SEROLOGICAL STUDIES ON PESTE DES PETITS RUMINANTS (PPR) IN SHEEP, GOATS AND CAMELS IN SOKOTO STATE, NIGERIA

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BY

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BEING A THESIS SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES, AHMADU BELLO UNIVERSITY, ZARIA IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF A DEGREE OF MASTER OF SCIENCE IN VETERINARY MICROBIOLOGY

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APRIL, 2013

DECLARATION

I hereby declare that the work in this thesis entitled '**SEROLOGICAL STUDIES ON PESTE DES PETITS RUMINANTS (PPR) IN SHEEP, GOATS AND CAMELS IN SOKOTO STATE, NIGERIA**' has been carried out by me in the Department of Veterinary Microbiology Under the supervision of Professors H. M. Kazeem, S.B. Oladele and M. Y. Fatihu. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this work was previously presented for another degree or diploma at this or any other institution.

.....	.....	.....
Name of student	Signature	Date

CERTIFICATION

This thesis titled "**SEROLOGICAL STUDIES ON PESTE DES PETITS (PPR) IN SHEEP, GOATS AND CAMELS IN SOKOTO STATE, NIGERIA**" by, MUHAMMAD BASHIR BELLO meets the regulations governing the award of the degree of Master of Science in Veterinary Microbiology of Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This work is dedicated to my parents, Dr. Bello Muhammad Tambuwal and Hajiya Fatima Bello Tambuwal, for the sound moral upbringing they have given me. May Allah in his infinite mercy and blessings reward them abundantly. Ameen.

.

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ABSTRACT

Peste des petits ruminant (PPR) is a highly contagious viral disease of small ruminants that is regarded as one of the most important militating factors against small ruminant production in Nigeria. Despite its endemicity in Sokoto State, scanty or no report of PPR exist in the State. Therefore, this study was aimed at determining the current serological status of the disease in sheep, goats and camels in Sokoto State, Nigeria. A total of 433 serum samples obtained from small ruminants (sheep and goats) in six randomly selected Local Government Areas (Tambuwal, Goronyo, Wurno, Kware, Bodinga and Sokoto South LGAs) in the State were examined for the presence of PPR antibodies using a monoclonal antibody-based competitive enzyme linked immunosorbent assay (c-ELISA). The sera were collected only from those small ruminants of not less than six months old and with no history of vaccination against PPR. The overall seroprevalence of PPR in small ruminants was 45.50% (197/433) while that of camel was 18.25% (25/137). Chi square test of independence at 95% confidence interval revealed a statistically significant difference in the seroprevalence rates among the LGAs sampled: Goronyo (56.72%), Tambuwal (59.10%), Bodinga (33.71%), Kware (53.24%), Wurno (18.60%) and Sokoto South (68.75%). Furthermore, sheep (52.41%) had a statistically greater seroprevalence rate than the goats (40.24%) sampled in this study ($p=0.015$). In addition, small ruminants aged 13-24 months had a significantly greater prevalence of PPR antibodies than other age groups considered in this study ($p=0.015$). However, While there was statistically significant difference in the seroprevalence rates of various breeds of goats considered in this study ($p= 0.0179$), in the sheep breeds, the difference was not statistically significant. The detection of PPR virus antibodies in small ruminants from all the LGAs sampled suggests

the wide distribution of the virus in the study area. In addition, the detection of PPR virus antibodies in the camel population sampled suggests that they are equally susceptible to PPR virus like sheep and goats. It is therefore recommended that PPR control programmes such as vaccination with PPR homologous vaccine should be intensified in the study area. Further studies on the isolation and molecular characterization of the virus in the study area are also recommended.

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LIST OF ABBREVIATIONS

AGID =	Agar gell immunodifusion test
cDNA =	Complementary Deoxy ribonucleic acid
CDV =	Canine distemper virus
CIE =	Counter immunoelctrophoresis
DDV =	Dolphin distemper virus
ELISA=	Emzyme linked immunosorbent assay enzyme linked immunosorbent assay
HA=	Haemagglutination test
HI =	Haemagglutination inhibition test
HRPO=	Horseradish peroxidase
MFH PPRV =	Matrix, Fusion and Hemmaglutinatin genes from peste des petits ruminants virus
NPC=	National Population Commission
PDV =	Phorcine distemper virus
PorDV =	Porpoise distemper virus
PPR =	peste des petits ruminants
RBC =	Red blood cell
RNA =	Ribonucleic acid
RPV =	Rinderpest virus
RT =	PCR- Reverse transcription polymerase chain reaction
RT =	PCR-ELISA- reverse transcription polymerase chain reaction
VNT =	Virus neutralisation test

CHAPTER ONE

INTRODUCTION

1.1 Backgroud of the Study

Peste des petits ruminants (PPR) is a highly contagious viral disease of small ruminants such as sheep and goats (Dhar *et al*., 2002), characterized by pyrexia (40-41^{0}C), purulent ocular/nasal discharge with congested conjunctiva, respiratory distress, sneezing, ulceration of mucous membranes of the mouth, diarrhea and gastroenteritis (Ozkul *et al.,* 2002). The disease is caused by an enveloped pleomorphic RNA virus which is a member of the Genus *Morbillivirus* belonging to the Family *Paramyxoviridae*. The virus is antigenically related to rinderpest, canine distemper, dolphin distemper, phorcine distemper, porpoise distemper and measles viruses (Barrett, 1999). Genetic characterization of the virus has grouped it into four distinct lineages: three from Africa and one from Asia. One of the African lineages is also found in Asia (Kerur *et al*, 2008). Cattle, buffalos and pigs can become infected, but there is little or no evidence of the disease associated with their infection (Chauhan *et al*., 2009). However, Khalafalla *et al.* (2010) reported an outbreak of the disease in camels. The disease could also be a natural infection of gazelles.

Peste des petits ruminants virus is easily transmitted by direct contact between the secretions and/or excretions of infected animals and nearby healthy animals (Ezeibe *et al.*, 2008). A substantial quantity of the virus is found in the ocular, nasal or oral secretions of infected goats and in the fecal material late in the disease (Abegunde and Adu, 1977). Fine infective particulate materials from these secretions and excretions are released into the air by the affected animals and when other animals in close contact inhale the droplets, they

are likely to be infected. Mortality in susceptible herd varies but may be as high as 90% (Abu-Elzien *et al*., 1990). Infection with the virus is usually magnified if complicated with other concurrent respiratory and/or gastro-intestinal infections.

The first description of Peste des petits ruminants dates back to the early 1940s in Ivory Coast (Gargadennec and Lalanne, 1942) although in Nigeria, Beaton (1930) reported the disease as rinderpestlike disease in goats in 1930. For a long time, PPR was incorrectly considered to be to a West African disease. However, with the advent of specific diagnostic tests that enable differentiation between PPR and similar diseases, the known geographical distribution expanded quickly between the late 1980s and the mid-1990s to cover all the countries in Africa between the Sahara and the Equator, the Near East and the Indian sub continent. Since the beginning of year 2000, PPR has emerged in other areas, probably because of the intensification of animal movements and because the disease is increasingly well known and diagnosed (EMPRESS, 2009). As of 2007, an estimated 1 billion sheep and goats in Africa and Asia were at risk of PPR (EMPRES, 2009).

1.2 Statement of Research Problem

Peste des petits Ruminants is known to be endemic in Nigeria. This has been confirmed by studies conducted by Kazeem (2001) and Shamaki *et al*. (2004). The disease is responsible for significant economic losses in the small ruminants industry in the endemic regions (Asim *et al*., 2009). It can quickly wipe out whole flocks either by itself or in combination with other diseases, such as pneumonia and gastrointestinal parasitism (Majiyagbe, 1985). Therefore, it has a direct impact on the livelihood of the rural poor and marginalised

segments of the society (Luka *et al*., 2011) since most disadvantaged households rely on small ruminants for food and cash security. Programs aimed at controlling the disease in ruminants have cost farmers, governments and aid agencies large sums of money. Hence, the disease remains a major constraint in the productivity of small ruminant in regions where it is endemic (Stem, 1993). However, despite all the negative economic consequences of PPR, there is paucity of information on the current status of the disease in sheep and goats in different parts of Sokoto State.

The camel was not reported as a possible natural host to PPR until in 1992 when Ismail *et al.* (1992) in Egypt, detected the infection through serology in Sudanese camels. Since then, several studies have been conducted on PPR in camels in Sudan, Ethiopia and other African countries (Roger *et al.,* 2001; Haroun *et al.,* 2002; Abraham *et al*., 2005; Khalafalla, *et al.,* 2010). However, very little work on PPR in camels has been done in Nigeria. Shamaki *et al.* (2004) reported the recovery of PPR virus RNA from tissues of camels with respiratory syndrome in Nigeria. In addition, Daneji *et al.* (1997) reported serological evidence of PPR in slaughtered camels in Sokoto State, Nigeria. However, the current status of the disease in camels in the state and its possible association with other epidemiologic variables (age and sex) is largely unknown. There is therefore need to study the prevalence of PPR virus antibodies from camels along with the small ruminants in Sokoto state so as to know their contribution to the epidemiology of peste des petits ruminants in the study area.

1.3 Justification of the Study

Sokoto State is one of the major livestock producing States in Nigeria (William *et al*., 2000). An estimated 3 million sheep, 5 million goats and 4600 camels were documented for Sokoto state making the State second in livestock production across the nation (MOCIT, 2002). Camels and small ruminants, such as sheep and goats, being among the major livestock reared in the State, serve as good sources of meat and milk. They contribute manure for crop production and also serve as food, commercial and cash security for many people in the rural areas (Brumby, 1990: Majiyagbe *et al.,* 1992). They are also used as gifts or sacrifices for traditional rituals and religious purposes, during Eid for Muslims. In addition, the skin of Sokoto Red goats is known for its superior quality as the source of Morrocan leather and its suitability for fine leather (Midau *et al.,* 2010).

During the drought conditions, camels are the animals least affected and can continue to be a reliable means of transport. They sustain the lives of the pastoralists in these harsh environmental conditions because they are the most efficient animals in converting fodder into energy for work and consumable products (Dawo, 2010).

Unfortunately, in many areas of Africa and Nigeria in particular, small ruminant production is seriously threatened by PPR. There is also increasing evidence that PPR is involved in respiratory disease complex in the camel (khalafalla *et al*., 2010). Control of the disease is therefore of paramount importance in camel and small ruminant production in the country. However, crucial to the effective control of the disease in Sokoto State is the adequate understanding of the epidemiology of the disease. Thus, it becomes imperative to

conduct a study on the current prevalence of the disease in the State so as to broaden the understanding of the epidemiology which will in turn help in the control of the disease and enhance all the benefits associated with small ruminants and camels in the State.

1.4 Aim and Objectives of the Study

The aim of the study was to determine the prevalence of PPR virus antibodies in sheep, goats and camels using the monoclonal antibody based competitive emzyme linked immunosorbent assay (c-ELISA) in Sokoto State. The specific objectives of the study were

1. To examine the sheep and goat sera obtained from selected Local Government Areas of Sokoto State for PPR virus antibodies using monoclonal antibody based c-ELISA.
2. To examine the sera obtained from camels slaughtered at Sokoto Municipal abattoir for the presence of antibodies to PPR virus using c-ELISA.
3. To determine the effect of age, sex, breed and species on the prevalence of PPR Virus antibodies in different parts of Sokoto State.

1.5 Research Questions

1. What is the current seroprevalence of Peste des petits ruminants (PPR) in sheep, goats and camels of Sokoto State?

2. Is the seroprevalence of PPR affected by age, sex, breed and species of animals studied?

CHAPTER TWO
LITERATURE REVIEW

2.1 The Disease Called Peste Des Petits Ruminants

Peste des petits ruminants (PPR), also known as Kata, psuedorinderpest, pneumoenteritis complex or stomatitis-pneumoenteritis syndrome is a severe and highly infectious viral disease of small ruminants caused by PPR virus, a *Mobilivirus* of the Family *Paramyxoviridae*. The disease is characterized by fever, erosive stomatitis, conjunctivitis, gastroenteritis, and pneumonia. The clinical disease resembles Rinderpest in cattle, which is acute, and after an incubation period of 3-6 days, the clinical symptoms become apparent, and include high rise of temperature, oral, occular and nasal discharges, necrotic stomatitis, severe pneumonia, dyspnoea, coughing, enteritis, severe diarrhoea followed by death (Ezeokoli, *et al.,* 1986; Pawaiya *et al.,* 2004).

The natural disease affects mainly goats and sheep, but is usually more severe in goats where it causes severe morbidity and mortality (Raghavendra *et al.,* 2000). Infection rates in sheep and goats increase with age, and the disease, which varies in severity, is rapidly fatal in young animals (Wosu, 1994). Generally, cattle are considered to be subclinically infected with the disease. However, in poor conditions it might be possible that cattle develop lesions following PPRV infection, clinical signs of which would be ascribed to rinderpest, because of the similarity of the two diseases clinically. Moreover, PPRV was isolated from an outbreak of rinderpest-like disease in buffaloes in India in 1995 (Govindarajan *et al*., 1997). It was also suspected to be involved in the epizootic disease that affected one-humped camels in Ethiopia in 1995–1996 (Roger *et al.,* 2001). Indeed PPRV antigen and PPRV nucleic acid were detected in some pathological samples

collected during that outbreak (Roger *et al.,* 2001), but no live virus was isolated. Cases of clinical disease have been reported in wildlife resulting in deaths of gazelles in captivity (Elzein *et al.,* 2004).

Before the recognition of PPR as a disease entity in small ruminants, there were historical records of outbreaks of Rinderpest like diseases in sheep and goats that did not cause disease in cattle. Furthermore, diagnostic tests that are capable of differentiating between the two viruses only became available in the past 20 years. Therefore, it was likely that, in the past, many cases of PPR were ascribed to RPV (Baron *et al*., 2011). The idea that PPR has been in existence as a distinct disease for a long time is supported by the phylogenetic tree of the morbilliviruses, which shows that both RPV and PPRV were a similar distance from their most recent common ancestor. Assuming both viruses mutate according to the same evolutionary 'clock', PPRV must have been in circulation for as long as RPV.

In all regions where PPR is endemic, it constitutes a serious threat to small ruminant production. The disease is said to be the fastest growing disease of small ruminants in developing countries (Baron *et al*., 2011). Therefore, it influences the livelihood of poor farmers, the main owners of sheep and goats. Hence its control is a major priority for programmes aimed at poverty alleviation.

2.2 Aetiology

The aetiology of the disease is a virus that is classified in the Order *Mononegavirales*, Family *Paramyxoviridae*, SubFamily *Paramyxovirinae*, Genus *Morbillivirus* on account of

its genetic similarity with other members of the Genus *Morbillivirus* that includes measles virus (MV), rinderpest virus (RPV), canine distemper virus (CDV) and a number of other viruses that infect aquatic mammals (Banyard *et al.,* 2010). The virus is genetically grouped into four distinct lineages (I, II, III, and IV) on the basis of partial sequence analysis of the fusion (F) protein gene (Dhar *et al*., 2002), despite the fact that only a single serotype has been reported. Lineage 1 and 2 are found exclusively in West Africa, whereas lineage 3 has been found in eastern Africa and Arabia. The fourth lineage is confined exclusively in the Middle East Arabia and Indian subcontinent (Shaila *et al.,* 1996).

2.2.1 Biology of Peste des petits ruminants (PPR) virus

2.2.1.1 *structure of PPR virus*

Peste des petits ruminants virus is an enveloped, pleomorphic particle containing single stranded RNA, approximately 16 kb long with a negative polarity as a genome (Barrett *et al.,* 2005). The genome of PPR virus is so far the longest of all the mobiliviruses, consisting of about 15,948 nucleotides. Intact virion has a diameter of about 130-390nm with the thickness of the ribonucleoprotein measuring approximately 14-23nm (Durojaiye *et al*., 1985). It is wrapped by a nuclear protein which is associated with two other proteins: the phosphoprotien (P) and the viral RNA dependant RNA polymerase (L). On the viral envelope are found two other viral proteins, Haemagglutin (H) and Fusion (F) proteins, which are very important for the induction of protective host immune response against the virus (Chauhan *et al*., 2009).

2.2.1.2 *structural proteins of the virus*

The virus encodes six structural proteins; nucleoprotein (N), phosphoprotein (P), matrix protein (M), fusion protein (F), hemagglutinin protein (H), large polymerase protein (L), and non-structural proteins V and C (Bailey *et al*., 2007). Among the structural proteins, N protein is antigenically the most conservative among the morbilliviruses and is highly immunogenic in spite of its internal location (Libeau *et al*., 1995). The F and H proteins are associated with the viral envelope where they are believed to play important roles in induction of protective immunity (Chauhan *et al*., 2009). The large (L) protein is the enzymatic component of the viral transcriptase and replicase. The L proteins are multi-functional and, in addition to their polymerase activity, have methylation, capping and polyadenylation activities (Lamb and Kolakofsky, 2001). The Matrix (M) proteins are basic membrane associated molecules that interact with surface glycoproteins in the lipid envelope as well as the virion ribonucleoprotein.

2.2.2 Physicochemical properties of the virus

The molecular weight of the genome is 5.8×10^6 while the diameter of the virion measures about 150-300nm. The virion is very sensitive to heat, lipid solvents, non- ionic detergents, formaldehyde and oxidising agents (Kingsbury, 1990). The virus is usually destroyed at 50^oC for 60 minutes or 37^oC for 2 hours. However, it survives for long periods in chilled and frozen tissues (OIE, 2009).

2.2.3 Lineages of PPR virus

Although, there is only one serotype of the virus (Barrett *et al.,* 1993), PPRV isolates on the basis of partial sequence analysis of the fusion (F) protein gene, can be grouped into four distinct lineages; Lineage 1 and 2 are found exclusively in West Africa, whereas lineage 3 has been found in eastern Africa and Arabia. The fourth lineage is confined exclusively in the Middle East Arabia and Indian subcontinent (Shaila *et al.,* 1996). Virulence of PPR lineages has been evaluated experimentally in West African goats. In that study, it was shown that virulence varies with lineage groupings: Lineage I (CI 89, Guinea Conakry, Bissau Guinea) caused peracute to acute PPR disease; Lineage II (Nigeria 75/1) caused mild to inapparent PPR disease; Lineage III (Sudan-Sennar) caused acute to mild PPR disease; Lineage IV (India-Calcutta) caused acute PPR disease (Couacy-Hymann *et al*., 2007). Perhaps the mildness of the virus (Nig 75/1) was what informed its use in the initial attenuation by multiple passages on vero cells to produce the current PPR vaccine (Diallo *et al*., 1989).

2.2.4 Antigenic and genetic relationship to other viruses

Peste des petits ruminants virus is antigenically related to Rinderpest, Canine distemper, Dolphin distemper (DDV), Phorcine distemper (PDV), Porpoise distemper (PorDV) and Measles viruses (Barrett, 1999). Studies on nucleotide homologies of PPRV and other mobiliviruses revealed regions of low and high nucleotide homology. Regions of high conservation include the L and M genes while the non-coding regions show very little similarity (Bailey *et al*., 2005). At the level of the nucleotide sequences, the genome of PPRV shows greatest similarity with that of RPV. In addition, full length genome sequence

shows that the N, V and H proteins of PPRV had close similarity with DDV, indicating a close antigenic relationship between the two viruses (Bailey *et al*., 2005). Indeed, several cross protection studies conducted concluded that PPR and rinderpest virus antibodies, as well as CDV and PDV, show some degree of cross protection (Gibbs *et al*., 1979). Tissue culture Rinderpest vaccine was the vaccine of choice for the control of PPR and was known to confer immunity against virulent PPR strains for one year. However, its use has been banned due to its interference with global Rinderpest eradication efforts because sheep and goats can be subclinically infected with Rinderpest virus.

2.3 History and Geographic Distribution of the Disease

The history of PPR dates back to 1942 when it was first described by Gargadennec and Lalane (1942) in Ivory Coast of West Africa. The existence of the disease was soon confirmed in Nigeria, Senegal and Ghana. Taylor and Abegunde (1975) described the first outbreak of the disease in Nigeria. For many years, the disease was thought to be restricted to the West African subcontinent until a disease of goats in the Sudan, which was originally diagnosed as rinderpest in 1972, was confirmed to be PPR (Banyard *et al*., 2010). The development of trade relations, transport and migration of wild animals susceptible to PPR is believed to be instrumental in the spread of PPR beyond the boundaries of west Africa (Kaukarbayevich, 2009).

The disease is known to be present in a broad belt of sub-Saharan Africa, Arabia, the Middle East and Southern Asia. It is currently believed to be endemic across much of West Africa. However, virus outbreaks are often poorly characterized due to the lack of reporting

systems and facilities within which to conduct molecular tests. Nevertheless, PPRV strains from both lineages I and II are believed to be currently circulating across West Africa although undoubtedly many outbreaks are not characterized at the molecular level. In Burkina Faso, antibody prevalence to PPRV of 28.5% has been reported in the north (Sow *et al*., 2008). Other cases of PPRV in sheep and goats populations have also recently been described in Nigeria (El-Yuguda *et al*., 2010).

2.4 Transmission

Generally, introduction of PPR into a flock may be associated with introduction of newly purchased animals, change in husbandary management towards intensification, change of weather such as the onset of rainy season or hammatan as well as history of recent movement or gathering together of sheep and/or goats of different ages with or without associated changes in housing and feeding (Abraham *et al*., 2005).

Indirect transmission of PPR virus seems to be unlikely in view of the low resistance of the virus in the environment and its sensitivity to lipid solvent (Lefèvre and Diallo, 1990). Transmission therefore requires close contact between the animals in the febrile state and the susceptible ones. The main routes of PPRV transmission are oral and aerosol (Couacy-Hymann *et al.*, 2009). The discharges from eyes, nose and mouth, as well as the loose faeces, contain large amounts of the virus. Fine infective droplets are released into the air from these secretions and excretions, particularly when affected animals cough and sneeze (Taylor, 1984; Bundza *et al*., 1988). When other animals inhale the droplets, they are likely to be infected (Ezeibe *et al.*, 2008).

However, although close contact is the most important way of transmitting the disease, it is suspected that infectious materials can also contaminate water and feed troughs and bedding, turning them into additional sources of infection. These particular hazards are probably fairly short-term since the PPR virus, like its close relative rinderpest, would not be expected to survive for long outside the host (FAO, 1999).

2.5 Host Range and Pathogenecity

Peste des petits ruminant is mainly a disease of small ruminants, particlarly goats and sheep. The virus exhibits different levels of virulence between sheep and goats. Goats are severely affected while sheep generally undergo a mild form (Lefèvre and Diallo, 1990). The virus has a higher affinity to caprine species than to ovine species. These findings suggest that the same virus capable of inducing high mortality in goats may not produce as severe an infection in sheep, but the reverse may not be true (Singh *et al*., 2004). The greater severity of the disease in goats may be attributed to a greater susceptibility of the goat population to infection with PPRV. The recovery rate of goats infected with PPRV is considerably less than that of sheep infected with the virus (Shankar *et al*., 1998). In addition, the fecundity of goats is higher than that of sheep, so newborn kids replace a large proportion of the goat population each year. Newborn animals become susceptible to PPRV infection at three to four months of age (Srivas and Gopal, 1996), corresponding with the natural decline in maternal antibodies (Saliki *et al*., 1993), and this large number of young susceptible stock may also account for the severity of PPRV infection in the goat population.

Cattle and pigs are known to be a dead end host and all attempt to induce clinical disease in adult cattle experimentally proved abortive. However, they undergo subclinical infection that protects them against subsequent challenge with virulent strain of Rinderpest virus (Taylor, 1984). They do not contribute to the epidemiology of the disease because of their inability to excrete the virus. Unlike cattle and pigs, PPR affect wildlife animals both under field condition and experimentally. The disease was induced experimentally in American white deer *(Odocoileus virginianus*) which was found to be susceptible (Hamdy *et al*., 1976) and a field outbreak was reported from a zoological collection (Furley *et al*., 1987). It caused a high mortality and severe disease in Dorcas Gazelles (*Gazella dorcas*), Nubian Ibex (*Capra ibex nubiana*), Laristan sheep (*Ovis orientalis laristani*) and gemsbok (*Oryx gazellaa*). Antelope and other small wild ruminant species can also be severely affected (Abu Elzein *et al*., 2004).

2.6 Peste Des Petits Ruminants as a Threat to Europe

Outbreaks of PPR have been reported in Morocco (Khalafalla *et al*., 2010) and Tunisia which are on the Edges of the European Union (Banyard *et al*., 2010). Historical exchanges of small ruminants exist between Morocco and Spain. This increases the threat of the movement of infected animals into the southern Europe. Similarly, antibodies to PPR virus have been detected in Algerian sheep in 2011. In addition, the disease has been endemic in Turkey, which is likely to become a member of the European Union in the future (Baron *et al*., 2011). The detection of PPRV in Turkey in 1996 raised the initial awareness and questioned the ability of the virus to spread to Europe (Ozkul *et al*., 2002). It is therefore

essential that Europe maintains surveillance of the disease in order to successfully contain the disease should it be imported (Minet *et al.*, 2009).

2.7 Clinical Manifestation of PPR

Clinical signs of PPR have been observed and reported by Couacy Hymann (2007) and Emikpe and Akpavie (2011). The clinical disease is acute, with a sudden onset of fever (lasting for 3-5 days), peaking on the second or third day at 40–42°C, before slowly returning to normal. With the onset of fever, the animals suffer loss of appetite and become severely depressed. An early watery nasal discharge develops and may become profusely catarrhal, containing mucus and pus (F.A.O., 1999). This can lead to encrustation, blocking of the nostrils and respiratory distress. The nasal lining may become necrotic and conjunctivitis with discharge from the eyes causes matting of the eyelids. Small areas of necrosis usually first appear on the lining of the lower gums. In severe cases, these spread rapidly to the dental pad, hard palate, cheeks and buccal papillae and tongue (including the anteriodorsal area). These areas of necrosis may increase in number and size and join together. The necrotic tissue sloughs, leaving irregular shallow erosions and remnant tags of necrotic epithelium. In some animals, the mouth lesions may be mild and heal within 48 hours. Such animals are likely to recover. In others however, the lining of the buccal mucosa becomes pale and coated with dying cells and, a foul smelling odour exudes from the mouth (Emikpe *et al*., 2011).

Affected animals breathe fast, sometimes so fast that they exhibit rocking movements with both the chest and abdominal walls moving as the animal breathes. Severely affected cases

show difficult and noisy breathing marked by extension of the head and neck, dilation of the nostrils, protrusion of the tongue and soft painful coughs. Most animals develop severe diarrhoea or dysentery about 2–3 days after the development of mouth lesions, resulting in rapid dehydration and loss of weight. The diarrhea is mostly preceded by a sudden drop of body temperature to normal. Secondary bacterial infections are common. Pregnant animals may abort (Abubakar and Khan, 2008). However, female goats which recover from PPR conceive as soon as they regain physiological normalcy (Eziegbe and Wosu, 1999). It has also been shown that PPR virus does not cause structural damage to the female reproductive system. Thus, the abortion observed in goats infected with PPR virus is most likely due to general debility (Eziegbe and Wosu, 1999).

The clinical disease may be complicated by many secondary pathogens. The most important secondary pathogens include *Pasteurella* spp causing pneumonic pasteurellosis and *Mycoplasma* which causes severe pneumonia. In addition, infections with *E. coli* produce a heat-labile enterotoxin which will increase the severity of the clinical signs and the mortality rate (Wohlsein and Saliki, 2006**)**. Death due to the disease usually occurs after a course of 4–12 days.

2.8 Pathology

2.8.1 Pathogenesis

The first event in the pathogenesis of PPR infection is the recognition of compatible receptors on the surface of host cells by the virus attachment proteins (Hemagglutin). Following attachment, the processed F protein mediates fusion of the viral envelope with

the plasma membrane at physiologic pH (Kingsbury, 1990). The virus is generally considered to be lymphotropic and epitheliotropic. Following infection, it localizes first replicating in the pharyngeal and mandibular lymph nodes as well as tonsil. Viremia may develop 2-3 days after infection and 1-2 days before the first clinical sign appears. Subsequently viremia results in dissemination of the virus to spleen, bone marrow and mucosa of the gastro-intestinal tract and the respiratory system (Scott, 1981). The most obvious feature of the pathogenesis of PPR is the profound but transient immunosuppression caused by the virus, thereby rendering the hosts more vulnerable to opportunistic infections (Diallo *et al*., 2007). The immunosuppression occurs as a result of virus-mediated destruction of lymphocytes in all lymphoid tissues, including the gut-associated lymphoid tissue (Peyer's patches). More so, the immune cells that support replication of PPR virus also produce numerous potent immunoregulatory cytokines after appropriate stimulation (MacLachlan *et al*., 2011).

2.8.2 Post mortem findings

The carcass may show moderate emaciation 7-days post infection with prominent bony projections especially those of the ribs and the pelvis. The nasal cavity is **c**ongested (reddened) and may be dry with erosions on the gums, soft and hard palates, tongue and cheeks and into the oesophagus. The respiratory airways contained moderate frothy exudate extending from the trachea to the bronchioles. There may be pulmonary congestion and oedema with focal consolidation of the anteroventral portion of the cranial lobe of the right lungs (Emikpe and Akpavie, 2011). The lung appears dark red or purple with areas

firm to touch, mainly in the anterior and cardiac lobes (evidence of pneumonia). Lymph nodes (associated with the lungs and the intestines) are soft and swollen.

In the gastro-intestinal system, ulcerative and erosive lesions are predominantly found. While the lesions are constantly found in the oral cavity, the rumen and abomasums are rarely affected. However, studies by Nusseiba *et al*. (2009) revealed that rumen, reticulum, omasum and abomasum were filled with foetid watery fluid. Lesions in the small intestine are generally moderate, being limited to small streaks of hemorrhages and, occasionally, erosions in the first portions of the duodenum and the terminal ileum. The large intestine is usually more severely affected, with congestion around the ileo-cecal valve, at the ceco-colic junction and in the rectum. In the posterior part of the colon and the rectum, discontinuous streaks of congestion "zebra stripes" form on the crests of the mucosal folds (Roeder *et al*., 1994).

2.8.3 Histopathologic findings

Peste des petits ruminants is most often characterised by an initial pulmonary congestion and oedema with mild interstitial lymphocytic infiltration with hyperplasia of the bronchial associated lymphoid tissue and goblet cell hyperplasia in the trachea. In the intestine, there is villous atrophy and matting while the lamina propria of the intestine is infiltrated with lymphocytes and the submucosal glands are necrotic (Emikpe and Akpavie, 2011). In lymph nodes, there is oedema in the cortex and medulla as well as infiltration of mononuclear cells and some giant cells in subcapsular areas and medullary sinuses. The spleen shows haemorrhage and haemosiderin pigment deposition (Nusseiba *et al*., 2009).

Generally, PPR virus causes epithelial necrosis of the mucosa of the alimentary and respiratory tracts marked by the presence of eosinophilic intracytoplasmic and intranuclear inclusion bodies. Multinucleated giant cells can be observed in all affected epithelia as well as in the lymph nodes (Brown *et al*., 1991).

2.8.4 Haematological and biochemical findings in PPR infection

Haematologic changes associated with PPR are those of lymphocytopaenia, elevated PCV, high RBC counts with normal levels of haemoglobin (Furley *et al*., 1987), monocytosis as well as eosinophilia. The lymphopenia observed during PPR infection could be attributed to lymphocyte trapping in the lymphoid tissues and it usually occurs in the early phase of induction of immune response following local virus replication (Rajak *et al*., 2005).

Daneji and Obi (1994) in their studies on blood biochemical changes in goats infected with PPR virus reported that there is a slight increase in total protein reflected especially in the globulin fraction. Other changes observed included icterus index and creatinine values consistent with decrease in haemoglobin, Blood Urea Nitrogen and Adenine/Guanine ratio which are indicative of slight hepato– renal involvement.

2.9 Differential diagnosis

Some of the diseases that can be confused with PPR include;

2.9.1 Pneumonic pasteurellosis: This is purely a respiratory disease. No diarrhoea or oral lesion found. In cases where PPR presents without oral lesions, diagnosis can only be

confirmed by detecting PPRV from tissues since isolating *pastuerella hemolytica* from lungs of sheep neither rules in Pasteurellosis nor rules out PPR infection (FAO, 1999).

2.9.2 Contagious caprine pleuro pneumonia (CCPP): Like PPR, it is characterised by fever, dyspnea and coughing, but however, no mouth lesions and diarrhoea are found. In addition, at post mortem, fibrin deposits are found on the lung and there is also adhesion of the lungs to the chest cavity.

2.9.3 Blue tongue: In this disease, fever, nasal discharge and oral lesions similar to those seen in PPR are observed. However, unlike PPR, bluetongue is characterised by edema of the head region as well as bluish discoloration of the oral cavity (Radostits *et al*., 2000).

2.9.4 Foot and mouth disease: The most important distinguishing features between FMD and PPR are the absence of breathing problems and diarrhoea, and the presence of marked lameness in FMD. In addition, the foul smelling odour that exudes from the mouth of PPR infected animals is usually not found in FMD

2.9.5 Contagious Ecthyma (ORF): The nodules seen in this disease resemble the thick scabs observed on the lips in PPR infection. However, pneumonia, diarrhoea and sometimes oral lesions are not usually observed in uncomplicated Orf (FAO, 1999).

2.10 Laboratory Diagonosis

2.10.1 Antigen detection methods

Various methods of detecting PPRV antigen have been used in the diagnosis of PPR. They include Virus Neutralisation test (VNT), Hemeagglutination (HA) tests, Immunocapture ELISA, counter immunoelectrophoresis (CIE) as well as Agar gel Immunodiffusion test (AGID). Hemaagglutination test is rapid, simple and has been used as a reliable comfirmatory test of PPR. Nusseiba *et al.* (2009) reported the use of HA for the diagonosis of PPR in Sudan. Similarly, Abubakar *et al*. (2011) reported that chicken RBCs show better results for HA than sheep RBCs and that HA test is more sensitive than AGID for detection of PPRV antigen, but Immunocapture-ELISA is the most sensitive of all three. Wosu (1991) reported a haemagglutination test for diagnosis of PPR in live animals. Haemagglutination inhibition (HI) test for definitive diagnosis of PPR has been reported by Wosu and Ezeibe (1991).

The immuno capture ELISA allows a rapid differential diagnosis of PPR or rinderpest viruses, and this is of great importance as the two diseases have a similar geographical distribution and may affect the same animal species. The Monoclonal antibodies used in immunocapture ELISA are directed against two non overlapping domain of the N-protein of PPR and RP, but the capture antibody detects an epitope common to both RP and PPR. The test is very simple, specific and sensitive, and can be performed on the precoated plates in less than 2 hours. In addition, the test can be carried out on samples which have not been kept under ideal conditions and where no viable virus is present (Libeau *et al*., 1994). Couacy-Hymman *et al*. (2009) used Immunocapture ELISA and detected PPR virus antigen at day 4 in ocular and nasal samples of goats infected with PPR virus. This suggests that immunocapture ELISA can be recommended as a useful method to

investigate PPR in small ruminants flocks at an early stage to prevent the spread of the disease.

Immunohistochemistry has also been used in the detection of PPR virus antigen from infected tissues. Saliki *et al.* (1994) reported the presence of PPR virus antigen in conjunctiva, tracheal, bronchial, bronchiolar, and ileal epithelial cells, type II pneumocytes, syncytial cells and alveolar macrophages. Similarly, Kumar *et al.* (2004) detected the presence of PPRV antigen in labial, intestinal, and bronchiolar epithelial cells, pneumocytes, macrophages and syncytial cells in lungs, and lymphoid (intact and necrotic) and reticular cells in lymphoid organs of goats experimentally infected with PPR virus.

2.10.2 Nucleic acid detection methods

2.10.2.1 *Polymerase chain reaction*

A reverse transcription PCR (RT-PCR) technique based on the amplification of the Nucleoprotein and Fusion protein genes has been developed for the specific diagnosis of PPR (Couacy-Hymann *et al*., 2002). It is one of the tests used in PPR diagnosis in many reference laboratories. This technique is very sensitive compared with other tests and results are obtained in 5 hours, including the RNA extraction. A multiplex RT-PCR, based on the amplification of fragments of N and M protein genes, has been reported (George *et al*., 2006). Another format of the N gene-based RT-PCR has also been described (Kumar *et al*., 2007). Instead of analysing the amplified product – the amplicon – by agarose gel electrophoresis, it is detected on a plate by ELISA through the use of a labelled probe. This new format, RT-PCR-ELISA, is ten times more sensitive than the classical RT-PCR. Thus,

RT-PCR-ELISA is the most appropriate test for the diagonosis of PPR in early phase of the disease from nasal and ocular swabs (Kumar *et al*, 2007).

2.10.2.2 *c-DNA probes*

Nucleic acid hybridization techniques using radiolabelled cDNA probes were developed in the early 1980s for the diagonosis of PPR and rinderpest. Inspite of its sensitivity however, the technique has not been used widely and routinely used due to the short half life of the isotopes as well as the constraints of handling the probes (Abubakar *et al.,* 2011). Non radiolabelled cDNA probes were also developed and were found to be very specific in differentiation of PPR from rinderpest (Pandey *et al*., 1992). However, they have reduced sensitivity (Diallo *et al*., 1995).

2.10.3 Antibody detection methods

Numerous serological tests have been employed for the detection of PPRV antibodies. These include: agar gel diffusion test, immunoelectrophoresis and recently competitive ELISA.

2.10.3.1 *c-ELISA*

Competative enzyme linked immunosorbent assay (c-ELISA) based on monoclonal antibodies for H-proteins and N-proteins were respectively developed by Anderson and Mckay (1994) and Libeau *et al*. (1995). The principle of the test is based on competition in binding to the antigenic epitopes between the monoclonal antibodies and PPR specific antibodies in the serum. The absorbance in PPR ELISA is converted to percentage of

inhibition (PI) using the formula: PI=100-(absorbance of the test wells/ absorbance of the mAb control wells) x 100. Sera showing PI greater than 50% are scored positive. The overall specificity of c-ELISA test was 98.4% with a sensitivity of 92.2% when compared with VNT. Because of the high sensitivity and specificity of c-ELISA. It has been reported that the test can be used even for samples not kept under ideal conditions (Munnir *et al*. 2009).

2.10.3.2 counter immunoelectrophoresis

Counter immunoelectrophoresis (CIEP) is the most rapid test for viral antigen detection and was first described for PPR diagonosis by Majiyagbe *et al*. (1984). It is carried out on a horizontal surface using a suitable electrophoresis bath, which consists of two compartments connected through a bridge (Majiyagbe, *et al.*, 1984). The apparatus is connected to a high-voltage source. Agar or agarose dissolved in 0.025 M barbitone acetate buffer is dispensed onto microscope slides in 3-ml volumes. From six to nine pairs of wells are punched in the solidified agar. The reagents are the same as those used for the AGID test. The electrophoresis bath is filled with 0.1 M barbitone acetate buffer. The pairs of wells in the agar are filled with the reactants: sera in the anodal wells and antigen in the cathodal wells. The slide is placed on the connecting bridge and the ends are connected to the buffer in the troughs by wetted porous paper. The apparatus is covered, and a current of 10–12 milliamps per slide is applied for 30–60 minutes. The current is switched off and the slides are viewed by intense light: the presence of 1–3 precipitation lines between pairs of wells is a positive reaction. There should be no reactions between wells containing the negative controls (Munir, 2011).

2.10.4. Virus neutralizing test

The virus neutralisation test (VNT) is sensitive and specific, but time-consuming and expensive. The standard neutralisation test is carried out in roller-tube cultures of primary lamb kidney cells or Vero cells when primary cells are not available. Serum against either PPR or RP may neutralise both viruses, but would neutralize the homologous virus at a higher titre than the heterologous virus. Therefore for differentiation purpose reciprocal cross neutralization is used (Taylor and Abegunde, 1979).

2.10.5 Virus isolation

Samples for virus isolation include heparinized blood, eye and nasal swabs (from live animals), and tonsil, mesenteric lymph nodes, spleen, section of colon and lung collected at post mortem. For successful isolation, samples must be collected during the hyperthermic phase (Lefevre, 1987) and submitted to the laboratory in cold ice. Different cell lines have been used for isolation of PPRV. The most widely used cell culture systems are primary lamb kidney and Vero cells. Cells, derived from African green monkey kidney are currently the most widely used cell line for PPRV and RPV. However, *Morbilliviruses* are now recovered and grown in SLAM cells (Ono *et al.*, 2001).

The CPE produced by PPRV can develop within 5 days and consists of cell rounding and aggregation culminating in syncytia formation in lamb kidney cells. In Vero cells, it is sometimes difficult to see the syncytia. If they exist, they are very small. However, in stained, infected Vero cells, small syncytia are always seen. Syncytia are recognised by a

circular arrangement of nuclei giving a 'clock face' appearance. Cover-slip cultures may give a CPE earlier than day 5. There are also intracytoplasmic and intranuclear inclusions. Some cells are vacuolated. Similar cellular changes may be seen in stained histopathological sections of infected tissues. After 5–6 days, blind passages should always be carried out as CPE may take time to appear.

2.11 Management of PPR

Specific chemotherapeutic agents for the treatment of PPR are not available. Palliative treatment such as anti PPR hyperimmune serum, antibiotics (Oxytetracyclines), antidiarrhoeas, and vitamins are often used to symptomatically treat the disease. In animals incubating the disease, administration of hyperimmune serum can greatly be of significance in fighting the disease (Nawathe, 1984). Also, the use of chemotherapeutic agents combined with hygiene, sanitation, isolation and quarantine of the infected animals has been reported to help in managing the disease. Ataja *et al*. (1997) investigated the efficacy of antibiotics in managing PPR infection in kids. In that study, only 20% was observed in 10 West African Dwarf infected kids that were treated using oxytetracycline 20mg/Kg superimposed on Advocin^{R} (Danofloxacin 1.25mg/Kg) for three days and Dextrose saline 5% (60mls/animal) twice for five days.

2.12 Control and Prevention of PPR

Being the major constraint to the livelihood of small scale farmers, the control of PPR becomes a priority. So far, vaccination is the most effective means of controlling the disease. Other measures believed to be instrumental in the control of PPR are movement

restriction of sheep and goats from affected areas, quarantine of affected animals, elimination of contact formites as well as disinfection of the affected premises (Abubakar *et al*., 2011).

Although, TCRV vaccine had been shown to be effective in protecting against PPR, a homologus vaccine was developed and confered immunity in sheep and goats for atleast 3 years (FAO, 1999). It is important to note that the use of TCRV vaccine to protect small ruminants against PPR is being discouraged because its use produces antibodies that can cross protect against rinderpest which compromises serosurveillance on rinderpest, thereby affecting the Global Rinderpest Eradication Programme (FAO, 1999).

A homologous vaccine for PPR was developed from the attenuation of Nig 75/1 PPRV strain (Diallo, 2004). Recently, Luka *et al*. (2011) reported that Nigerian strains of PPRV demonstrate 93-94% homology with PPRV homologous vaccine. Hence, there is no doubt that it is the vaccine of choice for the control of PPR in Nigeria. Over 98,000 sheep and goats were vaccinated from 1989-1995 using the vaccine. Interestingly, no unwanted side effects, such as abortion in pregnant animals, were recorded (Couacy-Hymann *et al*., 1995). In addition, the animals vaccinated with the attenuated vaccine were unable to transmit the challenge virus to the in-contact animals (Sen *et al*., 2010). However, the thermolabile nature of the vaccine may increase the likelihood of low sero-conversion following vaccination with the vaccine. Faris *et al*. (2012) reported a low seroconversion rate of 61%, indicating weak herd immunity in flocks of sheep and goats vaccinated using the homologous PPR vaccine. Thus there is a need to increase the thermostability of the

vaccine so as to boost the seroconversion rate following vaccination with vaccine. Baba *et al*. (2007) demonstrated that freeze drying the vaccine in an excipient-containing trehalose makes it very thermostable and it resists temperatures up to 45°C for a period of 14 days, with minimal loss of potency.

Several studies involving the development of new generation vaccines have been conducted. Reverse genetics has been successfully used by Parida *et al*., (2007) to develop a DIVA vaccine for RPV by swapping the RPV N gene with the N gene of PPRV. This DIVA vaccine was however not used during Rinderpest eradication programme because countries were extensively undertaking serosurvellance to confirm the absence of the disease. A rinderpest based chimeric vaccine was also developed by swapping the glycoprotein genes (F and H) between PPRV and RPV. This recombinant vaccine however grows poorly in cell culture (Das *et al.*, 2000). In order to improve the virus growth in the cell culture, M, F and H genes from RPV were replaced with those from PPRV. The resulting chimera not only grew well on the cell culture, but also protected goats against the virulent strains of PPRV (Mahapatra *et al.*, 2006). In addition, it was possible to differentiate vaccinated from infected animals using hemmagglutinin c-ELISA. This chimeric MFH PPRV marker vaccine may be of use to control PPRV, but field trials have never been conducted due to the ongoing rinderpest eradication campaign (Baron *et al*., 2011).

In order to reduce the economic costs of vaccination, there is a need to develop both a thermostable vaccine and a polyvalent vaccine for PPR and other important diseases of

small ruminants (Abubakar *et al*., 2011). In areas where multiple viral pathogens of small ruminants exist, development of multivalent vaccines capable of protecting against common viral pathogens will no doubt contribute to poverty alleviation. Multivalent vaccines are currently being developed that may both protect animals against several viral pathogens and enable vaccinated and infected animals to be distinguished using DIVA tests.

2.13 Immune Response to PPR Virus

Virulent PPR virus is known for its significant immunosuppressive effect as evidenced by reduced humoral immune response, marked leucopenia and lymphopenia (Rajak *et al*., 2005). Diallo *et al.* (2007) observed that animals challenged with the virulent PPR virus showed no detectable antibodies during the first week of the challenge indicating that the challenged virus has delayed the immune response of the host against PPR virus. Nevertheless, inspite of the profound immunosuppression caused by the virus, recovery from the infection is usually accompanied by the establishment of strong long lasting immunity in the infected host (Diallo *et al*., 2007). Although the immune response necessary for the recovery from PPR infection is poorly understood, it has been suggested that preferential long term activation of type2 CD4+ T cells could explain the immunity following recovery.

Goats immunized with a recombinant baculovirus expressing the H glycoprotein generated both humoral and cell-mediated immune responses (Sinnathamby *et al*., 2001). The responses generated against PPRV-H protein in the experimental goats are also RPV

crossreactive suggesting that the H protein presented by the baculovirus recombinant 'resembles' the native protein present on PPRV. Lymphoproliferative responses were demonstrated in these animals against PPRV-H and RPV-H antigens (Sinnathamby *et al*., 2001).

2.14 Antibody Dynamics Following Vaccination against PPR Virus and Duration of Maternal Antibodies in Young Ones

Vaccine against PPR has been shown to have higher antibody titres and confers immunity for more than 1 year. Following vaccination with PPR vaccine, there appears to be a sharp rise in the antibody titres on days 14 and 21. By the 45th day postvaccination, the mean antibody titre will have reached its peak and the PI values could be as high as 80% or even more (Rashid *et al*, 2010). Studies conducted by Awa *et al*. (2002) revealed that there was a sharp rise in the antibodies tire between ninth and the tenth month following vaccination. It was postulated that the sharp rise in the antibody titre could be as a result of exposure to the field virus which could lead to anamnestic response since the animals had earlier been vaccinated. This hypothesis however needs to be verified.

The protection of offspring against many infectious agents during the first months of life depends on the maternally acquired immunity through the ingestion of colostrum. The duration of maternal antibodies influences the optimal age for vaccination. Once acquired, the antibodies remain above the protection threshold for over three months after which the lambs and the kids become increasingly vulnerable to the disease. In a study conducted on the duration of maternal antibodies in lambs and kids, it was reported those 3 months after birth, 95% of lambs have lost their maternal antibodies (Bodjo *et al.,* 2006). The decline in

the antibodies titre corresponds to the increase in the susceptibility of the animals to the disease. This explains why the high mortality due to PPR is most often observed at 4-8 months of age. Another investigation on the duration of maternal antibodies specific to PPR in lamb and kid flocks demonstrated that they failed to be protected after 3.5 and 4.5 months in lambs and kids, respectively. Therefore, it was recommended that lambs and kids be vaccinated against PPR at 4 and 5 months of age, respectively (Awa *et al*., 2002).

2.15 Risk Factors Affecting the Occurrence of PPR

There are numerous environmental and animal related factors that affect the occurrence of PPR in small ruminants. Low environmental temperatures with high humidity, stress of migration as well as nutritional deficiencies, are known to influence the occurrence of PPR small ruminants. Ezeibe *et al*. (2008) reported the highest incidence of PPR during hammatan period when small ruminants are allowed to graze in fallows after crop havest.

In addition, trade in small ruminants, especially at markets where animals from different sources are brought into close contact with one another increases the opportunities of PPR transmission (FAO, 1999). Peste des petits ruminants is one of the diseases that affect trade where non-affected countries use technical regulations under animal health and sanitary control to stop imports from affected countries (Somaliland, 2009). Movement of animals and introduction of newly purchased animals from the market therefore play an important role in transmission and maintenance of the virus.

Mahajan *et al*. (2011) studied PPR in migratory and non migratory sheep and obtained a significantly higher seroconversion in the former than the later group. The stress of migration coupled with nutritional defieciency predisposes small ruminants to the PPR. Thus, El-rahim *et al*. (2005) reported that PPR is more severe in tired animals and those suffering from thirst and hunger. In addition, during nomadism, animals come in contact with local sheep and goat population from where they pick up the infection or spread disease if nomadic flock is pre-exposed. Therefore, migratory animals are considered to be risk factors that play an important role in the epidemiology of PPR.

Sex is also believed to be a risk factor for the occurrence of PPR in small ruminants. The difference in the seropositivity between males and females is however controversial in the literature. Luther *et al*. (2007) and Khan *et al*. (2008) reported a high seroprevalence in the females than in the males. However, Mahajan *et al*. (2012) reported a significantly higher seroprevalence of PPR in males when compared with females.

Age as a risk factor also affects the occurrence of PPR in small ruminants. Majiyagbe *et al*. (1992) showed that antibodies to PPR increase with age. As such, the disease is more fatal in goats of about 4-8 months of age when maternal antibodies might have disappeared since as the passive immunity becomes progressively lost, susceptibility to PPR infection increases (Abubakar *et al*., 2009).

Peste des petits ruminants is generally believed to be more severe in goats than in sheep. Sharma *et al*. (2007) reported a significantly higher morbidity and mortality in goats than

in sheep. Similarly, El-rahim *et al.* (2006) in their studies on outbreak of PPR in migratory sheep and goats reported the highest number of mortalities among the goats. Several serological studies may however reveal a higher prevalence of PPR virus antibodies among sheep than in goats. This could be attributed to a higher survival rate in the sheep. Because the disease is less severe in sheep, many of the infected sheep recover from the infection thereby increasing the seroprevalence (Mahajan *et al*. (2011).

Among the environmental factors affecting the occurrence of PPR, season is believed to be in the forefront. Ezeokoli *et al*. (1986) investigated the clinical and epidemiologic features of PPR and concluded that the disease is more common at the end of the dry season and the beginning of the rainy season. However, with the start of rains, the movement of animals is restricted due to the easy availability of local fodder. Nutritional status of the animals also gets improved during the rains. This may reduce disease transmission after the start of rains and during the period of easy availability of fodder (Taylor *et al.,* 1984).

2.16 Sero-Surveys of PPR Virus Antibodies and the Disease in Nigeria.

Beaton (1930) reported for the first time, the occurrence of PPR in Nigerian goats as a rinderpest like disease. Later, it was ascertained that the rinderpest like disease was PPR. In line with Beaton, in 1976, Hamdy and Dardiri (1976) reported the occurrence of PPR in Nigerian dwarf goats. Taylor and Abegunde (1979) conducted serological studies with PPR virus in Nigeria. Following the outbreak of the disease in western Nigeria, Durojaiye (1982) investigated the disease using AGPT technique. In that study, they demonstrated precipitating antibodies of 62.5%, 40% and 37.5% in goats naturally infected with the

disease in three different locations in the region. In another epidemiological study on PPR in small ruminants in southern Nigeria, Obi *et al*. (1983) reported seroprevalence of 52.3 and 47.2 percent seroprevalence of PPR neutralising antibodies in sheep and goats, respectively.

Various diagonostic tests have been used for the serosurvellence of PPR virus antibodies in Nigeria. Durojaiye and Taylor (1984) used Counterimmunelectrophoresis and neutralisation tests and concluded that CIEP was simple and could be used in serodiagonosis of PPR virus. Furthermore, Durojaiye (1987) concluded that indirect precipitation test was suitable for screening PPR virus antibodies in laboratories lacking facilities for neutralisation tests.

A more specific and sensitive diagnostic test for the detection of PPR virus antibodies was developed by Anderson and Mckay (1994). The test is monoclonal antibody based c-ELISA in which the antigen is Haemaglutinin protein of the PPR virus. Shamaki *et al*. (1995) Conducted sero survellence of PPR in some selected states of across the federation using c-ELISA test. In that study, prevalence rates of 40%, 40.6%, 7.7% and 38.4% were recorded for Plateau, Borno, Abia and Oyo States respectively. Similarly, Qadeer, (1998) reported a seroprevalence of 62.9% in goats from Adamawa states using the same technique. Luther *et al*. (2007) also reported a seroprevalence rate of 24.7% using the test.

Table 2.1: First report of PPR from different countries

S/No.	Country /	Reference
1	Ivory coast	Garagadennec and Lalane (1942)
2	Senegal	Mornet *et al.* (1956)
3	Chad	Provost *et al.* (1972)
4	Togo	Benazet *et al.* (1973)
5	Benin	Bourdin (1973)
6	Ghana	Boniwell and Gibbs (1976)
7	Nigeria	Hamdy *et al.* (1976)
8	Oman	Hedger *et al.* (1980)
9	Sudan	Ali and Taylor (1984)
10	Saudi Arabia	Furley *et al.* (1987)
11	India	Shaila *et al.* (1989)
12	Jordan	Lefevre *et al.* (1991)
13	Israel	OIE (1993)
14	Ethiopia	Roeder *et al.* (1994)
15	Kenya and Uganda	Wamwayi *et al.* (1995)
16	Pakistan	Amjad *et al.* (1996

(Source: Tiwari, 2004).

2.17 Economic Losses Due to PPR

Economic losses due to a disease could be estimated from the production losses (such as mortality) caused by the disease as well as the cost incurred in the control of the disease (Thombare and Sinha, 2009). PPR constitutes a significant threat to livestock production in developing countries. In endemic countries like Nigeria, PPR is considered to be the most important limiting factor in the development of the small ruminant industry because sheep and goats play an integral role in sustainable agriculture and employment. Hence, the economic losses associated with PPR are very enourmous.

Singh *et al*. (2004) estimated the economic losses due to PPR in India to be $39 million annually. Similarly in 2006, outbreak of PPR in Kenya was accompanied by severe socio economic losses on Food security and therefore impacted on the livelihood of the local population. The annual losses due to PPR in Kenya were estimated to be over $15 million (Baron *et al*., 2011). Economic losses due to PPR have also been estimated in Nigeria. Recently, Shamaki *et al.* (2004) estimated the economic losses due to PPR infection to be over 40 billion Naira. Thus it is clear that PPR brings great economic losses to countries where small ruminants constitute a significant livestock production.

2.18 Peste Des Petits Ruminants as a Candidate for Eradication

Sheep and goats are of much less individual value than cattle, and have a shorter working life, which means a faster turnover of animals. Consequently, more vaccines and personell to administer it in the field are required. Hence, there is a need to take into account, the socio economic benefit of PPR eradication.

With the impact of the disease on the livelihood of poor farmers, coupled with the ever expanding spread of the disease, the benefit of its eradication become greater. FAO (2009) published that there were good reasons to believe that the eradication of PPR was an achievable goal like rinderpest. Similar to rinderpest virus, there is only one serotype of PPRV. There are equally sensitive and specific diagnostic tests available for PPR and a similar safe attenuated homologous vaccine. Thus, with coodinated support from national and international bodies, and through survellance and seromonitoring over a period of 10-20 years, there is every possibility of eradicating PPR just like rinderpest was eradicated.

2.19 Distribution of Sheep and Goats Breed in Nigeria

2.19.1 Sheep breeds

2.19.1.1 *Balami*: The Balami is the largest bodied native sheep in Nigeria. As a pastoral animal it is confined to the semi-arid north, but it is favoured as a stall-fed breed by Muslims throughout the Nigerian Middle Belt. It is white and hairy with pendulous ears and a long thin tail; rams have a throat ruff and are horned but ewes are normally polled.

2.19.1.2 *Uda*: The Uda is slightly smaller-bodied than the Balami, although their size ranges overlap. It is easily recognised by a distinctive coat colour pattern; entirely brown or black forequarters and white behind. They are predominantly found in the northern fringes of Nigeria. Not much studies have been conducted on this breed. However, Haumesser and Gerbaldi (1980) studied traditionally-managed Uda flocks in Niger Republic.



Plate I: Balami breed of sheep: Large bodied with pendulous ears



Plate II :Uda breed of sheep: Black forequarters and white behind

2.19.1.3 *Yankasa*: The Yankasa breed has been the most extensively studied in Nigeria. The body colour is white with black patches around the eyes and sometimes on the feet. The muzzle and ears are usually black too. Rams have curved horns and a hairy white mane, and ewes are polled. Yankasa sheep have been recorded in all parts of Nigeria, though the populations attenuate towards the northern border and the sea-coast.

2.19.1.4 *West African Dwarf*: The West African Dwarf is a small-bodied, compact breed which may be all white, black, brown, or spotted black or brown on a white coat. Its variation in colour and patchy distribution make it difficult to distinguish clearly from the Yankasa. Adu and Ngere (1979) say that different types exist, mentioning the 'Pagan' variety on the Jos Plateau, and the 'Umuahia' variety near the Confluence, but there is no published account of such varieties.

2.19.2 Goat breeds

2.19.2.1 *West African Dwarf*: The WAD is usually black, although patched, pied, and occasionally all-white animals can be seen, even on the coast. Although they are stereotypically said to be native to the forest belts, their presence in Borno State and in adjacent Republics of Cameroon and Chad suggests that they were far more widespread until recently. They correspond to the West African Grassland Dwarf described for Cameroon by Ndamukong *et al*. (1989).

2.19.2.2 *Red Sokoto goats*: They are probably the most widespread and popular in Nigeria. It is the usual village goat in the northern two-thirds of the country although it is less



Plate III: Red Sokoto Goats



Plate IV: West African Dwarf goats: short legged and small bodied.

common with transhumant pastoralists. The Sokoto Red goat was the source of 'Morocco leather' known in Europe from the medieval period onwards. It acquired this name because it was transported across the Sahara by caravans controlled by Moroccan merchants. Their skins have coarse, thinly-spaced outer hairs and small sweat and wax glands and that they lacked fat.

2.19.2.3 *Sahelian breeds*: Sahel goats are very similar in appearance to the sheep with which they are often herded. The coat is white or dappled, the ears are pendulous and the legs are notably longer than other breeds. They are found along the northern border of Nigeria, particularly in Borno state. In Nigeria, the Sahel goat is generally the variety preferred by pastoralists.

2.20 Socio Economic Importance of Small Ruminants

Small ruminants form an integral and important component of the pattern of animal production in most rural communities. They are widely distributed in the rural, urban and peri-urban areas of this country and represent about 63.7% of total grazing domestic animals in Nigeria (Gefu, 2002). Sheep and goats are generally considered to be of great socio economic importance. Their importance is primarily associated with their small size, which is significant for the advantage of mankind as it favours low investments, small risk of loss and preference over large ruminants for food and reproductive efficiency and economic use of available land (Omoike *et al.*, 2006). They have lower feed requirements compared to cattle which allow them to be easily integrated into different farming system. They therefore play a significant role in the food chain and overall livelihoods of rural

households, where they are largely the property of women and their children (Lebbie, 2004).

Small ruminants produce a variety of foods, which are very useful for both urban and rural markets. With the exception of processed milk products, these food products are especially important for rural households. The meats from goats and sheep and also goat milk are very valuable for household nutrition and food security in the rural areas (Davendra, 1992). Apart from the anti-allergy properties in goat milk, other important characteristics of goat milk are the presence of higher levels of six of the ten essential amino acids and also monounsaturated, polyunsaturated and medium chain triglycerides which are all known to benefit human health (Posati and Orr, 1976).

In tropical Africa, small ruminants are reared mainly for four purposes, namely: Meat, milk, skin and wool, in their order of importance. According to the FAO (1982) tropical Africa has about one-sixth and about a third of the total world flock of sheep and goats, respectively. Within Africa, sheep and goats contributed 10.9% and 8.4%, respectively, of the total African meat supply. Furthermore, Sheep and goats in Africa produced 8.6% and 18.2% respectively, of the world total amount of milk produced from these two species and the production from both accounted for 13.6% of milk collected from small ruminants in the world.

CHAPTER THREE
MATERIALS AND METHODS

3.1 Study Area

Sokoto State is located between latitude 12 14^0N and longitude 4-6^0E in the Sudan Savanna zone of Nigeria. It forms boundaries with the republic of Niger to the north, Kebbi State to the west and southwest and Zamfara State to the east (NPC, 2006). It is divided into three geo-political zones, namely southern zone (Tambuwal, kebbe, Shagari, Yabo, Bodinga, Dange-shuni, Tureta), central zone (Sokoto south, Sokoto north, Wamakko, Kware, Binji, Tangaza, Wurno, Rabah) and eastern zone (Gwadabawa, Illela, Goronyo. Gada, Gudu, Silame, Sabon-birni and Isa) (M.L.G.C.A, 2009).

The state covers a total land area of about 32,000 square km with an estimated human population of 3,696,999 million (NPC, 2006), most of whom are farmers because of the sparse arable land in the state. A good percentage of the populace is engaged in animal husbandry. Annual rainfall is about 550mm and sets in from June to September. The harmattan period starts from October to February and the hot season comes from March to the end of May.

3.2 Sample Size

The size of the sample used in this cross sectional study was estimated using the formula according to Araoye (2003):

$$n = \frac{Z^2pq}{d^2}$$

Where

n= sample size

Z= standard normal deviation at 95% confidence interval (1.96)

p= prevalence rate from previous study; Daneji *et al*. (1997) reported 4% prevalence in camels and Kazeem (2001) obtained 16% and 11% for goats and sheep, respectively.

q= 1-p

d= allowable error (0.05)

Applying the above formula a minimum of 59, 207 and 150 samples were estimated for camels, goats and sheep respectively, for the study. However, 137, 246 and 187 samples for camels, goats and sheep respectively were used in this study.

3.3 Sampling Procedure

In this crossectional study, samples were obtained using a multistage sampling approach (Region-Local Government Area-Village-Flock) from September 2011 to March 2012. Accordingly, two (2) Local Government areas (LGAs) were randomly selected from each geo-political zone in the State for serum sampling. From each of the selected (LGAs), villages and flocks were conveniently selected based on vehicle accessibity and the consent of the flock owners. A total of 433 samples were collected from small ruminants (187 sheep and 246 goats) in all the sampling units in the study area. Camels were sampled at slaughter in Sokoto Metropolitan abattoir. Systematic sampling approach was used, whereby every third camel slaughtered was selected daily for serum sampling as previously described by Baba *et al*. (1990). A total of 137 serum samples were obtained from camels during the study period. Age, sex, species and breed of the animals were recorded at the point of sampling.

3.4 Inclusion Criteria

Small ruminants considered for sampling in this study were those with no history of vaccination against PPR. This was to eliminate the possibility of seropositivity due to vaccinal antibodies. Also, in order to avoid seropositivity due to maternal antibodies, only sheep and goats of not less than six months old were considered for sampling in this study.

3.5 Sample Collection

Five mls of blood sample was collected from the jugular veins of sheep and goats using sterile syringes with 21 gauge hypodermic needles. For camels, sera were obtained by taking blood samples at slaughter. The collected blood samples were allowed to clot to obtain sera. The collected sera were then transferred to sterile bottles and stored at -20^0C until examined.

3.6 Sample Analysis

The sera were examined for the presence of PPR virus antibodies using a monoclonal antibody based competitive enzyme linked immunosorbent assay (c-ELISA), in the PPR LAB of the Virus Research Division of National Veterinary Research Institute (NVRI), Vom.

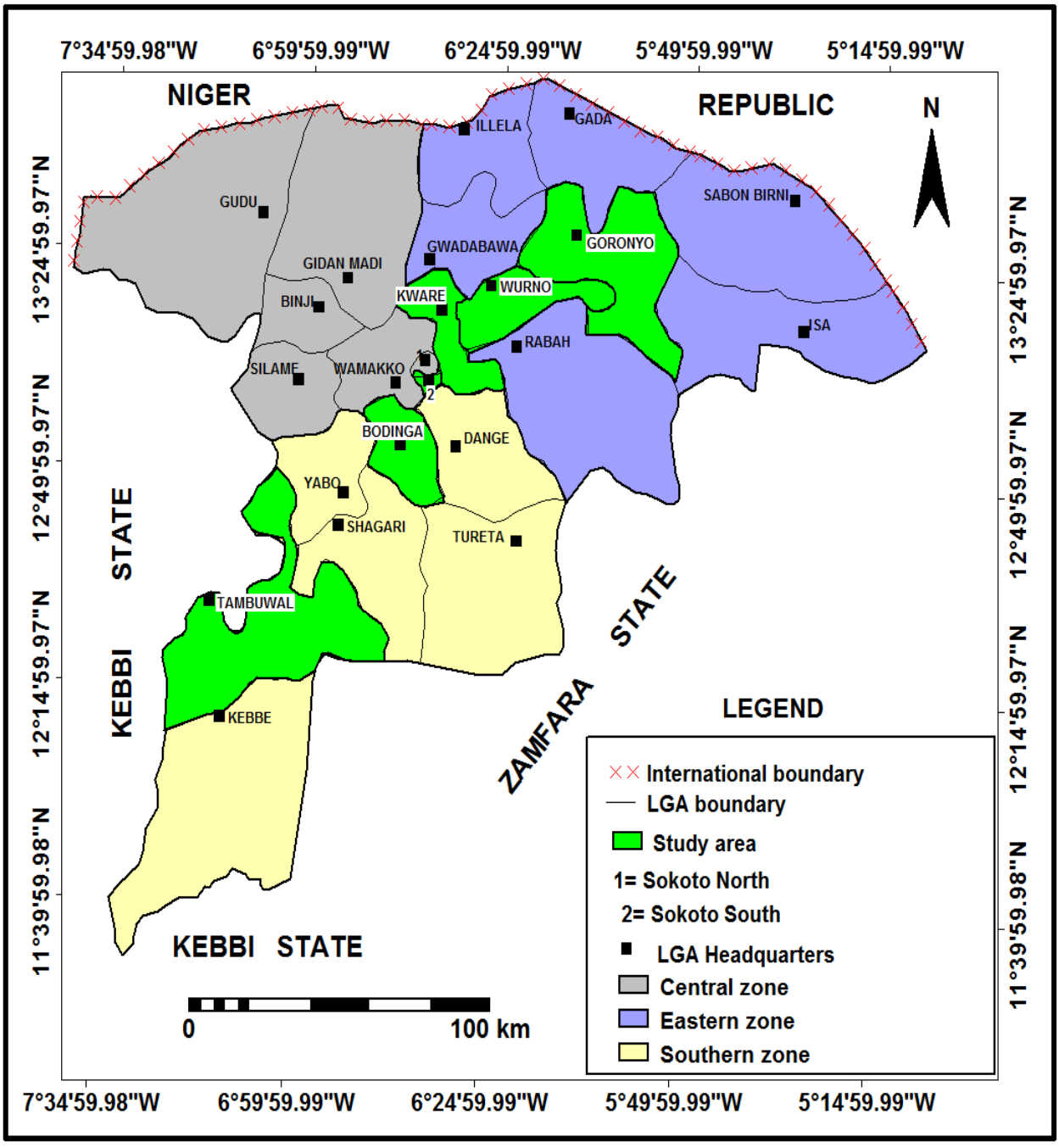


FIG.3.1: SOKOTO STATE SHOWING STUDY AREA

Source: Adapted and Modified from Administrative Map of Sokoto State, 2013

3.7 Principle of the Test

The c-ELISA used in this study detects IgG type of antibody. The principle of the test is based on the inhibition of binding of the mouse monoclonal antibodies (Mab), directed against the hemmagglutin antigen of the PPR virus, in the presence of a positive serum. The presence of antibodies to PPR virus in the test serum blocks the reactivity of the monoclonal antibody resulting in a reduction in the color following the addition of enzyme labelled antimouse antibody and substrate (Anderson *et al.*, 1990).

3.8 ELISA Kit

A competitive emzyme linked immunosorbent assay (c-ELISA) kit for the detection of PPR virus antibodies developed by the Institute of Animal Health, Pirbright U.K in collaboration with the Animal Production Unit, Agriculture Laboratory, Agencies Laboratory Division: Seiberssdorf, Australia was used in this study. The contents of the kit include:

- Micro ELISA plates
- Haemagglutin Antigen of PPR virus.
- Anti PPRV Monoclonal antibody supplied as freeze dried hybridoma cell culture supernatant.
- Rabbit antimouse immunoglobulin horseradish peroxidise (HRPO) conjugate,
- Strong positive control stock (C ++)
- Weak positive control stock (C+)
- Weak negative control stock (C-)
- Orthophenylenediamine (OPD) as chromogen stock and

- ❖ Perhydrit tablets as a substrate stock.

Coating, blocking and washing buffers as well as stopping solutions were prepared according to the kit supplier instructions.

3.9 Reagent Preparation

3.9.1 Antigen preparation: One vial of the freeze dried cell culture derived hemmaglutinin antigen of the PPR virus was reconstituted with 1mL of sterile water supplied with the kit and mixed gently until completely dissolved. The reconstituted antigen was then kept at -20^{0}C.

3.9.2 Anti PPR monoclonal antibody stock: The anti PPR monoclonal antibody supplied in freeze dried form was reconstituted by adding 1ml of the reconstitution diluents per each vial. The stock was then mixed gently until completely dissolved.

3.9.3 Conjugate stock: Once reconstituted, the rabbit antimouse immunoglobulin HRPO conjugate stock supplied with the kit was subdivided into 500uL aliquots in 1ml cryopreservation vials supplied, labelled and stored at 4^{0}C. 1 vial was used at a time until depleted.

3.9.4 Control serum stocks: Each control serum vial (C++, C+ and C-) was reconstituted with precisely 1mL of sterile water supplied with the kit and agitated gently until completely dissolved. The control serum stocks were stored in their original vials at -20^{0}C. One vial was used at a time until depleted before reconstituting the second vial.

3.9.5 Chromogen stock: One OPD tablet was dissolved in 75mL of locally produced distilled/deionised water, just before substrate/chromogen incubation step and stored in the dark at 4^0C.

3.9.6 Substrate stock: One hydrogen peroxide tablet was dissolved with 10mL of distilled water in the empty container supplied. The solution was then stored at 4^0C until depleted at which point a fresh stock should be prepared in the same manner.

3.9.7 Coating buffer: 0.01M of phosphate buffer saline (PBS) was dissolved in 1L of distilled water, labelled and stored at 4^0C.

3.9.8 Blocking buffer: This was prepared by the addition of 0.01M PBS supplemented with 0.1% Tween 20 to normal bovine serum.

3.9.9 Wash buffer: This was prepared by dissolving one PBS sachet in 1 litre of locally produced distilled water. It was then transferred to a wash fluid container followed by the addition of 4L of distilled water. The buffer was then mixed well and stored at room tempreture until used.

3.9.10 Stopping solution: This was prepared by the addition of 55mL of concentrated sulphuric acid to 945mL of locally produced distilled water. The solution was labelled and stored at room tempreture.

3.10 Test Procedure

The antigen (H-PPRV) was first diluted in coating buffer. 50µL of the diluted antigen was added to each well of the microtitre plate. The microplates were covered and placed in orbital plate shaker at 37^0C for 1 hour after which they were washed 3 times with washing buffer and blot dry. 40µL of the blocking buffer was added to all wells of the plate and 10uL volumes of the test and control sera were added to the appropriate wells. The monoclonal control wells received 10µL of the blocking buffer while 60µL of the blocking buffer was added to the conjugate control wells. All wells, except the conjugate control wells received 50µL of the Mab. The microplates were covered and placed on an orbital shaker at 37^0C for 1 hour after which they were washed 3 times and blot dry. All wells then received 50µL of antimouse conjugate and the sides of the plate were tapped to ensure that the conjugate working dilution was evenly distributed over the bottom of each well. The microplates were again covered and incubated for 1 hour at 37^0C with continous shaking. After 3 washings, 50µLof the chromogen/substrate mixture was added to all wells and after 10 minutes incubation at room temperature, color development was stopped by adding 50µL of stop solution to all wells. Optical density of the values was read at 492nm using ELISA reader.

3.11 Interpretation of the Test

The inhibition of binding of the monoclonal antibody in the presence of the test serum was expressed as percentage inhibition (PI) estimated from mean optical density using the formula.

$$PI = 100 - \left(\frac{\text{Optical density of the test wells }}{\text{Optical density of the Mab control wells}}\right) \times 100\%$$

Test sera demonstrating mean percentage inhibition values of 50% or greater were considered as positive to PPR virus antibodies. Test sera demonstrating mean percentage inhibition values less than 50% were considered to be negative to PPR virus antibodies.

3.12 Statistical Analysis

The data obtained from this study were subjected to descriptive statistics (Tables and charts), Chi-square test of independence and odds ratio (OR) to determine the association of the variables (age, sex, species and breed) with the presence of PPR virus antibodies. The value of $p<0.05$ was considered significant in this study. Descriptive statistics was carried out using Microsoft excel 2007. Chi square, odds ratio and confidence intervals were calculated using Graphpad instat software.

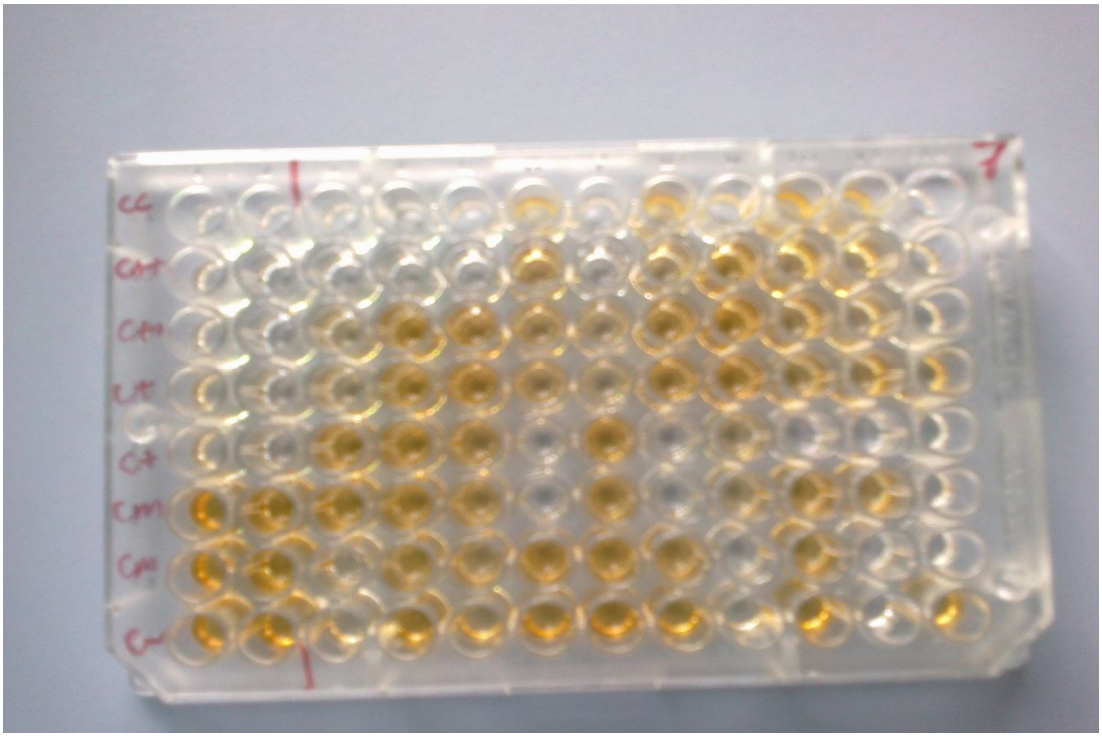


Plate V: c-ELISA microplate showing positive and negative reactions

CHAPTER FOUR

RESULTS

A total of 433 serum samples were obtained from small ruminants of not less than six months old and with no history of vaccination against PPR. Out of the total number of the samples obtained, 197 sera tested positive, making the overall prevalence of PPR virus antibodies in small ruminants in the study area to be 45.50%. Antibodies to PPR virus were detected in small ruminants from all the Local Government Areas (LGAs) sampled. The highest seroprevalence rate was reported in Sokoto south LGA (68.75%) followed in decreasing order of prevalence by Tambuwal LGA (59.10%), Goronyo LGA (56.72%), Kware (53.24%), Bodinga LGA (33.71%) and Wurno LGA (18.60%). There is statistically significant difference in the prevalence of PPR virus antibodies across the LGA sampled (Table 4.1).

Distribution of PPR virus antibodies among sheep and goats in the study area is summarized in Table 4.2. Of the 433 small ruminants sampled in this study, 246 (56.81%) were goats, while 187 (43.19%) were sheep. Out of the total number of 246 goats sampled, 99 (40.24%) were found to be positive for PPR virus antibodies. In the case of sheep, 98 (52.41%) out of 187 sampled were positive for PPR virus antibodies. This difference in the seroprevalence rates of PPR in sheep and goats was statistically significant (p=0.015)

Age distribution of PPR virus antibodies in small ruminants in the State has been determined in this study (Table 4.3). Of all the age groups, small ruminants aged 13-24 months had the highest seroprevalence rate of 52.49% while those aged over 24 months

Table 4.1: Distribution of PPR virus antibodies in small ruminants in some Local Government Areas of Sokoto State.

Local Government Area	Number sampled	Positive	Negative	Prevalence (%)
Goronyo	67	38	29	56.72
Tambuwal	66	39	27	59.10
Bodinga	89	30	59	33.71
Kware	77	41	36	53.24
Wurno	86	16	70	18.60
Sokoto South	48	33	15	68.75
Total	433	197	236	

Chi square (X^2) = 52.973,

Alpha 0.05, degrees of freedom = 5

$p<0.001$ (very significant)

Table 4.2: Distribution of PPR virus antibodies in sheep and goats in Sokoto State

Species	Number sampled	Positive	Negative	Prevalence (%)	Odds ratio	C.I
Sheep	187	98	89	52.41	1.64*	1.11-2.40
Goats	246	99	147	40.24	1.00 ref	
Total	433	197	236			

Chi square $X^2 = 5.86$

Alpha = 0.05, degree of freedom = 1

P = 0.0155 (considered significant)

Values with * are statistically significant

C.I = confidence interval

Table 4.3: Distribution of PPR virus antibodies in different age groups of small ruminants from Sokoto State

Age group (Months)	Number sampled	Positive	Negative	Prevalence
6-12	130	63	67	44.86
13-24	181	95	86	52.49
25-above	122	39	83	31.97
Total	433	197	236	

Chi square (X^2) = 13.032,

Alpha = 0.05

p=0.015 (significant)

had the least seroprevalence of 31.97%. At 95% confidence interval, there was statistically significant different seroprevalence rate among the age groups in this study (p=0.015).

Result for the sex- specific rates of PPR virus antibodies among small ruminants sampled is shown in Table 4.4. From the results, it is evident that females had a statistically greater seroprevalence rate of 49.80% compared to the males whose seroprevalence rate was estimated to be 39.08% (p=0.0358). Females were also found to be significantly more likely to have PPR virus antibodies than the males (OR=1.55).

Sheep and goats sampled in this study have been categorized into three distinct age groups: group 1 (6-12 months), group 2 (13- 24 months) and group 3 (over 2 years). Appendix I compares the seroprevalence rates of sheep and goats in different age groups. From the chat, it is clear that in all the categories, sheep had a greater seroprevalence rate than goats although statistically significant difference in the seroprevalence rates was only found in the age group 13- 24 months (p=0.002).

Prevalence of PPR virus antibodies in different sexes (males and females) of sheep and goats is compared in Appendix II. The results indicate that female sheep had a statistically greater seroprevalence rate (62.61%) than the female goats whose seroprevalence rate was 39.58% (p=0.004). In males however, there was no statistically significant difference between sheep and goats sampled in this study.

Table 4.4: Distribution of PPR virus antibodies in males and females small ruminants from Sokoto State

Sex	Number sampled	Positive	Negative	Prevalence	Odds ratio	C.I
Female	259	129	130	49.80	1.55*	1.05-2.29
Male	174	68	106	39.08	1.00ref	
Total	433	197	236			

Chi square X^2=4.406,

Alpha = 0.05

p=0.0358 (considered significant).

Values with * are statistically significant.

C.I = confidence interval

Tables 4.5 and 4.6 respectively show the distribution of PPR virus antibodies in different breeds of goats and sheep in the study area. Among the breeds of goats sampled, Red Sokoto goats had the highest seroprevalence rate of 47.52% followed by mixed breeds (33.33%) while sahelian breeds of goats had the least seroprevalence rate of 24.24%. The difference in the seroprevalence rates among the goats breeds was statistically significant (p=0.0179). In case of sheep, the highest seroprevalence rate was obtained in Uda breed (59.09%) followed by mixed and Balami breeds with seroprevalence rates of 49.21% and 41.67%, respectively. The difference was however not statistically significant at 95% confidence interval.

Prevalence of PPR virus antibodies in camels has also been determined in this study. Out of the 137 camels sera examined, 25 were positive to PPR virus antibodies making the seroprevalence of PPR in camels slaughtered in Sokoto abattoir to be 18.25%. Analysis of the sex specific prevalence of PPR in camels (Table 4.7) revealed that females (25.35%) had a significantly greater seroprevalence of PPR than the males whose seroprevalence rate was 10.61% (p=0.0443). Distribution of PPR virus antibodies in different age groups of camels slaughtered at Sokoto abattoir is shown in Table 4.8. From the Table, adult camels had a significantly greater seroprevalence of PPR than the young ones (p=0.0133).

Table 4.5: PPR virus antibodies distribution among different goats breeds in Sokoto State.

Breed	Number sampled	Positive	Negative	Prevalence
Red Sokoto	141	67	74	47.52
Sahel	33	8	25	24.24
Mixed	72	24	48	33.33
Total	246	99	147	

Chi square $X^2 = 8.046$

Alpha = 0.05, degrees of freedom = 2

P = 0.0179 (significant)

Table 4.6: Distribution of PPR virus antibodies among different breeds of sheep in Sokoto State.

Breeds	Number sampled	Positive	Negative	Prevalence (%)
Uda	88	52	36	59.09
Balami	36	15	21	41.67
Mixed	63	37	32	49.21
Total	187	98	99	

Chi square (X^2) = 3.50

Alpha = 0.05, degrees of freedom = 2

P = 0.1738 (not significant)

Table 4.7: Sex distribution of PPR virus antibodies in camels slaughtered in Sokoto Municipal abattoir.

Sex	Number sampled	Positive	Negative	Prevalence (%)	Odds ratio	C.I
Males	66	7	59	10.61	1.00^{ref}	
Females	71	18	53	25.35	2.86^{*}	1.11-7.39
Total	137	25	112			

Chi square $X^2 = 4.046$

Alpha = 0.05, degrees of freedom = 1.

P= 0.0443.

Values with * are considered significant

C.I= Confidence interval

Table 4.8: Age Distribution of PPR virus antibodies in camels Slaughtered in Sokoto Municipal abattoir.

Age-group (years)	Number sampled	Positive	Negative	Prevalence (%)	Odds ratio	C.I
Young (0-4 years)	77	8	69	10.39	1.00^{ref}	
Adults (over 4 yrs)	60	17	43	28.33	3.41^{*}	1.36-8.58
Total	137	25	112			

Chi square X^2= 6.125

Alpha = 0.0133, degree of freedom = 1

P= 0.0133

Values with * are considered significant

C.I= Confidence interval

Camel ageing (FAO, 1994)

Table 4.9: Percentage Inhibition (PI) in different species of animals sampled in Sokoto State

	Number of animals positive at various percentage inhibition values										
Species	**Percentage Inhibition (PI) values (%)**										**Total**
	50-55	56-60	61-65	66-70	71-75	76-80	81-85	86-90	91-95	96-100	
Goat	7	11	8	12	13	8	14	17	09	0	99
Sheep	9	15	13	8	9	18	10	12	04	0	98
camel	7	5	3	1	5	4	0	0	0	0	25
Total	23	32	25	23	27	30	21	30	11	0	222



Plate VI: Suspected case of PPR in a 1 year old Sokoto Red goat from Sokoto South LGA: Mucopurrulent occulonasal discharges.

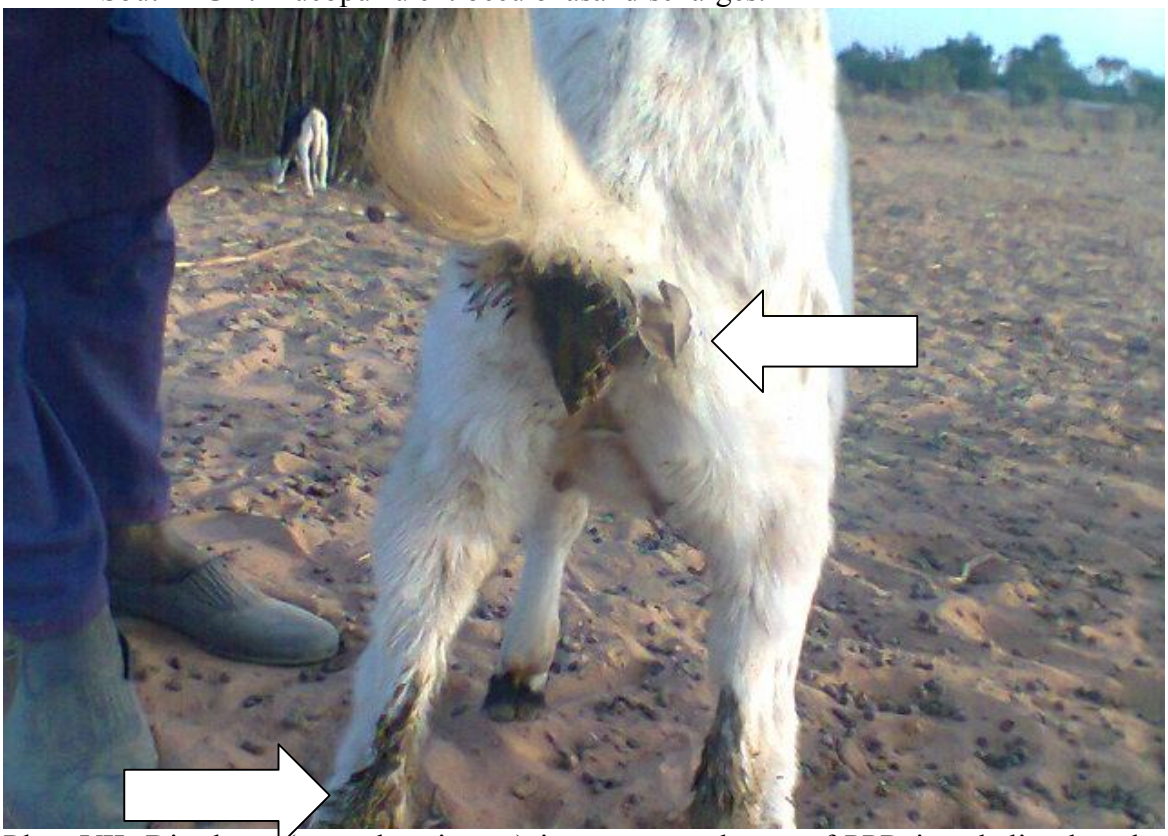


Plate VII: Diarrhoea (matted perineum) in a suspected case of PPR in sahelian breed of goat

CHAPTER 5

DISCUSSIONS

Peste des petits ruminants (PPR) is increasingly becoming important where small ruminants contribute in no small measure to food security. In Nigeria, PPR is regarded as the most important disease of small ruminants because the economic losses due to the disease are enormous. In addition, the disease is considered as a militating factor against livestock production in Nigeria (Shamaki *et al*., 2004).

Results from this study indicate that an overall prevalence of PPR virus antibodies in small ruminants in Sokoto State was 45.50%. This is in agreement with the 40.6% obtained by Shamaki *et al*., (1995) in Borno State, and slightly lower than 56.2% reported by Majiyagbe *et al*. (1992) in small ruminants producing areas of northern Nigeria. The high prevalence of PPR virus antibodies in the state could be attributed to the geographic location of the state. Sokoto State shares a common border with Niger republic which is also a PPR endemic country (Kaukarbayevich, 2009). Cross border nomadic movement of small ruminants from Niger Republic into the State could increase the chances of contact with local sheep and goats thereby facilitating the transmission of PPR. The high seroprevelence rate of PPR obtained in this study could also be attributed to the high population of livestock in the state, which may encourage crowding of animals and subsequent disease transmission.

Antibodies to PPRV were detected in small ruminants in all the LGAs sampled in this study. This confirms the endemic nature of PPR in the study area. In this study, the highest

prevalence of PPR virus antibodies was observed in Sokoto South LGA (68.75%), followed by Tambuwal LGA (59.10%) while the least seroprevalence rate of 18.60% was obtained in Wurno LGA (Table 1). The high seroprevalence rate of PPR obtained in Sokoto South LGA might have been due to the fact that, as of the point of sampling, most animals were showing clinical signs suggestive of ongoing outbreak of PPR. In other LGAs, however, samples obtained were mostly from apparently healthy sheep and goats.

Species specific prevalence of PPR virus antibodies has also been determined in this study (Table 4.2). Sheep (98/187) were observed to have significantly greater prevalence of PPR virus antibodies (52.41%) than the goats (99/246) whose PPR seroprevalence rate was 40.24% (p=0.0155) although only about 26% of the positive sheep had mean percentage inhibition values greater than 80% while more than 36% of the positive goats had that mean percentage inhibition value (Table 4.9). Similar results were obtained by (Mehmood *et al.* 2009) and Abraham *et al.* (2005). However, the results obtained in this study were contrary to the findings of Zahur *et al* (2011) who reported a significantly higher prevalence of PPR virus antibodies in goat than in sheep. In addition, this study further contradicts the findings of Kazeem (2001) who reported a higher seroprevalence rate of PPR virus antibodies in the goats than the sheep. The higher seroprevelence of PPR in sheep in this study should not be confused with the severity of the disease. Rather, it could be attributed to the higher survival rate in sheep than in goats. Majiyagbe, (1985) observed a significantly higher mortality due to PPR in goats compared to sheep. Because of this high mortality, most goats infected with PPR virus die prior to sampling. Conversely, most infected sheep survive the infection. Thus a large proportion of the sheep that have

recovered from previous infection will have detectable levels of circulating antibody in their serum. This may explain the significantly higher seroprevelence rate of PPR observed in sheep than in the goats in this study.

Age prevalence of PPR virus antibodies in small ruminants in the study area revealed a statistically significant difference among the three age groups considered in this study ($p=0.015$) (Table 4.3). Small ruminants aged between 13-24 months had the highest prevalence of PPR virus antibodies (52.49%) followed by those aged between 6-12 months (48.46%) while the least seroprevalence rate was obtained in those animals aged above 24 months. This to some extent agrees with the findings of Mahajan *et al.* (2012) who observed a significantly higher prevalence of PPR virus antibodies in animals aged above 12 months compared to those aged between 8-12 months. However, Majiyagbe *et al.* (1992) showed that PPR seroprevalence increases with age. Dams infected with PPR virus can passively transfer maternal antibodies to their young ones. Although the maternal antibodies progressively decay, they remain above the protective threshold for up to 4-5 months after which PPR vulnerability increases with age (Abubakar *et al.,* 2009). This increased PPR susceptibility with age after five months in small ruminants may explain the relatively lower seroprevalence rates obtained in small ruminants aged between 6-12 months when compared with those aged between 13-24 months in this study. However, low seroprevalence of PPR was observed in those small ruminants aged above 24 months in this study. It is possible that animals sampled in that category migrated from a less PPR endemic region and so had limited exposure to the field virus. That may explain the low seroprevalence rate observed in those animals.

Age group distribution of PPR virus antibodies in different species (sheep and goats) is shown in Appendix 1. Goats aged 6-12 months old had the highest prevalence rate followed by 13-24 months age group with the least prevalence rate obtained in goats of above 2 years old. The serological picture was somehow different in the sheep population where the highest seroprevalence rate was observed in those aged 13-24 months. However, in both species, it was observed that animals aged above 2 years old had the lowest seroprevalence rates of PPR virus antibodies.

Sex seroprevelence data obtained for small ruminants in this study indicates that the females were more likely (OR=1.55) to have PPR virus antibodies than male animals. Table 4.4 shows the distribution of PPR virus antibodies in males and female small ruminants sampled in this study. From the Table, it is evident that females had a significantly greater seroprevelence rate than their male counterparts (p=0.0358). When both sexes were compared in different species (Appendix II), it was observed that females sheep (62.61%) had a significantly greater prevalence of PPR virus antibodies than the female goats (39.58%). However, the reverse was the case in the males, where male goats had a greater seroprevalence rate than the male sheep (36.11%). This observation agrees with the findings by Luther *et al.* (2007) who reported a significantly higher seroprevalence rate of PPR virus antibodies in females than in male goats. Male animals are not usually kept in a flock for a long period of time. They are often sold out for meat at approximately 1-2 years of age, while the females remain in the flock for breeding purposes. Thus, female animals have a greater exposure time than males in the flock. This may explain the higher seroprevalence rate obtained in females in this study.

Three breeds of goats were sampled in this study. The distribution of PPR virus antibodies among the breeds is shown in Table 4.5. From the results, Red Sokoto goats (47.52%) appeared to have significantly greater prevalence of PPR virus antibodies than other breeds (Sahelian and Mixed) sampled in this study (p=0.0179). The result obtained in this study contradicts the findings of Qadeer (1998) who observed a significantly higher prevalence of PPR virus antibodies in mixed breeds (87.6%) than in pure breeds of goats. The higher seroprevalence obtained in Red Sokoto goats in this study could be explained by the dominance effect of the breed in the state. Red Sokoto goats are the most predominant breeds of goats available in Sokoto state. Other breeds of goats such as sahelian breeds are less popular in the State. Therefore, it is possible that since the disease is endemic in the State, most of the Sokoto red goats (which predominate in the state) had already develop antibodies against the disease making the seroprevalence rate higher in that breed of goats than other less popular breeds of goats in the state considered in this study. Genetic factors such as breed predisposition may also play a role in the susceptibility to PPR. It is possible that Red Sokoto goats are genetically more susceptible to PPR than the other breeds sampled in the study. This may also explain the higher seroprevalence rate of PPR in the breed. In the case of sheep, Uda, Balami and Mixed breeds constituted the breeds sampled in this study (Table 4.6). Results obtained indicate that uda breed had a greater seroprevalence rate of PPR than the other breeds considered in this study. However, the association was not statistically significant.

Although PPR is primarily a disease of small ruminants, several studies have confirmed the occurrence of the disease in camels (Ismail *et al*., 1992; Roger *et al*., 2000; Abraham *et al*., 2005; Khalafalla *et al.,* 2010). In this study, prevalence of PPR virus antibodies in camels slaughtered at Sokoto metropolitan abattoir was also determined. From the results obtained, 25 out of the total of 137 camels sampled turned out to be positive for PPR virus antibodies, giving a PPR seroprevalence rate of 18.2%. This is much higher than the result obtained by Daneji *et al.* (1997) who reported a seroprevalence rate of 4% in the same study area. In addition, Ismail *et al.* (1992) found 4.2% prevalence of PPR virus antibodies from 142 healthy camels using serum Neuralisation test. Camels are neither routinely vaccinated against PPR, nor against Rinderpest. Thus, the antibodies observed in this study must have been due to field challenge with PPR virus. Shamaki *et al.,* (2004) reported the recovery of PPRV antigen from camels slaughtered with respiratory syndrome in Sokoto abattoir. Therefore, there are good reasons to believe that PPR may occur in camels even though at a subclinical level.

On the basis of sex specific prevalence of PPR virus antibodies, female camels had a significantly greater PPR virus antibodies than the male camels sampled in this study ($p=0.043$). In addition, odds ratio analysis of the data revealed that the females were over two times more likely to have PPR virus antibodies than their male counterparts (OR=2.86). This is in line with the findings of Swai *et al.* (2011) who obtained similar results, although the association between the serologic positivity and the sexes in that study was not statistically significant. The reason for this difference is unclear. However, female animals are generally more subjected to stressfull condition physiologically than their male

counterparts. This makes them more susceptible to infections than the males. Therefore, it is not unlikely to obtain a greater PPR seroprevalence rate in females compared to the male camels slaughtered.

For the purpose of this study, the camels sampled were categorised into two groups: Young (0-4 Years) and Adults (Above 4 years). From the results obtained, camels aged above 4 years (28.3%) have a significantly greater PPR virus antibodies than the younger ones (10.39%) whose age falls between 0-4 years. The results further indicate that, the adult camels were over three times more likely to have PPR virus antibodies than the young ones (OR=3.41). Similar results were obtained by Swai *et al* (2011) who observed a greater PPR seroprevalenve rate in camels of 5-10 years age category. The result obtained in this study also agrees with the findings of Khalafalla *et al*. (2010) who reported that PPR is more severe in the adult camels particularly the pregnant and lactating camels than the young ones. It was argued that the high mortality observed in the mature and reproductively active camels in that study is a clear indication that PPR has a selective preference for reproductive age groups of camels.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 CONCLUSION

- The current seroprevalence of PPR in small ruminants in the study area is 45.50% while that of camels is 18.25%.
- The fact that antibodies to PPR virus were detected in all the LGAs sampled signifies the widespread nature of PPR and confirms the endemicity of the disease in Sokoto State.
- The antibodies to PPR virus observed in this study were neither due to vaccination nor maternal antibodies. They must have therefore been due to natural infection with field PPR virus.
- Since camels are neither routinely vaccinated against rinderpest, nor against PPR, the antibodies due to PPR observed in camels in this study were most probably due to field exposure to PPR virus. This further confirms the occurrence of the disease in camels.
- Epidemiologic variables such as age, sex, breeds and species may affect the outcome of the disease in the study area.

6.2 RECOMMENDATION

- Control efforts for PPR particularly vaccination with PPR homologous vaccine, should be intensified in Sokoto State

- In planning stategic control programmes for PPR in Sokoto State, the need to integrate camels in the epidemiological survellence of the disease becomes imperative.

- In order to enhance the knowledge of the epidemiology of PPR in Sokoto State, there is a need for an in depth study on the isolation and molecular characterization of PPR virus from small ruminants in the study area.

- Finally, more detailed study on the epidemiological role of camels in the spread of PPR in Sokoto State is also recommended. Future studies should also include testing for association between the serological positivity in camels and risk factors such as Nutritional status, presence of other concurrent infections, season of the year as well as management system of the camels.

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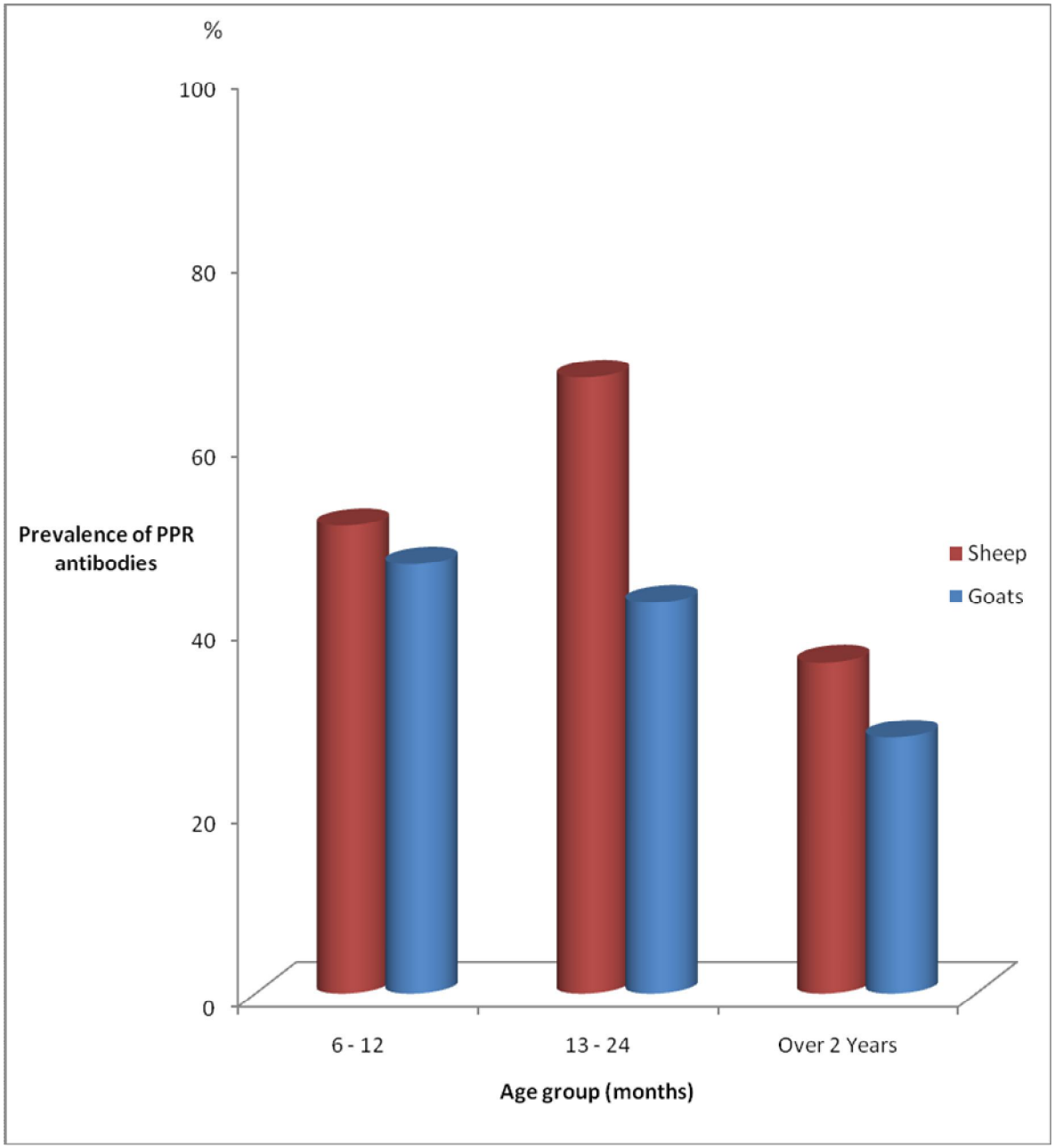
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APPENDICES

Appendix 1: Age distribution of PPR virus antibodies in different species of small ruminants in Sokoto State



Appendix II: Sex distribution of PPR virus antibodies in different species of small ruminant in Sokoto State.

