PESTE DES PETITS RUMINANTS MANIFESTATION IN SHEEP AND GOATS AND ITS PREVALENCE IN INCONTACT HERBIVORES IN KENYA

BY

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other university.

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This thesis has been submitted for examination with our approval as University supervisors.

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DEDICATION

This thesis is dedicated to my family.

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LIST OF ABBREVIATIONS

AGID	Agar gel immunodiffusion test
CCPP	Contagious caprine pleuropneumonia
cDNA	Copy deoxyribonucleic acid
CDV	Canine distemper virus
cELISA	Competitive enzyme linked immunosorbent assay
CIEP	Counter immunoelectrophoresis
CIRAD	Centre de cooperation internationale en recherché agronomique pour le developpement
CMV	Cetacean morbillivirus
CPE	Cytopathic effect
DMV	Dorphin morbillivirus
DNA	Deoxyribonucleic acid
DVS	Director of Veterinary Services
ELISA	Enzyme linked immunosorbent assay
GPS	Global positioning system
HA	Haemagglutinins
KWS	Kenya wildlife services
Mab	Monoclonal antibodies
MV	Measles virus
OIE	Office International des epizooties
PAGE	Polyacrylamide gel
PARC	Pan-African rinderpest campaign
PCR	Polymerase chain reaction
PCV	Packed cell volume

PMV	Phocine morbillivirus
PPR	Peste des petits ruminants
PPRV	Peste des petits ruminants virus
RBC	Red blood cells
RNA	Riboxynucleic acid
RPV	Rinderpest virus
RT-PCR	Reverse transcriptase polymerase chain reaction
RVF	Rift valley fever
TCID	Tissue culture infectious dose
VNT	Virus neutralisation test

ABSTRACT

Peste des petits ruminants (PPR) is a highly devastating viral disease of sheep and goats. PPR was confirmed to be occurring in Kenyan goat and sheep populations in March 2006 in Turkana district. The disease has since spread to other districts of Kenya. The confirmation and clinical presentation of PPR in the Kenyan goat and sheep populations as it occurred was hitherto not described. There was need to confirm that the outbreaks that occurred in Kenya were due to PPR as some of the signs that occur due to PPR are shared by other diseases. The aim of this study was to confirm the occurrence of PPR, document the clinical picture, management and risk factors that were associated with the PPR disease as it occurred in Kenya during the 2007-2008 outbreak. In addition the study sought to find out whether in-contact domestic and wild herbivores got natural exposure and infection with the PPR virus.

Occurrence of PPR was confirmed in goats by carrying out PCR test on ten nasal and ocular swab samples collected from Marakwet East district in May 2008. In order to determine the clinical picture, management and risk factors of PPR, 78 questionnaires were administered to livestock keepers in Tot division of Marakwet East district in November 2009. This was followed by determination of PPR seroprevalence by carrying out cELISA on cattle, camel, buffalo, giraffe and warthog serum samples. In total, 240 cattle serum samples were tested, 160 for camel, 98 for buffalo, 93 for warthog and 9 for giraffe. Thirty three goat serum samples were also tested to find out whether the virus had infected goats born five months after vaccination.

In this study, nasal and eye swabs from a female goat were positive for PPR RNA on PCR. The study further showed that Marakwet community largely practices pastoralism with sheep and goats being the most reared animals. Diseases were the biggest threat to livestock production amongst other challenges. Among the diseases

affecting the small stock contagious caprine pleuropneumonia (CCPP) topped the list, followed by heartwater and PPR came third. The clinical signs of PPR recorded included loss of appetite, ocular discharge, nasal discharge, diarrhoea, rough hair coat, coughing and death. There were no mouth lesions reported. The questionnaire survey also showed that 37.2% of livestock keepers opened up carcasses of animals dying of PPR. The post mortem signs observed included darkening or reddening of lungs, haemorrhages in the small intestines, impaction of the reticulum, oedema along the mesentry and the enlargement of lymphnodes. As a result of the disease, the study showed that livestock keepers and other players in the livestock industry suffered loss of income, loss of human food, loss of markets and losses arising from impact of imposed quarantines. The cELISA test results showed an overall PPR seroprevalence of 4.2% in cattle, 3.13% in camel and a locality specific PPR seroprevalence of 21.2%. Samples from the three wildlife species were negative for PPR antibodies.

The results of this study showed that the PPR disease as it occurred in Kenya was non-classical. This has the implication of poor recognition and reporting by livestock keepers. Continued surveillance mechanism therefore needs to be put in place to avoid disease flare up especially after a build up of a susceptible population. It is therefore recommended that vaccination against PPR be combined with the more prevalent diseases like CCPP. The results of this study further demonstrated that cattle and camel populations of Kenya got exposed to PPR virus and developed antibodies against this disease. As such, the incontact animals (cattle and camel) can be used as indicators (sentinel herds) for evaluation of increased PPR virus circulation among sheep and goat populations especially in the arid and semi-arid lands (ASALs) of Kenya where these different livestock species are herded together.

CHAPTER ONE

1.1 Introduction

Morbillivirus infections under which the peste des petits ruminants (PPR) virus falls have had a huge impact on both animals and human beings. The genus Morbillivirus constitutes highly contagious pathogens that have caused some of the most devastating viral diseases of animals and humans worldwide (Murphy *et al.*, 1999). They include the rinderpest virus (RPV), the peste des petits ruminants virus (PPRV), measles virus (MV) and canine distemper virus (CDV). Further, new emerging morbillivirus infections with significant ecological consequences for marine mammals have been discovered in the past decade. Phocid distemper virus (PDV) has been documented to occur in seals while cetacean morbillivirus (CMV) is found in dolphins, whales and porpoises (Barret *et al.*, 1993, Domingo *et al.*, 1990, McCullough *et al.*, 1991).

Morbilliviruses are enveloped, nonsegmented negative strand RNA viruses and constitute a genus within the family *Paramyxoviridae*. They cause fever, coryza, conjunctivitis, gastroenteritis, and pneumonia in their host species. The major sites of viral propagation are lymphoid tissues and acute diseases are usually accompanied by profound lymphopenia and immunosuppression, leading to secondary and opportunistic infections (Appel and Summers., 1995, Murphy *et al.*, 1999).

Peste des petits ruminants (PPR) is a highly contagious and infectious viral disease of domestic and wild small ruminants (Furley *et al.*, 1987). It is an economically significant disease of small ruminants such as sheep and goats (Dhar *et al.*, 2002). It was first described in Cote d'Ivoire in West Africa where it used to be identified as kata, pseudorinderpest, pneumoenteritis complex and stomatitis-pneumoenteritis

syndrome (Gargadennec and Lalanne, 1942, Braide, 1981). Taylor (1984) confirmed the existence of the disease in Nigeria, Senegal and Ghana. For many years it was thought that it was restricted to West Africa until a disease of goats in Sudan, which was originally diagnosed as rinderpest in 1972, was confirmed to be PPR (Diallo 1988). The realization that many of the cases diagnosed as rinderpest among small ruminants in India may, instead, have involved the PPR virus, together with the emergence of the disease in other parts of Western and Southern Asia (Shaila *et al.*, 1996), signified its ever-increasing importance. PPR has received growing attention because of its wide spread, economic impacts (Lefevre and Diallo, 1990) and the role it plays in complication of the ongoing global eradication of Rinderpest and epidemiosurveillance programmes (Couacy-Hymann *et al* ., 2002).

The disease in Eastern Africa

PPR is endemic in south-west Ethiopia in the Somali and Oromiya Regional States (USAID report, 2008). In Ethiopia, goats react more severely to PPR virus exposure compared to sheep and they exhibit striking clinical signs while sheep undergo a mild form of the disease. However, a similar profile of serological status is widely reported (Taylor, 1984). The disease continues to spread across pastoral and agropastoral areas of northwestern Kenya, northeastern Uganda, Southern Sudan and Somalia (USAID report, 2008). A serological survey by Swai *et al.*, (2009) in seven districts of the United Republic of Tanzania detected 49.5% and 39.8% antibodies to PPR by cELISA in goats and sheep respectively in six of the target seven districts, suggesting that infection has extended that far south.

Of the four known lineages of PPR virus, lineages 1 and 2 have been found exclusively in West Africa (Pronab Dhar *et al.*, 2002). PPR virus from an outbreak in Burkina Faso in 1999 (Pronab Dhar *et al.*, 2002) fell into the lineage 1 group. Viruses

of lineage 3 have been found in eastern Africa, where an outbreak in Ethiopia in 1996 was of this type (Pronab Dhar *et al.*, 2002). It is thought that the PPR disease within eastern Africa is caused by the PPR virus lineage 3 but its manifestation is influenced by many factors. Outbreaks for instance can be influenced by factors such as a decrease in veterinary services that include disease surveillance and vaccinations, the presence of new populations that have not been exposed to the virus and migration of infected animals into naive flocks. Although mortality rates and impact information has not been documented and compared within the eastern African countries, there is agreement that the PPR disease has had severe socio-economic consequences on food security and livelihoods (Pronab Dhar *et al.*, 2002). In Kenya, PPR disease was first diagnosed clinically and confirmed by polymerase chain reaction (PCR) in April 2006 in Turkana District but has since spread to other districts in the country (DVS, 2008; Ministry of Livestock Development, Nairobi; Kenya). At least 15 million sheep and goats are at risk of death from the disease in the affected areas, and in the worst-case scenario, the continued spread of the disease could spell a humanitarian crisis in Kenya's pastoral areas (DVS, 2008; Ministry of Livestock Development, Nairobi; Kenya). In Kenya, the clinical and pathological presentation of this disease in sheep and goats are yet to be described. There is also need to find out whether there is natural exposure of this virus to other in-contact domestic herbivores especially cattle and camel considering that the different animal species are herded together in the pastoral regions of the country. The in-contact herbivores can be used as indicators of a rise in the virus circulation given that they are not vaccinated whenever vaccination is carried out and there is high turnover of the small stock. The role wildlife might be playing in the maintenance of the virus in the East African goat and sheep populations and the prevalence of this disease in the affected wildlife species is not documented.

This study aims to describe the clinical manifestation of PPR in Kenya and find out its seroprevalence in in-contact herbivores other than sheep and goats.

PPR in Marakwet District of Kenya

Turkana district where the initial PPR outbreak was first reported and confirmed in 2006, borders West Pokot district to the south. West Pokot district was among the districts classified as PPR infected by the DVS, Ministry of Livestock development (MoLD) of kenya by June of 2008. The West Pokot district borders Marakwet East district to its south which was as well classified to be a PPR infected district (DVS, 2008; Ministry of Livestock Development, Nairobi; Kenya). PPR vaccinations were carried out from Turkana district southwards starting the month of October 2008. In West Pokot district, vaccinations were carried out in November 2008 while in Marakwet East district vaccination was carried out in December 2008 (Personal communication, Dr. Kiyeng, DVO Marakwet East November 2009). According to Dr. Kiyeng, vaccinations in Turkana and West Pokot districts were carried out to cover at least 80% of the small stock population because the disease had spread to most parts of the two districts. By the time vaccination was being carried out in Marakwet district, the disease had infected animals on the northern part of the district that border West Pokot. As such the vaccination approach in the district was different. The vaccination team was divided into 3 groups. The first group was to vaccinate from the northern parts of the district southwards in the infected area. The second group started the vaccination to the west of the district. This team was vaccinating on the areas bordering the Embuktu forest and the surrounding areas. These areas are high potential and the Merino sheep is the small stock species reared in large numbers. The DVO was addressing the issue of susceptibility in case the disease reached this area. The third group vaccinated by forming a buffer zone along the Tot division southern

border and the neighbouring Tirap division. This group vaccinated from this border northwards to meet with the first group. All the groups later joined to vaccinate the remaining parts of the district eastwards and southwards.

1.2 Hypothesis

The hypothesis of this study was that the Kenyan PPR virus did not present the classical clinical disease of PPR and that in-contact domestic and wild herbivores do not get natural exposure to the PPR virus.

1.3 OBJECTIVES

1.3.1 The Broad Objective

The broad objective of this study was to confirm the occurrence of PPR, describe the management and risk factors associated with the disease, determine the clinical presentation of PPR virus in sheep and goats under field situation and whether in-contact domestic and wild herbivores do naturally get exposed to the PPR virus in Kenya.

1.3.2 Specific Objectives

The specific objectives of the study were:

a) To confirm the occurrence of PPR disease in Marakwet East district of Kenya

b) To describe the management and risk factors associated with PPR

c) To determine the clinical presentation of the PPR disease in sheep and goats under natural conditions in the field as perceived by pastoralists

d) To determine the sero-prevalence of PPR in domestic herbivores

e) To determine the sero-prevalence of PPR in wildlife herbivores

1.4 Justification

PPR has had a profound impact on the livelihoods and food security of pastoral households in affected areas of Kenya (DVS, 2008; Nairobi). In particular, its high mortality and morbidity rates decimated stocks and productivity levels upon which pastoralists rely for food and income (DVS, 2008; Nairobi). Its spread also negatively impacted on the local and international livestock trade, reducing pastoral incomes even further (DVS, 2008; Nairobi). Areas of northwestern Kenya and northeastern Uganda are already highly food insecure, and PPR only exacerbated these conditions (USAID report, 2008). There is therefore need for the virus causing this disease to be studied in detail if proper control measures are to be instituted against it hence reducing its effects on the animals affected and eventually improving on the livelihoods of the pastoral communities. The Kenyan PPR field situation as far as PPR clinical disease was concerned has probably been non-classical (Personal communication, Dr. Kiyeng, DVO Marakwet East district 2009) in addition to the probability that the virus could be circulating in other domestic and wildlife herbivore species without causing clinical disease. In the pastoral regions of Kenya, sheep and goats are herded together with both cattle and camels (Miyuki Iiyama, 2006). If the later two domestic species do naturally get infected with PPR virus, they would act as indicator groups for the increased virus circulation among the vulnerable domestic species. In the wild, the buffalo and giraffe are known to be highly susceptible to the Rinderpest virus (Scott, 1964). Given the close association of the rinderpest virus to PPR virus, the buffalo and the giraffe are a good target for analysing the scenario as relates to PPR. The warthog is also susceptible to rinderpest (Plowright, 1988). It is included in this study due to the fact that it freely roams the Kenyan pastoral lands interacting with livestock.

CHAPTER TWO

2.0 Literature Review

2.1 The peste des petits ruminants (PPR) disease

2.1.1 Aetiology

PPR is caused by the PPR virus that was assumed for a long time to be a variant of rinderpest virus adapted to small ruminants. However, studies based on virus cross neutralization and electron microscopy showed that it was a morbillivirus that had the physicochemical characteristic of a distinct virus though biologically and antigenically related to RPV. It was also shown to be an immunologically distinct virus with a separate epizootiology in areas where both viruses were enzootic (Taylor, 1979a). The development of specific nucleic acid probes for hybridisation studies and nucleic acid sequencing have proved that PPR virus is quite distinct from rinderpest virus (Diallo *et al.*, 1989a). PPRV is in the *Morbillivirus* genus of the *Paramyxoviridae* family (Gibbs *et al.*, 1979). The *Morbillivirus* genus also includes other six viruses: measles virus (MV), rinderpest virus (RPV), canine distemper virus (CDV), phocine morbillivirus (PMV), porpoise distemper virus (PDV) and dolphin morbillivirus (DMV) (Barrett *et al.*, 1993a, Barrett, 2001). The virus has low resistance in the environment and it is highly sensitive to lipid solvents (Lefèvre and Diallo, 1990).

When viewed through electron microscope, morbilliviruses display the typical structure of *Paramyxoviridae* (see Figure 1): a pleomorphic particle with a lipid envelope which encloses a helical nucleocapsid (Gibbs *et al.*, 1979). The nucleocapsids have a characteristic herring-bone appearance. Morbilliviruses are linear, non-segmented, single stranded, negative sense RNA viruses with genomes approximately 15–16 kb in size and 200 nm in diameter (Norrby and Oxman, 1990).

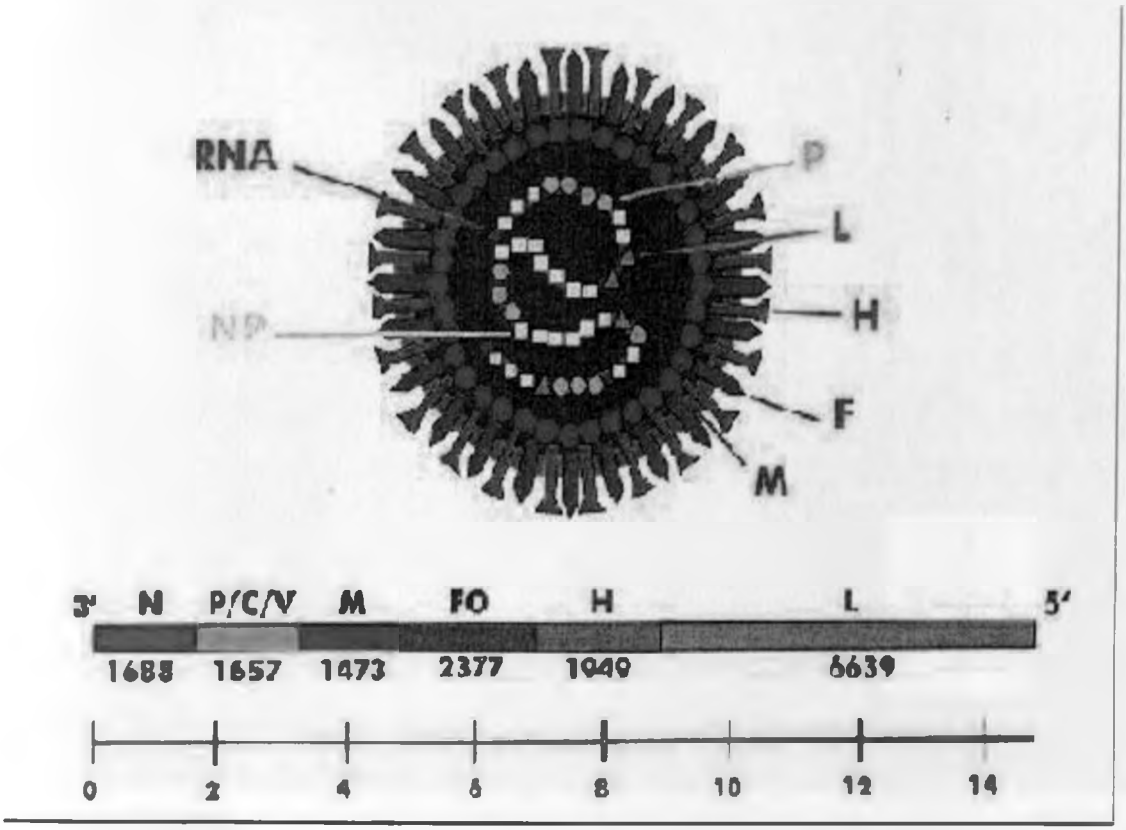
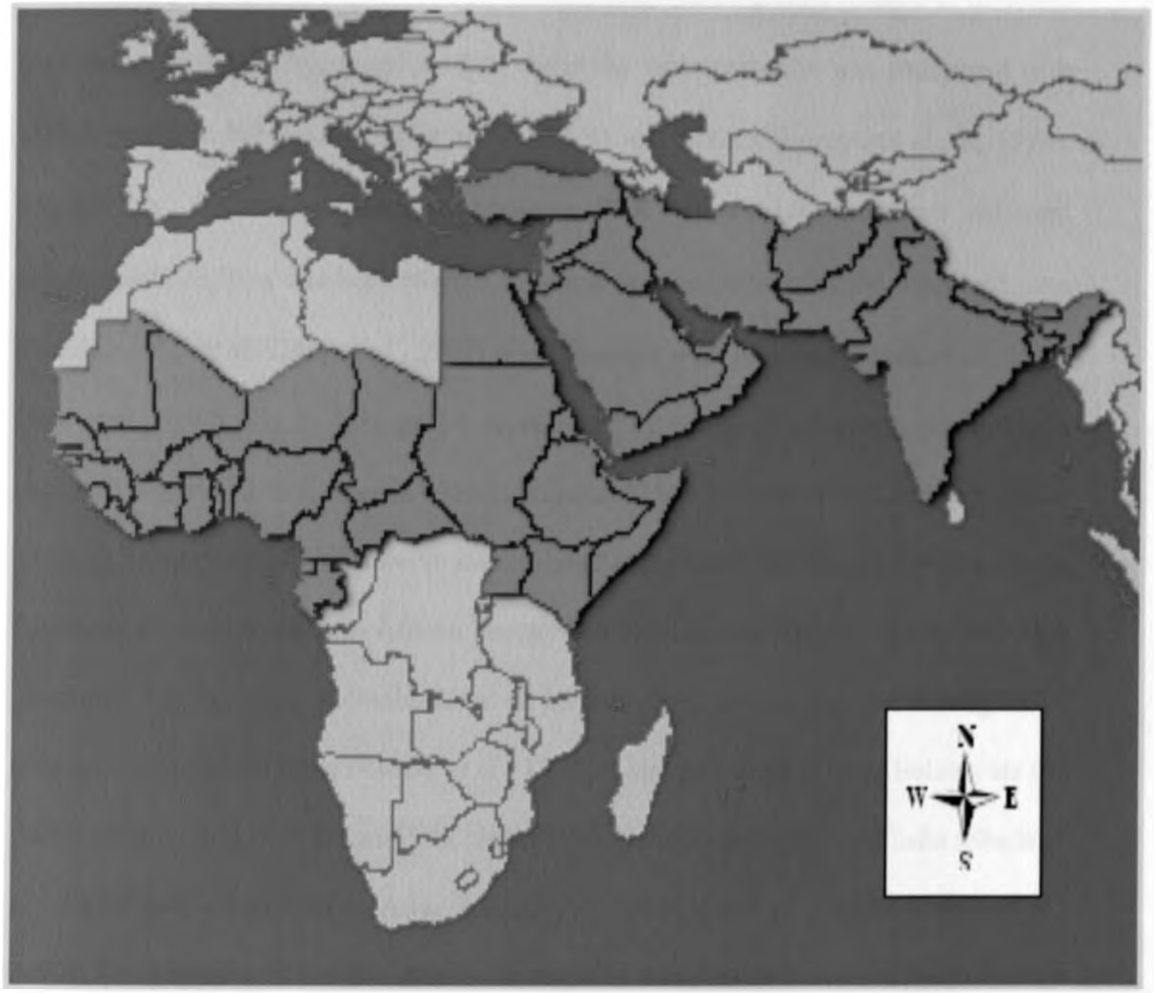


Fig:1 Genome of Morbilliviruses. (Ozkul *et al.*, 2002)

2.1.2 Geographical Distribution

PPR is known to be present in a broad belt of sub-Saharan Africa, Arabia, the Middle East and Southern Asia (Shaila *et al.*, 1996) as presented in map 1.

Map 1: A Map Showing the World PPR Distribution



Infected Zone

Source: *Recognizing peste des petits ruminants: A field manual. Rome, Italy: FAO 1999.*

Major outbreaks in Turkey and India in recent years have indicated a marked rise in the global incidence of PPR (Nanda *et al.*, 1996; Ozkul *et al.*, 2002; Shaila *et al.*, 1996). The virus was isolated in Nigeria (Taylor and Abegunde, 1979), Sudan (ElHag and Taylor, 1984), Saudi Arabia (Abu Elzein *et al.*, 1990), India (Shaila *et al.*, 1989, Nanda *et al.*, 1996) and Turkey (Ozkul *et al.*, 2002). Serological evidence of infection was shown in Syria, Niger and Jordan, while the virus presence was confirmed with cDNA probe in Ethiopia (Roeder *et al.*, 1994) and Eritrea (Sumption *et al.*, 1998), respectively. Genetic relationship between PPR viruses isolated from different geographical regions has been studied by sequence comparison of the F-protein gene (Shaila *et al.*, 1996, Dhar *et al.*, 2002). Four lineages were revealed (Shaila *et al.*, 1996, Dhar *et al.*, 2002) (Fig.2). Lineage 1 is represented by viruses isolated in Africa in 1970s (Isolates: Nigeria/1975/1, Nigeria/1975/2, Nigeria1975/3, Nigeria/1976/1 and Senegalese strain). Lineage 2 which includes viruses isolated in the late 1980s in West Africa (Ivory Coast and Guinea) is the only African lineage that did not cross the Red Sea to the Asian countries. Lineage 3 is a combination of isolates from Sudan (Isolate: Meilig/1972) (Diallo, 1988) and Ethiopia (Roeder *et al.*, 1994). Lineage 4 of PPR virus isolates are the Asian isolates; Israel/1994, Iran/1994, Nepal/1995, Bangledesch/1993 and India (Shaila *et al.*, 1996) and is confined to Asia. Recently, it was reported in Turkey (Ozkul *et al.*, 2002). The presence of the two African lineages in Asia beside a distinct Asian lineage may be taken as indication of the trade route of spread of the disease. The PPR lineages and their distribution are presented in figure 2.

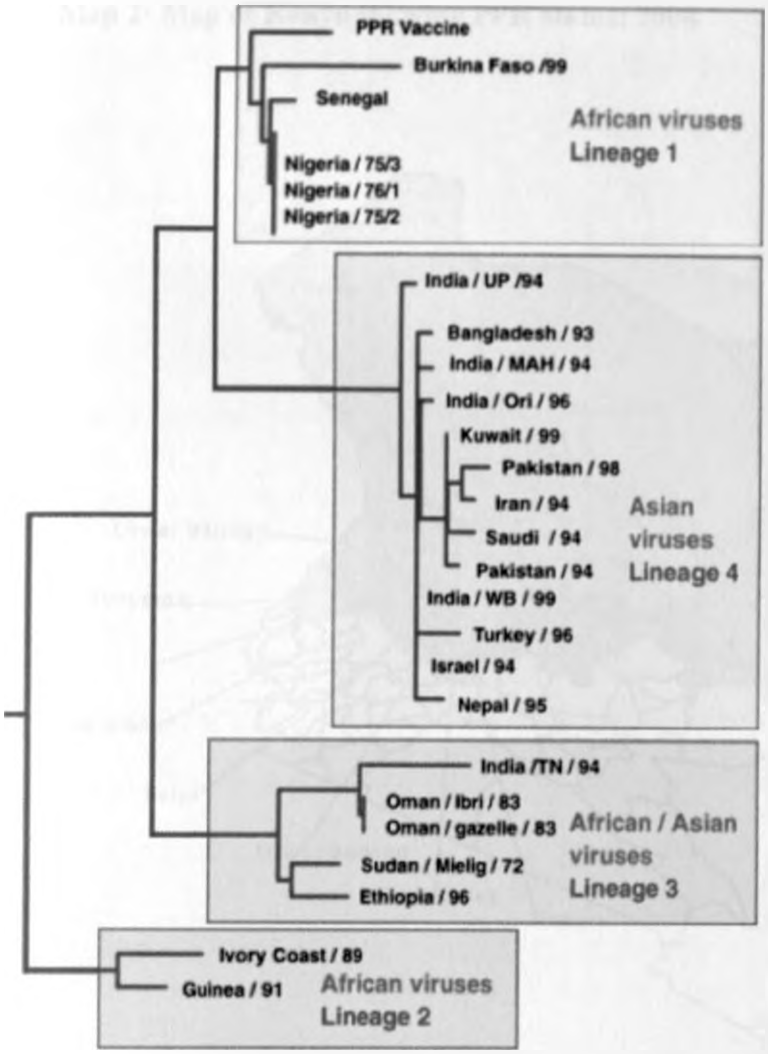
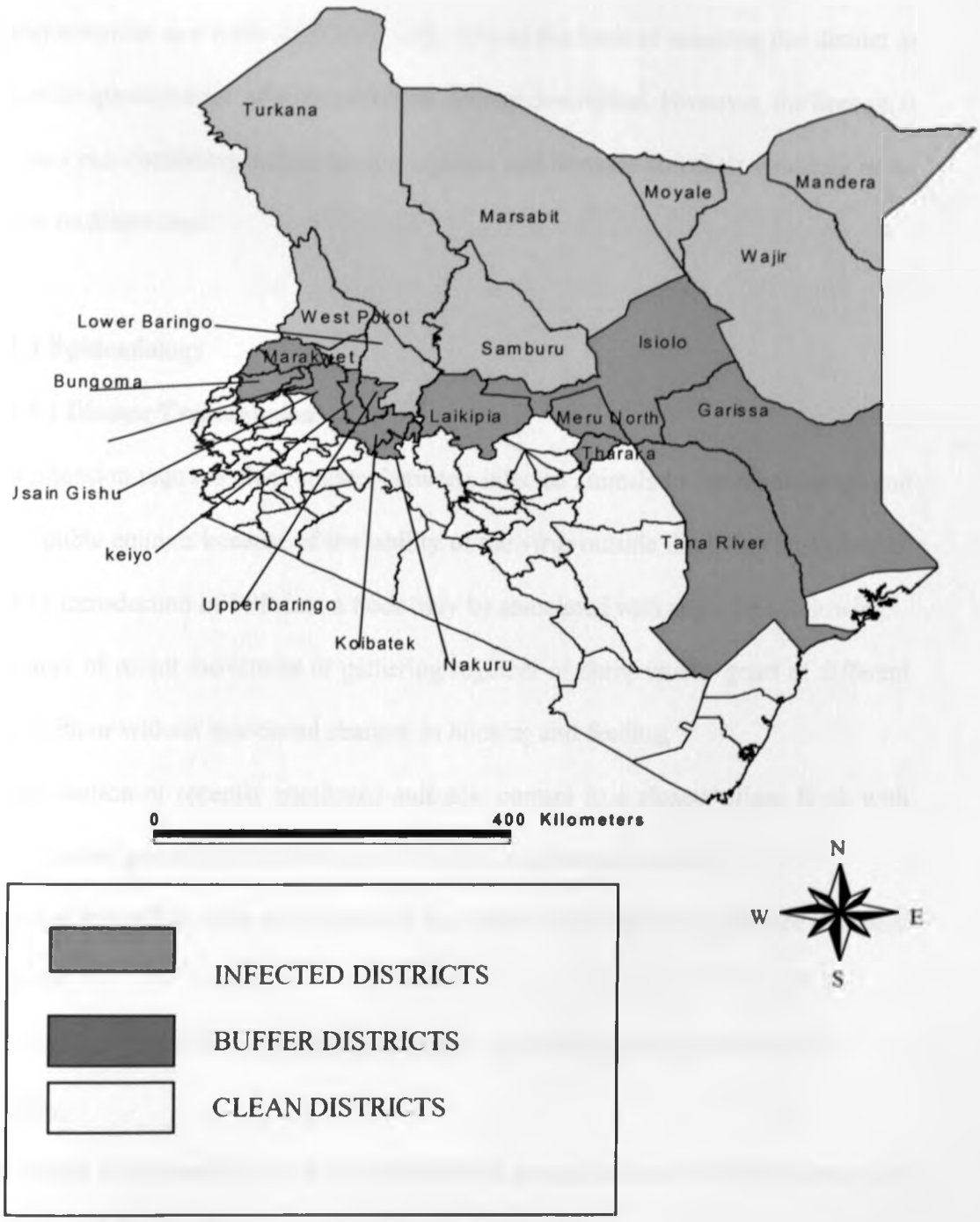


Fig. 2: Phylogenetic relationships of the Peste des petits ruminants virus isolates based on (F) protein gene (Ozkul *et al.*, 2002)

In Kenya the disease was detected in Turkana district in the year 2006 and has since spread to 16 districts such as West Pokot, North Pokot, Baringo, Samburu, Moyale, Marakwet, Marsabit, Mandera, Wajir, East Pokot, Laikipia West and Ijara (DVS report, June 2008). In these districts the disease has been associated with severe socioeconomic consequences to food security and livelihoods. In the DVS report of June 2008, seventeen other districts reflected as buffer districts on Map 2 below are either under suspicion or on high alert of infection and need to be protected.

Map 2: Map of Kenya showing PPR status; 2008



Source: GOK-DVS report (June, 2008) - Economic survey 2008.

For the Marakwet East district, the DVS report of June 2008 that mapped PPR as having occurred in the district together with the disease confirmation on samples taken from goats, processed and tested by Polymerase chain reaction as described under materials and methods in this work, formed the basis of selecting this district as a site for questionnaire administration for disease description. However, the lineage(s) of the virus circulating within Kenya, Uganda and Somalia and their virulence is/are yet to be determined.

2.1.3 Epidemiology

2.1.3.1 Disease Transmission

Transmission requires close contact between infected animals in the febrile stage and susceptible animals because of the lability of the virus outside the living host (Braide, 1981). Introduction of PPR into a flock may be associated with any of the following:

- history of recent movement or gathering together of sheep and/or goats of different ages with or without associated changes in housing and feeding;
- introduction of recently purchased animals; contact in a closed/village flock with sheep and/or goats that had been sent to market but returned unsold;
- change in weather such as the onset of the rainy season (hot and humid) or dry, cold periods,
- contact with trade or nomadic animals through shared grazing, water and/or housing;
- a change in husbandry (e.g. towards increased intensification) and trading practices (Roeder and Obi, 1999).

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 (Bundza *et al.*, 1988; Taylor, 1984). Animals in close contact inhale the droplets and are likely to become infected. 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 (Lefèvre and Diallo, 1990). The above named factors associated with introduction of PPR disease into a flock where animals from different sources are brought into close contact with one another, affords increased opportunities for PPR transmission (Roeder and Obi, 1999).

2.1.3.2 Host Range and Susceptibility

PPR is mainly a disease of goats and sheep. PPR 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). Breed may affect the outcome of PPR virus infection and its epidemiology. The Guinean goat breeds (West African dwarf, Iogoon, kindi and Djallonke) are known to be highly susceptible (Lefèvre and Diallo, 1990). This is in agreement with the finding that British goat and sheep breeds exhibited severe clinical reaction when infected experimentally while the Sudanese sheep breeds failed to develop a characteristic clinical response (El Hag and Taylor, 1984). The disease picture as far as species and breed differences are concerned within the east African region and Kenya in particular has not been documented.

Cattle and pigs are thought to be infected naturally but attempts to induce clinical disease in adult cattle experimentally failed (Gibbs *et al.*, 1979; Taylor, 1984). Seroneutralization test for the presence of PPR antibodies detected 4.2% in 142 camels (Ismail *et al.*, 1995). PPR affect wildlife: American white deer *(Odocoileus*

virginianus) was found to be susceptible (Hamdy *et al.*, 1976), 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) with death only reported by Furley *et al.*, (1987) in Dorcas gazelle *(Gazella dorcas)* and gemsbok (*Oryx gazella)* held in a zoological collection in the Arabian Gulf.

There are three members of the morbillivirus genus that infect susceptible wildlife in ecosystems that do contain domestic animals. These are rinderpest, peste des petits ruminants (PPR) and canine distemper. The wildlife hosts for rinderpest and PPR viruses are probably similar and all belong to the Order Artiodactyla (Scott, 1964).

Apart from the one reported incident (Furley *et al.*, 1987) of PPR, all the outbreaks of morbillivirus infections in wild Artiodactyla have been caused by rinderpest virus. Those that occurred up to the mid-1960s have been reviewed by Plowright (1982, 1985, 1988) and Scott (1981b). Since that time there have been very few records of rinderpest in wildlife. It occurred in 1982 in north Tanzania and in 1983 in Nigeria. Wildlife species were also reported to be affected in 1984 when the disease spread from either Chad or Sudan into the highly susceptible populations in the Central African Republic (Plowright, 1985).

Data on the susceptibility of wildlife in Africa has been obtained from the recorded histories of rinderpest outbreaks. The species most commonly affected were buffalo (*sylzcerus caffer*), eland (*Taurotragus oryx)* and warthog *(Phacuchuerus aethiupicus*) (Plowright,1982).

There is scanty information about the occurrence of PPR in African wildlife although the virus is now present in North-East, West and East Africa. Presumably all the antelope species are potentially susceptible for PPR. Some species occur in very large

numbers while others such as the gazelle *(Gazella spp.),* bushbuck *(Tragelaphus scriptus),* and duiker (*Cephalophus spp),* although not occurring in large numbers, are very widely distributed throughout the region and occur in most of the pastoral areas alongside sheep and goats. This suggests that PPR infection in antelope might become of much greater significance in the future. This could also apply to the Middle East where significant populations of some antelope species still occur in the nomadic pastoral areas (Anderson, 1995).

2.1.4 Disease Occurrence

In general, morbidity is common, particularly in fully susceptible goat populations. Milder forms of the disease may occur in sheep and partially immune goat populations (Lefèvre and Diallo, 1990). There are considerable differences in the epidemiological pattern of the disease in the different ecological systems and geographical areas. Though outbreaks in West Africa coincide with the wet rainy season, Opasina and Putt (1985) observed outbreaks during the dry season in two different ecological zones. A high morbidity of 90% accompanied with 70% case fatality was reported in goats from Saudi Arabia (Abu Elzein *et al.*, 1990).

Serological data from Nigeria revealed that antibodies occur in all age groups from 4-24 months indicating a constant circulation of the virus (Taylor, 1979b). In Oman the disease persisted on a year round basis maintaining itself in the susceptible yearling population (Taylor *et al.*, 1990). Therefore, an increase in incidence reflects an increase in number of susceptible young goats recruited into the flocks rather than seasonal upsurge in the virus activity, since its upsurge depend on the peak of kidding seasons (Taylor *et al.*, 1990). Moreover, the susceptibility of young animals aged 3 to 18 months was proved to be very high, being more severely affected than adults or unweaned animals (Taylor *et al.*, 1990).

2.1.5 Clinical Signs

Hamdy *et al.*, (1976) reported that PPR has a 3–4 day incubation period during which the virus replicates in the draining lymph nodes of the oro-pharynx before spreading via the blood and lymph to other tissues and organs including the lungs causing a primary viral pneumonia. Bundza *et al.*, (1988) reported an incubation period of PPR to be between two and six days. The predominant form of the disease is the acute form (Roeder *et al.*, 1994). The salient clinical signs start with sudden rise in body temperature to 39.5 - 41°C (Roeder *et al.*, 1994). A clear watery discharge starts to issue from the eyes, nose and mouth, later becoming thick and yellow as a result of secondary bacterial infection (Hamdy *et al.*, 1976). This agrees with Lefèvre (1987) who reported ocular and nasal discharges as the clinical signs that follow a temperature reaction. The serous to mucopurulent nasal discharge may crust over and occlude the nostrils and may lead to sneezing while the ocular discharges result in matting of the eyelids (Obi, 1984). One to two days after fever has set in, the mucous membranes of the mouth and eyes become very reddened (Hamdy *et al.*, 1976, Lefèvre, 1987). Taylor (1984) and Roeder *et al.*, 1994 reported epithelial necrosis following reddening of mucous membranes resulting in small pin-point greyish areas on the gums, dental pad, palate, lips, inner aspects of the cheeks and upper surface of the tongue. This is agreeable to the finding by Roeder and Obi (1999). The areas of epithelial necrosis increase in number and size and join together. The lining of the mouth is changed in appearance. It becomes pale and coated with dying cells and in some cases the normal membrane may be completely obscured by a thick cheesy material (Taylor, 1984). Braide (1981) reported that underneath the dead surface cells are shallow erosions such that rubbing across the gum and palate with a finger may yield a foul-smelling material containing shreds of epithelial tissue.

Affected animals breathe fast, sometimes so fast that they exhibit rocking movements with both the chest and abdominal walls moving as the animal breathes (Roeder *et al.*, 1994). Hamdy *et al.*, (1976) reported this as the commonly observed clinical sign before death. 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. They have obvious signs of pneumonia. Unlike RP, there is a definite but inconstant, respiratory system component (Brown *et al.*, 1991; Bundza *et al.*, 1988).

Whereas Roeder and Obi (1999) reported that diarrhoea will commonly appear about two to three days after the onset of fever, Obi (1984) indicates that in early or mild cases, diarrhoea may not be obvious. The faeces are initially soft and then watery, foul-smelling and may contain blood streaks and pieces of dead gut tissue (Roeder and Obi, 1999). Where diarrhoea is not an obvious presenting sign, insertion of a cotton wool swab into the rectum may reveal evidence of soft faeces which may be stained with blood (Obi, 1984). Due to diarrhoea, affected animals eventually become dehydrated with sunken eyeballs and death often follows within seven to ten days from onset of the clinical reaction (Hamdy *et al.*, 1976). Body temperature usually remains high for about 5-8 days, and then slowly returns to normal prior to recovery or drops below normal before death. Some animals will recover after a protracted convalescence (Roeder and Obi., 1999). The affected animals have lymphocytopenia, elevated PCV (above 60% while normal 35-45%), very high RBCs count while the level of haemoglobin and the white blood cell count is normal (Furley *et al.*, 1987). A common feature in later stages of the sub-acute disease is the formation of small nodular lesions in the skin on the outside of the lips around the muzzle. The exact cause of this is not known (Lefèvre, 1987).

2.1.6 Pathology

2.1.6.1 Pathogenesis

PPR virus, like other morbilliviruses, is lymphotropic and epitheliotropic (Scott, 1981). Consequently, it induces the most severe lesions in organ systems rich in lymphoid and epithelial tissues. The respiratory route is the likely portal of entry (Rowland *et al.*, 1969). After the entry of the virus through the respiratory tract system, it first replicates in the pharyngeal and mandibular lymph nodes as well as tonsil (Scott, 1981). Viremia may develop 2-3 days after infection and 1-2 days before the first clinical sign appears (Scott, 1981a). Subsequently viremia results in dissemination of the virus to spleen, bone marrow and mucosa of the gastro-intestinal tract and the respiratory system (Scott, 1981a).

2.1.6.2 Post mortem findings

The carcass of an affected animal is usually emaciated, the hindquarters soiled with soft/watery faeces and the eyeballs sunken (Scott, 1981a). The eyes and nose contain dried-up discharges. Lips may be swollen; erosions and possibly scabs or nodules in late cases (Scott, 1981a). The nasal cavity is congested (reddened) lined with clear or creamy yellow exudates and erosions (Bundza *et al.*, 1988). There are erosions on the gums, soft and hard palates, tongue and cheeks and into the oesophagus (Roeder and Obi, 1999).

The lung is dark red or purple with areas firm to the touch, mainly in the anterior and cardiac lobes (evidence of pneumonia) (Roeder *et al.*, 1994). Lymph nodes (associated with the lungs and the intestines) are soft and swollen (Roeder *et al.*, 1994). Abomasum is congested with lining haemorrhages (Bundza *et al.*, 1988).

The pathology caused by PPR is dominated by necrotizing and ulcerative lesions in the mouth and the gastro-intestinal tract (Roeder *et al.*, 1994). Erosion in the oral cavity is a constant feature (Roeder *et al.*, 1994). The rumen, reticulum and abomasum rarely exhibit lesions (Roeder *et al.*, 1994). Occasionally, there may be erosions on the pillars of the rumen (Roeder *et al.*, 1994). The omasum is a common site of regularly outlined erosions often with oozing blood (Roeder *et al.*, 1994). 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 (Roeder *et al.*, 1994). The large intestine is usually more severely affected, with congestion around the ileo-cecal valve, at the ceco-colic junction and in the rectum (Roeder *et al.*, 1994). In the posterior part of the colon and the rectum, discontinuous streaks of congestion termed "zebra stripes" form on the crests of the mucosal folds (Roeder *et al.*, 1994).

In the respiratory system, small erosions and petechiae may be visible on the nasal mucosa, turbinates, larynx and trachea (Hamdy *et al.*, 1976). Bronchopneumonia may be present, usually confined to the anteroventral areas, and is characterized by consolidation and atelectasis (Hamdy *et al.*, 1976).

2.1.7 Histopathology

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 (syncytia) can be observed in all affected epithelia as well as in the lymph nodes (Brown *et al.*, 1991). In the spleen, tonsil and lymph nodes, the virus causes necrosis of lymphocytes evidenced by pyknotic nuclei and karyorrhexis (Rowland *et al.*, 1971). Brown *et al.* (1991) using

immunohistochemical methods detected viral antigen in both cytoplasm and nuclei of tracheal, bronchial and bronchio-epithelial cell, type II pneumocytes, syncytial cells and alveolar macrophages. Small intestines are congested with lining haemorrhages and some erosions (Brown *et al.*, 1991). Large intestines (caecum, colon and rectum) have small red haemorrhages along the folds of the lining, joining together as time passes and becoming darker, even green/black in stale carcasses (Brown *et al.*, 1991).

2.1.8 Immunity

The surface glycoproteins hemagglutinin (H) and fusion protein (F) of morbilliviruses are highly immunogenic and confer protective immunity. PPRV is antigenically closely related to rinderpest virus (RPV) and antibodies against PPRV are both cross-neutralizing and cross protective (Taylor, 1979a). A vaccinia virus double recombinant expressing H and F glycoproteins of RPV has been shown to protect goats against PPR (Jones *et al.*, 1993) though the animals developed virus-neutralizing antibodies only against RPV and not against PPRV. Capripox recombinants expressing the H protein or the F protein of RPV or the F protein of PPRV conferred protection against PPR disease in goats, but without production of PPRV-neutralizing antibodies (Romero *et al.*, 1995) or PPRV antibodies detectable by ELISA (Berhe *et al*, 2003). These results suggested that cell-mediated immune responses could play a crucial role in protection. 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 cross-reactive suggesting that the H protein presented by the baculovirus recombinant 'resembles' the native protein present on PPRV (Sinnathamby *et al.*, 2001). Lymphoproliferative

responses were demonstrated in these animals against PPRV-H and RPV-H antigens (Sinnathamby *et al.*, 2001). N-terminal T cell determinant and a C-terminal domain harbouring potential T cell determinant(s) in goats was mapped (Sinnathamby *et al.*, 2001). Though the sub-set of T cells (CD4+ and CD8+ T cells) that responded to the recombinant protein fragments and the synthetic peptide could not be determined, this could potentially be a CD4+ helper T cell epitope, which has been shown to harbour an immunodominant H restricted epitope in mice (Sinnathamby *et al.*, 2001). Identification of B- and T-cell epitopes on the protective antigens of PPRV would open up avenues to design novel epitope based vaccines against PPR.

Recovered sheep and goats develop solid immunity and are unlikely to be infected more than once in their economic life (Taylor, 1984). Lambs or kids receiving colostrum from previously exposed dams or those vaccinated with RP tissue culture vaccine were found to acquire a high level of maternal antibodies that persist for 3-4 months. The maternal antibodies were detectable up to 4 months using virus neutralization test compared to 3 months with competitive ELISA (Libeau *et al.*, 1992).

PPR haemagglutinins (HA) titre of faecal extracts from 40 goats which recovered in Nigeria showed that all the goats shed the PPR virus HA antigen in their faeces for 11 weeks post recovery while nine goats continued shedding the viral antigen up to 12 weeks post recovery (Ezeibe *et al.*, 2008). Correlation existed between shedding of PPR virus HA antigen in faeces and time post recovery with $r = 0.7504$ ($p < 0.0\ 1$) (Ezeibe *et al.*, 2008).

The PPR virus antigen was excreted in faeces of both sick and recovered goats. Since the goats had recovered from clinical disease and some even conceived (Ezeibe and

Wosu, 1997), they were healthy goats. Shedding of PPR virus haemagglutinins in the faeces of these healthy goats suggested therefore that recovered goats may be shedding the PPR virus in their faeces (Ezeibe *et al.*, 2008). The shedding of PPR antigens by recovered goats may explain how the disease is maintained between seasons of low incidence and periods of high incidence. It may also help to explain the phenomenon that introduction of new sheep or goats into healthy PPR recovered flocks often leads to fresh outbreaks of PPR (Obi, 1980). The reduction in mean titre of PPR virus haemagglutinins shed in faeces with time post recovery ($r = 0.7504$) suggested that the animals' immune systems eliminated the infection with time following recovery. This may be due to regeneration of the lymphocytes destroyed by PPR infection (Olayele *et al.*, 1989).

Though PPR disease can be effectively controlled by RPV vaccine, rinderpest eradication programmes have been launched in many countries and if these campaigns are successful, *Office International des Epizooties* (OIE) recommends the cessation of vaccination of all the animals with RPV vaccine so that any residual foci of RPV could be identified. Under these circumstances, small ruminants could only be protected against PPR by using homologous attenuated vaccine. In addition, the successful use of an attenuated PPRV vaccine against RPV has been reported in goats, opening the possibility to use it as a differentiable vaccine for cattle (Couacy-Hymann *et al.*, 1995). Measles vaccine did not protect against PPR, but a degree of cross protection existed between PPR and canine distemper (Gibbs *et al.*, 1979).

PPR virulent virus causes marked immunosuppression evidenced by leukopenia, lymphopenia, and reduced early antibody response to both specific and non-specific antigen (Rajak *et al.*, 2005). These observations are predominant particularly during acute phase of disease (4–10 days post-infection). The immunosuppression induced

by this virus in its host is transient with the consequence of increased susceptibility to opportunistic infections and increased mortality. This immunosuppression effect is a resultant not only of the direct effect of the virus multiplication in lymphoid cells but also of the different strategies morbilliviruses, as many other viruses, have evolved to overcome the host immune defence system (Rajak *et al.*, 2005). On the other hand, the attenuated PPR vaccine virus induces only a transient lymphopenia without significantly affecting the immune response to nonspecific antigen or to itself during this period (Rajak *et al.*, 2005). Further, it could be demonstrated that the virulent PPR virus causes significant immunosuppression as indicated by reduced humoral antibody response to PPR virus, ovalbumin antigen as well as marked leukopenia and lymphopenia during active phase of infection (Rajak *et al.*, 2005).

2.1.9 Diagnosis

Goats and sheep can be infected with RP and PPR as well. Clinical differential diagnosis is not possible as similar disease is produced by both viruses in small ruminants. Therefore, tentative clinical diagnosis may have to be confirmed by laboratory analysis. Diagnosis of PPR may be performed by virus isolation, detection of viral antigens, nucleic acid sequencing and detection of specific antibody in serum.

2.1.9.1 Virus isolation

Samples for virus isolation include heparinized blood, eye and nasal swabs (from live animals), tonsil, mesenteric lymph nodes, spleen, section of colon and lung. For successful isolation, samples must be collected during the hyperthermic phase (Hamdy *et al.*, 1976) and submitted to the testing laboratory in cold ice. The most widely used cell culture systems are primary lamb kidney and ovine skin (Gilbert and

Monnier, 1962; Laurent, 1968, Taylor and Abegune, 1979) and Vero cells (Hamdy *et al.*, 1976).

The sensitivity of virus isolation technique could be increased when the virus is grown in lamb and goats kidney cells (Taylor, 1984). Vero cells are however widely used for their continuity and low liability of contamination. Appearance of cytopathic effects (CPE) may require at least 8-10 days or several blind passages. In Vero cells, the cytopathic effects (CPE) produced by PPRV consist of cell rounding, clumping into typical grape-like clusters, formation of small syncytia and appearance of long fine often anastomosing "spindle cells" (Hamdy *et al.*, 1976). Like other morbilliviruses, PPRV produces eosinophilic intracytoplasmic and intranuclear inclusion bodies both in primary cells (Laurent, 1968) and continuous cell lines (Hamdy *et al.*, 1976).

Once isolated in cell culture, a candidate PPRV may be identified by one of the three procedures:

• animal inoculation: PPR causes clinical disease in goats and sheep but not in cattle (Gibbs *et al.*, 1979);

• reciprocal cross neutralization (differential neutralization): PPRV is neutralized by both PPR and RPV reference sera, but is neutralized at greater titre with the homologous serum (Taylor and Abegunde, 1979; Taylor, 1979a);

• molecular techniques: cDNA probe, (Diallo *et al.*, 1989a ; Pandey *et al.*, 1992), electrophoretic profile in polyacrylamide gel (PAGE) (Diallo *et al.*, 1987) and PCR, (Barret *et al.*, 1993; Forsyth and Barret, 1995; Couacy-Hymann *et al.*, 2002).

2.1.9.2 Antigen detecting methods

2.1.9.2.1 Agar Gel Immunodiffusion Test

Agar gel immunodiffusion test (AGID) is widely used and can detect 42.6% of antemortem specimens and necropsy specimens (Obi, 1984; Abraham and Berhan, 2001). It can be used to test the presence of both antigen and antibodies and can give results within 2-4 hours when RP hyperimmune serum is used while it needs 4-6 hours with PPR hyperimmune serum (Obi, 1984). One of the important advantages of this test is that it is highly specific (92%), simple to conduct and easy to interpret. The test though can not differentiate between PPR and RP (Abraham and Berhan, 2001).

2.1.9.2.2 Counter immunoelectrophoresis

Counter immunoelectrophoresis (CIEP) uses the same principle as the AGID except that the gel is electrically charged to improve the sensitivity of the test (Abraham and Berhan, 2001).

2.1.9.2.3 ELISA for antigen detection

A monoclonal antibody-based sandwich ELISA was found to be highly sensitive in detection of antigen in tissues and secretions of infected goats (Saliki *et al.*, 1994). Another format of antigen ELISA which is more widely used is immunocapture ELISA (Libeau *et al.*, 1994). It utilizes MAb directed against the nucleocapsid protein (Libeau *et al.*, 1994). It can give a reliable result within two hours in precoated plates and from samples maintained at room temperature for a period of seven days with no more than 50% reduction in response (Libeau *et al.*, 1994). The immunocapture ELISA allows a rapid differential diagnosis of PPR or rinderpest viruses, and this is of great importance where the two diseases have a similar geographical distribution and may affect the same animal species. The detecting MAbs 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 (Libeau *et al.*, 1994). The test is very specific and sensitive and can detect $10^{0.6}$ $TCID_{50}$ per well for the PPR virus and $10^{2.2}$ $TCID_{50}$ for the rinderpest virus. This discrepancy between the two viruses in the assay may be due to a difference in the affinity of the detection antibody for the different N proteins. The main advantages of this assay are:

- Rapidity, it can be performed in a precoated plate in less than 2 hours;
- Specificity;
- Robustness, it can be carried out on samples which have not been kept under ideal conditions and where no viable virus is present;
- Simplicity (Libeau *et al.*, 1994).

The immunocapture ELISA is suitable for routine diagnosis of rinderpest and PPR from field samples such as ocular and nasal swabs (Libeau *et al.*, 1994).

2.1.9.2.4 cDNA probes

For the differentiation between PPR and RP, the use of [P^{32}]-labelled cDNA probes derived from the N-protein gene of the two viruses had been described (Diallo *et al.*, 1989a). cDNA probes can differentiate between the two viruses without need for virus isolation. cDNA directed against the matrix protein, fusion protein and phosphoprotein gene were found to cross hybridise to a much greater extent and were not suitable for use as discriminating probes (Diallo *et al.*,1989a). Unfortunately, this hybridization cannot be used widely because it requires fresh specimens and in addition to the short half life of [P^{32}], there are constraints with the handling of isotopes. Therefore, probes using non radioactive labels such as biotin (Pandey *et al.*, 1992) or dioxin (Diallo *et al.*, 1995) were developed. The biotin labeled cDNA was found to be as specific as the one using the radioactive label and more rapid in differentiation between PPR and RP (Pandey *et al.*,

1992). However, it was reported elsewhere, that the expected sensitivity had never been obtained using non-radioactive labels (Diallo *et al.*, 1995).

2.1.9.2.5 Reverse transcription polymerase chain reaction (RT-PCR)

Other than the conventional serological techniques and virus isolation normally used to diagnose morbillivirus infection in samples submitted for laboratory diagnosis, the polymerase chain reaction (PCR) has proved invaluable for analysis of field samples. Saiki and others (1988) first demonstrated this highly efficient means of amplifying *in vitro* a selected sequence flanked by two oligonucleotide primers of opposite orientation. The method consists of repetitive cycles of DNA denaturation, primer annealing and extension by a DNA polymerase effectively doubling the target with each cycle leading, theoretically, to an exponential rise in DNA product. The replacement of the polymerase Klenow fragment by thermostable polymerase derived from *Thermus aquaticus* (Taq) has greatly improved the usefulness of PCR. Using this system, a rate of amplification up to 10^7 to 10^9 times has been reported. The efficiency achieved actually can vary enormously and is dependent on factors such as the number of cycles, the quantity of the starting material, the length of the target DNA, the temperature conditions of annealing and priming, and the polymerase used. When the starting material is DNA, high purification of the nucleic acid is not necessary so the procedure is greatly simplified. These qualities have made the PCR one of the essential techniques in molecular biology today and it is starting to have a wide use in laboratory disease diagnosis. Since the genome of all morbilliviruses consists of a single strand of RNA, it must be first copied into DNA, using reverse transcriptase, in a reaction known as reverse transcription polymerase chain reaction (RT-PCR). RT-PCR has been shown to be useful for the rapid detection of morbillivirus-specific RNA in samples submitted for laboratory diagnosis (Shaila *et al.*, 1996). It has proved especially useful in identifying the new

morbilliviruses found in marine mammals (Barrett *et al.*, 1993b). Both genus-specific and universal morbillivirus primer sets have been produced that can be used to distinguish all known morbilliviruses (Forsyth and Barrett, 1995). Two sets of primers have been made, based on sequences in the 3' end of N genes (messenger sense), which are least conserved regions between the RP and PPRV viruses. They enable specific amplification of 300 base pair (bp) fragments for RPV and PPRV (Couacy-Hymann *et al.*, 2002).

Reverse transcription-polymerase chain reaction tests (RT-PCR) using phosphoprotein (P) universal primer and fusion (F) protein gene specific primer sets to detect and differentiate between PPR and RP were described (Barrett *et al.*, 1993b; Forsyth and Barret, 1995; Couacy-Hymann *et al.*, 2002). Currently a quantitative PCR (qPCR) also termed real time PCR is available (Light Cycler Probes). Quantitative PCR or real time PCR allows detection of the accumulation of PCR products during the amplification process in real time. This enables quantification of the number of templates present in the original sample before the PCR reaction has been started. This has the advantage of monitoring the PCR reaction process in real time, precisely measuring the amount of PCR products per cycle, combining amplification and detection and elimination of post-PCR interferences (Light Cycler Probes).

2.1.9.3 Serology

Many tests have been used for the demonstration of PPR antibodies in serum: virus neutralization test, agar gel diffusion test, immunoelectrophoresis and recently blocking and competitive ELISA.

2.1.9.3.1 Virus neutralisation 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. VNT is the most reliable test for detection of morbillivirus antibodies (Rossitter, 1994). 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).

2.1.9.3.2 cELISA

Competitive and blocking ELISA based on monoclonal antibodies specific for N-protein (Libeau *et al.*, 1995) and H-protein (Anderson and Mckay, 1994; Saliki *et al.*, 1994; Singh *et al.*, 2004) were developed for detection of antibodies in animal sera. These tests either use gradient purified virus or expressed antigens. In the N-protein cELISA, the serum antibodies and the MAb compete on specific epitope on nucleoprotein obtained from recombinant baculovirus. Though no cross reaction in N-protein cELISA was reported, a high level of competition up to 45% was observed among the negative (Libeau *et al.*, 1995). Despite the fact that neutralizing antibodies are not directed against the N-protein, but the H-protein (Diallo *et al.*, 1995), a correlation of 0.94 between VNT and cELISA was observed suggesting that the former was more sensitive (Libeau *et al.*, 1995). The relative sensitivity of this cELISA to VNT was 94.5, while the specificity was 99.4%. 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. The cELISA test can be useful in PPR and RP-free countries for the screening of animals either naturally infected or vaccinated with attenuated PPRV vaccine. It could also be used to differentiate infected animals from those vaccinated with F- and/or H-recombinant marker vaccines in areas where the virus is endemic, although weak cross-reactivity with RPV antibody was found in the competition immunoassay (Kang-Seuk Choi *et al.*, 2005). Competitive ELISA based on response to the H protein showed that animals vaccinated with chimeric vaccines were positive for PPRV-specific inhibition, whereas they remained negative for RPV-specific inhibition. Thus, the mAb tests based on the response to the H (Anderson & McKay, 1994) and N (Libeau *et al.*, 1992, 1995) proteins of RPV and PPRV could be used to distinguish between vaccinated and naturally recovered animals and also vaccinated animals that subsequently become infected (Barrett *et al.*, 2003).

2.1.10 Control and prophylaxis

There is no specific treatment against PPR (Roeder and Obi, 1999). Control of PPR in non infected countries may be achieved using classical measures such as restriction of importation of sheep and goats from affected areas, quarantine, slaughter and proper disposal of carcasses and contact fomites and decontamination of affected premises in case of introduction (Roeder and Obi, 1999). Control of PPR outbreaks can also rely on movement control (quarantine) combined with the use of focused ("ring") vaccination and prophylactic immunization in high-risk populations (Roeder and Obi, 1999).

Until recently, the most practical vaccination against PPR was based on the use of tissue culture adapted rinderpest vaccine (Taylor, 1979a). Vaccination of animals with

RP attenuated virus has been practiced for a long time. The tissue culture rinderpest vaccine (TCRV) at a dose of $10^{2.5}$ $TCID_{50}$ protected goats against PPR for 12 months and the animals were not able to transmit the infection following challenge with PPR virus (Taylor, 1979a), although the antigen was detected in lachrymal swabs from vaccinated animals after challenge with virulent virus (Gibbs *et al.*, 1979).

This vaccine was successfully used to control PPR in some countries in West Africa (Bourdin, 1973). It has been withheld from being used because of its interference with the Pan-African Rinderpest Campaign (PARC), since it is impossible to determine if seropositive small ruminants have been vaccinated or naturally infected with RPV (Schneider-Schaulies *et al.*, 2001). Sera from animals vaccinated with RP vaccine contain substantial level of RP antibodies with little or no cross neutralising antibodies to PPR but after challenge with PPR, neutralizing antibodies to PPR increase sharply (Taylor, 1979a). RP thermostable vaccine was developed for protection of goats against PPR (Stem, 1993). Homologous PPR vaccine attenuated after 63 passages in vero cell (Diallo *et al.*, 1989b) was used and produced a solid immunity for 3 years (Diallo *et al.*, 1995). The PPRV homologous vaccine was found to be safe under field conditions even for pregnant animals and it induced immunity in 98% of the vaccinated animals (Diallo *et al.*, 1995). The PPRV vaccine has been tried for protection of cattle against RP and it was found to be effective (Couacy-Hymann *et al.*, 1995).

The fact that PPRV neutralising antibodies were not detected in RPV vaccinated small ruminants before challenge has encouraged research on the development of a homologous PPR vaccine. In 1989 this goal was finally achieved by the successful attenuation of PPRV strain Nigeria 75/1 through serial passages on Vero cells (Diallo *et al.*, 1989). Several trials have demonstrated the efficacy of this vaccine on more

than 98,000 sheep and goats in the field between 1989 and 1996 (Diallo *et al.*, 2007). During those trials no unwanted side effects such as abortion in pregnant animals were recorded (Diallo *et al.*, 2007). It was also demonstrated that animals vaccinated with this attenuated PPRV were unable to transmit the challenge virus to in-contact animals (Diallo *et al.*, 2007). Anti-PPRV antibodies generated by vaccinated animals last for at least 3 years, the effective economic life of the small stock (Diallo *et al.*, 2007). PPRV Nigeria 75/1 belongs to lineage I (Diallo *et al.*, 1989). During the development process of the attenuated vaccine based on this virus, different PPRV strains were used as challenge viruses and all failed to induce disease in the vaccinated animals, result demonstrating the potential worldwide effective use of this vaccine to control PPR (Diallo *et al.*, 2007). The availability of a homologous vaccine for PPR is fortunate since the use of rinderpest vaccine in all animal species has now been discontinued worldwide. This is to ensure a rinderpest serologically negative ruminant population to allow for effective epidemiosurveillance of rinderpest disease to fulfil the OIE requirements needed to obtain the status of a rinderpest free country or zone. The attenuated homologous PPRV vaccine is now the only vaccine permitted for use in sheep and goats to protect them against PPRV infections (Diallo *et al.*, 2007).

As with all members of the family *Paramyxoviridae*, PPRV is very heat sensitive (Lefèvre and Diallo, 1990). This is a serious drawback to the efficient use of the live attenuated vaccine in areas which have hot climatic environments and at times coupled with poor infrastructure. Under these conditions it is difficult to maintain a cold chain to ensure the preservation of vaccine potency. This drawback was overcome by Worwall *et al.*, (2001) through the development of a thermotolerant vaccine freeze dried in the presence of a cryoprotectant containing trehalose. Under

these production conditions the vaccine is stable at 45 •C for 14 days with minimal loss of potency (Worwall *et al.*, 2001).

2.1.11 Economic Importance of PPR

The PPR epidemics can cause mortality rates of 50–80% in naive sheep and goats populations (Kitching, 1988). Due to the confusion with other diseases, the economic impacts of PPR are probably underestimated, but it is believed that PPR is one of the major constraints of small ruminant farming in the tropics (Taylor, 1984). Based on assumption that goats experience an outbreak every 5 years, Opasina and Putt (1985) estimated an annual sum ranging from 2.47£ per goat at high loss and 0.36 £ per goat at lowest. The loss due to PPR in Nigeria was estimated to be 1.5 million dollars annually (Hamdy *et al.*, 1976). The economic losses due to PPR alone in India have been estimated annually to 1,800 million Indian Rupees (39 millions US$) (Bandyopadhyay, 2002). An economic analysis for assessing benefits of vaccination against PPR in Niger revealed that such a programme was highly beneficial with an anticipated net present value (NPV) return in five years of 24 million USD following an investment of two millions USD. In Kenya, situational analysis by the ministry of Livestock and Fisheries Development (Department of Veterinary Services) put the annual loss, cost of production and control due to PPR to be Ksh. 1.1 Billion (USD 15.7 million) for the 2006 to 2008 outbreak (DVS report, June 2008) (see Table 1) and an estimated 15 million small stock to be at risk (see Table 2).

Table 1: Summary of annual production and control costs associated with PPR outbreak in Kenya (DVS report, June 2008)

Economic variable	Annual parameters
Number of Shoats infected by PPR	3,626,000
Number of Shoats dead from PPR	1,571,293
Number of animals vaccinated	2,500,000
Total cost of vaccination & other control costs (Ksh)	150,000,000
Farm-gate value of total meat loss (Ksh)	716,605,008
Farm-gate value of total milk loss (Ksh)	147,805,560
Total meat and milk loss (Ksh)	864,410,568
Total costs for milk, meat & control costs (Ksh)	**1,014,410,568**

Table 2: Estimated population of sheep and goats at risk (DVS report, June 2008)

	DISTRICT	GOATS	SHEEP	TOTAL
1.	Baringo	1,018,400	400,200	1,418,600
2.	Garissa	240,900	44,700	285,600
3.	Ijara	126,000	154,000	280,000
4.	Isiolo	230,000	260,000	490,000
5.	Kajiado	599,200	679,500	1,278,700
6.	Keiyo	78,800	110,500	189,300
7.	Kitui	538,800	16,800	555,600
8.	Koibateck	71,500	112,100	183,600
9.	Laikipia	226,800	42,900	269,700
10.	Lamu	88,400	20,800	109,200
11.	Machakos	235,000	95,100	330,100
12.	Makueni	297,600	103,600	401,200
13.	Mandera	360,000	239,600	599,600
14.	Marakwet East	83,500	180,000	263,500
15.	Marsabit	414,000	328,000	742,000
16.	Moyale	25,400	6,300	31,700
17.	Mwingi	334,400	44,300	378,700
18.	Narok	590,400	873,500	1,463,900
19.	North Meru	90,100	82,600	172,700
20.	Samburu	889,200	151,100	1,040,300
21.	Taita Taveta	133,500	61,700	195,200
22.	Tana River	368,300	280,000	648,300
23.	Tharaka	224,200	67,200	291,400
24.	Trans Nzoia	22,000	49,700	71,700
25.	Turkana	1,956,200	975,600	2,931,800
26.	Wajir	171,000	335,000	506,000
27.	West Pokot	300,900	346,300	647,200
	TOTAL	**9,714,500**	**6,061,100**	**15,775,600**

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 The Study Areas

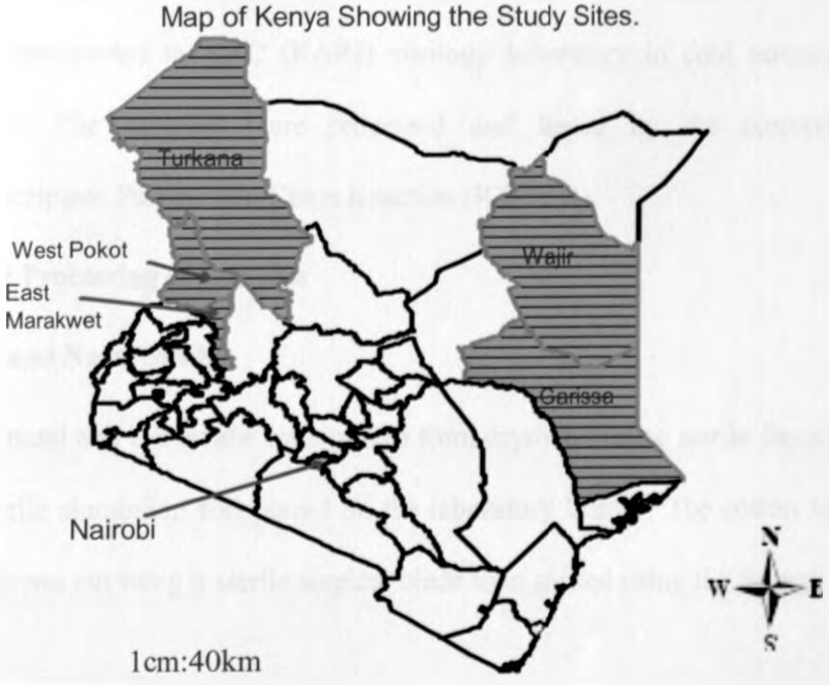
The study was undertaken in pastoral Turkana, West Pokot, Marakwet East, Wajir, and Garrisa districts of Kenya and the Tsavo and Meru national parks of Kenya (Map 3). The study areas are in agroecological zones IV, V and VI.

Table 3shows the figures of goat and sheep populations that were at risk in the districts of study in the year 2008 (DVS report, June 2008).

Table 3: Goat and Sheep populations in the districts of study

District	Goat Population	Sheep Population
Garissa	240,900	44,700
Marakwet East	83,500	180,000
Turkana	1,956,200	975,600
Wajir	171,000	335,000
West Pokot	300,900	346,300

Map 3: Map of Kenya showing districts where the samples for seroprevalence determination were collected and Marakwet East district where the samples for PCR were collected and questionnaires administered



All the districts apart from Garrissa reported cases of PPR during the 2007/2008 outbreak (DVS report, June 2008).

3.2 Confirmation of Occurrence of PPR by Reverse Transcriptase PCR in Marakwet East District

3.2.1 Sampling

In May 2008, a purposive disease search was carried out in in Marakwet East district. Nasal and eye swabs were collected aseptically in cryovials containing 0.5ml of Trizol from 10 PPR suspect goats in Kabetwa location, Tot division of Marakwet East district. The sampling was done purposively from goats that showed any clinical sign(s) that were similar to those known to occur due to PPR. The clinical picture was mainly depression, loss of appetite, a rectal temperature of between 39.9 °C and 41.3°C and diarrhoea in all the goats that were sampled and ocular and nasal discharges in only 5 of these goats. Temperature readings were done early in the morning before environmental temperature rose. There were no mouth lesions in all the goats examined. The goats from which samples were taken aged between six and eleven months. The eye and nasal swab samples taken were packed into sterile cryovials containing trizol and transported to VRC (KARI) virology laboratory in cool boxes containing ice packs. The samples were processed and tested by the conventional reverse transcriptase Polymerase Chain Reaction (RT-PCR).

3.2.2 Processing of Samples

Eye and Nasal Swabs

The nasal and eye swabs were picked from cryovials using sterile forceps and put onto a sterile aluminium foil placed on the laboratory bench. The cotton wool part of the swab was cut using a sterile surgical blade then picked using the forceps and put onto a

sterile syringe. Using the syringe piston, the cotton wool was squeezed and the fluid collected into the original cryovials.

3.2.3 RNA Extraction Using the Trizol Reagent Method

(RT-PCR protocol authored by Institute of Animal Health, Pirbright; 1994).

Each sample of the processed eye and nasal swabs was handled separately to avoid cross-contamination. 500µl of each sample was transferred onto a 2ml microcentrifuge tube. 1ml of the trizol reagent (Appendix 1) was added onto each tube and vortex mixed for 20 seconds. The samples were then left to stand at room temperature for 5 minutes. To each sample, 200µl of chloroform (Appendix 1) was added and vortex mixed for 20 seconds. The samples were left to stand at room temperature for 3 minutes. They were then centrifuged at 10,000 rpm for 15 minutes. The aqueous phase was collected and transferred into a fresh tube. 500µl of absolute ethanol (Appendix 1) was added and then mixed by vortexing for 20 seconds. The RNA was precipitated by storing at -20°C for 2 hours then pelleted by centrifuging at 10,000 rpm for 10 minutes and supernatant slowly discarded. The RNA pellet was washed by re-suspending in 1ml of 75% ethanol and centrifuged at 10,000 rpm for 10 minutes. This later step was repeated one more time. The supernatant was discarded and the RNA pellet air dried for 10-20 minutes at room temperature. The RNA pellet was re-dissolved in 50µl of TE buffer (Appendix 1) and kept at -20°C ready for the next step.

3.2.4 Reverse Transcription

(RT-PCR protocol authored by Institute of Animal Health, Pirbright; 1994).

To prepare for reverse transcription, the following solution and reagents were mixed in a thin walled 0.75ml microcentrifuge tube specifically designed for use in thermocyclers: 5µl RNA solution, 2µl of random hexanucleotide primer and 3µl of RNAase free water (Appendix 1) for every one tube reaction. The amount of one tube

reaction was multiplied by the ten ocular swab samples, ten nasal swab samples and two controls (positive and negative). This mixture was incubated at 70°C for 5 minutes on water bath to disrupt any RNA secondary structure. It was then cooled at room temperature for 10 minutes to allow primer annealing. Centrifuging followed at 2000 rpm for 10 to 20 seconds to ensure the sample is at the bottom of the tube. A reverse transcriptase master mix was prepared by adding the following reagents onto a separate tube: 4µl of 5X reverse transcriptase buffer, 2µl DTT (0.1M), 2µl bovine serum albumin, 1µl reverse transcriptase and 1µl of deoxynucleotidetriphosphates (10mM) (Appendix 1) for one tube reaction. The master mix was multiplied by the number of samples and two controls. To each RNA sample 10µl of the above prepared master mix was added, mixed gently and centrifuged for 10 to 20 seconds to ensure the sample was at the bottom of the tube. The tubes were left to stand at room temperature for 5 minutes then incubated in an incubator at 37°C for 60 minutes. After incubation at 37°C each sample was allowed to cool at room temperature then transferred to -20°C for the next step.

3.2.5 The PCR

(RT-PCR protocol authored by Institute of Animal Health, Pirbright; 1994).

For the PCR, a mixture of the following reagents was prepared: 5µl of 10X PCR buffer, 35µl of RNAase free water, 0.5µl Taq polymerase, 1µl dNTPs (10mM), 1µl of forward primer, 1µl of reverse primer and 1.5µl of Magnesium chloride (Appendix 1) for one tube reaction. The amount of the mixture was multiplied by the number of samples and two controls. To 5µl of the reverse transcriptase product, 45µl of the above master mix was added per sample tube. Gentle mixing was done followed by 10 to 20 seconds of centrifuging to ensure the mixture was at the bottom of the tube. The contents of the tube were overlaid with 50µl of mineral oil (Appendix 1). The tube with the contents

was placed onto a thermocycler machine that was programmed to carry out cycles in the following manner:

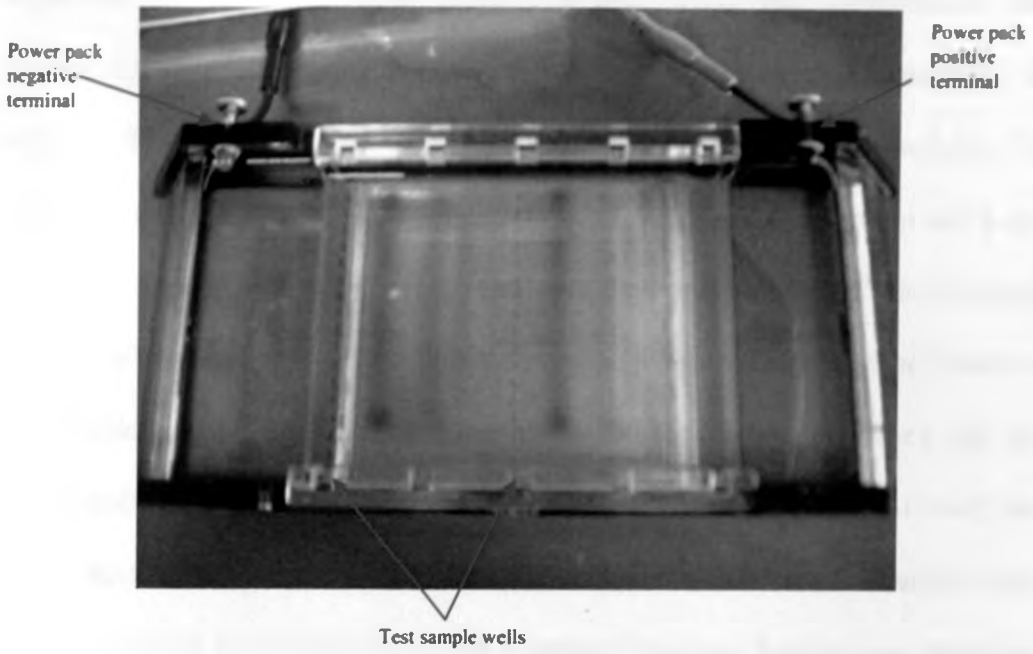
	Temperature	Time (Minutes)
Step 1	94°C	5
Step 2	50°C	1
Step 3	72°C	2
Step 4	94°C	1
Step 5	50°C	31
Step 6	72°C	10
Step 7	4°C	Store for next step

3.2.6 Analysis of RT-PCR Product (Gel Electrophoresis)

(RT-PCR protocol authored by Institute of Animal Health, Pirbright; 1994).

The Agarose gel was prepared as indicated under the preparation of reagents (Appendix 1). Eight µl of the reaction sample was removed from beneath the mineral oil overlay and 2µl of 5X gel loading buffer (Appendix 1) added and mixed. 10µl of each sample mixture was dispensed onto separate wells made on the gel using gel comb. The gel electrophoresis tank was flooded with 1X TBE buffer (Appendix 1) and connected to a power pack. The power pack voltage was set at 75V was run for 60 minutes. A photograph of the running of the gel electrophoresis is reflected in figure 3. After the electrophoresis, the results were read under ultra violet (UV) light machine and recorded onto the UV light machine camera photographic film (RT-PCR protocol authored by Institute of Animal Health, Pirbright; 1994).

Fig 3: Running of the Gel electrophoresis



3.3 Determination of the clinical presentation, management and risk factors associated with PPR Disease in the field

A Cross-sectional study

In the field the questionnaire reflected in appendix 2 was administered to livestock keepers of Tot division in Marakwet East district of Kenya in November 2009, eleven months after vaccination against PPR was carried out in the district. The number of questionnaires was determined using the formula by Goodchild *et al.*, (1994). The questionnaire was first tested by administering to fifteen members of the protozoology laboratory at the Veterinary Research Centre (KARI) Muguga. In the field, questionnaires were administered to 78 livestock keepers in the division in order to describe what was perceived as the presentation of PPR clinically and determine the risk factors that were associated with the disease. This was compared to the documented classical presentation of PPR. The local communities' participation was sought with the aim of coming up with the various livestock constraints within the

study area(s) and then narrowing down to PPR. The unit of questionnaire administration was the division (Tot). The sites where the questionnaire was administered were those lying along the all weather road because of accessibility as guided by the divisional livestock officer. A planning meeting was held with the Tot division livestock officer where the division was divided into ten portions and a site identified per portion as the meeting point with the livestock keepers. The livestock officer sent messages that he would be meeting the livestock keepers at the identified sites. While meeting the livestock farmers, the livestock officer explained who the questionnaire administrator was and the purpose of the questionnaire. At every site visited, farmers were systematically selected for questionnaire administration where the n^{th} individual was determined by the number of livestock keepers who turned up and eight questionnaires were the target for every site.

3.3.1 Questionnaire Sample Size Determination

Using the formula $n = pq/\{e/ Z_\alpha\}^2$ (Goodchild *et al.*, 1994) for binomial distribution where n is sample size, p the assumed prevalence, q is 1-p, e the allowed error and Z_α the normal deviate and assuming a 25% prevalence and error rate of 10% or 90% confidence level, the questionnaire sample size was determined to be:

$$0.25 \times 0.75 / (0.1 \times 1.96)^2 = 72$$

3.3.2 Generation of the Global Positioning System (GPS) Points

At every point within locations where sampling of livestock keepers for questionnaire administration was carried out, the GPS machine (GPS 45 Garmin Personal Navigator™, software 2.20) was switched on for the recording of the altitude, latitudes and longitudes readings of these places. These readings were used for the

generation of the map showing the areas within Tot division of Marakwet East district where questionnaires were administered.

3.4 Determination of Sero-prevalence of PPR among the domestic and wildlife species

Animals that were sampled were both from livestock and wildlife species. For the domestic animals; cattle, camels and goats were sampled while the buffalo, warthog and giraffe were sampled for the wildlife species.

3.4.1. Cattle

In the year 2008 the month of October and November, the Director of Veterinary Services (DVS) collected 15 cattle (*Bos indicus and Bos taurus*) blood samples (for serum) from every sub-location in Kenya for detection of Rinderpest. These samples were submitted to the Virology laboratory at the Veterinary Research Centre (KARI) Muguga in cool boxes containing ice for testing of rinderpest using cELISA kit. The samples were stored at -20°C. These were the samples that were used for the testing of occurrence of PPR antibodies in cattle. The districts from which cattle samples were tested were Turkana and West Pokot. Turkana district served as the portal of PPR entry to Kenya and West Pokot district neighbours Turkana district to the South. The cattle serum samples that were received from Turkana district were 142 whereas those received from West Pokot district were 98. For the two districts, all the serum samples were analysed to give the PPR scenario. The total number of cattle samples tested was 240. The DVS system has digitised the sub-location as the lowest unit of sampling and 15 animals randomly selected as the minimum recommended representative sample size. The sampling unit in this case was identified for the purpose of preparing an OIE dossier for Kenya's Rinderpest free status certification.

3.4.2 Camel

The camel (*Camelus dromedrius*) serum samples were kindly donated by the KAPP (Kenya Agricultural Productivity Programme) project CGS O6/IRS-LVST on the *Development of the camel milk chain in Wajir and Garissa.* The camel blood samples were collected at Garissa and Wajir slaughter houses between the month of September, 2008 and March, 2009. The camel blood samples were processed and stored stored at -20°C at the Department of Veterinary Pathology, Microbiology and Parasitology. Two hundred microlitres from each serum vial were aliquoted using a 200µl micropipette into microcentrifuge tubes with screw cups under a Bunsen burner flame to ensure sterility and packed onto a cool box with ice. The serum samples were transported in the cool box to the Virology laboratory at the Veterinary Research Centre (KARI) Muguga and stored at -20°C. The number of camel serum samples that was tested was 160.

3.4.3 Goats

During questionnaire administration in Marakwet East district in November 2009, blood samples were taken purposively from goats that were between five and nine months of age. The five months lower age limit was because PPR maternal antibodies wane by the fourth month and therefore there was need to exclude this group. The upper age limit of nine months was to ensure the animals from which blood samples were drawn were not born at the time of vaccination in the district which was eleven months before questionnaire administration. A total of 33 blood samples were taken. The blood samples were processed by centrifuging at 1500 rotations per minute to decant blood cells and obtain serum. The serum was packed into microcentrifuge tubes with screw cups and stored at -20°C ready for use.

3.4.4 Wildlife species

Serum samples from wildlife: 98 for buffalo (*Syncerus caffer*) , 93 for warthog (*Phacochoerus africanus*) and 9 for giraffe (*Giraffa camalopardalis*) collected from Meru and Tsavo national parks and Garissa district in July 2008 and August 2008 courtesy of the Director of Kenya Wildlife Services (KWS) and submitted to NVRC Muguga laboratories for cELISA test on Rinderpest were tested and analysed for PPR. The wildlife species from which blood for serum was drawn aged between 1-3 years. The calculated serum sample size was 144. The serum samples that got tested were 200. The buffalo serum samples were taken from the two national parks, warthog samples from Garrisa and Meru national park while the giraffe samples were from the Tsavo national park.

All serum samples were tested using N cELISA for PPR.

3.4.5 The Competitive ELISA (cELISA)

Test Sera

The sera were stored at-20 °C and therefore early before the start of the day's test, the sera were laid onto the operating bench for thawing at room temperature. The test sera were presented at the testing laboratory in cryovials.

The cELISA Test

The processed serum samples were subjected to the cELISA test. The standardised PPR antigen (virus) packed with the kit was reconstituted in coating buffer 1:3000. Fifty microlitres of the antigen was added to every well of microtitre flat-bottomed ELISA plates, sealed and incubated for one hour at 37°C on an orbital shaker. The plates were washed three times by filling and empting all wells with wash buffer and then blot dried on absorbent paper. After washing, all the wells were blocked using 40

microlitres of blocking buffer (contains coating buffer, Tween 20 and bovine negative serum). To the wells (F1, F2, G1, G2) that would carry monoclonal antibody control (Mab) an extra 10 microlitres of the blocking buffer will be added and extra 60 microlitres to the wells (A1, A2) that will carry the conjugate control. Volumes of 10 microlitres of the processed serum samples will be added in vertical duplicates to the test wells on the plate. 10 microlitres of the control sera was added to the respective wells on the control wells of the plate (strong positive to B1, B2, C1, C2, weak positive to D1, D2, E1, E2 and negative serum, to H1, H2 wells). Immediately a working dilution (as per manufacturer's instruction) of the monoclonal antibody was prepared in blocking buffer and 50 microlitres added to all wells of the plate except the conjugate controls (A1, A2). An incubation period of one hour at 37°C on orbital shaker followed, then washing using wash buffer for three times and blot drying. During the second incubation period a working dilution of conjugate in blocking buffer was prepared. 50 microlitres of the conjugate was added to all wells of the microtitre plate and incubated at 37°C for one hour on an orbital shaker. Before the end of this incubation, a working dilution of substrate (H_2O_2) and chromogen was prepared. 50 microlitres of the substrate-chromogen solution was added onto the first column of a separate plate termed blank plate that was used in blanking the ELISA reader machine. Similar amount was added onto all wells of the test plate. The test and blank plates were tapped briefly to ensure mixing, covered using aluminium foil to protect them from excessive light and left to stand for 10 minutes at room temperature for colour development. The reaction was stopped by adding 50 microlitres of 1M sulphuric acid to all wells of test plate and the first column of blanking plate. The plates were read at 492 NM (Nanometres) using the ELISA reader machine. The absorbance in PPR ELISA was converted to percentage of inhibition

(PI) using the formula: PI=100-(absorbance of the test wells/ absorbance of the Mab control wells) x 100.

Interpretation of Results

Monoclonal antibody control

For the monoclonal antibody control (Cm), before the PI values were calculated they were compared with the two intermediate optical density (OD) values (disregarding the highest and lowest values) of the Cm control to the lower and upper control limits were indicated on the ELISA data sheet. Both intermediate OD values must fall within these limits otherwise the plate(s) have to be rejected. The two intermediate values are used for calculation of the media Cm OD value and hence in subsequent PI calculations.

Monoclonal antibody (Cm), Strong positive (C++), Moderate positive (C+), Negative control serum (C-) and Conjugate (Cc) controls

For the monoclonal antibody control (Cm), conjugate control (Cc) and each serum control (C++, C+, C-), the replicate PI values were calculated and recorded on the ELSA data sheet. The replicate PI values for the Cm, C++, and C+ controls were compared to the upper and lower control limits indicated on the fact sheet and the criteria in table 4 used to accept or reject individual microplates.

Table 4: Replicate percentage inhibition values for monoclonal control, strong positive control and the weak positive control for cELISA test

Cm, C++ and C+ Control Data		
Replicate PI values		Status
IN	OUT	
4	0	Accept
3	1	Accept
2	2	Reject
1	3	Reject
0	4	Reject
IN – within UCL and LCL range OUT – outside UCL and LCL range		

Negative (C-) and Conjugate (Cc) controls

The replicate PI values for the C- and Cc controls were compared to the UCL and LCL indicated on the fact sheet and the criteria in table 5 used to accept or reject individual microplates.

Table 5: Replicate percentage inhibition values for the negative control and the conjugate control for cELISA

C- and Cc control data		
Replicate PI values		Status
IN	OUT	
2	0	Accept
1	1	Reject
0	2	Reject
IN – within UCL and LCL range OUT – Outside UCL and LCL range		

Acceptance of individual test sera data

The diagnostic threshold of this assay has been set at 50% inhibition (50 PI) of the monoclonal antibody control (Cm). To accept individual sera, both replicate PI values of a test serum must fall either above or below 50 PI.

Diagnostic interpretation of test sera data

Sera showing PI values greater than 50% were scored positive while those demonstrating PI values less than 50% were considered negative (Biological diagnostic supplies limited and FAO; cELISA for PPR).

3.4.6 Determination of Prevalence

Prevalence of PPR among the sampled population was calculated as the number of those animals that test positive divided by the total number of animals sampled.

3.4.7 Sample size determination

Within the study areas, the sample size (n) was determined using the formula by Martin *et al.*, (1987); $n = Z^2_{\alpha} pq / L^2$; where, n = sample size, z_{α} = normal deviate (1.96) at 5% level of significance, p = estimated prevalence, q = 1-p and L = precision of the estimate usually at 5%.

Taking p set at 0.16 for cattle (Abraham *et al.*, 2005) 0.042 for camel (Ismail *et al.*, 1995) and that for wildlife set at 0.105 (Ogunsanmi *et al.*, 2003) then n became;

Cattle: $1.96^2 \times 0.16 \times 0.84 / 0.05^2 = 206.5$

Camel: $1.96^2 \times 0.042 \times 0.958 / 0.05^2 = 61.8$

Wildlife: $1.96^2 \times 0.105 \times 0.895 / 0.05^2 = 144$

The outcome of the prevalence in the species studied was related to the results of PPR outbreaks reported elsewhere.

3.5 Data Analysis

The data collected was entered into the Microsoft Excel. Descriptive statistics of percentages, frequencies and means were carried out. The Chi Square test and 2X2 crosstabulations were employed. Percentages were carried out on numbers of male and female livestock keepers responding to the questionnaire, occupations of respondents, animal species reared by respondents, number of respondents employing various modes of grazing, the number of respondents reporting animal production challenges to various offices, numbers of small stock affected by PPR per respondent, the number of respondents indicating severity of PPR either in sheep or goats and by the sex of these two species. Percentages were also carried out on the numbers of small stock dieing of PPR and the seroprevalence of PPR in cattle, camel, goats and wildlife species. All the above variables onto which percentages were carried out

were entered in their frequencies. In addition, there are frequencies of the number of livestock keepers employing various modes of communication in disease reporting, the number of respondents reporting various animal production challenges, the important small stock diseases, signs of PPR, numbers of respondents reporting variation in species, sex and post mortem signs of PPR. The means of respondents' age and small stock death rate were recorded.

The chi square test was carried out to find significance difference in number of farmers who used various means of communication to report animal health challenges, the number of animal production challenges reported, the numbers of livestock keepers reporting signs of PPR and their similarity or differences in sheep and goats, numbers of farmers reporting similarity or difference of post mortem signs due to PPR in sheep and goats and differences in the numbers of losses attributed to PPR in the assessment of the impact this disease. There was 2x2 cross tabulation of the individuals who grazed livestock when various livestock production challenges were reported.

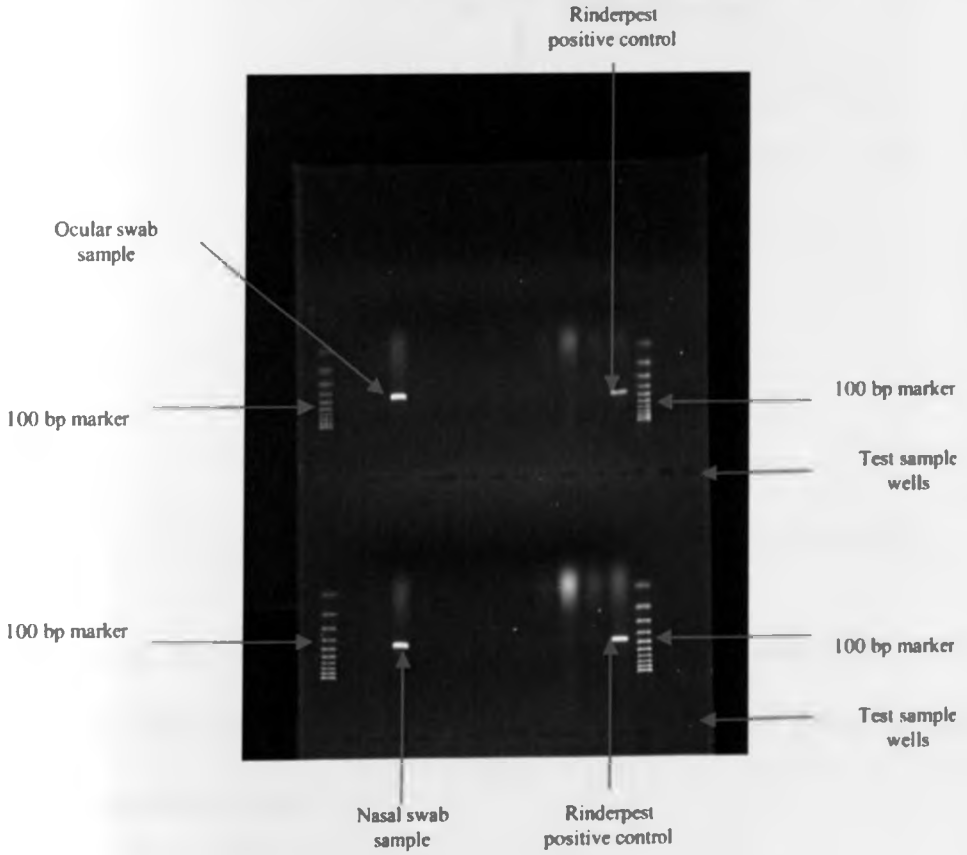
CHAPTER FOUR

4.0 RESULTS

4.1 Confirmation of PPR by Reverse Transcriptase PCR in Marakwet District

In this study, ocular and nasal swab samples collected from one of the 10 goats and labelled as female animal number three tested positive for PPR RNA on RT-PCR. The animal labelled number three from which the samples were taken was aged nine months and showed clinical signs of depression, loss of appetite, a rectal temperature of 41.3°C, ocular secretions, nasal secretions and diarrhoea. All animals sampled had no mouth lesions. The PCR results are shown in figure 4.

Fig 4: Results of RT-PCR product on illumination using UV light

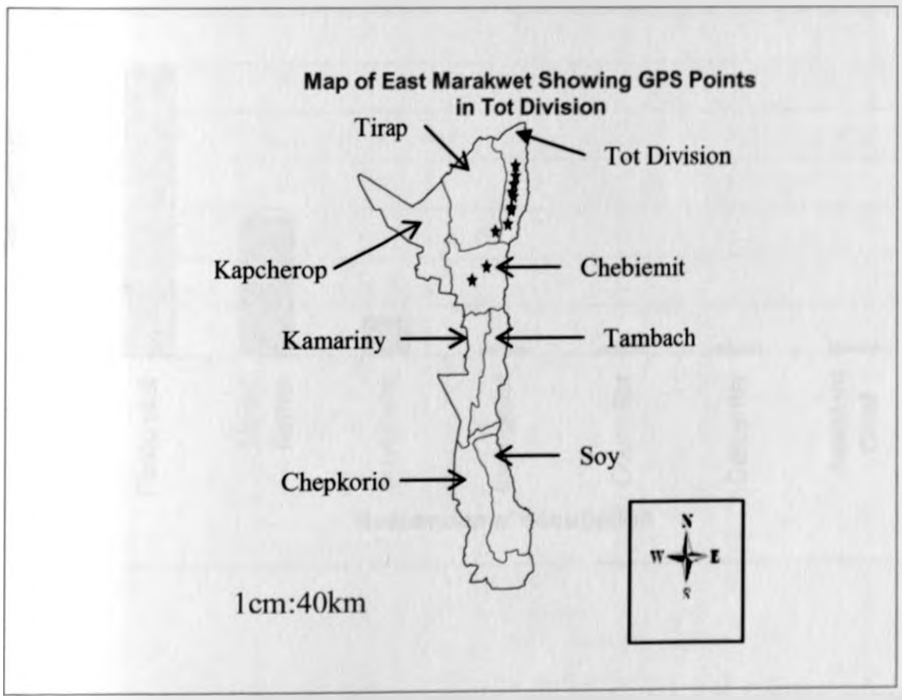


4.2 Clinical presentation, management and risk factors associated with PPR Disease in the field

Study Site

The map 4 below shows the points where the questionnaire was administered in Tot division of Marakwet East district. The points were generated by the Global Positioning System (GPS) machine and are reflected as stars in the map. The points generated are linear as the questionnaire administration was done along the all weather road.

Map 4: Map of Marakwet East district showing GPS points in Tot division



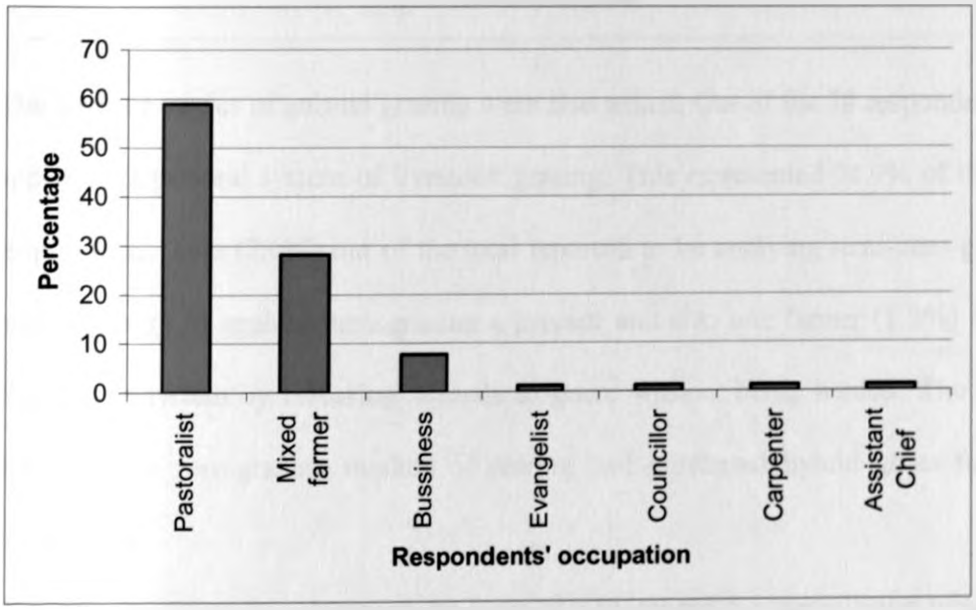
In the division, the youngest questionnaire respondent was 21 years while the oldest was seventy. The information on the age, numbers in terms of gender and marital status is summarized in table 6.

Table 6: Summary of Respondents' Information

Number of Respondents	Number of respondents by gender		Age of respondents				Marital status of respondents	
	Male	Female	Low	High	Mean	S.E.M	Married	Unmarried
78	70	8	21	70	43.62	1.551	75	3

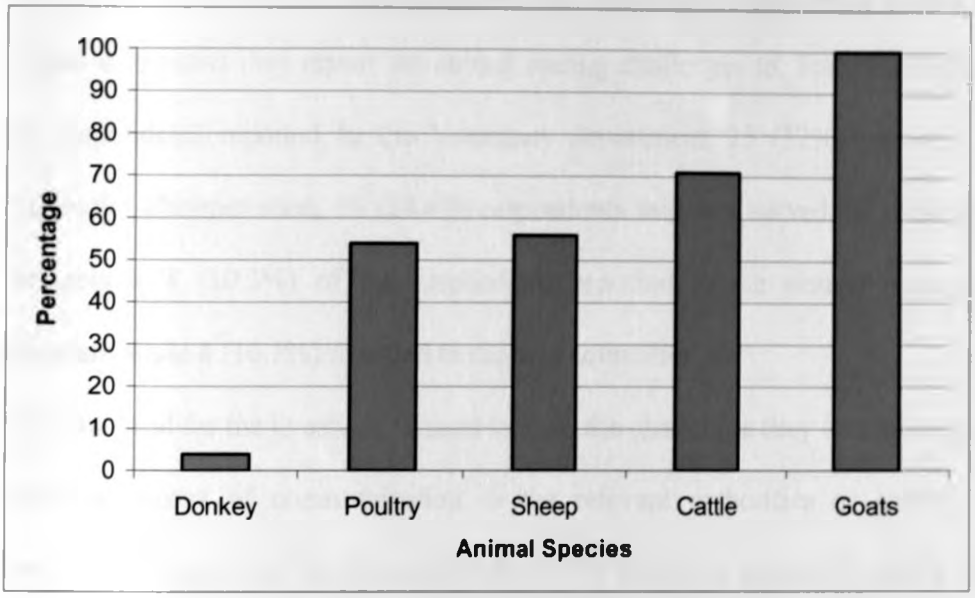
The people interviewed were of varying backgrounds as regards their occupation. The information as regards the number of respondents in each occupation category is presented in figure 5.

Fig 5: Respondents' Occupations and their Percentages



All the respondents under the above named categories were asked the animal species they reared. Fifty five (55) out of the 78 respondents reared cattle and this was a 70.5 percent of the livestock keepers interviewed. All the respondents (100%) reared goats. Sheep were reared by 44 (56%). Poultry were reared by 42 (54%) livestock keepers while the donkey was the least reared livestock species with 3 (3.8%) livestock keepers rearing it. The percentages of farmers rearing each animal species are presented in figure 6.

Fig 6: Livestock species reared and percentages of farmers rearing each species



The various modes of animal grazing were also asked. Out of the 78 respondents, 74 applied the pastoral system of livestock grazing. This represented 94.9% of the total respondents. Two (2.6%) out of the total reported to be applying semi-zero grazing, one farmer (1.3) applied zero grazing a percent and also one farmer (1.3%) applied free range system by releasing animals to graze without being herded. The farmer applying the zero-grazing method of rearing had purchased hybrid goats for milk production.

The questionnaire also addressed the issue of who grazed the animals and when. The categories of the people participating in the grazing were; the man of the house (husband/father), the woman of the house (wife/mother), the children, paid labour or either of these categories. The category where either of the family members or the paid labour was grazing was recorded by 43 livestock keepers, where the husband was grazing was recorded by 21 livestock keepers, grazing by the wife was recorded by 13 respondents, children were reported to graze by 11 respondents and one respondent's animals were grazed through paid labour.

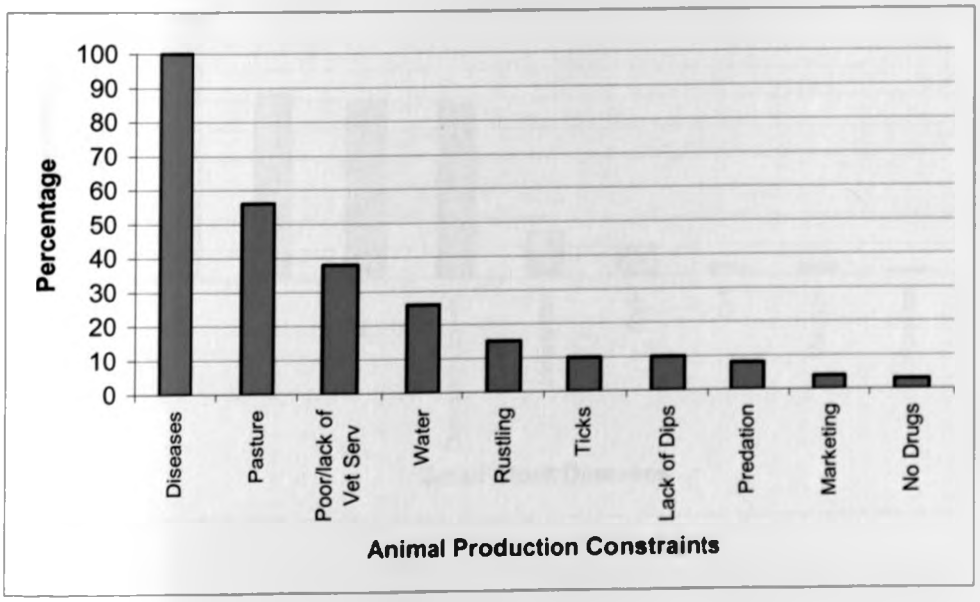
Livestock keepers face challenges in the course of day to day animal husbandry. During the questionnaire administration, the livestock keepers were asked of the offices or persons they report the animal rearing challenges to. Forty eight (62%) of the respondents reported to the Veterinary department, 25 (32%) reported to the Provincial administration, 19 (24.4%) respondents said they solved the challenges by themselves, 8 (10.3%) of the respondents reported to the nearest agrovet shop attendant while 8 (10.3%) reported to the area councillor.

With the need for the livestock farmers to have the challenges they face addressed, the mode or means of communication to the relevant authorities or animal health providers is important. In the questionnaire, the modes or means by which farmers relay their information was sought. Fifty four (69%) of the respondents indicated that they personally visited the relevant persons or offices for service provision. Ten (13%) of the respondents communicated through mobile phones and 1 (1.3%) of the total respondents indicated that he reports to the area animal health provider from the Ministry of livestock when he is on his normal duties. Thirteen (17%) of the respondents did not indicate whether they were reporting any animal health challenge to any authority. When the modes and/or means of communication were subjected to the Chi square test for significance in differences amongst these communication means, the P value was 0.001 ($p<0.05$, df 2), a highly significant difference in the number of farmers reporting through the various modes of communication.

In Tot division of Marakwet East district there were a number of livestock keeping constraints that livestock farmers reported. The constraints reported included disease occurrences, lack of pasture, poor or lack of veterinary services, lack of water, animal rustling or insecurity, ticks, lack of dips, predation, lack of markets or poor marketing and unavailability of drugs. All the livestock keepers (100%) reported disease

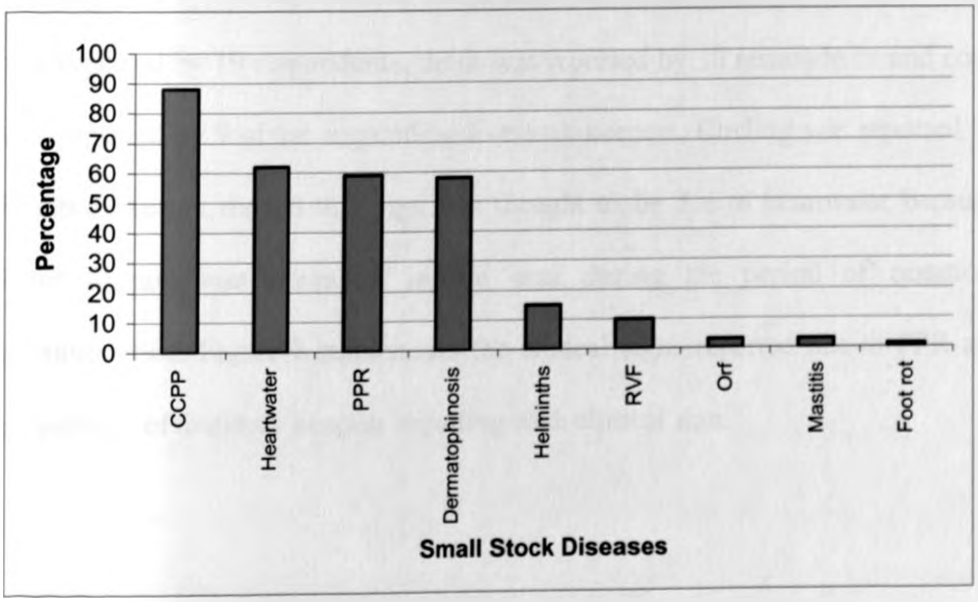
occurrence as their toughest challenge. Lack of pasture came second reported by 44 (56.4%) livestock keepers, 30 (38.5%) livestock keepers said there was poor or lack of veterinary services, lack of water was reported by 20 (26%) respondents, insecurity or animal rustling was reported by12 (15.4%) respondents, tick problem was reported by 8 (10.3%) respondents, lack of dips was also reported by 8 (10.3) livestock keepers, 6 (7.7%) livestock keepers reported predation to be a challenge, marketing of livestock was reported to be a challenge by 3 (3.8%) livestock keepers while unavailability of drugs was last reported by 2 (2.6%) livestock keepers. The percentages of livestock keepers reporting individual animal production constraints are reflected in figure 7. The chi square test of any significance difference in the numbers of livestock keepers reporting the constraints that the livestock farmers faced in Marakwet East district, Tot division, gave a P value of 0.001 ($p<0.05$, df 6) indicating a highly significant difference in the number of respondents reporting every livestock keeping constraint.

Fig 7: Animal Production Constraints and the percentages of livestock keepers reporting them



After the indication that diseases were the strongest challenge to livestock rearing in this division, livestock farmers were requested to list the diseases they considered as very important in sheep and goat keeping and in their order of importance. Contagious caprine pleuropneumonia (CCPP locally named Kipsasoy) topped the list as it was reported by 69 livestock keepers. Heartwater (Kiptalal) was second having been reported by 48 livestock keepers. Peste des petits ruminant (PPR with no local name) was third with 46 livestock keepers reporting it. Dermatophilosis (Kiburum) was reported fourth by 45 respondents, helminthosis (Soret) followed reported by 12 livestock keepers, Rift valley fever (RVF with no local name) had eight livestock keepers reporting it, orf (Kamuren) was reported by 2 livestock keepers , mastitis (Jemurungu) was reported by two livestock keepers and foot rot (Jepkelieny) which was reported by one livestock keeper. Figure 8 shows the percentages of livestock keepers who reported each of the diseases.

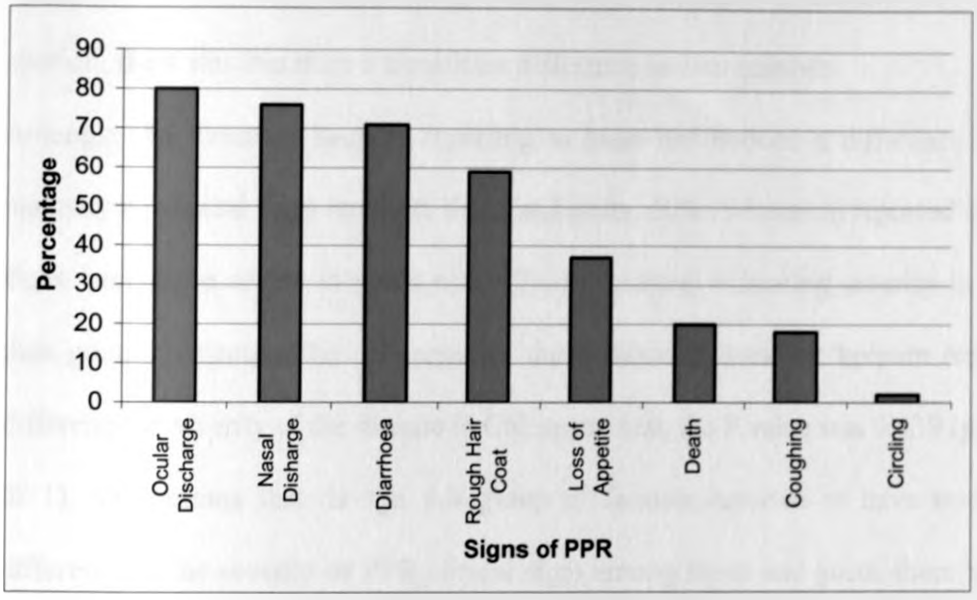
Fig 8: Small stock diseases and percentages of livestock keepers reporting each disease in Marakwet East district



Livestock keepers were asked as to whether the disease (PPR) of sheep and goats that was occurring in the neighbouring Turkana and West Pokot districts in the years 2007 and 2008 did occur in Marakwet East district as an outbreak. Livestock keepers referred to PPR as a mysterious disease. The number of farmers who reported that there was an outbreak was 51 and this was a 65.4% of the total. Twenty farmers reported to have had no outbreaks in their area. In the area where no outbreak was reported vaccination had been carried out before the disease spread to the area hence the small stock had developed immunity by the time the disease was reaching these areas.

Those livestock keepers that were reporting to have an experience of PPR in their sheep and goats reported that it started occurring from the December of 2007. They were asked of the signs they noticed among the sick animals. The signs are presented in the order of the frequency or entries made by the farmers. Lacrimation (ocular discharge) was reported by 41 out of the 51 livestock farmers who had knowledge of the disease. Nasal discharge was second reported by 39 livestock keepers. Diarrhoea was reported by 36 respondents, rough hair coat by 29 respondents, loss of appetite was reported by 19 respondents, death was reported by 10 respondents and coughing was reported by 9 of the responding livestock keepers. Circling was reported by one livestock keeper though this sign was thought to be due to heartwater because this latter disease was prevalent in the area during the period of questionnaire administration. Figure 9 summarises the clinical signs reported due to PPR and the percentage of livestock keepers reporting each clinical sign.

Fig 9: PPR clinical signs and the percentage of livestock keepers reporting them



Though these signs are captured in the order of their frequency, the manner in which they appeared during the disease progression was different. The initial sign reported was the loss of appetite. The affected animals then started shedding tears by the second day of not feeding. The ocular discharge could occur together with nasal secretion or the nasal secretion would appear a day later. By the fourth day, animals developed diarrhoea. The affected animals had a rough hair coat and by the sixth day were coughing. Death would result by the tenth day but some animals would recover with a protracted recovery period. The chi square test for significant difference in the number of farmers reporting individual signs of PPR disease that were reported had a P value of 0.001 ($p<0.05$, df 9), a highly significant difference.

The farmers were also asked as to whether the signs of PPR were similar or occurred with equal intensity amongst sheep and goats. Up to 33 of the farmers were noncommittal. Of the 45 farmers who gave an opinion, 30 of them said that the clinical signs were similar in both goat and sheep populations. This was a 66.7 % of those who had an opinion. Fifteen farmers out of the 45 reported that the signs had

different intensity amongst the small stock. When this report was subjected to a Chi square test, the P value was 0.025 ($p<0.05$, df 1). Amongst the farmers who had an opinion, there was therefore a significant difference in their number.

Amongst the livestock keepers reporting to have had noticed a difference in the intensity of clinical signs amongst sheep and goats, 60% (9 farmers) reported that the signs were more severe in goats with 40% (6 farmers) indicating severity in sheep than goats. Subjecting the difference in the number of livestock keepers reporting difference in severity of the disease to Chi square test, the P value was 0.439 ($p>0.05$. df 1). This means that though this group of farmers reported to have noticed a difference in the severity of PPR clinical signs among sheep and goats, there was no significant difference in the number of the livestock keepers giving out the opinion.

It was also interesting to find out whether it was males or females that were most affected by the disease among the sheep and goat populations. Forty six percent of the respondents (32 farmers) reported that the males were more severely affected by the disease while 53.8% of the respondents (39 farmers) indicated more severity in females. The P value after the chi square test on the numbers of the livestock keepers responding to either males or females undergoing a more severe disease was 0.463 ($p>0.05$, df 1), an indication that there was no significant difference in the number of livestock farmers reporting that either of the small stock sex was more severely affected by the PPR disease.

PPR is known to cause deaths. In the administration of this questionnaire, the question as to whether PPR caused deaths in this region was posed. Out of 51 farmers, 47 (92%) reported that PPR caused deaths while 4 (8%) reported no deaths due to PPR. The mean mortality rate was 32.17.

The questionnaire sought to document whether livestock keepers opened up dead animals and if they could describe the post-mortem lesions they observed. Twenty nine of the livestock keepers agreed to have opened up the carcasses. Livestock keepers reported that they were fearful of opening up animal carcasses as they had experienced rift valley fever (RVF) disease not a so distant past (2006/2007) before PPR surfaced. During the occurrence of the RVF, farmers were advised not to open up dead animals and as such they extended this advice during the occurrence of PPR which was a new disease. However, from the livestock farmers who had opened up the dying or dead animals, the post-mortem lesions were recorded. There were 22 farmers recording reddish or dark lungs. Ten reported to had observed blood lining the surfaces of the small intestines (haemorrhages), six of the respondents said the animals had lost blood (anaemic), two reported that the reticulum was full of ingesta (impacted), two observed watery secretion (oedema) along the mesentry and one respondent reported enlargement of lymphnodes. The P value for any significance difference in the numbers of the observed post-mortem signs was 0.002 ($p<0.05$, df 5). The difference in the number of observations made for each post-mortem sign recorded was significant.

In the questionnaire, it was also sought whether the post-mortem signs recorded were similar in both sheep and goats. Twenty one of the 29 respondents who opened up the carcasses reported that the post-mortem signs were similar in both sheep and goats. Six respondents said the signs were occurring differently while two were noncommittal.

Due to occurrence of PPR, livestock keepers were requested to report what they had observed was the impact of the disease after it occurred. Thirty three livestock keepers observed a direct loss of flock. Loss of income was reported by 31 livestock

keepers, loss of food by 30 livestock keepers, loss of markets was reported by 10 respondents while losses due to quarantine was reported by 5 respondents. As to whether the number of the livestock keepers reporting each category of the losses was significantly different from the others, the Chi square test gave a P value of 0.001 ($p<0.05$, df 4). This meant there was a highly significant difference in the number of livestock keepers who had observed the five categories of the losses reported for assessment of the impact of PPR disease.

During statistical analysis, cross tabulations (Appendix 4) of husbandry practices versus the observations made were also carried out. Cross tabulation of who grazed the animals when the various livestock constrains were noted indicated a high count of 19 disease observations (out of 30) when the husband/father grazed. This was compared with the cross tabulation of who grazed the animals when various small stock diseases occurred. In the later cross tabulation, PPR prevalence (30 counts out of 49) was highest when either of the family members (father/husband, mother/wife and children) grazed the animals. The cross tabulation of who grazes the animals versus the various PPR clinical signs observed showed that PPR signs could be reported by any family member grazing the animals. Diarrhoea was reported more (8 out of 39) where respondents indicated that the wife grazed animals most of the times while lacrimation was reported more (5 out of 39) when children grazed. However, nasal discharge that had the highest counts (26 out of 39) was observed when either of the family members grazed.

4.3 The Sero-prevalence of PPR among the in-contact domestic and wild herbivores

4.3.1 Sero-prevalence in Cattle

All cattle from which samples were drawn were aged between two and three years. Table 7 summarises the number of samples tested per district and based on sex of the animal, the number of sera that tested positive per district and by sex plus the percentages of the positive sera per district and overall. Occurrence of seroconversion in cattle is an indicator that cattle do naturally get infected by the PPR virus without clinical disease. The outcome of a higher seroconversion in Turkana district than West Pokot could be a result of either the virus losing its infectivity as the disease spread down south or a change in husbandry practices such that there was minimal interaction of cattle and small ruminants.

4.3.2 Sero-prevalence in camel

All camels from which samples were tested were above three years in age. The outcome of the camel sera testing by cELISA is presented in table 7. The fact that the positive camel serum samples were for camels from Wajir district concurs with the DVS mapping of the PPR disease situation with Garrisa having experienced no outbreak but was one of the districts that were at high risk.

4.3.3 Sero-prevalence in Goats

Since PPR vaccination campaign was carried out in November and December of 2008 for the affected and the risk districts in Kenya, blood samples were taken from goats that were five to nine months old in Marakwet district during questionnaire administration in November 2009. A total of 33 goats were sampled. The results of the goat sera testing by cELISA are presented in table 7. Detection of PPR antibodies

in goats older than five months and being unvaccinated could be a pointer to the virus still being in circulation in sheep and goat populations in the country.

4.3.4 Sero-prevalence in Wildlife

All the 200 wildlife serum samples tested negative to the PPR virus antibodies when run using the competitive ELISA kit (see table 7).

Table 7: Summary of Sero-prevalence in In-contact herbivores

Animal species	Variable	Category and number of samples	Number positive	Percentage	Overall Percentage
Cattle	District	Turkana N=142	8	5.6%	4.2%
		West Pokot N=98	2	2%	
	Animal sex	Male (125)	3	2.4%	
		Female (115)	7	6.1%	
Camel	Slaughter house	Garissa N=72	0	0.0%	3.13%
		Wajir N=128	5	3.9%	
Goats	Animal sex	Male (12)	2	16.7%	21.2%
		Female (21)	5	23.8%	
Wildlife	Animal species	Buffalo (98)	0	0.0%	0.0%
		Warthog (93)	0	0.0%	
		Giraffe (9)	0	0.0%	

CHAPTER FIVE

5.0. DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 DISCUSSION

In this study, samples for RT-PCR were taken from animals that had a temperature reaction as a prerequisite clinical sign. This was because during the incubation and hyperthermic phase the virus replicates in the draining lymph nodes of the oro-pharynx before spreading via the blood and lymph to other tissues and organs and get excreted in the ocular and nasal secretions (Hamdy *et al.*, 1976). However, the secretion of this virus in secretions is intermittent. Animals that were showing clinical signs like diarrhoea or nasal secretions without a temperature reaction were not sampled as these signs could occur due to other husbandry challenges. Ocular and nasal swab samples taken were preserved in trizol, a chemical that enables preservation of the RNA structure.

In this study, the ocular and nasal swab samples from the female goat labelled number three were positive for PPR RNA on RT-PCR test. This animal had clinical signs of depression, loss of appetite, temperature reaction of 41.3°C, ocular secretions, nasal secretions and diarrhoea. There were no mouth lesions observed in this animal. This probably meant that either the goat was in the early stages of the PPR disease progress or the virus that had infected this animal was not causing PPR clinical disease that occurred with mouth lesions. At the time of sample taking (May 2008), the disease was new in the district (Personal communication, Dr. Kiyeng, DVO Marakwet East, May 2008). Positive results of both the ocular and nasal swabs on RT-PCR were confirmatory as known and specific PPR primers were used. This is because the primers that were used for the positive control and the samples were specific. The PPR virus fusion gene segment that was recognised by the specific primers used for running

this RT-PCR test is highly conserved and is known to be 448 base pairs (bps). Similarly the Rinderpest virus fusion gene segment that was used as the positive control is highly conserved but also known to be 448 base pairs. The marker was 100 base pairs. As a result the marker would run four and half times ahead of the PPR sample segment and the Rinderpest positive control segment. The PPR sample segment and the Rinderpest positive control segment were recorded to be at the same level because they were of equal base pairs. The inclusion of the marker that ran four and half times ahead of the test samples and the positive control of known base pairs that was at the same level with the positive test sample at the point of results reading plus the use of specific primers for the test, is confirmatory that indeed the sample contained PPR RNA at the start of sample processing and RNA extraction (RT-PCR protocol authored by Institute of Animal Health, Pirbright; 1994).

Elsewhere PCR has been used for detection of PPR virus isolates grown in Vero cells (Couacy-Hymann *et al.*, 2002) at CIRAD whereas Forsyth and Barrett (1995) evaluated PCR for the detection and characterisation of rinderpest and peste des petits ruminants viruses for epidemiological studies. However, PCR has not been used as a screening test and therefore disease prevalence including that for PPR has been based on tests that can handle large quantities of samples, are less expensive and that are readily available. Such tests include the antibody detecting ELISAs. The confirmation of the PPR virus in Marakwet East district formed the background of choosing the district as a study area for disease description.

In this study, the questionnaire was administered to 70 male respondents and 8 female respondents. This in part can be explained by the fact that it is the men who drive livestock into the grazing fields as captured by Cheserak (2005) and as such therefore, there was a higher likelihood of meeting more men grazing animals than women. Also

in the Marakwet community, men respond to the issues that have to do with families and the general public than women (Cheserak, 2005). In the pastoral communities, family members get exposed to livestock husbandry at an early age (Cheserak, 2005). In this community, the questionnaires were administered to randomly selected individual so long as he or she was an adult, married or unmarried.

The number of the unmarried respondents was three while the married respondents formed the majority being seventy five. In the Marakwet community, it is the young unmarried family members, mostly young men, who drive livestock far into the grazing fields (Cheserak, 2005). Since this study was carried out purposively by targeting areas not far from the all whether roads along which also homesteads are built, there is the possibility that the people grazing in the fields around these areas were the elderly men while the young men were far into the fields.

The occupations of the people of Tot division were varied. The larger portion of respondents was made of livestock keepers being 46 of the 78 respondents. There were mixed farmers keeping livestock while having mango farms and growing vegetables by the river beds. These were 22 of the total respondents. This agrees with the recorded fact that the Marakwet community largely practices agro-pastoralism (Miyuki Iiyama, 2006). Also there were businessmen, a councillor, a carpenter and an assistant chief. One common factor amongst all these groups of people was that they reared livestock.

The species of animals kept by the respondents were varied. The task of capturing the actual number of each animal species kept by the livestock keepers was quite difficult. The farmers were therefore asked to indicate which animal species they reared. From the results it was clear that the goat was the most reared animal species in the division. The study shows that sheep and goats are the livestock species with the

highest numbers of the livestock reared in this community and therefore they play a significant role in the daily livelihood and the economy of these livestock keepers. Goats provide milk while the two species get slaughtered for red meat that is domestically consumed and also dominate livestock trade markets for cash economy. It is therefore important that the sheep and goat populations alongside the other livestock species are well taken care of for the sustenance of the pastoral livelihoods. It is also known that these two species and especially the goats are good browsers. Given that the pastoral communities of Kenya are in arid and semi-arid lands (ASALs) of the country characterised by low rainfall, the sheep and goat populations therefore improve the coping mechanisms for the pastoral communities. This agrees to the finding by Miyuki Iiyama, (2006) that the Marakwet community does depend on livestock for their livelihoods.

The Marakwet community of Tot division largely practices pastoral grazing. Up to 94.9% percent of the respondents (74) reported to drive their animals to the grazing grounds in the morning until a return in the evening. Two respondents indicated to have been practicing semi-zero grazing with the animals either grazing within the homestead or being fed while in animal sheds. One farmer practiced zero grazing by feeding animals only indoors. The farmers practicing both semi-zero grazing and the zero-grazing had acquired improved breeds of goats. One farmer practiced free range kind of grazing whereby he released his animals to the neighbourhood in the morning and they would return by themselves in the evening. There being other practices of animal grazing other than pastoral grazing agrees with the finding by Mizutani *et al.*, (2005) that currently livestock keepers do practise zero or semi-zero grazing with the introduction of exotic/crossbreed cattle and dairy goats.

The mode of animal grazing is important for disease spread. Of recognition is the pastoral grazing that was practiced by the majority of farmers and the free range grazing. The only difference between these two was that in the pastoral system there was a person looking after the animals in the grazing fields, otherwise the grazing fields were common. This mode of grazing allows for animals from various backgrounds to interact in the grazing fields and as such transmission of disease causing organisms from infected to non-infected animals (Roeder and Obi, 1999).

Pastoral grazing in the ASALs of Kenya carries with it certain characteristics. During the short rainy seasons, there is plenty of water in springs and seasonal rivers for human and animal consumption as well as pasture for animals (Cheserak, 2005). During such times, animals are grazed near homesteads. As the rains subside, availability of pasture dwindles and rivers start to dry up. Wells of water are only found in certain locations of the seasonal river banks or animals are fed at watering points built by the government or non-governmental organisations (Cheserak, 2005). It is during these periods of dry spell that animals from far locations will meet in the fields where little pasture is available and/or at the few watering points. This is so because allocation of pastures for the dry spells and watering points for the dry periods is done by the clan elders (Cheserak, 2005). This meeting at pasture and watering points affords animals of varying backgrounds a contact and as such an enabling environment for disease spread. This is believed to have been a major contributor to the quick spread of PPR among the small stock of the pastoral communities of Kenya and the Eastern African region as a whole.

Though Miyuki Iiyama, (2006) noted that in the pastoral communities it is the men who in most instances get involved in animal husbandry, the study shows that livestock grazing could be carried out by the man as the head of the family, his wife,

children or paid labour, depending on availability of any of these individuals. For the individuals who grazed the animals, an entry where either of the family members or the paid labour grazed the animals was included. On an individual capacity, it is the man who had the highest entry by the respondents being 21 followed by the wife with 13 entries. Children who basically grazed when not in school had 11 entries while paid labour had only one entry. However, instances where either of the family members got involved had the highest recording 43 respondents out of the total of 78 respondents. This means that since any of these groups get involved at some point in the animal husbandry, impacting knowledge on livestock diseases such as PPR should target all of them. This is important when designing extension materials to impact knowledge on such a disease.

Disease reporting is a crucial component of disease surveillance, containment and eventual control. The persons or the offices to whom or which the farmers report their livestock challenges or constraints therefore are important. Though the highest number of respondents indicated to be reporting animal health issues to the Ministry of Livestock development officials (48 out of 78), a relatively high number did report to the Provincial administration (25 out of 78) basically the chief and his assistant. There were 8 respondents reporting to the area councillor with a similar number (8) reporting to the nearest agrovet shop. With this recognition that the farmers have these varied persons or offices to report to, it is imperative therefore that disease surveillance, containment and control is integrated to involve all the players in the livestock sub-sector.

It is because of disease reporting that the questionnaire sought to find out the most applied mode or mechanism through which the farmers reported the animal health or husbandry challenges. Whereas up to 54 of the respondents indicated to personally

visit the relevant persons or offices, 10 of the respondents reported or communicated by mobile phones. With the advancement in the communication industry, continued availability of mobile phones and radio stations airing contents in the Kenyan different dialects, it is envisaged that these advancements will be gainful in disease risk communication and control strategies. This can improve the knowledge base even to the farmers who confirmed not to be reporting their animal health issues to any authority.

The livestock sector constraints in the Tot division of Marakwet East district were quite varied. Out of the 78 questionnaires administered, all respondents reported occurrence of diseases as a challenge. Others were lack of pasture, poor or lack of veterinary services, lack of water, animal rustling/insecurity, ticks, lack of dips, predation, lack of markets and unavailability of veterinary drugs. An important finding of this study is the fact that during pasture and water unavailability, animal rustling and sale at markets, animals from various locations are brought together and this promotes disease dissemination to susceptible animals. This combined with the lack or poor veterinary services including timely vaccinations renders disease containment and control difficult.

Amongst the animal health issues captured, a record of sheep and goat diseases occurring in the area and in their order of importance was made. Contagious caprine preulopneumonia was the highest recorded disease followed by heartwater and PPR came third. Others were dermatophilosis, helminths, rift valley fever, orf, mastitis and foot rot. As regards PPR, 51 farmers agreed that the 2007/2008 PPR disease occurred as an outbreak while 20 farmers did not observe an outbreak. Others were noncommittal. The farmers reporting the outbreak were on the areas bordering West Pokot which borders the larger Turkana district from where the disease had started.

The study further captured the clinical signs that were observed by livestock keepers during the occurrence of PPR. Lacrimation or the shedding of tears was reported by 41 livestock keepers while nasal discharge was second reported by 39 livestock keepers. Diarrhoea was third most reported clinical sign with 36 livestock keepers reporting it. Going by these reports, the first three signs can be said to have almost equal strength in terms of observations. These three signs were followed by a rough hair coat reported by 29 livestock keepers, loss of appetite 19, death reported 10 times and coughing reported by 9 livestock keepers. Arranged as per the appearance of clinical signs during the progressive PPR clinical disease as observed by farmers, loss of appetite was the initial clinical sign. This was followed by issuance of ocular discharge either together with the nasal discharges or nasal discharges occurring hours or a day after ocular discharge. Diarrhoea was reported after occurrence of the nasal discharge followed by a rough hair coat. Coughing preceded death or recovery. From these findings, it can be agreed that though there was no clinically occurring disease at the time of questionnaire administration, the farmers could vividly remember the clinical picture of the PPR disease. The classical documented PPR disease starts with marked depression during which time the affected animal has a temperature reaction (Hamdy *et al.*, 1976) and hence does not feed. One to two days later the animals start shedding tears and producing nasal discharges (Roeder and Obi, 1999). In this study, signs of ocular and nasal discharges were reported with almost equal entries following the loss of appetite. By the third day the animal starts to diarrhoea (Roeder and Obi, 1999). This was the fourth observed clinical sign observed in this study and is agreeable to the finding by Roeder and Obi, (1999). It is due to the reluctance to feed and diarrhoea with the eventual loss of body condition that the livestock keepers observed the rough hair coat. During occurrence of clinical PPR, pneumonia is almost

an accompanying complication (Roeder *et al.*, 1994). It was due to the pneumonia that coughing was reported. Hamdy *et al.*, (1976) reports this as the commonly observed clinical sign before death. Death occurs in seven to ten days or the animal recovers after a protracted period with a solid immunity to the disease (Hamdy *et al.*, 1976; Roeder *et al.*, 1994; Roeder and Obi, 1999). The farmers' description of the PPR clinical disease agrees to a large extent to the documented disease. However, the classical disease occurs with mouth lesions in form of epithelial necrosis (Taylor, 1984 and Roeder *et al.*, 1994) but in this study these were not reported. The PPR disease as it occurred in Kenya therefore had a mild clinical presentation.

As to whether the reported clinical signs were occurring similarly or with equal intensity amongst sheep and goat populations, most of the respondents were of the opinion that there was no difference in the occurrence of clinical PPR in the two species. Only 15 out of 45 livestock keepers recorded to have observed a difference in the clinical disease among sheep and goats. The P value for those recording similarity in clinical picture and those who observed a difference was 0.025. This was a significant difference in terms of the entries made for each category. However, the difference in those recording severity of the disease in goats (9 farmers) versus those who observed severity in sheep (6 farmers) was not significant. The P value for this difference was 0.439 at the 95 confidence level. The results of this study therefore indicate no difference in clinical PPR disease that occurred in Kenya among the sheep and goat flocks. Previous studies have shown that goats are severely affected while sheep generally undergo a mild form of PPR disease (Lefèvre and Diallo, 1990).

In terms of the sex that farmers perceived was more severely affected by the disease, 32 farmers reported that males were more affected whereas 39 observed a more severe disease in females of both sheep and goats. When the significance of this

difference was sought, the P value given after Chi square test was 0.463. This means therefore that there was no significant difference in the severity of PPR clinical disease in either sex of the affected sheep and goat populations. Disease control strategies should therefore target both sexes with equal measure. Studies relating to the severity of PPR in males compared to females in sheep and goat populations were unavailable.

Farmers lost their animals due to PPR with the mean mortality recorded being 32. However, it was observed that farmers were more conversant with clinical signs than post-mortem signs. This was partly because as expected farmers should not open up animal carcasses while on the other hand the knowledge base of what to look for or observe might be limited. Only 29 out of 78 farmers admitted to have opened dead animals. The lesions that were recorded included reddish to dark lungs, occurrence of blood on the surface (haemorrhages) of small intestines, animals seemed to have no blood (anaemia), reticulum was full of ingesta (impacted), mesentry had watery appearance (oedema) and the lymphnodes were enlarged. When compared to the classical documented PPR post mortem signs (Roeder *et al.*, 1994), most of the described signs namely the darkening of lungs that is indicative of pneumonia, haemorrhages in small intestines, oedema along the mesentry and enlargement of the lymph nodes were agreeable. The reported impacted reticulum and anaemia could be as a result of concurrent infections.

With the occurrence of the PPR disease, farmers could well spell out the impact of this disease. There was a direct loss of small stock, reduced income due to unavailability of livestock for sale, loss of animal protein due to reduced availability of small stock for slaughter, loss of markets due to the condition of animals and the losses attributable to the imposition of quarantines. Recognition and eventual control

of PPR will ensure therefore that the players in the small stock chain will not suffer the impact of this disease.

Cross tabulation of who grazed the animals when various animal health challenges were reported was carried out. Diseases as an animal production challenge was reported more in entries where it was the man of the house who grazed the animals most of the times. However, narrowing to the diseases, PPR reporting was highest when either of the family members was grazing the animals. This would therefore mean that all the family members had knowledge on PPR but when it came to disease reporting, this was done in most instances by the man of the house. This was well captured on the cross tabulation of who grazed the animals versus the PPR clinical signs observed. Diarrhoea was highest in entries when the woman of the house grazed the animals, shedding of tears had higher observations when the children grazed while nasal discharge had the highest counts when either of the family members grazed.

The occurrence of PPR antibodies in cattle is a clear indicator that cattle do naturally get infected by the PPR virus without running a clinical disease. The seroprevalence of PPR in cattle was 4.2% with the highest number of those testing positive being serum samples for cattle from the Larger Turkana district. The seroprevalence in the neighbouring West Pokot district was lower either because the disease was put out pretty fast or cattle were herded far away from homesteads that never occasioned the mixing of cattle and infected small stock for them to get infected. The higher PPR seropositivity in female cattle would mean probably females were more susceptible to the virus than males. In addition, female cattle have a longer productive life than their male counterparts hence a longer exposure period. However, the factors contributing to this observation need more studies. Previous studies on seropositivity variation in cattle as relates to their sex were unavailable. From the questionnaire, it can be

concluded that the virus that was spread into West Pokot and hence the one infecting sheep and goats in Marakwet district caused a milder disease especially so because farmers were not reporting any mouth lesions and the post mortem lesions reported were also less severe than the documented classical disease. This mild form of the virus was therefore probably not highly infective to cattle. The seroprevalence reported in this study was lower than that reported in Ethiopia where cattle had an overall PPR seroprevalence of 9% (Abraham *et al.*, 2005). The higher PPR seroprevalence in Ethiopian cattle could be explained in part by the fact that PPR has been endemic in Ethiopia for a longer period of time (Taylor, 1984) compared to Kenya

The study results show that camels had a PPR seroprevalence of 3.13%. This compares with an overall 3% PPR seroprevalence that was reported in camels in Ethiopia (Abraham *et al.*, 2005). All the camel samples that tested positive on the competitive ELISA test were from Wajir district of Kenya. This finding agrees with the Kenya Director of Veterinary services mapping of the districts that experienced PPR outbreaks in the year 2008 (DVS report, June 2008) with Wajir being among the infected districts. Camel samples from Garissa tested negative. The district experienced no outbreaks but was marked as a buffer district.

The confirmation that Kenyan cattle and camel populations got infected with PPR virus during the outbreaks allows for development of sentinel cattle and camel herds in the PPR high risk districts of Kenya to be used for surveillance and indicators of increased virus circulation amongst goat and sheep populations.

The serosurvey for goats in Tot division was aimed at finding out the PPR status of the small stock that were born after vaccinations were carried out in 2007 and 2008. The age was also raised to be above five months to avoid detection of maternal

antibodies. Blood samples were taken in November 2009 from animals that were between five and nine months old. There was a locality specific PPR antibody prevalence of 21.2% in goats. The goat was the most reared small stock species in this area and sheep were not sampled. The 21.2% seroprevalence in the goats compares to the locality specific PPR seroprevalence that was recorded in Ethiopia of 22% (Abraham *et al.*, 2005). The present study showed a higher occurrence of PPR seropositivity in female goats than male goats. This indicates a higher susceptibility for females compared to males but this requires further studies.

The wildlife serum samples from buffalo, giraffe and warthog tested negative on the competitive ELISA test. These serum samples were collected from the Tsavo and Meru national parks of Kenya and Garissa district of Kenya. The negative results agrees to the Kenya DVS mapping of the PPR presence in the country as these regions were mapped to either be PPR free or fell under the buffer zone. However, there is need to target wildlife animal species that occur in areas that were mapped by the DVS to be PPRV infected and where there is small stock and wildlife interaction. Elsewhere, 4 out of 38 sera collected from the grey duiker (*Sylvicapra grimmia*) in Osun state of Nigeria tested positive for PPR virus antibodies after being run by cELISA (Ogunsanmi *et al.*, 2003).

5.2 CONCLUSIONS

1. The small stock disease that was occurring as outbreaks in Marakwet East district in 2008 and against which vaccination was carried out in December 2008 was confirmed to be PPR by RT-PCR.

2. The management and risk factors that were associated with the occurrence of PPR in Marakwet East district are: the pastoral mode of animal grazing, poor mechanism of disease reporting, low uptake of advancement in communication technology including use of mobile phones in disease reporting, extremes of weather changes and patterns, lack of or poor veterinary services, animal rustling and lack of structured markets and marketing.

3. The clinical presentation of PPR as it occurred in sheep and goats and recorded by livestock keepers in Marakwet East district was non-classical.

4. PPR antibodies were confirmed to occur in cattle and camel serum samples tested using cELISA.

5. PPR antibodies were not detected in wildlife species: buffalo, giraffe and warthog after serum sample testing using cELISA.

5.3 RECOMMENATIONS

1. The study showed that sheep and goat alongside the other livestock species are central to sustenance of the pastoral livelihoods. PPR threatens this livelihood by devastating sheep and goat populations. Therefore mechanisms for surveillance, timely diagnosis, disease risk communication and containment need to be put into place.

2. The results of this study further showed all family members in the Marakwet community get involved in the animal husbandry. Therefore impacting knowledge on livestock diseases such as PPR should target all of them. This is important when designing extension materials to impact knowledge on such a disease.

3. The study showed that the farmers report animal health challenges to different persons or offices. It is therefore imperative that disease surveillance, containment and control should be integrated to involve all the players in the livestock sub-sector.

4. The PPR clinical picture described in this study is of a non-classical disease. The implications are that the livestock keepers may report it minimally. It is therefore important to put a continuous surveillance system into place rest there is a build up of a large naive population and hence a severe disease flare up. Vaccinations can also be combined with the more prevalent diseases like CCPP for the farmers to be more motivated to present their animals for vaccinations.

5. The serology results confirmed that Kenyan cattle and camel populations got infected with PPR virus during the outbreaks. This allows for development of sentinel

cattle and camel herds in the PPR high risk districts of Kenya to be used for surveillance and as indicators of increased virus circulation amongst goat and sheep populations.

6. PPR seroprevalence was higher in both female cattle and female goats. The factor(s) contributing to this observation need further studies.

CHAPTER SIX

6.0 References

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CHAPTER SEVEN (7.0)

7.0 Appendices:

7.1 Appendix 1: The RT-PCR Reagents and Equipment

RNA Extraction

Trizol: Contains phenol and acid-guanidine isothiocyanate (Invitrogen)

Chloroform (BDH Analar)

Absolute ethanol (Prolabo)

Tris ethylene diamine tetra-acetic acid (EDTA) buffer (TE buffer) pH 8 (Invitrogen)

Double distilled water (Laboratory made)

Reverse Transcription

Random hexanucleotide primers (Invitrogen)

RNAase/DNAase free water (Gibco)

Reverse transcriptase buffer (5X) (Invitrogen)

0.1M Dithiothreithol (DTT) (Invitrogen)

Bovine serum albumin (acetylated) (Invitrogen)

Reverse transcriptase enzyme (Invitrogen)

Deoxynucleotidetriphosphates (dNTPs) 10 mM (Invitrogen)

Polymerase Chain Reaction (PCR)

PCR buffer (10X) (Invitrogen)

DNAase/RNAase free water (Gibco)

Taq Polymerase enzyme (Invitrogen)

Deoxynucleotidetriphosphates (dNTPs) 10 mM (Invitrogen)

Magnesium chloride (50mM) (Invitrogen)

PPR specific forward and reverse primers F1b, F2d (Sigma Genosys)
-Forward primer sequence (F1b): 5'AGTACAAAAGATTGCTGATCACAGT
-Reverse primer sequence (F2d): 5' GGGTCTCGAAGGCTAGGCCCGAATA

Rinderpest specific forward and reverse primers F3b, F4d (Sigma Genosys)

Mineral oil (Sigma)

Gel Electrophoresis

Agarose (Molecular grade) (Sigma)

TBE buffer (Tris, boric acid and EDTA) pH 7.4

1. Tris base (Sigma)
2. Boric acid (Sigma)
3. EDTA (BDH Analar)

Loading buffer (Sigma)

1. 0.25% Bromophenol blue
2. 0.25% Xylene cyanol
3. 40% (w/v) Sucrose in water

Ethidium bromide (Sigma)

DNA Standard marker (100 base pairs) (Invitrogen)

Preparation of Reagents

1. Random hexanucleotide primers working dilution 1:40. The random hexanucleotide primers were diluted by adding 1µl to 39µl of DNAase/RNAase free water.

2. Bovine serum albumin (acetylated) working dilution 1:50. Bovine serum albumin (acetylated) was diluted by adding 1µl to 49µl of DNAase/RNAase free water.

3. Reverse transcriptase enzyme working dilution 1:200. 1µl of the enzyme was added onto 199µl of DNAase/RNAase free water.

4. PPR specific forward and reverse primers working dilution 1:10. 1µl of each set of the primers is added onto 9µl of DNAase/RNAase free water.

DNA Standard marker working solution. This is a mixture of 20 µl of the DNA marker, 90 µl of DNAase/RNAase free water and 30 µl of loading buffer.

Buffers

TE buffer was commercially availed ready for use.

Reverse transcriptase buffer (5X) was commercially availed ready for use.

PCR buffer (10X) was commercially availed ready for use.

TBE buffer 10X: Tris base 10.8g, EDTA 5.5g and 7.4g were added onto 1 litre of double distilled water.

Loading buffer was commercially availed ready for use.

Agarose gel

1.5% Agarose	0.75g
TBE (1X)	50ml
Ethidium bromide (10mg/ml)	5µl

Agarose was boiled in the 1X TBE buffer until it was completely dissolved. It was then left to cool at room temperature to 60°C and Ethidium bromide was added. The mixture was poured onto a gel tank on the electrophoresis machine that had appropriate gel combs fixed. The comb was to make wells on the gel onto which the PCR product was to be loaded. The gel was allowed to set at room temperature.

The RT-PCR Equipment

Adjustable single channel pipettes

Vortex mixing machines

Microcentrifuges

A -20°C freezer

Safety cabinets

Water bath 70°C

Incubator 37°C

Thermocycler

Microwave

Electric weighing balance

Electrophoresis machine

Ultra violet light machine

7.2 Appendix 2: Questionnaire for farmers.

UNIVERSITY OF NAIROBI, DEPARTMENT OF VETERINARY PATHOLOGY, PARASITOLOGY AND MICROBIOLOGY AND THE KENYA AGRICULTURAL RESEARCH INSTITUTE, VETERINARY RESEARCH CENTRE MUGUGA.

A Questionnaire on the clinical and pathological manifestation of PPR in Kenya.

Questionnaire serial number
Date
Enumerator's name

Respondent's Information:
Name of livestock farmer
Age
Sex
Marital status
Occupation
Location
Division
District
Agroecological zone
Community

What type of animals do you rear? (tick the ones reared)

Cattle
Goats
Sheep
Camels
Donkeys
Poultry
Others (specify)

How do you graze your animals?

1. Pastoral
2. Rotational grazing
3. Semi-zero grazing
4. Zero grazing

Who among the family members graze which animals?

Whenever your animals have a problem(s), whom do you notify?

What are your ways of notifying or communicating animal health problems to the authorities?

1.
2.
3.
4.
5.

What do you consider as five (5) most important problems facing livestock keeping in your area?

1.
2.
3.
4.
5.

Amongst sheep and goats which five (5) diseases do you consider very important? (Arrange the diseases in their order of importance)

1.
2.
3.
4.
5.

There has been a disease of sheep and goats that was occurring as outbreaks, has it occurred in this area?

Yes..... No..... (tick where applicable)

If yes for the above question, what is the name of this disease in the local language?

Amongst sheep and goats, how does this disease start and what is the progress?

Were the signs of this disease in goats similar to those in sheep?

Yes..... No.....

If no for above question, what is/was different?

Which animals were more affected by the disease?
Sheep..... Goats..... (tick where appropriate)

Does this disease cause deaths?
Yes..... No..... (tick where appropriate)

Did you open the carcasses of dead animals?
Yes..... No..... (tick where appropriate)

If yes to above question, how did the opened up carcasses appear?

Was the appearance of opened up sheep and goat carcasses similar?
Yes..... No..... (tick where appropriate)

If no to above question, what difference was noted?

How has the occurrence of this disease affected livestock keepers and the livestock industry?

7.3 Appendix 3: The cELISA Reagents

Control Sera

AntiPPRV strong antibody positive (C++) serum

AntiPPRV weak antibody positive (C+) serum

AntiPPRV antibody negative (C-) serum

All control sera were whole caprine sera supplied with the PPR cELISA test kit, freeze dried and stored at +4°C (Biological diagnostic supplies limited and FAO; cELISA for PPR).

Monoclonal Antibody

Mouse antiPPRV monoclonal antibody was supplied with the PPR cELISA test kit as freeze dried hybridoma cell culture supernatant and stored at +4°C (Biological diagnostic supplies limited and FAO; cELISA for PPR).

Antispecies Conjugate

Horseradish peroxidase (HRP) conjugated in rabbit antimouse immunoglobulin in liquid form was supplied with the cELISA kit and stored at +4°C (Biological diagnostic supplies limited and FAO; cELISA for PPR).

Coating Buffer

Phosphate buffered saline (PBS) in powder form (Sigma Aldrich Chemicals), stored dry at room temperature.

Wash and Blocking Buffer

Phosphate buffered saline (PBS) in powder form (Sigma Aldrich Chemicals), stored dry at room temperature.

Blocking Detergent

Tween 20 in liquid form stored at room temperature (Sigma Aldrich Chemicals).

Substrate

Hydrogen peroxide tablets, stored dry at +4°C (Sigma Aldrich Chemicals).

Chromogen

Ortho-phenylenediamine (OPD) tablets were in a blister pack and stored at +4°C (Sigma Aldrich Chemicals).

Reconstitution diluent

Pyrogen-free, deionized water supplied with the kit was used for the test. All bottles that were used had been autoclaved to ensure sterility and were stored at +4°C (Biological diagnostic supplies limited and FAO; cELISA for PPR).

Stopping Solution

Concentrated sulphuric acid (Sigma Aldrich Chemicals). This was to be diluted.

Reagents and Sample preparation

The PPRV antigen stock

The freeze dried PPR virus supplied in a vial was reconstituted with precisely 1ml of the reconstitution diluent and mixed gently until it was completely dissolved. This was stored at -20 °C.

The AntiPPRV Monoclonal antibody stock

The freeze dried AntiPPRV Monoclonal antibody supplied in a vial was reconstituted with precisely 1ml of the reconstitution diluent and mixed gently until it was completely dissolved. This was stored at -20 °C.

Antispecies Conjugate stock

The rabbit anti-mouse immunoglobulin conjugate stock was subdivided into 500µl aliquots in 1ml cryopreservation vials that were supplied, labelled and stored at +4°C. The content of each vial was used at a time until depleted.

The control Serum stock

The freeze dried control sera in separate vials were reconstituted with precisely 1ml of the reconstitution diluent supplied with the kit, agitated gently to ensure they completely dissolved and stored at -20 °C.

Chromogen stock

One OPD tablet was dissolved in 75ml of laboratory produced deionized water just before the substrate/chromogen incubation step of the cELISA PPR test and stored at +4°C for immediate use. The pH of the chromogen was 5.4. (The recommended pH is between 5 and 6). The remaining chromogen stock after a days work was stored at -20 °C in the dark (covered with a foil paper).

Substrate stock

One hydrogen peroxide tablet was placed in an empty bottle (supplied) and dissolved in 10ml of laboratory produced deionised water. This gave a 3% solution. The solution was stored at +4°C.

Coating Buffer

The contents of one satchet of the powdered phosphate buffered saline was dissolved in a sterile bottle containing 1 litre of laboratory produced deionised water. The resultant solution was a 0.01M phosphate buffered saline (PBS) with a pH of 7.4. The PBS was labelled and stored at +4°C.

Blocking Buffer

Onto the 0.01M PBS at the pH of 7.4, 0.1% (v/v) Tween 20 and 0.3% (v/v) normal serum (the negative control) was added. Fresh blocking buffer was reconstituted every day the test was run and therefore the amounts of the three components making up the blocking buffer depended on the anticipated number of test plates to be run. The blocking buffer had a pH of 7.4.

Wash Buffer

One satchet of the PBS powder was emptied onto a sterile bottle containing 1 litre of laboratory produced deionised water. This was well dissolved on a magnet orbital shaker and a further 4 litres of the deionised water added to make a 0.02M phosphate buffered saline that had a pH of 7.4. The wash buffer was stored at room temperature and was transferred onto a wash fluid container at the point of washing the test plates.

The Stopping solution

27.5ml of concentrated sulphuric acid was slowly added onto 472.5ml of laboratory prepared deionised water to make a 1M sulphuric acid. This was labelled and stored at room temperature.

7.4 Appendix 4: Crosstabulations

Who grazes the animals * Problems

Crosstab			Problems				Total
			Poor/lack Vet Serv	No Drugs	Disease	Ticks	
Who grazes the animals	Wife	Count	4	0	0	0	4
		% within Who grazes the animals	100.00%	0.00%	0.00%	0.00%	100.00%
		% within Problems	100.00%	0.00%	0.00%	0.00%	13.30%
		% of Total	13.30%	0.00%	0.00%	0.00%	13.30%
	Children	Count	0	1	0	0	1
		% within Who grazes the animals	0.00%	100.00%	0.00%	0.00%	100.00%
		% within Problems	0.00%	100.00%	0.00%	0.00%	3.30%
		% of Total	0.00%	3.30%	0.00%	0.00%	3.30%
	Husband	Count	0	0	19	0	19
		% within Who grazes the animals	0.00%	0.00%	100.00%	0.00%	100.00%
		% within Problems	0.00%	0.00%	100.00%	0.00%	63.30%
		% of Total	0.00%	0.00%	63.30%	0.00%	63.30%
	All	Count	0	0	0	6	6
		% within Who grazes the animals	0.00%	0.00%	0.00%	100.00%	100.00%
		% within Problems	0.00%	0.00%	0.00%	100.00%	20.00%
		% of Total	0.00%	0.00%	0.00%	20.00%	20.00%
Total		Count	4	1	19	6	30
		% within Who grazes the animals	13.30%	3.30%	63.30%	20.00%	100.00%
		% within Problems	100.00%	100.00%	100.00%	100.00%	100.00%
		% of Total	13.30%	3.30%	63.30%	20.00%	100.00%