ADDIS ABABA UNIVERSITY
FACULTY OF VETERINARY MEDICINE

ASSESSMENT OF THE EPIDEMIOLOGY OF PESTE DES PETITS RUMINANTS (PPR) AND POST VACCINAL SEROCONVERSION RATE OF PPR-VIRUS IN SMALL RUMINANTS OF AWASH FENTALE WOREDA, AFAR NATIONAL REGIONAL STATE, ETHIOPIA

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A thesis submitted to the school of Graduate Studies of Addis Ababa University, in the partial fulfillment of the requirements for the Degree of Master of Veterinary Science in Tropical Veterinary Epidemiology

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LIST OF ABBREVIATIONS

AAPBMDA	Animal, Animal Products and By-products Market Development Authority
AGID	Agar gel immunodiffusion
AHS	African horse sickness
ANRS	Afar National Regional State
AU	African Union
B-ELISA	Blocking enzyme-linked immunosorbent assay
CBPP	Contagious bovine pleuropneumonia
CCPP	Contagious caprine pleuropneumonia
cDNA	Complementary deoxyribonucleic acid
CDV	Canine distemper virus
C-ELISA	Competitive enzyme-linked immunosorbent assay
CIEP	Counter immunoelectrophoresis
CMV	Cetacean morbillivirus
CPE	Cytopathic effects
DNA	Deoxyribonucleic acid
EARO	Ethiopian Agricultural Research Organization
ELISA	Enzyme-linked immunosorbent assay
FAO	Food and Agricultural Organization of the United Nations
FMD	Foot and mouth disease
GDP	Gross Domestic Product
GIT	Gastrointestinal tract
GPS	Global Positioning System
GREP	Global Rinderpest Eradication Project
HS	Haemorrhagic septicemia
MAbs	Monoclonal antibodies
masl	Meters above sea level
mbsl	Meters below sea level
MEDaC	Ministry of Economic Development and Cooperation
MoARD	Ministry of Agriculture and Rural Development
N	Nucleaocapsid protein

NVI	National Veterinary Institute
PANVAC	Pan African Veterinary Vaccine Center
PARC	Pan African Rinderpest Campaign
PCR	Polymerase chain reaction
PDV	Phocine distemper virus
PI	Percentage colour inhibition
PMV	Porpoise morbilli virus
PPR	Peste des petits ruminants
PRRV	Peste des petits ruminants virus
RPV	Rinderpest virus
$TCID_{50}$	Tissue culture infectious dose fifty
TCRV	Tissue culture rinderpest vaccine
VNT	Virus neutralization test

ABSTRACT

A study was conducted in small ruminants in Awash Fentale *Woreda* Afar National Regional State, from September 2006 to June 2007, to determine seroprevalence of peste des petits ruminants, to investigate outbreak of the disease and assess postvaccinal seroconversion to the virus. From a total of 1,239 small ruminants, 879 goats and 360 sheep sampled 21(1.70%) animals were found to be positive using competitive ELISA. The seroprevalence in goats was 2.28% and in sheep 0.28%. The difference in the seroprevalence rate of PPR between goats and sheep was statistically significant ($p<0.05$). The overall and the village level seroprevalence were very small but since animals included in this study had no history of PPR vaccination, the seroprevalence result indicated the circulation of the virus in the population. In addition to its importance in detecting the virus circulation, this seroprevalence study indicated indirectly the poor herd immunity level. A number of small ruminants in the area, which were not vaccinated, showed characteristic symptoms of PPR and serological result from these groups was higher than the previous seroprevalence report. From 238 small ruminants (197 goats and 41 sheep) sampled from suspected outbreak, 84 (42.6%) goats and only 3 (7.3%) sheep were found to be seropositive. The seroprevalence between sheep and goats was statistically different ($p<0.05$). Animal health professionals and CAHWs vaccinated small ruminants in the study area against PPR. Among the vaccinated small ruminants a total of 1,096 were resampled from the same villages to see sroconversion. The seroconversion estimate of animals vaccinated was 61.1% but there was difference between sheep and goats. The rate of postvaccinal seroconversion in goats (68%) was higher and statistically different ($p<0.05$) compared with sheep (47.6%). But no difference in the post vaccinal seroconversion rate was seen between animals vaccinated by professional animal health workers and CAHWs ($p>0.05$).

Key words: goats, sheep, PPRV, seroprevalence, seroconversion, outbreak investigation, postvaccination

1. INTRODUCTION

The growing demand for meat and milk to developing world, changing functions of livestock and changing consumers perspectives are the major driving forces in the global livestock sector. The movement of people from rural areas to urban centers will increase the demand for food of animal origin. By the year 2020 the global population is projected to consume about 120 million tons of meat above the current consumption (Ibrahim and Olaloku, 2000).

Livestock perform multiple functions in the Ethiopian economy by providing food, input for crop production and soil fertility management, raw material for industry, cash income as well as in promoting saving, fuel, social functions, and employment. Various estimates show that the livestock sub-sector contributes 12–16% of the total and 30–35% of agricultural GDP, respectively (MEDaC, 1998; AAPBMDA, 1999). The sector's contribution to national output is underestimated because traction power and manure for fertilizer are not valued. Contributing 12–15% of total export earnings, the sub-sector is the second major source of foreign currency through export of live animals, hides and skins (MEDaC, 1998). The sector also employs about one-third of the country's rural population (EARO, 2000).

According to the data from Livestock Marketing Authority (2002), the livestock population of Ethiopia is 35.1, 24 and 18 million heads of cattle, sheep and goats, respectively. The sheep and goats supply more than 30% of the domestic meat consumption and generate cash and hard currency from export of meat, live animals and skin (Ibrahim and Olaloku, 2000).

Animal diseases have a significant impact on household food security. Some of these diseases wipe out the entire herds and threaten the livelihoods of the farmers. They contribute to a general decline in the productivity and have been determinant factors for poverty in rural communities. The major causes of economic loss and poor productivity in livestock is the prevalence of a widerange of diseases such as contagious bovine pleuropneumonia (CBPP), foot and mouth disease (FMD), contagious caprine pleuropneumonia (CCPP), peste des petits ruminants (PPR), African horse sickness (AHS) and haemorrhagic septicemia (HS) and parasites. The direct loss due to mortality is estimated at 8-10% for cattle, 14-16% for sheep flock and 11-13% for goat per annum. Indirect economic losses occur through slow growth, low fertility and decreased work

output. Three major impacts of diseases are: socio-economic 85% (primarily production losses and control costs incurred by the poor), zoonotic (for those diseases transmissible from animals to humans) and national average of 15% a combination of marketing impacts on the poor with public-sector expenditures on disease control. Therefore, improving health and productivity would provide an important opportunity for increasing food security and could be a promising and cost effective way of stimulating the national economy (Abraham, 2005).

Peste des petits ruminants (PPR) is an acute, highly contagious and infectious viral disease of sheep, goats and wild small ruminants characterized by fever, anorexia, necrotic stomatitis, diarrhea, ocular and nasal discharge and respiratory distress. It was first described in Cote d'Ivoire in West Africa in 1942 (Gargadennec and Lalanne, 1942). Investigators soon confirmed the existence of the disease in Nigeria, Senegal and Ghana. For many years it was thought that it was restricted to that part of African continent until a disease of goats in Sudan, which was originally diagnosed as rinderpest in 1972, was confirmed to be PPR (EMPRES, 1999; Radostits *et al*., 2002).

Peste des petits ruminants has received a growing attention because of its widespread, 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).

In Ethiopia, PPR was suspected on clinical grounds to be present in goat herds in Afar region in 1977 (Pegram and Tereke, 1981). However, the presence of the virus was only confirmed in 1991 with cDNA probe in lymph nodes and spleen specimens collected from an outbreak in a holding land near Addis Ababa. The disease in this outbreak caused more than 60% mortality and was characterized by ocular and nasal discharges, mouth lesions, pneumonia, gastroenteritis and diarrhea (Abraham *et al*., 1991, Roeder *et al*., 1994). In a serological survey carried out in 1997 at Debre Zeit abattoirs high prevalence of antibodies was reported (Yayehrade, 1997). The national serosurveillance carried out in 1999 has indicated seroprevalence of 5.7% in goats and sheep from different regions of the country (MoARD, 2004). The etiologic agent of PPR was isolated from tissue samples collected from suspected outbreaks of PPR in Arsi Zone of Ethiopia (Gelagay *et al.,* 2003). Abraham *et al.* (2005) have reported the overall seroprevalence of PPR as

3% in camels, 9% in cattle, 9% in goats and 13% in sheep in different parts of Ethiopia including Afar Region.

The existence of PPR virus in Ethiopia has remarkable economic impact on livestock production and export. Hence, in order to complement the effort being done in promoting livestock health in Ethiopia and export of their products, systematic control strategies need for appropriate intervention.

There are only few studies conducted on the seroprevalece of PPRV in Ethiopia in general and even lesser in Afar region in particular. No study with the aim of detecting post-viccination seroconversion rate of peste des petits ruminants virus (PPRV) vaccine yet conducted in Ethiopia. Measuring the herd immunity level indirectly indicates the proportion of vulnerable population to infection. Therefore, the current study was conducted to enhance the knowledge in the epidemiology of PPR in the *Woreda* and assess effectiveness of vaccination in terms of seroconversion rate.

Therefore the objectives of this study are:

- To determine the seroprevalence of PPR in goats and sheep of Awash Fentale *Woreda*, Afar National Regional State
- To assess risk factors associated with the epidemiology of the disease
- Assess post vaccinal seroconversion rate of homologous PPRV vaccine and factors affecting seroconversion rate in Awash Fetale *Woreda.*

2. LITERATURE REVIEW

2.1. History

Peste des petits ruminants (PPR) was first described in Côte d'Ivoire in West Africa (Gargadennec and Lalanne, 1942). Investigators soon confirmed the existence of the disease in Nigeria, Senegal and Ghana. For many years it was thought that it was restricted to that part of African continent until a disease of goats in Sudan, which was originally diagnosed as rinderpest in 1972, was confirmed to be PPR (EMPRES, 1999; Radostits *et al*., 2002). It used to be named as 'kata', psuedo-rinderpest, pneumoenteritis complex and stomatitis-pneumenteritis complex. Goats are usually more severely affected than sheep (Saliki, 1998).

PPR was suspected on clinical grounds to be present in goats herd in Afar region of eastern Ethiopia in 1977 (Pegram and Tereke, 1981). Moreover, serological and clinical evidences were reported by Taylor (1984). However, the presence of the virus was only confirmed in 1991 with cDNA probe in lymph nodes and spleen specimens collected from an outbreak in a holding land near Addis Ababa. The disease probably was introduced into Ethiopia in 1989 in the Southern Omo river valley from where it moved eastward to Borena region and then northwards along the Rift valley to Awash (Abraham *et al*., 1991; Roeder *et al*., 1994). The disease became endemic in goats (Abraham and Berhan, 2001).

2.2. Aetiology

For many years, PPR virus was considered as a variant of RPV, specifically adapted for goats and sheep that had lost its virulence for cattle. It is now known that the two viruses are distinct though closely related antigenically (Saliki**,** 1998; Radostits *et al*., 2002). PPRV is in the *Morbillivirus* genus of the *Paramyxoviridae* family. Morbillivirus is linear, non-segmented, single stranded, negative sense RNA virus with genomes approximately 15–16 kb in size and 200 nm diameter (Murphy *et al.,* 1999). Virus members of this group have six structural proteins: the nucleocapsid protein (N), which encapsulates the virus genomic RNA, the phosphoprotein (P), which associates with the polymerase (L for large protein), the matrix (M) protein, the fusion (F) and the haemagglutinin (H). The *Morbillivirus* genus also includes other viruses such as measles virus

(MV), rinderpest virus (RPV), canine distemper virus (CDV), phocine morbillivirus (PMV), porpoise distemper virus (PDV), dolphin morbillivirus (DMV) (Barrett, 2001) and equine influenza recently described in Australia (Radostits *et al*., 2002). Of the four known lineages of PPR virus, lineage 1 and 2 viruses have been found exclusively in West Africa. Virus from an outbreak in Burkina Faso in 1999 fell into the lineage 1 group. Viruses of lineage 3 have been found in East Africa, Arabian Peninsula and in southern India. Lineage 4 of PPR virus includes the isolates from Israel/1994, Iran/1994, Nepal/1995, Bangeldesch/1993 and India (Shaila *et al*., 1996). Lineage 4, hence, is confined to Asia. Recently, it was also reported in Turkey (Dhar *et al.*, 2002; Ozkul *et al*., 2002).

2.3. Nature of the virus and its resistance

As other members of *Paramyxoviridae* particularly *Morbilllivirus* genus, PPR virus contains non segmented, single stranded RNA genome of negative polarity, with a lipid envelope containing surface glycoprotein, which mediate entry and exit of the virus from its host cell. This virus replicates in intracytoplasmic position of the host cell (Murphy *et al*., 1999). Because of its envelope, PPR virus is sensitive to chemicals such as alcohol, ether, detergents; and disinfectants including phenol and sodium hydroxide 2% for 24 hours. However, the virus survives at a temperature of 60^{o}C for 60 minutes and it is stable between pH 4 and 10. The virus also survives for a long period in chilled and frozen tissues (OIE, 2002).

2.4. Epidemiology

2.4.1. Geographical distribution

Peste des petits ruminants virus (PPRV), originally endemic in west Africa has spread across most African countries south of the Sahara and north of the equator, the Arabian Peninsula, and in most of the Middle Eastern countries such as Jordan, Israel, and Lebanon, the Near East, and South Asia such as Bangladesh as far as Turkey (Shaila *et al*., 1996; Saliki, 1998; Ozkul *et al*., 2002).

Countries that have imported small ruminants from these areas are advised to investigate thoroughly any disease syndrome characterized by disturbed breathing, discharges from the eyes, nose and mouth, sores in the mouth and diarrhea in order to rule out PPR (EMPRES, 1999).

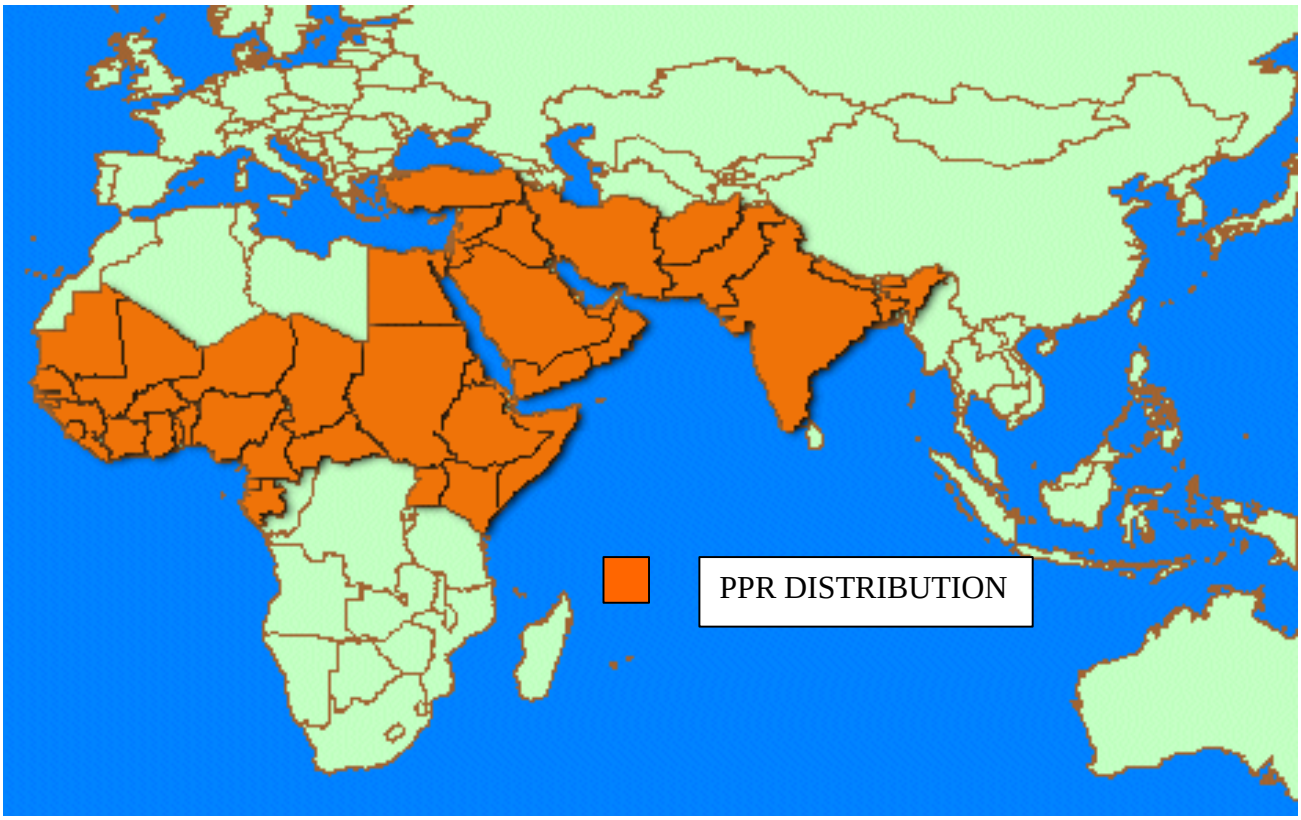

Figure 1. Probable global distribution of PPR virus infection (EMPRES, 1999).

2.4.2. Host range

The natural disease affects mainly goats and sheep, but it is usually more severe in goats where it causes heavy losses and is only occasionally severe in sheep. It is generally admitted that cattle can only be infected subclinically. Rossiter and Wardley (1985) reported that virulent PPRV strains can replicate in bovine lymphocytes, although less efficiently than in sheep and goat cells. Considering the immunosuppressive effect of PPRV as all other morbilliviruses, it may therefore be possible, depending on the age, and physical state of the host animal, that PPRV can

occasionally overcome the innate resistance of large ruminants and lead to the development of clinical signs similar to rinderpest. This may explain the clinical signs that had occurred in buffalo and camels following PPRV infection. This ability of PPRV to infect large ruminants could pose a serious threat to cattle population in PPR endemic areas which, with the success of the global rinderpest eradication programme, are no longer vaccinated against rinderpest and so do not possess cross-protective immunity against this virus (Diallo *et al*., 2007). PPRV was isolated from an outbreak of rinderpest-like disease in buffaloes in India in 1995 (Govindarajan *et al.,* 1997). PPRV was also suspected to be involved in the epizootic disease that affected one-humped camels in Ethiopia in 1995–1996 (Roger *et al*., 2000). PPRV antigen and PPRV nucleic acid were detected in some pathological samples collected during that outbreak, but no live virus was isolated. A case of clinical disease has been reported in wildlife resulting in deaths of gazelles (*Gazella dorcas*), ibex (*Capra ibex nubiana*), gemsbok (*Oryx gazella*) and Laristan sheep (*Ovis orientalis laristanica*) (Furley *et al*., 1987). The American white-tailed deer (*Odocoileus virginianus*) can be infected experimentally (Hamdy and Dardiri, 1976).

2.4.3. Transmission

For PPRV to spread, close contact between infected and susceptible animals or contact with contaminated fomite is needed. Large amounts of the virus are present in nasal discharge, coughed secretions, diarrheic feces, tears and other body excretions and secretions. Infection is mainly through inhalation but could also occur through the conjunctiva and oral mucosa (Radostits *et al*., 2002). Fine infective droplets are released into the air from all secretions and excretions of sick and incubating animals particularly when affected animals sneeze or cough (OIE, 2002; Radostits *et al*., 2002).

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 source of infection. These particular hazards are, however, probably short-term since the PPR virus, like its close relative rindepest, would not be expected to survive for long outside the host (Saliki, 1998; EMPRES, 1999).

2.4.4. Risk factors

Goats are more susceptible to PPR than sheep. The age group between four months and one year of age is most susceptible. Trade in small ruminants, at markets where animals from different sources are brought into close contact with one another, affords increased opportunities for PPR transmission, as does the development of intensive fattening units. In a particular flock, the risk of an outbreak is greatly increased when a new flock is introduced or when animals are returned unsold from livestock markets where they may have readily contracted the disease (EMPRES, 1999; Radostits *et al*., 2002). The severity of clinical signs depends on the virulence of the strains (Couacy-Hymann *et al*., 2007)

2.4.5. Morbidity and mortality

Infection rates of PPR in sheep and goats of susceptible population are generally high and can be up to 90% of the flock during outbreaks (OIE, 2002). The incidence rate in an enzootic area may be low but exists continuously. When the susceptible population builds up, periodic epizootics (outbreaks) occur, that receive more attention than usual. Case fatality rates are much higher in goats (55-85%) than sheep (less than 10%). Morbidity and mortality can be as high as 100% and 90%, respectively. When associated with other diseases such as capripox, mortality can be 100% (Dhar *et al*., 2002). There is no seasonal variation in the prevalence of the disease but since maternal antibodies are lost at about 4 months of age, the number of susceptible animals is likely to increase 3 to 4 months after peak kidding and lambing season (Radostits *et al*., 2002).

2.5. Pathogenesis

The main characteristic of the pathogenesis of PPRV infection, as for all other morbilliviruses, is the profound but transient immunosuppression induced by this virus in its host 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 defense system (Diallo *et al*., 2007)

PPR virus, like other morbilliviruses, is lymphotropic and epitheliotropic. 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. After the entry of the virus through the respiratory tract system, it localizes first in the pharyngeal and mandibular lymph nodes as well as tonsil. Viremia may develop 2-3 days after infection and 1-2 days before the first clinical sign appears. Subsequently, viremia results in dissemination of the virus to spleen, bone marrow and mucosa of the gastro-intestinal tract and the respiratory system (Scott, 1981). Death may occur from severe diarrhea and dehydration, before respiratory lesions become severe or is hastened by concurrent diseases (Radostits *et al*., 2002).

2.6. Clinical signs

Peste des petits ruminants (PPR) has four clinical forms in goats and sheep. The incubation period is 2-10 days, but most commonly 4-5 (OIE, 2002).

Table 1.Clinical signs of peste des petits ruminants

Clinical form	Clinical signs of peste des petits ruminants
Acute	-It is the most common form -Sudden high fever (40-41°C), remaining high for 5-8 days; will return to normal before recovery or drop below normal before death -Serous nasal discharge, becoming muco-purulent; can crust over and occlude nostrils -Purulent ocular discharge with congested conjunctiva; can encrust, cementing eyelids together -Bronchopneumonia -Necrosis and ulceration of mucous membrane and inflammation of gastrointestinal tract leading to severe, non hemorrhagic diarrhea -Respiratory distress, including dyspnea and sneezing in an attempt to clear nose -Excessive salivation -Anorexia -Severe dehydration and emaciation followed by hypothermia -Death usually occurs after 5-10 days -Abortion - Mortality rate can reach 100% -Secondary infections may be activated and complicate clinical signs
Peracute	Frequent in goats
Subacute and chronic	-Pneumonia -Develops over 10-15 days -Inconsistent symptoms

Source: Saliki (1998) ,EMPRES(1999), OIE (2002).

2.7. Necropsy findings

Table 2. Necropsy findings of peste des petits ruminants

Organ system	Necropsy findings of peste des petits ruminants
Digestive system	-Inflammatory and necrotic lesions in mouth and gastrointestinal tract -Erosive stomatitis in inside of lower lip and adjacent gum -Lesions on hard palate, pharynx, and upper third of esophagus in severe cases -Rumen, reticulum, and omasum rarely have lesions -Erosions on pillars of rumen -Abomasum often oozes blood -Small intestine lesions usually moderate -Extensive necrosis of Peyer's patches, resulting in severe ulceration -Large intestine features congestion around iliocecal valve, at cecocolic junction, and in rectum -"Zebra stripes" (discontinuous streaks of congestion) in posterior part of colon and rectum
Respiratory system	-Small erosions and petechiae visible on nasal mucosa, turbinates, larynx, and trachea -Pleuritis, resulting in hydrothorax
Other systems	-Slightly enlarged and congested spleen -Enlarged, congested, and edematous lymph nodes throughout body -Erosive vulvovaginitis may exist

Source: Saliki (1998).

2.8. Differential diagnosis

Other diseases that could cause diarrhea or pneumonia in sheep and goats may pose diagnostic challenge but history of recent introduction of new stock and clinical and post mortem findings of stomatitis, enteritis and syncytial giant cell pneumonia are typical of PPR (Radostits *et al*., 2002). In addition to rinderpest, other conditions that should be considered in differential diagnoses include: contagious caprine pleuropneumonia, bluetongue, pasteurellosis, contagious ecthyma, foot and mouth disease, heartwater, coccidiosis and mineral poisoning (OIE, 2002).

2.9. Disease economy

PPR is an important animal disease which now threatens the billion-strong small ruminant population in Africa, the Middle and Near East, South-West and Central Asia. Its economic importance was highlighted in a report on an international survey carried out by Perry *et al.* (2002). In this they identified PPR as one of the priority animal diseases whose control is considered important for poverty alleviation in Western Africa and Southern Asia. In the same survey, it was also pointed out that PPR is still a poorly recognized disease, particularly with regard to epidemiological features such as transmission dynamics under different production systems. A great deal of more research into this aspect of the disease is urgently required. The fact that PPRV can infect cattle, buffaloes and camels gives PPR an even higher priority, particularly in the current situation where vaccination against rinderpest in cattle has been stopped (Diallo *et al.,* 2007).

Peste des petits ruminants is regarded as the most important disease of goats in West Africa where these animals are the major source of protein (Radostits *et al*., 2002). The PPR epidemics can cause mortality rates of 50–80% in naive sheep and goat 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 the 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 economic losses due to PPR alone in India have been estimated annually to 1,800 million Indian Rupees (39 million US$) (Bandyopadhyay, 2002).

2.10. Diagnosis

A major thrust of the developments in diagnostic virology has been towards rapid methods that provide a definitive answer in less than 24 hours or even in the course of the initial examination of the animal. The best viral diagnostic methods fulfill five prerequisites: speed, simplicity, sensitivity, specificity and low cost (Murphy *et al*., 1999).

2.10.1. Clinical and necropsy findings

A provisional diagnosis of PPR can be made from epidemiological and clinical features (Table 1). Any disease characterized by discharges, diarrhea, and deaths with breathing problems in goats and/or sheep, but not in-contact cattle, with mainly the adult being affected and dying, must arouse a suspicion of PPR. The observation of characteristic post-mortem changes (Table 2) would further strengthen the provisional diagnosis (Libeau *et al*., 1995).

2.10.2. Sample collection

The collection of samples at the correct time is important to achieve a diagnosis by virus isolation. Samples should be obtained in the acute phase of the disease when clinical signs are still apparent. In live animals, swabs are made of the conjunctival discharge and from the nasal and buccal mucosae. During the very early stage of the disease, whole blood is also collected in anticoagulant for virus isolation, polymerase chain reaction (PCR) and hematology. At necropsy, lymph nodes, especially the mesenteric and bronchial lymph nodes, lungs, spleen and intestinal mucosae should also be collected aseptically, chilled on ice and transported under refrigeration (OIE, 2004). All samples should be shipped fresh (not frozen) on ice with 12 hours after collection (Saliki, 1998). There are several important points to observe when using the services of a laboratory.

1. Provide epidemiological and clinical details with the samples.

2. Always sample several animals in an outbreak.

3. Keep samples cool during transfer to the laboratory (preferably on melting ice) and reduce the time in transit to the minimum

4. Mark sample bottles carefully with an indelible pen and record details of each sample's origin for submission to the laboratory.

Goats and sheep can be infected with RP and PPR as well. Clinical differential diagnosis is not possible as both viruses in small ruminants produce similar disease. 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, and nucleic acid sequencing and detection of specific antibody in serum (Ozkul, 2002).

2.10.3. Virus isolation

Even when diagnosis has been carried out by rapid techniques, the virus should always be isolated from field samples in tissue cultures for further studies (Lefevre and Diallo, 1990).

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 and submitted to the testing laboratory in ice. The most widely used cell culture systems are primary lamb kidney (Taylor and Abegunde, 1979) and Vero cells (Hamdy *et al*., 1976).

Appearance of cytopathic effects (CPE) may require at least 8-10 days or several blind passages. In vero cells, the cytopathic effects 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". Like other morbilliviruses, PPRV produces eosinophilic intracytoplasmic and intranuclear inclusion bodies both in primary cells 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 (Diallo *et al*., 1995).
- 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).
- Molecular techniques: cDNA probe, (Pandey *et al*., 1992), electrophoretic profile in polyacrylamide gel (PAGE) (Diallo *et al*., 1987) and PCR (Forsyth and Barrett, 1995; Couacy-Hymann *et al*., 2002).

2.10.4. Detection of viral antigens and nucleic acid

Different techniques have been used to detect the virus or viral antigens; these include: agar gel immunodiffision (AGID), counter immunoelectrophoresis (CIEP), immunocapture ELISA, indirect or passive haemagglutionation, immunofluorescence or immunoperoxidase staining, complement fixation, virus isolation in cell culture and many more (Diallo *et al*., 1995).

Agar gel immunodiffusion test

These techniques are based on the ability of antibody to form precipitin lines specifically with the antigen. After optimal development of precipitin patterns, the plate can be read directly or stained for considerable improvement in sensitivity. Usually, 1-3 precipitin lines will develop between the serum and the antigen within 18-24 hours. Positive reactions show lines of identity with the positive control antigen (Talwar, 1983). It can be used to test the presence of both antigen and antibodies (OIE, 2004). One of the important advantages of this test is that it is highly specific (92%), though it cannot differentiate between PPR and RP (Obi, 1984). Therefore, there is a need for a rapid, specific and sensitive virus detection test (Diallo *et al*., 1995).

Counter immunoelectrophoresis

Counter immunoelectrophoresis (CIEP) is the most rapid test for viral antigen detection (Durojaiye, 1982). It is carried out on a horizontal surface using a suitable electrophoresis bath, which consists of two compartments connected through a bridge. The apparatus is connected to a high-voltage source. Agar or agarose (1–2%, w/v) dissolved in 0.025 M barbitone acetate buffer is dispensed on to microscope slides in 3-ml volumes. From six to nine pairs of wells are punched in the solidified agar. The reagents are the same as those used for the AGID test. The electrophoresis bath is filled with 0.1 M barbitone acetate buffer. The pairs of wells in the agar are filled with the reactants: sera in the anodal wells and antigen in the cathodal wells. The slide is placed on the connecting bridge and the ends are connected to the buffer in the troughs by wetted porous paper. The apparatus is covered, and a current of 10–12 milliamps per slide is applied for 30–60 minutes. The current is switched off and the slides are viewed by intense light: the presence of 1–3 precipitation lines between pairs of wells is a positive reaction. There should be no reactions between wells containing the negative controls (OIE, 2004).

ELISA for antigen detection

The ELISA, which gained in popularity for serology, can now also be applied to the detection of virus antigens (Saliki *et al*., 1994; Singh *et al*., 2004). In immunocapture ELISA (sandwich ELISA), the sample to be analyzed is first allowed to react with the detection antibody and the immunocomplex formed will be captured by a second antibody (capture Ab) previously adsorbed onto the ELISA plate surface. One of the antibodies can be polyclonal and the other a monoclonal. Using a polyclonal antibody for capture increases the sensitivity of the assay, due to its high avidity for the Ag, while use of a monoclonal antibody for detection gives the test greater specificity. However, both the capture and detection antibodies used can also be monoclonal. In that case, the detection antibody needs to be labeled with a reporter molecule, for example biotin (Libeau *et al.,* 1994). The assay can be carried out in a single step: the Ag plus the biotinylated detection MAb and the conjugate (streptavidinperoxidase) are mixed and incubated together in the ELISA plate precoated with the capture Ab (Diallo *et al.,* 1995). 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 and simplicity. The immunocapture ELISA is suitable for routine diagnosis of rinderpest and PPR from field samples such as ocular and nasal swabs (Diallo *et al*., 1995).

Complementary DNA probes (cDNA)

The advent of genetic engineering has opened a new approach to disease diagnosis by focusing on the genome of the pathogen. The spectrum of diseases that can be diagnosed is thus broadened since organisms, which are difficult to cultivate, can be more easily identified. Different genes of both rinderpest and PPR viruses have now been cloned and used to develop diagnostic tests based on nucleic acid hybridization. The principle of this technique is the ability of two complementary single stranded nucleic acids to associate and form a stable duplex through the formation of a large number of hydrogen bonds. To detect this association, one of the nucleic acid strands (the probe) has to be libeled in some way. This technique has been used to differentiate rinderpest and PPR virus infections using cDNA clones corresponding to their respective N genes as probes. Using this method with [^{32}P] labeled probes; PPR has been detected for the first time in India (Shaila *et al*., 1989). Although radiolabelled probes are very sensitive, they have many drawbacks

which do not favor their use in routine diagnostic test which include: short half life (14 days), health hazard due to radioactivity, and need for special equipment to protect users (Diallo *et al.*, 1995). These led to the development of non-radioactive probes for the diagnosis of rinderpest and PPR, using either biotinylated DNA (Pandey *et al*., 1992) or digoxigenin-labelled oligonucleotides. This kind of probe not only has a long shelf life, but also is also safe to handle (Diallo *et al*., 1995).

Reverse transcription polymerase chain reaction (RT-PCR)

Conventional serological techniques and virus isolation are normally used to diagnose morbillivirus infection in samples submitted for laboratory diagnosis. However, such techniques are not suitable for use on decomposed tissue samples. A more efficient rinderpest and PPR diagnostic test, based on nucleic acid technology, is the polymerase chain reaction (PCR). 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 thermo stable 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 (Diallo *et al*., 1995).

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 two-step 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). Saliki and others (1994) first demonstrated the efficiency of amplifying in vitro a selected sequence flanked by two oligonucleotide primers of opposite orientation. 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. The procedure is sensitive and rapid, taking only 5 hours to complete, including the RNA extraction (Diallo *et al*., 1995; Forsyth and Barrett, 1995; Couacy- Hymann *et al*., 2002).

2.10.5. Detection of antibodies

Because of cross reaction between RPV and PPRV anti-sera, differentiation is essential when sera from sheep and goats are tested for PPR since RPV could also be circulating in these species. To enable these differential testing of sera many tests have been used for the demonstration of PPR antibodies in serum: virus neutralization test, agar gel diffusion test, immunoelectrophoresis and recently blocking ELISA (B-ELISA) and competitive ELISA (C-ELISA) tests have been developed for specific seromonitoring of each infection (Diallo *et al*., 1995).

Virus neutralization

Previously the main technique used to detect specific antibody to PPR was virus neutralization test (VNT). The virus neutralization test is sensitive and specific, but time-consuming and expensive. The standard neutralization 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. Serum against either PPR or RP may neutralize both viruses, but would neutralize the homologous virus at a higher titer than the heterologous virus (Rossiter, 1994). The drawbacks have led to a search for alternatives to the VNT for seromonitoring of RP and PPR viruses (Diallo *et al*., 1995).

ELISA for antibody detection

Due to their simplicity, high sensitivity, and economy, several competitive enzyme-linked immunosorbent assays (C-ELISAs) and blocking-ELISA (B-ELISA) have been recognized as suitable systems for use for diagnosis and seroepidemiological surveillance. They target the hemagglutinin (H) protein (Singh *et al*., 2004) or nucleocapsid (N) protein (Libeau *et al.,* 1995). The C-ELISA procedure consists of at least four reaction steps, including adsorption of the antigen onto a solid phase, competitive binding of a serum-monoclonal antibody (MAb) mixture to the antigen, detection of the MAb bound to the antigen, and the substrate reaction. This rapid C-ELISA is a simple, fast, reliable, and inexpensive tool for diagnostic and epidemiological purposes (Diallo *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 C-ELISA was observed suggesting that the former was more sensitive (Libeau *et al*., 1995). The relative sensitivity of this C-ELISA to VNT was 94.5%, while the specificity was 99.4%. Both blocking ELISA and C-ELISA detecting anti-N or anti-H antibodies are based on competition between an anti-N or anti-H monoclonal antibody (MAb) and serum antibodies, but in case of blocking ELISA the test sera are preincubated with antigen and then incubated with the MAb. The sensitivity and specificity of the H-blocking ELISA were found to be 90.4% and 98.9%, respectively (Saliki *et al*., 1993). PPR C-ELISA using MAb directed against the H-protein cross reacted to some extent with rinderpest, while RP C-ELISA is specific, therefore an animal was assumed to have experienced RP if it is positive in both PPR and RP ELISA (Anderson and McKay, 1994).

The anti-H RP C-ELISA has been successfully used for serological monitoring of post vaccination herd immunity in the Pan African Rinderpest Campaign (PARC) project to control and eradicate rinderpest from the African continent and which later became part of the FAO Global Rinderpest Eradication Project (GREP). Other tests, such as CIEP, AGID, and indirect fluorescent antibody test have been described, but remain of little interest compared with the VN and ELISA (Abraham, 2005).

2.11. Control and progress in the development of PPR vaccine

2.11.1. Treatment

There is no specific treatment against the disease. However, valuable sick animals in the early stage of the disease should be isolated and given hyperimmune serum, which may be obtained from cattle immunized against rinderpest. Supportive treatment includes fluid therapy for dehydration (Radostits *et al*., 2002). In addition drugs that control bacterial and parasitic complications may be used to reduce mortality rates. Specifically, oxytetracycline and chlorotetracycline are recommended to prevent secondary pulmonary infections (Saliki *et al*., 1998; OIE, 2002).

2.11.2. Progress in the development of PPR vaccine

Information with regard to the immune response necessary for recovery from or for protection against infection to PPR, as well as of rinderpest, is lacking. However, for the control of this disease, vaccines were developed or are on the process of development following the same strategy as for the other morbilliviruses (Diallo *et al.*, 2007).

Tissue culture live attenuated RPV vaccine

Considering the closely antigenic relationship between RPV and PPRV, live attenuated rinderpest tissue culture vaccine was tested in goats for vaccination against PPR. Evaluation of the serological immune response of these vaccinated animals demonstrated the presence of neutralizing antibodies against RPV but not against PPRV, or only on few occasions. However, all animals resisted to PPRV challenge and that was accompanied with rising levels of PPRV neutralizing antibody activity (Taylor, 1979). This result indicates probably that some replication of PPR challenge virus occurs in animals vaccinated with this RP vaccine prior to its clearance by the rinderpest-primed immune response. Despite the possible transient replication of PPRV in vaccinated animals, rinderpest attenuated vaccine was successfully used to protect small ruminants against PPR disease, a protection which lasts for at least one and probably three years (Taylor, 1979; Taylor *et al*., 1990). The use of RP vaccine to protect small ruminants against PPR is now contraindicated because of the production of antibodies to RP, which compromise sero surveillance for RP and that intern complicate the global rinderpest eradication programs (EMPRESS, 1999).

Homologous live attenuated PPRV vaccine

The fact that PPRV neutralizing was not detected in RPV vaccinated small ruminants before challenge has encouraged continuing research on the development of a homologous PPR vaccine. In 1989 this goal was finally achieved by successful attenuation of PPRV strain Nigeria 75/1 through serial passages on Vero cells. Several trials have demonstrated the efficacy of this vaccine on more than 98,000 sheep and goats in the field between 1989 and 1996. During those trials no unwanted side effects such as abortion in pregnant animals were recorded. It was demonstrated also that animals vaccinated with this attenuated PPRV were unable to transmit the

challenge to in-contact animals. Anti-PPRV antibodies generated by vaccinated animals last for at least 3 years, the effective economic life of the animals. PPRV Nigeria 75/1 belongs to lineage I. During the development process of 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 species of animals has now been discontinued worldwide (OIE, 2004). This is to ensure a serologically negative ruminant population to allow for effective epidemiosurveillance of rinderpest disease to fulfill 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). The homologous PPRV Nigeria 75/1 developed in CIRAD-EMVT, and distributed to veterinary vaccine producing countries by PANVAC, an AU center of excellence, Debre-Zeit, Ethiopia. The prospects of control using homologous attenuated vaccine are very important because PPR need to be differentiated from rinderpest, particularly as the Global RP Eradication Program proceeds towards the anticipated eradication of rinderpest by the year 2010 (OIE, 2004).

As with all members of the family *Paramyxoviridae*, PPRV is very heat sensitive and this is a serious drawback to the efficient use of the live attenuated vaccine in the endemic areas, which have hot climatic environments. In addition these regions usually have poor infrastructures and under these conditions it is difficult to maintain a cold chain to ensure the preservation of vaccine potency. This drawback was overcome through the development of a thermotolerant vaccine freeze dried in the presence of a cryoprotectant containing trehalose (Worral *et al*., 2001). A similar vaccine was recently produced in India using a local strain of PPRV (Sarkar *et al*., 2003). Under these production conditions the vaccine is stable at 45 ^{o}C for 14 days with minimal loss of potency. PPR control will benefit greatly from the use of this thermostable form of the attenuated vaccine (Diallo *et al*., 2007).

A major disadvantage when using classical live attenuated vaccine is that the antibody responses they induce in animals cannot be distinguished from those following a natural infection. This

makes sero-epidemiosurveillanceof the disease impossible in endemic areas where a vaccination programme has or is being implemented. A way to combine activities, vaccination and serosurveillance, for the better management of the disease would be the use of DIVA vaccines, the acronym used for vaccines which enable *d*ifferentiation between *i*nfected and *v*accinated *a*nimals (van Oirschot, 1999).

Recently genetically engineered recombinant vaccines are currently undergoing field trials. A work to protect small ruminants against both capripox and PPR using recombinant capripox vaccine where these two economic important diseases share the same geographical distributions is underway (Berhe *et al*., 2003).

2.11.3. Eradication

Eradication is recommended when PPR appears in new areas. Methods that have been successfully applied for RP eradication in many areas would be appropriate for PPR. These should include quarantine, slaughter and proper disposal of carcasses and contact fomites, decontamination, and restriction on importation of sheep and goats from affected areas (Saliki, 1998). The disease can be prevented by avoiding introduction of new stock from unknown sources, especially animals bought at livestock markets. In addition, animals returned unsold from markets should be segregated unless the entire herd or flock has been vaccinated (Radostits *et al*., 2002). However, where the disease is endemic and small ruminants are kept in large numbers, quarantine and segregation are not realistic means of controlling the disease. As such, vaccination is the preferred method of control (Nawathe, 1984).

2.12. The epidemiology of PPR in Ethiopia

2.12.1. Disease history

Peste des petits ruminants was probably introduced into Ethiopia in 1989 in the Southern Omo river valley from where it moved eastward to Borena region and then northwards along the Rift valley to Awash (Abraham *et al*., 1991; Roeder *et al*., 1994).

2.12.2. Disease surveillance and confirmation

Peste des petits ruminants was suspected on clinical grounds to be present in goat herds in Afar Region of eastern Ethiopia in 1977 (Pegram and Tereke, 1981). Moreover, serological and clinical evidences were reported by Taylor (1984). However, the presence of the virus was only confirmed in 1991 with cDNA probe in lymph nodes and spleen specimens collected from an outbreak in a holding land near Addis Ababa (Abraham *et al*., 1991; Roeder *et al*., 1994).

In a serological survey carried out in 1997 at Debre Zeit abattoirs high prevalence of antibodies was reported (Yayehrade, 1997). The national serosurveillance carried out in 1999 has indicated a seroprevalence of 5.7% in goats and sheep from different regions of the country (MoARD, 2004).

The etiologic agent of PPR was isolated from tissue samples collected from suspected outbreaks of PPR in Arsi Zone of Ethiopia (Gelagay *et al.,* 2003). Abraham *et al*. (2005) have reported the overall seroprevalence of PPR as 3% in camels, 9% in cattle, 9% in goats and 13% in sheep in different parts of Ethiopia including Afar region (Table 3).

Table 3. Summary of prevalence of PPR in Ethiopia

Region	Prevalence (%)		Type of Test	Reference
	Goats	Sheep		
Afar	Not Known	Suspected		Pegram and Tareke, (1981)
Near Addis Ababa	Not Known	Confirmed	c-DNA probe	Abraham *et al*.(1991)
Arsi	53%	33%	Ic-ELISA	Roger and Bereket, (1996)
Afar	67%	0%	Ic-ELISA	Roger and Bereket, (1996)
North Shewa	17%	12%	C-ELISA	Gelagay(1996)
Konso	7%	0%	C-ELISA	Gelagay (1996)
Debre Zeit Abattoir	-	46.7%	Ic-ELISA	Yayehrade (1997)
North Wello	5.7%	3.0%	C-ELISA	Mohammed (2001)
East Shewa	13.9%	12.6%	C-ELISA	Mohammed (2001)
Arsi	Confirmed	-	Tissue culture, ELISA	Gelagay *et al.* (2003)
National survey		5.7%	C-ELISA	MoARD (2004)
Afar, Borena, E. Shoa, Gambella Jijiga	9%	13%	C-ELISA	Abraham *et al.(*2005)
Afar (Gewane)	-	6.1%	C-ELISA	Dawit (2006)
Afar(Awash Fentale)	0.88%	1.05%	C-ELISA	Getahun (2006)

As part of the control activities, a serological survey was undertaken in randomly identified areas of the country so as to determine the magnitude of PPR and identify areas of higher risk. A total of 13,849 sera were collected from 72 districts of seven regional states of the seven regional veterinary laboratories.

The laboratory analysis of the serum samples using competitive ELISA has been undertaken at the National Animal Health Research Center (NAHRC), Sebeta (Table 4).

Table 4. Results of PPR serological survey and number of *Woredas* involved in 1999

Region	No. of *Woredas* included in the survey	No. of samples collected	No. of samples positive	Seroprevalence (%)
Afar	8	1504	238	15.80
Amhara	20	6643	279	4.20
B/Gumuz	6	253	10	3.95
Oromia	11	2282	22	0.96
SNNPRS	15	1902	21	1.10
Somali	4	465	99	21.29
Tigray	8	800	129	16.13

Source: MoARD (2004).

The above table indicated that regions with higher number of lowland *Woredas* were more affected in 1999. Most of the *Woredas* with sero-prevalence of greater than 10% were found in Afar and Somali Regional States. This observation was further investigated statistically to compare the PPR sero-reaction between *Woredas* in an altitude of greater than 1,800 masl and lessr than 1,800 masl (Table 5). A statistically significant result was obtained for higher occurrence of PPR in *Woredas* below an altitude of 1800 masl than in *Woredas* above 1800 masl ($p=0.02$) (MoARD, 2004).

Table 5. Status of *Woredas* for PPR seroprevalence under two categories of altitude ranges

Status of Woredas	Altitude <1800(masl)	Altitude >1800(masl)	
Positive for PPR	33 (78.6%)	16 (53.3%)	49 (68.0%)
Negative for PPR	9 (21.4%)	14 (46.7%)	23
Total	42	30	72

Source: MoARD (2004).

In the year 2001, a total of 2,815 sera samples from camels, cattle, goats and sheep from different parts of the country were collected and tested. The animals had not been vaccinated against rinderpest or PPR. In this study the antibody prevalence was 10% in camels in Afar and PPR antibody was not detected in camels in Jijiga. In East Shoa the highest antibody prevalence was 16% in sheep. Antibody seroprevalences detected in camels, cattle, goats and sheep confirmed natural transmission of PPR virus under field conditions. This is the first report of PPR antibody detection in cattle and camels in Ethiopia (Abraham *et al.*, 2005).

Table 6. Serological results of PPR in Ethiopia

Locality	Camels		Cattle		Goats		Sheep	
	Samples	Positive (%)	Samples	Positive (%)	Samples	Positive (%)	Samples	Positive (%)
Afar	400	10	400	15	NA	NA	396	16
Borena	160	0	200	5	200	0	160	2
E. Shoa	NA	NA	110	5	100	0	111	16
Gambella	NA	NA	120	16	42	22	48	23
Jijiga	68	0	80	5	100	15	120	7
Total	628		910		442		835	

NA: Serum samples were not collected

Source: Abraham *et al,* (2005).

2.12.3. PPR outbreak reports

As part of the livestock disease surveillance system of the country, disease outbreak reports are collected monthly from all *Woredas* of the country, which helps to fully understand the epidemiology of diseases. Based on these reports, the occurrence of PPR in Ethiopia from 1996 to 2004 is summarized (Table 7).

From recent outbreaks of contagious diseases that occurred in Arsi, North Shoa (adjacent to Afar Regional State) and Amhara Regional State, clinical samples including lymph nodes, spleen and

intestine were submitted to NAHRC for confirmation. The involvement of the virus in these outbreaks was confirmed by PPR virus immunocapture ELISA (MoARD, 2004).

Table 7.Number of new outbreaks of PPR in Ethiopia according to the annual report of 1996-2004

Year	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total
1996	0	1	1	1	1	2	1	1	0	0	4	0	12
1997	1	7	2	6	0	2	1	1	2	0	0	0	22
1998	1	1	2	0	6	1	0	0	0	8	3	2	24
1999	0	0	0	0	0	0	0	0	0	1	0	0	1
2000	0	0	0	0	0	0	0	0	0	1	0	0	1
2001	0	0	0	0	0	0	0	0	1	0	0	1	2
2002	0	0	2	1	0	0	0	0	2	0	0	0	5
2003	0	2	2	0	2	1	0	1	1	3	0	0	12
2004	2	3	0	1	5	1	0	2	0	2	2	3	21
Total	4	14	9	9	14	7	2	5	6	15	9	6	100

Source: OIE (2005).

2.12.4. Government control strategies

Vaccination of small ruminants is the most cost-effective and practical strategy for the control of PPR in the country (MoARD, 2004). The vaccine that is being produced in Ethiopia is a homologous PPR vaccine. It is mentioned that this vaccine confers a protective immunity for a period reaching for at least three years (OIE, 2004).

A national control programme has started against PPR, which will last for three years consecutive years starting from 2004. The programme began with vaccination of sheep and goat population using a homologous vaccine. The vaccination campaigns will be executed strategically in those *Woredas* considered to be endemic by the regional states including their neighbors based on the results of the 1999 serological survey and outbreak disease reports. During these campaigns a

total of 22428624 goats and sheep will be vaccinated against PPR in all regional states except Addis Ababa, Dire Dawa, Gambella and Harari (Table 8).

Table 8. Small ruminant population versus the planned vaccination coverage for PPR

Region	Small ruminant population	Planned PPR vaccination	% coverage	Vaccine dose distributed in 2004/5	% of vaccine dose distributed in 2004/5
Tigray	2446338	1965000	80	1291200	65.7
Afar	467841	374273	80	280000	74.8
Amhara	9136189	7308951	80	2366755	32.4
Oromia	8865984	7092787	80	886095	12.5
Somali	1029381	823505	80	488620	59.4
B/Gumuz	259242	207394	80	40985	19.8
SNNP	5820893	4656714	80	641300	13.8
Gambella	92822	0	0	0	-
Harari	24872	0	0	0	-
D/Dawa	125022	0	0	0	-
A/Ababa	97215	0	0	10980	-
Total	28365799	22428624	80	6005935	26.8

Source: Central Statistics Authority (2003), MoARD (2004).

3. MATERIALS AND METHODS

3.1. Study area

The study was conducted in Awash Fentale *Woreda* of Afar National Regional State (ANRS). The Region is one of the four major pastoral areas in Ethiopia and is located in the northeastern part of the country bordering with the Republic of Dijbouti in the east, Amhara and Tigray regions in the west, Oromia Region in the south and Eritrea in the north. The land coverageof the region is 10, 086 km sq. The altitude varies from about 1500 masl to as low as 120 mbsl in Dallol Depression.

Awash Fentale is located at 225 km from Addis Ababa in the northeast. It is a lowland area with an altitude range of 739 to 931 masl and with annual average rainfall of 450 mm. The average minimum and maximum temperature is 21°C and 38°C, respectively. The dominant tree species of the area is acacia with different shrubs.

3.2. Livestock production system

Afar National Regional State is one of the nine regional states and one of the four major pastoral regions in Ethiopia. The regional population is estimated to be 1.2 million of which 90% are pastoralists and 10% agro-pastoralists. The majority of the land is rocky and the annual precipitation is low (150-500 mm/annum) which makes crop cultivation unsuitable. People in the region therefore depend mainly on livestock production for their livelihood. The livestock population in the ANRS is estimated as 703,424 cattle, 1,003,000 heads of sheep, 2, 014,418 heads of goats, 301,733 camels and 16,976 donkeys (CSA, 2003). Commonly kept livestock include cattle, goats, sheep, camels and donkeys. Milk from cattle, goat and camel is an important component of paternalists' family diet.

In Awash Fentale *Woreda*, the current study area the livestock production system is specifically semi-sedentary type of pastoral production where pastoralists do have permanent homestead but temporal migration in search of feed and water for their livestock during drought. Recently agro-

pastoral production system has been started using irrigation assisted by the government and some Non Governmental Organizations (NGOs).

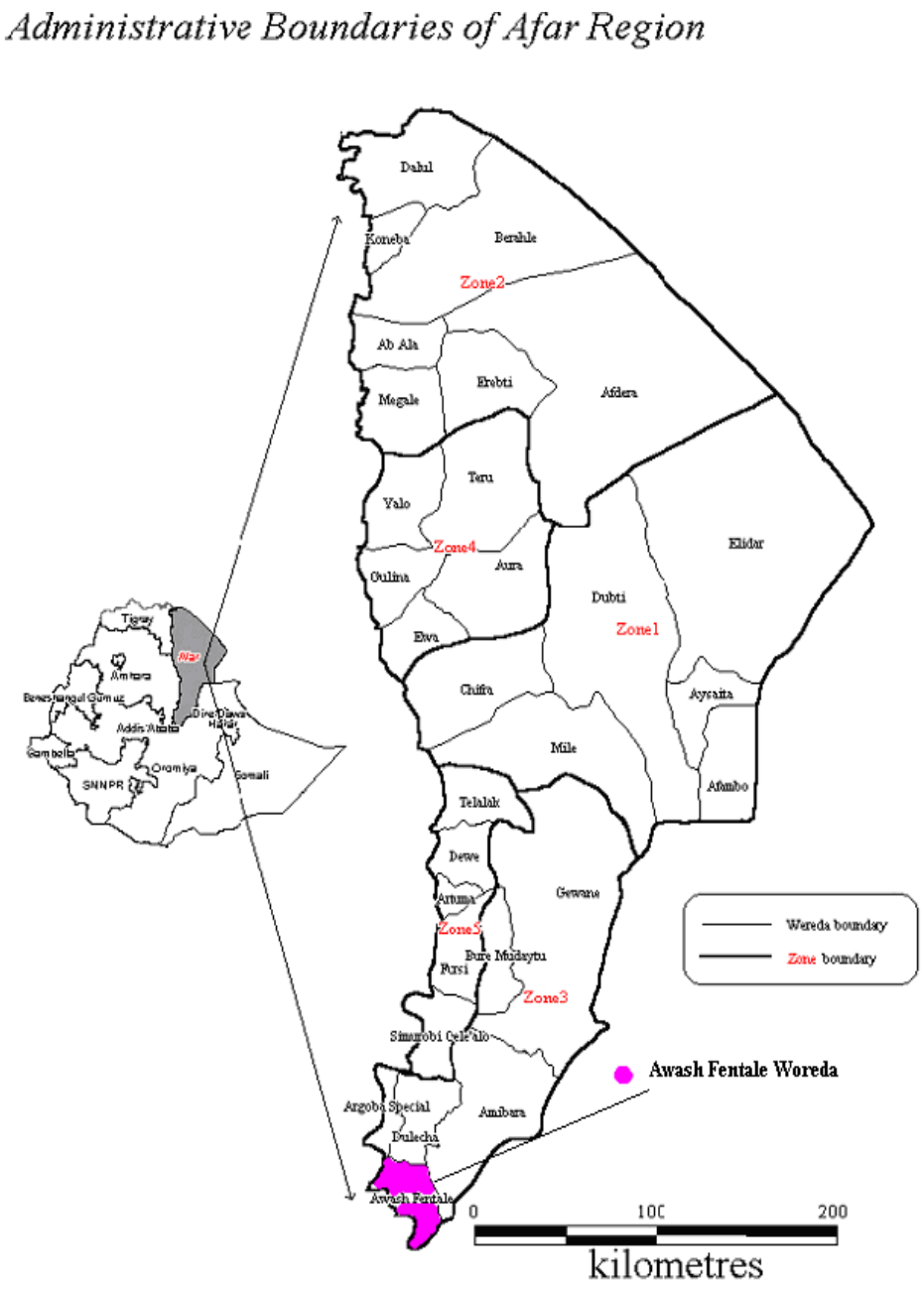

Figure 2. Map of study area.

3.3. Study animals and sampling

The study population was both sheep and goats found in Awash Fentale *Woreda,* ANRS. Pastoralists in the area rear their livestock using free grazing system, and goats are the 2nd dominant species following cattle in terms of population size. Though they are less in number compared with goats, sufficient numbers of sheep are found in the area. Both sheep and goats found in the area are local breeds, which are of *Adal* and *Danakil* breeds and thought to have entered Ethiopia from Yemen and Saudi Arabia (FARM-Africa, 1996). Pastoralists maintain high proportion of breeding females because of their need for milk. The mean flock size owned goats is 41 (SD 24) and adult male: female ratio of goats is 1: 42 (FARM-Africa, 1996). Small ruminants are usually herded by children and occasionally by women. Kids and lambs are kept in small houses and adults return to thorn enclosures for protection during night. Besides their use as dietary source to pastoralists' family, sheep and goats are considered as consumer and market goods, which are frequently sold and traded for grains and basic household goods of primary necessity

A total of 2,573 animals were included during the study period for three different objectives.

3.3.1. Seroprevalence study

A total of 1,239 animals were sampled from 10 different villages using combinations of simple random sampling, to select villages and flocks, and cluster sampling to select animals. Animals in the randomly selected flocks (animals belonging to the same) were all sampled (cluster sampling). Forty eight flocks were visited to collect sera and animals possessed by one owner were considered as cluster

Due to lack of information about between cluster variance, the method suggested by Thrusfield (1995) was used. According to this method, the standard deviation of the flock prevalence was guessed (i.e. the average difference expected between an individual cluster prevalence and the overall mean cluster prevalence) and then squared to give between cluster variance component (Thrusfield, 1995).

Table 9. Estimates used for estimation of the design effect

Woredas	Prevalence/ Sheep	Prevalence/ Goat	Mean Prevalence	Standard deviation	Between cluster variance V_c	d	n
Gewane	0	0.06	0.025	0.001	0.000001	0.05	30
Awash Fentale	0.01	0.01				0.05	30

Source: (Dawit, 2006; Getahun, 2006)

The sample size was determined according to Thrusfield (1995) following one- stage cluster sampling method for infinite population with 95% confidence level, 5% desired absolute precision and expected mean prevalence 50% (0.50) to increase sample size using the formula:

$$g = \frac{1.96^2 (nV_c + P_{exp}(1 - P_{exp}))}{nd^2}$$

g = no. of cluster to be sampled = 19

P_{exp} = expected mean prevalence =0 .50

n = predicted number of animal per cluster = 30

d = desired absolute precision = 0 .05

V_c = between cluster variance =0 .000001

To increase the precision the number of clusters was increased to 48. Increase in the number of clusters rather than the sample size increases the precision (Thrusfield, 1995).

3.3.2. Outbreak investigation

A total of 238 animals showing PPR like clinical signs such as pyrexia, severe muco-purulent

nasal and ocular discharges, erosive or necrotic lesions on the mouth, respiratory distress and diarrhea were sampled (Annex 4). These animals were not vaccinated.

3.3.3. Postvaccinal serocoversion rate

Following a minimum of two weeks post vaccination days, that has been done together with the *Woreda's* veterinary professionals and Community Animal Health Workers (CAHWs); a total of 1,096 animals were sampled in the same villages sampled during seroprevalence study, using systematic random sampling method to select flocks. Nearly an equal number of animals were selected from the randomly selected flocks to see differences in factors that could affect seroconversion rate.

3.4. Study design

3.4.1. Seroprevalence survey

A cross-sectional epidemiological study was conducted to determine the seroprevalence of PPR in the study area. Risk factors associated with the epidemiology of the disease such as age, species and sex were recorded. Information about previous PPRV vaccination was gathered from the *Woreda's* Pastoralists and Agricultural Development Office. According to the information gathered, none of the animals from which the samples were collected had a history of PPR vaccination.

3.4.2. Outbreak investigation

Observational study on clinical cases has been conducted during the occurrence of an outbreak. Samples for laboratory examination were collected from individuals that exhibited clinical signs suspected to be PPR, or at least one animal from the flock has died or recovered from suspected PPR. Anti-mortem samples (nasal swabs and/or blood using heparinized tube) were collected from active cases with the aim of virus isolation and sera from flocks possessing sick or recovered animals with a history of ill health were collected for serological investigation. Ris

factors such as species, age of the animal and vaccination status was recorded. The characteristic signs seen (Annex 4) and complaints of deaths by the owner were enumerated.

3.4.3. Postvaccinal serocovertion rate

A cross-sectional design was used to assess postvaccination seroconversion rate and factors that can affect the rate of seroconversion. Information about whether individual animals sampled are included or not in the vaccination campaign was gathered from animal owners.

3.5. Data collection

3.5.1. Sample collection

Sera: Blood was collected by jugular- veinpuncture using vacutainer tubes and needles. The blood was kept to clot, after which, sera were separated by decanting into sterile test tubes The tubes then, were kept in a container packed with ice during transportation.

Swab: Samples of nasal secretion were obtained using cotton swabs. The swabs were inserted into the nasal cavity, and swirled around to collect nasal secretion. The swab was broken off, inserted into sterile tube containing virus transport media and antibiotic to suppress bacterial growth, and kept in ice during transportation.

Unclotted blood: Unclotted blood was collected from the jugular vein puncture using heparinized vacutainer tube and sterile needle.

Postmortem specimens: No post-mortem sample was collected because there was no fresh tissue sample.

3.5.2. Laboratory diagnosis

Competitive ELISA for detection of PPR antibodies: The N nucleocapsid (N) protein-based competitive ELISA (C-ELISA) was used for all the three serological investigations. Monoclonal antibodies, specific for N protein of PPR virus was used to detect antibodies in sera that was collected during seroprevalence study, assessment of post vaccination seroconversion rate and seroconversion following suspected PPR outbreak. The material used for competitive C-ELISA kit was obtained from CIRAD-EMVET, France. The test was carried out according to the manufacturer's protocol (Annex 3) and sera with more than 50 % colour inhibition were considered positive. ELISA plates were read using Titertek Multiskan Photometer (Labsystems, Shandon, Finland) ELISA reader with an inference filter of 492 nm. The optical density (OD) values were converted to percentage inhibition (PI) using the following formulae.

Formula for calculation of percentage inhibition of the test sera

$$PI = 100-\left(\frac{\text{OD of test serum x100}}{\text{Mean OD of MAb}}\right)$$

OD = optical density, MAb = monoclonal antibody

The data expressed in optical density for monoclonal antibodies control, and the data expressed in PI for the other four controls (negative, strong positive, weak positive and conjugate controls) were used to determine whether or not the test has been performed within acceptable limits of variability and thereafter whether or not to accept the test result for any given microtiter plate.

3.5.3. Questionnaire survey

The preferred types of livestock reared

Five villages each having seven informants, consisting of community elders, Community Animal Health Workers (CAHWs) and women were selected from these villages and asked the type of

animals they have and also to compare their livestock preference based on their own criteria by proportional piling method.

Livestock disease scoring

The same informants used for livestock preference comparison were asked about the most important sheep and goat diseases and to characterize them

Based on the information gathered from the informants the above four diseases were compared using mortality and morbidity as indicators by proportional piling method.

3.6. Data management and analysis

The data that were collected from the field, during sample collection and laboratory analysis results were coded, organized and arranged in Microsoft Excel spread sheet computer program and analyzed by appropriate statistical programmes.

Based on the types of data generated simple descriptive, Pearson's chi-square, Fisher’s exact test, odds ratio were considered using Inetrcoll Stata 7.0/2001.

4. RESULT

4.1. Seroprevalence of PPR using competitive ELISA (C-ELISA)

From a total of 1,239 small ruminants, 879 goats and 360 sheep, sampled from the study area only 21 animals consisting of 1 sheep and 20 goats were found to be seropositive. The overall seroprevalence of 1.70% and the village level seroprevalence values were small (Table 10).

Table 10. Seroprevalence of PPR in 10 villages of Awash Fentale *Woreda*

Name of Village	n	No. seropositives	Seroprevalence (%)	95%CI
Yello	217	2	0.92	0-2.2
Ededas	59	2	3.39	0-8.1
Keda - Melkadura	89	2	2.2	0-7.0
Faris Gubi	179	2	1.12	0-2.7
Hadia Habur	91	3	3.30	0-7
Kamp	91	2	2.20	0-5.3
Hayukele - Boloyta	124	3	2.24	0-5.2
Unda Bolyta	213	2	0.94	0-2.2
Erifoda	106	3	2.83	0-6
Unda Ilalla	70	0	0	0

n = number of small ruminants sampled, CI = confidence interval

Among ten villages included in the seroprevalence survey, one village (Unda Ilalla) was found to be seronegative for PPRV antibody. In the nine other villages, the value ranges from 0.92% to 3.39% (Table 10). The seroprevalence values in the entire villages did not show statistically significant difference ($p>0.05$).

Table 11. Seroprevalence of PPR in sheep and goats sampled from ten villages of Awash Fentale *Woreda*

Name of Village	Sheep		Goats	
	n	No. seropositiv (%)	n	No. seropositive (%)
Yello	77	0 (0)	140	2(1.43)
Ededas	37	0 (0)	22	2(9.10)
Keda Meldadura	35	0 (0)	54	2(3.70)
Faris Gubi	33	0 (0)	146	2(1.37)
Hadia Habur	11	0 (0)	80	3(3.75)
Kamp	55	1 (1.82)	36	1(2.28)
Hayukele Boloyta	45	0 (0)	79	3(3.80)
Unda Bolyta	34	0 (0)	179	2(1.12)
Erifoda	19	0 (0)	87	3(3.45)
Unda Ilalla	14	0 (0)	56	0(0)

n= number of animals sampled

The seroprevalence rate of PPR in sheep in all villages was zero (Table 11) but only in one village goats seroprevalence found to be zero and in the rest nine villages it was with in range of 1.12% - 9.1%

Table 12**.** Comparison of seroprevalence of PPR between sheep and goats

Species	n	No.seropostive	Seroprevalence (%)	95%CI	OR	p-value
Goats	879	20	2.28	1.3-3.3	8.4	0.013
Sheep	360	1	0.28	0-0.82		
Total	1239	1218	21(1.70)			

n = number sampled, OR = odds ratio, CI = confidence interval

The difference in seroprevalence value of PPR between goats and sheep was statistically significant ($p<0.05$). The probability of goats being seropositive to PPRV was 8.4 times higher than sheep (OR=8.4) (Table 12).

Table 13. Seroprevalence of PPR in goats and sheep at different variables

Species	n	Seropositives(%)	p-value
Goat			
≤6m	55	1(1.82)	0.717
6-12m	162	2(1.23)	
>12m	662	17(2.57)	
Sheep			
≤6m	15	0(0)	1.000
6-12m	74	0(0)	
>12m	271	1(0.37)	
Goats			
Male	57	1(1.75)	1.000
Female	822	19(2.31)	
Sheep			
Male	28	0(0)	1.000
Female	332	1(0.3)	

The presence of association between seroprevalence of PPR virus with age and sex was tested (Table13). But PPR seroprevalence among age groups in both goats and sheep was not statistically significantly different ($p>0.05$). Similarly, statistically significant difference between male and female animals both in goats and sheep was not observed ($p>0.05$).

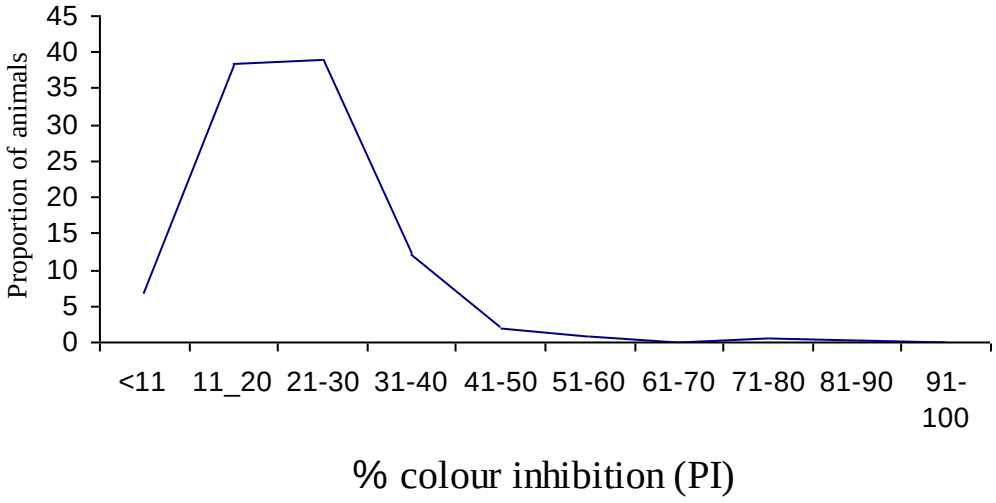

Figure 3. The pattern of percentage colour inhibition (PI) value of competitive ELISA as in direct measure of serum antibody level in sheep and goats.

The percentage colour inhibition values obtained from competitive ELISA (Figure 3) clearly showed that the majority of the small ruminant population sampled had seronegative value (PI<50 %) during seroprevalence study.

4.2. Outbreak investigation

During the initial seroprevalence survey, no characteristic clinical signs of PPR were observed and all small ruminants sampled were apparently healthy. Two months after the initial survey, a characteristic sign of PPR was seen in some goats and sheep (Annex 4) and deaths were also complained by the animal owners. These animals were not vaccinated against PPRV. The over all seroprevalence during this suspected PPR out break was 36.6 % (Table14).

Table 14.Seroprevalence of PPR during an outbreak, suspected to be PPR

Name of Village	Specie				Seroprevalence in sheep and goats
	Goat		Sheep		
	n	No.seropostive(%)	n	No.seropostive(%)	
Ededas	16	8 (50)	5	2 (40)	47.6
KedaMelkadoro	26	23 (88.5)	1	0 (0)	85.2
Kamp	30	4 (13.3)	24	0 (0)	7.4
Unda Boloyta	44	38 (86.4)	1	1 (100)	86.7
Erifoda	13	3 (23.1)	1	0 (0)	21.4
Unda Ilalla	68	8 (11.8)	9	0 (0)	10.4
Overall	197	84 (42.6)	41	3 (7.3)	36.6

n = number of animal suspected for PPR, %= seroprevalence

The seroprevalence of PPR in these villages ranges from 7.4 % to 86.7 %. The overall seroprevalence in goats was 42.6 % while in sheep it was 7.3 % (Table 14).

Table 15. Seroprevalence of PPR in sheep and goats of Awash Fentale *Woreda* during suspected PPR outbreak

Species	n	No.seropostives	Seroprevalence (%)	95%CI	OR	P-value
Goats	197	84	42.6	35.7 - 49.6	9.4	0.000
Sheep	41	3	7.3	0 - 15.6		
Total	238	87	36.6			

No. = number, CI = confidence interval

A statistically significant difference between seroprevalence rate of PPR virus in goats (42.6%) and sheep (7.3%) was observed ($p<0.05$). The seroprevalenve rate of PPRV was 9.4 times higher in goats compared with sheep during that suspected PPR outbreak (OR = 9.4) (Table 15).

Table 16. Comparison of seroprevalence value of PPRV antibody in sheep and goats at different ages in suspected PPR out break in Awash Fentale *Woreda*

Species	n	No.seropositive (%)	95% CI	p-value
Sheep				
≤6m	8	0(0)	0	1.000
6-12m	15	1(6.7)	0-20	
>12m	18	2(11.1)	0-27.2	
Goats				
≤ 6m	49	27(55.1)	40.7-69.5	0.125
6-12m	37	14(37.8)	21.4-54.2	
> 12m	111	43(38.7)	29.5-47.9	

m = age in months, CI= confidence interval

Statistically significant difference was not observed among the different age groups both in goats and sheep (p>0.05) (Table 16).

Table 17.Comparison of seroprevalence rate between male and female small ruminants during the suspected PPR outbreak

Species	n	No. eropositives	Seroprevalence(%)	p-value
Goats				
Female	171	74	42.3	0.644
Male	26	10	38.5	
Sheep				
Female	37	3	8.1	1.000
Male	4	0	0	

Statistically significant different was not observed in seroprevalence rate of PPRV in both species between sexes (p>0.05) (Table 17).

4.3. Postvaccination seroconversion (herd immunity) to PPRV

After the initial seroprevalence survey there was vaccination campaign against PPRV. In the campaign two groups of animal health workers were involved. These were professionals, which was a group consisting veterinarians, assistant veterinarians and animal health technician. The second group was Community Animal Health Workers (CAHWs).

After a minimum of two weeks post vaccination days, a total of 1,096 sera were collected from the ten villages, which were part of the initial seroprevalence survey. From l, 096 sera, 726 were from goats and the rest from sheep. All animals sampled were vaccinated using attenuated homologous PPRV Nigeria 75/1 developed in CIRAD-EMVT, and distributed to veterinary vaccine producing countries by PANVAC, an AU center of excellence, Debre-Zeit, Ethiopia.

Table 18. Post vaccinal seroconversion rate in different villages of Awash Fentale *Woreda*

Name of Village	n	Seropostives	Seroconversion (%)	p-value
Yello	129	82	63.6	0.000
Ededas	120	58	48.3	
Keda Melkadura	109	97	89.0	
Faris Gubi	138	80	58.0	
Hadia Habur	138	77	56.0	
Kamp	86	38	44.2	
Hayukele Boloyta	129	115	89.1	
Unda Boloyta	93	64	68.8	
Erifada	89	30	33.7	
Unda Illala	65	29	44.6	
Total	1096	670	61.1	

The rate of postvaccinal seroconversion ranges between 33.7 % in village Erifoda to 89.1 % in Hayukele Boloyta. There was statistically significant difference in the seroconversion rate of the vaccination against PPRV in the10 villages resampled ($p<0.05$) (Table 18).

Table 19. Seroconversin rate in vaccinated goats and sheep of Awash Fentale *Woreda*

Species	n	Seropositives	%seropostive	95%CI	p-value
Goats	726	494	68	64.6,71.4	0.000
Sheep	370	176	47.6	42.5,52.7	
Total	1096	670	61.1		

The percentage of goats that seroconverted following vaccination against PPRV was 68 % which was greater than the figure in sheep (47.6). The difference in seroconversion rate between goats and sheep was statistically significant ($p<0.05$) (Table 19).

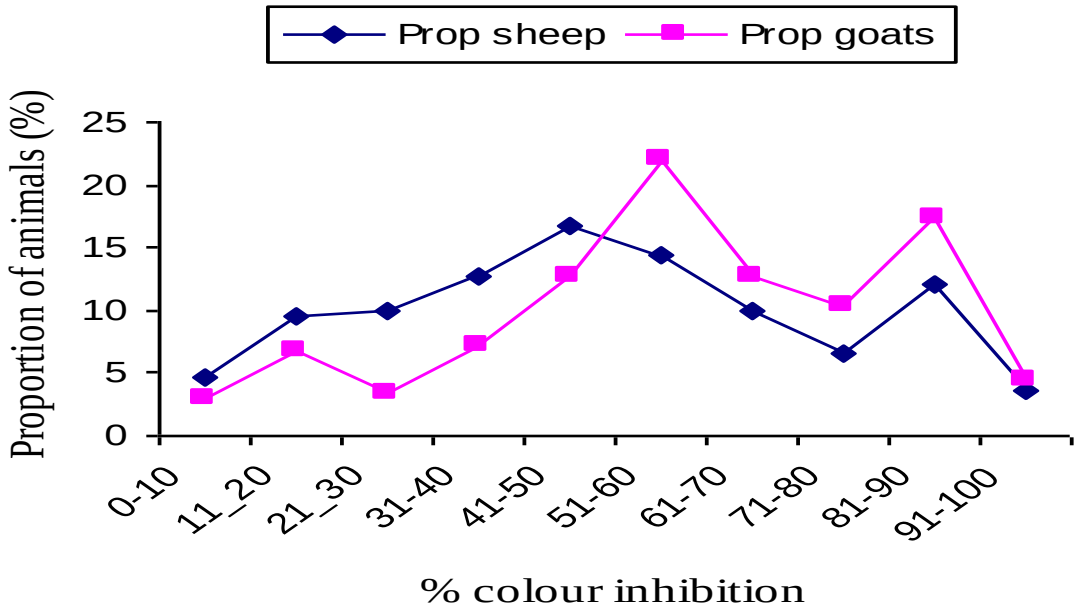

Figure 4. Comparison of post viccinal serum antibody level in sheep and goats.

The competitive ELISA used in the present study to detect the presence of antibody to PPRV in vaccinated sheep and goats was able to clearly differentiate between seropositive and seronegative animals. However, considerable differences were observed between vaccinated goats and sheep population when the result of competitive ELISA was plotted as frequency of the percent colour inhibition (Figure 4).

Table 20. Postvaccination seroconversion rate in sheep and goats among different age groups

Species	n	seropostives	%seropostive	p-value
Goats				
≤ 6m	27	19	70.4	0.277
6-12m	109	67	61.5	
> 12m	590	408	69.2	
Over all	726	494	68.0	
Sheep				
≤ 6m	19	9	47.4	0.503
6-12m	103	54	52.4	
> 12m	248	113	45.6	
Over all	370	176		

There was no statistically significant difference in the post vaccination seroconversion rate among different age groups both in goats and sheep ($p>0.05$) (Table 20).

Table 21. Difference in seroconversion rate due status of vaccinators

Variable	n	Seropostives	%seropostive	p-value
Goats				
CAHWs	150	102	68	0.99
Professionals*	576	392	68.1	
Sheep				
CAHWs	129	68	52.7	0.15
Professionals	241	108	44.8	

Professinal* represent veterinarians, assistant veterinarians, and animal health technicians

The presence of association between seroconversion and who vaccinated the small ruminants was tested. There was no statistically significant difference between small ruminants vaccinated by CAHWs and professionals ($p>0.05$) (Table 21).

4.4. Questionnaire result

Preferred types of livestock reared

About 35 pastoralists from five different villages of Awash Fentale *Woreda* were asked in-group to rank their livestock based on their own criterion. Their preference was nearly similar among all villages and the mean preference of all villages used to rank their livestock (Table 22).

Table 22. Preferred types of livestock reared in five pastoral villages of Awash Fentale *Woreda*

Livestock species reared		Percent preference of livestock reared
English name	Local name	(rank)
Goats	Woder	31(2^{nd})
Camel	Gali	40(1^{st})
Cattle	Lah	21(3^{rd})
Sheep	Elli	6(4^{th})
Donkey	Denen	2 (5^{th})

Livestock disease scoring

The same informants also listed down the most important sheep and goat disease in their villages. They characterized these diseases differently. If we take Woder Sele they said that it kills within short period after the animal became ill, it is new disease and seen for the first time around nine years, it has similarity with the disease called Yagurri, (RP) which was controlled by mass vaccination and the second disease (Yagurri) was seen in cattle. This new disease seen in goats and also in sheep has similarity with the disease occurred before last year in camel in that area, and it causes both respiratory and GIT problems. It can kill many animals at a time (high mortality).

The first four small ruminant diseases that were mentioned as important in all the five villages were ranked using mortality and morbidity as indicators (Table 23).

Table 23. Ranking important sheep and goat diseases in Awash Fentale *Woreda*.

Local name of the disease	Mortality rank (%)	Morbidity rank (%)
Solis	4 th (13)	4 th (5)
Gublo	2 nd (25)	2 nd(28)
Wodre Sele	1st (45)	1 st (57)
Kibris	3 rd (17)	3 rd (10)

5. DISCUSSION

The overall seroprevalencce rate of PPR (1.70%) in sheep and goats in this study was much more less than the previous studies in the Afar National Regional State and other parts of the country. But in one seroprevalence study conducted in the same region, the result was relatively less than the current situation (0.94%) (Getahun, 2006). Similarly, in the same year as Getahun, Dawit (2006) reported 6.1% seroprevalence of PPR in Gewane *Woreda* of the same region. In the serological survey conducted by Ministry of Agriculture and Rural Development, PPR seroprevalence in Afar region was 15.80% (MoARD, 2004). This result was higher than the current figure.

The reasons for the low seroprevalence observed in this study might be the time of sampling in that this survey was conducted during a period when all small ruminants' flock included being apparently healthy, no characteristic PPR clinical symptoms during sampling. The sample size could also be the reason for the low seroprevalence where in this study a total of 1,239 smallruminants versus 687 in Dawit (2006) report. Currently there is no diagnostic test that can differentiate serum antibodies origin, being vaccial or due to natural infection of PPRV. But in the current seroprevalence study no PPR vaccination was given, hence, seropositive animals were due to natural PPR virus circulation in the population under study.

The low seroprevalence signifies the vulnerability of the flock in the event of an outbreak; where 98.3% of the population was seronegative (vulnerable) to PPRV infection, this is an important point to be considered from contagious nature PPRV transmission.

The seroprevalence was higher in goats than in sheep. This result agrees with other reports in the country where PPR seroprevalence was higher in goats than in sheep. Roger and Bereket (1996) reported the seroprevalence values as high as 53% and 33% in goats and sheep, respectively, in Arsi. Gelagay (1996) also reported 17% in goats and 12% in sheep in North Shoa. Mohammed (2001) reported seroprevalence of PPR in North Wollo as 5.7% and 3.0% in goats and sheep, respectively. However, Abraham *et al.* (2005) reported a contradictory seroprevalence result to the current result where the seroprevalence of PPR in sheep (13%) was higher than in goats (9%)

in a survey conducted in Afar, Borena, E. Shoa, Gambella and Jijiga. However, a similar profile of serological status beween sheep and goats was widely reported (Taylor, 1984).

During the outbreak, suspected to be PPR, the seroprevalence reached 36.6% from sera collected from small ruminants that were recovered, sick or at least one animal died in the flock. All animals here were from unvaccinated group. The village level seroprevalence in this case was as high as 86.7% where the previous result being 0.94% in the same village (Unda Boloyta). The seroprevalence value in this investigation could indicate that the characteristic signs observed and deaths complained by the owner were due to PPRV. In addition to the signs and deaths in that specific group of animals, they were not included in the vaccination campaign against PPR given just after the initial seroprevalence study. The low seroprevalence observed is an indication of low herd immunity, which in turn is associated with vulnerability of animals during the outbreak.

The difference in the seroprevalence values recorded between sheep and goats during the suspected PPR outbreak was supported by the previous reports in different parts of the country. Roader *et al.* (1994) reported that during PPR outbreak in land holding near Addis Ababa in goats purchased from southwest Ethiopia, only goats but not sheep were clinically affected. In the current outbreak tentatively assumed to be PPR, the proportion of goats seropositive following the outbreak was higher than sheep by 9.4 times. The difference might have resulted from the number of samples taken based on clinical history and observation which was 198 goats versus 41 sheep.

In the suspected PPR outbreak, all age groups of goats were found to be equally susceptible to PPRV infection. Both male and female goats were also found to be equally susceptible. The same was true for sheep though they were less affected than goats. This shows that in an area with low herd immunity to PPRV, all small ruminants regardless of age and sex are susceptible to infection.

The herd immunity level against PPR was very low in the study area prior to vaccination. However, arise in herd immunity had been seen after vaccination campaign. The immune response, never confer absolute protection and is never equal in all members of vaccinated population. Since the immune response is influenced by a number of factors, the range of

immune response in a large random population of animals tend to follow a normal distribution. Most animals respond by mounting an average immune response, a few will mount a strong immune response, and a small proportion will mount a very poor immune response. Therefore it is impossible to guarantee 100% protection in a large population (Tizard, 1996). The difference in seroconversion rate between goats and sheep was seen. The reason for the difference might effective administration of the vaccine where goats are relatively easy to administer the vaccine than sheep (because of wool they have). There might be other reason, which is not yet understood in this study. The value recorded in this study was lower than the herd immunity targeted figure to rinderpest (greater than 85%) (Roader, 2005), which is another member of morbillivirus, closely related to PPRV.

Like the previous vaccination campaign against RP in Afar (Catley, 1999), CAHWs were involved in current PPR vaccination campaign and no difference in the herd immunity was seen between animals vaccinated by CAHWs and professionals. This implies that in remote pastoral communities marginalized from services and skilled manpower in animal health, the herd immunity to PPRV could be improved by involving CAHWs (PARC, 1996). The herd immunity reported in Afar cattle vaccinated by CAHWs against RP was 83% (Mariner, 1996), which was greater than the current value in PPR (61.1%). The difference might be due to difference in the nature of the vaccine where rinderpest vaccine used was thermostable while PPRV vaccine administered in this study was thermolabile.

Thermostability of live vaccine can be defined in terms of its shelf life at ambient temperature simulated with field situation. In pastoral areas including Awash Fentale *Woreda*, there are a number of factors, which could potentially affect the effectiveness of thermolabile vaccines. These include the high ambient temperature, poor infrastructure such as electric power to maintain the cold chain and others all reducing the efficacy of such vaccines. Studies are in need to be conducted on vaccines to be used in developing countries where vaccine quality suffers due to in transportation and storage facilities owing to frequent power failures and untrained manpower (Sarkar *et al.*, 2003).

Besides the thermolabile nature of the homologous PPRV vaccine used, vaccination coverage in the current study the might be less than RP vaccination. There was also a difference in the frequency of vaccination, in which PARC was given yearly so that the herd immunity build up as the frequency increased but in the current PPRV vaccination, it was the first of its kind in the population studied.

There were some small ruminants, which were not included during vaccination campaign due to temporal movement but meanwhile suffered from suspected PPR outbreak. Some pastoralists were not willing to vaccinate their animals. There are two main reasons for vaccination campaign failure in pastoral areas. The first is that the organization of the campaign does not take into account the basic constraints facing the local population. The other is that vaccination was offered at inappropriate times, in inappropriate places or in a manner that does not allow the owners to participate. The other cause of poor vaccination campaign results is a failure to install confidence in the beneficiaries as to the value of the campaign so that animal owners would be unwilling to vaccination (Mariner, 1996).

The rate of seroconversion in those vaccinated animals included in this study might be higher than determined using the assay due to the diagnostic tests used (C-ELISA) being less sensitive than VN test. It has been reported that goats having a VN titer of 1:4 for PPR antibody were protected against an experimental challenge (Sreenivasa *et el*., 2004). However, such goats were rarely positive for PPR virus antibody employing C-ELISA. Further more, a considerable population of PPR vaccinated sheep and few goats falling in VN titer range of 1:8-1:16 and sometimes over 1:32 with a definite antibody protection level could not be detected in the C-ELISA test (Singh *et al.*, 2004).

The effectiveness of the vaccine could be confirmed based on observations seen during the outbreak where only unvaccinated animas were suffering and dying due to that ill health, which was suspected to be PPR. Economic modeling in Niger suggested that the introduction of a vaccination campaign in the 1980s was a sound decision in terms of animal health and potential economic returns (Stem, 1993). The use of a thermostable vaccine would further reduce costs. In northern Cameroon, field evaluation of a homologous vaccine against PPR showed that it significantly reduced mortality in vaccinated flocks. An economic analysis, which evaluated the

cost of the vaccine, the potential for increased production and the current market prices for livestock, suggests that vaccination may accrue considerable economic benefit to herders (Martrenchar *et al*., 1999).

6. CONCLUSION AND RECOMMENDATIONS

The seroprevalence of PPR in sheep and goats of Awash Fentale *Woreda* was low in the current study. Difference in the seroprevalence rate between sheep and goats was seen. No difference was observed in seroprevalence rate among different age groups in both goats and neither between sexes. These animals were not vaccinated against PPRV. Therefore, the seroprevalences in this study could result only from field infection with PPR virus. The low seroprevalence value indicates poor herd immunity and hence, the majority of animals were vulnerable to PPR virus infection. The low herd immunity was followed by suspected PPR outbreak observed in the study area.

Small ruminants, which were sick and showing stomatitis pnemointeritis complex signs, were most likely due to PPRV, were not vaccinated. All age groups of goats that were not vaccinated were affected by this outbreak. This shows that there was no maternally transferred antibody in kids and lambs.

Community Animal Health Workers could be efficiently used in animal health services including vaccination and their efficiency with regard to effectiveness to vaccination could be improved by enhancing thermotolerance nature of the vaccine.

Based on the above conclusions, the following recommendations are generated:

- Like other infectious diseases, detail epidemiological study of PPR is essential to undertake control program.
- Low seroprevalence signifies poor herd immunity which intern shows the vulnerability of the population hence vaccination in such population is recommended.
- Lambs and kids from unvaccinated dams should be vaccinated at their early age.
- If mass vaccination campaign is used to control PPR, sero-monitoring of antibodies in vaccinated animals should be carried out using appropriate tests to assess the success of vaccination.
- The vaccination campaigns should be done on time and the cold chain mechanism should be strictly applied.

- In such economically important diseases as PPR, it is important to assess the effect of vaccination, the efficiency of vaccination in protecting sheep and goats against the disease PPR under field condition in terms of mortality and morbidity rates, which could provide an estimate of the direct economic benefit of vaccination.
- Presently there are no diagnostic tests that can distinguish between vaccinal and wild type of PPRV; one can't determine the origin (vaccinal or natural infection) of antibodies in goats and sheep. This may be the stimulating force for developing marker vaccine for PPR so that thorough research should be conducted to solve this problem.

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8.ANNEXS

Annex 1. Altitude and GPS coordinate of the study area

Name of village	GPS coordinate in degree		Elevation metes above sea level
	N	E	
Erifoda	$9^0$14.61'	39°59.69'	790
Keda Melkadura	$9^0$12.81'	40°1.44'	739
Unda Boloyta	$9^0$13.74'	40° 0.33'	770
Unda Elalla	$9^0$13.10'	39°58.94'	801
Hayukele Boloyta	$9^0$12.24'	40°0.88'	787
Camp	$9^0$15.11'	40°3.60'	739
Yello	$9^0$15.83'	40°3.77'	736
Hadiya Habur	$9^0$14.25'	40°1.25'	776
Ededas	$9^0$14.02'	40°3.32'	741
Faris Gubi	9^0 13.15'	40°3.15'	750

Annex 2. Summary of animals sampled during seroprevalence study

Name of village	No.goats sampled	No.sheep sampled	Total
Yello	90	39	129
Ededas	85	35	120
Keda Melkadura	81	28	109
Faris Gubi	88	50	138
Hadia Habur	83	55	138
Kamp	54	32	86
Hayukele Boloyta	78	51	129
Unda Boloyta	58	35	93
Erifoda	51	38	89
Unda Ilalla	58	7	65
Total	726	370	1,096

Annex 3. Test protocol for C-ELISA

1. N-PPR antigen must be diluted in PBS according to a dilution factor, which depends of the antigen batch number. Add 50µl of antigen solution to all wells of the plate; tap the side of the micro plates to ensure that the antigen is evenly distributed over the bottom of each well. Incubate 1 hour at 37 ^{0}C on an orbital shaker.
2. Wash plates three times in washing buffer and blot dry.
3. Add 45 µl of blocking buffer to all wells of the plate. Then add a further:
   - 5 µl of blocking buffer to the monoclonal control wells (F1, F2, G1 and G2).
   - 55µl of blocking buffer to the conjugate control well (A1 and A2).
   - 5 µl of test serum to test wells
   - 5 µl of strong positive control to control wells (B1, B2, C1 and C2).
   - 5 µl of weak positive serum to control wells (D1, D2, E1 and E2).
   - 5 µl of negative serum to control wells (H1 and H2)
   - 50 µl of monoclonal antibody diluted 1/100 in blocking buffer to all wells of the plate except the conjugate control (A1 and A2).
   - Incubate 1 hour at 37 ^{0}C on orbital shaker.
4. Wash the plates three times and blot dry.
5. Add 50 µl of anti-mouse conjugate diluted 1/1000 in blocking buffer.
   Incubate 1 hour at 37 ^{0}C
6. Wash the plates three times as before
7. Prepare OPD solution by adding just before use H_2O_2 3% solution. The final substrate/ chromogen solution must be colorless and kept until use 4^{0}C in the dark. Add 50 µl of substrate/chromogen mixture in each well. Color development in stopped after 10 mn by adding 50 µl of 1 M H_2SO_4. Read with an ELISA reader at 492 nm. Use a blanking plat (i.e. column 1 filled with substrate and stopping solution).
8. The inhibition of MAb binding in the presence of serum is expressed as percentage inhibition (PI), calculate from Mean OD values using the formula:

PI= 100-[(OD of the test wells/OD of the Cm wells)x 100]

Sera showing PI greater that 50% are considered to be PPR positive.

ELISA Plate layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	Cc	Cc	1	5	9	13	17	21	25	29	33	37
B	C++	C++	1	5	9	13	17	21	25	29	33	37
C	C+	C+	50									
D	C+	C+				25						
E	Cm	Cm										
F	Cm	Cm					35					
G	Cm	Cm						22				
H	C-	C-										

Notes Cc: Conjugate controls (no serum/no monoclonal antibody)

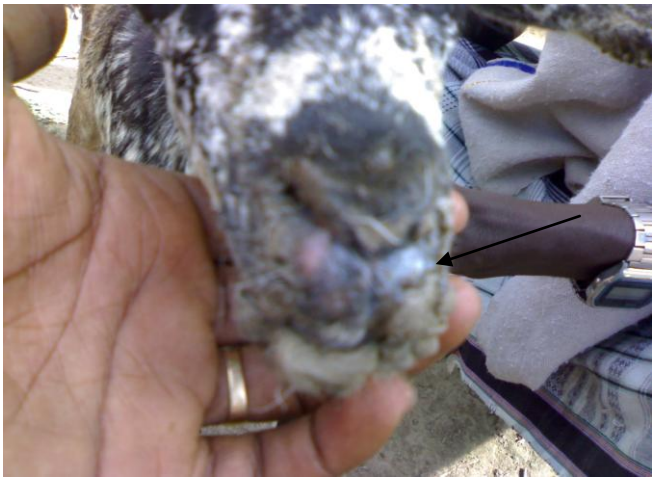
C++: strong positive

Cm: Monoclonal antibody

C+: Weak Positive

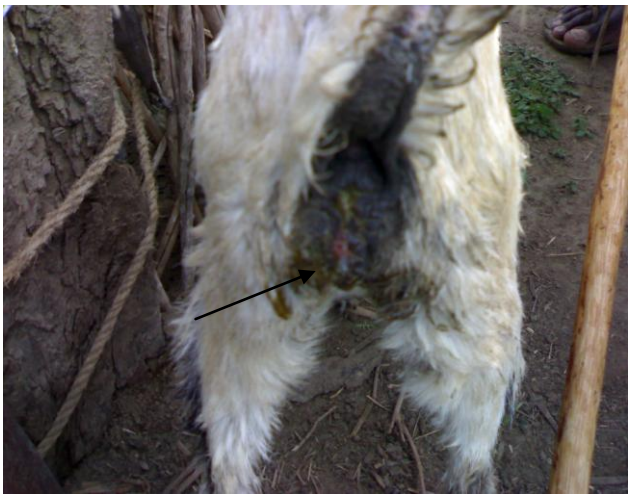
C-: Negative Serum Control

Annex 4. Characteristic signs observed during suspected PPR outbreak



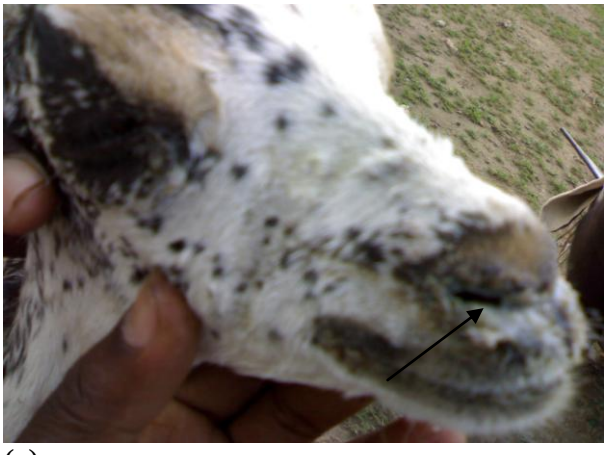
(a)

(b)



(c)

(d)



(e)

(f)

Nasal discharge (a,b,e); diarrhea(c,d); oral lesion(f)

9. CURRICULUM VITAE

Personal data

Name	Faris Delil
Date of Birth	May 22, 1980 G.C. (1972 E.C.)
Place of birth	Addis Ababa
Sex	Male
Nationality	Ethiopian
Marital status	Unmarried
Children	No
Religion	Orthodox Tewahido
Language Proficiency	Amharic, Guraginga and English
Contact	Phone (Mobile) -0911 179647
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Educational background

1980-1987 E.C.	Ener Elementary School and Meger Weira Junior Secondary school (SNNPRS)
1988-.1991 E.C.	Menelik II Senior Secondary School (Addis Ababa).
1992 E.C.	Addis Ababa University, Faculty of Science, Addis Ababa (Fresh man courses).
1993-1997 E.C.	Addis Ababa University, Faculty of Veterinary Medicine, Debre Zeit (Courses of general Veterinary medicine).
1998-1999 E.C.	Addis Ababa University, Faculty of Veterinary Medicine, Debre Zeit (Master of Veterinary Science in Tropical Veterinary Epidemiology).

Research paper

- A study on endo and ectoparasites of young cattle in Hollota and its surroundings DVM Thesis (2005) FVM, AAU, (Unpublished).

Other papers

- Marketing and Management problems as major constraint to skin and hide production in Ethiopi. Seminar paper (2004).
- Diagnostic and control approaches of peste des petits ruminants. Seminar paper on Masters of Veterinary Science (2006).

Additional trainings and certificates

- Computer literacy: Basic computer application software courses and internet utilisation.

Work experience

- Pear education in HIV/AIDS (Certificate by ISAPSO) during under graduate study in Addis Ababa University Faculty of Veterinary Medicine, Debre Zeit
- As externship student from September, 2004-June, 2005 in Hollota, Ethiopian Agricultural Research Center, abattoir work, laboratory, clinical work activities in the Hollota District
- Laboratory experience in competitive ELISA during the Master research work at National Veterinary Institute, Debre Zeit, Ethiopia.
- Field experience during the Master research work in Awash Fentale *Woreda*, Afar National Regional State (one of the pastoral regions of Ethiopia)

10. SIGNED DECLARATION SHEET

I the undersigned, declare that the thesis is my original work and has not been presented for a degree in any university and that all sources of material used for the thesis have been duly acknowledged.

Name: Faris Delil

Signature.....

Date of submission.....

This thesis has been submitted for the examination with our approval as advisors

	Signature
Dr. Yilkal Asfaw (DVM, MSc, Asst. Professor)	_____
Dr. Kelay Belihu (DVM, PhD, Asst. Professor)	_____
Dr. Berhe Gebreegziabher (DVM, MVSc, PhD)	_____