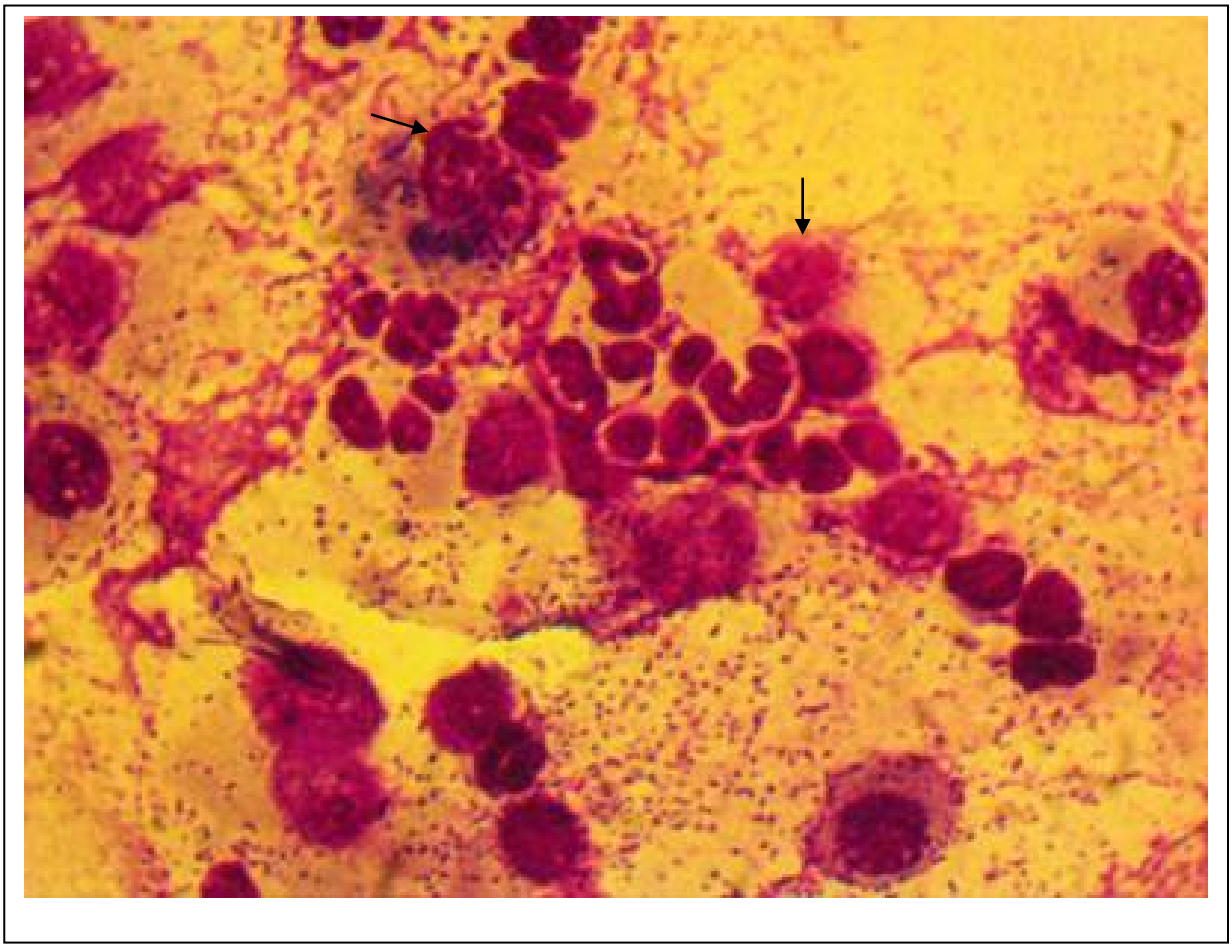


Plate 4.3. Photomicrograph from cytology of bronchoalveolar lavage fluid showing degenerate neutrophils (arrow) and numerous bacteria rods.
Giemsa stain, x1000.

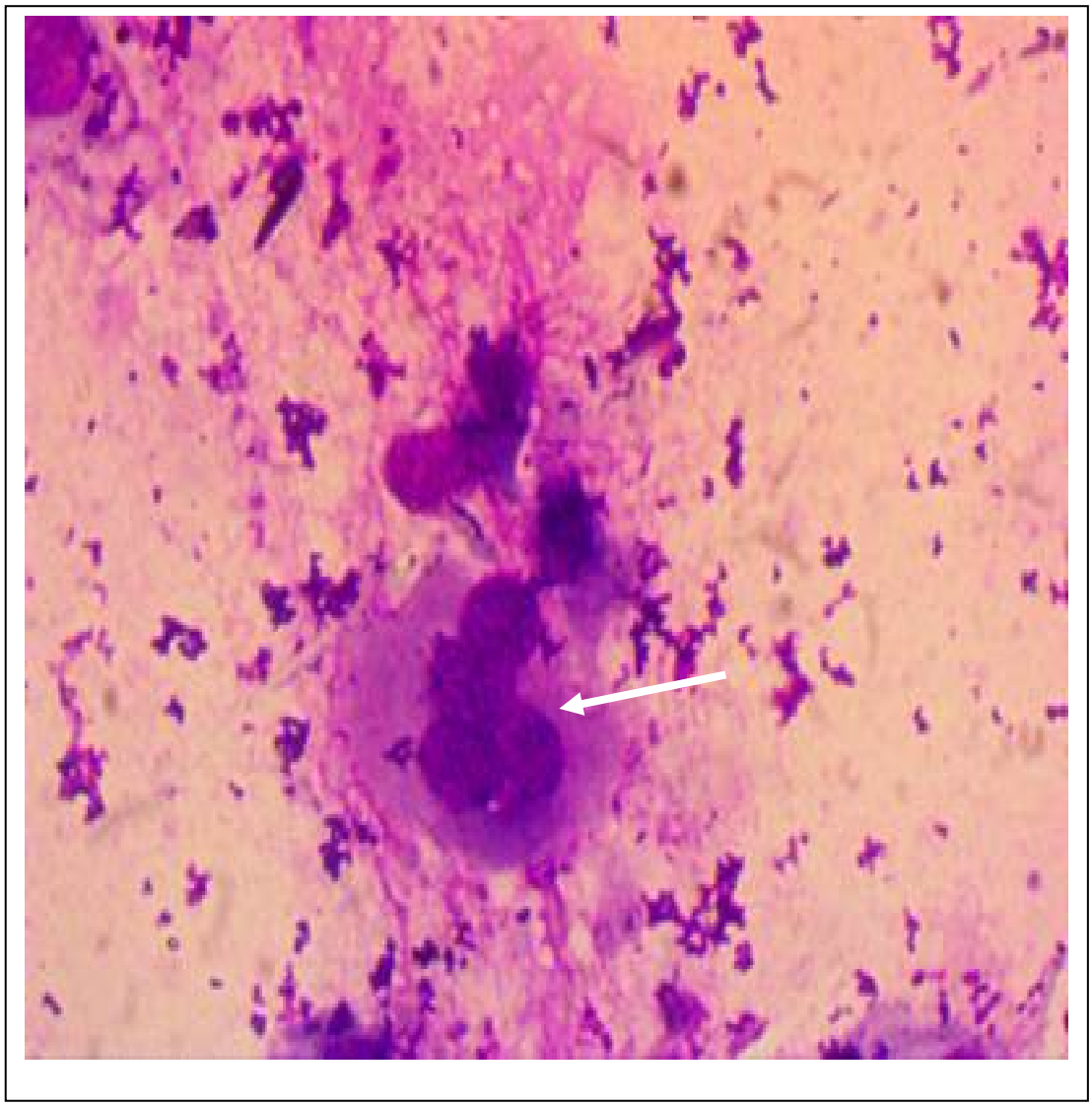


Plate 4.4. Photomicrograph from cytology of bronchoalveolar lavage fluid showing a giant cell (arrow). Giemsa stain, x1000.

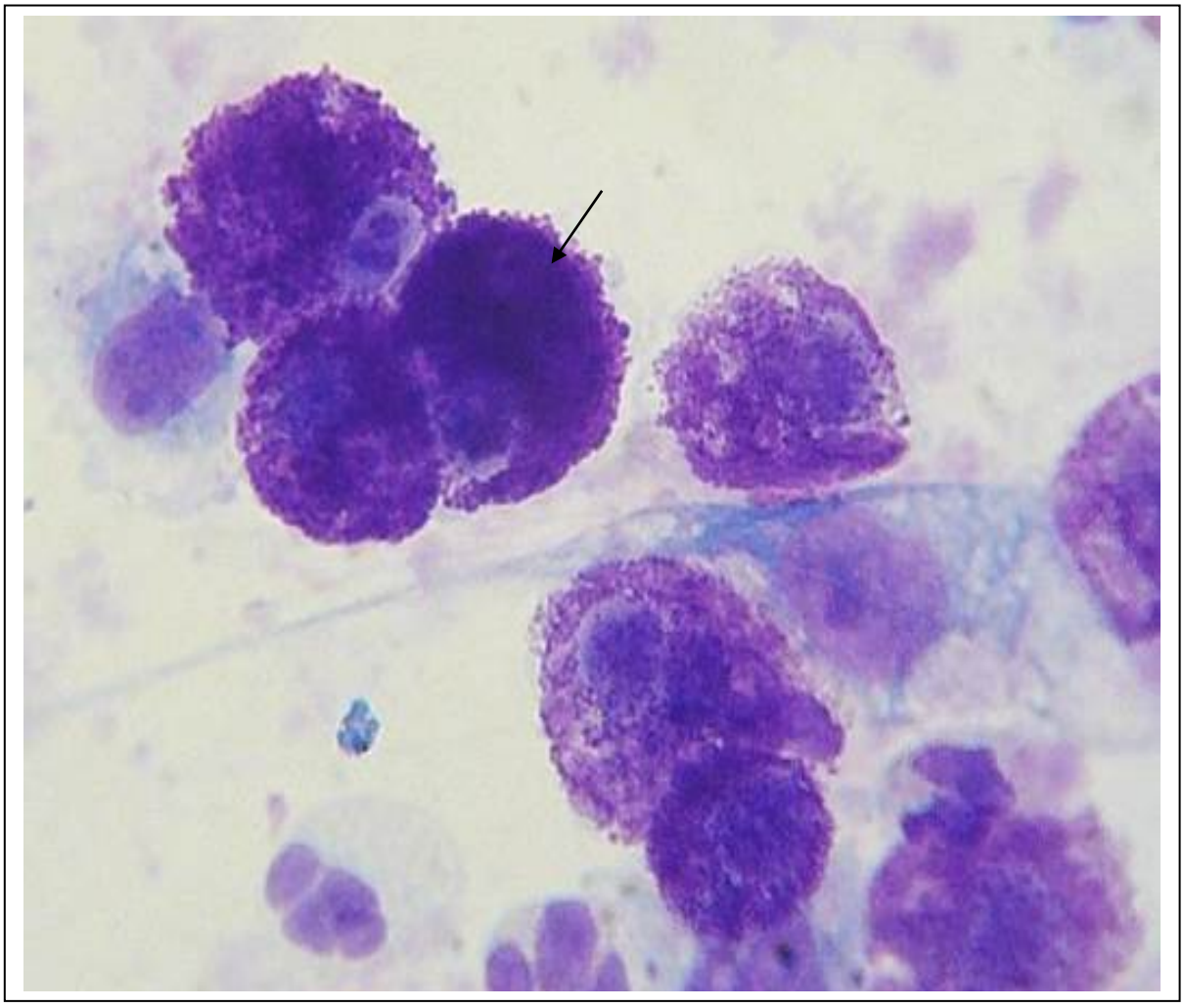


Plate 4.5. Photomicrograph from cytology of bronchoalveolar lavage fluid showing a mast cells (arrow), epithelial cells and few other granulocytes. Toluidine blue stain, x1000.

4.4 DISCUSSION

The work described the haemogram and bronchoalveolar fluid cellular dynamics in normal and pneumonic goats. The findings will form basis and prediction of diagnosing pneumonia in goats early enough to reduce the mortality of goats and economic loss to farmers. The predictos and markers established include neutrophil counts, MN ratio in bronchoalveolar lavage fluid, white blood cell counts and differentials.

The goats were graded for different typres of pneumonia which was also correlated with the cellular changes in the bronchoalveolar fluid and blood picture. The severity of the tissue damage and pulmonary lesion is also reflected in the leukocytosis and neutrophilia in the haemogram and the increased neutrophil counts in the bronchoalveolar lavage. The haemogram changes were similar findings of Daramola et al (2005) and Ezeasor et al (2015) who reported baseline haematological in normal and pneumonic nigerian goats. The leukocytosis in the pneumonic goats correlated positively with the level of tissue injury and or inflammation and even stress.

Lee et al. (2015) emphasized the clinical application of bronchoalveolar lavage fluid analysis in diagnosis of pulmonary diasese. This study has equally underscored this importance and highlighted the usefulness of leucocyte differential as a predictor of cellular/tissue damage in the lungs. There was a strong correlation of MN ratio and pattern of pneumonia in the goats. The high MN ratio was aslo reported in cattle and goats (Tenuche et al. 2013; Ezeasor et al. 2015).

The increases in the BALf cells was associated with congestion, oedema and inflammation in the lungs (McKane, 2010). It was also associated with presence of mucus in the lavage fluid. This can be as a result of mucous cell response in the bronchial tree and catarrhal inflammation (Lee et al. 2015). The cell counts in lavage fluid is important for concise information of the pulmonary homeostasis.This study further established the baseline data for bronchoalveolar lavage in goats, showing levels below 5% of neutrophils, less than 2% of mast cells and less than 0.5% eosinophils as normal for BALf in goats. The ranges for macrophages are high reflecting their importance in pulmonary clearance. More over, high lymphocytes and macrophage counts were reported in cattle (Taniuchi et al. 2009). Counts above 10% for neutrophil in the lavage fluid is very much indicative of pneumonia in the goat.

Some factors need to be considered for accurate interpretation and cytological diagnosis of bronchoalveolar findings. Expertise and amount of saline infused into the lung may influence the accuracy of cell counts, while presence of catarrh exudate can also trap cells in the lavage fluid.

Mast cells are located throughout the respiratory tract, both in the airways and in the alveoli. Within the distal airways they are mostly located beneath the respiratory epithelium superficial to the basement membrane (Lamb and Lumsden, 1982), although occasionally they are observed between epithelial cells and may abut directly on to the bronchial Lumen (Jeffery et al. 1984). Mast cells are also found in association with alveolar capillaries and form a component of the alveolar walls, where they constitute 1.6-2.1% of the surface area (Fox et al. 1981). Not surprisingly therefore mast cells and basophil like cells have been identified in bronchoalveolar lavage fluid" and presumably originate from both the distal conducting airways and the alveoli (Agius et al. 1985). Mast cells contribute to the lesion and inflammation of the lung through it chemical mediators and chemotactic substances released from the granular content or synthesized denovo.

The importance of BALf in clinical diagnosis of pneumonia was reviewed by Espinasse (1991) and McKane (2010). The indications include signs of respiratory distress, dyspnea, harsh lung sounds and mucopurulent nasal discharges (Dawson et al. 2005). The changes in colour, turbidity and content are important (Alen, 1992; Blodörn et al. 2015). It adds to the diagnostic value of clinical eaxamination and possibly radiography in the live animal or patient, as it can also serve for purposes of cultural isolation of infectious causes.

In conclusion, BAL should be considered in the diagnositic workup of pulmonary diseases in livestock and goats in particular for early detection and control of caprine pneumonia. It will of diagnostic value especially in the tropics. Macrophage-neutrophil ratio and leukocytosis in addition to clinical examination will predict presence of pneumonia in goats.

CHAPTER FIVE

5.0 EVALUATION OF OXIDATIVE STRESS IN CAPRINE BRONCHOALVEOLAR LAVAGE FLUID OF PNEUMONIC AND NORMAL LUNGS

5.1 INTRODUCTION

Respiratory diseases in sheep and goat have been established from exposure to stressors which may include long period of starvation and transportation, housing and weather followed by invasion of bacterial and viral infectious agents (Emikpe et al. 2013; Rahal et al. 2014). Exposure to these stressful conditions cause excessive release of reactive oxygen species and radicals with potential membrane damaging effects (Samarghandian et al. 2013).

Oxidative stress involves oxidative modification by reactive oxygen species of biomolecules (proteins, nucleic acids, and lipids). It induces a variety of organ dysfunction as a result of shift in either free radicals and homeostatic enzymes in cells and tissues (Naito et al. 2010, Kalyanaraman, 2013).The Pro-oxidant promotes oxidation while antioxidants checkmates the activities of these pro-oxidants. Oxidative stress may also result from defects in expression of the genes controlling antioxidant enzymes (Nazief et al. 2009; Kataria et al. 2010). The respiratory pathogens survive insults from reactive species generated by the host through detoxification mechanisms using denovo or dietary antioxidant processes. The processes involved in enzymatic detoxification mechanisms and other adaptive mechanism are controlled by gene expression (Kalyanaraman, 2013; Omobowale et al. 2015).

Research in the area of oxidative stress in pneumonic pathology still requires attention especially in small ruminants. Hitherto, evaluations of oxidative stress in animals require estimation of certain blood biomarkers that reflect the oxidative profile of affected cases (Pilania et al. 2013), as was done in PPR virus infected sheep (Kataria et al. 2012). The assay of these markers in BAL may be a more sensitive indicator of respiratory diseases than blood. Hence, this study evaluates for the first time to the best of our knowledge the oxidative stress parameters in bronchoalveolar lavage fluid of healthy and pneumonic Nigerian goats.

5.2 MATERIALS AND METHODS

5.2.1 Study Animals

Two hundred goats of different pneumonic status and normal were randomly sampled cross sectionally. The source of animals and pattern of pneumonia have been described.

5.2.2 Bronchoalveolar lavage fluid

The collection of BALf was also described previously in the last chaper. The stored supernatant at -20^{o}C was retrieved for analysis of oxidative stress. One hundred and ninety two (192) BALf samples were made alvaible for kinentic and nonkinetic oxidative reactions for evaluation of antioxidants, Myeloperoxidase, lipid peroxidation and total protein. The procedure was carried at the Veterinary Biochemistry Department, University of Ibadan.

5.2.3 Oxidative Biochemical assays

5.2.3.1 Evaluation of Protein content: Protein content in each BALf was evaluated using Biuret method (Gornall et al 1949). Cu^{2+} precipitates were prevented by addition of potassium iodide into the test sample. The following reagents were prepared;

- Phosphate buffer (0.1M, pH 7.4) using 35.822g of disodium mono hydrogen phosphate (Na_2HPO_4, sourced from BDH Chemical Limited, England) and 15.603g of sodium dihydrogen phosphate salt (NaH_2PO_4) dissolved in 900ml of distilled water, adjusted to 1L at pH 7.4.
- 1.2% Potassium Chloride using 1.15g KCL (BDH Chemical Limited, England) dissolved in distilled to 1000ml store at 4°c. 0.2M NaOH using 8g of sodium hydroxide (BDH, England) dissolved in distilled water to 1L.
- Stock Bovine Serum Albumin (standard)- 20mg of BSA (Sigma Chemical Co., USA) dissolved in 2ml distilled water to give a stock solution of 10mg protein/ml.
- Biuret Reagent- 3g of $CuSO_4$. $5H_2O$ (BDH Chemicals, England) were dissolved in 500ml of 0.2M NaOH. 5g of potassium iodide, KI (BDH Chemicals, England) was added and the solution made up to a litre with 0.2M NaOH.

Protein Standard Curve: Different dilutions of the stock solution containing 2-10mg protein/ml were made. 4ml of biuret reagent was added into 1ml of each protein standard solution in a test tube, at 25^{o}C for 30 minutes. The optical densities of the solutions were read on a spectrophotometer at 540nm. A curve of the optical densities against protein concentration was plotted.

50µL of BALf supernatant was added to 100µL of biuret reagent in the microtitre plate. The plate was left for 30min at room temperature before read on the spectrophotometer at a wave length of 490nm using distilled water as blank. The readings were extrapolated on the total protein standard curve.

5.2.3.2 Measurement of superoxide dismutase (SOD): Activity profile of dismutase in the homogenates was as described by Misra and Fridovich (1972).

- 0.05M Potassium Phosphate buffer (pH 7.8). 6.97g of K_2HPO_4 and 1.36g KH_2 PO_4 dissolved in 900ml of distilled water diluted to 1 litre at pH 7.8.
- 0.05M Carbonate buffer (pH 10.2)- 14.3g of Na_2CO_3 $10H_2O$ and 4.2g of $NaHO_3$ were dissolved in 900ml of distilled water 1 litre at pH 10.2.
- 0.3mM Adrenaline- 0.0137g of adrenaline (epinephrine) dissolved in 200ml-distilled water diluted to 250ml and prepared afresh.

Procedure: Each well of the microtitre plate contained 20µL BALf, 250µL carbonate buffer, 300µL of acidified reconstituted epinephrine. The change in absorbance was observed every 30s for 180s at 490 nm wave length..

5.2.3.3 Measurement of Glutatathione: The reduced glutathione (GSH) was measured following method of Jollow et al (1994).

250µL of 4% sulfosalicylic acid was added to 250µL of BALf in test tube, the tube was spinned at 4000 rpm for 5 minutes. 20µL of the supernatant was aliquoted into wells of the microtitre plates, and 180µL of Ellman's reagent (containing 0.04 g of DTNB in 100 mL of 0.1M phosphate buffer, pH 7.4). The absorbance of the reaction was read on the spectrophotometer at a wavelength of 405nm against distilled water as blank.

1ml of sample was diluted in 9ml of distilled water to make a 1 in 10 dilution. An aliquot of the diluted sample was added to 2.5ml of 0.05M carbonate buffer (pH 10.2) to equilibrate in the spectrophotometer and the reaction started by the addition of 0.3ml of freshly prepared 0.3mM adrenaline to the mixture which was quickly mixed by inversion. The reference cuvette contained 2.5ml buffer, 0.3ml of substrate (adrenaline) and 0.2ml of water. The increase in absorbance at 480nm was monitored every 30 seconds for 150 seconds.

Calculation

A_0 = absorbance after seconds ;

A_3 = absorbance 150 seconds

$$\text{Increase in absorbance per minute} = \frac{A_3 - A_0}{2.5}$$

$$\% \text{ inhibition} = \frac{\text{increase in absorbance for substrate X 100}}{\text{increase in absorbance of blank}}$$

1 unit of SOD activity was given as the amount of SOD necessary to cause 50% inhibition of the oxidation of adrenaline to adrenochrome during 1 minute.

5.2.3.4 Colorimetric Estimation of Hydrogen Peroxide (H_2O_2): The hydrogen peroxide generation was evaluated according to Woff's (1994). The well of the microtitre plate contained 100µL of 0.1M phosphate buffer (pH 7.4), 50µL of Ammonium ferrous sulphate, 20µL of sorbitol, 10µL of Xanthine Oxidase (XO), 25µL of sulphuric acid and 50µL of BALf. The plate was vortexed slightly change in colour (pink colour. The plate was left for incubation at 25^{o}C for 30 minutes. The reaction absorbance was read at 490nm wavelength using distilled water as blank. The H_2O_2 standard curve was used for extrapolation of H_2O_2 level generated.

5.2.3.5 The myeloperoxidase (MPO) activity was evaluated as described by Xia and Zweier (1997). The well of the microtitre plate contained 200uL O-dianisidine mixture and 10uL of BALf. The reaction absorbance was monitored at 0, 30 and 60 seconds at 450nm wavelengths. Different amount of H_2O_2, ranging from 10 to 100 µmoles was taken in small test tubes and 2ml of dichromate/acetic acid was added to each. Addition of the reagent instantaneously produced an unstable blue precipitate of perchromic acid. Subsequent heating for 10 minutes in a boiling water bath changed the colour of the solution to stable green due to formation of

chromic acetate. After cooling at room temperature, the volume of the reaction mixture was made to 3ml and the optical density measured with a spectrophotometer at 570nm. The concentrations of the standard were plotted against absorbance.

5.2.3.6 Estimation of reduced glutathione (GSH) level: The method of Beutler et al (1963) was followed in estimating the level of reduced glutathione (GSH).

Principle- The reduced form of glutathione comprises in most instances the bulk of cellular non-protein sulfhydryl groups. This method is therefore based upon the development of a relatively stable (yellow) colour when 5′,5′ – dithiobis - (2-nitrobenzoic acid, DTNB) (Ellman's reagent) is added to sulfhydryl compounds. The chromophoric product resulting from the reaction of Ellman reagent with the reduced glutathione, 2-nitro-5-thiobenzoic acid possesses a molar absorption at 412nm. Reduced glutathione is proportional to the absorbance at 412.

GSH working standard-40mg GSH was dissolved in 0.1M phosphate buffer, pH 7.4, and made up to 100ml with the same.

0.1M Phosphate buffer (pH 7.4)- a) First 0.1M K_2HPO_4 $12H_2O$ was prepared by dissolving 0.992g in 200ml of distilled water. b) 0.1M KH_2PO_4. $2H_2O$ (MW=156.03) was prepared by dissolving 1.946g in 200ml of distilled water.

Finally 0.1M phosphate buffer was prepared by adding 200ml of (a) to 100ml of (b) and the pH adjusted to 7.4 with drops of concentrated HCl or NaOH as the case may be.

Ellman Reagent [5′, 5′-Dithiobis- (2-nitrobenzoate) DTNB]: a) This was prepared by dissolving 0.04g of Ellman reagent in 0.1M Phosphate buffer and made up to 200ml.

Precipitating Solution- 4% of sulphosalicyclic acid ($C_7H_6O_6S.2H_2O$ MW=254.22) was prepared by dissolving 4.8g of sulphosalicyclic acid in 120ml of distilled water. This reagent is stable for approximately three weeks at 40C.

Calibration of GSH standard curve: Serial dilutions of the GSH working standard were prepared as shown in the table below.

To each was added 4.5ml of Ellman reagent. GSH was proportional to the absorbance at 412nm (as well as at 430nm using colorimeter). The readings were taken before five minutes.

This is because the colour is stable for at least 5 minutes after the addition of Ellman reagent. After 10 minutes of standing, there is frequently a loss of 1 to 2% of the colour. However, an additional delay of 5 – 15 minutes will result in only a small error. Each sample was prepared in duplicate. A graph of optical density against concentration was plotted (5.2.4).

Half ml **(**0.5ml) of sample was placed into the tubes and 0.5ml of the precipitating solution was mixed with sample. The mixture was centrifuge at 4,000rpm for 5minutes. Take 0.5ml of the supernatant and put in another test tube, add 4.5ml of Ellman's reagent to the supernatant in the tube. Read at 412nm against blank as distill water.

A blank was prepared with 2ml of the 0.1M phosphate buffer, 3ml of diluted precipitating solution (3parts to 2 parts of distilled water), 1ml of the above mixture is added to 4.5ml Ellman's reagent. The optical density was measured at 412nm. GSH was proportional to the absorbance at that wavelength and the estimate was obtained from the GSH standard curve.

5.2.3.7 Assessment of lipid peroxidation: Lipid peroxidation was determined by measuring the formation of thiobarbituric acid reactive substances (TBARS) according to the method of Varshney and Kale (1970).

Principle- Under acidic condition, malondialdehyde (MDA) produced from the peroxidation of fatty acid membranes and food products react with the chromogenic reagent, 2-thiobarbituric acid (TBA) to yield a pink coloured complex with maximum absorbance at 532nm and fluorescence at 553nm. The pink chromophore is extractable into organic solvents (butanol).

30% Trichloroacetic acid (TCA)- 6g of TCA (CCl3COOH) was dissolved in distilled water and made up to 20ml.

0.75% Thiobarbituric acid (TBA)- This was prepared by dissolving 0.15g of TBA in 0.1M HCl and made up to 20ml.

0.15M Tris-KCl buffer (pH 7.4)- 1.15g of KCl and 2.36g of Tris base were dissolved separately in distilled water and made up to 100ml. The pH was then adjusted to 7.4.

The well of the microtitre plate contained 400µL of Tris-KCl, 125µL of 30% TCA, 100µL of BALf and 125µL of 0.75% TBA prepared in 0.2 M HCL. The plate was incubated at 80°C for 45 min in water bath. It was cooled on ice and centrifuged at 3000 rpm for 15 min. The reaction absorbance was read against distilled water as blank at wavelength of 490nm.

The level of lipid peroxidation (units/milligram protein) was valued on a molar extinction coefficient of 1.56 × 10^{5} /M/cm.

$$\text{MDA (units/mg protein)} = \frac{\text{Absorbance x volume of mixture}}{E_{532nm} \text{ x volume of sample x mg protein}}$$

5.2.3.8 Glutathione peroxidase assay

Sodium azide (NaN_3; 10mM)- 0.0325g of sodium nitrite dissolved in 50ml distilled water.

Reduced glutathione (GSH 4mM)-0.0123g of reduced GSH dissolved in 100ml phosphate buffer

Hydrogen peroxide (H_2O_2; 2.5mM)- 28µL hydrogen peroxide dissolved in 100ml distilled water.

Tricholoroacetic acid (TCA, 10%)- 2g of TCA was dissolved in 20 ml of distilled water

Di-potassium hydrogen orthophosphate (K_2HPO_4; 0.3M)-5.23g of di-potassium hydrogen orthophosphate was dissolved in 100mls of distilled water.

5'-5'-dithiobis-(2-dinitrobenzoic acid) DTNB- 0.04 DTNB dissolved in 100ml phosphate buffer.

Phosphate buffer- 0.992g of K_2HPO_4 and 1.946g of KH_2PO_4 were dissolved with 200ml of distilled water and adjusted to pH of 7.4 according to Rotruck et al (1973).

The glutathione peroxidase (GPX) was estimated as described by Beutler et al. (1963). The tube contained 250µL 0.1M phosphate buffer (pH, 7.4), 50µL of Sodium azide, 100µL of GSH solution, 100µL of H_2O_2, 250µL of BALf and 300µL of distilled water. The tube was incubated at 37°C for 5 min in water bath. Now 250µL of TCA was added to the tube before centrifugation at 3000 rpm for 5 min. 50µL of the supernatant was aliquoted into well of the microtitre plate, 100µL of K_2PHO_4 and 50µL of DTNB were added. The reaction absorbance was evaluated at a wavelength of 405nm with distilled water as blank.

The whole reaction mixture was incubated at 37°C for 3 minutes after which 0.5ml of TCA was added and thereafter centrifuged at 3000rpm for 5 minutes. To 1ml of each of the supernatants, 2mls of K_2HPO_4 and 1ml of DTNB was added and the absorbance was read at 412 nm against a blank. Glutathione peroxidase activity was observed by plotting the standard curve and the concentration of the remaining GSH was extrapolated from the curve.

GSH consumed = 245.34 – GSH remaining

Glutathione peroxidase activity = $\frac{H_2O_2 \text{ CONSUMED}}{\text{mg PROTEIN}}$

5.2.3.9 Glutathione transferase (GST) activity: The glutathione transferase (GST) activity was estimated via the conjugation of 1-chloro, 2,4-dinitrobenzene (CDNB) with reduced glutathione (Mannervik, 1985). A unit of enzyme will conjugate 10.0 nmol of CDNB with reduced glutathione per minute at 25^{o}C. The changes in absorbance was monitored at a wavelength of 405nm.The rate of the linear reaction was set at Δ405/min. The absorbance Δ405/min for the blank reaction was subtracted from the absorbance Δ405/min for each BALf sample reaction. The molar extinction coefficient of CDNB was 0.0096 μM-1/cm. Thus, GST activity = [(Adjusted Δ405/min)/ 0. 0096 μM-1/cm] x (1.0 ml /0.1 ml) x sample dilution = U/ml.

5.2.4 Statistical analysis

Data from the variables was presented as Mean ± SEM, and compared using Pearson correlation, T-test, ANOVA and Duncan multiple range test (DMRT) at 5% significance.

5.3 RESULTS

5.3.1 Pattern of pneumonia

One hundred and ninety two (192) BALf were biochemically analyzed for this study. Of the 192 BALf; 35 were of normal lungs, 29 of congestion and oedema, and 72 of bronchopneumonia, 29 of broncho-interstitial pneumonia, 22 of interstitial pneumonia and 3 of granulomatous pneumonia.

5.3.2 Pro-oxidant and anti-oxidant parameters

The total protein (TP) concentration in the BALf of goats with histologically normal lung was 9.56±0.7 mg/ml. It was significantly lower than that in congestion and oedema (11.6±1.1 mg/ml), bronchopneumonia (16.7±1.7 mg/ml), broncho-interstitial pneumonia (13.5±1.7

mg/ml), interstitial pneumonia (15.4±3.5 mg/ml) and granulomatous pneumonia (15.2±4.8 mg/ml).

The measure of Glutathione peroxidase (GPx) activity was 24.1±1.5units mg^{-1} protein in the BALf of normal goats. There were slight reduction in the GPx activity from congested and oedematous (21.4±1.7 unit mg^{-1} protein), bronchopneumonia (18.9±1.4 unit mg^{-1} protein), broncho-interstitial pneumonia (20.1±1.8 unit mg^{-1} protein), interstitial pneumonia (22.3±2.7 unit mg^{-1} protein) and granulomatous pneumonia (20.4±4.9 unit mg^{-1} protein). The difference was significant in BALf from goats with bronchopneumonia ($p<0.05$).

The level of reduced glutathione (GSH) was 9.9±0.3 µgmL^{-1} form BALf of normal lungs. There were slight decrease to 9.4±0.2 µg mL^{-1} in congested and oedematous lungs, 9.7±0.1o µg mL^{-1} in bronchopneumonia, 9.5±0.1 µg mL^{-1} in broncho-interstitial pneumonia, but similar in interstitial pneumonia (9.9±0.3 µg mL^{-1}) and granulomatous pneumonia (10.2±0.4 µg mL^{-1}).

The activity of Glutathione transferase (GST) was 8.0±1.1 unit mg^{-1}protein in the BALf from normal lungs. It decreased in congested and oedematous lungs (6.5±1.0 unit mg^{-1}protein), bronchopneumonia (7.0±0.8 units mg^{-1}protein), least in broncho-intersttial pneumonia (6.3±0.9 unit mg^{-1}protein) but similar in interstitial pneumonia (8.6±1.1 units mg^{-1}protein) and granulomatous pneumonia (8.1±3.3 unit mg^{-1}protein).

Similar pattern was also observed in Superoxide dismutase (SOD) activity; 4.1±0.3 unit mg^{-1}protein in normal, 3.6±0.3 units mg^{-1}protein in congestion, decreasing further to 3.2±0.2 unit mg^{-1}protein in bronchopneumonia, 3.4±0.3 unit mg^{-1}protein in broncho-interstitial pneumonia, 3.7±0.4 unit mg^{-1}protein in interstitial pneumonia and 3.4±0.8 unit mg^{-1}protein in granulomatous pneumonia.

Hydrogen peroxide generation was least in the BALf of normal lungs (3.6±0.5µmol mg-1 protein). It increased to 5.0±0.4 µmol mg-1 protein in congestion and oedema, 4.9±0.3 µmol mg-1 protein in bronchopneumonia, 5.0±0.6 µmol mg-1 protein in broncho-interstitial pneumonia, 5.2±0.4 µmol mg-1 protein in interstitial pneumonia and 5.6±0.7 µmol mg-1 protein in granulomatous pneumonia.

Malondialdehyde (MDA) levels was low in normal (2.3±0.1 µmol/mL), oedematous lungs (2.1±0.1 µmol/mL), bronchopneumonia (2.3±0.1 µmol/mL), remarkably high in broncho-interstitial pneumonia (4.43±0.1 µmol/mL), 2.2±0.1 µmol/mL in interstitial pneumonia, and 3.0±0.1 in granulomatous pneumonia.

Myeloperoxidase (MPO) activity was least in BALf from histologically normal lungs (2.0±0.2 µmol/min) but significantly higher in congestion and oedema (16.0±0.1µmol/min), bronchopneumonia (20.0±0.1 µmol/min), broncho-interstitial pneumonia (30.0±0.2 µmol/min), interstitial pneumonia (10.0±0.1 µmol/min) and granulomatous pneumonia (11.0±0.1 µmol/min).

The pattern of the antioxidants in BALf from examined goats is shown in figure 1 while that of the pro-oxidants is shown in figure 5.3.2. There was also significant positive correlation of BALf pro-oxidant assay to the type and severity of pneumonia (+0.65, $p<0.05$).

Table 5.3.1. Prooxidant and Antioxidant levels in the normal and pneumonic BALf

Lung	TP mg/ml	GPX	GSH	GST	SOD	H_2O_2	MDA	MPO*
		units mg^{-1}	µg mL^{-1}	unit $mg^{-1}p$	unit $mg^{-1}p$	µmol mg^{-1} p	µmol/mL	µmol/min
Normal	9.56±0.7[a]	24.1±1.5[a]	9.9±0.3[a]	8.0±1.1[a]	4.1±0.3[a]	3.6±0.5[a]	2.3±0.1[a]	2.0±0.2[a]
Oedema	11.6±1.1[a]	21.4±1.7[a]	9.4±0.2[a]	6.5±1.0[b]	3.6±0.3[a]	5.0±0.4[b]	2.1±0.1[a]	16.0±0.1[b]
Br pn	16.7±1.7[b]	18.9±1.4[b]	9.7±0.1[a]	7.0±0.8[a]	3.2±0.2[a]	4.9±0.3[b]	2.3±0.1[a]	20.0±0.1[b]
Br In Pn	13.5±1.7[a]	20.1±1.8[a]	9.5±0.1[a]	6.3±0.9[b]	3.4±0.3[a]	5.0±0.6[b]	4.43±0.1[b]	30.0±0.2[c]
Int pn	15.4±3.5[b]	22.3±2.7[a]	9.9±0.3[a]	8.6±1.1[a]	3.7±0.4[a]	5.2±0.4[b]	2.2±0.1[a]	10.0±0.1[b]
Gran pn	15.2±4.8[b]	20.4±4.9 [a]	10.2±0.4[a]	8.1±3.3[a]	3.4±0.8[a]	5.6±0.7[b]	3.0±0.1[a]	11.0±0.1[b]

Values with different superscripts are significantly different within column
Br Pn- Bronchopneumonia; Br In- Bronchointerstitial pneumonia; Int pn- Interstitial pneumonia; Gran pn- Granulomatous pneumonia
TP- Total Protein; GPx- Glutathione peroxidase; GSH- Reduced glutathione; GST- Glutathione transferase; H_2O_2- Hydrogen peroxide; MDA- Malondialdehyde; MPO- Myeloperoxidase; SOD- Superoxide dismutase

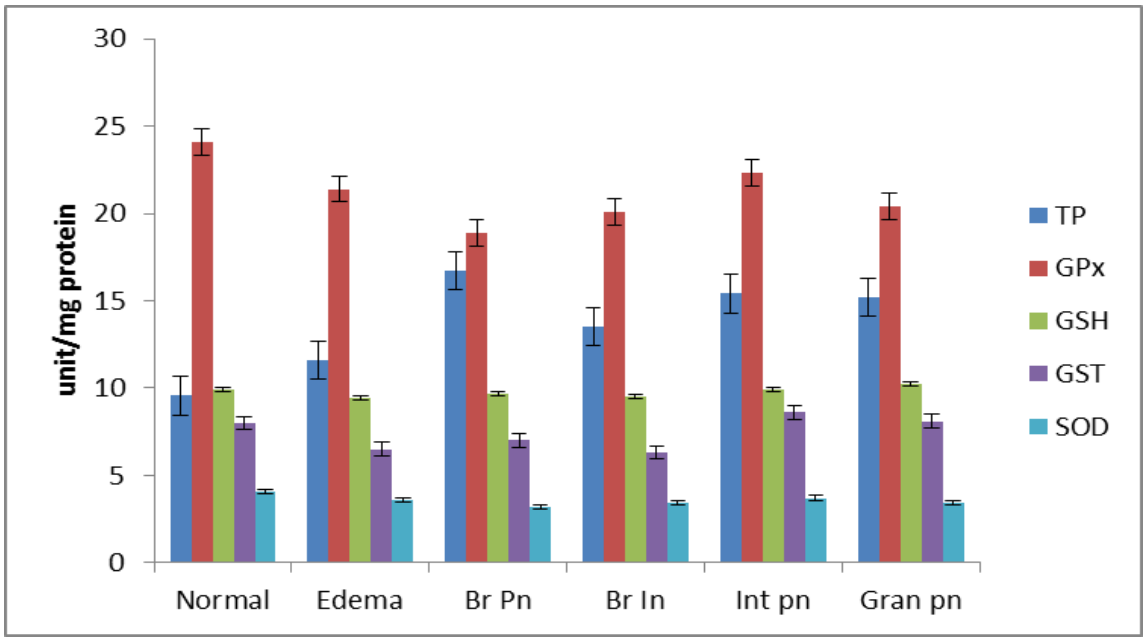


Figure 5.3.1: Changes in oxidants levels in BALf of examined goats with normal and pneumonic lungs

Br Pn- Bronchopneumonia; Br In- Bronchointerstitial pneumonia; Int pn- Interstitial pneumonia; Gran pn- Granulomatous pneumonia
TP- Total Protein; GPx- Glutathione peroxidase; GSH- Reduced glutathione; GST- Glutathione transferase; SOD- Superoxide dismutase

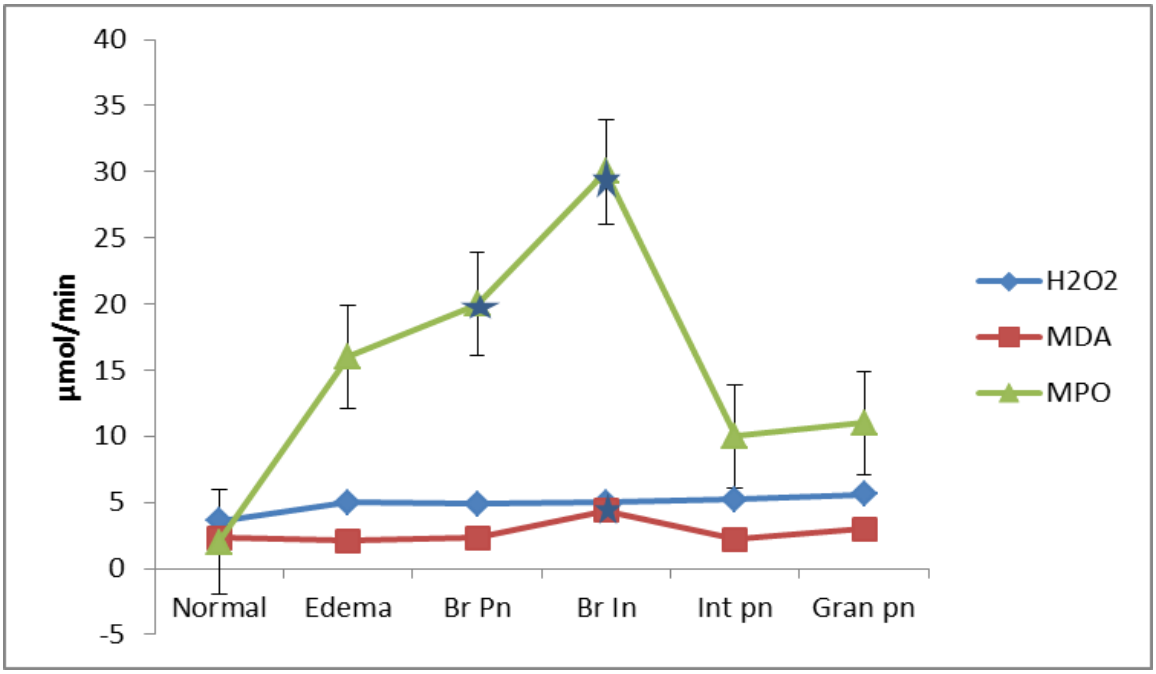


Figure 5.3.2: Changes in pro-oxidants levels in BALf of examined goats with normal and pneumonic lungs

Br Pn- Bronchopneumonia; Br In- Bronchointerstitial pneumonia; Int pn- Interstitial pneumonia; Gran pn- Granulomatous pneumonia

H_2O_2- Hydrogen peroxide; MDA- Malondialdehyde; MPO- Myeloperoxidase.

5.4 DISCUSSION

This study determined for the first time to the best of our knowledge the levels of pro-oxidants and antixoidants in bronchoalveolar lavage fluid (BALf) in Nigerian goats. Hitherto, the available few studies on oxidative stress in goats and ruminants quantified the markers in plasma. However, this study quantified oxidative stress biomarkers including GST, SOD, GPx, MDA, H_2O_2 and MPO in BALf of normal and pneumonic goats with different histologic classifications.

The MDA and SOD values obtained in BALf of goats in this study are not too different from that of blood of sheep and goats reported by various workers (Kataria and Kataria 2012, Tanko et al. 2016). In goats, the MDA and SOD values of 250 nmol/mL and 80µM were reported in goats in humid tropical environment (Tanko et al. 2016), while Forcados et al (2016) reported 4.68 ± 0.26 and 6.09 ± 0.69 (nmol/mL) for MDA, 4.35 ± 0.13 and 3.67 ± 0.12 (µmol/L) for Glutathione in non-grazing and grazing cattle in similar environment. Kataria and Kataria (2012) reported 167.98±10.00 kU L^{-1} and 294.22±9.91 kU L^{-1} for SOD; 5.87±0.10 µmol L^{-1} and 3.10±0.06 µmol L^{-1} for Glutathione from healthy and pneumonic sheep affected Pest des petits ruminants respectively in India.

The difference in oxidative parameters from BALf observed in pneumonic lungs was significant, this could be associated with the severity of inflammation and cellular damage commonly observed in bronchopneumonia and broncho-interstitial with destruction of epithelial cells and fibrinous reaction from vascular damage. This clearly showed that the measure of MDA, H_2O_2 and MPO correlated with the inflammatory reactions and mechanisms of cellular response.

The role of antioxidant should therefore be stressed especially in ruminants, where vitamin supplement may not be part of treatment because it is assumed that forage taken could adequately supply enough antioxidants. It should be noted also that pneumonia induces loss of condition and anorexia in these animals thereby further depleting antioxidant levels which are known to limit dangerous effects of ROS generation in living tissues in disease state.

The protein concentration of BALf in the pneumonic was significantly different to that observed in non-pneumonic goats. This is unique and quite contrasting to the observations of

Lewis et al (1993) in pneumonic, asthmatic and normal human patients. The significant decrease in most of the enzymes (GPx, GST and SOD) indicates an overwhelming exhaustion of these enzymes. Consequently there would be damage to macromolecules, cells, tissues and organ dysfunction with health implications on the affected animal. Significant reductions were observed in broncho-interstitial pneumonia and bronchopneumonia.

Myeloperoxidase activity is unregulated in inflammation as well as in oxidative stress because of the increased phagocytic activity and release of reactive oxygen species (ROS) from neutrophils (Dhiman et al. 2009). The high MPO values recorded in bronchopneumonia and broncho-interstitial pneumonia further corroborate cellular damage and local neutrophilic inflammation. This is similar to findings of Schmekel et al (1990), who linked origin of myeloperoxidase in bronchoalveolar lavage to local neutrophilic reaction. The increases in H_2O_2 generation and MDA levels may have underscored the cellular injury, membrane lipid peroxidation and acute inflammatory reactions induced by pathogens (bacterial and/or viral) of the lung. More so, effect of ROS in the pathogenesis of viral infections has been emphasized (Schwarz, 1996; Gil et al. 2004). The cellular injury, damage of tissues and inflammation induced by respiratory pathogens makes them potent pro-oxidant agents (Kataria and Kataria, 2012). They are in turn eliminated by specific cell defense mechanisms which may also generate reactive oxygen species (Romero et al. 1995). This may explain the MDA levels which increased in goats with broncho-interstitial pneumonia. Although Nisbet et al (2007) suggested a shift towards pro-oxidant production in the pathology of Pest des petits ruminant (PPR) virus. This may be accentuated in bacterial complicated viral pneumonia.The findings further corroborate the assertion of Schawrz, (1996) who suggested the application of antioxidants in the therapy of viral infections due to the generation of reactive metabolites (Peterhans, 1997).

Dyer *et al*. (1989) also examined oxidative respiratory burst activity in lavage-procured bovine pulmonary alveolar macrophages where it was shown that non-stimulated alveolar macrophages released a minimal quantity of superoxide anion and had small amounts of glucose flux through the pathways of energy metabolism. This could be aggravated in severe pneumonic conditions and massive neutrophilic influx, as was observed from H_2O_2 generation, MDA and MPO levels in the pneumonic goats. Furthermore, the impact of

oxidative stress in virus infections could the related to the nature of viral replication in cells, growth and packaging of proteins (Peterhans, 1997). The changes in antioxidants and enzymes levels in the goats explain the reductive effects of free radicals in caprine pneumonia complex. The influence of ROS may have contributed to the severity of cellular injury in broncho-interstitial pneumonia and bronchopneumonia.

It is apparent that antioxidant enzymes assay may not reflect the level of oxidative stress because of the their endogenous and dietary sources which naintains the threshold level in time (Almroth, 2008). Nonetheless, prooxidant levels andor reactive oxygen species are better indicators..

Thus, periodic assessment of oxidative stress in small ruminants is important with supplements of antioxidants in pneumonic animals. In humans, Israel and Gougerot-Pocidalo (1997) already showed the influence of antioxidant molecules as good therapeutics. The histological pattern of disease linked to the BALf may not have strengthened the attribution of causality. However, this study has further compared the histological findings with BALf redox changes in pneumonia and its contribution to the pathology and fatality in small ruminants. Thus, the management of caprine pneumonia should incorporate antioxidant supplementation to correct the imbalance in pro-oxidant and antioxidant levels.

In conclusion, oxidative stress markers in BALf may serve as predictors of pneumonia especially in the early stages. Oxidative stress plays a role in pneumonia and there is need to closely study it in complicated pneumonia for control of caprine pneumonia complex.

CHAPTER SIX

6.0 IMMUNOHISTOCHEMICAL DETECTION OF SOME VIRAL AND BACTERIAL RESPIRATORY PATHOGENS ASSOCIATED WITH CAPRINE PNEUMONIA IN NIGERIA

6.1 INTRODUCTION

Pneumonia has been established as a cause of mortalities in ruminants throughout the world (Yener et al. 2005). Pneumonic conditions have been reported in sheep and goats (Caswell and Williams, 2007; Çeribasi et al. 2016). However, the causative agents were rarely determined and many viruses associated with respiratory system diseases in ruminants have been implicated in natural and experimental studies in small ruminants (Thiry et al. 2006). Previous investigations have shown that Mycoplasma spp., *Pasteurella multocida (Pm*) and *Mannheimia haemolytica* (*Mh*) were the most important non-viral agents associated with caprine pneumonia (Gourlay and Barber, 1960; Odugbo et al. 2003; Mohammed, 2009); but there is very little information on the pathogenesis and detection of the anitigens in goats.

Respiratory diseases in these animals are infectious in nature caused by viruses (Brogden et al. 1998; Çeribasi et al. 2016), or the viruses as it were disrupt the pulmonary defence mechanisms for entry of secondary or opportunistic organisms (Emikpe et al. 2011).

In Nigeria, pathogens including bacteria (Ojo et al. 1971; Tijanni et al. 2012), mycoplasma and viruses have been implicated in pneumonia of sheep and goats but only Emikpe et al (2011) suggested the possible complication of Mannheimia and PPR virus in caprine pneumonia. Pneumonia complex in small ruminants has assumed a great significance in the face of the immense measures being put in its control. Hitherto, PPR virus was considered very important (Brown et al. 1991) but the different control measures instituted has not being able to effectively arrest the mortality arising from pneumonia in sheep and goats, while the roles of other possible viruses are yet unknown.

PI-3 virus has been associated serologically with natural respiratory tract infections in goats (Obi and Ibu, 1990), and clinical signs of respiratory embaraament was reported in parainfluenza pneumonia (Allen et al. 1978; Bryson et al. 1978; Ellis, 2010).

On the other hand, respiratory syncytial viral pneumonia is an important condition in humans and in ruminants (Eleraky et al. 2001; Ceribasi et al. 2013). The three strains in ruminants have structural and antigenic similarities (Alansari and Potgieter, 1994; Bunt et al. 2005). The diagnoses of these viruses have been limited by macroscopic and histopathological findings due to similar pulmonary lesions since most respiratory viruses are known to destroy ciliary activity and markedly decrease mucociliary clearing in the respiratory tract (Jericho, 1983; Ceribasi et al. 2014). Ellen et al (2001), Gulbahar et al (2002), and Gershwin (2012) demonstrated the antigens in lung tissues in other climes, the need for such a study in poor resource setting of tropical Africa cannot be over emphasised.

A few natural pneumonic cases related to some these agents are knwon in small ruminants (Lawal et al. 2011; Çeribasi et al. 2016) however the role of other viruses other than that caused by PPRV has not been fully established in caprine pneumonia in Nigeria. Attempts were made in demonstrating the Mannheimia antigens in lungs of experimentally challenged WAD goats (Emikpe and Akpavie, 2011). An immunohistochemical studies on pulmonary lesions in calves naturally infected with *Mannheimia spp* was published by Haritani et a (1989) and Narita et al (2000), and in goats by (Yener et al. 2009) in Turkey. More so, Zamri-Saad et al (1991) described the pathology of Pasteurellosis in transport stressed and dexamethasone treated goats, but none has been able to demonstrate the pathogen in goats.Therefore, the study objective was to elucidate involvement of PPR, PI3 and RS viruses and also demonstrate the antigens of Pasteurella and Mannheimia in the pathology of caprine pneumonia from formalin-fixed, paraffin embedded caprine lung tissues.

6.2 MATERIALS AND METHODS

6.2.1 Study Animals

The animals were selected across breeds (Red Sokoto RS; West African dwarf WAD; Sahelian) and ages from one to four years.

6.2.2 Pathology

150 lung samples from goats already characterized macroscopically and microscopically were selected randomly for this present study. The selection was from already macroscopic and microscopically characterized pneumonic caprine lungs comprising broncho-interstitial, interstitial pneumonia, bronchopneumonia, and normal lungs. The selection was also across age and season.

6.2.3 Formalin-killed vaccine (*Bacterine*)

A colony of *M. haemolytica and P. multocida* were each inoculated into 5 mL of Tryptone Soy Broth (TSB) for incubation at 37°C for 18–24 h in shaking incubator. The broth cultures were spinned at a speed of 6000 rpm at a temperature of 4°C for 15 minutes. Afterwards the top was discarded, for recovery of cell pellets at the bottom which was washed in acetone twice. Also, the pellets were washed twice with diethyl ether. The pellet was re-suspended in 1% formalin saline. Few drops of Tween 80 was included to the suspension at a final concentration of 3%–4%. The procedure was repeated three times with saline solution. The inactivated bacterial cells were now suspended to a final concentration of 4×10^9 cells/mL.

6.2.4 Raising of polyclonal antibody in rabbits

This was done following methods of Diker et al (2000). The antigen used was the attenuated PPR (Nig/75) vaccine locally produced in Vom, Nigeria, and bacterines. 1.0ml of 1:1V/v mixture of vaccinal/bactine antigens and Freunds complete adjuvant (FCA) was inoculated each into New Zealand white rabbits in duplicates (n=6). Four weeks later, with 0.5ml antigen without FCA. Two further boosters were given at one week intervals. The rabbit was test bled three weeks after the last booster injection and its sera tested using Haemagglutination

inhibition test. The sera were stored in 1ml aliquots at -20^{o}C. Pre-immune sera of the rabbits were used as a control and negative labelling was observed in known positive samples.

6.2.5 Monoclonal antibodies

PI3 Monoclonal antibodies (Cat No: MAI- 7316) and RSV Monoclonal antibodies (Cat No: MAI- 7286) both raised in mouse with goat specificity were sourced from Thermo Scientific USA (thermofisher.com/antibodies). They were supplied in cold chain under optimal conditions.

6.2.6 Immunohistochemical detection

Immunohistochemical test for the antigens was performed on paraffin-embedded tissue as described by Meyerholz et al (2004). The staining procedure used the Avidin-Biotin peroxidase kit (M IHC Select Detection System, HRP/DAB, Merck, Germany. LOT: 2775482). Thin sections (4µ) of the lungs were cut in triplicates, floated and mounted on APES (3-aminoethoxypolypropylene) charged glass slides.

The slides with tissue sections were rehydrated by placing 2x in xylene (10 min each), 2x in 100% ethanol (10 min each), 1x in 95% ethanol (5 min), 1x in 70% ethanol (1 min), 1x in 50% ethanol (1 min) and finally rinsed in distilled water (5 min).

Antigen retrieval was heat-induced epitope retrieval (HIER), by immersing the tissues in Sodium citrate buffer for 8minutes at 60^{o}C.

Primary antibody concentration for each of the monoclonal antibodies was set at 4µg/ml while 1:100 dilutions was set for the polyclonal antibody.

Antigen staining (1^{o} Antibody)- a moisture chamber was prepared before surrounding the tissue on glass slide with barrier pen. Peroxidase activity was blocked in the tissues using 3% H_2O_2 in Methanol for 5minutes. The tissues were incubated in serum blocking reagent for 30minutes, rinsed before incubated in primary antibody overnight at 4^{o}C.

Antigen staining (2^{o} Antibody)- the slides were rinsed 3x in wash buffer 5 min each before incubated with biotylated secondary antibody for 30 min. The slides were rinsed 3x in wash buffer 5min each, incubated in ABC (avidin-biotin complex) reagent for 30min and rinsed 3x in wash buffer 5min each. 3,3'-Diaminobenzidine (DAB) chromogen solution was added to the slide for few seconds and monitored for intensity before rinsing in water. The slides were

counterstained in Mayer's haematoxylin, rinsed in water bofore dehydration in 50% ethanol, 70% ethanol, 95% ethanol, 2x 100% ethanol, 2x xylene, mounted with DPX and coverslipped for examination.

The known normal goat lungs were used as negative controls. For positive controls, negative sera were used instead of the immune rabbit serum and monoclonal antibodies on the pneumonic lung tissues.

Grading technique: Micrographs of the stained tissues was obtained using the digital camera (Amscope MU900) connected to the Olympus (C2X) binoculars. The images were quantified for staining intensity using reciprocal intensity of localized viral antigens on the open source Fiji (ImageJ) software (Nguyen et al. 2013). The optical density was calculated using the fomular;

$$OD = \log_{10}(\text{max reciprocal intensity} / \text{mean reciprocal intensity})$$

Cuf off for the OD values was set at 50. Immuno-positive staining was then graded as weak (50-100), moderate (101-200) and strong (>200).

6.2.7 Statistical analysis

The number and percentages of the caprine lung samples positive and negative for the three different viral and two bacterial antigens were estimated in percentages. The OD values are in Mean±SEM. The means were analysed inferentially with ANOVA at $\alpha=0.05$.

6.3 RESULTS

6.3.1 Signalment of the goats

In all, 22 were one-year old, 59 two-year old, 57 three-year old and 12 four-year old. 57 were from dry season (October to March) and 93 from wet season (April to August). Ninety three percent (93%) of the goats were male and only 7% female. Red Sokoto breed was 81, West African Dwarf breed 47 and Crosses 22. In terms of age; 22 of the goats were within a year, 58 two years, 54 three years and 16 above three years old. Sixty two (62) of the goats were examined in the dry season and 88 in wet season. Nine (9) of the goats were female and 141 males.

6.3.2 Pattern of pneumonia

Of the 150 goats randomly selected for this study, 61 were broncho-interstitial pneumonia, 25 interstitial pneumonia, 42 bronchopneumonia, 12 bronchiolitis and 10 normal caprine lung samples.

6.3.3 Immunohistochemical stained antigens

Out of the 61 caprine lungs with broncho-interstitial pneumonia, 57 of those lungs showed immuno-positivity to the PI3, RSV and or PPR viral antigens while 4 were negative. Of the 25 caprine lungs with interstitial pneumonia, 23 showed immuno-positivity while 2 were negative. Of the 42 caprine lungs with bronchopneumonia, 23 also showed positive immunostaining while 19 were negative. Ten 10 out of the caprine lungs with bronchiolitis showed immunostaining and 2 negative. All the histologically normal lungs were negative for immunostaining. Thus in all, 113 were positive and 37 negative for immunostaining.

The specific immunostaining was brownish staining of the cells which demonstrates or show the location of the antigens in the epithelium of the bronchi, bronchioles,alveoli, pneumocytes, giant cells and inflammatory cells. The intensity varied with the distribution of the lesion and antigens with very slight reaction in vessels while the bronchial, bronchiolar epithelium and

the luminal exudates with macrophages in the bronchial associated lymphoid tissue stained strongly.

The distribution of the immune-positive samples to the three viral antigens and the corresponding staining intensities is shown in Table 6.1.

Twenty three percent (23%) of the pneumonic caprine lung samples showed positive immune-staining to PI3 (plate 6.2) viral antigen only. Ten percent (10%) were positive for RSV antigen (plate 6.3) only while 34% were positive for PPR viral antigen (plate 6.1) only. Eight percent (8%) showed immunostaining for the multiple respiratory viral infections in these goats.

The distribution of immuno-staining in the different pattern of caprine lungs is shown in Table 6.2. RSV was slightly higher in bronchopneumonia while PPR was observed more in broncho-interstitial pneumonia and bronchiolitis. The optical densities (OD) varied according to the degree of staining intensities. The mean OD for the different viruses is shown in Table 6.3. The reciprocal intensities directly correlated with increasing staining intensity ($p<0.05$).

The bacterial agents are on the apical border of the airway epithelium especially in the bronchioles and respiratory bronchioles (plates 6.4, 6.5), on the alveolar epithelium and alveolar macrophages (plate 6.6). The distribution of the goats showing immune-positive staining is shown in Table 6.5. Thirty (20%) goats did not show antigens for either *Pm* and *Mh*, and 120 (80%) was positive for the bacterial antigens. The percentage positive and intensities of the immunostaining for the bacterial antigens was more in the wet season ($p<0.05$) and in goats two years above. The RS breed was significantly immunopositive for the bacterial antigens as compared to the other breeds ($p<0.05$). There was slight increase in the number of goats immunopositive for *Pm* than *Mh* (table 6.5). However, there was strong immunostaining for *Mh* (OD= 213±23) than *Pm* (152±13) ($p<0.05$).

6.3.4 Effect of Age, season and breed on occurrence of viral antigens

Age: PI3 and RSV viral antigens were mostly observed in young animals while PPRV were predominantly in older animals.

Season: slight increases were observed in the prevalence of both PI3 and RSV in the lung tissues of the goats lungs during dry season, while spread of PPR viral antigens was even.

Breed: Of the three different breeds, PI-3 viral antigen was more in RS, RSV antigen was only in RS and while PPR viral antigen was equally located in lung tissues from RS, WAD but more in WAD goats (table 6.4).

Table 6.1. Distribution of viral antigens and staining intensities in the caprine lungs

Antigen positivity	No.	Staining intensity		
		Weak	Moderate	Strong
PI3 only	34 (23%)	14	16	8
RSV only	15 (10%)	6	5	4
PPR only	51 (34%)	12	18	21
PI3 & RSV	3 (2%)		2	1
PI3 & PPR	5 (3%)	1	2	2
RSV & PPR	3 (2%)	0	3	0
PI3 RSV & PPR	2 (1%)	1	1	0
	113 (75%)			

Table 6.2. Distribution of immuno-staining in the different pattern of caprine lungs examined

	PI3	RSV	PPR	PI3 & RSV	PI3 & PPR	RSV & PPR	PI3 RSV & PPR	Negative	Total
Broncho-Interstitial	19	4	29	0	0	3	2	4	61
Interstitial	10	4	9	0	0	0	0	2	25
Bronchopneumonia	5	7	3	3	5	0	0	19	42
Bronchiolitis	0	0	10	0	0	0	0	2	12
Normal	0	0	0	0	0	0	0	10	10
Total	34	15	51	3	5	3	2	37	150

Table 6.3: Staining intensities of the IHC staining to the viral antigens

VIRUS		Mean±SE	p-value
PI3	Positive	55.6±11.1	0.003
	Negative	15.3±2.9	
RSV	Positive	47.6±12.3	0.001
	Negative	10.6±1.6	
PPR	Positive	59.2±12.6	0.000
	Negative	10.6±2.0	

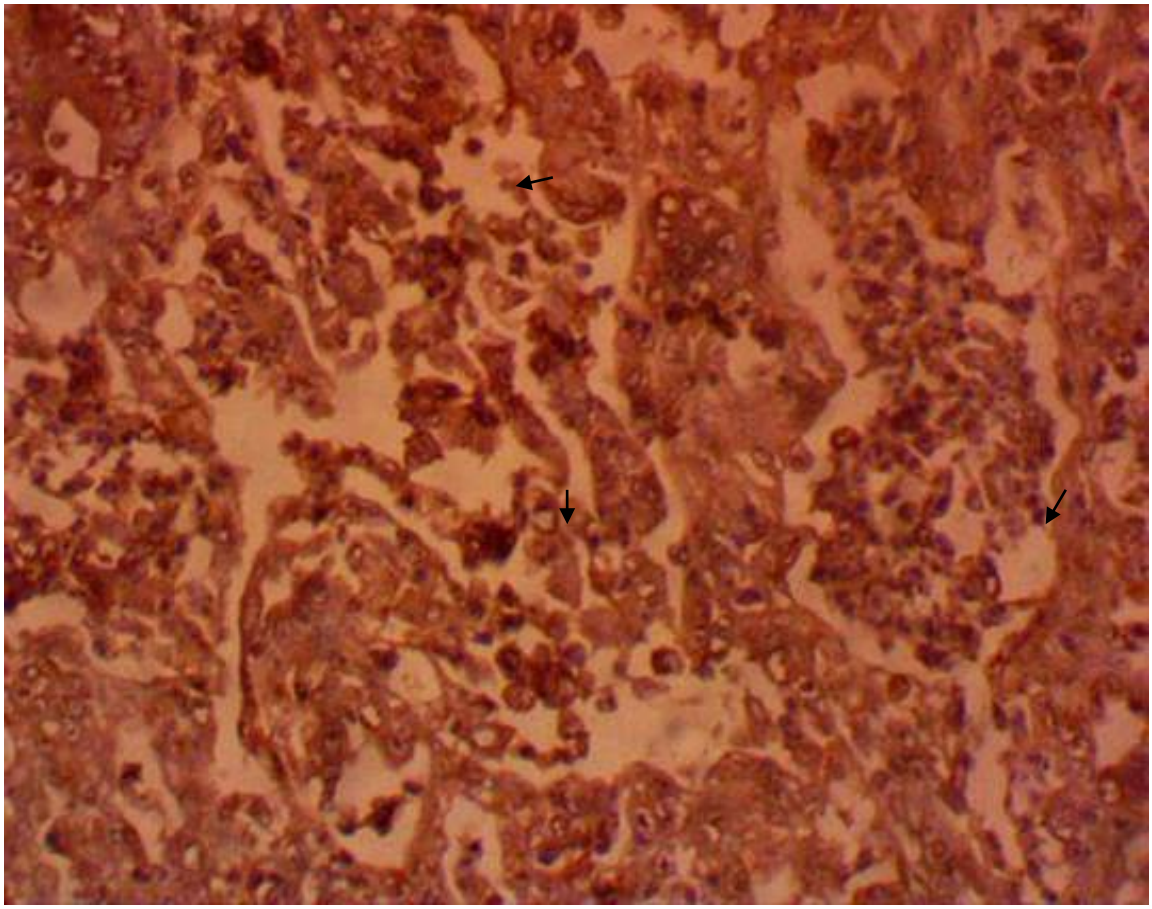


Plate 6.1:Epithelial cells of bronchial mucosae, bronchial glands and intra luminar exudates showing PPR antigens (arrows) in goats. ABC HRP counterstained with haematoxylin. X400

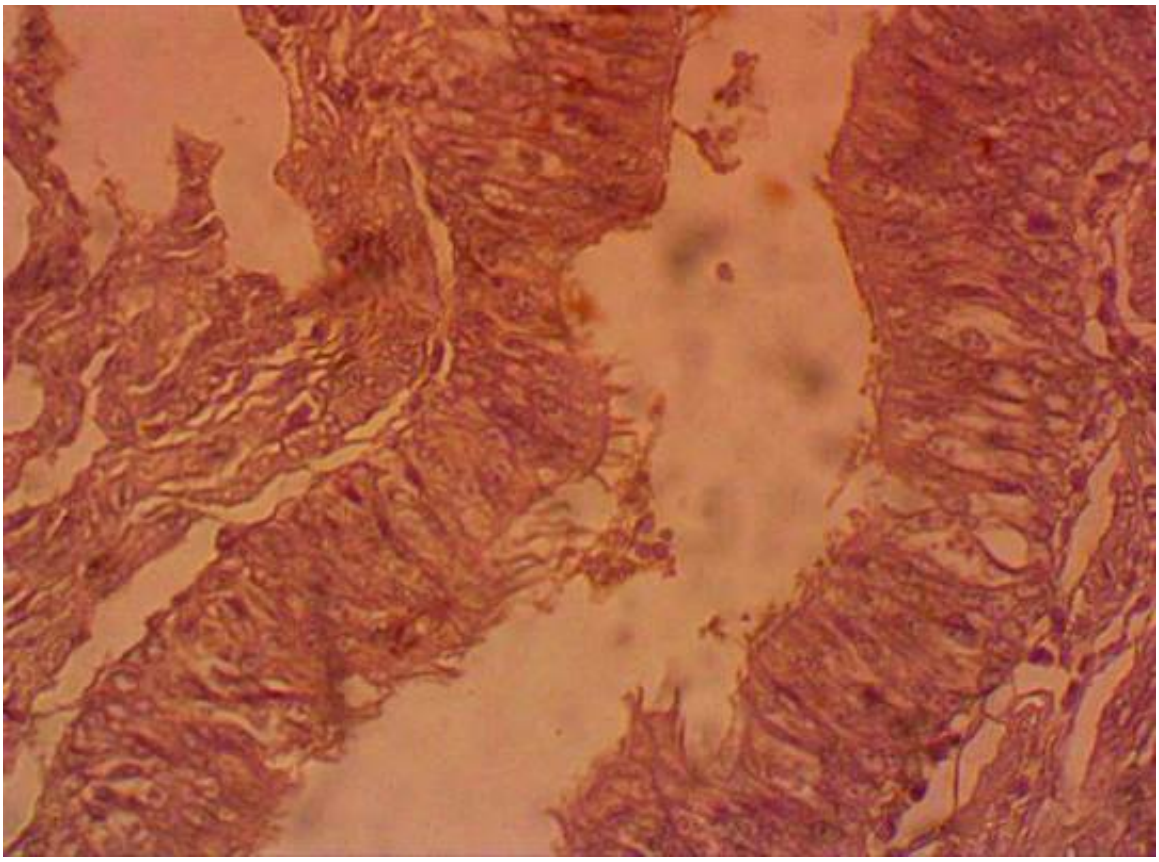


Plate 6.2 :Bronchial epithelial, bronchial associated lymphoid tissue and intra luminar exudates showing PI3 antigens in goats. ABC HRP counterstained with haematoxylin. x400.

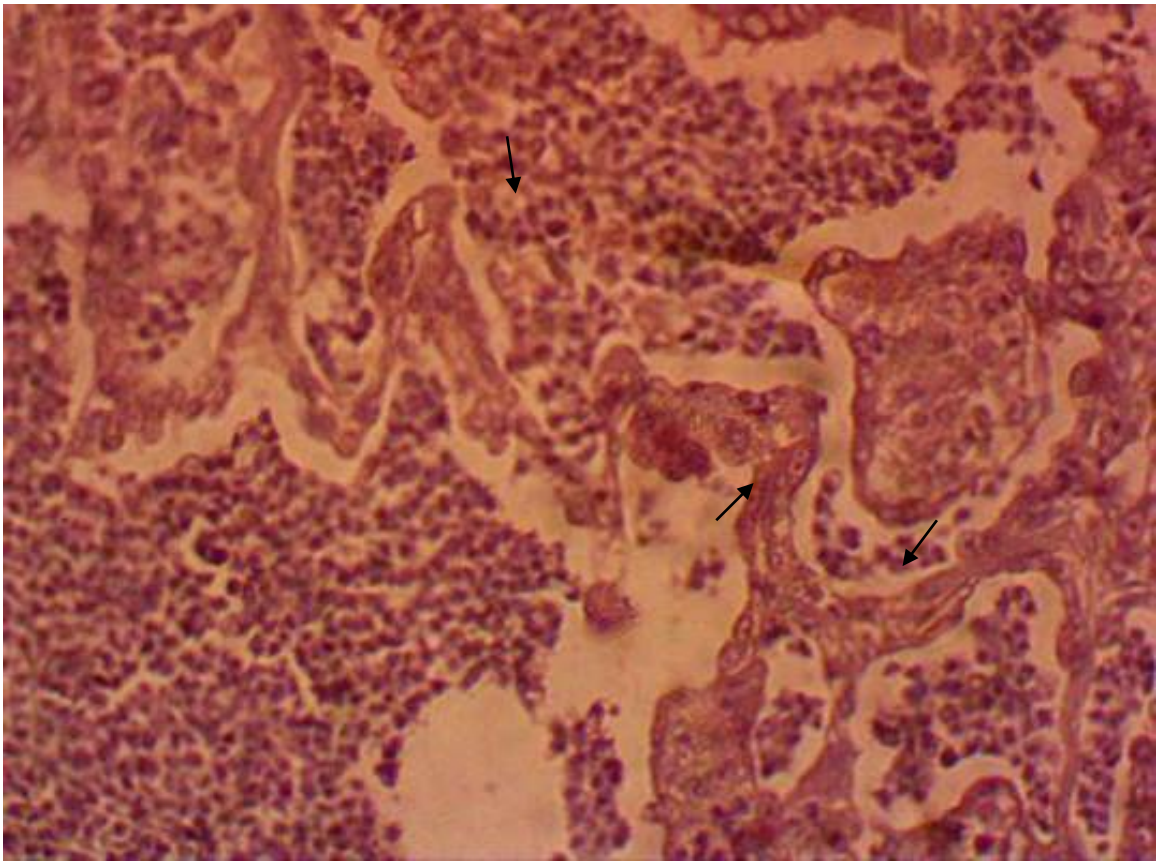


Plate 6.3: Positive immunostaining to RSV antigen in pneumocytes and macrophages, bronchial epithelial, and giant cell in goats ABC method HRP with haematoxylin. x400.

Table 6.4: Distribution of the viral antigens based on age, season and breed in pneumonic caprine lungs

	PI3	RSV	PPR	PI3 & RSV	PI3 & PPR	RSV & PPR	PI3 RSV &PPR	Negative	Total
one year	6 (4%)	4 (3%)	16 (11%)	0	0	0	0	12 (8%)	38 (25%)
two year	13 (9%)	3 (2%)	18 (12%)	3 (2%)	3 (2%)	0	0	3 (2%)	43 (29%)
three year	9 (6%)	6 (4%)	17 (11%)	0	0	3 (2%)	2 (1%)	20 (13%)	57 (38%)
four year	6 (4%)	2 (1%)	0	0	2 (1%)	0	0	2 (1%)	12 (8%)
Total	34 (23%)	15 (10%)	51 (34%)	3 (2%)	5 (3%)	3 (2%)	2 (1%)	37 (25%)	150
Dry	20 (13%)	9 (6%)	26 (17%)	3 (2%)	5 (3%)	3 (2%)	0	9 (6%)	57 (38%)
Rainy	14 (9%)	6 (4%)	25 (17%)	0	0	0	2 (1%)	28 (19%)	93 (62%)
Total	34 (23%)	15 (10%)	51 (34%)	3 (2%)	5 (3%))	3 (2%)	2 (1%)	37 (25%)	150
RS	20 (13%)	15 (10%)	21 (14%)	3 (2%)	3 (2%)	2 (1%)	0	17 (11%)	81 (54%)
WAD	10 (7%)	0	23 (15%)	0	0	0	2 (1%)	12 (8%)	47 (31%)
Sahelian	4 (3%)	0	7 (5%)	0	2 (1%)	1 (1%)	0	8 (5%)	22 (15%)
Total	34 (23%)	15 (10%)	51 (34%)	3 (2%)	5 (3%)	3 (2%)	2 (1%)	37 (25%)	150

RS-Red Sokoto goat; WAD- West African dwarf goat; Sahelian

Table 6.5: Pattern and Distribution of PM and MH antigens in caprine lungs in Nigeria

	Negative	*Mh*	*Pm*	*Mh + Pm*	Total	p-value
Broncho-Interstitial	9 (6%)	9 (6%)	21 (14%)	2 (1%)	41 (27%)	
Interstitial	5 (3%)	9 (6%)	13 (9%)	0	27 (18%)	
Bronchopneumonia	11 (7%)	24 (16%)	25 (17%)	12 (8%)	72 (48%)	0.03
Normal	5	5 (3%)	0	0	10 (7%)	
	30 (20%)	47 (31%)	59 (39%)	14 (9%)	150 (100%)	
one year	3 (2%)	0	13 (9%)	6 (4%)	22 (14%)	
two year	12 (8%)	19 (13%)	21 (14%)	6 (4%)	58 (39%)	
three year	14 (9%)	19 (13%)	21 (14%)	0	54 (36%)	0.01
four year	1 (1%)	9 (6%)	4 (3%)	2 (1%)	16 (11%)	
	30 (20%)	47 (31%)	59 (39%))	14 (9%)	150 (100%)	
Female	2 (1%)	5 (3%)	0	2 (1%)	9 (6%)	
Male	28 (19%)	42 (28%)	59 (39%)	12 (8%)	141 (94%)	0.00
	30 (20%)	47 (31%)	59 (39%)	14 (9%)	150 (100%)	
Dry	9 (6%)	24 (16%)	21 (14%)	8 (5%)	62 (41%)	
Rainy	21 (14%)	23 (15%)	38 (25%)	6 (4%)	88 (59%)	0.01
	30 (20%)	47 (31%)	59 (39%)	14 (9%)	150 (100%)	
RS	16 (11%)	33 (22%)	38 (25%)	2 (1%)	89 (59%)	
WAD	9 (6%)	9 (6%)	21 (14%)	6 (4%)	45 (30%)	0.02
Sahelian	5 (3%)	5 (3%)	0	6 (4%)	16 (11%)	
	30 (20%)	47 (31%)	59 (39%)	14 (9%)	150 (100%)	

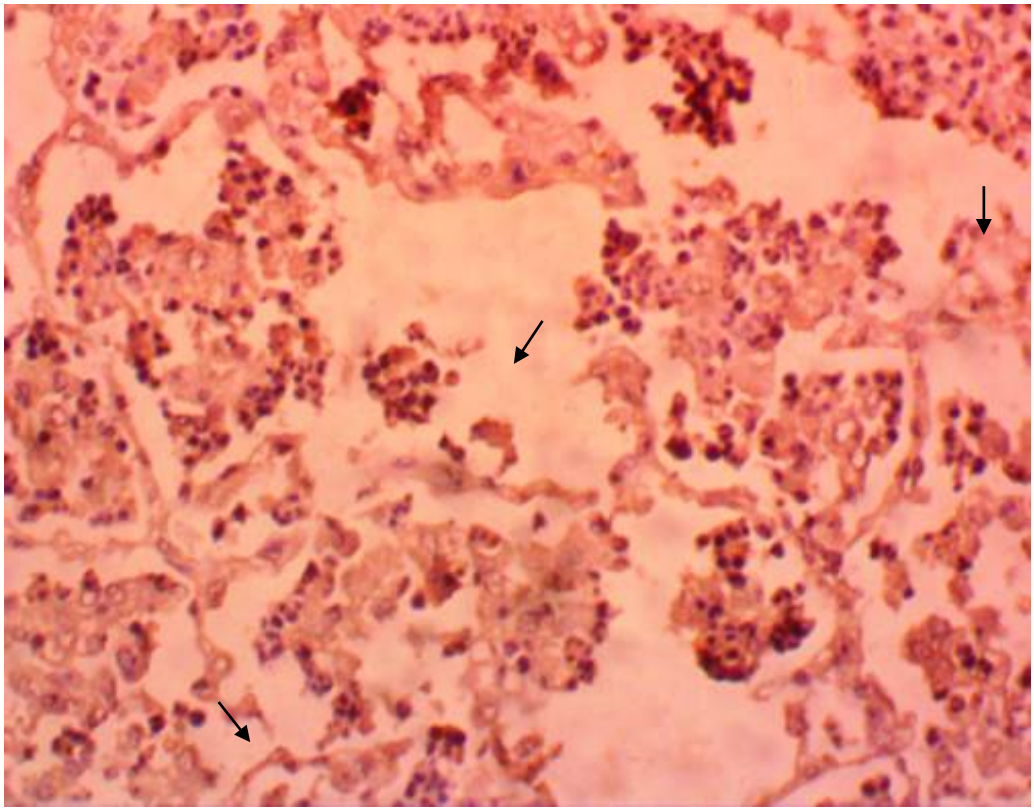


Plate 6.4. Immunodetection of *Mannheimia haemolytica* antigens in bronchiolar cells, luminal exudate and alveolar macrophages (arrows). ABC HRP x400.

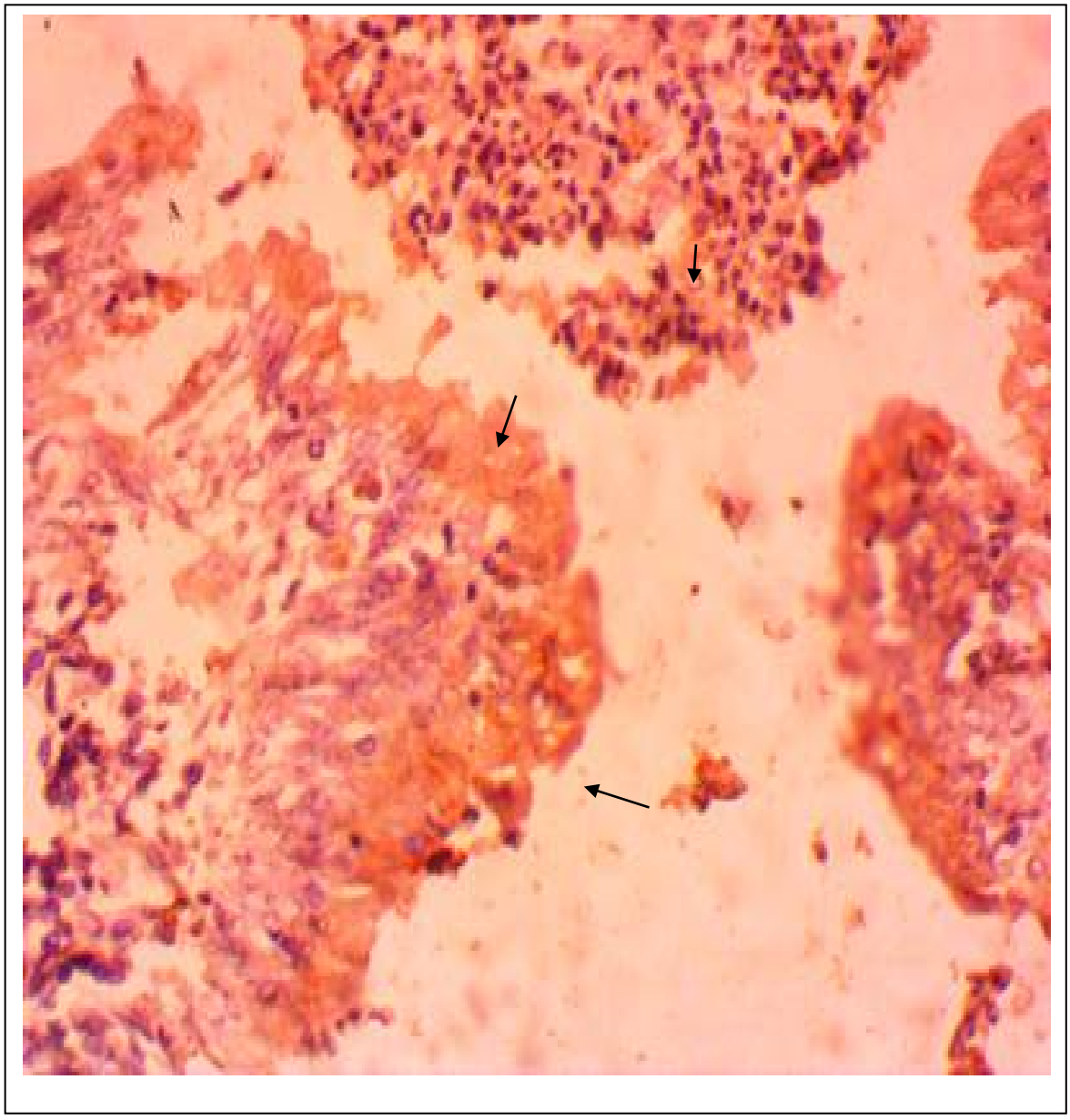


Plate 6.5. Immunodetection of *Pasteurella multocida* antigens on the bronchiolar epithelial cells and luminal exudate (arrows). ABC HRP x400.

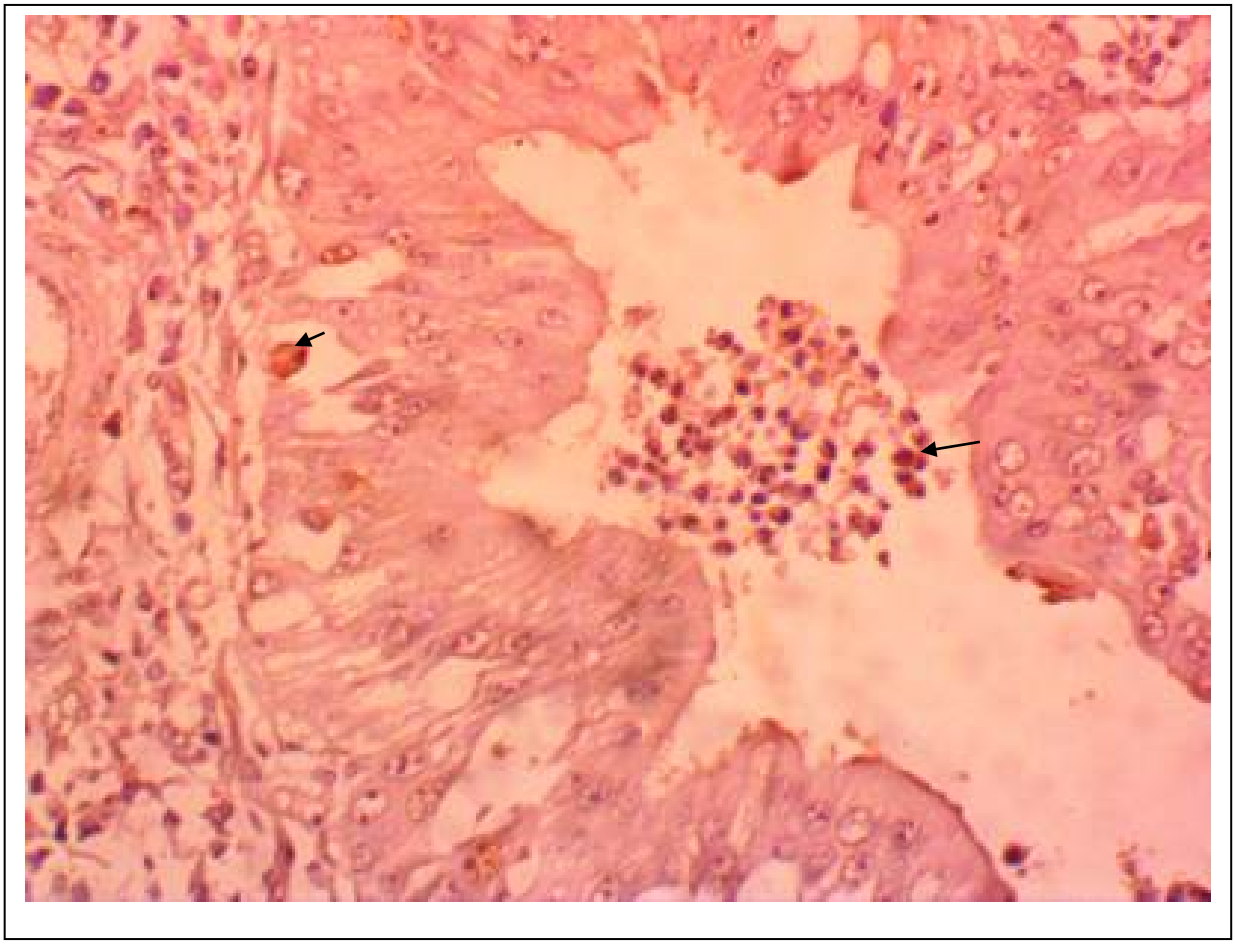


Plate 6.6. Immunodetection of *Pasteurella multocida* antigens on the bronchiolar epithelial cells (arrows) and alveolar macrophages. ABC HRP x400.

6.3.5 Virus bacteria interaction in the pneumonic goats

Of the 150 caprine lungs, 141 (94%) were positive for the pathogens and 9 (6%) negative. Viral (PPR, PI3 & RSV) antigens were detected in 113 (75%) and bacterial (*Mh* & *Pm*) antigens were in 120 (80%) of the lungs. 37 (25%) were negative for the viral antigens and 30 (20%) negative for the bacterial antigens (Table 6.6).

More than one pathogen were detected in 108/141 pneumonic goats. There were virus-bacteria antigens in 106/108 of the pneumonic goats. PPRv and Mh co-infection was the highest (20/106), others include PI3v-Pm (18/106), PPRv-Pm (12/106), PI3v-*Mh* (10/106) and RSV-*Mh* (9/106).

For sole infection, PPR viral antigen alone was in 15 (10%). The antigens of other pathogens were below 5 except for Pm antigen (19).

The morphological changes observed in the airways and air spaces varied from degeneration and necrosis to inflammation with presence of giant cells (Table 6.7).

Table 6.6: Interaction of viral and bacterial antigens in caprine pneumonia in Nigeria

		Bacterial antigens				
		Negative	*Mh*	*Pm*	*Mh* + *Pm*	Total
Viral Antigens	PI3v	4 (2%)	10 (7%)	18 (12%)	2 (1%)	34 (23%)
	RSV	0	9 (6%)	3 (2%)	3 (2%)	15 (10%)
	PPR	15 (10%)	20 (13%)	12 (8%)	4 (3%)	51 (34%)
	PI3 + RSV	1 (0.7%)	1 (0.7%)	1 (0.7%	0	3 (2%)
	PI3 + PPR	0	3 (2%)	2 (1%)	0	5 (3%)
	RSV+ PPR	0	0	3 (2%)	0	3 (2%)
	PI3 + PPR+ RSV	1 (0.7%)	0	1 (0.7%)	0	2 (1%)
	Negative	9 (6%)	4 (3%)	19 (13%)	5 (3%)	37 (25%)
	Total	30 (20%)	47 (31%)	59 (39%)	14 (9%)	150

PI3v- Parainfluenza 3 virus, RSV- Respiratory syncytial virus, PPRv- Pestes des petits ruminants virus, Mh- *Mannheimia haemolytica*, Pm- *Pasteurella multocida*.

Table 6.7: Lesion score in the viral and bacterial induced caprine pneumonia

	Lesion Score	Morphological changes
PI3	1	Bronchiolar epithelial necrosis
PI3 + Mh	2	Bronchopneumonia moderate diffuse
PI3 + Pm	1	Bronchiolar epithelial necrosis
PI3 + Mh + Pm	2	Bronchopneumonia moderate diffuse
RSV + Mh	2	Bronchopneumonia moderate diffuse
RSV + Pm	1	Bronchiolar epithelial necrosis
RSV + Mh + Pm	2	Bronchopneumonia moderate diffuse
PPR	3	Bronchointerstitial pneumonia
PPR + Mh	5	Diffuse fibrinous severe bronchopneumonia
PPR + Pm	3	Bronchointerstitial pneumonia
PPR + Pm + Mh	5	Diffuse fibrinous severe bronchopneumonia
PI3 + RSV + Mh	4	Diffuse fibrinous severe bronchopneumonia
PI3 + RSV + Pm	4	Diffuse fibrinous severe bronchopneumonia
PI3 + RSV + PPR	3	Bronchopneumonia moderate diffuse
PI3 + RSV + PPR + Pm	4	Bronchointerstitial pneumonia
Mh	2	fibrinous bronchopneumonia
Pm	1	Bronchopneumonia moderate diffuse
Mh + Pm	5	Diffuse pleuropneumonia

0= no observable lesion
1= degeneration of epithelial cells
2= degeneration and necrosis of epithelial cells
3= degeneration and necrosis of epithelial cells + GCs
4= degeneration and necrosis of epithelial cells + GCs + inflammatory cells
5= suppurative/purulent inflammation

6.4 DISCUSSION

The study detected and described immunohistochemical localisation of some viral and bacterial antigens in the caprine pneumonia complex in Nigeria. It has also been able to underscore the importance of other viruses other than PPR especially RSV and PI3 in caprine pneumonia complex in Nigeria, and also the first to demonstrate the antigens in the caprine lungs in our environment. It has butressed the studies of Ojo (1975, 1976), Ikede (1975, 1976) and Emikpe et al. (2011).

This study showed that PPR is still endemic with detection of PPRV antigen in 34% of the pneumonic caprine lungs. There was more involvement in the WAD and little or no influence of age and season. Hitherto, from most previous studies, the giant cell pneumonia was often attributed to PPR virus but with the present knowledge these may also be due to other viruses or their synergistic effect; as it was observed in the RS and cross breeds of goats. The intensity of the immuno-staining was strong in bronchial, bronchiolar epithelium, macrophages (alveolar, intravascular and interstitial) and giant cells. This clearly underscores the role of PPR virus in the susceptibility of goats to pulmonary consolidation. The synergistic influence of Pest des petits ruminants virus and the other viral agents needs further investigation however the subtle complication of PPRV by *Mannheimia haemolytica* in caprine pneumonia was evident.

The differential diagnosis of PPRV can be achieved through viral isolation and differential neutralization with specific antisera (Taylor and Abegunde, 1979), animal inoculation (Gibbs et al. 1979), or estimation of electrophoretic mobility of the nucleocapsid protein from infected cells (Diallo et al. 1987). All the three methods have shortcomings which include, need for cell culture facilities and well preserved specimen that may be unattainable in many poor resource countries like Nigeria where PPR occurs hence the need for antigen detection in tissues using immunohistochemistry as described in this study to surmount these challenges (Brown et al. 1991). The changes in the caprine lungs were similar to that reported in cattle (Jericho and Langford, 1982) and sheep (Davies et al. 1982) and goat (Emikpe et al. 2010).

The presence of PI-3 viral antigens was detected in 23% of the pneumonic caprine lungs with high occurrence of the antigens in the young and RS goats which may be due to immunosuppression of other agents or the dry weather where most of the goats were obtained. This finding is unprecedented in our environment where previous reports showed a serological evidence of PI-3 (Obi and Ibu, 1990). The result is comparable to 28.30% recorded in goats in Turkey (Ceribasi et al. 2012) but lesser to 66.6% of the caprine lungs examined by immunohistochemistry in the regions of Bitlis and Van, Turkey (Yener et al. 2005) and higher than 5.8% reported in sheep from Turkey (Gulbahar et al. 2002). The strong immunostaining on the bronchial, bronchiolar epithelia and BALT coupled with the macrophage hyperplasia in the BALT, cytoplasm of syncytial giant cells and less frequently in the pneumocytes. Thus, Parainfluenza-3 virus contribute to the pathogenesis of caprine pneumonia in Nigeria as reported elsewhere (Malone et al. 1988; Ceribasi et al. 2012).

Many factors such as climate, time of infection and amount of infective virus, age of sampled animals, management and nutritional conditions might have important role in the differences of infection incidence (Gencay and Akca, 2004). For instance, in a serological study carried out showed higher seropositivity of PI-3 infection in goats in spring (Turan and Bolat, 1999). In this study however, that young animals had more PI3 viral antigens could possibly be as a result of lack of maternal or acquired antibodies. Likewise, the higher occurrence in the dry season may show that dust and dry weather may contribute to spread of the parainfluenza agent as suggested by Gencay and Akca (2004). Our findings differ slightly from Rosadio et al (2011) and Chakraborty et al (2014) with the distribution of PI3 Viral antigens more in bronchointestial pneumonia.

Hitherto, RSV was also investigated based on serological methods (Yesilbag and Gungor, 2009). However, our study revealed for the first time the evidence of RSV antigen in goat lung in tropical African environment. The source of infection with this virus may not be unconnected to the extensive management of sheep, goat and cattle across the country which may account for the occurrence in RS goat since RSV causes respiratory disease in calves (Ames, 1993; Sarmiento-Silva et al (2012). Hence the need to characterized RSV in small ruminants.

However, the timing and nature of sampling needs to be considered, to prevent inactivation of immunogenic viral epitopes in tissues fixed with formalin (Caswell and Williams, 2007; Ceribasi et al. 2013). Comparable similar effect season and age were observed for RSV and PI3V however the influence of sex could not be evaluated as most of the animals examined were males.

Immunohistochemical detection of the *Pm* and *Mh* antigens has been able to definitely confirm the roles of Pasteurella and Mannheimia in caprine pneumonia, as was suggested by findings of Ojo (1975) and recently by Emikpe et al (2011). The 80% immunopositive lung samples for PM and MH underscores the importance of these pathogens in caprine pneumonia. *Pm* was slightly higher than *Mh* probably due to the role of transport stress. However, the intensity of the staining for *Mh* as compared Pm may further buttress the virulence of the pathogens. Similar protein profiles cross-species immune response have been reported between *P. multocida* and *M. haemolytica*, and considerable induction of humoral and secretory IgA titers were observed in Mh vaccination (Roier et al. 2013). The immune response was actually based on the outer membrane proteins. There were strong correlations between the clinical signs and the severity of lesions as was also observed by Zamri et al (1996). The positive rate of the bacterial antigen detected (79%) similar to that (88%) detect from naturally occurring pneumonic tissues of calves (Haritani et al.1989).

Respiratory diseases had been associated with viral infection, stress conditions and other secondary or co-infections resulting in severe clinical observations (Haanes et al. 1997; Maidana et al. 2012). There are few reports of multiple viral pathogens involvement in caprine pneumonia. In this study, multiple viral aetiologies involving PI3, RSV and PPR with antigens of these viruses in leucocytes, pneumocytes within alveolar spaces and lateral borders of bronchiolar epithelial cells. 8% of the pneumonic caprine lungs showed evidence of PI3, RSV and PPR viral antigens. Though, PPR is still endemic but this study has further shown and highlighted the role of PI3 and RSV in caprine pneumonia. Most of the giant cell pneumonia attributed to solely PPR may have been due to these other viruses or their synergistic effect. Their involvement is proven with the intensity of the immunostaining and the presence of the antigens in the exudates of the alveolar spaces, on the pneumocytes, pulmonary macrophages, bronchi and bronchioles and respiratory

airway epithelium especially in the less purulent form (Narita et al. 2000; Nguyen et al. 2013).

The importance of *Mh* in caprine and ovine pneumonia cannot be overemphasized (Emikpe et al. 2011; Odugbo et al. 2003). However, this study has also underscored the equal importance of *Pm* in caprine pneumonia. This may not be unconnected to stressful conditions like long distance transportation from different part of the country. Thus, for adequate control of caprine pneumonia, these agents should be incorporated into conventional vaccine. The efficacy of a novel (attenuated live vaccine) containing both *M. haemolytica* serotype A1 and *Pasteurella multocida*, was evaluated in calves challenged with *M. haemolytica* serotype A6. Although the challenge was more severe than expected, vaccinated calves had reduced clinical scores, lower mortality, and significantly lower lung lesion scores (Crouch et al. 2012). Vaccines to prevent animal infections caused by *M. haemolytica* and *P. multocida* are commercially available, but not all vaccines have consistently shown benefits in feedlot programs (Fulton, 2009; Griffin et al. 2010).

In conclusion, the immunohistochemical staining employed in this study clearly showed that the presumption that giant cell pneumonia could be associated mainly to PPR in sub Saharan Africa may have not taken cognizance of PI3 and RSV as possible candidates for such findings hence much work is desired in the use of immunohistochemical technique especially in a poor resource setting for a more precise diagnosis, which is a very important tool in the control of caprine pneumonia especially in sub-Saharan Africa. Thus, knowledge of their importance in caprine pneumonia and appropriate characterization of their antigenic epitopes would enhance immune response in goats.

CHAPTER SEVEN

7.0 PROTECTIVE EFFECT OF INTRANASAL PESTE DES PETITS RUMINANTS VIRUSAND *BACTERINE* VACCINATIONS: CLINICAL, HAEMATOLOGICAL, SEROLOGICAL, OXIDATIVE STRESS AND PATHOLOGICAL CHANGES IN CHALLENGED GOATS

7.1 INTRODUCTION

Respiratory diseases are associated with infectious, managements and commingling among animals. The disease may be as a result of viral and bacterial infections (Hodgson et al. 2005; Emikpe et al. 2013). The anatomic features in ruminant lung reduce collateral ventilation and effectiveness of the innate immune response, thus resulting in reduced clearance and ensuing inflammation in the lower respiratory tracts. Our preliminary studies **o**n goats from different regions of Nigeria have shown the dynamics, pattern and type, and risk factors of pneumonia in goats.

Vaccination via the intranasal route has been shown to induce strong and generalized mucosal immunity in the goat (Emikpe et al. 2010; Ezeasor et al. 2015). None the less, caprine pneumonia has defied all the attempts at chemotherapy and use of some available vaccine hitherto, probably due to the bacterial complications of primary viral infection (Emikpe and Akpavie, 2011) or presence of multiple pathogens in the causation of pneumonia in goat. In Nigeria, the current vaccination for peste des petits ruminants PPR has met with myriad challenges and continuous endemicity of pneumonia in goats.

Vaccinal application and use forms one of the historical success of medicine to infectious diseases. And mucosal immunity is an important of defence system against invading pathogens (Ogra et al. 2001). The important viral agents including PPR, Parainfluenza-3 and Respiratory syncytial viruses in caprine pneumonia have been reported in our environment, while role of bacterial agents including *Mannheimia haemolytica* (*Mh)* and *Pasturella multocida* were also reported. However, PPRV and *M. hemolytica* have been

responsible for higher percentages of the pneumonic lesions in the goats. Despite the advances in vaccinology, there is need for update on vaccination strategies against caprine pneumonia.

There is much to be known on enhancing mucosal immune response of the goat especially as it relates to vaccine protection. Ezeasor et al. (2015) observed clinical improvement on intranasal vaccination of PPR in goats while that against *Mh* was not protective (Tenuche et al. 2013). The natural conditions of pneumonia in goats is quite complex because most infections on the field are complicated.

This present study is set to elucidate and evaluate the Protective effect of intranasal PPRV and *Mh* vaccination. This is to proffer different vaccinal strategies and framework needed in the prevention and control of endemic caprine pneumonia in Nigeria.

7.2. MATERIALS AND METHOD

7.2.1 Ethical approval

The study was give approval by the institutional ethical review commiter, University of Ibadan Animal Care Use and Research Ethics Committee. It was assigned UI-ACUREC/17/0060. The guidelines on use of animals were duly followed and the animals were handled humanely to avoid pain.

7.2.2 Experimental Animals

Sixteen West African Dwarf goats aged 6 to 8 months (Lasisi et al. 2002) were acquired from a recognised breeding farm for this study. They weighed between 4.5 to 6 kg. The animals were housed in fly proof pen in the animal house. Upon arrival, they were acclimatized for 14 days and monitored clinically for changes in temperature, respiratory rate, body condition etc. Their blood, sera and faecal samples were taken for screening of blood parasites (cytology), PPR virus (ELISA) and helminthes (floatation) respectively.

The animals were provided fresh water daily, and maintained on ration of bean husks, and maize bran and forages (*Cyandon plectostacyus*) during acclimatization and period of experiment. They were further conditioned for four weeks before start of experiment.

7.2.3 Experimental Design

The study followed a randomized complete block design, whereby the animals were randomly grouped A-D and vaccinated within two weeks using PPRV linage 1 vaccine (Nig/75), *M. hemolytica* and *P. multocida* bacterines. The groups comprised 4 goats each tagged on the neck for easy identification.

7.2.3.1 PPR virus Vaccine

The lyophilized PPRv vaccine which is of the linage 1 (Nig/75) was sourced from National Veterinary Research Institute (NVRI), Vom Nigeria.

7.2.3.2 Bacterine preparation

A colony of each of *M. haemolytica and P. multocida already* isolated from pneumonic goats were each inoculated into 5mL of TSB, for incubation at 37°C for 18–24 h in shaking incubator. The broth cultures were spinned at a speed of 6000 rpm at a temperature of 4°C for 15 minutes. Afterwards the top was discarded, for recovery of cell pellets at the bottom which was washed in acetone twice. Also, the pellets were washed twice with diethyl ether. The pellet was re-suspended in 1% formalin saline. Few drops of Tween 80 was included to the suspension at a final concentration of 3%–4%. The procedure was repeated three times with saline solution. The inactivated bacterial cells were now suspended to a final concentration of 4×10^9 cells/mL.

7.2.3.3 Vaccination schedule

The animals were vaccinated as indicated in each group below. 1ml PPRv vaccine and 1ml of the bacterine were sprayed intranasally to each goat. The animals were now observed for three weeks post vaccination.

A. PPR + Mannheimia haemolytica (*Mh*)
B. PPR + Pasteurella multocida (*Pm*)
C. PPR only
D. Control (negative)

7.2.3.4 Experimental challenge

All the vaccinated animals were housed together under the same roof. A known infected WAD with apparent clinical signs of PPR was introduced into the pen mimicking field condition of the virus (Radostis et al. 2006; Oma et al. 2016). All the animals were allowed to comingle with clinical observations taken daily for another five weeks post infection.

7.2.4 Clinical studies

The respiratory rate was taken for each goat when calm, by placing a hand on its side if necessary, the inhalation or expansion (rises and falls) of the chest wall was counted each for 60 seconds. The quality of the respirations were noted (normal respiration rate for an adult goat is 10 to 30 breaths per minute, and for a kid it is 20 to 40 breaths per minute).

The body temperature was taken and recorded from the rectum using the thermometer. The general body condition was evaluated and score systemically as described by Battaglia (2001) on a scale of 1-5 (amount of fat and muscle at key anatomical points); 1= very thin (poor), 2= thin (fair), 3= normal (good), 4=fat (obese) and 5= (very obese). However, the goats were either scored as good, fair or poor.

4ml of blood was collected weekly from each goat by jugular venipuncture using a clean sterile disposable syringe and needle into plain and heparinised tubes for serological and haematological analysis respectively.

The haematological studies were carried out manually as outlined by Jain (1986). Thin blood smears were made on glass slides, fixed in absolute methanol and stained with Romanousky Giemsa. The slides were viewed for blood parasites. Packed cell volume (PCV) determination was done using the Hawksky microhaematocrit centrifuge set at 10,000 r.p.m for 5minutes. PCV capillary tubes were read using the graphic reader. Plasma Protein concentration was determined using the Goldberg refractometer (TS meter, American Optical Scientific Instrument Division Buffallo, N.Y. U.S.A). Total Red Blood and White Blood Cell Counts were done using the improved Hawksley haemocytometer.

Enzyme linked immunosorbent assay (ELISA) for the determination of serum antibodies in goats: PPR c-ELISA kit (CIRAD-EMVT, Montpellier, France) designed to detect antibodies directed against the N protein of PPRV was used.

Negative/Positive cut-off O.D. value: The average of negative control plus 3 times standard deviation (3 x SD) was taken as the cut-off value. This was determined separately for each plate.

Positive Test Animals- An animal was considered positive for PPRV antibody. This was used to express the proportion of positive animals.

7.2.5 Pathological evaluations

7.2.5.1 Sacrificed animal was put to sleep using pent-barbiturate intravenously at 130mg/kg body weight. The carcasses were necropsied for detailed examination of the lungs. Samples from the Lymph nodes, Lung, Spleen and Intestines were taken.

7.2.5.2 Gross evaluation of pneumonia and histological examination was as described earlier.

7.2.5.3 Bronchoalveolar lavage fluid analysis followed same procedures of our earlier studies.

7.2.5.4 Tissue from the mediastinal lymph nodes, lungs, and spleen were taken for microbiological examination following standard proceures and as described earlier.

7.2.5.5 Immunohistochemistry procedure was the ABCkit (M IHC Select Detection System, HRP/DAB, Merck, Germany. LOT: 2775482) with slight modification of the procedure and as described previously.

7.2.6 Statistics: Data presented in frequencies, percentages, Mean±SD and using appropriate parametric statistics at 5% significance.

7.3 RESULT

7.3.1 *Clinical Observations*

Group A (PPRV + *Mh* vaccinated): There was no remarkable change in the weight of the goats (Figure 7.1). The goats showed slight pyrexia in the 1st week post vaccination, but which normalized 4 days later. There was a mild serous ocular discharge, and the body condition was good.

Group B (PPRV + *Pm* vaccinated): The animals did not show signs of weakness until around 11th day post infection. There was moderate pyrexia, serous to purulent ocular discharge. One of the goat had a fair body condition score due to roughness of hair coat and dehydration.

Group C (PPRV only): Two of the goats showed dyspneoa due to the severe occluding catarrhal discharge from the nostrils and copious salivation. There was pyrexia on 5th day post infection, muco-purulent ocular discharge, weakness, dehydration and two had diarrheoa after two weeks. A mortality was recorded on the 22nd day post infection.

Group D (Unvaccinated/control): There was pyrexia which peaked 4 days post exposure. The goats were dehydrated due to the severe diarrheoa observed 1 week post-exposure. There was mucopurulent ocular discharges, palor of mucous membranes, crusting and congestion of the conjunctiva, anorexia, dyspneoa and erosion on the dental pad. Two goats died at 13th and 28th days post infection.

Plate 7.1a. Goat vaccinated with PPR+Mh active few days post infection.

Plate 7.1b. Goat with muco-purulent oculonasal discharge (yellow arrow), swollen face, rough hair coat (white arrow) and diarrhoeic faeces (red arrow) few days post infection.

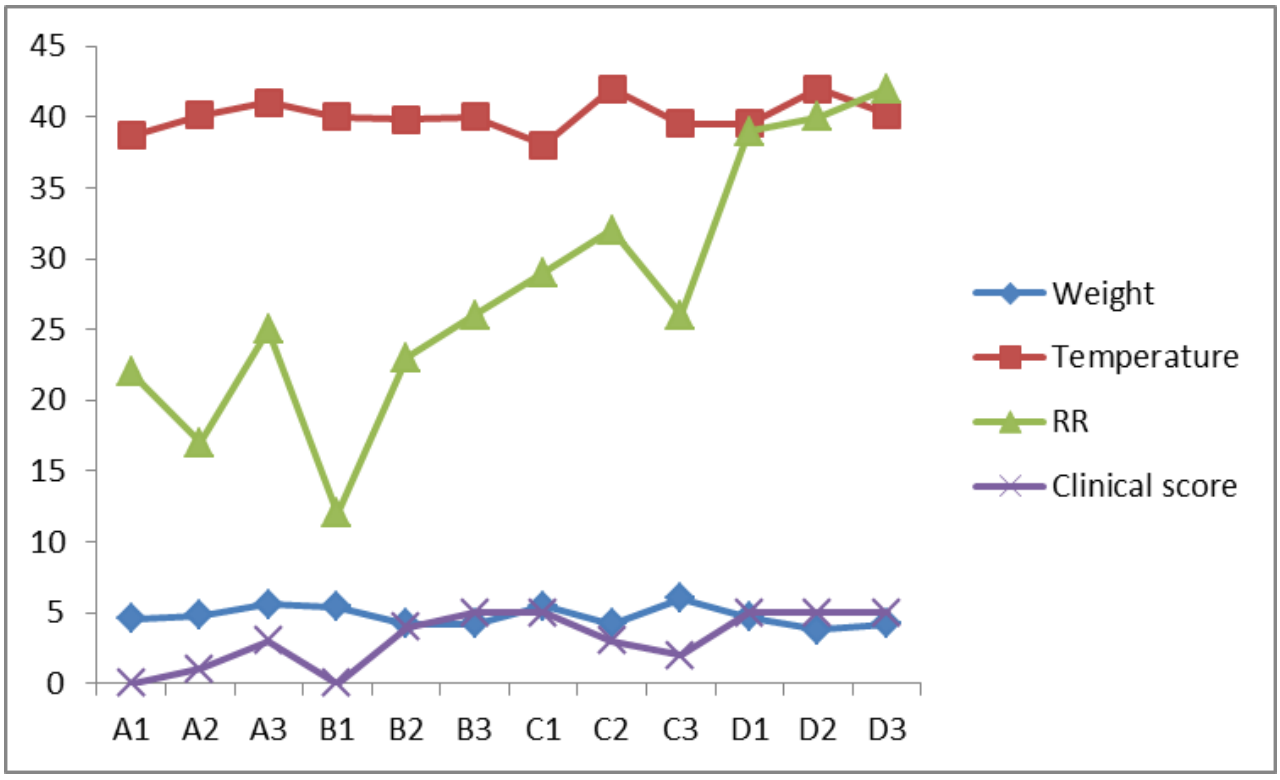


Figure 7.1 Clinical examination parameters from the experimental goats

7.3.2. Haematological observations

Group A (PPRV + *Mh* vaccinated): The haematocrit, haemoglobin concentration and red cell counts were with reference range before and after vaccination, and 6weeks PI. The leucocytes and platelets were also within reference range except with slight leukocytosis observed 6weeks PI ($\alpha>0.05$).

Group B (PPRV + *Pm* vaccinated): The erythrogram, leucogram and platelet counts were also in range. (Table 7.1).

Group C (PPRV only): There was slight anaemia, moderate leucocytosis and moderate neutrophilia ($\alpha<0.05$).

Group D (Unvaccinated/control): There was marked anaemia, moderate leukopaenia, thrombocytopaenia, lymphocytopaenia, neutrophila and marked hypoproteinaemia ($\alpha<0.05$).

7.3.3. Serum Protein and Oxidative stress parameter changes

Group A (PPRV + *Mh* vaccinated): The plasma protein, albumin and immunoglobulin values were within range range and not different in the goats. The SOD, GSH, and GPx activities decreased slightly, while MPO content, MDA and H_2O_2 generation increased ($\alpha>0.05$).

Group B (PPRV + *Pm* vaccinated): The plasma protein, albumin and immunoglobulin values were within range range (Table 7.2). The SOD, GSH, and GPx activities decreased slightly, while MPO content, MDA and H_2O_2 generation increased moderately ($\alpha<0.05$).

Group C (PPRV only): There was hypoglobulinaemia, decreased SOD, GSH, and GPx activities, increased MPO content, MDA and H_2O_2 generation ($\alpha<0.05$).

Group D (Unvaccinated/control): There was marked hyoproteinaemia, hypoalbuminaemia and hypoglobilinaemia ($\alpha<0.05$). General reductions in SOD, GSH, and GPx activities, and increased MPO content, MDA and H_2O_2 generation ($\alpha<0.05$).

7.3.4 Serology

The Percentage inhibition (PI) titre of PPR antibodies in the goats is shown in Figure 7.2. The highest titre was in the goats vaccinated with PPRV + Mh, and the least titre in unvaccinated goats ($\alpha<0.05$).

Table 7.1: Haematological changes in the experimental goats exposed to PPR infection

		PCV * %	HB * g/dl	RBC * x10^3/µL	WBC X10^3/µL	PLT X10^3/µL	LYM x10^3/µL	NEUT x10^3/µL	M0N x10^3/µL	EOS * x10^3/µL	NL *	MCV * fL	MCH * pg	MCHC * g/dl
	A	32.3±2.3	10.5±0.9	11.6±0.7	7.0±1.4	1.4±0.3	5.0±1.2	1.7±0.2	0.1±0.0	0.1±0.0	0.4±0.0	27.9±0.9	9.0±0.4	32.4±0.7
Pre Vacc	B	33.0±2.7	10.9±1.2	11.3±0.6	9.6±0.9	1.4±0.2	6.4±0.6	2.7±2.4	0.2±0.1	0.3±0.1	0.4±0.0	29.1±1.7	9.6±0.7	32.8±1.1
	C	33.0±2.9	10.9±1.1	12.6±0.7	13.5±1.4	1.9±0.1	8.8±0.7	3.9±1.1	0.3±0.1	0.4±0.2	0.5±0.1	26.7±3.7	8.8±1.3	32.9±0.8
	D	28.3±3.2	9.3±1.3	10.7±0.3	9.5±0.5	1.4±0.3	6.4±0.3	2.7±0.3	0.3±0.0	0.2±0.1	0.4±0.1	26.4±2.5	8.7±1.0	32.8±1.1
	A	29.0±1.7	9.2±0.4	10.6±0.3	13.3±4.6	2.5±0.3	7.9±2.2	4.7±2.1	0.3±0.1	0.4±0.1	0.5±0.1	27.5±1.1	8.7±0.2	31.8±0.7
Post	B	33.7±1.8	11.0±0.6	12.3±0.6	11.4±3.9	2.8±0.1	7.7±2.4	3.2±1.3	0.2±0.1	0.3±0.1	0.4±0.1	27.5±0.4	9.0±0.1	32.7±0.2
Vacc	C	30.7±1.2	10.1±0.6	11.0±0.7	13.3±3.5	2.6±0.3	8.3±2.2	4.5±1.3	0.2±0.1	0.3±0.1	0.6±0.1	28.1±0.7	9.2±0.2	32.8±0.3
	D	33.3±0.7	10.9±0.3	12.0±0.4	9.6±2.2	3.4±0.4	6.5±1.5	2.8±0.8	0.2±0.1	0.2±0.1	0.4±0.0	27.8±0.4	9.1±0.0	32.8±0.3
	A	30.0±1.5	8.5±1.0	8.6±1.3	14.4±2.1	2.0±0.5	6.6±0.5	6.8±1.6	0.6±0.1	0.4±0.1	1.0±0.2	20.1±1.3	6.7±0.1	33.5±2.6
Post	B	27.7±0.9	8.6±0.2	9.6±0.6	11.2±1.3	1.7±0.3	5.6±0.9	5.0±0.2	0.4±0.1	0.2±0.1	0.9±0.1	18.7±2.1	6.4±0.3	34.5±2.3
Infect	C	19.3±0.3	6.5±0.2	9.9±0.4	17.5±2.1	2.1±0.3	6.5±1.2	7.4±0.8	0.4±0.1	0.2±0.0	0.9±0.1	19.6±1.1	6.5±0.2	33.5±1.4
	D	11.7±6.0	3.4±1.7	4.9±2.4	3.8±3.9	1.1±0.6	1.0±2.2	2.2±1.6	0.4±0.2	0.2±0.1	0.5±0.3	16.0±8.2	4.6±2.3	19.4±9.7

PCV- packed cell volume, HB- haemoglobin concentration, RBC- red blood cell count, WBC- white blood cell count, PLT- platelets, LYM- lymphocyte, NEUT- neutrophil, NL- neutrophil-lymphocyte ratio, MCV- mean corpuscular volume

Table 7.2: Protein and Oxidative stress parameter changes in the experimental goats exposed to PPR infection

		TP * g/dl	ALB * g/dl	IG * g/dl	SOD unit $mg^{-1}p$	GSH $\mu g\ mL^{-1}$	GPx unit $mg^{-1}p$	MPO µmol/min	MDA µmol/mL	H_2O_2 µmol mg^{-1} p
Pre vacc	A	8.4±0.1	3.5±0.1	4.9±0.1	11.0±2.6^a	9.0±2.5^a	16.0±4.5^a	1.1±0.5^a	1.0±0.1^a	1.5±0.3^a
	B	7.9±0.3	3.1±0.3	4.8±0.0	11.5±3.0^a	8.9±3.0^a	16.4±5.5^a	1.8±1.0^b	1.2±0.1^a	1.0±0.5^b
	C	7.8±0.5	3.1±0.4	4.7±0.4	9.9±2.8^a	11.0±3.0^a	16.0±4.0^b	1.2±4.0^b	0.8±0.1^a	2.4±0.4^b
	D	8.2±0.3	3.5±0.3	4.7±0.2	9.9±2.5^a	10.9±3.0^a	15.8±4.8^a	2.8±1.0^c	1.0±0.1^b	1.5±0.7^b
Post Vacc	A	8.4±0.1	3.5±0.1	4.9±0.1	10.0±2.5^a	8.8±2.1^a	15.8±4.2^a	1.1±0.5^a	1.0±0.1^a	1.5±0.3^a
	B	7.9±0.3	3.1±0.3	4.8±0.0	11.3±3.1^a	9.1±2.7^a	18.0±5.6^a	1.8±1.0^b	1.2±0.1^a	1.0±0.5^b
	C	7.8±0.5	3.1±0.4	4.7±0.4	10.7±3.1^a	10.0±2.8^a	16.9±4.1^b	1.2±4.0^b	0.8±0.1^a	2.4±0.4^b
	D	8.2±0.3	3.5±0.3	4.7±0.2	9.9±2.8^a	10.0±3.1^a	17.2±5.8^a	2.8±1.0^c	1.0±0.1^b	1.5±0.7^b
Post Infec	A	8.5±0.3	3.6±0.1	4.9±0.2	9.8±1.8^a	8.9±1.5^a	16.4±2.3^a	2.2±1.5^a	1.0±0.1^a	3.2±2.2^a
	B	7.3±0.4	3.2±0.4	4.1±0.0	6.4±0.8^a	6.4±0.6^a	12.6±1.9^a	14.8±2.9^b	1.8±0.1^a	8.0±2.5^b
	C	7.8±0.3	4.7±1.7	3.0±1.5	4.8±0.4^a	3.9±0.2^a	9.9±2.5^b	18.1±4.4^b	1.8±0.1^a	8.4±2.5^b
	D	4.7±2.4	1.9±1.0	2.8±1.4	5.4±0.9^a	3.6±0.3^a	9.4±2.2^a	24.5±3.0^c	2.1±0.1^b	9.5±2.9^b

TP- total protein, ALB- albumin, IG- immunoglobulin, SOD- Superoxide Dismutase, GSH- Reduced Glutathione, GPx- Glutathione peroxidase, MPO- Myeloperoxidase, MDA- Malondialdehyde, H_2O_2- Hydrogen peroxide

7.3.5. Bronchoalveolar lavage fluid cellular and oxidative stress changes

Group A (PPRV + *Mh* vaccinated): There was increase in macrophage (54.7%) and lymphocyte counts (42.7%) fig . The antioxidant activities (GPx, GSH, GST and SOD) were high while prooxidant content was very low (Table 7.3).

Group B (PPRV + *Pm* vaccinated): There was increase in neutrophil counts (33.3%). Total protein and MPO content increased significantly ($\alpha<0.05$).

Group C (PPRV only): There was increase in neutrophil counts (33.3%). The total protein content increased, antioxidant enzymes reduced, while the proxidants increased significantly ($\alpha<0.05$).

Group D (Unvaccinated/control): There was increase in neutrophils, and plasma cell counts. Also, the total protein content increased, antioxidant enzymes reduced, while the proxidants increased significantly ($\alpha<0.05$).

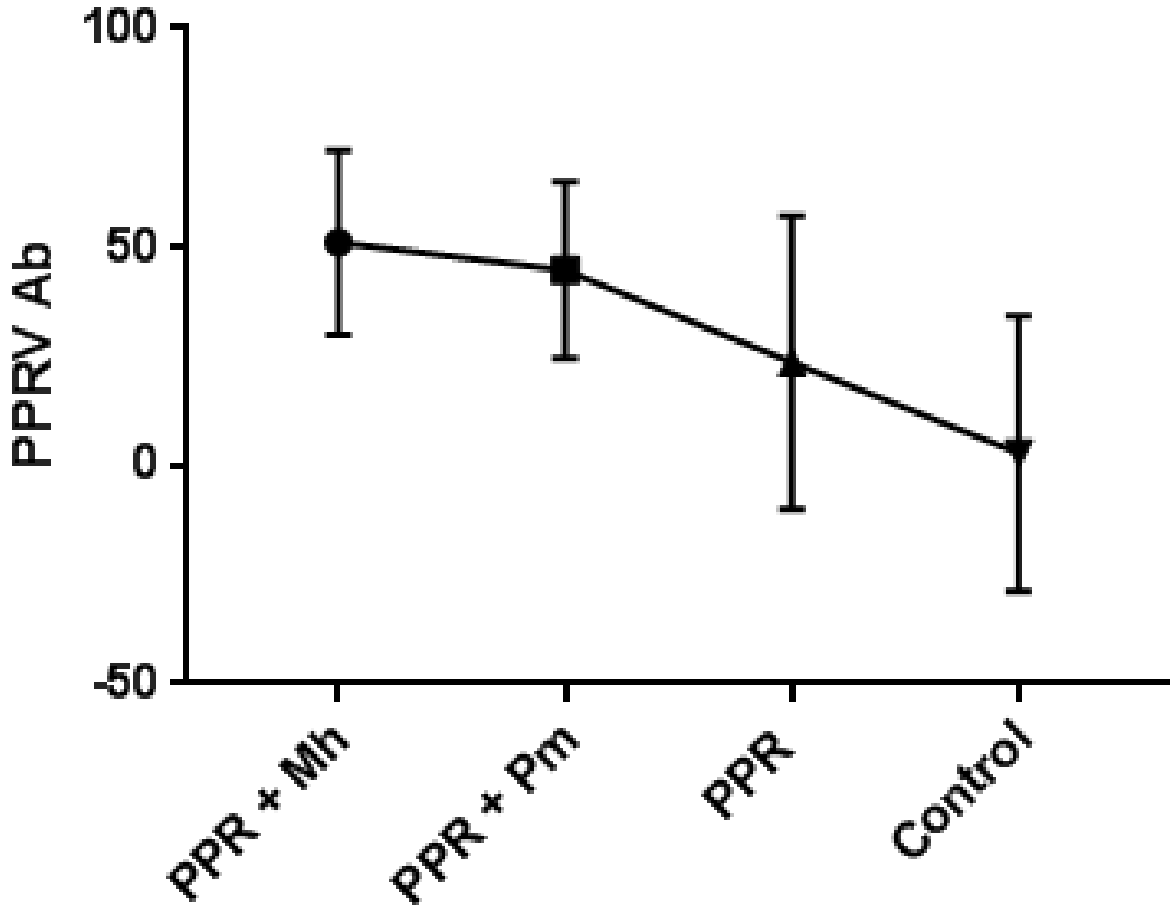


Figure 7.2: Percentage inhibition (PI) titre of PPR antibodies from sera samples of the goats using cELISA.

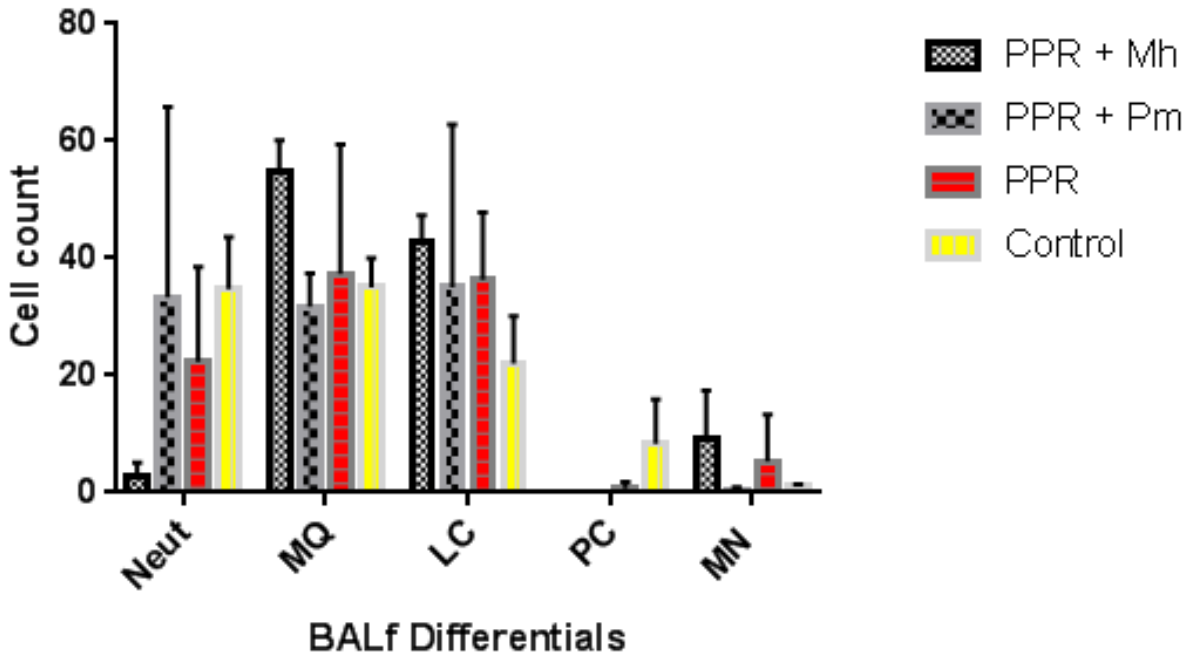


Figure 7.3. Bronchoalveolar lavage fluid cellular changes in the experimental goats

Table 7.3:Bronchoalveolar lavage fluid oxidative stress

Group	TP mg/ml	GPX units mg^{-1}	GSH µg mL^{-1}	GST unit $mg^{-1}p$	SOD unit $mg^{-1}p$	H_2O_2 µmol mg^{-1} p	MDA µmol/mL	MPO* µmol/min
A	9.56 ± 0.7^{a}	24.1 ± 1.5^{a}	9.9 ± 0.3^{a}	8.0 ± 1.1^{a}	4.1 ± 0.3^{a}	3.6 ± 0.5^{a}	2.3 ± 0.1^{a}	2.0 ± 0.2^{a}
B	11.6 ± 1.1^{a}	21.4 ± 1.7^{a}	9.4 ± 0.2^{a}	6.5 ± 1.0^{b}	3.6 ± 0.3^{a}	5.0 ± 0.4^{b}	2.1 ± 0.1^{a}	16.0 ± 0.1^{b}
C	16.7 ± 1.7^{b}	18.9 ± 1.4^{b}	9.7 ± 0.1^{a}	7.0 ± 0.8^{a}	3.2 ± 0.2^{a}	4.9 ± 0.3^{b}	2.3 ± 0.1^{a}	20.0 ± 0.1^{b}
D	13.5 ± 1.7^{a}	20.1 ± 1.8^{a}	9.5 ± 0.1^{a}	6.3 ± 0.9^{b}	3.4 ± 0.3^{a}	5.0 ± 0.6^{b}	4.43 ± 0.1^{b}	30.0 ± 0.2^{c}

TP- total protein, ALB- albumin, IG- immunoglobulin, SOD- Superoxide Dismutase, GSH- Reduced Glutathione, GPx- Glutathione peroxidase, MPO- Myeloperoxidase, MDA- Malondialdehyde, H_2O_2- Hydrogen peroxide

PPR + Mh vac
PPRV + Pm vac
PPRV only
Unvaccinated control

7.3.6. Pathology

The consolidation pattern and lesions are shown in figure 7.4 and Table 7.4 respectively.

Group A (PPRV + *Mh* vaccinated): The lungs were collapsed and pinkish (normal) except a goat with patchy consolidation on the middle lobe. The alveoli were normal microscopically (figure 7.2).

Group B (PPRV + *Pm* vaccinated): The lungs showed patchy or lobular consolidation on the cranial lobes, and a few inflammatory cells in the alveolar spaces (figure 7.3).

Group C (PPRV only): There was a severe fibrinous pleuropneumonia adhering to the rib cage, and the lungs showed cranioventral consolidation affecting upto 68% of the cranial lobes. Micrroscopically, fibrinous exudate and inflammatory cells comprising chiefly neutrophils and macrophages occupied the brochiolar lumen and alveoli (figure 7.4)

Group D (Unvaccinated/control): The lungs were diffusely dark red (plate 7.5a) and consolidated (62%), there were also patches/lobular consolidation including the 85% of the assessory lobe (plate 7.6b). Micoscopically, there were numerous inflammatory and fibrinous exudate and giant cells in the alveoli and interstitial spaces (plate 7.6b).

7.3.7. Bacteria Isolation

A few organisms were isolated from the sacrificed goats after exposure to PPR infection (Table 7.5). *Mannheimia haemolytica* was cultured from the pneumonic lesions of goats vaccinated only with PPR and unvaccinated (Plate 7.6), while *Pasteurella multocida* and *Escherichia coli* were the other agents identified.

7.3.8. Immunolocalisation of the antigens

Mannheimia haeomolytica antigens were located in alveolar macrophages and inflammatory exudate (Plate 7.7). PPR viral antigens were detected in bronchiolar epithelium (plate 7.6) and flooding the alveolar epithelial cells and leucocytes in the pneumonic lungs (Plate 7.7).

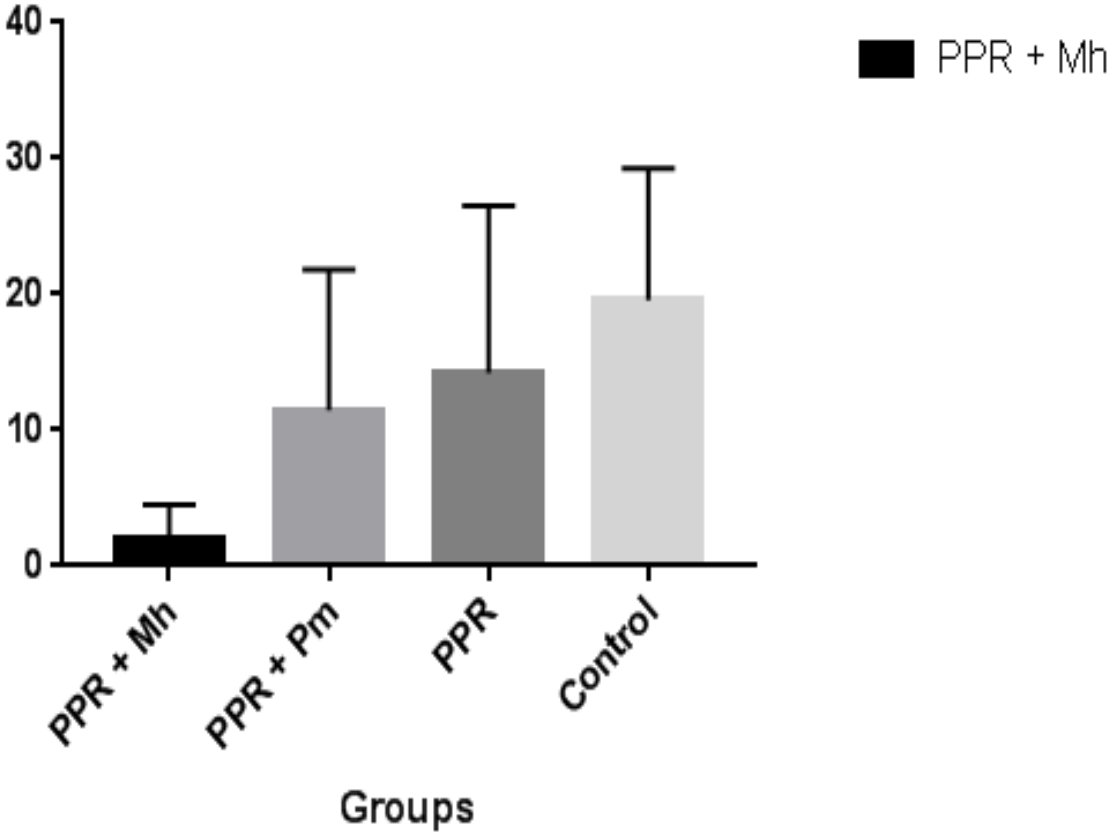


Figure 7.4: Lung consolidation score in the experimental goats A-D

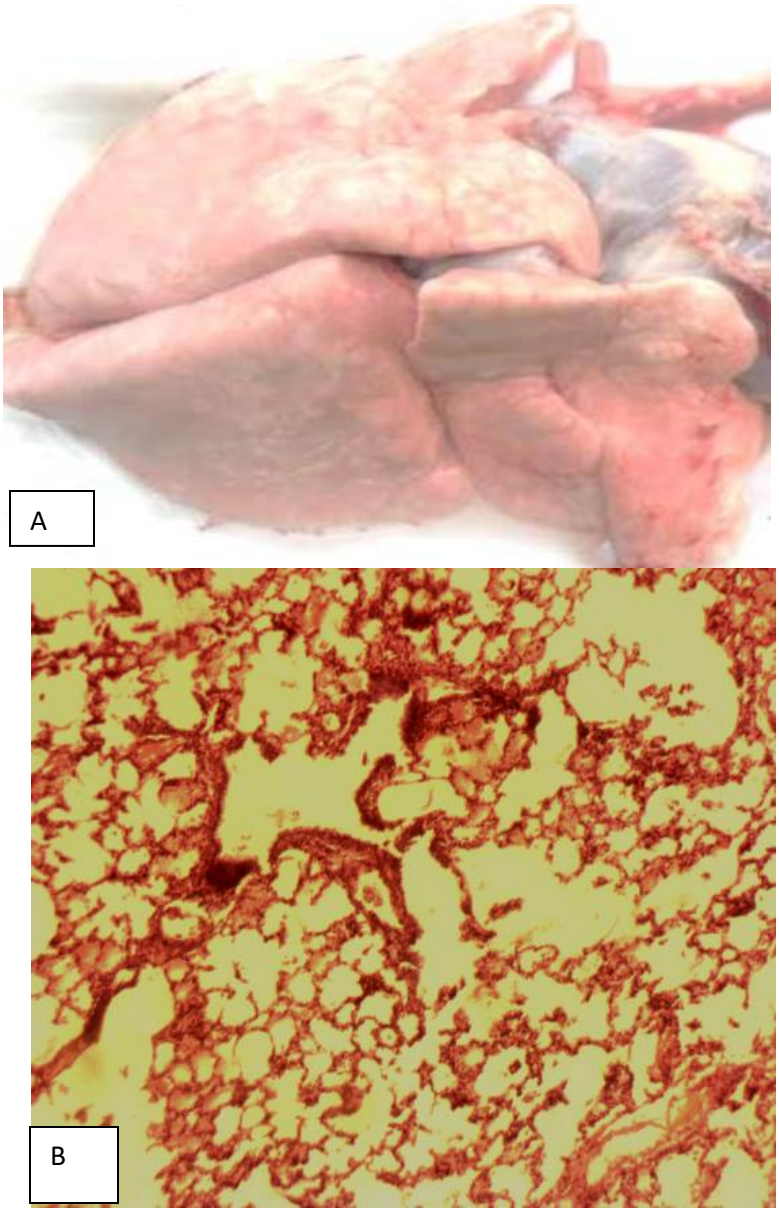


Plate 7.2- Normal lung from a goat vaccinated with PPR + *Mh* and exposed to PPR infection. A- Gross and B- Microscopic. (HE x100).

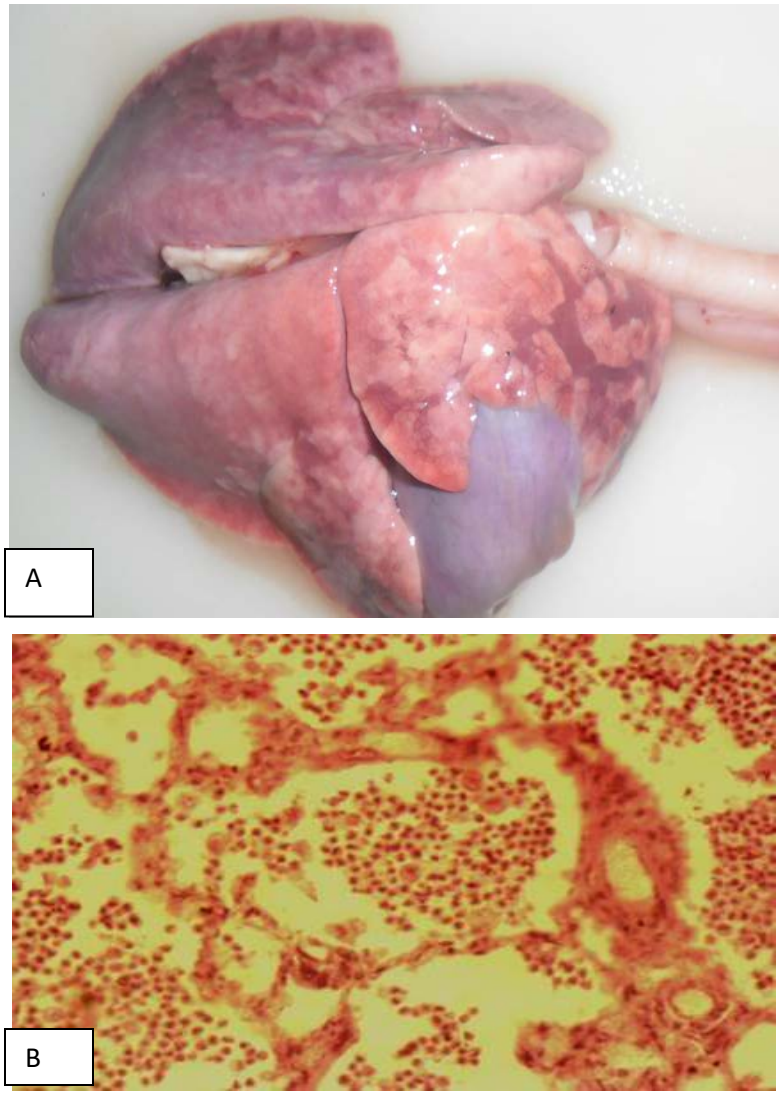
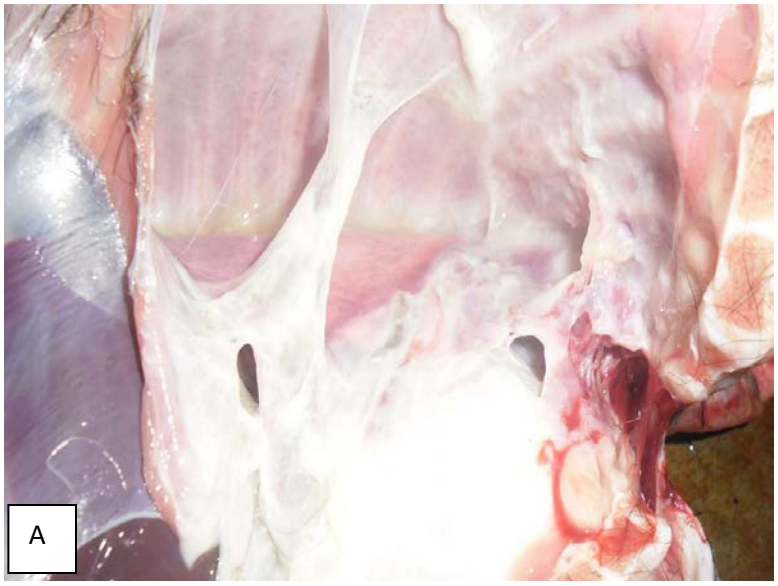


Plate 7.3- Lungs with patchy lobar consolidation (A) due to moderate infiling of alveolar spaces by neutrophils and macrophages (B) from goat vaccinated with PPR + *Pm* and exposed to PPR infection. HE x100



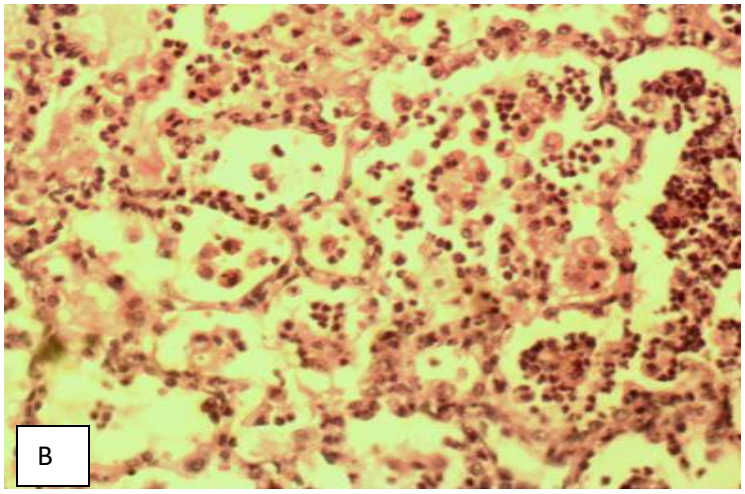
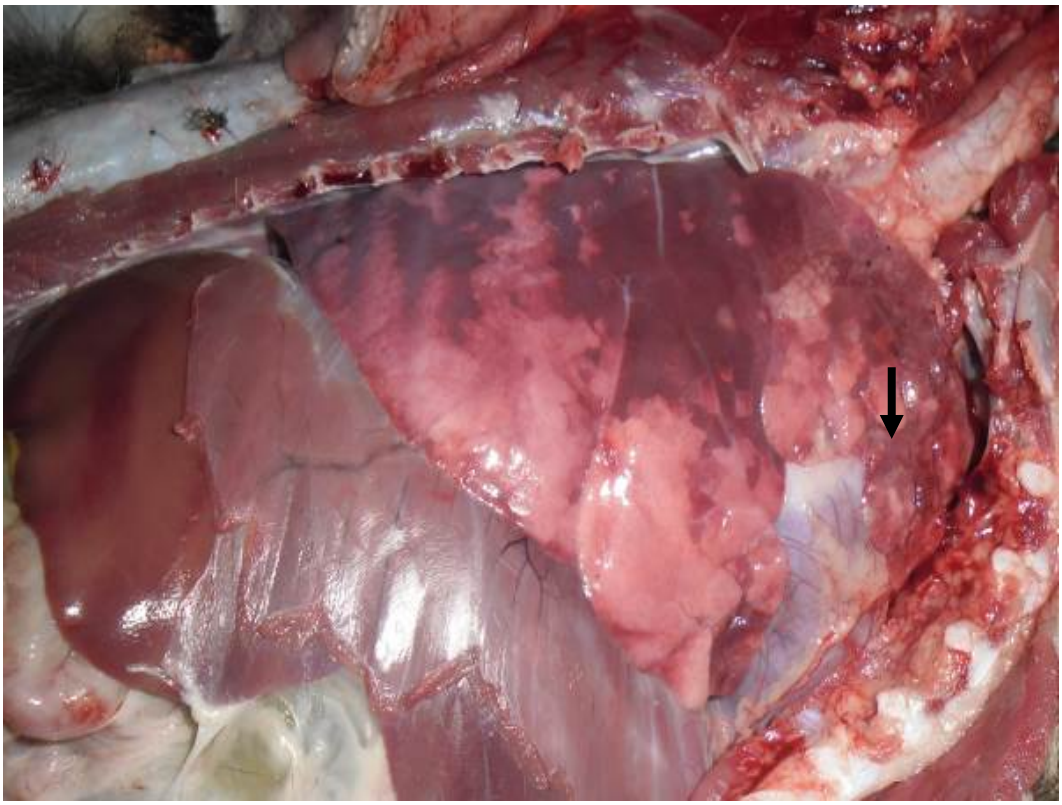


Plate 7.4: -Lung with marked adhesive fibrinopleuropneumonia from a goat vaccinated and exposed to PPR infection (A). There are neumrous fibrinous and cellular exudate in alveolar spaces (B). HE x100



Plates 7.5a: Diffuse pneumonia and cranio-ventral consolidation of the lung (arrow) from an unvaccinated goat exposed to PPR infection .

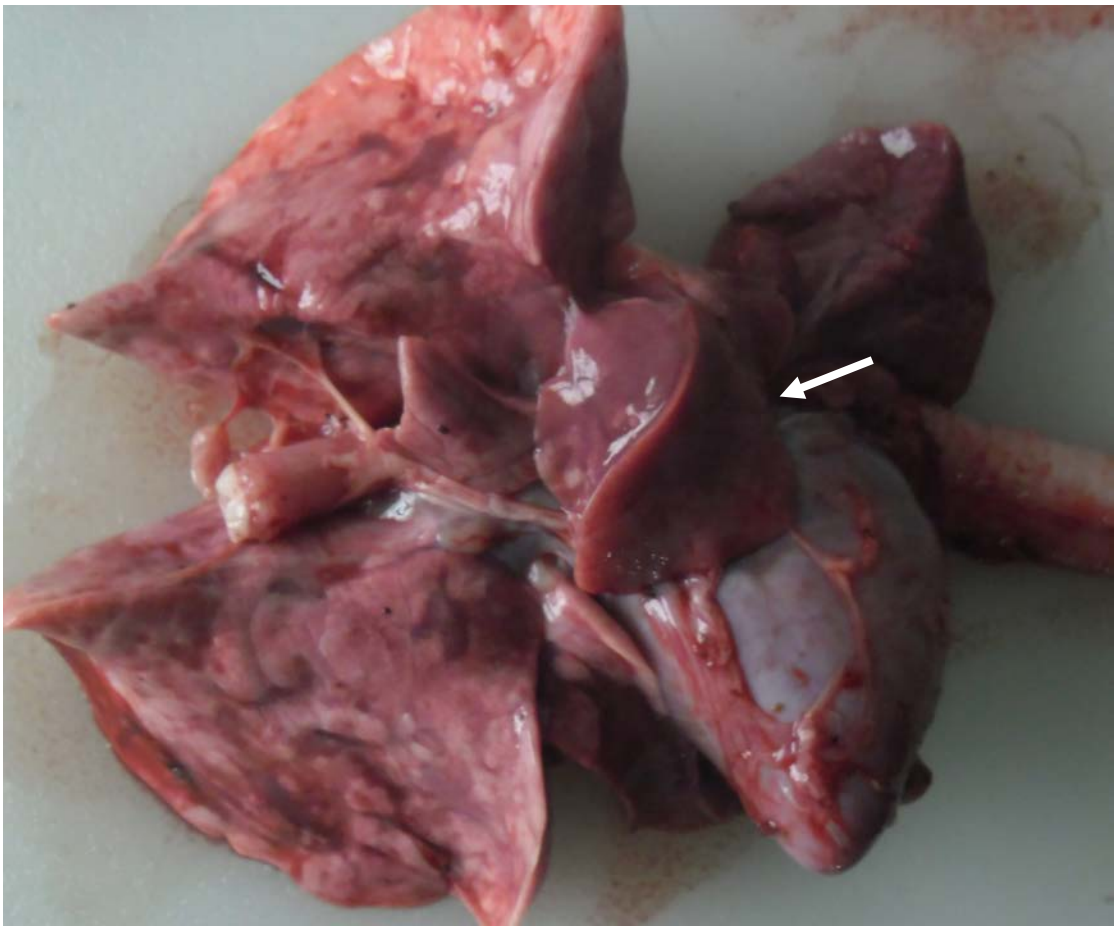
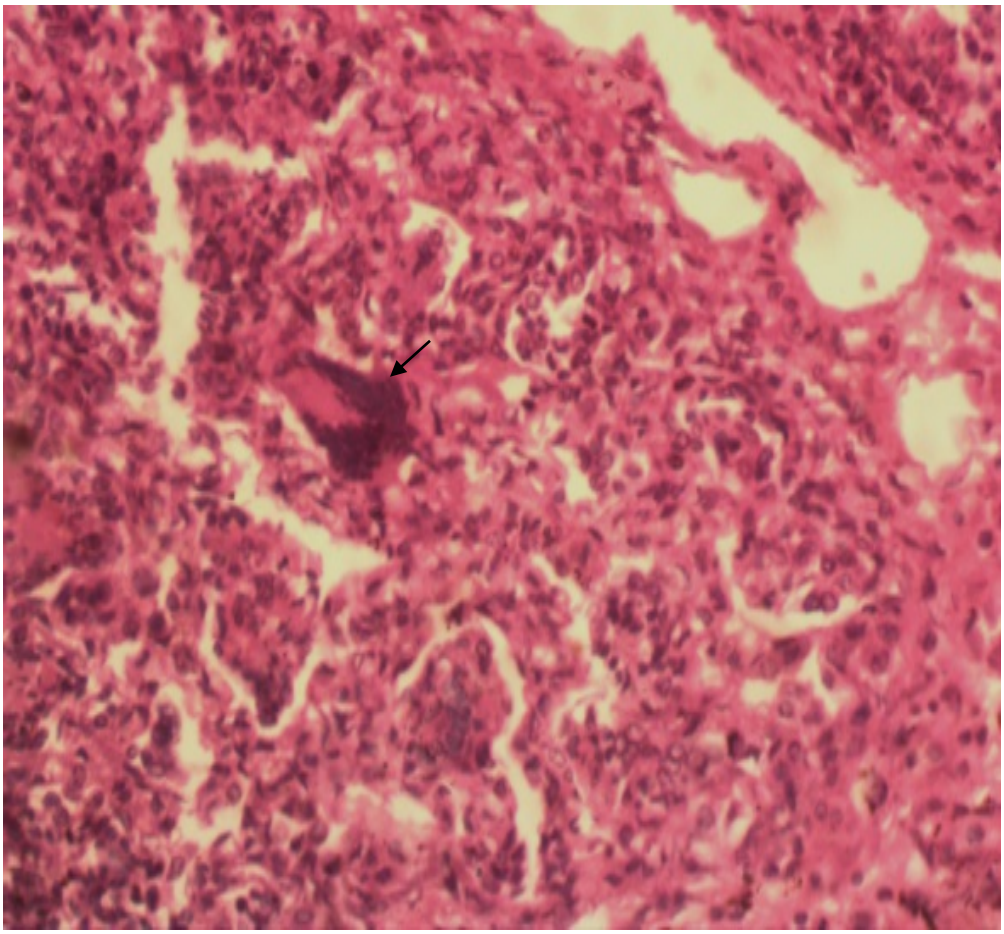


Plate 7.5b. The lungs are dark red, non-collapsed and firm, and the assessory lobe is 96% consolidated (arrow), in a goat unvaccinated and exposed to PPR infection.



Plates 7.5c: Broncho-interstitial pneumonia with giant cells (arrow) and fibrinopurulent exudate in lung from an unvaccinated goat exposed to PPR infection . HE x400

Table 7.5. Bacterial pathogens isolated microbiologically from the goats in the different groups exposed to natural PPR infection

Isolate	A	B	C	D
E. coli	+	++	-	-
Pasteurella sp.	+	+	+	+
Mannheimia h.	_	++	+++	+++

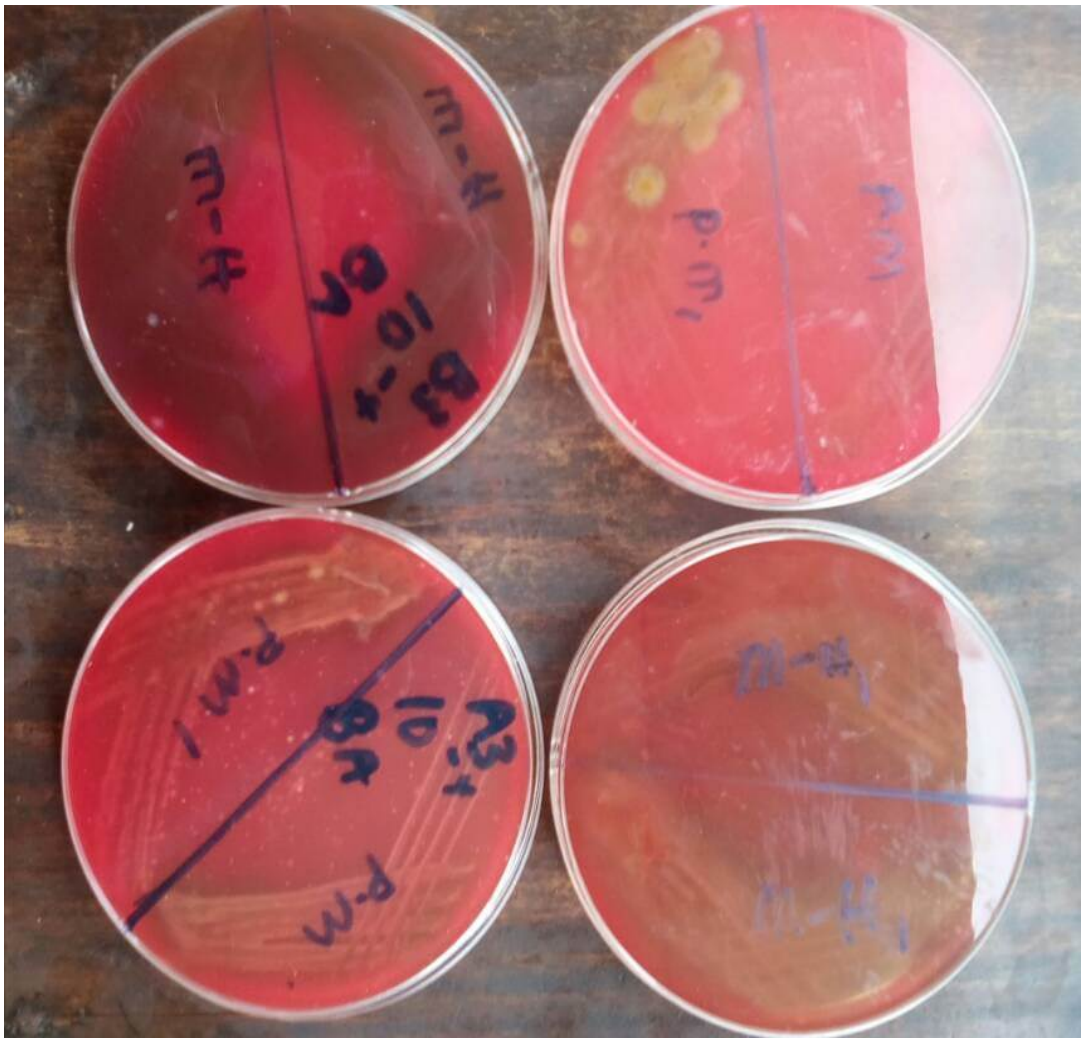


Plate 7.6:Petri dishes showing colonies of the bacterial growth on blood agar

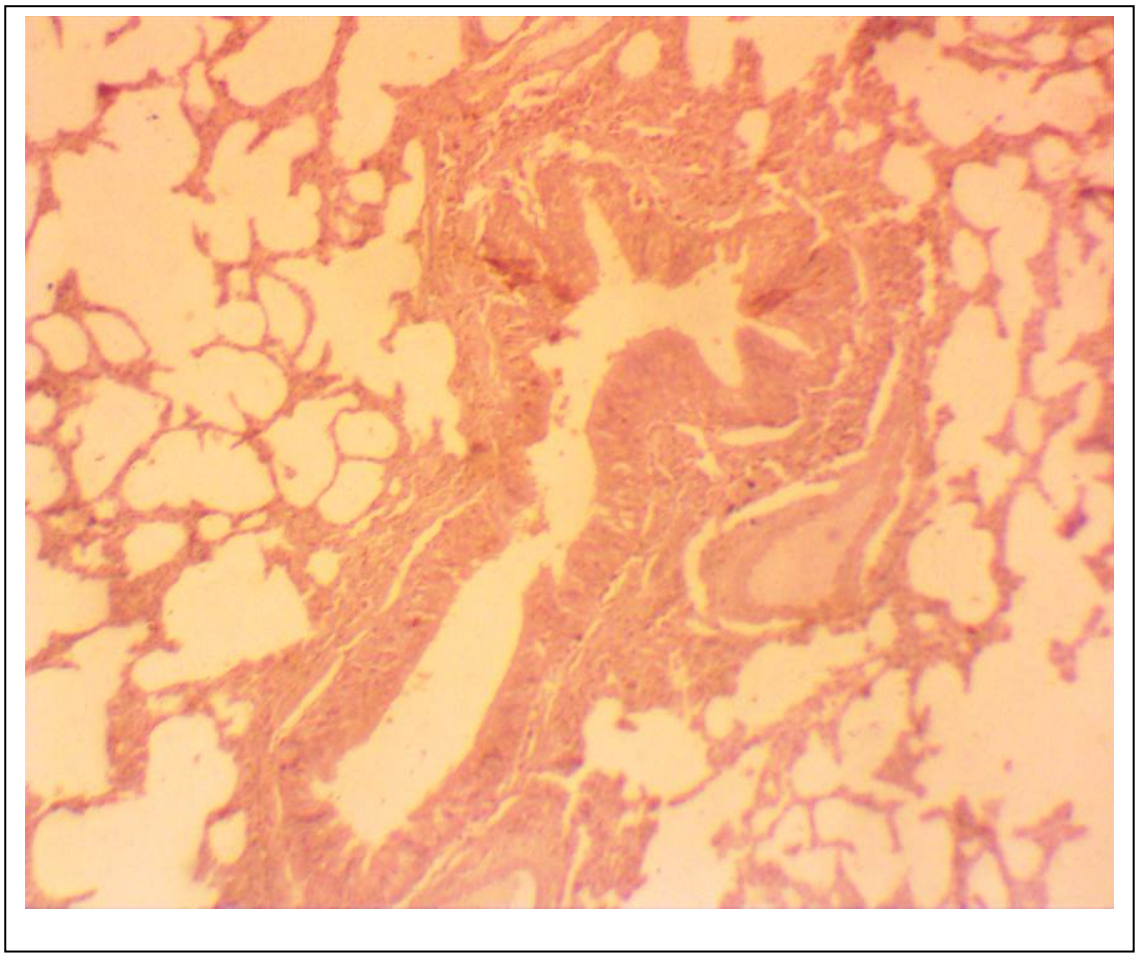


Plate 7.7: Immunolocalisation of PPRV antigens in bronchiolar epithelium of goat in PPR+MH vaccination challenged. ABC HRP counterstained with haematoxylin. x400

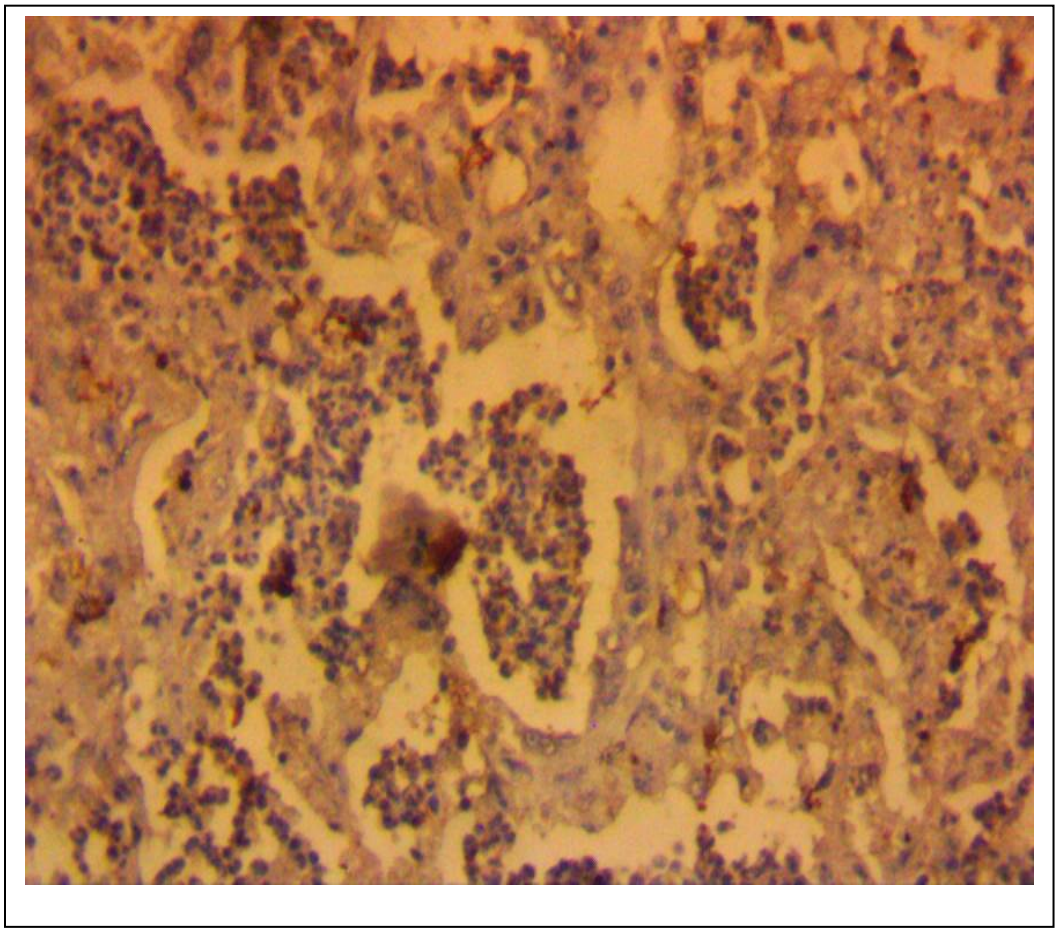


Plate 7.8: Immunolocalisation of Mannheimia haemolytica antigens in pneumonic lung from PPR only vaccinated goat infected with PPR. ABC HRP counterstained with haematoxylin. x400

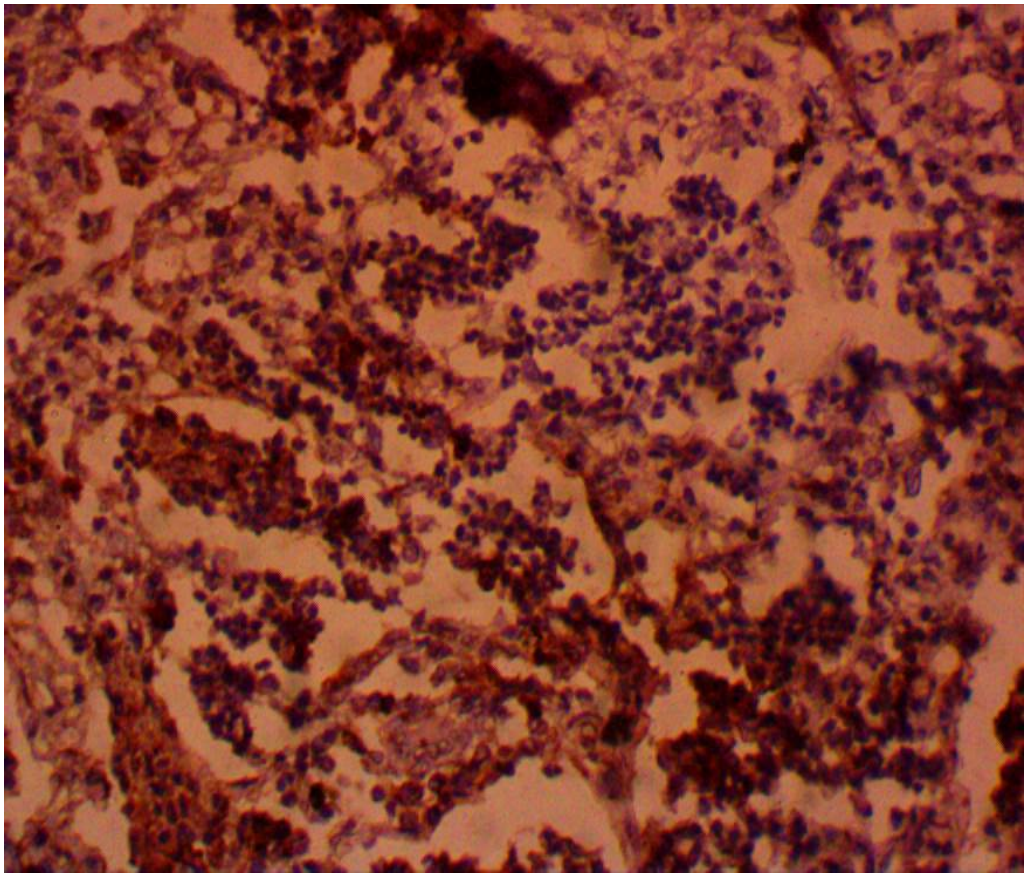


Plate 7.9: PPRV antigens in pneumonic lung from an unvaccinated goat.. ABC HRP/DAB counterstained with haematoxylin. x400

7.4 DISCUSSION

This present study evaluated the Protective effect of intranasal PPRV and *Mh* vaccination to challenged goats. Viral complicated bacterial pneumonia is very fulminating in small ruminants and the losses of income (Hu et al. 2012). The intranasal combined PPR and Mh vaccines elicited better protection against clinical PPR disease.

The clinical changes observed in the infected goats were similar to earlier findings in both experimental and natural infections of PPR in goats (Obi, 1984; Aytekin et al. 2011; Emikpe and Akpavie, 2011). All the unvaccinated goats (D) showed severe signs of respiratory distress due to the pulmonary consolidation and blockage of the nasal passages and airways. The progressive decline in the body weight 2 weeks post infection was largely due to the dehydration and anorexia.

The anaemia, leucopaenia and haemoconcentration observed in goats with clinical pneumonia were to that reported (Obi et al. 1983; Olaleye, 1985; Ezeasor et al. 2014). The dehydration in the infected animals probably contributed significantly to the weakness of the animals.

The intranasal vaccination with combined PPRV vaccine and Mh bacterine protected the goats better than other schedules in the groups of comingled goats. This may be because of the strong mucosal response elicited (Ezeasor et al. 2015) or their synergistic activity on the induction of immune response antigenically. Pneumococcal vaccination of mice through the intranasal route boosted the mucosal immunity against *Streptococcus pneumoniae* (Wu et al. 1997). *Mh* bacterine immunization was not protective to goats in our environments (Emikpe et al. 2013; Tenuche et al. 2015), but it is now clear that a combination of PPRV vaccine and the bacterine provided a better protection to goats.

The reason to comingle the animals with introduction of a sick animal was justified by the fact that outbreaks of PPR occur when new stock is introduced into a naïve flock (Radostits et al. 2006; Oma et al. 2012). More so, commingling results in fulminant PPR because the virus are in excretions and secretions of the sick animals. PPRV causes severe necrotizing and haemorrhagic enteritis, evident clinically as diarrhea and anaemia (Olaleye, 1985; Sahinduran et al. 2012). Also, the leukopenia, monocytopenia, and lymphopenia arises because the virus replicates in lymphoid tissues using signaling lymphocyte adhesion molecules (SLAM). The

findings were similar reports of Aikhuomobhogbe and Orheruata (2009) and Sahinduran et al (2012).

Enhanced mucosal response can be assessed by the expression of S-IgA antibody (Ogra et al. 2001). This may have been reflected in the hyperglobulinaemia observed in the PPR + Mh vaccinated animals which showed better immune response. However, the expression comprise antibody isotypes, cytokine, complement and adaptive cells.

The diagnosis of Pest des petits ruminants is on the characteristic clinical signs, gross lesions, histopathological changes and laboratory confirmation by virus isolation, serology or genome detection (OIE, 2009). Most laboratory methods require collection of clinical and post mortem materials of high quality which needs to be transported in a cold chain system from distant places. Sometimes this is hard to achieve especially when PPR outbreaks occur in remote and inaccessible parts.

The significance haematological indices in domestic species has been emphasised (Anosa and Isoun, 1979). Daramola et al. (2005) and Addass et al (2010) documented haemogram indices of indigenous Nigerian goats. Oxidative stress is a growing field of research in small ruminant medicine and has a great role in various diseases processes. The understanding of the role of oxidant and antioxidants in physiological and pathological conations (Donia et al. 2014) is very important. Respiratory rate, pulse rate and rectal temperature are significantly higher ($p \leq .005$) in pneumonic goats in compared with healthy one the same findings were recorded by (Radostitis et al. 2007) in pneumonic goats. The activity of CAT, SOD and GPx in pneumonic goats in compared with healthy one, the same findings were recorded by (Kataria et al. 2007) in goats infected with PPR. In this study higher serum activity of CAT could be due to higher rate of formation of hydrogen peroxide (Kataria et al. 2010) that indicating higher oxidative stress. On the other hand (Donia et al. 2014) were recorded lower serum activity of CAT in pneumonic sheep. Super oxide dismutase enzyme activity was higher in pneumonic goats in this study and the same result was recorded by (Pilania et al. 2013), while (Donia *et al.,* 2014) were recorded lower serum activity of SOD in pneumonic sheep. SOD is responsible for scavenge of super oxide radicals which are results during various metabolic pathways. It catalyzes the destruction of super oxide into oxygen and hydrogen peroxide (Kataria *et al.,* 2012 c). Also researches (Rana et al. 2013) may haveve used SOD as one of

oxidative stress biomarkers. Glutathione peroxidase serum activity in this study was higher in pneumonic goats, while (Donia et al. 2014) were recorded lower serum activity of GPx in pneumonic sheep. Increased activities of serum enzymes of oxidative stress (CAT, SOD and GPx) signified the presence of oxidative stress in order to combat excessive production of free radicals (Pilania et al. 2013; Kataria et al. 2012; Nisbet et al. 2007). It can be said that pneumonia in goats results in excessive production of free radicals and so in balance between oxidant and antioxidant system in body (Pilania et al. 2013; Kataria et al. 2012).

Examination of all the carcasses showed that gross and Histopathological lesions were limited to the respiratory, lymphoid tissues and alimentary organs. However, the respiratory lesions observed were severe/fulminant than those reported by other workers. This may be because of the commingling or the exclusion of antibiotics during the conditioning period, as reported by Olaleye (1985).The diagnostic precision of immunoperoxidsae is resourceful in poor resource settings. Samples from goat with clinical PPR or lesion homogenate can be a rich source of viral antigen, this was used for erythrocyte agglutination test (Wosu (1985). This test was adopted in the detection of PPR in a flock antemortem. HI test is quite concise and diagnostic (Wosu and Ezeibe, 1991).

In conclusion , Intranasal combined PPR and Mh vaccines elicited better protection against pneumonia in goats. This vaccinal strategy will provide framework needed in the prevention and control of endemic caprine pneumonia in Nigeria.

CHAPTER EIGHT

GENERAL DISCUSSION, CONCLUSSION, CONTRIBUTION TO KNOWLEDE AND RECOMMENDATION

8.1 GENERAL DISCUSSION

Small ruminants across Africa remain at risk of endemic, exotic and newly emerging pathogens that cause morbidity and economic losses. The diseases of the respiratory and the alimentary systems constitute major causes of losses in these livestock (Ameh et al. 2000).

This study elucidated and determined the risk and predisposing factors, haematological and BALf cellular changes, pro-oxidants and antixoidants in bronchoalveolar lavage fluid (BALf), immunohistochemical detection of some viral and bacterial antigens in pneumonia complex in Nigerian goats. The consolidation pattern in goats in the tropics and influence of host factors were described. Markers of pneumonia includes M:N and leukocytosis. The study also quantified oxidative stress biomarkers including GST, SOD, GPx, MDA, H_2O_2 and MPO in BALf of normal and pneumonic goats with different histologic classifications. the measure of MDA, H_2O_2 and MPO correlated with the inflammatory reactions and mechanisms of cellular response. Detected other importance viruses other than PPR especially RSV and PI3 in caprine pneumonia complex in Nigeria, and also the first to demonstrate the antigens in the caprine lungs in our environment. Meanwhile, detection of the *Pm* and *Mh* antigens has been able to definitely confirm the roles of Pasteurella and Mannheimia in caprine pneumonia.

This study elucidates the interaction of respiratory viruses and bacteria in caprine pneumonia in Nigeria. It further buttressed the findings of Brown et al. (1991) and Saliki et al. (1994) in experimental induced caprine pneumonia and nature of the lesions in PPRv infected goats (Kumar et al. 2004), and *M. haemolytica* lesions of infected goats (Haritani et al. 1987; Yener et al. 2009). We also reported immunohistochemical detection of PPR, PI3 and RSV in pneumonic lungs of goats in our environment.

Polymicrobial diseases are lethargic in most cases as they are elusive to control (Brodgen and Guthmiller 2002). The mechanisms involved in multiple pathogen infections include immunosuppression of the host due to morphological, functional or stress induced; colonization of pathogen to mucosa surface followed by inflammatory reactions (Brogden 2002).

Our findings further agree with observations from pneumonia of alpaca neonates (Rosadio et al. 2011, Cirilo et al. 2012, Guzmán et al. 2013). Respiratory viruses aid colonization of bacteria to mucosal epithelium and the ensuing severe inflammation. The effect of transport stress, helminthosis, population density, and or age has been observed in Mannheimiosis (Jasni et al. 1991; Zamri-Saad et al. 1996; Brogden et al. 1998). The high prevalence of *Pm* in the goats may not be unconnected to the influence of stress. More so, the co-existence of these pathogens especially, Mh underscores the possible reversal of virulence in the respiratory tract. These associated factors have been highlighted in experimental pasteurellosis of goats by Zamri Saad et al. (1989, 1991).

It has become evident that caprine pneumonia is a complex of multiple pathogens in our environment. This may have contributed to the elusiveness of pneumonia control in this important livestock and ruminants generally.

We further evaluated the Protective effect of intranasal PPRV and *Mh* vaccination in goats exposed to PPRV, and showed that Intranasal combined *PPR and Mh* vaccines elicited better protection against pneumonia in goats. This combined vaccination will reduce the bearing/landing cost and enhancement of the mucosal response. This vaccinal strategy will provide framework needed in the prevention and control of endemic caprine pneumonia in Nigeria.

Sudden climatic changes, gathering and handling, and infections have been recognized as predisposing causes to bacterial respiratory infections in small ruminants (Tijanni et al. 2012; Kumar et al. 2014). *PPRV*, other paramyxoviruses, *Mannheimia haemolytica*, and *Pasteurella multocida* are important in goats in this environment. They can cause primarily, act synergetically and or co-infect in ruminants (Damassa et al. 1992; Lin et al. 2008; Nicholas et al. 2008; Emikpe and Akpavie, 2011; Tijjani et al. 2012). Moreso, the mucosal response

induce the effector arms of the specific immune responses but this has not been fully exploited due to the physical and chemical barriers that inhibit immune system activation.

Studies on the pathophysiology of stress and how it disrupt the dynamics of nasal flora and induces pulmonary disease in ruminants are important. In the light of these, there is urgent need to consider developing potent vaccines multivalent in nature to effectively protect our animals from the adverse effect of stress on the immune response of animals.

8.2 CONCLUSION

Pneumonia is a major condition of goats in Nigeria. These findings have shown the dynamics in the pattern of pneumonia in Nigerian goats, and bronchopneumonia is the most prevalent. Body condition, age and breed are important risk factors to pneumonia in goats in our environment. There is need to further characterize and or quantify the nature of the host responses, virulence determinants of the pathogens and environmental influences on the pattern of pneumonia.The role of innate immune response in the goat is reflected by the presence of alveolar macrophages in BAL fluid. Outbreaks of PPR from commingling is fulminant because infectives virion excretions and secretions of the sick animal. Combined vaccination strategies and intranasal vaccination would elicit better response for optimal mucosal immunity. BAL fluid analysis would compliment diagnostics to pneumonia. The mucosal immune system should be harnessed for vaccines, and development of multivalent vaccine to elicit potent and long-lasting immune responses.

Further studies are required to investigate these complex interactions of infectious organisms in caprine pneumonia complex for proper prevention and control of factors than may contribute to the condition and alleviatinon of the problem. There is need to characterize the prevailing strains of PPRV in our environment and also isolation or molecular detection of these other respiratory pathogens is equally important for prompt and effective design of appropriate vaccinal candidates to takle the endemic caprine pneumonia in Africa.

More so, there is need for improved knowledge on morphometric evaluation of the different breeds of goats in Africa. This can underscore innate adaptions and predisposal of the different breeds to pulmonary consolidation. Survey of the different caprine vaccines and their

antigenicities needs further investigation across the countries in Africa. Nonetheless, there is need for an update on population and distribution of goats across Africa.

8.3 CONTRIBUTIONS TO KNOWLEDGE

We have been able to establish the fact that caprine pneumonia is still an important problem in goats and elucidated the associated factors involved in the endemicity of pneumonia in Nigerian goats. These factors complicate the severity of pulmonary consolidation. and causes impedeance of pulmonary clearance and defence mechanisms.

We also quantified oxidative stress in bronchoalveolar lavage in goats for the first time to the best of our knowledge in our environment. Thus, underscoring the importance of maintaining levels of antioxidants in susceptible goats.

We also identified markers of pneumonia in goats including clinical signs, blood and BALf cellular changes.

We demonstrated some of the causal agents of caprine pneumonia from the pneumonic lung tissues. Reporting for the first time, to the best of our knowledge, RSV and PI3Vviral antigens in the goats.

The study equally identified the interactions of microbial pathogens in precipitation of pulmonary lesions in caprine pneumonia. Which shall also iInform development of vaccines and vaccination strategies for effective control of caprine pneumonia.

Lastly, for the immediate alleviation of problems associated with caprine pneumonia, susceptible goats and young goats should be vaccinated intranassaly with PPRV and *Mannheimia haemolytica* vaccines for prompt elicitation and enhancement of mucosal immune response.

8.4 RECOMMENDATIONS

Goat production and health can be harnessed through effective vaccination of young and susceptible goats againt viral and bacterial causes of pneumonia. Host, environmental and management factors are equally important in small ruminat pneumonia. The mucosal immune system should be harnessed for vaccine administration and optimal immune response through clonal differentiation of mature lymphoid cells and associated lymphoid aggregates. Predisposing factors to pneumonia in goat include body score, age and season. Immunohistochemical staining clearly showed evidence of PI3 and RSV in giant cell pneumonia in goat. Combined vaccination strategies should adopted in controlling caprine pneumonia. Antioxidant therapy and intranasal vaccination with multivalent vaccine incorporating Peste des Petits Ruminants virus, *Mannheimia haemolytica* and other identified pathogens: *Pasturella multocida*, Para-Influenza-3 virus and Respiratory syncytial virus are recommended as an effective approach to the control of caprine pneumonia in Nigeria.

REFERENCES

Abdullah, K. M., Shewen, P. E. and Mellors, A. 1992. A neutral glycoprotease of Pasteurella haemolytica A1 specifically cleaves O-sialoglycoproteins. Infection and Immunity 60: 56−62.

Abegunde, A. and Adu, F. D. 1977. Excretion of the virus of peste des petits ruminants by goats. *Bull Anim Hlth Prod Afr*, 25: 207-311.

Abera, T. Thangavelu, A., Chandran, N. D. Joy and Raja, A. 2014. A SYBR Green I based real-time RT-PCR assay for specific detection of Peste des petits ruminants virus. *BMC Veterinary Research* 10: 22-7

Abubakar, M., Arshed, M. J., Zahur A.B., Ali, Q. and Banyard, A. C. 2012. Natural infection with Peste des petits ruminants virus: a pre and post vaccinal assessment following an outbreak scenario. *Virus Research* 167: 43–47.

Ackermann, M. R., Derscheid, R., Roth, J. A. 2010. Innate Immunology of Bovine Respiratory Disease. *Vet Clin North Am Food Anim Pract*. 26(2): 215–228.

Ackermann, M. R. 2005. Lamb Model of Respiratory Syncytial Virus–Associated Lung Disease: Insights to Pathogenesis and Novel Treatments ILAR Journal, 55 (1), doi: 10.1093/ilar/ilu003

Addass, P. A., Midau, A. and Babale, D. M. 2010. Haemato-biochemical findings of indigenous goats in Mubi, Adamawa State, Nigeria. J. Agric. Sci. 6: 14-16.

Ademosun, A. A. 1985. Contributions of research to small ruminants production in Nigeria. In *Small Ruminant Production in Nigeria.* Adu, I. F., Osinowo, O. A., Taiwo, B. B . A., Alhassan, W. S. (Eds.), Nigeria, Zaria, 6—10th October, NAPRI Shika, 18—34.

Ademosun, A. A. 1988. Trends in small ruminant production for the last two decades and its future in West and Central Africa. Keynote address. *In* Improvement small ruminants (K.O. Adeniji, ed.). Organization of African Unity, Nairobi, 18-22.

Adombi, C. M., Lelenta, M., Lamien, C. E., Shamaki, D., Koffi, Y. M., Traore, A., Silber, R., Couacy-Hymann, E., Bodjo, S. C., Djaman, J. A., Luckins, A. G. and Diallo, A. 2011. Monkey CV1 cell line expressing the sheep-goat SLAM protein: a highly sensitive cell line for the isolation of peste des petits ruminants virus from pathological specimens. *Journal of Virological Methods* 173: 306–313.

Adu, F. D. and Nawathe, D. R. 1981. Safety of tissue culture rinderpest vaccine in pregnant goats. *Tropical Animal Health and Production* 13: 166.

Adu, F. D. and Joannis, T. E. 1984. Serum-virus simultaneous method of immunisation against Peste des petits ruminants. *Tropical Animal Health and Production* 16: 119–122.

Agarwal, S. K. and Marshall, G. D. 2001. Stress effects on immunity and its application to clinical immunology. *Clin Exp Allergy* 31:25-31

Agius, R. M., Godfrey, R. C., Holgate, S. T. 1985. Mast cell and histamine content of human bronchoalveolar lavage fluid. *Thorax*. 40:760-767

Ahmed, K., Jamal, S. M., Ali, Q. and Hussain, M. 2005. An outbreak of peste des petits ruminants in goat flock in Okara, Pakistan. *Pakistan Veterinary Journal*. 25: 146–148.

Ah-Tye, C., Schwartz, S. Huberman, K., Carlin, E. and Moscona. A. 1999. Virus-receptor interactions of human parainfluenza viruses types 1, 2, and 3. *Microb. Pathog*. 27:329–336.

Aich, P., Babiuk, L. A., Potter, A. A., Griebel, P. 2009. Biomarkers for prediction of bovine respiratory disease outcome. OMICS. A Journal of Integrative Biology 113:199–210.

Aikhuomobhogbe, P. U. and Orheruata, A. M. 2009. Haematological and blood biochemical indices of West African dwarf goats vaccinated against Pestes des petit ruminants (PPR). *African Journal of Biotechnology* 5: 743–748.

Akpavie, S.O., Okoro, H.O., Salaam, N.A. and Ikheloa, J.O. 1991. The pathology of adult bovine lung in Nigeria. Tropical Veterinarian. 9:151-161.

Alansari, H. and Potgieter, L. N. 1994. Molecular cloning and sequence analysis of the phosphoprotein, nucleocapsid protein, matrix protein and 22K (M2) protein of the ovine respiratory syncytial virus. *J. Gen. Virol.* 75: 3597-3601.

Alexandre, G., Beauville, S., Asselin de Bienville, Y., and Shitalou, E. 2002. La ch`evre multifonctionnelle dans la soci´et´e antillaise. Ethnozootechnie. 70: 35–52.

Allen, E. M., Pirie, H. M., Selman, I. E., and Snodgrass, D. R. 1978. Some characteristics of a natural infection by parainfluenza-3 virus in a group of calves. *Res Vet Sci.* 24:339–46.

Allen, J. W., Viel, L., Bateman, K. G., Nagy, E., Røsendal, S, and Shewen P. E. 1992. Serological titers to bovine herpesvirus 1, bovine viral diarrhea virus, parainfluenza 3 virus, bovine respiratory syncytial virus and Pasteurella haemolytica in feedlot calves with respiratory disease: associations with bacteriological and pulmonary cytological variables. *Can J Vet Res.* 56:281–8.

Allen, J. W., Viel, L., Bateman, K. G., Rosendal, S., Shewen, P. E.,and Physick-Sheard, P. 1991. The microbial flora of the respiratory tract in feedlot calves: associations between nasopharyngeal and bronchoalveolar lavage cultures. *Can J Vet Res.* 55:341.

Allen, J. W., Viel, L., Bateman, K. G., Rosendal, S., Shewen, P. E. 1992. Cytological findings in bronchoalveolar lavage fluid from feedlot calves: associations with pulmonary microbial flora. *Can. J. Vet Res.* 56(2):122-126.

Al-Majali, A. M., Hussain, N. O., Amarin, N. M. and Majok, A. A. 2008. Seroprevalence of, and risk factors for Peste des petits ruminants in sheep and goats in Northern Jordan. *Preventive Veterinary Medicine* 85: 1–8.

Almroth, B. C 2008. Oxidative damage in fish used as biomarkers in field and laboratory studies. Department of Zoology/Zoophysiology, University of Gothenburg, Box 463, 405 30 Göteborg, Sweden.

Al-Qudah, K. M., Al-Majali, A. M. and Obaidat, M. M. 2008. A study on pathological and microbiological conditions in goats in slaughterhouses in Jordan. *Asian J Animal Vet Adv*. 3: 269-274.

Al-Tarazi, Y. H. M and Daghall, G. J. K. 1997. Nasal carriage of *Pasteurella haemolytica* serotypes by the sheep and goats in the Jordan. *Trop. Anim. Hlth. Prod.* 29:177-179

Ameh, J. A., Egwu G. O., and Tijjani, A. N. 2000. Mortality in Sahelian goats in Nigeria. *Prev. Vet. Med.* 44:107-111.

Ames, T. R., Markham, R. J. F., Opuda-Asibo, J., Leininger, J. R., and Maheshwaran, S. K. 1985. Pulmonary response to intratracheal challenge with *Pasteurella haemolytica* and Pasteurella multocida. Canadian Journal of Comparative Medicine. 49: 395–400

Ames, T. R. 1993. The epidemiology of BRSV infection. *Vet. Med. 88*: 881–885.

Amjad, H., Qamar Ul, I., Forsyth, M., Barrett, T. and Rossiter, P.B. 1996. Peste des petits ruminants in goats in Pakistan. *Veterinary Record* 139: 118–119.

Anderson, J. and McKay, J. A. 1994. The detection of antibodies against Peste des petits ruminants virus in cattle, sheep and goats and the possible implications to Rinderpest control programmes. *Epidemiology and Infection.* 112: 225–231.

Angen. O., Thomse, J., Larsen, L. E., Larsen, J., Kokotovic, B., Heegaard, P. M. H., and Enemark, J. M. D. 2009. Respiratory disease in calves: microbiological investigations on trans-tracheally aspirated bronchoalveolar lavage fluid and acute phase protein response. Vet Micro. 137(1–2):165–171.

Angen, O., Mutters, R., Caugant, D. A., Olson, J. E. and Bisgaard, M. 1999. Taxonomic relationships of the *Pasteurella haemolytica* complex as evaluated by DNA-DNA hybridizations and 16S rRNA sequencings with proposal of *Mannheimia haemolytica gen. nov., comb. nov., Mannheimia granulomatis comb. nov., Mannheimia glucosidal sp. nov., Mannheimia ruminalis sp. nov. and Mannheimia varigena sp. nov*. *International Journal of Systematic Bacteriology*. 49: 67−86.

Animal Health Australia 2009. *Disease strategy: Peste des petits ruminants (Version 3.0)*. Australian Veterinary Emergency Plan (AUSVETPLAN). Primary Industries Ministerial Council, Canberra, Australia.

Aniţă. A., Aniţă, D., Răileanu, C., and Savuţa, G. 2015. Detection of Parainfluenza Type 3 Virus Antigens in Goats. *Bulletin UASVM Veterinary Medicine.* 72(1): 27-29

Anosa, V.O. and Isound, I.I. 1979. Haematological studies of domestic animals in Nigeria. *ZBC Vet.* 25: 64-66.

Aruni, A. W., Lalitha, P. S., Mohan, A. C., Chitravelu, P. and Anbumani, S. P. 1998. Histopathological study of a natural outbreak of peste des petitis ruminants in goats of Tamilnadu. *Small Ruminants Research* 28: 233–240.

Ashley, B. D., Noone, M. A., Dwarakanath, D. and Manlick, H. 2004. Fatal *Pasteurella dagmatis* peritonitis and septicaemia in a patient with cirrhosis: A case report and review of the literature. *Journal of Clinical Pathology*. 57: 210−212.

Atmaca, H. T. and Kul, O. 2012. Examination of epithelial tissue cytokine response to natural Peste des petits ruminants virus (PPRV) infection in sheep and goats by immunohistochemistry. *Histology and Histopathology* 27: 69–78.

Atta-Ur-Rahman, Ashfaque, M., Rahman, S.U., Akhtar, M. and Ullah, S. 2004. Peste des petits ruminants antigen in mesenteric lymph nodes of goats slaughtered at D.I. Khan. *Pakistan Veterinary Journal.* 24: 159–160.

Atwal, O. S., Masoodi, M. A and Aziz, S. H. 1979. PAS-positive inclusions in the alveolar Type II cells of the caprine lung. *Res. Vet. Sci.* 27: 5-8.

Awa, D. N., Njoya, A. and Ngo Tama, A. C. 2000. Economics of prophylaxis against Peste des petits ruminants and gastrointestinal helminthosis in small ruminants in north Cameroon. *Tropical Animal Health and Production.* 32: 391–403.

Aytekin, I., Mamak, N., Ulucan, A., Kalinbacak, A. 2011. Clinical, Haematological, Biochemical and Pathological Findings in Lambs with Peste des Petits Ruminants. *Kafkas Üniversitesi Veteriner Fakültesi Dergisi1*7: 349-355

Baker, J. C., Werdin, R. E., Ames, T. R., Markham, R. J. and Larson, V. L 1986. Study on the etiologic role of bovine respiratory syncytial virus in pneumonia of dairy calves. *J. Am. Vet. Med. Assoc. 189*: 66–70.

Bakke, T. and Nostvold, S. 1982. An investigation of ovine pneumonia in four herds from central Norway. I. Prevalence of pneumonia and microbiological findings. *Acta Veterinaria Scandinavia.* 23: 248−258.

Balamurugan, V., Sen, A., Venkatesan, G., Yadav, V., Bhanot, V., Riyesh, T., Bhanuprakash, V. and Singh, R. K. 2010. Sequence and phylogenetic analyses of the structural genes of virulent isolates and vaccine strains of Peste des petits ruminants virus from India. *Transboundary and Emerging Diseases* 57: 352–364.

Balamurugan, V., Sen, A., Venkatesan, G., Rajak, K.K., Bhanuprakash, V., and Singh, R.K., 2012. Study on passive immunity: Time of vaccination in kids born to goats vaccinated against Peste des petits ruminants. *Virol. Sin. 27*: 228–233.

Bamford, A.I., Adair, B.M., and Foster, J.C. 1995. Primary cytotoxic response of bovine peripheral blood leukocytes to parainfluenza type 3 virus infection. *Vet Immunol Immunopathol* 45:85–95.

Bandyopadhyay, S.K. 2002. The economic appraisal of PPR control in India. In: *14th Annual Conference and National Seminar on Management of Viral Diseases with Emphasis on Global Trade and WTO Regime*. Indian Virological Society, New Delhi, India.

Banyard, A.C., Parida, S., Batten, C., Oura, C., Kwiatek, O. and Libeau, G. 2010. Global distribution of peste des petits ruminants virus and prospects for improved diagnosis and control. *Journal of General Virology* 91: 2885–2897.

Blackall, P. J., Fegan, N., Chew, G. T. I. and Hampson, D. J. 1998. Population structure and diversity of avian isolates of *Pasteurella multocida* from Australia. *Microbiology* 144: 279–289.

Blier, P. 2014. Fish Health: An Oxidative Stress Perspective. *Fish Aquac J* 5: e105. doi: 10.4172/2150-3508.1000e105

Blodörn, K., Hägglund, S., Gavier-Widen, D., Eléouët, J., Riffault, S., Pringle, J., Taylor, G., and Valarcher, J. 2015. A bovine respiratory syncytial virus model with high clinical expression in calves with specific passive immunity. BMC Vet Res. 11: 76. Doi: 10.1186/s12917-015-0389-6

Bloom, W. and Fawcett, D.W. 1976. Textbook of Histology, 9th edn., Saunders, Philadelphia and London, pp. 629-651

Bodjo, S.C., Couacy-Hymann, E., Koffi, M.Y. and Danho, T. 2006. Assessment of the duration of maternal antibodies specific to the homologous peste des petits ruminant vaccine 'Nigeria 75/1' in Djallonke lambs. *Biokemistri* 18: 99–103.

Bonniwell, M.A. 1980. The use of tissue culture Rinderpest vaccine to protect sheep and goats against Peste des petits ruminants in the Ashanti region of Ghana. *Bulletin de l'Office Internationale des Epizooties* 92: 1233–1238.

Boudreaux, C. M. 2004. A novel strategy of controlling bovine pneumonic pasteurellosis: Transfecting the upper respiratory tract of cattle with a gene coding for the antimicrobial peptide cecropin B. M. Sc. Thesis, Louisiana State University, USA.

Boxer, E.L., Nanda, S.K. and Baron, M.D. 2009. The Rinderpest virus non-structural C protein blocks the induction of type 1 interferon. *Virology* 385: 134–142.

Boyazoglu, J., Hatziminaoglou, I., Morand-Fehr, P. 2005. The role of the goat in society: past, present and perspectives for the future. *Small Ruminant Research* 60: 13–23

Brogden, K. A, Guthmiller, J. M., editors. Polymicrobial Diseases. Washington (DC): ASM Press; 2002 Part II, Polyviral Diseases. P 71-109.

Brogden, K.A., Lehmkuhi, H.D. and Cutlip, R.C. 1998. Pasteurella haemolytica complicated respiratory infections in sheep and goats. *Veterinary Research*. 29(3-4): 233–54.

Brown, C. 2008. Hemorrhagic septicemia, in *Foreign Animal Diseases*, United States Animal Health Association, Boca Raton, Fla, USA, pp. 297–300.

Brown, C. C., Mariner, C., and Olander, H.J 1991. An immunohistochemical study of the pneumonia caused by Peste des petits ruminants virus. *Veterinary Pathology* 28: 166–170.

Bryson, D.G., McNulty, M.S., Ball, H.J., Neill, S.D., Connor, T.J., and Cush, P.F. 1979. The experimental production of pneumonia in calves by intranasal inoculation of parainfluenza type-3 virus. *Vet Rec* 105:566–73.

Bryson, E.G., McFerran, J.B., Ball, H.J., and Neill, S.D. 1978. Observations on outbreaks of respiratory disease in housed calves. 1. Epidemiological, clinical and microbiological findings. *Vet Rec* 103:485–9.

Caswell, J.L. and Williams, K. 2007. The respiratory system. In: Jubb K.V.F., Kennedy P.C., Palmer N. (eds.): Pathology of Domestic Animals, 5th ed. MAXIE M.G. (eds). Saunders Elsevier, Edinburgh, UK, 594-622.

Çeribaşi, S., Özkaraca, M., Çeribaşi, A.O. and Özer, H. 2013. Immunohistochemical detection of Peste des Petits Ruminants (PPR) viral antigens in pneumonic caprine lungs in Elazig region. Fırat Üniversitesi Sağlık Bilimleri Vet.Derg.. 27(2): 99 – 103.

Çeribaşi, A.O., Özkaraca, M., Çeribaşi, S. and Özer, H. 2014. Histopathologic, immunoperoxidase and immunofluorescent examinations on natural cattle pneumonia originated from Parainfluenza type 3, Respiratory Syncytial virus, Adenovirus type 3 and Herpesvirus type 1. *Revue Med.Vet.* 165:201-212.

Çeribaşi, A.O., Çeribaşi, S. and Özkaraca, M. 2016. Immunohistochemical detection of bovine herpesvirus type 1 and bovine adenovirus type 3 antigens in frozen and parafinised lung sections of pneumonic sheep and goats. Veterinarski Arhiv. 86(1):9-21.

Chakraborty, S., Kumar, A., Tiwari, R., Rahal, A., Malik, Y., Dhama, K., Pal, A., and Prasad, M. 2014. Advances in Diagnosis of Respiratory Diseases of Small Ruminants.

Hindawi Publishing Corporation Veterinary Medicine International Volume 2014, Article ID 508304, 16 pages ttp://dx.doi.org/10.1155/2014/508304

Chauhan, H. C., Chandel, B. S., Kher, H. N., Dadawala, A. I., and Agrawal, S. M. 2009. Peste des petits ruminants virus infection in animals. *Veterinary World.* 2(4): 150–155

Cirilo, E.C., Manchego, A.S. and Rivera, H.H. 2012 Viral and bacterial coexistence in acute pneumonia in neonatal alpacas. Rev Inv Vet Perú 23 (3): 317-335

Cole, P., Turton, C, Lanyon, H., and Collins, J. 1980. Bronchoalveolar lavage for preparation of free lung cells: technique and complications. *Br J Dis Chest* 74:273-8.

Couacy Hymann, E., Bodjo, C., Danho Koffi, M.Y.T., Libeau, G., and Diallo, A. 2007a. Early detection of viral excretion from experimentally infected goats with Peste Des Petits Ruminants virus. *Preventive Veterinary Medicine*. 78: 85–88.

Couacy Hymann, E., Bodjo, C., Danho, T., Libeau, G. and Diallo, A. 2007b. Evaluation of the virulence of some strains of Peste Des Petits Ruminants virus PPRV in experimental infected West African dwarf goats. *The Veterinary Journal* 173: 178–183.

Dadas, R. C., Muthuchelvan, D., Pandey, A. B. et al. 2012. Development of loop-mediated isothermal amplification (LAMP) assay for rapid detection of peste des petits ruminants virus (PPRV) genome from clinical samples. *Indian Journal of Comparative Microbiology, Immunology and Infectious Diseases*, 33: 7–13

Dalton, T. P., Shertzer, H. G. and Puga, A. 1999. Regulation of gene expression by reactive oxygen. Annual Review of Pharmacology and Toxicology. 39: 67-101.

DaMassa, A. J., Wakenell, P. S. and Brooks D. L. 1992. Mycoplasmas of goats and sheep. *Journal of Veterinary Diagnostic Investigation* 4(1): 101–113.

Daramola, J.O., Adeloye, A.A., Fatoba, T.A. and Soladoye A.O. 2005. Haematological and biochemical parameters for West African Dwarf goats. *Livestock Research for Rural Development.* 17:8. Retrieved from:http://www.cipav.org.co/lrrd/lrrd 17/8/dara 17095.htm

Dawson. S., Else, R.W., Rhind, S.M., Collie, D.D. 2005. Diagnostic value of cytology of bronchoalveolar fluid for lung diseases of sheep. *Vet. Rec.* 157(15):433-6.

Dey, S., Singh, V.P., Kumar, A.A., Sharma, B., Srivastava, S.K. and Singh, N. 2007. Comparative sequence analysis of 16S rDNA gene of *Pasteurella multocida* serogroup B isolates from different animal species. *Res Vet Sci.* 83(1):1-4.

Dhama K., Karthik, K., Chakraborty, S., Tiwari, R., Kapoor, S., Kumar, A., and Thomas, P. 2014. Loop-mediated isothermal amplification of DNA (LAMP)-a new diagnostic tool lights the world of diagnosis of animal and human pathogens: a review. *Pakistan Journal of Biological Sciences.* 17(2):51–166.

Dhama K., Wani, M. Y., Tiwari, R .and Kumar, D. 2012. Molecular diagnosis of animal diseases: the current trends and perspectives. *Livestock Sphere.* 1(5): 6–10.

Dhar, P., Sreenivasa, B.P., Barrett, T., Corteyn, M., Singh, R.P. and Bandyopadhyay, S.K. 2002. Recent epidemiology of Peste des petits ruminants virus (PPRV). *Veterinary Microbiology*. 88:153–159.

Dhiman, M., Estrada-Franco, J.G., Pando, J.M., Ramirez-Aguilar, F.J., Spratt, H., Vazquez-Corzo S., Perez-Molina, G., Gallegos-Sandoval, R., Moreno, R. and Garg, N.J. 2009. Increased myeloperoxidase activity and protein nitration are indicators of inflammation in patients with Chagas′ disease. *Clin Vaccine Immunol.*16:660-6.

Diallo, A., Barrett, T., Barbron, M., Meyer, G. and Lefevre, P.C. 1994. Cloning of the nucleocapsid protein gene of peste-des-petits-ruminants virus: relationship to other morbilliviruses. *Journal of General Virology* 75: 233–237.

Diallo, A., Minet, C., Berhe, G., Le Goff, C., Black, D.N., Fleming, M., Barrett, T., Grillet, C. and Libeau, G. 2002. Goat immune response to capripox vaccine expressing the hemagglutinin protein of Peste des petits ruminants. *Annals of the New York Academy of Sciences.* 969: 88–91.

Diallo, A., Taylor, W.P., Lefevre, P.C. and Provost, A. 1989. Attenuation d'une souche de virus de la peste des petits ruminants: candidat pour un vaccin homologue vivant. *Revue d'élevage et de médecine vétérinaire des pays tropicaux* 42: 311–317.

Diamond, G., Zasloff, M., Eck, H., Brasseur, M, Maloy, W.L. and Bevins C. L. 1991. Tracheal antimicrobial peptide, a cysteine-rich peptide from mammalian tracheal mucosa: Peptide isolation and cloning of cDNA. *Proc Natl Acad Sci* 88:3952–3956.

Durojaiye, O.A. 1984. Detection of the antigen of the Peste des petits ruminants virus in tissues by indirect immunofluorescence technique. *Nigerian Vet J*. 13: 77-80.

Durojaiye, O.A., Taylor, W.P. and Smale, C. 1985. The ultrastructure of peste des petits ruminants virus. *Zentralblatt Fur Veterinarmedizin Reihe B* 32: 460–465.

Dyer, R.M., Erney, S., Spencer, P., Benson, C.E 1989. Oxidative metabolism of the bovine alveolar macrophage. *Am J Vet Res.* 50(4):448-54.

Eleraky, N.Z., Kania, S.A., Potgieter, L.N. 2001. The ovine respiratory syncytial virus F gene sequence and its diagnostic application. *J. Vet. Diagn. Invest.* 13: 455-461

Ellis, J.A. 2010. Bovine Parainfluenza-3 Virus. *Vet Clin Food Anim.* 26:575–593 doi:10.1016/j.cvfa.2010.08.002

Elsheikh, H.M., Hassan, S.O. 2012. Pneumonia in Goats in Sudan. *International Journal of Animal and Veterinary Advances*. 4(2): 144-145.

Emikpe B.O, Jarikre T.A and Eyarefe O.D (2013). Retrospective Study of Disease Incidence and Type of Pneumonia in Nigerian Small Ruminants in Ibadan, Nigeria. *Afr. J. Biomed. Res. Vol.16; 107 - 113*

Emikpe B.O. and Akpavie S.O. (2010): The prevalence of peste des petits ruminants virus antibodies in goats from selected rural and urban communities in Ibadan Nigeria. *Bull Animal Prod Health Africa*, 58 (2): 147-153.

Emikpe B.O. and Akpavie S.O. (2011): The clinicopathologic effects of *Peste des petits ruminants* virus in experimentally infected West African dwarf goats. *Small Rumin Res*, 95 (2, 3): 168-173 doi:10.1016/j.smallrumres.2010.09.009

Emikpe B.O., (2009). The role of Mannheimia hemolytica in the pathology of experimental Pestes Des Petits Ruminants virus infections in West Africa dwarf goats Ph.D Thesis, University of Ibadan.

Emikpe BO, Akpavie SO. The pattern of distribution of pneumonia in experimental Peste des petits ruminants Virus (PPRV) and/or Mannheimia hemolytica infection in West African Dwarf goats. *International Journal of Morphology* 2010; 28 (2): 563–568.

Emikpe BO, Tanko PN, Onilude OM, Sabri MY (2013) The influence of dexamethasone treatment and successive road transport stress on the occurrence of caprine

pneumonia in a hot humid tropical environment. Vet world6(8): 497-501, doi:10.5455/vetworld.

Emikpe BO. Sabri MY, Akpavie SO and Zamri-Saad M (). Experimental infection of Peste des Petit Ruminant virus and Mannheimia haemolytica A2 in goats: immunolocalisation of Mannheimia haemolytica antigens. Vet Res Commun DOI 10.1007/s11259-010-9425-y

Emikpe, B. O., Akpavie, S. O., Zamri-Saad, M., Sabri, Y. M . (2011) Histopathology and immu nodetection of *Mannheimia hemolytica* antigens in experimental caprine pneum onia. Folia Veterinaria 55 (1): 32—37

Emikpe, B.O., Ezeasor, C.K., Sabri, M.Y., and Anosa, V.O. (2013). Clinicopathological evaluation of intranasal, subcutaneous and intramuscular routes of vaccination against intratracheal challenge of *Peste des petits ruminants* virus in goats. *Small Ruminant Research* Vol. 113, (1): 290-296

Encyclopedia Mythica, 2004. Lindemans, M.F. (Ed.), Mythologies (http://www.pantheon.org/cgi bin/search.pl?nocpp=1and-Realm=mythicaandMatch=1andterms=goat accessed on 11 May 2015).

Espinasse, J., Alzieu, J. P., Papageorgiou, C., Beguin, J. C. & Van Gool, F. (1991). Use of transtracheal aspiration to identify pathogens in pneumonic calves. Veterinary Record 129, 339

Ezeasor CK, Emikpe BO, Anosa VO (2015) Haematological changes associated with intranasal and parenteral routes of vaccination against Peste des petits ruminants in West African dwarf goats. Comp Clin Pathol 24:189–192. DOI 10.1007/s00580-014-1920-z

Ezeasor CV, Emikpe BO, Yussof SM, Afusat J, Anosa VO (2013) Cellular responses in the respiratory tract following intranasal administration of Peste des petits ruminant vaccine in goats. *Int. J. Morphol., 31(2)*:687-692.

Ezeibe, M.C., Okoroafor, O.N., Ngene, A.A., Eze, J.I., Eze, I.C. and Ugonabo, J.A. (2008) Persistent detection of peste de petits ruminants antigen in the faeces of recovered goats. *Tropical Animal Health and Production* 40: 517–519.

Ezeokoli, C.D., Umoh, J.U., Chineme, C.N., Isitor, G.N. and Gyang, E.O. (1986) Clinical and epidemiological features of peste des petits ruminants in Sokoto Red goats. *Revue d'élevage et de médecine vétérinaire des pays tropicaux* 39: 269–273.

Feldman, S. A., R. M. Hendry, and J. A. Beeler. 1999. Identification of a linear heparin binding domain for human respiratory syncytial virus attachment glycoprotein G. J. Virol. 73:6610–6617.

Felix, N (2013). Current scenario and control initiatives for PPR at global, regional and country level according to the risk factors and socioeconomic impact. In Proceedings of the Second Regional Conference on Progressive Control of Peste Des Petits Ruminants in South Asia, Kathmandu, Nepal, 19–20 December 2013.

Fenner E, Paul J G, Frederick A, and Rudplf-Rott M. 1996. Veterinary virology. 2nd Edn., Academic Press, New York

Fentahun T. and Woldie M. (2012) Review on Peste Des Petits Ruminants (PPR)," *European Journal of Applied Sciences* 4(4): 160–167

Fernandez, A., J. Oros, J. L. Rodriguez, J. King & J. B. Poveda, 1996. Morphological evidence of a filamentous cilia-associated respiratory (CAR) bacillus in goats. Veterinary Pathology, 33, 445−447.

Field B N, Knipe D M, Howley P M 1996. Parainfluenza Viruses. In: Fields Virology, Fields B N, Knipe D M and Howley P M (Eds.). 3rd Edn., Lippincott-Raven Publications, Philadelphia, pp: 1205-1241.

Fink SL, Cookson BT (2005). Apoptosis, pyroptosis, and necrosis: mechanistic description of dead and dying eukaryotic cells. Infect Immun 73:1907–16.

Fishaut M, Tubergen D, McIntosh K (1980). Cellular response to respiratory viruses with particular reference to children with disorders of cell-mediated immunity. J Pediatr 96:179–86.

Forcados GE, Lohlum AS, Usman Y, Tondo BK and Atiku AA (2016). Changes in serum biochemical parameters and oxidative stress biomarkers in grazing cattle.. Comp Clin Pathol. DOI 10.1007/s00580-016-2298-x

Fox B, Bull TB, Guz A. Mast cells in the human alveolar wall: an electromicroscopic study. J Clin Pathol 1981;34: 1333-42.

Frank GH, Marshall RG (1971). Relationship of serum and nasal secretion neutralizing antibodies in protection of calves against parainfluenza-3 virus. Am J Vet Res 32:1707–13.

Frank GH, Marshall RG (1973). Parainfluenza-3 virus infection of cattle. J Am Vet Med Assoc 163:858–60.

Fulton RW, Purdy CW, Confer AW, Saliki JT, Loan RW, Briggs RE, Burge LJ (2000): Bovine viral diarrhea viral infections in feeder calves with respiratory disease: interactions with Pasteurella spp., Parainfluenza-3 virus, and Bovine respiratory syncytial virus. Canadian Journal of Veterinary Research 64: 151–159

Gafer J A M, Hussein H A, Reda I M (2009). Isolation and Characterization of PI-3 Virus from sheep and goats. International Journal of Virology 5(1): 28-35

Gagea M, Bateman KG, van Drumel T, BJ McEwen, S Carman (2006). Diseases and pathogens associated with mortality in Ontario beef feedlots. J Vet Diagn Invest 18:18–28.

Galbraith, S.E., McQuaid, S., Hamill, L., Pullen, L., Barrett, T. and Cosby, S.L. (2002) Rinderpest and peste des petits ruminants viruses exhibit neurovirulence in mice. *Journal of Neurovirology* 8: 45–52.

Galbraith, S.E., Tiwari, A., Baron, M.D., Lund, B.T., Barrett, T. and Cosby, S.L. (1998) Morbillivirus downregulation of CD46. *Journal of Virology* 72: 10,292–10,297.

Gale C (1968). Bovine parainfluenza-3 immunization procedures. J Am Vet Med Assoc 152:871–80.

Garcin D, Taylor G, Tanebayashi K, et al (1998). The short Sendai virus leader region controls induction of programmed cell death. Virology 243:340–53.

Gates GA, Cesario TC, Ebert JW, et al (1970). Neutralising antibody in experimentally induced respiratory virus infection in calves. Am J Vet Res 31:217–24.

Genest, P., and P. Daniel. 1966. Genomic modifications in cell line cultures chronically infected with a myxovirus. Proc. Soc. Exp. Biol. Med. 123:722–725.

Getty, R., (1975). In: Sisson and Grossman, The Anatomy of the Domestic Animals, 5thedn.. Saunders, Philadelphiaand London, pp. 113-144,916936.

Ghosh, S., Parvez, M.K., Banerjee, K., Sarin, S.K. and Hasnain, S.E. (2002) Baculovirus as mammalian cell expression vector for gene therapy: an emerging strategy. *Molecular Therapy* 6, 5–11.

Gibbs, E.P., Taylor, W.P., Lawman, M.J. and Bryant, J. (1979) Classification of peste des petits ruminants virus as the fourth member of the genus Morbillivirus. *Intervirology* 11: 268–274.

Gil L, Martinez G, Tapanes R, Castro O, Gonzalez D and Bernardo L (2004). Oxidative stress in adult dengue patients. *Amer. J. Trop. Med. Hyg*. 71; 652–657.

Gilbert, Y., and Monnier, J. Adaptation of the virus of peste des petits ruminants virus to tissue cultures. *Revue Elev. Med. Vet. Pays Trop*. 1962, *15*, 321–335.

Gonzalez C.T., Maheswaran S.K. (1993). The role of induced virulence factors produced by Pasteurella hemolytica in the pathogenesis: review and hypothesis. British Veterinary Journal 149:183-193

Gornall AG, Bardawill CJ, David MM (1949). Determination of serum proteins by means of the biuret reaction. J Biol Chem., 177:751-66.

Gourlay, R. N. & L. Barber, 1960. A strain of Pasteurella haemolytica isolated from goats in Uganda. Journal of Comparative Pathology, 70, 211−216.

Govindarajan, R., Koteeswaran, A., Venugopalan, A.T., Shyam, G., Shaouna, S., Shaila, M.S., Ramachandran, S (1997). Isolation of peste des petits ruminants virus from an outbreak in Indian buffalo (bubalus bubalis). *Vet. Rec.*, *141*, 573–574.

Graham DA, McShane J, Mawhinney KA, I. E. McLaren, B. M. Adair, M. Merza (1998). Evaluation of a single dilution ELISA system for detection of seroconversion to bovine viral diarrhea, bovine respiratory syncytial virus, parainfluenza-3 virus, and infectious rhinotracheitis virus: comparison with testing by virus neutralization and hemagglutination inhibition. J Vet Diagn Invest 10:43–8.

Grubor B, Meyerholz DK, Ackermann MR. (2006) Collectins and cationic antimicrobial peptides of the respiratory epithelia. Vet Pathol 43:595–612.

Grune, T., Jung, T., Merker, K. and Davies, K., J.A. (2004). Decreased proteolysis caused by protein aggregates, inclusion bodies, plaques, lipofuscin, ceroid, and 'aggresomes'

during oxidative stress, aging, and disease. International Journal of Biochemistry and Cell Biology. 36, 2519-2530.

Gulbahar M.Y., Cabalar M., Erturk A. 2002. Detection by immunoperoxidase tecnique of parainfluenza type-3 virus and respiratory syncytial virus antigens in naturally occuring pneumonia in lambs. *J. Fac. Vet. Med. Yuz. Yil.* 13: 74-77.

Guzmán, K. M., Rosadio, R. A, Maturrano, L H, Manchego, A. S. 2013. Association of bacterial and viral agents in acute pneumonia in young alpacas. Rev Inv Vet Perú 24(4): 524-536

Haanes, E.J.; Guimond, P.; Wardley, R. The bovine parainfluenza virus type-3 (BPIV-3) hemagglutinin/neuraminidase glycoprotein expressed in baculovirus protects calves against experimental BPIV-3 challenge. Vaccine 1997, 15: 730–738.

Hagglund, S.; Svensson, C.; Emanuelson, U.; Valarcher, J.F.; Alenius, S (2006). Dynamics of virus infections involved in the bovine respiratory disease complex in Swedish dairy herds. *Vet. J. 172*: 320–328.

Haines DM, Kendall JC, Remenda BW, Breker-Klassen MM, Clark EG. (1992). Monoclonal and polyclonal antibodies for immunohistochemical detection of bovine parainfluenza type 3 virus in frozen and formalin-fixed paraffin-embedded tissues. J Vet Diagn Invest 4:393–9.

Halliwell, B. and Gutteridge, J. M. C. (1999). Free radicals in biology and medicine. Oxford University Press, Oxford.

Hamdy, F.M., Dardiri, A.H., Nduaka, O., Breese, S.R.J. and Ihemelandu, E.C. (1976) Etiology of the stomatitis pneumcenteritis complex in Nigerian dwarf goats. *Canadian Journal of Comparative Medicine* 40: 276–284.

Haritani M, Ishino S, Oka M, Nakazawa M, Kobayashi M, Narita M,Takizawa T. 1989. Immunoperoxidase evaluation of pneumonic lesions in calves naturally infected with Pasteurella haemolytica. Nippon Juigaku Zasshi 51 (6) 1137–1141

Haritani M, Nakazawa M, Oohashi S, Yamada Y, Haziroglu R, Narita M. (1987). Immunoperoxidase evaluation of pneumonic lesions induced by Pasteurella haemolytica in calves. American Journal of Veterinary Research 48(9):1358–62.

Harlow E, Lane D (1999) Using Antibodies: a Laboratory Manual. Cold Spring Harbor Laboratory Press, NY

Hart, S.P., (2001). Recent perspectives in using goats for vegetation management in USA. J. Dairy Sci. 84, 170–176.

Harwood PF, Gravel LJ, Mahony TJ (2008). Identification of two distinct bovine parainfluenza virus type 3 genotypes. J Gen Virol 89:1643–8.

Heaney, J., Barrett, T. and Cosby, S.L. (2002) Inhibition of in vitro leukocyte proliferation by morbilliviruses. *Journal of Virology* 76: 3579–3584.

Henrickson K. J (2003). Parainfluenza Viruses. Clinical microbiology reviews. p. 242–264

Hensley, K. and Floyd, R. A. (2002). Reactive Oxygen Species and Protein Oxidation in Aging: A Look Back, A Look Ahead. Archives of Biochemistry and Biophysics. 397, 377-383

Herbert, R.; Baron, J.; Batten, C.; Baron, M.; Taylor, G. Recombinant adenovirus expressing the haemagglutinin of Peste des petits ruminants virus (PPRV) protects goats against challenge with pathogenic virus; a DIVA vaccine for PPR. *Vet. Res.* 2014, *45*, 24.

Hilwig, R. W., J. G. Songer & C. Reggiardo, 1985. Experimentally induced pneumonic pasteurellosis: dose response relationships and protection against natural infection in calves. American Journal of Veterinary Research, 46, 2585−2587.

Hodgson, J.L. 2006. Collection and interpretation of trachealwash and bronchoalveolar lavage for diagnosis of infectious and non-infectious lower airway disorders. Proceedings Of The 9th International Congress Of World Equine Veterinary Association Jan. 22 - 26, 2006 - Marrakech, Morocco.

Hodgson, P. D., P. Aich, A. Manuja, K. Hokamp, F. M. Roche, F. S. L. Brinkman, A. Potter, L. A. Babuik & P. S. Griebel, 2005. Effect of stress on viral-bacterial synergy in bovine respiratory disease: Novel mechanism to regulate inflammation. Comparative and Functional Genomics, 6, 244−250.

Hopkins B.A., T.H.M. Huang, and L.D. Olson, “Differentiating turkey postvaccination isolants of *Pasteurella multocida* using arbitrarily primed polymerase chain reaction,” *Avian Diseases*,vol. 42, no. 2, pp. 265–274, 1998.

Hore DE (1966). Isolation of ovine strains of parainfluenza virus serologically related to type 3. Vet Rec 79:466–7.

Ihemelandu, E.C., Nduaka, O. and Ojukwu, E.M. (1985) Hyperimmune serum in the control of peste des petits ruminants. *Tropical Animal Health and Production* 17, 83–88.

Ikede, B.O. 1977. The pattern of respiratory lesions in goats and sheep in Nigeria, Part I- lesions in goats. *Bull Anim Health Prod Afr*, 16: 49-59.

Ikede, B.O. 1978. The pattern of respiratory lesions of goats and sheep in Nigeria, Part II – lesions in sheep. *Bull Anim Health Prod Afr,* 26 (2): 172-185.

Iniguez, L., (2004). Goats in resource-poor systems in the dry environments of West India. Central Asia and the inter-Andean valleys. Small Rumin. Res. 51, 137–144.

Israel N and Gougerot-Pocidalo MA (1997). Oxidative stress in human immunodeficiency virus infection. *Cell Mol.Life. Sci*., 53:864-870.

Jagtap, S.P., Rajak, K.K., Garg, U.K., Sen, A., Bhanuprakash, V., Sudhakar, S.B., Balamurugan, V., Patel, A., Ahuja, A., Singh, R.K. and Vanamayya, P.R. (2012) Effect of immunosuppression on pathogenesis of peste des petits ruminants (PPR) virus infection in goats. *Microbial Pathogenesis* 52, 217–226.

Jain NC (1986). Haematological principles In: Schalms Veterinary Hematology, Jain, N.C. (Ed.). 4th Edn. Lea and Febiger, Philadelphia, PA., USA., pp: 256-273.

Jarikre T.A., Akpavie S.O. and Bello K. (2013): Outbreak of Contagious Bovine Pleuropneumonia in Ibarapa. *Tropical. Veterinarian* 31(1): 20-29

Jasni S., Zamri-Saad M., Mutalib A.R. and Sheikh-Omar A.R. (1991): Isolation of *Pasteurella haemolytica* from the nasal cavity of goats. *Br Vet J*, 147 (4): 352-355.

Jeffery PK, Corrin B. Structural analysis of the respiratory tract. In: Bienenstock J, ed. Immunology of the lung and upper respiratory tract. New York: McGraw-Hill, 1984:1-27.

Jericho KW, Langford EV. Aerosol vaccination of calves with Pasteurella haemolytica against experimental respiratory disease. *Canadian Journal of Comparative Medicine* 1982; *46(3):* 287-92.

Jericho, K. W. F. & E. V. Langford, 1978. Pneumonia in calves produced with aerosols of bovine herpesvirus 1 and Pasteurella haemolytica. Canadian Journal of Comparative Medicine, 42, 269−277.

Jericho, K.W.F. Histological changes in the respiratory tract of calves exposed to aerosols of bovine herpesvirus 1 and *Pasteurella haemolytica*. J. Comp. Pathol. 1983, 93: 73-82.

Jetteur P., Thiry E., Pastoret P.P.: Serological survey of IBR, CHV-2, PI-3, bovine respiratory syncytial virus and rinderpest virus in sheep and goats in Zaire. *Rev. Elev. Med. Vet. Pays. Trop.*, 1990, 43, 435-437.

Jones, L., Giavedoni, L., Saliki, J.T., Brown, C., Mebus, C. and Yilma, T. (1993) Protection of goats against peste des petits ruminants with a vaccinia virus double recombinant expressing the F and H genes of Rinderpest virus. *Vaccine* 11, 961–964.

Jones, R. Baskerville, A. and Reid, L., (1975). Histochemical identification of glycoproteins in pig bronchial epithelium a) Normal b) Hypertrophied from enzootic pneumonia. J. Pathol., 116: l-Il.

Kahwa C.K.B., and Purton M. (1996) Histologica1 and histochemical study of epithelial respiratory tract in adult goats. SmallRuminantResearch 20: 181-186

Kahwa, C.K.B., (1992). An ultrastructural and histological study of the epithelium of the respiratory tract in the neonatal and adult goat. Ph.D. Thesis. University of Glasgow.

Kalyanaraman B (2013). Teaching the basics of redox biology to medical and graduate students: Oxidants, antioxidants and disease mechanisms. Redox Biology 1: 244–257

Kapil S, Basaraba RJ (1997). Infectious bovine rhinotracheitis, parainfluenza-3, and respiratory coronavirus. Vet Clin North Am Food Anim Pract 13:455–69

Karron RA, Collins PL. (2007) Parainfluenza viruses. In: Knipe DM, Howley PM, editors. 5th edition, Fields virology, vol. 1. Philadelphia: Lippincott, Williams & Wilkins; p. 1497–526.

Kataria AK, Kataria N (2012). Evaluation of Oxidative Stress in Sheep Affected with Peste des petits ruminants. Journal of Stress Physiology & Biochemistry., 8(4): 72-77

Kataria N, Kataria AK, Maan R and Gahlot AK (2010). Evaluation of oxidative stress in brucella infected cows. *Journal of Stress Physiology & Biochemistry*. 6; 19-31.

Kaul, D. and Ogra, P. L. (1998). Mucosal responses to parenteral and mucosal vaccines. *Dev. Biol. Stand.* 95: 141-146.

Kelly, W.R., 1984: Veterinary Clinical Diagnosis. Third edition published by Bailliete Tindall. London Pp. 312-335.

Khin, M. N.; Zamri-Saad, M.; Noordin, M. M. and Effendy, A. W. M. (2009) Effect of intranasal attenuated *Pasteurella multocida* B:2 on haemorrhagic septicaemia in calves. *Online J. Vet. Res., 13(2)*:65-72,

Kılıc A & Kalender H & Eroksuz H & Muz A and Tasdemir B (2013).Identification by culture, PCR, and immunohistochemistry of mycoplasmas and their molecular typing in sheep and lamb lungs with pneumonia in Eastern Turkey. *Trop Anim Health Prod* DOI 10.1007/s11250-013-0394-3

Kılıc A, Kalender H, Eroksuz H, Muz A, Tasdemir B. Identification by culture, PCR, and immunohistochemistry of mycoplasmas and their molecular typing in sheep and lamb lungs with pneumonia in Eastern Turkey'. *Tropical Animal Health and Production* 2013; DOI 10.1007/s11250-013-0394-3

Kim, J. C. S., 1977. Immunological injury in shipping fever pneumonia of cattle. American Journal of Pathology, 100, 109−111.

Kimman, T. G., Zimmer, G. M., Straver, P. J. & De Leeuw, P. W. (1986) Diagnosis of bovine respiratory syncytial virus infections improved by virus detection in lung lavage samples. American Journal of Veterinary Research 47, 143-147

Kirschvink N (2008). Respiratory function in cattle: impact of breed, heritability, and external factors. Dtsch Tieraertzl Wschr 115:265–270.

Kiyono, H. and Fukuyama, S. (2004). The mucosal immune system: features of inductive and effector sites to consider in mucosal immunization and vaccine development. *Nat. Rev. Immunol.* 4: 699-710.

Komatsu T, Takeuchi K, Yokoo A, et al (2004). C and V proteins of Sendai virus target signalling pathways leading to IRF-3 activation for the negative regulation of interferon-beta production. Virology 325:137–48.

Kul, O., Kabakci, N., Atmaca, H.T. and Ozkul, A. (2007) Natural peste des petits ruminants virus infection: novel pathologic findings resembling other morbillivirus infections. *Veterinary Pathology* 44: 479–486.

Kul, O., Kabakci, N., Ozkul, A., Kalender, H. and Atmaca, H.T. (2008) Concurrent peste des petits ruminants virus and pestivirus infection in stillborn twin lambs. *Veterinary Pathology* 45: 191–196.

Kulkarni, D.D., Bhikane, A.U., Shaila, M.S., Varalakshmi, P., Apte, M.P. and Narladkar, B.W. (1996) Peste des petits ruminants in goats in India. *Veterinary Record* 138: 187–188.

Kumar N, Maherchandani S, Kashyap K, Singh SV, Sharma S, Chaubey KK and Ly H (2014). Peste Des Petits Ruminants Virus Infection of Small Ruminants: A Comprehensive Review. *Viruses 6,* 2287-2327; doi:10.3390/v6062287

Kumar P., Tripathi B.N., Sharma A.K., Kumar R., Sreenivasa B.P., Singh R.P., Dhar P and Bandyopadhyay S.K. (2004) Pathological and immunohistochemical study of experimental Pestes des petits ruminants virus infection in goats. Journal Veterinary Medicine B 51: 153–159

Kumar S, Vihan VS, Deoghare PR. Economic implication of diseases in goats in India with reference to implementation of a health plan calendar. Small Ruminant Research 2003, 47: 159–164

Kumar, A., Singh, S.V., Rana, R., Vaid, R.K., Misri, J. and Vs, V. (2001) PPR outbreak in goats: epidemiological and therapeutic studies. *Indian Journal of Animal Sciences* 71: 815–818.

Kwiatek, O., Minet, C., Grillet, C., Hurard, C., Carlsson, E., Karimov, B., Albina, E., Diallo, A. and Libeau, G. (2007) Peste des petits ruminants (PPR) outbreak in Tajikistan. *Journal of Comparative Pathology* 136: 111–119.

Laegreid WW, Taylor SM, Leid RW, R.M. Silfiow (1989). Virus-induced enhancement of arachidonate metabolism by bovine alveolar macrophages in vitro. J Leukoc Biol 45:283–92.

Lamb D, and Lumsden A. Intraepithelial mast cells in human airway epithelium: evidence for smokinginduced changes in their frequency. Thorax 1982;37: 334-42.

Lamb, R.A.; Parks, G.D (2007). *Paramyxoviridae*: The Viruses and their Replication. In *Fields Virology*, 5th ed.; Knipe, D.M.; Howley, P.M., Eds.; Lippincott Williams & Williams: Philadelphia, PA, USA, 2: 1449–1496.

Lasisi O.T., Ojo N.A., and Otesile E.B. Estimation of age of cattle in Nigeria using rostral dentition. *Tropical Veterinarian* 20 (4) 204 – 208

Lawal A., Lasisi O.T., Emikpe, B.O., and Ogundipe G.A.T. (2011): Outbreak of *Peste des Petits Ruminants* in West African Dwarf Goats in Eruwa, Southwestern Nigeria. *Nigerian Vet J*, 32 (4), 331-335

Lawal-Adebowale O.A. (2012). Dynamics of Ruminant Livestock Management in the Context of the Nigerian Agricultural System, Livestock Production, Dr. Khalid Javed (Ed.), ISBN: 978-953-51-0814-6, InTech, DOI: 10.5772/52923. Available from: http://www.intechopen.com/books/livestock-production/dynamics-of-ruminant-livestock-management-in-the-context-of-the-nigerian-agricultural-system

Lebbie, S.H.B., (2004). Goats under household conditions. Small Rum. Res. 51, 131–136.

Lee W, Chung WS, Hong K, Huh J (2015). Clinical Usefulness of Bronchoalveolar Lavage Cellular Analysis and Lymphocyte Subsets in Diffuse Interstitial Lung Diseases. Annals of Laboratory Medicine. 35(2): 220–225. Doi: 10.3343/alm.2015.35.2.220

Lee, C. W. & P. E. Shewen, 1996. Evidence of bovine immunoglobulin G1 (IgG1) protease activity in partially purified culture supernate of Pasteurella haemolytica “A1”. Canadian Journal of Veterinary Research, 60, 127−132.

Lefevre, P.C. and Diallo, A. (1990) Peste des petits ruminants. *Revue Scientifique et Technique* 9: 935–981.

Lefevre, P.C., Diallo, A., Schenkel, F., Hussein, S. and Staak, G. (1991) Serological evidence of peste des petits ruminants in Jordan. *Veterinary Record* 128: 110.

Lemen RJ, Quan SF, Witten ML, Sobonya RE, Ray CG,. Grad R (1990). Canine parainfluenza type 2 bronchiolitis increases histamine responsiveness in beagle puppies. Am Rev Respir Dis 141:199–207.

Lerch, R.A.; Anderson, K.; Wertz, G.W. Nucleotide sequence analysis and expression from recombinant vectors demonstrate that the attachment protein G of bovine respiratory

syncytial virus is distinct from that of human respiratory syncytial virus. *J. Virol.* 1990, *64*, 5559–5569.

Lerch, R.A.; Stott, E.J.; Wertz, G.W. Characterization of bovine respiratory syncytial virus proteins and mRNAs and generation of cDNA clones to the viral mRNAs. *J. Virol.* 1989, *63*, 833–840.

Libeau, G., Diallo, A., Calvez, D. and Lefevre, P.C. (1992) A competitive ELISA using anti-N monoclonal antibodies for specific detection of rinderpest antibodies in cattle and small ruminants. *Veterinary Microbiology* 31: 147–160.

Libeau, G., Prehaud, C., Lancelot, R., Colas, F., Guerre, L., Bishop, D.H. and Diallo, A. (1995) Development of a competitive ELISA for detecting antibodies to the peste des petits ruminants virus using a recombinant nucleoprotein. *Research in Veterinary Science* 58: 50–55.

Liggitt D, Huston L, Silflow R, J. EVERMANN, and E. TRIGO. (1985). Impaired function of bovine alveolar macrophages infected with parainfluenza-3 virus. Am J Vet Res 46:1740–4.

Lillie BN, Keirstead ND, Squires EJ, Hayes MA. Gene polymorphisms associated with reduced hepatic expression of porcine mannan-binding lectin C. Dev Comp Immunol 2007;31:830–846.

Lopez, A. 2012. Respiratory system, thoracic cavity and pleura. Chapter 5 In: Pathologic basis of Veterinary disease, 5th edn, eds M. D. McGavin, & J. Zachary, Mosby-Year Book Inc., pp. 425−595.

Iovannitti, B., Pirie, H.M. and Wright, N.G., (1985). A scanning electron microscopic study of the lower respiratory tract in calves and adult cattle. Res. Vet. Sci., 38: 80-87.

Lovell, M. A., Ehmann, W. D., Mattson, M. P. and Markesbery, W. R. (1997). Elevated 4-Hydroxynonenal in Ventricular Fluid in Alzheimer's Disease. Neurobiology of Aging. 18, 457.

Lu, S. C. (1999). Regulation of hepatic glutathione synthesis: current concepts and controversies. FASEB J. 13, 1169-1183.

Luka, P.D., Erume, J., Mwiine, F.N. and Ayebazibwe, C. (2012) Molecular characterization of peste des petits ruminants virus from the Karamoja region of Uganda (2007–2008). *Archives of Virology* 157: 29–35.

Lyon, M. , C. Leroux, T. Greenland, J. Chastang, J. Patet, and J.- F. Mornex, (1997)"Presence of a unique parainfluenza virus 3 strain identified by RT-PCR in visna-maedi virus infected sheep," *Veterinary Microbiology*, 57(supplement 2-3): 95–104,

Mahajan S., R. Agrawal, M. Kumar, A. Mohan, and N. Pande, (2013) Comparative evaluation of RT-PCR with sandwich-ELISA for detection of Peste des petits ruminant in sheep and goats," *Veterinary World* 6(6): 288–290

Mahapatra, M., Parida, S., Baron, M.D. and Barrett, T. (2006) Matrix protein and glycoproteins F and H of Peste-des-petits-ruminants virus function better as a homologous complex. *Journal of General Virology* 87: 2021–2029.

Maidana, S.S.; Lomonaco, P.M.; Combessies, G.; Craig, M.I.; Diodati, J.; Rodriguez, D.; Parreno, V.; Zabal, O.; Konrad, J.L.; Crudelli, G.; Mauroy, A.; Thiry, E.; Romera, S.A. Isolation and characterization of bovine parainfluenza virus type 3 from water buffaloes (Bubalus bubalis) in Argentina. BMC Vet. Res. 2012, 8, 83

Majid, A., (1986). A combined histological, histochemical and scatning electron microscopical study of the canine respiratory tract. Ph.D. Thesis, University of Glasgow.

Mak, T.W. and Saunders, M.E. 2006.The Immune Response: Basic and Clinical Principles. Elsevier Academic Press 30 Corporate Drive, Suite 400, Burlington, MA 01803, USA. Pages 739-780

Mallipeddi S.K., Samal S.K.: Analysis of the ovine respiratory syncytial virus (RSV). *J. Gen. Virol.*, 1993, 74, 2787-2791.

Mallipeddi, S.K.; Samal, S.K. Sequence variability of the glycoprotein gene of bovine respiratory syncytial virus. *J. Gen. Virol.* 1993, *74*, 2001–2004.

Mallipeddi, S.K.; Samal, S.K. Structural difference in the fusion protein among strains of bovine respiratory syncytial virus. *Vet. Microbiol.* 1993, *36*, 359–367.

Mariassay, A.T. and Plopper, C.G., (1983). Tracheobronchial epithelium of the sheep. I. Quantitative light microscopic study of the epithelial cell abundance and distribution. Anat. Rec., 205: 263-215.

Masot A.J., Gomez L., Martînez M.S., Gazquez A., Redondo E.: Experimental infection of lambs with bovine respiratory syncytial virus : pathological and haematological studies. *Rev. Méd. Vét.*, 1993, 144, 773-780.

Masot, A.J.; Kelling, C.L.; Lopez, O.; Sur, J.H.; Redondo, E (2000). In situ hybridization detection of bovine respiratory syncytial virus in the lung of experimentally infected lambs. *Vet. Pathol. 37*: 618–625.

Massaro, G.D., Davis, L. and Massaro, D., (1984). Postnatal development of the bronchiolar Clara cells in the rats, Am. J. Physiol., 247: C195-C203.

Matrosovich MN, Matrosovich TY, Gray T, Roberts NA, Klenk HD (2004). Neuraminidase is important for the initiation of influenza virus infection in human airway epithelium. J Virol 78:12665–7.

Mavrogenis, A., Sinapis, E., (2003). A review on goat production in the East and South Mediterranean region.Wageningen Academic Publishers, Wageningen. European Association for Animal Production (EAAP) Publication No. 99, pp. 279–288.

McFerran JB, McNulty MS (1981). Aids to diagnosis of virological diseases. Br Vet J 137:455–63.

McGowan S.E. (2014). The formation of pulmonary alveoli. Chapter four In The Lung (Second Edition), Development, Aging and Environment. Academic Press Pg 65-84.

McKane SA (2010) Tracheal wash and bronchoalveolar lavage: sampling technique and fluid interpretation. Proceedings of the 49th British Equine Veterinary Association Congress BEVA Sep. 8 – 11, 2010 Birmingham, United Kingdom. Reprinted in IVIS with the permission of BEVA.

Mellado M., Foote R.H. and de Tellitu J.N. (1991): Effect of age and season on mortality of goats due to infections and malnutrition in northeast Mexico. *Small Rumin Res*, 6 (1): 159-166.

Meng, X., Dou, Y., Zhai, J., Zhang, H., Yan, F., Shi, X., Luo, X., Li, H. and Cai, X. (2011) Tissue distribution and expression of signaling lymphocyte activation molecule receptor to peste des petits ruminant virus in goats detected by real-time PCR. *Journal of Molecular Histology* 42: 467–472.

Miller, M. W. (2001): Pasteurellosis, p. 330– 339. In E. S. Williams and I. K. Barker (ed.), Infectious diseases of wild mammals. Iowa State University Press, Ames, IA.

Minakshi, G. C. Chaturvedi, P. Sarthi, and P. Tomar, "Detection of P. multocida antibody in the serum of cattle by dot immunobinding assay and Rose Bengal test," in *Laboratory Manual on Recent Approaches in Immunodiagnostics for Livestock and Poultry Diseases*, pp. 114–116, 2000.

Minka, N.S., Ayo, J.O., Sackey, A.K.B., and Adelaiye, A.B. (2009) Assessment and scoring of stresses imposed on goats during handling, loading, road transportation and unloading, and the effect of pretreatment with ascorbic acid. *Livest. Sci* 125: 275-282.

Minnich LL, Ray CG (1987). Early testing of cell cultures for detection of haemadsorbing viruses. J Clin Microbiol 25:421–2.

Misra HP, Fridovich I (1972). The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. J Biol Chem., 247; 3170-5.

Mitchell GB, Al-Haddawi MH, Clark ME, **ME Clark, J D. Beveridge and J L. Caswell**. Effect of corticosteroids and neuropeptides on expression of defensins by tracheal epithelial cells. Infect Immun 2007;75(3):1325–1334.

Mitra-Kaushik, S., Nayak, R. and Shaila, M.S. (2001) Identification of a cytotoxic T-cell epitope on the recombinant nucleocapsid proteins of Rinderpest and Peste des petits ruminants viruses presented as assembled nucleocapsids. *Virology* 279: 210–220.

Mohamed, R. A. and E. B. Abdelsalam, 2008. A review on pneumonic pasteurellosis (respiratory mannheimiosis) with emphasis on pathogenesis, virulence mechanisms and predisposing factors. Bulg. J. Vet. Med., 11, No 3, 139-160.

Mohammadi GR, Nazifi S, Rezakhani A, Esmailnejad Z 2007. Effect of transportation stress on blood and bronchoalveolar lavage fluid components in calves. *Comp. Clin. Pathol*, *16*:85-95.

Morck, D. W., T. G. Raybould, S. D. Acres, A. Babiuk, J. Nelling & J. W. Costerton, 1987. Electron microscopic detection of glycocalyx and fimbriae on the surface of Pasteurella haemolytica. Canadian Journal of Veterinary Research, 51, 83−88.

Morein B (1970). Immunity against parainfluenza-3 virus in cattle; immunglobulins in serum and nasal secretions. Int Arch Allergy 39:403–14.

Mori, I., K. Nakakuki, and Y. Kimura. 1996. Temperature-sensitive parainfluenza type 1 vaccine virus directly accesses the central nervous system by infecting olfactory neurons. J. Gen. Virol. 77:2121–2124.

Morris, J. A., R. E. Blount, Jr., and R. E. Savage. 1956. Recovery of cytopathogenic agent from chimpanzees with coryza. Proc. Soc. Exp. Biol.Med. 92:544–594.

Moussallem, T.M., Guedes, F., Fernandes, E.R., Pagliari, C., Lancellotti, C.L., De Andrade, H.F. Jr and Duarte, M.I. (2007) Lung involvement in childhood measles: severe immune dysfunction revealed by quantitative immunohistochemistry. *Human Pathology* 38: 1239–1247.

Munir M. (2013). Peste des Petits Ruminants Virus. Chapter 5 in. *Mononegaviruses of Veterinary Importance Vol. I: Pathobiology and Molecular Diagnosis* (ed. M. Munir). CAB International pp 65- 98.

Munir M., S. Zohari, and M. Berg, (2013) "Current advances in molecular diagnosis and vaccines for Peste des Petits Ruminants," *Springer Briefs Animal Science*, vol. 2013, pp. 105–133,

Munir, M. (2013) Role of wild small ruminants in the epidemiology of peste des petits ruminants. *Transboundary and Emerging Diseases* doi: 10.1111/tbed.12052.

Munir, M., Abubakar, M., Zohari, S. and Berg, M. (2012a) Serodiagnosis of Peste des Petits Ruminants Virus. In: Al-Moslih, M. (ed.) *Serological Diagnosis of Certain Human, Animal and Plant Diseases*. InTech, Croatia, pp. 37–58.

Munir, M., Siddique, M. and Ali, Q. (2009) Comparative efficacy of standard AGID and precipitinogen inhibition test with monoclonal antibodies based competitive ELISA for the serology of Peste des Petits Ruminants in sheep and goats. *Tropical Animal Health and Production* 41: 413–420.

Munir, M., Zohari, S., Berg, M. Genome organization. In *Molecular Biology and Pathogenesis of Peste Des Petits Ruminants Virus*; Springer: Heidelberg, Germany, 2013; pp. 1–18.

Munir, M., Zohari, S., Saeed, A., Khan, Q.M., Abubakar, M., Leblanc, N. and Berg, M. (2012b) Detection and phylogenetic analysis of peste des petits ruminants virus isolated from outbreaks in Punjab, Pakistan. *Transboundary and Emerging Diseases* 59, 85–93.

Nadel, J.A., Davis, B. and Phipps, R.J., (1979). Control of mucus secretion and ion transport in airways. Ann. Rev. Physiol., 41:369-381.

Nahed, A.K., Hanan, S.A., Hanan, M.S., Laila, A.S. and Fatma, S.M. (2004) The production and evaluation of a standard diagnostic peste des petits ruminants (PPR) hyperimmune serum prepared from the Egyptian antigen (Egypt 87). *Egyptian Journal of Immunology* 11: 9–14.

Nanda, Y.P., Chatterjee, A., Purohit, A.K., Diallo, A., Innui, K., Sharma, R.N., Libeau, G., Thevasagayam, J.A., Bruning, A., Kitching, R.P., Anderson, J., Barrett, T. and Taylor, W.P. (1996) The isolation of peste des petits ruminants virus from northern India. *Veterinary Microbiology* 51: 207–216.

Nguyen, D.H.; Zhou, T.; Shu, J.; Mao, J.H. Quantifying chromogen intensity in immunohistochemis-try via reciprocal intensity. *Cancer InCytes* 2013, 2(1): 1-4.

Nickel R, Schummer A, Seiferle E (1973). The viscera of domestic mammals. Translated and revised by Sack WO. Verlag Pawl Parey Berlin. Pg 252

Nisbet C, Yarim GF, Gumusova SO and Yazici Z (2007). Investigation of the antioxidative metabolism in sheep with *peste des petits*ruminants. *Acta. Vet. (Beograd)*. 57; 351-356.

Obi, T.U. 1984. Clinical and epidemiological studies on PPR in sheep and goats in Southern Nigeria. Ph.D Thesis, University of Ibadan.

Obi, T.U., Ojo, M.O., Durojaiye, O.A., Kasali, O.B., Akpavie, S. and Opasina, D.B. (1983) Peste des petits ruminants (PPR) in goats in Nigeria: clinical, microbiological and pathological features. *Zentralblatt Für Veterinarmedizin B* 30: 751–761.

Obi, T.U.; Ibu, J. Parainfluenza type 3 (PI-3) virus infection in goats in Nigeria. Small Rumin. Res 1990, 3: 517–523.

Odugbo, M. O., I. E. Odama, J. U. Umoh & L. H. Lombin, 2004b. The comparative pathogenicity of strains of eight serovars and untypable strains of Mannheimia

haemolytica in experimental pneumonia of sheep. Veterinary Research, 35, 661−669.

Odugbo, M. O., J. O. Okpara, S. A. Abechi & P. R. Kumbish, 2004a. An outbreak of pneumonic pasteurellosis in sheep due to Mannheimia (Pasteurella) haemolytica serotype 7. The Veterinary Journal, 167, 214−215.

Odugbo, M. O., Odama, L. E., Umoh, J. U ., Makinde A. A., (2003): Serotypes of *Pasteurella hemolytica* from pneumonic lungs of sheep in Northern Nigeria. *Small Ruminant Res.,* 48: 239—243.

OdugboM.O.; Odama, L.E.; Umoh, J.U. and Lombin, L.H. (2004): The comparative pathogenicity of strains of eight serovars and untypable strains of *Mannheimia haemolytica* in experimental pneumonia of sheep. Vet Res. 2004;35(6):661-9.

Ogunbiyi PO, Black WD, Eyre P (1988). Parainfluenza-3 virus-induced enhancement of histamine release from calf lung mast cells–effect of levamisole. J Vet Pharmacol Ther 11:338–44.

Ogunnariwo, J. A. & A. B. Schryvers, 1990. Iron acquisition in Pasteurella haemolytica: Expression and identification of a bovine-specific transferrin receptor. Infection and Immunity, 58, 2091−2097.

Ohlson, A.; Emanuelson, U.; Traven, M.; Alenius, S (2010). The relationship between antibody status to bovine corona virus and bovine respiratory syncytial virus and disease incidence, reproduction and herd characteristics in dairy herds. *Acta Vet. Scand. 52*: 37.

Ohlson, A.; Heuer, C.; Lockhart, C.; Traven, M.; Emanuelson, U.; Alenius, S (2010). Risk factors for seropositivity to bovine coronavirus and bovine respiratory syncytial virus in dairy herds. *Vet. Rec. 167*: 201–66.

OIE Manual of Diagnostic tests and Vaccines for Terrestrial Animals 2009 Peste des petits Ruminants Pp 1-6.

OIE. World Organisation for Animal Health.Contagious Caprine Pleuropneumonia. Terrestrial Manual. 2008. Chapter 2.7.6, pp: 1000-1012.

Ojo MO (1971). A review of microbial diseases of goats in Nigeria. *Bull. Epizootic Disease Afr.* pp. 5-13.

Ojo, M. O., 1975. Caprine pneumonia. III. Biochemical characterization and serological types of pasteurellae. Nigerian Journal of Animal Production, 2, 216−221.

Ojo, M. O., 1976. Caprine pneumonia in Nigeria: Epidemiology and bacterial flora of normal and diseased respiratory tracts. Tropical Animal Health and Production, 8, 85−89.

Olaleye, O.D. (1985). Haematological and Serum Biochemical changes in experimental peste des petits ruminants virus infection in West African Dwarf Goats (Fouta Djallon). M.V.Sc. Dissertation, Department of Veterinary Pathology, University of Ibadan.

Oma VS, Tråvén M, Alenius S, Myrmel M and Stokstad M. (2016). Bovine coronavirus in naturally and experimentally exposed calves; viral shedding and the potential for transmission. Virology Journal 13:100 pp 1-11 DOI 10.1186/s12985-016-0555-x

Omobowale TO, Oyagbemi AA, Oyewunmi OA, and Adejumobi OA (2015). Chemopreventive effect of methanolic extract of *Azadirachta indica* on experimental *Trypanosoma brucei* induced oxidative stress in dogs. Phcog Res 7:249-58

Oseni, S.O. and Ajayi, B.A. (2014) Phenotypic Characterization and Strategies for Genetic Improvement of WAD Goats under Backyard Systems. *Open Journal of Animal Sciences*, 4, 253-262. http://dx.doi.org/10.4236/ojas.2014.45032

Osman NA, Ali AS, Rahman MEA, Fadol MA (2009) Pathological, Serological and Virological findings in goats experimentally infected with Sudanese Peste des Petits Rumminants virus isolates. Journal of General and Molecular Virology 1: 001-006.

Ostanello F., Dottori M., Gusmara C., Leotti G., Sala V., (2007). Pneumonia disease assessment using a slaugtherhouse lung-scoring method. *Journal of veterinary medicine*, 5, 70-75.

Ostrowski S, Thiaucourt F, Amirbekov M, Mahmadshoev A, Manso-Silván L, Dupuy V, Vahobov D, Ziyoev O, Michel S. Fatal Outbreak of *Mycoplasma Capricolum* Pneumonia in Endangered Markhors. *Emerging Infectious Diseases* 2011. 17(12:):2338-2341 DOI: http://dx.doi.org/10.3201/eid1712.110187

Otto, B. R., A. M. Verweij-Van-Vught & D. M. Maclaren, 1992. Transferrin and heme compounds as iron sources for pathogenic bacteria. Critcal Reviews in Microbiology, 18, 217−233.

Pack, R.J., Al-Ugaily, L.H. and Morris, G., (1981). The cells of the tracheobronchial epithelium of the mouse: A quantitative light microscopy and electron microscopy study. J. Anat., 132: 71-84.

Parida, S., Mahapatra, M., Kumar, S., Das, S.C., Baron, M.D., Anderson, J. and Barrett, T. (2007) Rescue of a chimeric rinderpest virus with the nucleocapsid protein derived from peste-des-petits-ruminants virus: use as a marker vaccine. *Journal of General Virology* 88, 2019–2027.

Parrott, R. H., H. W. Kim, J. O. Arrobio, D. S. Hodes, B. R. Murphy, C. D. Brandt, et al. 1973. Epidemiology of respiratory syncytial virus infection in Washington, D.C. Am. J. Epidemiol. 98:289–300.

Pastoret, P. (2006) Rinderpest: A general introduction. In *Rinderpest and Peste Des Petits Ruminants Virus: Plague of Large and Small Ruminants*; Biology of Animal Infections; Academic Press: London, UK,; pp. 1–12.

Patel, A., Rajak, K.K., Balamurugan, V., Sen, A., Sudhakar, S.B., Bhanuprakash, V., Singh, R.K. and Pandey, A.B. (2012) Cytokines expression profile and kinetics of peste des petits ruminants virus antigen and antibody in infected and vaccinated goats. *Virologica Sinica* 27: 265–271.

Peacock C. (2005). Goats—A pathway out of poverty. Small Ruminant Research 60, 179–186

Peacock, C.P., (1996). Improving Goat Production in the Tropics. A Manual for Development Workers. FARM-Africa/Oxfam, Oxford.

Peterhans E (1997). Oxidants and antioxidants in viral diseases: Disease mechanisms and metabolic regulation. *J. Nutr.,*. 127; 962S- 965S.

Petersen, K. D.; Christensen, H.; Bisgaard, M. and Olsen, J. E. (2001): Genetic diversity of *Pasteurella multocida* fowl cholera isolates as demonstrated by ribotyping and 16S rRNA and partial atpD sequence comparisons. Microbiology 147: 2739–2748.

Pirie, H.M. 1979. Respiratory diseases of animals. Thomson Litho Ltd., East Kilbride, Scotland. Pp 78-89.

Pirie, M.E.S., (1990). An ultrastructural and histological study of the equine respiratory tract in health and disease. Ph.D Thesis, University of Glasgow.

Plopper, C.G., Halsebo, J.E., Berger, W.S., Sonstegard, K.S. and Nettesheim, P., (1983). Distribution of Clara cells in intra- and extra-pulmonary airways of the rabbit. Exp. Lung Res., 5: 79-98.

Plopper, C.G., Heidsiek, J.G., Weir, A.J., St. George, J.A. and Hyde, D.M., (1989). Tracheobronchial epithelium in the adult Rhesus monkey: A quantitative histochemical and ultrastructural study. Am. J. Anat., 184: 3140.

Plopper, C.G., St. George, J.A., Nishio, S.J., Etchison, J.R. and Nettenshein, P., (1984). Carbohydrate cytochemistry of tracheobronchial airway epithelium of rabbit. J. Histochem. Cytochem., 32: 209-218.

Pope, R.A.; Parida, S.; Bailey, D.; Brownlie, J.; Barrett, T.; Banyard, A.C. Early events following experimental infection with peste-des-petits ruminants virus suggest immune cell targeting. *PLoS One* 2013, *8*, e55830.

Porter, D. D., G. A. Prince, V. G. Hemming, and H. G. Porter. 1991. Pathogenesis of human parainfluenza virus 3 infection in two species of cotton rats: *Sigmodon hispidus* develops bronchiolitis, while *Sigmodon fulviventer* develops interstitial pneumonia. J. Virol. 65:103–111.

Probert M, Stott EJ, Thomas LH, et al (1978). An inactivated parainfluenza virus type 3 vaccine; the influence of vaccination regime on the response of calves and their subsequent resistance to challenge. Res Vet Sci;24:222–7.

Qia D, Janke BH, Elankumaran S (2010). Complete genomic sequence and pathogenicity of two swine parainfluenza 3 isolates from pigs in the United States. J Virol 84:686–94.

Qin, J., Huang, H., Ruan, Y., Hou, X., Yang, S., Wang, C., Huang, G., Wang, T., Feng, N., Gao, Y. and Xia, X. (2012) A novel recombinant Peste des petits ruminants–canine adenovirus vaccine elicits long-lasting neutralizing antibody response against PPR in goats. *PLoS ONE* 7, e37170.

Quinn, P. J., B. K. Markey, M. E. Carter, W. J. Donnelly & F. C, Leonard, 2002. Veterinary Microbiology and Microbial Disease, Blackwell Science, pp. 137−143.

Raaperi, K., Bougeard, S., Aleksejev, A., Orro, T., and Viltrop, A (2012). Association of herd BRSV and BHV-1 seroprevalence with respiratory disease and reproductive performance in adult dairy cattle. *Acta Vet. Scand. 54*: 4.

Radostits, O. M., C. C. Gay, K. W. Hinchcliff, and PD Blood (2006). Veterinary Medicine: A Textbook of Diseases of Cattle, Sheep, Pigs, Goats and Horses, 10th edn, W. B. Saunders.

Raghavendra, L., Setty, D.R.L. and Raghavan, R. (1997) (2000) Clinico-biochemical changes in sheep and goats experimentally infected with Vero cell adapted peste-des petits ruminants (PPR) virus. *International Journal of Animal Sciences* 15: 171–173.

Rahaman, A., Srinivasan, N., Shamala, N. and Shaila, M.S. 2003. The fusion core complex of the peste des petits ruminants virus is a six-helix bundle assembly. *Biochemistry* 42: 922–931.

Rajak, K.K., Sreenivasa, B.P., Hosamani, M., Singh, R.P., Singh, S.K., Singh, R.K. and Bandyopadhyay, S.K. 2005. Experimental studies on immunosuppressive effects of peste des petits ruminants (PPR) virus in goats. *Comparative Immunology, Microbiology and Infectious Diseases* 28: 287–296.

Ramirez-Romero, R. and Brogden, K. A. 2000. The potential role of the Arthus and Shwartzman reactions in the pathogenesis of pneumonic pasteurellosis. *Inflammation Research.* 49:(3) 98-101.

Renukaradhya, G.J., Sinnathamby, G., Seth, S., Rajasekhar, M. and Shaila, M.S. 2002 Mapping of B-cell epitopic sites and delineation of functional domains on the hemagglutinin-neuraminidase protein of peste des petits ruminants virus. *Virus Research* 90: 171–185.

Reynolds HY 2000. Use of bronchoalveolar lavage in humans—past necessity and future imperative. Lung 178:271-293

Roger, F., Diallo, A., Yigezu, L.M., Hurard, C., Libeau, G., Mebratu, G.Y., Faye, B 2000. Investigation of a new pathological condition of camels in Ethiopia. *J. Camel Pract. Res.*, *2*: 163–166.

Romero, C.H., Barrett, T., Kitching, R.P., Bostock, C. and Black, D.N. 1995. Protection of goats against peste des petits ruminants with recombinant capripoxviruses expressing the fusion and haemagglutinin protein genes of rinderpest virus. *Vaccine* 13: 36–40.

Rosadio R., E. Cirilo, A.Manchego, and H. Rivera, 2011. Respiratory syncytial and parainfluenza type 3 viruses coexisting with Pasteurella multocida and Mannheimia

hemolytica in acute pneumonias of neonatal alpacas. *Small Ruminant Research*. 97(1–3): 110–116

Rossiter, P.B. and Taylor, W.P. 1994 Peste des petits ruminants. In: Coetzer, J.A.W., Thomson, G.R. and Tustin, R.C. (eds) *Infectious Diseases of Livestock*, vol. 2. Oxford University Press, Cape Town, South Africa.

Roth J, Bendayan M, Orci L 1978 Ultrastructural localization of intracellular antigens by the use of protein A gold complex. J Histochem Cytochem 26:1074 1081

Rottoli P., and Bargagli E. 2004. Is bronchoalveolar lavage obsolete in the diagnosis of interstitial lung disease? Curr Opin Pulm Med 10:320

Rudolph KM, Hunter DL, Rimler RB, E. F. Cassirer, W. J. Foreyt, W. J. DeLong,. G. C. Weiser, and A. C. S. Ward 2007. Microorganisms associated with a pneumonic epizootic in Rocky Mountain bighorn sheep (Ovis canadensis canadensis). *J Zoo Wildl Med* 38:548–58.

Sabri, M.Y., Shahrom-Salisi, M., Emikpe, B.O. 2013. Comparison prior and post vaccination of inactivated recombinant vaccine against Mannheimiosis in Boer Goats Farm in Sabah. *J Vaccines Vaccin* 4:173. doi:10.4172/2157- 7560.1000173

Sahinduran, S., Albay, M.K., Sezer, K., Ozmen, O., Mamak, N., Haligur, M., Karakurum, C. and Yildiz, R. 2012. Coagulation profile, haematological and biochemical changes in kids naturally infected with peste des petits ruminants. *Tropical Animal Health and Production* 44: 453–457.

Saliki J. T., Brown C. C., House J. A., Dubovi E. J 1994. Differential immunohistochemical staining of peste des petits ruminants virus and rinderpest antigens in formalin-fixed, paraffin embedded tissues using monoclonal and polyclonal antibodies. Journal Veterinary Diagnostic investigations 6: 96–98

Saliki, J.T., House, J.A., Mebus, C.A. and Dubovi, E.J. 1994. Comparison of monoclonal antibody-based sandwich enzyme-linked immunosorbent assay and virus isolation for detection of peste des petits ruminants virus in goat tissues and secretions. *Journal of Clinical Microbiology* 32: 1349–1353.

Salt JS, Thevasagayam SJ, Wiseman A, and A. R. Peters 2007. Efficacy of a quadrivalent vaccine against respiratory diseases caused by BHV-1, PI3V, BVDV and BRSV in experimentally infected calves. Vet J 174:616–26.

Samarghandian S, Borji A, Afshari R, Delkhosh MB, Gholami A 2013. The effect of lead acetate on oxidative stress and antioxidant status in rat bronchoalveolar lavage fluid and lung tissue. Toxicol Mech Methods., 23(6):432-6. doi: 10.3109/15376516.2013.777136.

Saravanan P., R. P. Singh, V. Balamurugan et al., 2004. Development of aNgene-based PCR-ELISA for detection of Peste-des-petitsruminants virus in clinical samples. *Acta Virologica*. 48(4): 249–255.

Saravanan, P., Sen, A., Balamurugan, V., Rajak, K.K., Bhanuprakash, V., Palaniswami, K.S., Nachimuthu, K., Thangavelu, A., Dhinakarraj, G., Hegde, R. and Singh, R.K. 2010. Comparative efficacy of peste des petits ruminants (PPR) vaccines. *Biologicals* 38: 479–485.

Sarmiento-Silva R E, Nakamura-Lopez Y and Vaughan G (2012). Epidemiology, Molecular Epidemiology and Evolution of Bovine Respiratory Syncytial Virus. *Viruses 4*: 3452-3467; doi:10.3390/v4123452

Sato M, Wright PF (2008). Current status of vaccines for parainfluenza virus infections. Pediatr Infect Dis J 27:S123–5.

Schalm, O. W., Jain, N.C and Carrol, E.J. (1975). Veterinary Haematology, 3rd Edn., Lea and Febiger, Philadelphia, pp. 197-199

Schmekel B, Karlsson SE, Linden M, Sundström C, Tegner H, Venge P (1990). Myeloperoxidase in human lung lavage. I. A marker of local neutrophil activity. Inflammation., 14(4):447-54.

Schrijver RS, Kramps JA, Middel WG, WGJ **Middel**, M Maris-Veldhuis (1995). Bovine respiratory syncytial virus replicates minimally in bovine alveolar macrophages. Arch Virol 140: 1905–17.

Schwarz KB 1996. Oxidative stress during viral infection: a review. *Free Radic. Biol. Med.*21:641-649.

Sendow I, Syafriati T, Wiedosari E, Selleck P 2002. Infection of Parainfluenza type 3 (PI-3) as one of the causative agent of pneumonia in sheep and goats. IJAV 7(1):62-68.

Shaikh, F.Y.; Crowe, J.E., Jr. 2013. Molecular mechanisms driving respiratory syncytial virus assembly. *Future Microbiology Journal 8*: 123–131.

Shaila, M.S., Purushothaman, V., Bhavasar, D., Venugopal, K., and Venkatesan, R.A 1989. Peste des petits ruminants of sheep in India. *Vet. Rec.*, *125*: 602.

Shaila, M.S., Shamaki, D., Forsyth, M.A., Diallo, A., Goatley, L., Kitching, R.P. and Barrett, T. 1996. Geographic distribution and epidemiology of peste des petits ruminants virus. *Virus Research* 43: 149–153.

Shankar, H., Gupta, V.K. and Singh, N. 1998. Occurrence of peste des petits ruminants like diseases in small ruminants in Uttar Pradesh. *Indian Journal of Animal Sciences* 68: 38–40.

Sharp, J.M.; Nettleton, P.F. 2007. Acute respiratory virus infections. In: Diseases of Sheep. (Aitken I. D., Ed.), Blackwell Publishing, pp. 207-208.

Shewen PE, and Wilkie BN. 1985. Evidence for the Pasteurella haemolytica cytotoxin as a product of activity growing bacteria. American Journal of Veterinary Research 46: 1212–1214.

Shewen, P. E. and J. A. R. Conlon, 1993. Pasteurella. In: Pathogenesis of Bacterial Infections in Animals. 2nd edn., eds C. L. Gyles & C. O. Thoen, Iowa State University Press, Ames, U.S.A.

Shoo M.K 1989. Experimental bovine pneumonic pasteurellosis: Review. Veterinary Records 124: 141–144

Singh R. P., Saravanan P., Sreenivasa B. P., Singh R. K., and Bandyopadhyay S. K. 2004. Prevalence and distribution of peste des petits ruminants virus infection in small ruminants in India," *OIE Revue Scientifique et Technique*, 23(3): 807–819

Singh, R.P., Sreenivasa, B.P., Dhar, P., Shah, L.C. and Bandyopadhyay, S.K. 2004. Development of a monoclonal antibody based competitive-ELISA for detection and titration of antibodies to peste des petits ruminants (PPR) virus. *Veterinary Microbiology* 98: 3–15.

Sinnathamby, G., Renukaradhya, G.J., Rajasekhar, M., Nayak, R. and Shaila, M.S. 2001. Immune responses in goats to recombinant hemagglutinin-neuraminidase glycoprotein of Peste des petits ruminants virus: identification of a T cell determinant. *Vaccine* 19: 4816–4823.

Sinnathamby, G., Seth, S., Nayak, R. and Shaila, M.S. 2004. Cytotoxic T cell epitope in cattle from the attachment glycoproteins of rinderpest and peste des petits ruminants viruses. *Viral Immunology* 17: 401–410.

Slauson DO, Ley JC, Castleman WL, Neilsen, N.R 1987. Alveolar macrophage phagocytic kinetics following pulmonary parainfluenza-3 virus infection. J Leukoc Biol 41:412–20.

Solis-Calderon JJ, Segura-Correa JC, Aguilar-Romero F and Segura-Correa, VM 2007. Detection of antibodies and risk factors for infection with bovine respiratory syncytial virus and parainfluenza virus-3 in beef cattle of Yucatan, Mexico. Prev Vet Med 82:102–10.

Spicer, S.S., Chakrin, L.W., Wardel. J.R. and Kendrick, W., 1983. Histochemical properties of the respiratory tract epithelium in different species. Am. Rev. Respir. Dis., 128: 520-528.

Spilki, F.R., Almeida, R.S., Domingues, H.G., D'Arce, R.C.F., Ferreira, H.L., Campalans, J., Costa, S.C.B. and Arns, C.W 2006. Phylogenetic relationships of Brazilian bovine respiratory syncytial virus isolates and molecular homology modeling of attachment glycoprotein. *Virus Res. 116,* 30–37.

Srinivas, R.P. and Gopal, T. 1996. Peste des petits ruminants (PPR): a new menace to sheep and goats. *Livestock Advisor* 21: 22–26.

Stephensen, E., Sturve, J. and Forlin, L. 2002. Effects of redox cycling compounds on glutathione content and activity of glutathione-related enzymes in rainbow trout liver. Comparative Biochemistry and Physiology Part C. 133, 435-442.

Stilwell G, Matos M, Carolino N, and M. S. Lima 2008. Effect of a quadrivalent vaccine against respiratory virus on the incidence of respiratory disease in weaned beef calves. Prev Vet Med 85:151–7.

Stine, L.C.; Hoppe, D.K.; Kelling, C.L. 1997. Sequence conservation in the attachment glycoprotein and antigenic diversity among bovine respiratory syncytial virus isolates. *Vet. Microbiol. 54*, 201–221.

Stott EJ, Thomas LH, Collins AP, et al 1980. A survey of virus infections of the respiratory tract of cattle and their association with disease. J Hyg (Camb) 85: 257–70.

Streatfield, S.J. 2005. Plant based vaccines for animal health. *Rev sci tech Off Int Epi* 24(1):189–199

Svitek, N. and Von Messling, V. 2007. Early cytokine mRNA expression profiles predict Morbillivirus disease outcome in ferrets. *Virology* 362, 404–410.

Taniuchi N, Ghazizadeh M, Enomoto T, Matsuda K, Sato M, Takizawa Y, Jin E, Egawa S, Azuma A, Gemma A, Kudoh S, Kawanami O. 2009. Evaluation of Fractional Analysis of Bronchoalveolar Lavage Combined with Cellular Morphological Features. *International Journal of Medical Sciences* 6(1):1-8

Tanko NP, Emikpe BO, Sabri MY. 2016.Changes in haematological parameters and oxidative stress response of goats subjected to road transport stress in a hot humid tropical environment. *Comp Clin Path.* 25: 285-93

Taylor WP, Abegunde A 1979. The isolation of peste des petits ruminants virus from Nigerian sheep and goats. Research in Veterinary Science 26: 94-96

Taylor, W.P. 1979. Protection of goats against peste-des-petits-ruminants with attenuated rinderpest virus. *Research in Veterinary Science* 27: 321–324.

Ten Have-Opbroek, A.A.W., Otto-Verbeme, C.J.M., Dubbeldam, J.A. and Dykman, J.H., 1991. Proximal border of the human respiratory unit, as shown by scanning and transmission electron microscopy and light microscopical cytochemistry. *Anat. Rec.* 229: 339-354.

Tenuche O, Emikpe BO, Sabri MY 2013. Cellular and mucosal immune responses in the respiratory tract of Nigerian goats following intranasal administration of inactivated Recombinant *Mannheimia hemolytica* bacterine. *Niger. J. Physiol. Sci. 28: 121–125*

Thiaucourt F., Manso-Silvan L., Salah W., Barbe V., Vacherie B., Jacob D., Breton M., Dupuy V., Lomenech A., Blanchard A., Sirand-Pugnet P. 2011. Mycoplasma mycoides,

from “mycoides mall Colony” to “capri”. A microevolutionary perspective. *BMC Genomics* 12:114.

Thiry, J.; Keuser, V.; Muylkens, B.; Meurens, F.; Gogev, S.;Vanderplasschen, A.; Thiry, E. Ruminant alphaherpesviruses related to bovine herpesvirus 1. Vet. Res. 2006, 37, 169-190.

Thomas LH 1973. Observations on the role of viruses in pneumonia of calves. Vet Rec 93:384–8.

Thombare, N.N. and Sinha, M.K. 2009. Economic implications of Peste des petits ruminants (PPR) disease in sheep and goats: a sample analysis of district Pune, Maharastra. *Agricultural Economics Research Review* 22: 319–322.

Thomson, R. G., 1974. Pathology and pathogenesis of the common diseases of the respiratory tract of cattle. *Canadian Veterinary Journal.* 15, 249−251.

Thomson, R. G., S. Chander, M. Savan and M. L. Fox, 1975. Investigation of factors of probable significance in the pathogenesis of pneumonic pasteurellosis in cattle. Canadian Journal of Comparative Medicine, 39,194−207.

Tijjani AN, Ameh JA, Gambo HI, Hassan SU, Sadiq MA, and Gulani I 2012. Studies on the bacterial flora and pathologic lesions of caprine pneumonic lungs in Maiduguri North-Eastern Nigeria. *African Journal of Microbiology Research* 6(48): 7417-7422

Toth TE, Frank GH 1988. Bovine parainfluenza-3 virus proteins recognized by antibodies of aerosol-exposed calves. Am J Vet Res 49:1945–9.

Truong, T.; Boshra, H.; Embury-Hyatt, C.; Nfon, C.; Gerdts, V.; Tikoo, S.; Babiuk, L.A.; Kara, P.; Chetty, T.; Mather A. 2014. Peste des petits ruminants virus tissue tropism and pathogenesis in sheep and goats following experimental infection. *PLoS One*, *9*, e87145.

Valarcher J.F., Schelcher F. and Bourhy H 2000. Evolution of bovine respiratory syncytial virus. *J. Virol. 74*: 10714–10728.

Valarcher J.F., Taylor G. 2007. Bovine respiratory syncytial virus infection. *Vet. Res.38*: 153–180.

van Wyke Coelingh KL, Winter CC, Tierney EL, S.L. Hall, W.T. London, H.-W. Kim, R.M. Chanock, B.R. 1990. Antibody responses of humans and nonhuman primates to

individual antigenic sites of the hemagglutininneuraminidase and fusion glycoproteins after primary infection or reinfection with parainfluenza type 3 virus. J Virol 64:3833–43.

Vangeel I, Ioannou F, Riegler L, Salt J.S., Harmeyer S.S 2009. Efficacy of an intranasal modified live bovine respiratory syncytial virus and temperature-sensitive parainfluenza type 3 virus vaccine in 3-week-old calves experimentally challenged with PI3V. Vet J 179:101–8.

Varshney R, and Kale R.K. 1990. Effects of calmodulin antagonists on radiation-induced lipid peroxidation in microsomes. *Int J Radiat Biol.* 58:733-43.

Waret-Szkuta A., Roger F., Chavernac D., Yigezu L., Libeau G., Pfeiffer D.U. and Guitian J. 2008. Peste des petits ruminants (PPR) in Ethiopia: analysis of a national serological survey. *BMC Veterinary Research* 4: 34.

Welsh R.D, Dye L.B, Payton M.E. and Confer A.W. 2004. Isolation and antimicrobial susceptibilities of bacterial pathogens from bovine pneumonia: 1994-2002. *J Vet Diagn Invest*, 16: 426-431.

West K, Petrie L, Haines DM, Cortese V, Ellis JA. 1999. The effect of formalin-inactivated vaccine on respiratory disease associated with bovine respiratory syncytial virus infection in calves. Vaccine 17:809–20.

Winsor L. 1994. Tissue processing. In Woods A and Ellis R eds. *Laboratory histopathology*. New York: Churchill Livingstone. 4.2-1 - 4.2-39.

World Organisation for Animal Health (OIE), *World Animal Health Information Database (WAHID)* (2004). –Contagious caprine pleuropneumonia. Chapter 2.4.6. *In* Manual of Diagnostic Tests and Vaccines for Terrestrial Animals, 5th Ed. OIE, Paris, 623-635. Also available at: http://www.oie.int/eng/normes/mmanual/A_00072.htm (accessed on 10 February 2015).

World Organization for Animal Health (OIE), *World Animal Health Information Database (WAHID) [Database Online] List of Countries By Sanitary Situation: Haemorrhagic Septicemia*, World Organization for Animal Health (OIE), Paris, France, 2009, http://www.oie.int/wahis/public.php?page=disease status lists.

Wosu, L.O. 1994. Current status of peste des petits ruminants (PPR) disease in small ruminants. A review article. *Stud. Research in Veterinary Medicine* 2: 83–90.

Wosu, L.O. and Ewuzie, L.I. 1985. Heamagglutination – Inhibition (HI) Test for Rinderpest antibody with Measles virus in Nigeria. *Nigeria Veterinary Journal, 14*: 1, 59 – 60.

Wosu, L.O., Ezeibe, M.C.O., 1991. Haemagglutination inhibition technique for definitive diagnosis of PPR virus specific antibody. *Biomedical. Letters*. 47: 173– 177

Yarim G.F., Nisbet C., Yazıcı Z. and Gumusova S.O. 2006. Elevated serum total sialic acid concentrations in sheep with peste des petits ruminants. *Medycyna Weterynaryjna* 62: 1375–1377.

Yener Z, Ilhan F, Ilhan Z, and Saglam Y.S 2009. Immunohistochemical detection of Mannheimia (Pasteurella) haemolytica antigens in goats with natural pneumonia. Veterinary Research Communication 33:305–313.

Yener Z., Saglam Y.S., Temur A. and Keles H. 2004. Immunohistochemical detection of peste des petits ruminants viral antigens in tissues from cases of naturally occurring pneumonia in goats. *Small Ruminant Research* 51: 273–277.

Yener Z., Sağlam Y.S., Timurkaan N., Ilhan F. 2005. Immunohistochemical detection of parainfluenza type 3 virus antigens in paraffin sections of pneumonic caprine lungs. J Vet Med A Physiol Pathol Clin Med., 52(6): 268-71.

Yesilbag K., Gungor B. 2008. Seroprevalence of bovine respiratory viruses in North-Western Turkey. *Trop. Anim. Health Prod. 40*: 55–60.

Yesilbag K., Gungor B. 2009. Antibody prevalence aganist respiratory viruses in sheep and goats in North- Western Turkey. *Trop. Anim. Health Prod.*, 41: 421-425.

Yunus, M. and Shaila, M.S. 2012 Establishment of an in vitro transcription system for peste des petits ruminant virus. *Virology Journal* 9: 302.

Zamri S.M., Effendy W. M., Maswati M.A., Salim N. and Sheikh O.A.R. 1996. The goat as a model for studies of pneumonic pasteurellosis caused by Pasteurella multocida. British Veterinary Journal, 152: 453−458.

Zamri Saad M., Maswati M.A., Effendy A.W.M., Salim N.B. and Sheikh-Omar A.R. 1996. Establishment of Pasteurella haemolytica A2 in the lungs of goats following intratracheal exposure. *Journal Veterinary Malaysia* 8:15–19

Zamri, S.M., Jansi S., Nurida A.B. and Sheikh O.A.R. (1991). Experimental infection of dexamethazone treated goats with Pasteurella haemolytica “A2”. British *Veterinary Journal* 147: 565−568.

Zamri-Saad M. and Effendy A.W.M. 1999. The Effects of Dexamethasone on the Response of Bronchus- associated Lymphoid Tissue to Intranasal Administration of Formalin-killed Pasteurella haemolytica A2 in Goats. Veterinary Research Communications, 23: 467–473

Zamri-Saad M., Effendy A.W., Israf D.A, Azmi M.L. 1999. Cellular and humoral responses in the respiratory tract of goats following intranasal stimulation using formalin-killed *Pasteurella haemolytica* A2. *Vet. Microbiol. 65(3)*:233-40.

Zamri-Saad M., Sharif H., Basri K. 1989. Microbiological and pathological evaluation of vaccination against naturally occurring caprine pasteurellosis. Veterinary Records 124: 171–172

Zecchinon L., Fett T. and Desmecht D. 2005. How Mannheimia haemolytica defeats host defense through a kiss of death mechanism. *Veterinary Research* 36: 133−156.

Zhang L, Bukreyev A, Thompson CI, Watson B, Peeples ME, Collins PL, Pickles RJ. (2005). Infection of ciliated cells by human parainfluenza virus type 3 in an in vitro model of human airway epithelium. *J Virol* 79:1113–24.

APPENDIX

Attachment 1- Reaearch ethics approval certificate for the study

ANIMAL CARE USE AND RESEARCH ETHICS COMMITTEE (ACUREC)

UNIVERSITY OF IBADAN

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E.mail: **animaluseresearch@gmail.com / animaluseresearch@yahoo.com**

Our Ref: Assigned number: UI-ACUREC/17/0060 Your Ref: .....

Date: .....

NOTICE OF FULL APPROVAL AFTER FULL COMMITTEE REVIEW

Re: Clinico-Pathological Evaluation of Causal Agents and Their Roles in Pneumonia Complex in Goats

Name of Principal Investigator: Dr Theophilus Aghogho JARIKRE

Address of Principal Investigator: Department of Veterinary Pathology
Faculty of Veterinary Medicine
University of Ibadan, Ibadan

Date of receipt of valid application: 13/06/2017

Date of meeting when final determination on ethical approval was made: **14/07/2017**

This is to inform you that the research described in the submitted protocol, have been reviewed and *given full approval by the* UI-ACUREC.

This approval dates from **14/07/2017 to 13/07/2018.** If there is delay in starting the research, please inform U1-ACUREC so that the dates of approval can be adjusted accordingly.
Note that no activity related to this research may be conducted outside of these dates. It is expected that you submit your annual report as well as an annual request for the project renewal to the UI-ACUREC at least four weeks before the expiration of this approval in order to avoid disruption of your research.

The National Code for Health Research Ethics requires you to comply with all institutional guidelines, rules and regulations and with the tenet of the Code including ensuring that all adverse events are reported promptly to the UI-ACUREC. *No changes are permitted in the research without prior approval by the UI-ACUREC except in circumstances outlined in the code.* The UI-ACUREC *reserves the right to conduct compliance visit to your research site without previous notification*

You are to note that **UI-ACUREC** reserves the right to monitor and conduct compliance visit to your research site without previous notification.

.....

Prof. S.I.B. Cadmus
Chairman, UI-ACUREC

Attachment 2- Some of the comingled goats housed in the experimental animal house.

Table A1: Clinicopathological observations in the experimental goats
ND- nasal discharges; OD- ocular discharges; Drh- Diarrhea;

	WT (KG)			TEMP (^{0}C)			CLINICAL SIGNS					
	nitial	**PV**	**PI**	**Initial**	**PV**	**PI**	**Initial**	**PV**	**PI**	**Clinical s c o r e**	**Respiratory**	**PM**
A1	4.8	4.9	4.6	36	40	38.7	-	-	-	0	22	-
A2	4.8	4.9	4.8	37	39.5	40.1	-	-	Serous OD	1	17	Normal
A3	6	6.1	5.6	38	40.5	41	-	-	Drh	3	25	-
B1	5.2	5.4	5.4	38	39.8	40	-	OD	-	0	12	Normal
B2	4.9	5.1	4.2	37	39.8	39.8	-	-	OD, Drh	4	23	Fibrinous pn
B3	5.6	5.3	4.2	39	40	40	-	OD, ND	ND, OD, Drh, Dull	5	26	Interstitial Pn
C1	5.7	6	5.5	37	38.4	38	-	-	ND, OD, Drh, Dull	5	29	Fibrinous pn
C2	4.6	4.7	4.2	38.2	40	42	-	-	ND, OD, Dull	3	32	Interstitial Pn
C3	5.9	6	6	38.1	39.2	39.5	-	-	Purulent OD	2	26	-
D1	6	6	4.7	38.3	39.9	39.5	-	-	ND, OD, Drh, We ak	5	39	Interstitial Pn
D2	4	4.2	3.8	37.9	40	42	-	OD	ND, OD, Drh, Dull	5	40	Broncho Pn
D3	5	5	4.2	39	40.1	40.2	-	-	ND, OD, Drh, Dull	5	42	Fibrinous pn

1. **Jarikre T.A.,** Emikpe B.O., Morenikeji O.A., Akpavie S.O. 2016). Pattern and associated risk factors of caprine pneumonia in Nigeria. *Asian Pacific Journal of Tropical Diseases* 6(3): 179-183
2. **Jarikre T.A.**, Emikpe B.O., Ohore O.G., Akinremi T.A. and Akpavie S.O. (2016). Bronchoalveolar Lavage Fluid Cellular and Haematological Changes in Different Types of Caprine Pneumonia. *Niger. J. Physiol. Sci. 31(June 2016)*
3. Jarikre T.A. , G.O. Ohore , A.A. Oyagbemi, B.O. Emikpe (2017). Evaluation of oxidative stress in caprine bronchoalveolar lavage fluid of pneumonic and normal lungs. International Journal of Veterinary Science and Medicine 5 (2017) 143–147
4. **Jarikre TA** and Emikpe BO (2017): First report of immunohistochemical detection of Peste des petit ruminants, parainfluenza 3 and respiratory syncytial viral antigens in lungs of Nigerian goats, Journal of Immunoassay and Immunochemistry, **38**(5): 1-14 DOI: 10.1080/15321819.2017.1349669.
5. Jarikre TA, Alao OS, Emikpe BO. Cultural and immunohistochemical evaluation of bacterial agents in caprine pneumonia in Nigeria. Comparative Clinical Pathology (Springer) (In press).

MANUSCRIPTS UNDER REVIEW

1. Jarikre TA and Emikpe BO. Immunohistochemical evidence of viruses-bacterial interactions in caprine pneumonia in Nigeria: Implications for combined vaccines
2. Jarikre TA and Emikpe BO. Intranasal Peste des petits ruminants virus and *Mannheimia hemolytica* vaccination: Clinical, haematological, serological and serum oxidative stress changes in challenged goats.

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Pattern and associated risk factors of caprine pneumonia complex in Nigeria

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ABSTRACT

Objective: To investigate the pattern of lung consolidation in natural infections and identify the risk factors associated with caprine pneumonia in Nigeria so as to elucidate and aid the understanding of caprine respiratory disease complex in Nigeria.

Methods: A total of 700 goats were examined before slaughtering between March 2014 and July 2015. Ante mortem evaluation for physical characteristics, body condition, breed and sex, gross morphometry for estimation of the percentage of lung consolidation and histopathology was performed according to standard techniques. Data were presented in percentages, mean ± SEM and subjected to non-parametric analysis.

Results: The results showed that 50.5% of goats belonged to the breed of West African Dwarf, 35.4% Red Sokoto (RS) and 14.1% Sahelian. As for the age distribution, 5.7% of them were one year old, 30.7% were two years old and 63.6% were above two years old. The overall prevalence of pneumonia was 59.7%. The mean lung consolidation score was 8.1 ± 0.5, consolidation for male was 7.8 ± 0.5 and 21.4 ± 7.7 for female ($P < 0.05$). There was more consolidation on the right lung and caudal lobes, and RS showed the highest prevalence of consolidation. There was a positive correlation of contralateral lobar consolidation in the pneumonic animals, while significant effect of sex and body scores on the lung consolidation was observed ($P < 0.05$). The types of pneumonia observed were bronchopneumonia (41.6%), bronchointerstitial pneumonia (8.7%), interstitial pneumonia (7.9%), verminous pneumonia (0.5%), granulomatous pneumonia (0.9%) and normal lungs (40.3%).

Conclusions: Sex, breed and body scores were observed to be risk factors associated with caprine pneumonia in Nigerian goats. In this study, transport stress may be responsible for the high consolidation in RS. This information will help to increase the knowledge on the pathogenesis and the risk factors that often aggravate the prevalence of pneumonia in goats.

1. Introduction

Goats are important commodities to a large segment of the world's population as a source of meat, milk and skin. Goats (*Capra hircus*) are descendants from the species *Capra aegragus*, and the first ruminant to be domesticated[1]. The economic importance of goats cannot be overemphasized because they serve as a source of income for the poor, because they are comparably cheap to manage and serve as essential livestock in rural households[2]. However, goats are on the receiving end of adverse environmental influences, stressful managemental practices and conditions which predispose to respiratory diseases.

Pneumonia is no doubt a major constraint to small ruminant production worldwide[3], causing severe morbidity manifested physically as unthriftiness or weight loss[4] and large scale mortality in domestic and wild goats[1-5]. Pattern of pulmonary consolidation or pneumonia serves as a reliable clinical parameter in auscultation[6]. The pattern of pulmonary consolidation has been reported in experimental conditions. Emikpe and Akpavie[7] reported a mean pulmonary consolidation score of 6.83 in West African Dwarf (WAD) goats, 5%–60% pulmonary consolidation score in aerosol-vaccinated calves[8] and 32% pulmonary consolidation score in Markoz goats[9], but little is known regarding the influence of age, sex, body score and breed on the

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All experimental procedures involving animals were conducted in accordance to international guides for use and care of experimental animals and approved by University of Ibadan animal care use in research and experiment committee.
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Full Length Article

Evaluation of oxidative stress in caprine bronchoalveolar lavage fluid of pneumonic and normal lungs

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ABSTRACT

Research in the area of oxidative stress in pneumonic pathology still requires attention especially in small ruminants especially with the use of bronchoalveolar lavage (BAL) which may be a more sensitive indicator of respiratory diseases than blood. This investigation evaluates the role of oxidative stress in the pathogenesis of caprine pneumonia using BAL fluid (BALF) for morbidity and pneumonic goats. A BALF from 150 goats (without pneumonic histopathology) had been characterized using standard techniques) was biochemically assayed for anti-oxidants and pro-oxidants Malondialdehyde (MDA), hydrogen peroxide generation (H_2O_2), superoxide dismutase (SOD) and reduced glutathione (GSH) contents were measured to ascertain the radical activity in the BALF. Superoxide dismutase (SOD), Glutathione transferase (GST) and Glutathione peroxidase (GPx) activity were also determined colorimetrically. There were significant increases in the BALF concentrations of MDA, H_2O_2 and SOD with decreases in GSH level and SOD activity in the pneumonic goats ($P < 0.05$). There was also significant correlation of BALF oxidative assay to the type and severity of pneumonia. The levels of MDA, H_2O_2 and SOD increased significantly ($P < 0.05$) in bronchopneumonia and bronchointerstitial pneumonia than other pneumonic conditions and normal lungs. The management of caprine pneumonia should often incorporate antioxidant supplementation to correct the imbalance in pro-oxidants and anti-oxidant levels.

1. Introduction

Respiratory diseases in sheep and goat have been established from exposure to stressors which may include long period of starvation and transportation, bleeding and weaning followed by invasion of bacterial and viral infectious agents [1,2]. Exposure to these stressful conditions leads to excessive production of free radicals and reactive species with potential to enhance damaging effects [3].

Oxidative stress involves oxidative modification by reactive oxygen species of biomolecules (proteins, nucleic acids, and lipids). It induces a variety of organ dysfunction as a result of imbalance between the pro-oxidant and anti-oxidant levels in cells and tissues [4,5]. The pro-oxidants promotes oxidation while anti-oxidants checkmates the activities of these pro-oxidants. Oxidative stress may also result from defects in expression of the genes controlling anti-oxidant enzymes [6].

The respiratory pathogens survival results from reactive species generated by the host through detoxification mechanisms using enzymatic and non-enzymatic processes. The anti-oxidant enzymes are involved in enzymatic detoxification mechanisms and other adaptive mechanisms controlled by gene expression [6,7].

Research in the area of oxidative stress in pneumonic pathology still requires attention especially in small ruminants. Hitherto, evaluations of oxidative stress in ovine is regulated estimation of certain blood biomarkers that reflect the oxidative profile of affected cases [8,9], as was done in Peste des petits ruminant (PPR) viral infected sheep [10]. The assay of these markers in BAL may be a more sensitive indicator of respiratory diseases than blood. Hence, this study evaluates for the first time the oxidative stress parameters in bronchoalveolar lavage fluid of healthy and pneumonic goats.

2. Materials and methods

2.1. Ethical approval

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed, and also

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First report of immunohistochemical detection of Peste des petit ruminants, parainfluenza 3 and respiratory syncytial viral antigens in lungs of Nigerian goats

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ABSTRACT

This study determined the of involvement of PPR, PI3, and RS viruses in the pathology of caprine pneumonia across Nigeria. 150 goats were selected randomly. PI3 and RSV monoclonal antibodies and PPR polyclonal antibody were used for the immunolocalization of the antigens. Histologically, 61 of the goats had broncho-interstitial pneumonia, 23 had interstitial pneumonia, 43 had bronchopneumonia, 13 had bronchiolitis, and 10 were normal. PPR, PI3, and RS viral antigens were demonstrated in intact and desquamated bronchial, bronchiolar epithelial cells, macrophages, leukocytes, pneumocytes, and giant cells. 29% of the caprine lungs had positive immuno-staining to PI3 viral antigen, 10% were positive for RSV antigen while 36% were positive for PPR viral antigen. 8% showed immunostaining for the two and or three respiratory viral antigens in the goats. PI3 and RSV antigens were more in the young goats, red sokoto breed and during the dry season. This is the first report of immunohistochemical detection of PPR, PI3 and RS viral antigens in caprine lungs in Nigeria. These findings underscore the importance of PI3 and RSV viruses in the control of caprine pneumonia in Nigeria.

KEYWORDS
caprine pneumonia; parainfluenza 3 virus; peste des petits ruminants virus; respiratory syncytial virus; Nigeria

Introduction

Pneumonia is a leading cause of mortality in ruminants throughout the world.[1] Respiratory tract infections have been demonstrated in sheep and goats.[2–4] However, the causative agents were rarely determined and many viruses associated with respiratory disease conditions in ruminants have been implicated in natural and experimental infections in sheep and goats.[5]

Respiratory viruses including parainfluenza 3 (PI-3) virus, adenovirus type 6 and respiratory syncytial virus (RSV), and to a lesser extent bovine adenovirus type 2, ovine adenovirus types 1 and 5, and reovirus type 1 cause respiratory infections and pneumonia in ruminants.[6,4] More importantly, these viruses also dramatically increase the susceptibility of

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