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CONTRIBUTION TO THE KNOWLEDGE OF THE EPIDEMIOLOGY OF PESTE DES PETITS RUMINANTS IN WOLLO AND EAST SHEWA ZONES OF ETHIOPIA

by

Elzein Bashir Mohammed Ali

December, 2001

FREIE UNIVERSITÄT BERLIN - ADDIS ABABA UNIVERSITY

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A thesis submitted in partial fulfillment for the Degree of Master of Science in Tropical Veterinary Epidemilogy at the Freie Univesität Berlin and Addis Ababa University

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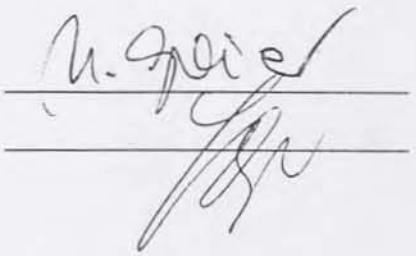

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Abbreviations and acronyms

µl	Microlitre
Ab	Antibody
AGID	Agar gel immunodiffusion test
ANOVA	Analysis of variance
AS	Active Surveillance
C-ELISA	Competitive enzyme immunosorbent assay
FAO	Food and Agriculture Organisation
Ic-ELISA	Immunocapture enzyme immunosorbent assay
PCR	Polymerase chain reaction
PPR	Peste des petits ruminants
PPRV	Peste des petits ruminants virus
RP	Rinderpest
RPV	Rinderpest virus
RPS	Retrospective panel study
RVET	Retrospective analysis of veterinary service records
NPV	Net present value
IRR	Internal rate of return
nm	Nanometer
RBC	Red blood cells
CPE	Cytopathic effect
SD	Standard deviation
F-protein	Fusion protein
Vero cell	African green monkey kidney cell line
CIEP	Counter immuno-electrophoresis
PAGE	Polyacrylamide gel electrophoresis

Fractions were used throughout the thesis to indicate the proportions except in the abstract where percentages were used instead.

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ABSTRACT

A study was carried out to estimate the prevalence of peste des petits ruminants (PPR) in East Shewa, North Wollo and South Wollo in Ethiopia and to assess potential risk factors of the disease and the economic feasibility of control by vaccination. The study consisted of three parts, retrospective panel study (RPS) based on the analysis of a combination of serum bank samples and samples collected during active search for the disease (AS), an active search for the disease with ultimate aim of virus isolation and antigen detection and a retrospective analysis of data collected by the veterinary service (RVET). This study was designed to estimate the level of sero-prevalence in the three zones, different age groups, different sex groups and species, the spatial distribution of the disease, risk factor associated with the disease occurrence and the economic feasibility of conducting a control programme. The result of testing 1472 sera with competitive enzyme linked immunosorbent assay (C-ELISA) was used to estimate the sero-prevalence.

Sera selected from a serum bank established by the National Animal Health Research Centre that comprised 4000 sera collected during the year 2000 and sera collected during the active search for the disease were tested with (C-ELISA). A sero-positivity of 13.4%, 4.3% and .4% was detected in E.Shewa, N.Wollo and S.Wollo zones, respectively. The difference in prevailing sero-positivity in the three zones was assessed with Fisher's exact test and was found significant ($p<.001$). The wide variation in sero-positivity between Wollo areas and E.Shewa was attributed to the presence of different patterns of disease dictated by the natural and ecological characteristics of the two areas. The examination of samples based on the serum bank and samples collected during the active search for the disease yielded different estimates for sero-positivity. In the serum bank samples estimates of 16.8% and 4.3% were obtained in E.Shewa and N.Wollo respectively, while in the field based samples estimates of 7.9% and 8.3% were obtained in E.Shewa and N.Wollo respectively. In S.Wollo only one animal was found positive in the serum bank. The range of sero-positivity in the different villages based on the serum bank based sera was 0-35% and it was 2.9-15% in the field based samples. A different coverage of village and districts in RPS and AS serological study was suggested as a possible explanation. Correlation of the disease by herds was assessed visually with a histogram and a kernel density trace initially. The intracluster correlation coefficient was deduced by

ANOVA. An intracluster correlation of .198, that necessitated the inflation of the variance of the sero-positivity by a factor of 5.194, was detected in E.Shewa zone. In N.Wollo zone an intracluster correlation of .023, that necessitated the inflation of the variance of the sero-positivity by a factor of 2.094, was detected. The adjustment for herd correlation accounted for the interdependence among herd mates and produced wider confidence intervals for sero-positivity.

No significant differences (p>. 05) in the sero-conversion in the different age groups and species was detected in North Wollo and E.Shewa with chi-square. A significant difference (p<. 05) in the sero-positivity in female and male animals was found with chi-square (p<. 001) in E.Shewa. However, this difference became insignificant after adjustment for intraherd correlation. Logistic regression was fitted with three significant factors (the source of the serum, the zone and the sex groups) for sero-positivity (P<. 019). Female (male odds ratio=. 156), E.Shewa (N. Wollo odds ratio=. 269, S. Wollo odds ratio=. 02) and the serum bank sera (field based sample odds ratio=. 525) could be proposed as possible explanatory factors for sero-positivity. However, no adjustment for intracluster correlation was made. Estimation of the true prevalence by considering the assumed sensitivity (90.4%) and specificity (98.9%) revealed that PPR C-ELISA underestimated the sero-positivity in E.Shewa where the apparent sero-positivity was 13.4% and the true prevalence was 13.8%. The same test overestimated the sero-positivity in N.Wollo where the apparent sero-positivity was 4.3% and the true prevalence was found to be 3.6%. Testing of 20 specimens including post-mortem specimens with Immunocapture ELISA and agar gel immunodiffusion test yielded no positive result therefore no further laboratory work was done to isolate the virus. A considerable level of agreement was found between questionnaire outcomes and serology results when aligned together and plotted as scatterplot.

Analysis of the veterinary service records highlighted the adverse effects of PPR on small ruminants. PPR was found to affect .72% of sheep and 3.3% of goats and it caused the death of .3% and 2% of these herds upon its introduction into the herd. It caused the death of 44.7% and 65% of the affected sheep and goats, respectively. A significant difference (p<. 001) between the proportion of affected, deaths among affected and average death rates due to PPR was established in sheep and goats. Both the veterinary service records and the questionnaire outcomes indicated that PPR occurred in all months of the year with the peak of outbreaks

between October and March. Peak of kidding and waning of maternal immunity were hypothesised as a main determinant for the time trend of the disease, since the peak of kidding is confined to the months April to August while the outbreaks used to occur as mentioned before. Therefore, September was suggested as the optimum month for launching annual vaccination campaigns.

Economic assessment of the vaccination policy was done using decision tree analysis. Vaccination against PPR was found to be economically beneficial, except when the total estimate for sero-positivity in sheep was used to rate the probability of infection (.049). The value assigned for sheep (84 birr) and goats (50 birr) was found to be the main factor that led to the low expected return. Though the expected return was found marginal (.23-2.02 Birr per animal) given that the cost of vaccination is 2.5 birr per animal, the vaccination could be justified in order to eliminate any speculated role for small ruminants in complication of rinderpest eradication programmes.

1.INTRODUCTION AND OBJECTIVES

1.1.Introduction

Ethiopia has got a very large livestock population of which sheep and goats represent almost 60%. 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 (Alemayehu and Fletcher, 1991).

Peste des petits ruminants (PPR) is a contagious, viral disease that affects both domestic and wild small ruminants (Furley *et al.*, 1987). It was first identified in Ivory Coast in West Africa in 1940. However currently morbidity and mortality is attributed to PPR in most of sub-Saharan Africa north of the equator, Arabian peninsula, middle East, India, Pakistan, Nepal and Turkey (OIE, 2000). Four lineages of PPR virus that reflect the geographical location of isolation were identified with molecular biological methods, polymerase chain reaction and virus nucleic acid sequence (Shaila *et al.*, 1996). PPR is transmitted by direct contact between animals, hence its incidence is associated with introduction of new animals into flocks and aggravated with close confinement (Taylor, 1984). Two forms of the disease were recognised, an epidemic form that causes high mortality and morbidity and an endemic form in which the virus circulates around the year maintaining itself in the new-born kids (Lefevre and Diallo, 1990). However it is believed that an increase in incidence is associated with the peak of kidding and waning of maternal antibodies (Taylor, 1984). The severity of the disease varies among the different age groups, breed and species; young animals and dwarf African breed of goats' susceptibility is well known (Lefevre and Diallo, 1990, Taylor *et al.*, 1990). PPR causes a wide havoc in sheep and goats and its economic impacts were well defined in West Africa (Hamdy *et al.*, 1976, Stem, 1991). In areas where the epidemic form of the disease prevails, it causes high mortality and morbidity (60%-80%), but where the endemic form persists, the PPR acts as a predisposing factor opening the door for secondary bacterial infection such as pasteurellosis (Abu ElZein *et al.*, 1990, Lefevre and Diallo, 1990). Apart from its economic impacts PPR plays an important role in the complication of Rinderpest (RP) control and eradication programs especially in the

Indian subcontinent where RP affects small ruminants. In Africa this situation is not common though serological evidence were detected in western Kenya (Rossiter *et al.*, 1982) and the isolation of the virus from a goat was reported from Sudan (Babiker, 1973, ElHag and Taylor, 1988). It was assumed for a long time that PPR did not infect cattle under field condition. But a recent study disclosed (Anderson and Mackay, 1994) that cattle undergo silent infection of PPR that interfere with immunity inherent to RP vaccination leading to underestimation of herd immunity level against RP when assessed by sero-monitoring methods.

PPR was suspected on clinical grounds to be present in goats herd in Afar region of Eastern Ethiopia in 1977 (Pegram and Tereke, 1981, cited by Roeder *et al.*, 1994). Moreover the presence of serological and clinical evidence was reported by Taylor (Taylor, 1984). However, the presence of the virus was confirmed in 1990 with c-DNA probe in lymph node and spleen specimens collected from an outbreak in a holding land near Addis Ababa. The source of the animal and probably the disease in this outbreak which resulted in 60% mortality was southwest Ethiopia (Awassa, Yabeo, Borena and Hagaree mariam) (Roeder *et al.*, 1994). PPR virus was isolated from a sheep and was classified in lineage one in 1994/1995 (Roger *et al.*, 1997).

Though the presence of PPR virus was confirmed in Ethiopia, the pattern of the disease needs to be further explored to identify the prevalence of the disease in the different districts and production systems, susceptibility of different breeds and age groups. Thus control of PPR will be justified as a disease which has economical and health drawback that affect the producer and the national economy. Furthermore the temporal distribution need to be predicted in order to pinpoint the ideal time for control measures application.

1.2. Study Objectives

1.2.1.The overall objective

♦Contribution to the knowledge of the epidemiology of PPR in East Shewa, North and South Wollo zones of Ethiopia.

1.2.2.Specific Objectives

1) To estimate sero-prevalence and to assess the potential risk factors (age, species, sex, and season).

2) To gain knowledge about the spatial distribution of the disease.

3) To isolate and characterise the PPRV circulating in Ethiopia.

4) Determination of the economic feasibility of vaccination as a control measure.

2. LITERATURE REVIEW

Peste des petits ruminants is an infectious, contagious disease of domestic and wild ruminants (Furley *et al.*, 1987). It causes a wide havoc in small ruminants in a quite number of African and Asian countries (Shaila *et al.*, 1996). It was first identified in Ivory Coast (Gardenne and Lanlane, 1942 cited by Taylor, 1984) in West Africa where it used to be described with such varied name as Kata, Psuedo-rinderpest, Pneumoenteritis complex and Stomatitis-pneumenteritis syndrome (Hamdy *et al.*, 1976, Braide, 1981). Recently it has received a growing attention because of its wide spread, economic impacts (Leverfevre and Diallo, 1990) and the role it plays in complication of Rinderpest (RP) control and surveillance programmes (Anderson and MacKay, 1994).

2.1. Causative agent:

PPR is caused by a virus that was assumed for a long time to be a variant of Rinderpest adapted to small ruminant (Gibbs *et al.*, 1979). Studies based on virus cross neutralisation and electron microscopy showed that it was a morbillivirus that had the physiochemical characteristic of a distinct virus though biologically and antigenically related to RPV (Gibbs *et al.*, 1979). The extent of antigenic homology between PPRV and the other morbilliviruses is not greater than the homology already exists among them (Gibbs *et al.*, 1979). Subsequent studies based on molecular biology techniques established the marked difference between the two viruses and substantiated its classification along with canine distemper virus (CDV), measles virus (MV), phocine distemper virus (PDV) and porpoise morbillivirus (PV) in the morbillivirus genus (Haas and Barrett, 1996, Barrett *et al.*, 1993, Diallo *et al.*, 1987). A newly isolated virus that affected horses and humans in Australia (Westbury *et al.*, 1995), was classified initially as a morvillivirus, but later on it was found that it could not be allocated to any of the known genera of paramyxoviridae (Williamson *et al.*, 1998).

Morbilliviruses are RNA viruses of 16 kilobase and 200nm diameter (Barret *et al.*, 1993). When viewed through electronmicroscope morbilliviruses display the typical structure of

paramyxoviridae: a pleomorphic particle with a lipoprotein envelope which encloses a helical nucleocapsid that contains the non segmented negative sense RNA genome (Gibbs *et al.*, 1979). Morbilliviruses genome is divided into six transcriptional units encoding two non structural protein (V and C protein) and six structural proteins that include the two surface glycoprotein: hemagglutinin (H-protein) and fusion protein (F protein), the matrix protein (M), nucleoprotein (N) and the phoshoprotein (P) (Barret *et al.*, 1993, Haffar *et al.*, 1999). The phosphoprotein (P) forms the polymerase complex with the large protein (L) of RNA genome with which is associated and functions in transcription and replication (Diallo *et al.*, 1994, Barret *et al.*, 1993, Haffar *et al.*, 1999). A universal morbillivirus primer set, which can amplify all morbilliviruses, was derived from the P-protein (Foryth and Barret, 1995). The N-protein encapsides and protects the RNA (Diallo *et al.*, 1987, Barret *et al.*, 1993). It is almost identical in mobility in polyacrylamide (60K) to that of CDV and MV but different from that of RPV (66-68) (Diallo *et al.*, 1987). The sequencing of PPRV N-protein revealed that PPRV N-protein consists of 1662 nucleotides, while that of both MV and RPV is 1683 nucleotides. Therefore it was deduced that PPRV separated from RPV which was supposed to be the archivirus long before MV (Meyer and Diallo, 1994). Comparison of the nucleic acid and protein sequences of all the morbilliviruses nucleoproteins indicated the presence of two major subgroups; the first subgroup included CDV and PDV and the other subgroup RPV, MV and PPRV which was found slightly more related to CDV and PDV than MV and RPV (Meyer and Diallo, 1994). The M-protein appears to play a central role in the formation of the virions which are liberated from the infected cells by budding; defects in the M-protein leads to persistent, non productive virus infection that was observed in measles and CD (Haffar *et al.*, 1999, Barret *et al.*, 1993). Gene encoding the matrix protein of the PPRV consists of 1466 nucleotides with an open reading frame capable of encoding basic protein of 335 amino acids (Hafar *et al.*, 1999). The hemagglutinin and fusion proteins are responsible for attachment and fusion of host cell membrane, respectively. The H-protein determines host specificity because it recognises receptors on the cell membrane and anchors the virus to them, so antibodies directed against it prevent attachment (Devireddy *et al.*, 1999, Meyer and Diallo, 1999). The F-protein fuses the virus envelope to the host cell membrane, releases the nucleocapsid and facilitates the spread of the virus from cell to cell by inducing syncytia. Thus both F-protein and H-protein play a vital role in cytopathology of virus and infection initiation (Barret, 1999, Devireddy *et al.*, 1999, Meyer and Diallo, 1995, Haffar *et al.*, 1999). The F-protein gene of PPR was found to be 2321 nucleotides long excluding poly (A) tail, it was shorter than RPV and MV F-protein gene by 28 and 56 nucleotide respectively (Meyer and Diallo, 1994).

The comparison of nucleic acid sequences of different morbilliviruses F-gene revealed that the 5-end sequence of the mRNA is specific to each virus, therefore a set of primers was derived from this part for specific identification of each virus (Meyer and Diallo, 1994, Barret and Foryth, 1995). The H-protein of PPRV induces neutralising antibodies and it is assumed to be the least antigenically cross-reactive protein (Barret *et al.*, 1993). With exception of MV H-protein, the PPRV H-protein is the only morbillivirus that hemagglutinates RBCs while the F-protein lysis chicken RBCs (Devireddy *et al.*, 1999).

The immunological relationship between PPRV and RPV was demonstrated by precipitating antibodies, complement fixing antibodies and cross protection between the two viruses (Hamdy *et al.*, 1976, Gibbs *et al.*, 1979). However high neutralising antibody titers occur in the homologous system rather than in heterologous system (Taylor and Abegunde, 1979). A degree of cross protection between CDV and PPRV was reported while no cross protection existed between PPR and MV (Gibbs *et al.*, 1979). Moreover epitopes on the internal virion proteins of RP which were absent from PPR virus were identified with radio-immunoprecipitation specific monoclonal antibodies (McCullough *et al.*, 1991). PPR virus is sensitive to ether and acid and can easily be destroyed with lipid solvent (Lefevre and Diallo, 1990). It can be cultured and produce cytopathic effects with a clock-faced cyncytia in a variety of cell cultures including sheep and goats kidney, vero cells and bovine kidney (Gibbs *et al.*, 1979). However, difficulties in propagation and assessing of syncytia in bovine kidney cell culture were reported (Taylor and Abegunde, 1979). Sheep and goats cells cultures were considered the most sensitive for isolation and assay of the virus (Gibbs *et al.*, 1979, Taylor and Abegunde, 1979), though Rossiter *et al.* (1985) reported that CPE is much easier to detect in vero cell when used in micro-neutralisation.

2.2. Geographical distribution

PPR originally was described in West Africa, but currently it is known to be responsible for morbidity and mortality throughout most of sub-Saharan Africa north of the Equator, the Arabian peninsula, India and many other countries in Asia (Lefevre and Diallo, 1990, Shaila *et al.*, 1996). The virus was isolated in Senegal, Ivory Coast, Guinea (Shaila *et al.*, 1996), Nigeria (Taylor and Abugunde, 1979), Sudan (ElHag and Taylor, 1984), Saudi Arabia (Abu ElZein *et al.*, 1990), India (Shaila *et al.*, 1989, Nanda *et al.*, 1995), Egypt, Israel, Turkey and Pakistan

(OIE, 2000). Serological evidence were detected in Syria, Niger and Jordan (OIE, 2000), while the virus presence was confirmed with cDNA probe and indirect fluorescent antibody technique in Ethiopia (Roeder *et al.*, 1994) and Eritrea (Sumption *et al.*, 1998), respectively. The similarity of PPR and RP always causes confusion and leads to erroneous diagnosis in Asia, however two Sudanese isolates from goats originally identified as RP, ten years latter were found to be PPR viruses (ElHag and Taylor, 1984).

The genetic relation among PPR viruses isolated from different geographical regions was studied by sequence comparison of the F-protein gene of 19 PPR viruses. Phylogenetic analysis based on the former study revealed that there were four biological lineages or groups (Shaila *et al.*, 1996). Lineage one is represented by viruses isolated in Africa in the 1970s (Sudan/Sennar/1972, Nigeria/1975/1, Nigeria/1975/2, Nigeria1975/3, Nigeria/1976/1 and Senegalese strain). It was assumed that lineage one was confined to Africa but recently the same lineage has been isolated from a domestic buffalo (Bubalus bubalis) in India (Govindarajan *et al.*, 1997). Therefore lineage two, which includes viruses isolated in the late 1980s in West Africa (Ivory Coast and Guinea) is the only African lineage that did not cross the sea to the Asian countries (if we assume West Africa as the cradle of PPR). Since lineage three is a combination of isolates from Sudan (Meilig /1972), Oman (Ibri/1983) and southern India (Kulkarni *et al.*, 1996). Though in a comparative study based on neutralisation test and polyacrylamide gel electrophoresis analysis of the nucleocapsid protein of the Oman isolate and the African isolates including Sudan/Meilig/1972, it was concluded that the Oman isolate was not identical to them (Taylor *et al.*, 1990). Lineage four of PPR virus isolates, which includes the Asian isolates from Israel/1994, Iran/1994, Nepal/1995, Bangeldesch/1993 and northern India (Shaila *et al.*, 1996), is confined to Asia. The presence of the two African lineages in Asia beside a distinct Asian lineage may be taken as indication of the route of spread of the disease.

2.3. PPR in Ethiopia

PPR was suspected on clinical grounds to be present in goat herd in Afar region of Eastern Ethiopia in 1977 (Pegram and Tereke, 1981, cited by Roeder *et al.*, 1994). Moreover the presence of serological and clinical evidence was reported by Taylor (1984). However the presence of the virus was confirmed in 1991 with cDNA probe in lymph nodes and spleen specimens collected from an outbreak in a holding land near Addis Ababa. The source of the

animals and probably the disease in this outbreak which resulted in 60% mortality was Southwest Ethiopia (Awassa, Yabeo, Negele, Borena and Haree mariam (Roeder *et al.*, 1994). According to information precited in the internet (Radiscon, 2000) PPR 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. The disease regained its epidemic status between 1994-1996 and moved northward into central Afar region and eastward into Ogaden region. In a sero-survey carried out in 1997 at Debre Zeit abattoir high prevalence of antibodies with a variation among the different production systems was reported (Yayehrade, 1997). Sero-prevalence of antibodies was detected in 85.7%, 32.9% and 43.2% of the samples originated from the nomadic, sedentary farmers and mixed farms respectively (Yayehrade, 1997). The same investigator detected antigen with immunocapture ELISA in 12 samples out of 33. The previous study established an overall sero-prevalence of 46.7% and 32.2% in sheep and goats respectively with a prevalence of 100% in both Somali region (80 samples) and Arsi (50 samples). This result was inconsistent with the finding of Roger and Bereket who reported 53% and 33% in goats and sheep of Arsi, 67% and 0% in goats and sheep of Afarland, 17% and 12% in goat and sheep of North Shewa and 7% and 0% in goat and sheep of Konso (Gelagay, 1996). In an outbreak of a previously unknown respiratory disease in camel, PPRV antigen was identified with immunocapture ELISA (Ic-ELISA) in specimens collected from a morbid camel (Roger *et al.*, 1997). Unfortunately all attempts to isolate the virus in vero and lamb lung cells failed and negative results were obtained with PCR when RPV and PPRV usual primers were used. However the amplification of a fragment was successful when a couple of primers from a conserved region of the RPV and PPRV N-protein was used. Despite the PCR and the Ic-ELISA positive results, virus isolation was not successful and two other pathogens (Strept. equi was isolated and mycoplasma was identified with Dot-blot) were identified in specimens from the same outbreak (Yigezu *et al.*, 1995). During the same outbreak and from the same area PPRV was isolated from a sheep and classified in lineage one.

An increase in virus activity in October and March was proposed based on detection of sero-conversion (Yayehrade, 1997, Gelagay, 1996).

2.4. Epidemiology of PPR

2.4.1. Disease transmission

PPR spreads by direct contact mainly (Braide, 1981), substantial amount of the virus is shed in the ocular, nasal and oral secretion and the faeces of sick animals (Bundza *et al.*, 1988). The virus was also demonstrated in the saliva and the urine (Obi and Patrick, 1984). While infected fomites such as bedding may contribute to the onset of an outbreak, most contact infection occurs through inhalation of infectious aerosol that probably produced by combination of sneezing and coughing at the height of the disease (Taylor, 1984). Indirect transmission seems to be unlikely in view of the low resistance of the virus in the environment and its sensitivity to lipid solvent (Lefevre and Diallo, 1990).

2.4.2. Host range

PPR mainly is a disease of small ruminants; it affects both sheep and goats. Goats are severely affected while sheep undergo a mild form of the disease. In Asia PPR is confused with RP which is well known to be adapted to small ruminants there, a thing which is uncommon in Africa though serological evidence in western Kenya (Rossiter *et al.*, 1982) and the isolation of the virus from a goat in Sudan (Babiker, 1973, ElHag and Taylor, 1988) were reported. Cattle and pigs are known to be a dead end host and all attempt to induce clinical disease in adult cattle experimentally failed; they undergo a silent or subclinical infection that protect them against subsequent challenge with virulent strain of RP (Gibbs *et al.*, 1979, Taylor, 1984). However clinical disease was produced experimentally in calves and raised temperature, erosion and death were clearly demonstrated (Mornet et al, 1956 cited by Taylor, 1984).

It has been assumed for a long time that PPR does not affect cattle in contact with small ruminants infected with PPR under field condition. However, a recent report has revealed that cross infection occurred in cattle in contact with sheep and goats infected with PPR and antibodies due to this silent infection interfere with the RP vaccine induce immunity leading to

underestimation of herd immunity level against RP when assessed by competitive ELISA in sero-monitoring programme (Anderson and Mackay, 1994). PPR also affects wildlife animals both under field condition and experimentally. The disease was induced experimentally in American white deer (Odocoileus Virginianus) which was found to be susceptible (Daridari and Hamdy, 1975) and a field outbreak was reported from a zoological collection in Alain (Furley *et al.*, 1987). It caused a high mortality and severe disease in Dorcas Gazelles (Gazella dorcas), Nubian Ibex (Capra ibex nubiana), Laristan sheep (Ovis orientalis laristanica) and gemsbok (Oryx gazella). Subclinical involvement of Nigale (Tragelaphinae) was suspected. In another report from Saudi Arabia PPR was suspected on clinical and serological base in Gazalle and deer (Abu ElZein *et al.*, 1990). Isolation of PPR virus from a domestic buffalo (Bubalus bubalis) in Nadu Tamil state in Southern India (Govindarajan *et al.*, 1997) extended the host range of PPR to include the domestic buffalo. Camels also can be held as a potential host for PPR, though the Koch postulates in camel respiratory disease outbreak has not yet been fully fulfilled (Roger *et al.*, 1997). Serological evidences for the presence of PPR antibodies were detected in 4.2% of 142 healthy camels with sero-neutralisation test (Roger *et al.*, 1997).

2.4.3. Pattern of the disease

There are considerable differences in the epidemiological pattern of the disease in the different geographical areas. In the humid Guinean zone where PPR occurs in an epidemic form, it may have dramatic consequences with morbidity of 80%-90% accompanied with a mortality between 50 and 80% (Lefevre and Diallo, 1990). While in arid and semi-arid regions, PPR is seldomly fatal but usually occurs as a subclinical or inapparent infection opening the door for other infections such as Pasteurellosis (Lefevre and Diallo, 1990). In southern Nigeria the owners claimed that the disease used to occur in waves every 3 to 5 years and it caused morbidity of 62% and case mortality of 54%. This is in contrast to the records of Ibadan university which showed that PPR may be encountered around the year except in January and February with half of the cases in the wet season (Taylor, 1984). Though it was believed that outbreaks in West Africa coincide with the rainy season, Opasina and Putt (1985) observed outbreaks during the dry season in two different ecological zones. However this picture of the disease is different in East and North Nigeria where large epidemics may occur with distinct inter-epizootic period (Taylor, 1984). Investigation of three outbreaks in South-west Nigeria showed overall attack rate of 42.4%, 13.7% and 37.1 and case fatality rate of 86.9%, 41% and 63.9% respectively (Opasina and Putt, 1985). In India morbidity and case fatality reach 10 and

25% respectively in flocks of indigenous sheep (Shaila *et al.*, 1989), however PPR was associated with an outbreak that caused 96% case fatality in domestic buffalo in South India (Govindarajan *et al.*, 1997). A high morbidity of 90% accompanied with 70% case mortality was also reported from Saudi Arabia (Abu ElZein *et al.*, 1990). While the average case fatality in a number of African countries (Ivory Coast, Egypt, Mali) was estimated to be 30%, 16%, 41%, 27% for the years 85, 86, 87, 88, respectively (Lefevre and Diallo, 1990), sero-prevalence of 53% and 63% in sheep and goats respectively was reported from Niger (Stem, 1993) and 30-40% prevalence of antibodies suggesting a previous exposure and recovery was reported from Nigeria (Taylor, 1984). Sero-prevalence of 23% and 24% in sheep and goats respectively was reported from Oman, with an increase in antibodies prevalence with age a thing that could be taken as indication of the endemicity of the disease (Taylor *et al.*, 1990). Serological data from Nigeria revealed that antibodies occur in all age groups from 4-24 months indicating a constant circulation of the virus (Taylor, 1984), a finding that was established in Oman where infection persisted on a year round basis maintaining itself in the susceptible yearling population (Taylor, 1990). Therefore an increased incidence reflects an increase in number of susceptible young goats recruited into the flocks rather than seasonal upsurge in the virus activity since its upsurge pend on the peak of kidding (Taylor, 1990). Moreover the susceptibility of young animals aged 3 to 18 months was proved to be very high; being more severely affected than adults or unweaned animals (Taylor *et al.*, 1990). It was also reported that prognosis improved with increasing age (Lefevre and Diallo, 1990). Furthermore statisticai significant differences were observed in three different outbreaks between attack rates in different age groups with animals 5-8 months being the worst (Opasina and Putt, 1985). However in an outbreak in Saudi Arabia all age groups and both sexes were involved (Abu ElZein *et al.*, 1990). Several authorities reported that outbreaks were facilitated by closed confinement of animals and introduction of new stock into the existing flock (Braide, 1981, Taylor, 1984). In addition to that high level of sero-prevalence was detected in animal living in restricted enclosure compared to animal with limited restriction where high prevalence was observed in the latter (Taylor *et al.*, 1990). Also a large outbreak associated with the movement of large animals during the major festival in Kumsi region of Ghana was observed (Taylor, 1984). In Africa sheep generally are held to be less severely affected though serological data revealed that infection is equally common (Taylor, 1979a). Also an outbreak with a high mortality in sheep in Nigeria and Ghana was reported by Taylor (1984) who hypothesised that sheep possessed an innate resistance to the clinical effects of disease, but occasional field strains could overcome this resistance and produce high mortality.

Breed may affect the epidemiology of the disease. The Guinean breeds (West African dwarf, Iogoon, kindi and Djallonke) are known to be highly susceptible (Lefevre and Diallo, 1990). This is in agreement with the finding that British breeds exhibited severe clinical reaction when infected experimentally while the Sudanese breeds failed to develop a characteristic clinical response (ElHag and Taylor, 1984). No significant difference in the case fatality rate and attack rate between sex groups was observed (Opasina and Putt, 1985). Variation in the different ecological zones, can be explained by greater resistance of paramyxoviridae in regions of low relative humidity which means that PPR will survive longer in dry regions (Lefevre and Diallo, 1990).

2.5. Clinical signs

After the entry of the virus through the respiratory tract system, it localises first in the pharyngeal and mandibular lymph nodes as well as tonsil. 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, cited by Bundza *et al.*, 1988).

PPR is an acute or subacute disease of goats and sheep characterised by fever, erosive stomatitis, conjunctivitis, gastro-enteritis and pneumonia (Taylor, 1984). The course of the disease takes 5-6 days, with an incubation period of 5 days (Lefevre and Diallo, 1990, Braide, 1981, Taylor, 1984). The first indication of infection is a pyrexia, with a body temperature between 40-42°C, followed by anorexia and general signs of gross illness and subsequently by irritation of the pharynx and larynx, ulcerative gingivitis and diarrhoea (Braide, 1981). There are also mucopurulent discharges from the nostrils, conjunctivitis and ulceronectrotic stomatitis (Braide, 1981) and enterocolitis similar to that caused by RP (Bundza *et al.*, 1988). During the febrile phase oral lesion, diarrhoea and dyspnoea were the prominent signs (Brown *et al.*, 1991). In an outbreak in a zoological collection in Alain, with a few exceptions animals were found dead and the affected animals suffered or appeared to have suffered from diarrhoea, often blood stained, dark and putrid. The affected animals had lymphocytopenia, elevated PCV (above 60% while normal 35-45%), very high RBCs count while the level of haemoglobin and the white blood cells was normal (Furley *et al.*, 1987). Other clinical signs observed in PPR cases included tachypnoea, dyspnoea, corneal opacity, engorgement and erosion of the vulva

and soft moist, productive cough (Obi *et al.*, 1980, Brown *et al.*, 1991). The mean respiratory rate (plus or minus the standard deviation) reported from an outbreak in Olukosi in Nigeria was 79±7 (range 30-120), while 55±3 was reported from another place in Nigeria (Obi *et al.*, 1983). The clinical picture in India is not different, however kids showed only diarrhoea and nasal discharges before death (Kulkarni *et al.*, 1996). Conjunctivitis and corneal opacity was also observed, a symptom, that was assumed not to be a feature of PPR but mostly caused by secondary infection with Moraxella (Obi et al, 1983). In the only reported outbreak of PPR in domestic buffalo; congestion of conjunctiva, profuse salivation and depression were the only clinical signs observed. Buffalo calves infected developed only pyrexia 6 days after infection and died 30 days later exhibiting haemorrhagic and oedematous abomastitis in postmortem (Govindarajan *et al.*, 1997). Generally it is thought that PPR induces initial damage in the pulmonary region that may be modified by secondary bacteria such as Pasteurella haemolytica, Staphyloccus pyogene and E.coli (Brown *et al.*, 1991, Lefevre and Diallo, 1990). Also adenoviruses were isolated along with PPRV from two goats died from PPR in separate outbreaks (Gibbs and Taylor, 1977), but according to the authors adenoviruses, as commensal had no significant role in epidemiology and pathogenesis of the disease.

Necrosis of the alimentary tract epithelium, degeneration and proliferation in respiratory epithelium, depression of lymphoid tissues and the occurrence of intranuclear and intracytoplasmic inclusion bodies in epithelial tissues were constant features in histological sections (Braide, 1981, Bundza *et al.*, 1988). Brown *et al.* (1991), using immunohistochemical methods, detected viral antigen in both cytoplasm and nuclei of tracheal, bronchial and bronchiolar epithelial cells, type II pneumocytes, cyncytial cells and alveolar macrophages.

Based on the postmortem lesions encountered in dead or necropsised animals three clinical form of PPR were described (Braide, 1981). The peracute form results in death with no visible postmortem lesion other than congestion of the ileo-caecal valve and secondary pneumonia. The acute form presents with congestive stomatitis, acute gastritis and oedema of the lymph nodes (Braide, 1981). A more broader classification identified almost the same three form of the disease was proposed by Lefevre and Diallo (1990) as follows

1-Hyperacute form

Occurs frequently in goats in which elevated temperature is the first sign after two days incubation period, followed by apathy, anorexia, nasal and lachrymal discharges. There may be

constipation during the first days that gives the way to profuse diarrhoea. The prognosis is not good and it leads to death within 5-6 days.

2-Acute form

Incubation period is 3-4 days and the initial stages are identical with those of hyperacute form accompanied with sero-mucous nasal discharges, which become mucopurulent. Erosive and ulcerative involvement and complication which depend on the bacterial superinfection particularly P.haemolytica (Mannheimia haemolytica) or P.multocida type A may be observed. Also latent parasitic infection such as coccidiosis, piroplasmosis and trypansomosis may be reactivated.

3-Subclinical or Inapparent forms

This form seems to be prevalent in certain regions where the local breeds and animals have innate resistance. The disease course is 10-15 days with inconstant symptoms. At the late stage pulmonary involvement usually occurs with a dry cough, which rapidly become loose. Some papules and pustules similar to those of contagious ecthyma may appear, but the infection in general is complicated by respiratory diseases.

2.6. Immunity

Sheep and goats are unlikely to be infected more than once in their economic life (Taylor, 1984). Kids receiving colostrum from previously exposed does or does vaccinated with RP tissue culture vaccine were found to acquire a high level of maternal antibodies that persist for 3-4 months. Therefore the age of 3 months was proposed as optimum time for vaccinating kids (Ata *et al.*, 1989). However another study showed that the maternal antibodies were detectable up to 4 months using virus neutralisation test compared to 3 month with competitive ELISA (Libeau *et al.*, 1995). Measles vaccine did not protect against PPR, but a degree of cross protection existed between PPR and canine distemper (Gibbs *et al.*, 1979).

2.7. Diagnosis

2.7.1. Antigen detecting methods

Various techniques were applied in the diagnosis of PPR. The most commonly used were virus neutralisation, agar gel diffusion test, virus isolation in cell culture and immunohistochemistry But these techniques were either time consuming, labour intensive, expensive or lack specificity and were unable to differentiate between PPR and RP because they used polyclonal antibodies (Diallo *et al.*, 1995). However the development of an immunocapture (Libeau *et al.*, 1995) and a sandwich ELISA (Saliki *et al.*, 1994) overcame many of these obstacles and facilitated the differention between PPR and RP which used to rely on virus isolation and its characterisation with several tests including differential neutralisation (Abegunde and Taylor, 1979) and electrophoretic profile in polyacrylamide gel electrophoresis (PAGE) (Diallo *et al.*, 1987). In PAGE the differentiation was based on the estimation of migration of nucleoprotein from infected cells (Diallo *et al.*, 1987). A cDNA probe was developed for differentiation between PPR and RP (Diallo *et al.*, 1989, Pandey *et al.*, 1992), but polymerase chain reaction (Foryth and Barret, 1995) proved to be more useful and sensitive in differentiation between PPR and RP and easy to perform (Diallo *et al.*, 1995). Biological characterisation of PPR virus isolates by inoculation of sheep and goats, which show a characteristic profile of clinical signs and cattle, which undergo silent infection, was practised for a long time.

2.7.1.2. Agar gel immunodiffusion test

It is widely used and can detect PPR antigen in 42.6% of antemortem specimens and necropsy specimens (Obi and Patrick, 1984). It can be used to test the presence of both antigen and antibodies and can detect antigen in 54.4% of ocular and buccal specimens and 63.6% of the nasal and faecal samples. Moreover it was observed that it could detect antigen in 75-100% of specimens collected 4-5 days after the onset of fever and could give results in 2-4 hours when RP hyperimmune serum was used while it needed 4-6 hours with PPR hyperimmune serum (Obi and Patrick, 1984). Agar gel immunodiffusion (AGID) is a very simple and cheap test that can be performed in any laboratory and even in the field. Standard PPR viral antigen is

prepared from mesenteric or bronchial lymph nodes, spleen or lung materials ground as 1/3 suspensions in buffered saline and centrifuged at 500g for 10-20 minutes. After that the supernatant fluids are stored in aliquots at -20°C (OIE, 2000) and can be retained for 1-3 years. Control antigen is prepared similarly from normal tissues. Standard antiserum is made by hyperimmunising sheep with 5 ml of PPR virus with a titer of 104 TCID50 (50% tissue culture infective dose) per ml given at weekly intervals for 4 weeks. The animals are bled 5-7 days after the last injection (Obi and Patrick, 1984). Standard RP hyperimmune antiserum is also effective in detecting PPR antigen (OIE, 2000). Results are obtained in 1 day, but the test is not sensitive enough to detect mild forms of PPR. Test protocol can be found in the material and methods.

2.7.1.2. Counter immunoelectrophoresis

Counter immunoelectrophoresis (CIEP) is the most rapid test for viral antigen detection. 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 onto microscope slides in 3ml 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 (Obi and Patrick, 1984; OIE, 2000).

2.7.1.3. ELISA for antigen detection

A monoclonal antibody (mAb) based sandwich ELISA which employed monoclonal antibodies directed against the H-protein of PPRV was found to be highly sensitive in detection of antigen in tissues and secretions of infected goats (Saliki *et al.*, 1994). When it was compared with virus isolation in vero cell using 89 paired samples of tissues and secretion from six experimental animals, it was found more sensitive (71.9% in ELISA versus 65.2% in Virus isolation, $p<.05$) (Saliki *et al.*, 1994). It was used to detect antigen in lachrymal, nasal and oral

swabs, peripheral blood lymphocyte 6 days post infection and from brain, tonsil, lung, mesenteric lymph nodes, liver, spleen, small intestine, colon, caecum and kidney 8 days post infection (Saliki *et al.*, 1994). Another format of antigen ELISA which is more widely used is immunocapture ELISA. It utilised mAb directed against the nucleocapsid protein (Libeau *et al.*, 1995). It can give a reliable result within one hour in precoated plates and from samples maintained at room temperature for a period of seven days with no more than 50% reduction in response (Libeau *et al.*, 1995). The immuncapture ELISA (Ic-ELISA) allows a rapid differential identification of PPR or rinderpest viruses, and this is of great importance as the two diseases have a similar geographical distribution and may affect the same animal species (OIE, 2000). The detecting mAbs used in Ic-ELISA are directed against two non overlapping domains of the N-protein of PPR and RP, but the capture antibody detects an epitope common to both RP and PPR (Libeau *et al.*, 1995). The test is very specific and sensitive, it can detect 100.6 TCID50/well for the PPR virus and 102.2 TCID50 for the rinderpest virus.

2.7.1.4. Virus isolation

PPRV could be grown on different types of cell cultures. The sensitivity of virus isolation technique could be increased when the virus is grown in lamb and goats kidney cells (Taylor, 1984). Vero cell is widely used for its continuity and low liability of contamination; bovine kidney cells (BK) are also used though Taylor (1979) failed in isolation of PPRV Nigerian strain 75/1 initially but later on he succeeded in adapting it to the BK. T-lymphoblast cell line transformed by Theileria parva was used for RP isolation (Rossiter *et al.*, 1992). It proved to be more sensitive when compared to other cell culture system and gave a result within 24 hours (at least 6 days for other cells culture). It gave also a similar result with PPRV (Rossiter *et al.*, 1992).

2.7.1.5. cDNA probe

For the differentiation between PPR and RP, the use of [P^{32}]-labelled cDNA probe derived from the N-protein gene of the two viruses has been described (Diallo *et al.*, 1989). It could differentiate between the two viruses without need for virus isolation. cDNA directed against the matrix protein, fusion protein and phosphoprotein gene were found to cross hybridise to a much greater extent and were not suitable for use as discriminating probe (Diallo *et al.*, 1989). Unfortunately this hybridisation can not be used widely because it can only be used for fresh

specimens in addition to the short half life of [P^{32}] and the risk of handling isotopes (Diallo *et al.*, 1995, Pandey *et al.*, 1992). Therefore probes using non-radioactive materials such as biotin (Pandey *et al.*, 1992) or digoxin (Diallo *et al.*, 1995) as a label were developed. The biotin labelled cDNA was found to be as sensitive as the one used the radioactive label and more rapid in differentiation between PPR and RP (Pandey *et al.*, 1992). However it was reported that the expected sensitivity had never been obtained using non-radioactive labels (Diallo *et al.*, 1995).

2.7.1.6. Reverse transcription polymerase chain reaction (RT-PCR)

Reverse transcription-polymerase chain reaction test (RT-PCR) using phosphoprotein (P) universal primer and fusion (F) protein gene specific primer sets to detect and differentiate between PPR and RP were described (Foryth and Barret, 1995). It was found to be sensitive and could detect antigen 4 days post infection (immunocapture ELISA 6 days). It could be used for diagnosis once the RNA has been extracted from the sample. After extraction the RNA genome of PPR virus has first to be copied into cDNA using reverse transcriptase enzyme prior to amplification. Another set of primers has been prepared based on the sequence of the 3' end of the gene coding for the N protein (OIE, 2000). With these primers, specific amplification of 300 base pair fragment is possible. The specificity of the amplified fragments is confirmed by restriction enzyme digestion (Rsa 1 cuts only RP and PPR virus fragments) or by hybridisation with a non-radioactive oligonucleotide used as a probe. The PCR is sensitive and results are obtained in 5 hours including the RNA extraction (OIE, 2000). PCR can be used to amplify the virus RNA in gum and lachrymal swabs transported in phosphate buffer containing penicillin, streptomycin and fugizone, whole blood and post-mortem specimens. In brief, PCR can be carried out as follows

RNA isolation

RNA is extracted using the rapid acid quanidinium thiocyanate method (Forsyth and Barrett 1995). 1gram of lymphoid tissues is finely minced and homogenised in 4ml of denaturing solution (solution D) containing quanidinium thiocyanate, sodium citrate and ß-2-mercaptoethanaol before the addition of sodium acetate and subsequent extraction with phenol-chloroform-isoamyl alcohol. RNA is precipitated by the addition of 2.5 volume of cold ethanol. For the lachrymal, mouth and gum swab samples 1ml is vortexed in 2ml of solution D before a similar extraction and precipitation of the RNA.

Peripheral lymphocytes from 5 to 10ml of whole blood are purified on Ficoll gradients and the cell pellets taken up in 0.4 ml of solution D and extracted using the above method.

Reverse transcription

-RNA (1-5 µg) is heated at 70°C for 5minutes with either 75ng random hexanucleotide mix or 10ng P or F gene specific primer.

-The mixture is cooled to room temperature for 10min and reverse transcribed with 50mM Tris-HCl, 75 mM KCl, 10mM DTT, 3mM $MgCl_2$, 500µM dNTPs, acetylated bovine serum albumin (1mg/ml) and 200 units MoMuLV reverse transcriptase at 37°C for 45min.

-With the random primer reverse transcription, a 5µl aliquot of the reaction is taken for amplification whereas with the P or F gene-specific primers the total RT is used.

-The cDNA is amplified in a mixture containing 20Mm Tris-HCl (pH 8.4), 50mM KCl, 1.5mM $MgCl_2$, 200µM dNTPs, 2.5U DNA Tag polymerase and 100pM P (mRNA sense) or F (virus RNA) gene-specific primers set.

UPPF, 5′-ATGTTTATGATCACAGCGGT 3′ at position 374-393

UPPR, 5′-ATTGGGTTGCACCACTTGTC-3′ at position 783-802

PPRV F1, 5′-ATCACAGTGTTAAAGCCTGTAGAGG 3′ at position 777-801

PPRV F2, 5′-GAGACTGAGTTTGTGACCTACAAGC 3′ at position 1124-1148

RPV F3, 5′-GGGACAGTGCTTCAGCCTATTAAGG 3′ at position 815-839

RPV F4, 5′-CAGCCCTAGCTTCTGACCCACGATA 3′ at position 1162-1186

The nested F-gene specific primers are:

PRRV F1, 5′-ATGCTCTGTCAGTGATAACC 3′ at position 802-821

PRRV F1, 5′-TTATGGACAGAAGGGACAAG 3′ at position 1092-1110

RPV F3, 5′- GCTCTGAACGCTATTACTAAG 3′ at position 842-862

RPV F3, 5′- CTGCTTGTCGTATTTCCTCAA 3′ at position 1056-1076

–The thermal cycle for the above mixture is:

Steps 1: 1 cycle-95°C for 5 min.

Step 2: 30 cycles-94°C for 1 min, 70°C for 2min.

Step 3: 70°C for min.

-5µl of the PCR product is run on a 1.5% agarose gel containing 2.5µg/ml ethidium bromide and visualised under UV light for the presence of DNA bands of the expected size.

2.7.2.Serology

Many tests were used for demonstration of PPR antibodies in serum, microvirus neutralisation test, agar gel diffusion test, immunoelectrophoresis, counter-currentosmophoresis and recently blocking and competitive ELISA.

2.7.2.1. Virus neutralisation

This test is sensitive and specific, but it is time-consuming and expensive. The standard neutralisation test is carried out in roller-tube cultures of primary lamb kidney cells or vero cells when primary cells are not available (OIE, 2000). Serum against either PPR or RP may neutralise both viruses, but would neutralise the homologous virus at a higher titer than the heterologous virus. Therefore for differentiation purpose reciprocal cross neutralisation is used (Abegunde and Taylor, 1979). Previous studies suggested that 1 in 20 dilution of serum would be higher than the heterologous titer of most sera to either PPR or RP viruses (Taylor, 1979a). The test can be carried as follow (OIE, 2000)

i) Dilute inactivated sera in a two-fold dilution series and mix with a stock of virus suspension containing approximately 103 TCID50/ml.

ii) Incubate the virus/serum mixtures either for 1 hour at 37°C or overnight at 4°C.

iii) Inoculate 0.2 ml of the mixture into each of five roller tubes, followed immediately by 1 ml of vero cell suspension in growth medium at a rate of 2x105 cells/ml.

iv) Incubate the sloped tubes for 3 days at 37°C.

v) Discard the tubes showing virus-specific CPE; replace the medium in the remaining tubes with maintenance medium and roll the tubes for a further 7 days. The virus challenge dose is acceptable if it falls between 101.8 and 102.8 TCID50/ tube. Any detectable antibody at a dilution of 1/8 is considered to be positive.

2.7.2.2. ELISA

Competitive and blocking ELISAs based on monoclonal antibodies specific for N-protein (Libeau *et al.*, 1995) and H-protein respectively (Anderson *et al.*, 1991, Saliki *et al.*, 1993) were developed for detection of antibodies in animal sera. Two test using PPR mAb specific for H-

protein have been described, a competitive ELISA (Anderson *et al.*, 1991) and a blocking ELISA (Saliki *et al.*, 1993). In the N-protein C-ELISA, the serum antibodies and the mAb compete on specific epitope on nucleoprotein obtained from spodoptera frugipeda (Sf9) infected with PPR nucleoprotein recombinant baculovirus. The relative sensitivity of this method to VNT was 94.5, while the relative specificity was 99.4%. Despite the fact that neutralising antibodies are not directed against the N-protein, but the H-protein (Diallo *et al.*, 1995) a correlation of .94 between VNT and the C-ELISA was observed (Libeau *et al.*, 1995). Though no cross-reaction in N-protein C-ELISA was reported, a high level of competition up to 45% was observed among the negative (Libeau *et al.*, 1995). Both blocking ELISA and C-ELISA are based on competition between mAb and serum antibodies, but in case of blocking ELISA the test sera are preincubated with a solid phase PPR-H antigen and then incubated with mAb (Saliki *et al.*, 1993). The sensitivity and specificity of the 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 *et al.*, 1991, Anderson and Mckay, 1994). Majayabe (1994) reported a cross-reaction of 11.4% of 702 samples collected from apparently normal and non vaccinated sheep and goats, but 0% (0/30) in small ruminants and 55% in bovine sera (21/38) was reported (Anderson and Mckay, 1994). The absorbance in PPR ELISA is converted to percentage of inhibition (PI) using the formula

$$PI=100-\frac{\text{absorbance of the test wells}}{\text{absorbance of the mAb control wells}}X100$$

Sera showing PI greater than 50% are designated positive.

2.8. Prophylaxis

Immunisation of small ruminants with lymph node and spleen materials containing virulent virus inactivated with 1.5-5% chloroform was tried and the animals were immune to subsequent challenge 18 months later (Braide, 1981). Vaccination of animals with RP tissue culture vaccine has been practised for a long time, the vaccine protected the animals for 12 months and the animals were not able to transmit the infection following challenge with PPR virus (Taylor,

1979b), though the antigen was detected in lachrymal swabs from vaccinated animals after challenge with virulent virus (Gibbs *et al.*, 1979). Sera from animals vaccinated with RP vaccine contain substantial level of RP antibodies with little or no cross-neutralising antibodies to PPR but after challenge with PPR, neutralising antibodies to PPR increase sharply. The same thing was observed in dogs vaccinated against CDV with MV vaccine which was explained as some sort of delayed hypersensitivity between MV and CDV (Taylor, 1979b). Also RP thermostable vaccine was used for protection of goats against PPR (Stem, 1993). But now homologous PPR vaccine attenuated after 63 passage in vero cell is used and it produces a solid immunity for 3 years (Diallo *et al.*, 1992). However it was reported previously that considerable residues of virulence were detected after 32, 42, even 65 serial passage in embryonic lamb kidney cells (Benazet, 1973, cited by Taylor, 1979b). But the homologous vaccine was found to be safe under field condition even for pregnant animals and it induced immunity in 98% of the vaccinated animals (Diallo *et al.*, 1992). The same vaccine has been tried for protection of cattle against RP and it was found very effective (Couacy-Hymann *et al.*, 1995).

2.9. Disease economy

Due to the confusion with other diseases, the economic impact of PPR are underestimated, but it is believed that PPR is one of the major constraints of small ruminants in the tropic (Taylor, 1984). The loss due to PPR in Nigeria was estimated to be 1.5 million dollar annually (Hamdy *et al.*, 1976). An economic analysis for assessing benefits of vaccination against PPR in Niger revealed that such a programme was highly beneficial with an anticipated net present value (NPV) return in five years of 24 millions dollars internal rate of return (IRR>900%) following an investment of two millions dollars (Stem, 1993). Based on assumption that goats experienced an outbreak every 5 years, Opasina and Putt (1985) estimated an annual sum ranging from 2.47£ per goat at high loss and .36 £ per goat at lowest loss could be profitably spent in the successful prevention of the disease.

3.MATERIAL AND METHODS

3.1. Study design

The study consisted of three parts. A retrospective panel study (RPS) based on the analysis of serum bank and the data obtained through a questionnaire means, an active surveillance (AS) with ultimate aim of antigen detection and virus isolation and a retrospective analysis of data collected by the veterinary service (RVET).

3.1.1. Retrospective panel study (RPS)

It was a retrospective panel study not a cross sectional study, because it was based mainly on the results of testing serum bank (1073 sera). The sera collection dates span over time interval, so the final sample did not refer to a population at one given point of time. Retrospective means historic sampling dates back into the past. Panel means a collection of samples whereby we did not have control about the sampling procedures and purpose of the sampling was not related to our study. Since the parameters to be cross-classified in the analysis were not considered during the sample collection, post-hoc analysis of the data generated by testing 1073 sera previously collected from the three administrative zones (East Shewa, North and South Wollo) of Ethiopia. RPS aimed at estimation of sero-positivity in E.Shewa, North and South Wollo zones, the sero-positivity in the different strata of the population and the spatial distribution of the disease in the three zones. RPS was based on the outcome of testing sera selected from the serum bank at the National Animal Health Research Centre at Sebeta. The sera were collected during the year 2000 by veterinary service in the three zones in order to identify the sero-prevalence of contagious caprine pluerapneumonia (CCP) and Rift Valley Fever (RVF). It was stored in deep freeze at –20 C° at the National Animal Health Research Centre in Sebata. 1073 sera were selected randomly by the technicians at the National Animal Health Research Centre from a total of 3990 sera that covered the three zones.

The sera selected from the serum bank in addition 399 sera collected during the active search for the disease were tested with C-ELISA. The combined sera result was used to estimate the sero-positivity and the potential risk factors associated with its distribution.

Table 1 Summary of the serum samples tested in East Shewa, North Wollo and South Wollo in the combined sample (RPS and AS)

District	Total	Sheep	Goats	Female	Male	<=1 year	1-2 years	2-3 Year	>3 years
East Shewa	620	222	396	538	78	89	181	193	144
North Wollo	584	301	283	471	108	23	52	269	240
South Wollo	268	250	16	243	24	4	10	152	101
Total	1472	773	695	1253	210	117	238	615	491

a. Species missing identification were 2 (E.Shewa), 2 (S.Wollo).

b. Sex missing identifications were 4 (E.Shewa), 5 (N.Wollo), 1 (S.Wollo).

c. Age missing identifications were 13 (E.Shewa), 1 (S.Wollo).

3.1.2. Active surveillance (AS)

This part of the study aimed at collecting data from the owners or workers on one hand and pave the way for identification of the virus lineage circulating in Ethiopia on the other hand. Unfortunately no clinical cases were found and the isolation and characterisation of the virus was not possible. During the active search for the disease 399 sera were collected, these sera were used in combination with the serum bank sera to determine the sero-posititivity in the three zones and the different strata of the population and the risk factor associated with its distribution. The study area was selected based on the previous reports to the veterinary service. In this phase of the study specimens for laboratory examinations were collected from individuals that exhibited the clinical signs in the three zones, while the herd owners or workers were interviewed. Lachrymal, nasal and faecal swabs and necropsy specimens were collected from cases according to the following definition of the disease:

PPR is a severe, fast-spreading disease of mainly domestic small ruminants. It is characterised by the sudden onset of depression, fever, discharges from the eyes and nose, sores in the mouth, disturbed breathing and cough, foul-smelling diarrhoea and death (FAO 1999).

The specimens were transferred on melting ice to the laboratory where parallel testing was carried out. All field specimens were tested with both Agar gel immunodiffusion test (AGID) (see section 3.3.4.) and immunocapture ELISA test (see section 3.3.5.) for detection of PPR antigen The specimens were processed with Ic-ELISA because AGID is less sensitive and can not differentiate between RP and PPR antigen. A form that provided information about the number of cases, herd size, species affected, mortality, morbidity..etc, was submitted with the specimen. (Annex 1). Table 2 displays the specimens collected from suspected cases during the active search for the disease. The villages covered during the active search for the disease are shown in annex 4.

Table 2 The specimens collected during the active search for the disease

ID	Specimen	Location	Species	ID	Specimen	Location	Species
1	ES	Kobo	Goat	8	NS and LS	Kotabar-Towea	Sheep
2	NS	Heik-Alasha	Sheep	9	FS	Kotabar-Towea	Sheep
3	FS	Bati-Burka	Goat	10	Lung and spleen	Bati-Burka	Goat
4	FS	Kobo-Manderfa	Goat	10	Mediastinal L.node	Bati-Burka	Goat
5	NS	Kobo	Goat	10	Prescapular L.node	Bati-Burka	Goat
6	NS and FS	Kotabar-Towea	Sheep	11	NS and LSl	Chafa Robit	Sheep
7	NS and FS	Adami tulli-Arba	Goat	12	NS and FS	Fentale	Goat

ES= eye swab, NS= nasal swab, FS=faecal swab, LS=lacrymal swab

3.1.3.Restrospective analysis of veterinary service records (RVET)

Data about the pattern of the disease and its frequency were deduced from the veterinary service records for the last three years. The analysis of veterinary service records was conducted in order to identify the impact of the disease and to obtain information to determine the economic feasibility of launching a control programme. Records for 18 outbreaks were found in the veterinary service headquarter database in the Ministry of Agriculture, Addis Ababa. All the 18 outbreaks were reported to the OIE. Detailed account about these outbreaks can be found in annex 2. This data was used to calculate the average proportion of affected animals, average proportion of death among affected animals and average proportion of death and to identify the time trend of the disease. The time trend of the disease was compared with the questionnaire-generated data.

3.2. Description of study site and sampling procedure

3.2.1.Description of study site

This study was conducted in East Shewa zone and the former province of Wollo, which is currently divided into North, and South Wollo zones. Serum samples that covered the three zones were tested for sero-positivity estimation of PPR antibodies. 1073 sera were selected from the serum bank at the national research centre in Sebeta and 399 sera were collected during the active search for the disease. Moreover a questionnaire was filled by the owners of the herds whose herds were examined for the presence of clinical cases in the three following zones

East Shewa

It is located between 8°9′ and 9°45′ N latitude and 38°45′ and 40°18′ E longitude with an area of 12754 Km^2. The altitude of the region ranges between 700 meter to 1300 meter above sea level. The livestock population is comprised of 989270 cattle, 178010 sheep, 313940 goats, 125137 equines and 18282 camels (Agricultural Samples survey 1999).

Wollo

South and North Wollo cover an area of 21200 and 28600 Km^2 respectively with three agro-ecolgical zones. Only the higher altitude (2500-3500) is inhabited purely by sedentary farmers, other areas are inhabited by sedentary, semi-nomadic and nomadic pastoralists subsisting on livestock raising. The sheep population is 437580 and 1018740 in North and south Wollo respectively. While goats population is 215550 and 518860 respectively with a range of 10-15 animals per house hold in the lowland and 20-100 animals in the high land, however a communal grazing field of 2500 heads in Gimba and Quana areas in Wollo were reported by Sisay (Sisay 1997). Sheep and goats population is shown in Table 3.

Table 3 The population in the zones of Wollo and E.Shewa

Zone	Goats Population	Sheep Population
East Shewa	313940	178010
South Wollo	518860	1018740
North Wollo	215550	437580

Source: (Agricultural Samples survey 1999)

3.2.2. Sampling techniques and sample size determination

1073 serum samples from the three zones were selected from the veterinary service serum bank at the national research centre in Sebata. Moreover another 399 sera were collected during the active search for the disease in the different villages. These sera were tested by competitive ELISA (c-ELISA) for determination of the sero-positivity of antibodies. The data obtained was used in the retrospective panel study (RPS). The Epi-info software formula for resampling from bank serum was used for determination of the sample size (Dean *et al.*, 1994).

Equation 1 Formula for calculation sample size from the serum bank

$$n = \frac{z^2 (1-P)P}{d^2}$$

$z=1.96$

d= absolute of precision =. 04

P= expected prevalence was 40%

n= 576

To account for the non-perfect diagnostic test the following correction was done using assumed sensitivity (Se=. 904) and specificity (Sp=. 989).

$$n = \frac{576}{(Se+Sp-1)^2} = 653$$

But since there was no information about the purpose of sampling, 1073 samples was selected to increase the precision and to compensate for non-random selection. It was selected from a total number of 4000 serum samples available in the serum bank for the Wollo and East Shewa by the technicians. Both the sensitivity (Se=. 904) and specificity (Sp=. 989) of the C-ELISA test were considered in the estimation of the sample size, their estimation was based on previous finding (Saliki *et al.*, 1993). Equation 1 was used for calculation of the sample size from the serum bank.

In the active surveillance (AS) simple cluster sampling was used. Villages' herds were covered on a rumour base, otherwise an even distribution of villages in the province and the districts was sought. The later strategy was followed in E.Shewa where four villages were picked from every district in the zone. In Wollo zone survey activity was dominated with active hunt for the disease. In every village all animals were examined for the presence of one or two of the clinical signs. During the active search for the disease 399 sera were collected. The sera were collected from apparent healthy animals. Animals sampled were caught by the members of our team and animals' attendants who were instructed to bring any animal.

Due to lack of information about between cluster variance, the method suggested by Thrusfield was used (Thrusfield, 1995). According to this method the standard deviation of the herd prevalence was established (i.e. the average difference expected between an individual cluster prevalence and the overall mean cluster prevalence) to obtain a crude estimate of variance component (Thrusfield, 1995). In order to establish the standard deviation and hence the between cluster variance, the finding of a previous study in the three zones was used (Yayherade, 1997). The prevalence of PPR in different herds in two locations in both East Shewa and Wollo zone were used to estimate the overall prevalence and the standard deviation, which was squared to calculate between cluster variance. Thus 48 herds in each zones were

examined to detect the presence of the disease. The study results used are shown in Table 4 while equation 2 used for calculation of the sample size. Within each cluster selected, an average number of 28 sera were collected from sheep and goats.

Table 4 Estimates used for estimation of the design effect

Zones	Prevalence/ sheep	Prevalence/goat	Mean Prevalence	Standard deviation	Between cluster variance V_c	P_{exp}	D	N	G
East Shewa	.152	.10	.2704	.1436	.02	.40	.04	100	48
	-	.35				.40	.04	100	
Wollo	-	.45				.40	.04	100	
	30%					.40	.04	100	

Source of information: Yayherade, 1997.

Equation 2 Formula for calculation the number of cluster to be sampled

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

G = no. of clustered to sampled = 48

P_{exp} = expected mean prevalence = .2704

n = predicted number of animal per cluster = 100

d = desired absolute precision = .04

V_c = between cluster variance =. 02

3.3.1.Samples for laboratory work

Sera: The blood was collected by jugular venipuncture using evacuated vacutainers and it was kept overnight to clot, after which the sera were separated from the clotted blood by centrifugation. The sera were stored at -20 C° until use.

Swab: lachrymal swabs were collected using swab of absorbent cotton wool. The swabs were inserted into the conjunctival sac and swirled around to collect tears. The swab was broken off into sterile tube. The nasal and faecal specimens were collected also using the same type of swab.

Unclotted blood: It was collected by the jugular venipuncture using heparinized vacutainers (vacutainers contains the anticoagulant heparin).

Post-mortem specimens: Mediastinal and mesenteric lymph nodes, portion of the spleen and the lungs were collected aseptically from an euthanised goat suspected to be infected with PPR. The goat was selected from flocks of sheep and goats in Burka village with clinical sign similar to those of PPR. All specimens collected during the active search for the disease were kept in melting ice till their delivery to the national animal health research centre at Sebeta.

3.2.3. Competitive ELISA (C-ELISA) for detection of PPR

C-ELISA based on monoclonal antibodies specific for H-protein of PPR virus was used to detect antibodies in 1073 sera that were selected from the bank serum and 399 sera collected during the active search for the disease. Complete C-ELISA kits produced by the world reference laboratory for Rinderpest in Pirbright and distributed by BDSL company (www.bdsl.com) was used. The test was carried out according to the protocol supplied by the manufacturer and samples with more than 50% percentage inhibition were designated positive. The ELISA plates were read with Titertek multiskan reader with an inference filter of 492 nm. The reader was connected to computer loaded with ELISA Data Information (EDI) software (Joint FAO/IAEA, 1993). EDI software was used to automate the reading and calculation of percentage of inhibition (PI). Phosphate buffer solution .002M was used for washing and the

blocking buffer was prepared from phosphate buffer .01M plus .3% (v/v) normal bovine serum plus .1% (v/v) tween 20. The test protocol is summarised in Table 5 and the ELISA layout is shown in Figure 1.

Table 5 Summary of the C-ELISA protocol

C-ELISA test protocol (Joint FAO/IAEA programme, 1993)

Assay Steps	Assay Condition			
	Incubation Time	Incubation Temperature	Plate Shaking	Wash Step
1) Coat PPR Antigen	1 hour	37°C	Yes	⌟3x
2) Add Test Sera & Anti-PPR Mab	1 hour	37°C	Yes	⌟3x
3) Add conjugate	1 hour	37°C	Yes	⌟3x
4) Add Substrate & chromogen	10 min	37°C	No	
5) Add Stopper	none	room temp	tap to mix	
6) Read Reaction.	(492nm filter must be in plate reader)			

	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+	2									
D	C+	C+	2									
F	Cm	Cm	3									
E	Cm	Cm	3									
G	Cm	Cm	4									40
H	C-	C-	4									40

Figure 1 C-ELISA Plate layout

All controls were supplied by the manufacturer

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

C++: strong positive

C+: Moderate Positive

Cm: Monoclonal antibody

C-: Negative Serum Control

The PI for controls and test samples was calculated according to equation 3 and 4 respectively. The data expressed in optical density and PI for monoclonal antibody controls and the data expressed in PI for the other four 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 microplate.

Equation 3 Formula for calculation of the percentage inhibition of the control in C-ELISA

$$PI = 100 - \frac{\text{Mean OD of Control}}{\text{Mean OD of Cm}} X100$$

Equation 4 Formula for calculation of percentage inhibition of the test sera

100-(Mean OD of test serum/ Mean OD of Cm)*100

$$PI = 100 - \frac{\text{Mean OD of test serum}}{\text{Mean OD of Cm}} X100$$

3.3.4.Agar gel immunodiffusion test (AGID)

It is the easiest and cheapest test that can be conducted to detect the PPR virus antigen in antemortem and necropsy specimen with sensitivity up to 42.6%. However it can not be used to differentiate between RP and PPR, thus Ic-ELISA was used in order to increase its sensitivity and specificity (Obi *et al.*, 1984, Libeau, 1994).

Kits provided by BDSL company and produced in Pirbright laboratory were used. The test was carried out according to the manufacturer's instruction. The cotton part of the lachrymal and nasal swab was compressed in a 2cc syringe with 100µl of .01 M buffer saline just before the test. The test was carried out as follows

i) 1% agar was dispensed in normal saline, containing sodium azide as a bacteriostatic agent, into petri dishes (6 ml/5 cm dish).

ii) Wells were punched in the agar following a hexagonal pattern with a central well. The wells were 5 mm in diameter and 5 mm apart.

iii) The central well was filled with positive antiserum, one peripheral well with positive antigen, and one well with negative antigen, all supplied with the test kits. The remaining wells were filled with test materials.

iv) Usually, 1-3 lines will develop between the serum and antigens within 18-24 hours (Taylor, 1979a).

3.3.5. Immunocapture ELISA (Ic-ELISA)

Ic-ELISA that employed monoclonal antibodies directed against the virus nucleocapsid protein of PPR virus was used to detect the antigen in the lachrymal, nasal and oral swab and spleen (Libeau *et al.*, 1994). The Ic-ELISA kits provided by CIRAD-EMVT/France were used. The cotton part of the lachrymal and nasal swab was removed with sterile scalpel and then compressed in a sterile 2cc syringe with 100µl of .01 M buffer saline just before launching the test. The post-mortem specimens were minced with sterile sand and 100µl of .01 M buffer saline using a scissors and pestle in a sterile mortar. The test was carried out according to the manufacture's protocol with slight modification. According to the manufacturer's layout samples were supposed to be tested for both RP and PPR, but specimens in this study were tested only for PPR. The steps of the test were

i) Microtitre ELISA plates were coated with 100µl (1/100) of a capture mAb that could react with both RP and PPR.

ii) After washing, 50 µl of the sample suspension was added to all wells and control wells were filled with blocking buffer.

iii) Immediately, 25 µl of a detecting biotinylated mAb (1/1000) for PPR and 25 µl of streptavidin-peroxidase (1/3000) were added to all well.

iv) The plates were incubated at 37°C for 1 hour with constant agitation.

v) After three vigorous washes, 100 µl of ortho-phenylenediamine (OPD in hydrogen peroxide) were added and the plates were incubated for 10 more minutes at room temperature.

vi) The reaction was stopped by the addition of 100 µl of 1 N sulphuric acid, and the absorbance is measured at 492 nm on multiscan Titertek ELISA reader.

The cut-off was calculated from each blank (PPR blank) as two times the mean absorbance values of control (blocking buffer). The washing was done using a manual pipette because the antigen was so sticky.

3.4.1.Questionnaire

A questionnaire was administered with the aim of collecting data from the herd owners to be matched with veterinary service records and sero-surveillance results. The questions covered aspects that would cast light on the seasonality of the disease, breed and species susceptibility, age effect and the importance of the disease for the producer. The recall data was analysed to identify the time trend of the disease, its frequency and impacts. The questionnaire format is shown in annex 2.

Table 6 Questionnaire database layout

Variable	Meaning	Codes
Prodsystem	Production system	Production system:0=nomadic,1=sedentary, 2=mixed
Disepre-sence	Presence of PPR disease	0=No, 1=Yes, 2= somewhat(some die during treatment), 9= disease not known
Status	Status of interviewed	1=owner, 2=worker
Orales	Knowledge about disease with oral and muzzle	lesion:1=present, 0=absent
Watdiacou	Knowledge about disease with watery diarrhoea and cough	1=present, 0=absent
Nasladis	Knowledge about disease with nasal and lacrimal discharge	1=present, 0=absent
Dpresen	Presence of a disease with the above sign	1=present, 0=absent
Frequenc	Frequency of occurrence	1=yearly, 2=always there, 3=every 2 years, 4=every 3 years, 5=only once, 9=disease not known
Season	Season of occurrence	0=dry season, 1=rainy season, 2=spring (April-June), 3=always, 9=disease not known
Specsus	Species highly susceptible	1=Goat, 2=Sheep, 3=both, 9=disease is not known
insource	Source of income	1=Selling animal, 2=Cultivation, 3=Other, 4=selling milk, 5= both 1& 2
Vacneed	The need for vaccine	0=No, 1=Yes, 2=I do not know, 9=disease not known
Vacpay	Payment for vaccine	0=No, 1=Yes,2=I do not know, 9=disease not known
Sagesusc	Susceptibility among sheep	1=<6 month, 2=6-18m, 3=>18 m, 4=all, 5=non susceptible, 9= I do not know
Gagesusc	Susceptibility among goats	1=<6 month, 2=6-18m, 3=>18 m, 4=all , 5=non susceptible, 9= I do not know
Treateff	Efficiency of treatment	0=No, 1=Yes, 2= somewhat(some die during treatment),9= disease not known

3.5.Data storage and management

Laboratory results, questionnaire data and veterinary service records data were entered and stored in separate database in both Ms-Access database and Ms-Excel spreadsheet. Data were screened for proper coding, missing data and entry error was corrected. The data was transferred to stata software (Stata, 2000).

3.6.Analysis

3.6.1. Sero-positivity study based on C-ELISA results

Initially sero-positivity based on C-ELISA results was calculated in the total sample, the three zones, the age groups, sheep, goats, sex groups and the sero-positivity of the mentioned strata in the three zones. Extreme variability in the sero-positivity in the three zones was observed that rendered any attempt to pool the samples in the three districts and generate overall estimates for the total sample meaningless. Moreover a wide variation in herd effect (intracluster correlation) in the three zones was observed. Therefore the notion of estimating parameters for the whole sample variables was dropped. The sero-positivity was calculated for serum bank based sera and the field collected sera separately and as a combined sample.

3.6.1.1. Sero-positivity estimation

The sero-positivity of antibodies was estimated after Thrusfield (1995). Equation 5 was used to calculate the following sero-positivity:

Sero-positivity in the three administrative areas.

2. Sero-positivity in the different species (sheep and goats) in the three zones
3. Sero-positivity in the sex group (females and males) in the three zones.
4. Sero-positivity in the different age groups (4 age groups) in the three zones.

Equation 5 Formula for calculation sero-positivity

$$\text{Sero-positivity} = \frac{\text{Number of sero-positive sera}}{\text{Number of sera tested}}$$

3.6.1.2. Confidence Interval (C.I.) Calculation

Equation 6 (Collet, 1999) which is used for calculation of the exact confidence interval for binomially distributed data was used to calculate confidence interval for species, sex groups and age groups within each zone. 100(1-α)% confidence interval with α equal to .05 was established. In equation 6 P_L and P_U are the upper and lower limits of the confidence interval and $F_{a, b}(P)$ denotes the 100 pth percent point of the F-distribution on a and b degrees of freedom. They refer to the smallest and largest binomial probabilities for which the occurrence of the observed proportion y/n has a probability that is at least equal to α/2. Stata statistics package (Stata, 2000) was used to calculate the confidence interval.

Equation 6 Formula for calculation of the confidence interval for bionomially distributed data

$$P_L = y\,[y+(n-y+1)F_{2(n-y+1),2y}(\alpha/2)]^{-1} \qquad (6)$$

$$P_U = (y+1)[y+1+(n-y)/F_{2(y+1),2(n-y)}(\alpha/2)]^{-1}$$

3.6.1.3. Adjustment for herd effect and intracluster correlation

Using a cluster-sampling scheme for serum samples collection and the contagious nature of PPR led to heterogeneity in the distribution of the outcome variable (sero-positivity). A preliminary visual assessment of the sero-positivity distribution using a histogram and a cosine kernel density trace revealed the presence of a clear correlation within herds. Stata software (Stata, 2000) was used to plot the histogram and density curve. The bin interval for the histogram was calculated as $2\sqrt{n}$ (Stata, 2000-graphic reference). Intracluster correlation may occur when herds are sampled but individual animal's responses are assessed and is very likely when data from contagious diseases are handled (McDermott *et al.*, 1994). The intracluster correlation resulted in violation of the assumption of independence on which many statistical methods are based. Spurious statistical inference could be reached and the null hypothesis is easily rejected because the variance is underestimated and narrow confidence intervals would

be calculated. Many methods were used to adjust for intracluster correlation for proportion data. Fixed and random models using mixture likelihood, overdispersion model and post-hoc adjustment using inflation factor were used for intracluster correlation adjustment (McDermott *et al.*, 1994). The post-hoc adjustment was used in this study. Equation 7 was used to calculate the intracluster correlation and thereafter the inflation factor. The sum of mean square between cluster (MSB) and within cluster (MSW) was derived from one way analysis of variance (ANOVA) (McDermott *et al.*, 1993). The ANOVA was run in Stata (Stata, 2000). The inflation factor was calculated according to equation 8 where ρ referred to intracluster correlation and n to the mean size of the cluster. The factor was used to inflate the variance and thereafter the confidence interval.

Equation 7 Formula for calculation of intracluster correlation

$$\text{Intracluster correlation } \rho = \frac{(\text{MSB-MSW})}{\text{MSB}+(n-1)*\text{MSW}}$$

Equation 8 Formula for calculation of the inflation factor

The inflation factor $C=1+\rho(n-1)$ (8)

The variance under sample cluster sampling = C* The variance under sample random sampling

3.6.1.4. Adjustment for test misclassification

Equation 9 was used to calculate the true prevalence (Rogan and Gladen, 1978). There was no estimation for specificity and sensitivity for the ELISA kits used for antibody detection, neither during its validation (Anderson *et al.*, 1991) nor with the kits work sheet. Therefore two options were available, either to ignore adjustment for test misclassification or to use estimates for sensitivity (Se) and specificity (Sp) of PPR ELISA developed by other laboratories. The parameter estimates of a blocking ELISA developed by Saliki and other (1993) were used. This ELISA used a similar mAb directed against the H-protein, but it used a different format (blocking ELISA) and a different dilution. The sensitivity and specificity of C-ELISA based on the aforementioned paper were .904 and .989, respectively. The sero-positivity detected in the combined sample was used as the apparent prevalence (AP) in equation 9.

Equation 9 Formula for calculation of true prevalence

$$\text{True sero-prevalence}=\frac{AP+Sp-1}{Se+Sp-1}$$

3.6.2. Comparison of mean prevalence values

Chi-square was used to test the null hypothesis that there was no significant difference between the sero-positivity in sheep and goats, female and male and the different age groups in the East Shewa and North Wollo zones and the sero-positivity in the three zones. In case of small sample size (less than 5 per cell) Fisher's exact test was used instead of chi-square. Because the cases were not evenly distributed stratification by zone was adopted for risk factor effects assessment. South Wollo was excluded because there was no sufficient number of sero-positive animals (one case). The underlying assumption of chi-square includes independence of individuals within group comparison. Due to intracluster correlation this assumption was violated in this data. Adjustment for the herd effect was done using the method suggested by Donnner (1993). The chi-square statistic was divided by the inflation factor calculated in the previous section. Equation 10 was used for adjustment.

Equation 10 Formula used for adjustment of chi-square statistics for herd effect

$$\text{Adjusted chi-square}=\frac{X^2}{C}$$

3.6.3. Risk factor identification

Logistic regression was used to identify the risk factors that might affect the distribution of sero-positivity. The logistic regression was run using Stata software (Stata, 2000). A multivariate model was run to identify the most important factors and to fit the final model. Before fitting the model, all the missing values for all the variables were removed in order to have a fixed degree of freedom. The logistic regression model was fitted with all the assumed risk factors (sex, age, species, source of sample (serum bank or field based) and zonecode, dummy variables for categorical data (the age groups and zonecode) were created using Stata's command xi. According to stata reference manual H-O (PP. 209) the command lfit computes goodness-of-fit test, either using the Pearson chi-square test or the Hosmer-Lemeshow test. lfit command, typed without options, presents the Pearson chi square goodness-of-fit test for the estimated model. The Pearson chi-square is a test of the observed against expected number of responses using cells defined by the covariate patterns. Stata (2000) reports the wald test, which

is the ratio of the estimated regression coefficient to its standard error (b/Se(b)). When the ratio of coefficient to its standard error was within the range (-2,2), the respective variable was dropped from the model. The final model was fitted with the animal status (positive or negative) as the dependent variable and zonecode (which zone), sex and source of the sera (serum bank or field base sera) as the independent variable.

3.6.4.Questionnaire data analysis

PPR could be confused with other disease such as Pasturellosis, Goatpox, Ecthyma and Maedi visna. Therefore the data generated by the questionnaire was taken with caution. All the variables in the questionnaires were summarised in the three zones without preliminary assessment (annex 7). The questionnaires in which the owners described two clinical sign or more in East Shewa and three clinical sign in Wollo area were considered eligible for analysis unless there were other indications that could exclude PPR as a causative agent. This resulted in 34 questionnaires out of 70 (49%) questionnaires in East Shewa and 48 questionnaires out of 100 (48%) questionnaires in Wollo. Variables such as time of kidding, need and payment for vaccine were not affected by misleading results of disease identification (recall bias or misclassification bias); therefore all responses were considered (100 and 70 questionnaires). Identification of the time of kidding was used to pinpoint the time when there were so many susceptible animals assuming that the disease was endemic. It was compared with last date of occurrence of the disease and veterinary records, thereafter it was use to identify the optimum vaccination time (section 7).

3.6.5.RVET data analysis

Veterinary service records were used to calculate the average proportion of affected animals, average proportion of death among affected animals and average proportion of death in the outbreak and to identify the time frame of the disease. Chi-square was used to compare the frequency of its occurrence in sheep and goats. The time trend of the disease was compared with the questionnaire-generated data. The frequency and distribution of the event over the months of the year were identified and plotted. It was plotted versus the kidding time and annual variation according to the questionnaire. Equations 11-13 were used to extract the different estimates.

Equation 11 Formula for calculation of average proportion of death among affected

$$\text{Average proportion of death among affected animals} = \frac{\text{number of death due to PPR}}{\text{number of infected animals with PPR}}$$

Equation 12 Formula for calculation of average proportion of death

$$\text{Average proportion of death due to PPR} = \frac{\text{number of deaths due to PPR}}{\text{average number of population at risk}}$$

Equation 13 Formula for calculation of average proportion of affected animals

$$\text{Average proportion of affected animals} = \frac{\text{number of animals infected with PPR}}{\text{average number of population at risk}}$$

3.6.6. Comparison between AS, RSP and RVET results

The extent of agreement between the questionnaire outcome and the serology results was explored using a scatterplot. Dots were connected with smooth lines to ease visual inspection without any implication about interpreted values for non-observed time points. Based on the outcome of the questionnaire and the veterinary records the annual variation of PPR was plotted. The different questionnaire variables were summarised and the extent of agreement in the questionnaire outcome was compared in the three zones.

3.6.7. Decision tree analysis

Decision tree analysis provides a structured approach which allows full evaluation of the alternative options available and the likely economic outcomes of these decisions (Ngategize, 1985). There were numerous applications for this technique in veterinary literature particularly related to the evaluation of different treatment options for various diseases (Dargatz and Salman, 1990, Fetrow *et al.*, 1985). It was also used to evaluate the economic impact of disease control (Carpenter *et al.*, 1987, Seargent, 1992). This method was used to identify the economic feasibility of conducting a control programme using PPR vaccine. The objectives of the decision tree were

-To evaluate the economic feasibility of conducting a control programme for PPR through vaccination means.

-To identify the optimum price of vaccination, yet it is profitable for the owner (break even point).

The assumption underlying the model were as follow

a) Since small ruminants' milk is not sold, only unfavourable direct effects were considered, loss only due to death.

b) Loss due to mortality was estimated based on the price of a live animal, different age groups (young, old) and sex groups were assigned different prices and the average of these prices was obtained for fitting the model. An animal value was the value of a live animal, or a cost of a dead animal.

c) Only healthy animals are supposed to be vaccinated during vaccination campaign.

d) Vaccination of animals once through their economic life was assumed to be sufficient for inducing solid immunity (Diallo *et al.*, 1992).

e) The expected return from vaccination was calculated as the expected monetary value (EMV) for vaccinating minus EMV for not vaccinating for a given set of parameter estimates.

f) The break-even point for a programme is the percentage animal loss that needed to be prevented to cover the cost of the programme.

g) The estimate of the cost of RP vaccination in Ethiopia (Tambi *et al.*, 1999) was used to estimate cost of PPR vaccination. The previous paper assessed the economic impact of rinderpest control in a number of African countries including Ethiopia. The labour, transport and other logistic expenses were included as a part of cost of vaccination.

The programme Data 3.5 (data 3.5, 1999) was used to set up and evaluate the decision trees. The structure of the decision tree used in this study is shown in Figure 13. Working from the left, the tree starts from a decision node, in this case providing two decision options, to vaccinate against PPR or not to vaccinate. The probabilities used in this model were deduced as followed

1. Probability of a vaccinated animal to be non immune, was estimated based on the finding of Gopillo (1996) who reported that only 70% of the animals vaccinated (ear punched animals) against Rinderpest (RP) were found positive when tested with ELISA. This estimation was used as a probability for PPR vaccine efficiency because both RP and PPR are morbilliviruses, both vaccines need a cold chain, the animal population is overlapping and vaccination is going to be carried by the same vaccination teams (government).
2. The rate of exposure and infection was estimated based on the estimation of sero-positivity in this study.
3. Proportion of death among affected animals' estimate deduced from the veterinary service records (section 3.6.5.) was used as the probability of death among affected animals.

4. To estimate the loss due to mortality and the loss evaded, the monetary value of animals was estimated based on a market survey in Debre Berhan and Shino areas.
5. The decision tree was constructed for naïve, susceptible animals with no previous exposure to the disease. Though may not appear realistic, it simulates the real situation in Africa where mass vaccination of all the animals regardless of their immunological status is carried out. However only healthy animals are supposed to be vaccinated no sick animal would be vaccinated. The probability used for estimation of the immunity induced by the vaccine was deduced from a similar situation where sporadic outbreaks used to occur from time to time and annual vaccination are carried out (Gopilo, 1996).

The model was run for sheep and goats separately based on prevalence estimates extracted from the total sample and from E.Shewa zone. Thus four decision trees were constructed to represent the four situations. Sensitivity analysis using three-way sensitivity command and tornado diagram was carried out for the variable rate of infection, immunity induced by the vaccine, vaccine price and value of the animal. The three way sensitivity analysis quantified the change in expected monetary due to change in the three variables simultaneously within a range specified by the investigator. The three way sensitivity analysis was carried out using the variables immunity, rate of infection and value of the animal, it was also carried out using the variables rate of infection, vaccine price and animal value at the same time. Different ranges were issued for the variable rate of infection (.004-. 5), immunity induced by the vaccine (.6-.9), goat price (40-100 Birr), sheep price (50-150 Birr) and vaccine price (0-20 Birr). The tornado diagram is a form of one way sensitivity analysis brought together in a single graph.

4. RESULTS

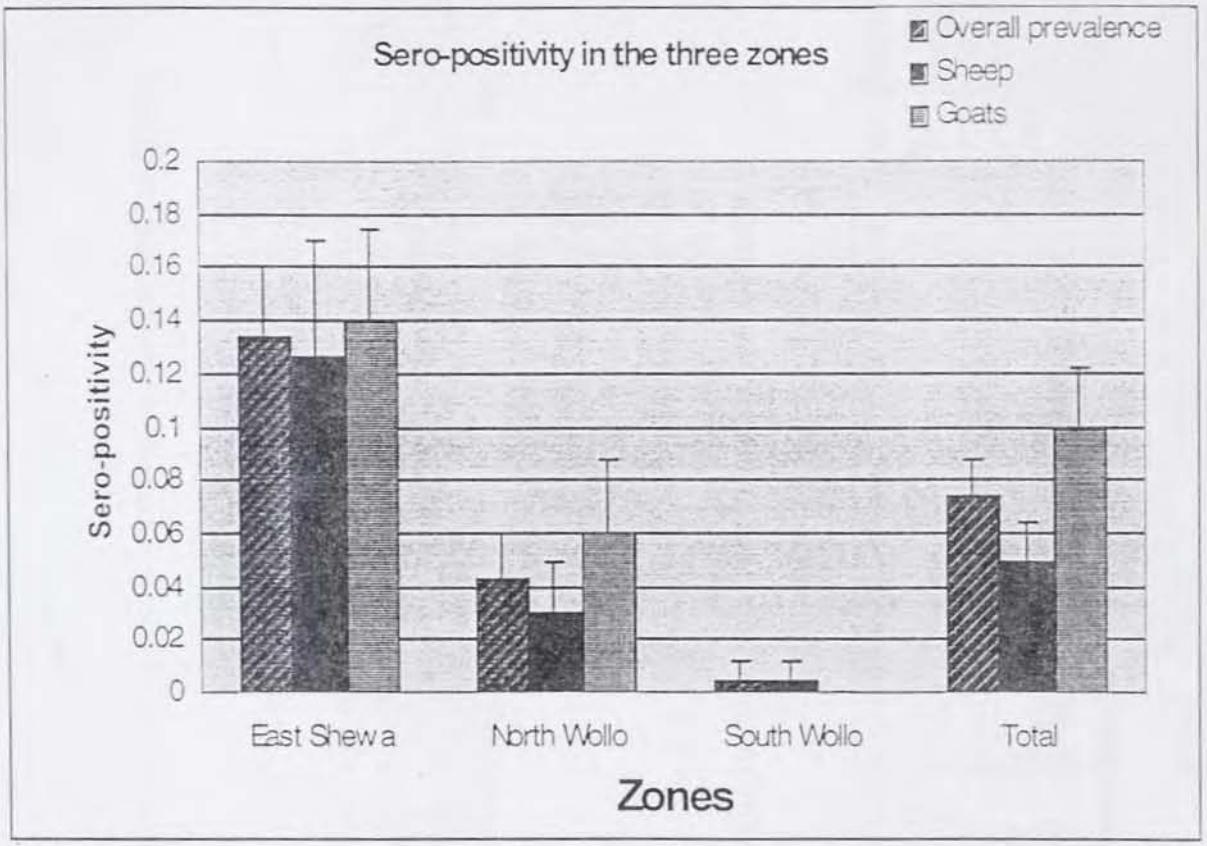
4.1. Serological results obtained by C-ELISA

4.1.1.Sero-positivity estimation

Two estimates for the sero-positivity in the three zones were obtained based on the source of the samples. Estimates based on serum samples originated from serum bank sera (RPS) are shown in Table 7 and estimates based on field samples (AS) are displayed in Table 8 while estimates based on the combined samples are shown in Table 9. Based on the combined sample high sero-positivity of .134 was detected in E.Shewa and sero-positivity of .043 and .004 in North and South Wollo, respectively. Similar estimates for sero-positivity in goats and sheep of E.Shewa were obtained. They were .139 and .126 for goats and sheep respectively. In North Wollo sero-positivity of .057 and .03 was observed in goats and sheep, respectively. A sero-positivity of .183 and .167 was detected in females and males of E.Shewa and a sero-positivity of .046 and .028 was detected in female and male of North Wollo, respectively. In the four age groups sampled in E.Shewa sero-positivity of .112, .094, .166 and .17 were established, while sero-positivity of 0, .01, .041 and .053 were detected in the same age groups of North Wollo. A high sero-positivity in animals more than two years (.166-. 17) was encountered in E.Shewa and zero sero-positivity was detected in animals less than one year in N.Wollo. The distribution of the sero-positivity in the different strata is reflected in the length of the bars in Figures 2 and 3. Stratification of the whole samples by zone was done in order to visualise the wide variation in sero-positivity in the three zones. The distribution of the sero-positivity in different species, sex group and age groups within each zone is shown in Tables 10, 11 and 12.

Sero-positivity based on the total samples of the serum bank (RPS) results was estimated to be .0792. A similar estimate of .0602 was obtained from sera collected during the active search (AS) for the disease. However different estimates for the sero-positivity in E.Shewa and N.Wollo were obtained from the results of the serum bank based samples and field based samples. In the serum bank samples estimates of .1684 and .0431 were obtained in E.Shewa and N.Wollo, respectively, while in the field based samples estimates of .0792 and .0833 were

obtained in E.Shewa and N.Wollo respectively. The range of herds' sero-positivity in the serum bank sera was 0-. 35 and it was .029-. 15 in the field samples. In Adama district of E.Shewa zone the sero-positivity range according to the serum bank sera was .1-. 4 and .049 according to the field samples. Two districts of E.Shewa (Busato and Arsi Negele) were covered by the field samples but not by the serum bank sera and the Adaa liben district was covered only by serum bank sera. Sero-positivity range in Fentale according to the serum bank was 0-. 7 (5 villages) and 0 sero-positivity according to the field based samples. The range of herds' sero-positivity in the district of Bati in N. Wollo was .0196-. 143 according to serum bank sera and .025-. 119 according to the field sample. The districts of Delante in N.Wollo and the district of Debrasina in South Wollo were covered by the serum bank samples only, while the district of Waldiya in N.Wollo and districts of Kutabar and Heik in South Wollo were covered by the field samples only.



*Exact 95% C.I. are overlaid.

Figure 2 Sero-positivity in the three zones based on the combined samples (RPS and AS)

Table 7 Sero-positivity in the three zones based on serum bank samples

District	Total sample	Total +ve	positivity	Sheep	Sheep +ve	Positivity	Goats	Goat +ve	Positivity	Female	Female+ve	Positivity	Male	Male+ve	positivity
East Shewa	380	64	0.1684	153	24	0.1569	226	40	0.1770	344	60	0.1744	36	4	0.1111
North Wollo	464	20	0.0431	259	8	0.0309	205	12	0.0585	378	18	0.0476	81	2	0.0247
South Wollo	229	1	0.0043	229	1	0.0043	np	0	0	212	1	0.0047	17	0	0
Total	1073	85	0.0792	641	33	0.0515	431	52	0.1206	934	79	0.0846	134	6	0.0448

a. Species missing identification were 1 animal (E.Shewa), b. Sex missing identification were 5 animals (N.Wollo), np=no animal tested

Table 8 Sero-positivity in the three zones based on field samples

District	sample	Total +ve	positivity	Sheep	Sheep +ve	Positivity	Goats	Goats +ve	Positivity	Female	Female+ve	Positivity	Male	Male+ve	Positivity
East Shewa	240	19	0.0792	69	4	0.0580	170	15	0.0882	194	19	0.0979	42	0	0
North Wollo	120	5	0.0417	42	1	0.0238	78	4	0.0513	93	5	0.0538	27	0	0
South Wollo	39	0	0	21	0	0	16	0	0	31	0	0	7	0	0
Total	399	24	0.0602	132	5	0.0379	264	19	0.072	319	24	0.075	75	0	0

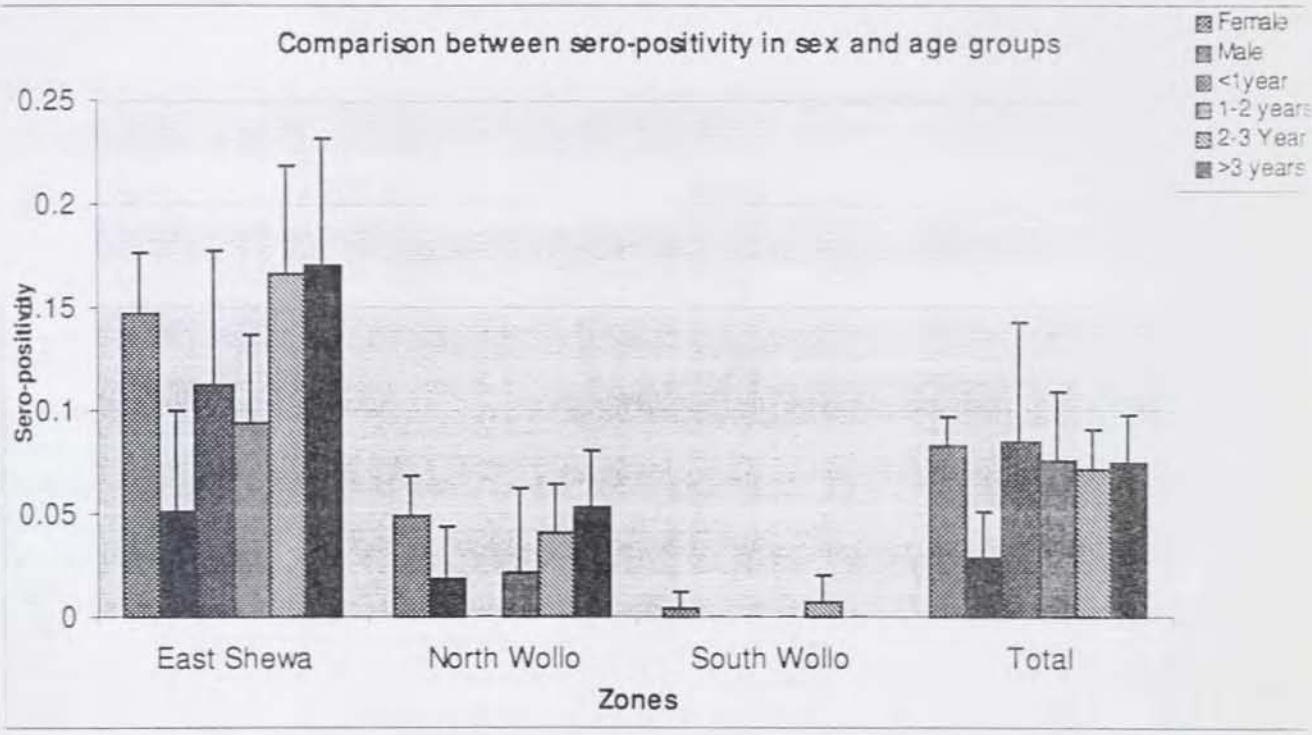
a. Species missing identification were 1 animal (E.Shewa), 2 animals (S.Wollo), b. Sex missing identification were4 animals (E.Shewa) 1 animals (S.Wollo).

Table 9 Sero-positivity in the three zones with its different strata based on the combined samples (RPS and AS)

District	Total	Total +ve	Positivity	Sheep	Sheep +ve	Positivity	Goats	Goats +ve	Positivity
East Shewa	620	83	0.1339	222	28	0.1261	396	55	0.1389
North Wollo	584	25	0.0428	301	9	0.0299	283	16	0.0565
South Wollo	268	1	0.0037	250	1	0.004	16	0	0
Total	1472	109	0.0740	773	38	0.0492	695	71	0.1022

Species missing identification were 2 animal (E.Shewa), 2 animals (S.Wollo)

b. Sex missing identification were 4 animals (E.Shewa) 5 animals (N.Wollo) 1 animal (S.Wollo)



*Exact 95% C.I. are overlaid

Figure 3 Sero-positivity in the sex and age groups in the three zones based on the combined samples (RPS and AS)

Table 10 Sero-positivity in E.Shewa and the different age groups, species and sex groups based on the combined sample (RPS and AS)

Variable	Sera tested	Positive	Sero-positivity
Sheep	222	28	0.1261
Goats	396	55	0.1388
Female	538	79	0.1468
Male	78	4	0.05128
<=1 year	89	10	0.1124
1-2 years	181	17	0.0939
2-3 Year	193	32	0.1658
>3 years	144	24.	0.1667
Total	620	83	0.1339

Missing identification a) Species 2 animals b) Sex 4 animals c) Age 13 animals

Table 11 Sero-positivity in N.Wollo and the different age groups, sex groups and species based on the combined sample (RPS and AS)

Variable	Sera tested	Positive	Sero-positivity
Sheep	301	9	0.0299
Goats	283	16	0.0565
Female	471	23	0.0488
Male	108	2	0.01852
<=1 year	23	0	0
1-2 years	52	1	0.0192
2-3 Year	269	11	0.0409
>3 years	240	13	0.0542
Total	584	25	0.0428

Missing identification a) Sex 5 animals

Table 12 Sero-positivity in S. Wollo and the different age groups, sex groups and species based on the combined sample (RPS and AS)

Variable	Sera tested	Positive	Sero-positivity
Sheep	250	1	0.004
Goats	16	0	0
Female	243	1	0.0041
Male	24	0	0
<=1 year	4	0	0
1-2 years	10	0	0
2-3 Year	152	1	0.0066
>3 years	101	0	0
Total	268	1	0.0037

Missing identification a) Species 2 animals b) Sex 1 animals c) Age 1 animals

4.1.2.Confidence interval estimation

A confidence interval of .107-.161 was established for PPR sero-positivity in E.Shewa. However, a wider confidence interval was established among the animals 2-3 years (.1135-. 2259) and animals greater than three years (.1087-. 2314). A confidence interval between .0279-.0625 was established for sero-positivity in N.Wollo zone. Detailed account of the confidence intervals for the different strata in the different zones can be found in Tables 13 and 14.

Table 13 Confidence interval for different age groups, sex groups and species in E.Shewa based on the combined sample (RPS and AS)

Variable	Sero-positivity	St. Dev.	Confidence interval
Sheep	0.1261	0.0223	0.0855-0.1771
Goats	0.1388	0.0174	0.1064-0.1769
Female	0.1468	0.0152	0.1178-0.1793
Male	0.05128	0.025	0.0141-0.1261
<=1 year	0.1124	0.0335	0.0552-0.1969
1-2 years	0.0939	0.0217	0.0557-0.1461
2-3 Year	0.1658	0.0268	0.1163-0.2259
>3 years	0.1667	0.0311	0.1098-0.2378
Total	0.1339	0.0137	0.1081-0.1632

Table 14 Confidence interval for different age groups, sex groups and species in North Wollo based on the combined sample (RPS and AS)

Variable	Sero-positivity	St. Dev.	Confidence interval
Sheep	0.0299	0.0098	0.0138-0.056
Goats	0.0565	0.0137	0.0327-0.09019
Female	0.0488	0.0099	0.0312 -0.0724
Male	0.01852	.01297	.00225-.0653
<=1 year	0	uc	uc
1-2 years	0.0192	0.0210	0.0005-0.1129
2-3 Year	0.0409	0.0120	.0205-.0717
>3 years	0.0542	0.0143	0.0284-0.0887
Total	0.0428	.00838	.0279-.0625

Uc=not allowed

4.1.3. Herd effect assessment

The herd effect on sero-positivity estimate based on the combined sample (RSP and AS) was explored visually using a histogram, a kernel density trace and the scatterplot shown in Figures 4, 5, 6. A Correlation of sero-positivity by herd was striking. Among the herds sampled in E.Shewa, N.Wollo and S.Wollo 21%, 41% and 88% of the herds have a zero sero-positivity. Of the herd sampled in E.Shewa, N.Wollo and S.Wollo, respectively 7.1%, 33% and 12% had sero-positivity between .01 and .05. 18% and 16% of the herd sampled in E.Shewa and N.Wollo had a sero-positivity between .5 and .1. 29% and 16% of the herd sampled in E.Shewa and N.Wollo had a sero-positivity between .1 and .2. However 25% of the herds sampled in E.Shewa had a sero-positivity between .2 and .7. The range of sero-positivity in the different villages of the three zones was between 0 and .7 in E.Shewa, between 0 and .143 in N.Wollo and only one case was detected in S.Wollo in the village of Endias in Debrasina district (warda). Furthermore the relation between herd size and sero-positivity was assessed visually using scatterplot. High frequencies of disease occurrence were found in herd with medium size. The disease seems to occur mostly in cluster sample with 20 animals (19 out 25) followed by cluster with 40 animals. Detailed account of herds tested, their size, source of the sera and location can be found in Table 15.

Table 15 Distribution of sero-positivity in the different villages (herd) within the different districts

No.	Location (Zone-District-Village)	Cases	Sample	Sero-positivity	Source o [a]
1	E. S. Adami tulli-Abjafa	6	20	0.3	1
2	E. S. Adami tulli-Mego	2	20	0.1	1
3	E. S. Adami tulli-Mechafera	7	20	0.35	1
4	E.S. Adami tulli-Walak A.P.A	1	20	0.05	1
5	E. S. Adami tulli-Batele	0	20	0	1
6	E. S. Adaa Liben-Borertinop A	1	20	0.05	1
7	E. S. Adaa Liben-Haroricha	2	20	0.1	1
8	E. S. Adaa Liben-Gichaegerenbabo	0	20	0	1
9	E. S. Adaa Liben-Laep P.A Lugo	1	20	0.05	1
10	E. S. Adaa Liben-Filtino	2	20	0.1	1
11	E.S. Adama-Mekasa	8	20	0.4	1
12	E.S. Adama-Dabi Dengore	2	20	0.1	1
13	E.S. Adama-Kelento	6	20	0.3	1
14	E.S. Adama-Gedemsa	2	20	0.1	1
15	E.S. Fentale-Kesesi	14	20	0.7	1
16	E.S. Fentale-P.A.Weyba	0	20	0	1
17	E.S. Fentale-P.A.Alaka	10	20	0.5	1
18	E. S. Fentale-P.A.Kobo	0	20	0	1
19	E. S. fentale-P.A.Legabenti	0	20	0	1
20	N. W. Bati-Bina	5	58	0.08620	1
21	N. W. Bati-Coarero	3	59	0.0508	1
22	N. W. Bati-Woyofelna	5	42	0.11902	1
23	N. W. Bati-Chachatu	2	42	0.0476	1
24	N. W. Bati-Salemere	3	80	0.0375	1
25	N. W. Bati-Fura	2	80	0.025	1
26	N. W. DelantaDawente-Sehye mewcha	0	33	0	1
27	N. W. DelantaDawente-Shola Derdria	0	40	0	1
28	N. W. DelantaDawente-Cheule kutri	0	30	0	1
29	S. W. Debrasina-Endhias	1	40	0.025	1
30	S. W. Debrasina-cheffe Bello (26)	0	34	0	1
31	S. W. Debrasina-Tewa	0	39	0	1
32	S. W. Debrasina-Betaso	0	40	0	1
33	S. W. Debrasina-Dilefire.	0	39	0	1
34	S. W. Debrasina-Worke	0	37	0	1
35	E.Shewa-Adami-Marmasa	2	41	0.0488	2
36	E.Shewa-Busato-Feto	3	20	0.15	2
37	E.Shewa-Busato-Danore Teo	1	19	0.0527	2
38	E.Shewa-Busato-Gassi	2	19	0.1053	2
39	E.Shewa-Fentale	0	26	0	2
40	E.Shewa-Arsi Negele	4	20	0.2	2
41	E.Shewa-Adami tulli-Oetu	3	20	0.15	2
42	E.Shewa-Adami tulli-Arba kebele	1	34	0.0294	2
43	E.Shewa-Adami tulli-Forme	3	41	0.0732	2
44	S. Wollo-Heik-Hitacha	0	28	0	2
45	N.Wollo-Bati-Salmera	1	51	0.0196	2
46	N.Wollo-Bati-Burka	4	28	0.1429	2
47	N.Wollo-Waldiya-Kobo	0	41	0	2
48	S.Wollo-Kutabar-Alasha	0	11	0	2

Source of the sera: 1=serum bank 2=Field base sample, np=no positive

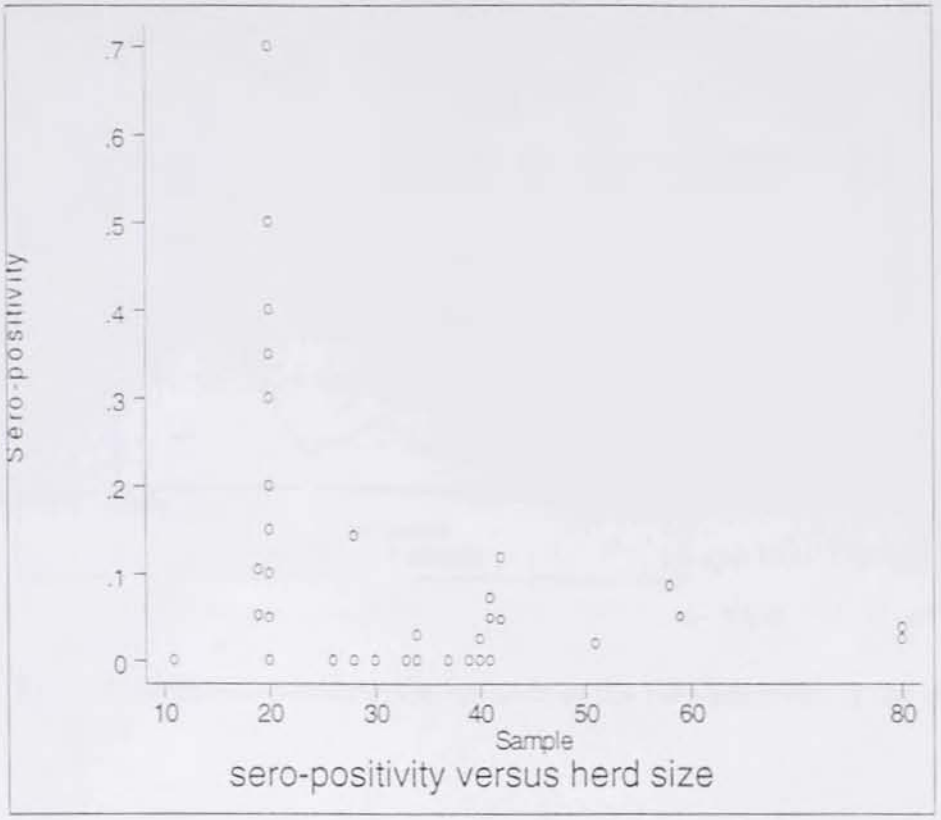


Figure 4 The distribution of the sero-positivity versus the flock size

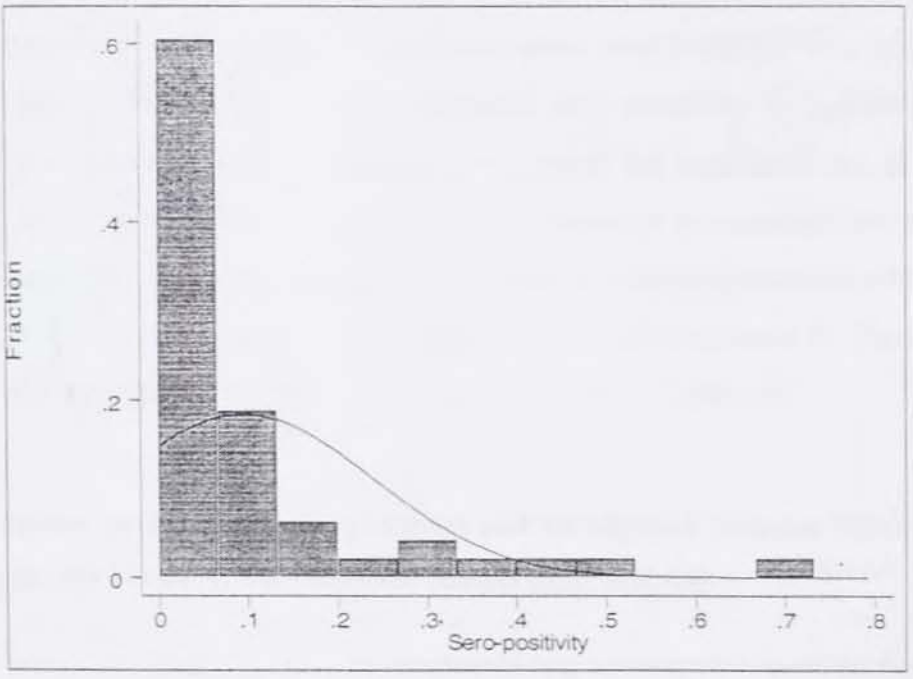


Figure 5 The distribution of sero-positivity in the different flocks

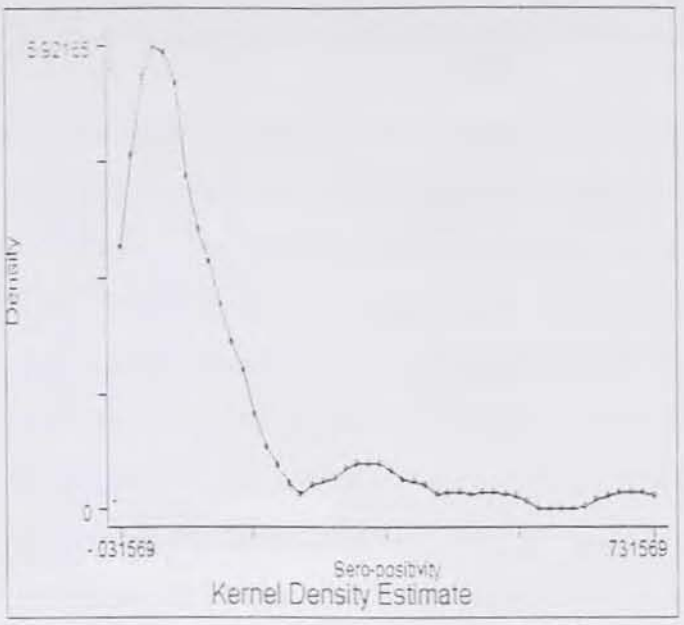


Figure 6 the kernel density trace estimate of the sero-positivity in the different villages' flocks

4.1.4. Adjustment for test misclassification

The estimated true prevalence in E.Shewa was found to be .137 for assumed sensitivity and specificity of C-ELISA, while the apparent sero-positivity was .134. Thus C-ELISA test used to detect PPR antibodies underestimated sero-positivity in E.Shewa where high sero-positivity was detected. The same test overestimated the sero-positivity in N.Wollo where an adjusted prevalence of .03583 was calculated compared to apparent sero-positivity of .0430. .11 sero-positivity was identified as the threshold in the study beyond which the test overestimated the true prevalence and below which the test underestimated it. The calculated true prevalence in the three zones and the total sample is shown in Table 16.

Table 16 Apparent sero-positivity and its adjusted estimate (True prevalence) based on the C-ELISA result for the combined sample (RSP and AS)

District	Total	Total +ve	Sero-positivity*	True prevalence
East Shewa	620	83	0.1339	0.1377
North Wollo	584	25	0.0428	0.0358
Total sample	1472	109	0.0740	0.07055

* Assumed Sensitivity=. 904 and Specificity=. 989 for the C-ELISA

4.1.5. Adjustment for herd effect and cluster sampling

The variance of the different estimates was inflated with a design factor (Equation 8) that accounted for the herd effect and the cluster sampling. An intracluster correlation (Equation 7) of .198391, that necessitated the inflation of the variance of the sero-positivity by a factor of 5.194, was detected in E.Shewa zone. In N.Wollo zone an intracluster correlation of .022944, that necessitated the inflation of the variance of the sero-positivity by a factor of 2.0937, was detected. A low intracluster correlation of .00375 was detected in S.Wollo zone that rendered accounting for the herd effect unnecessary. Wider confidence intervals were established for the different estimates in E.Shewa, N.Wollo and the total sample, the different confidence intervals are shown in Tables 18, 19 and 20. The intracluster coefficients are shown in Table 17. The graph in Figure 8 explains how the intracluster correlation underestimated the variance in the combined sample and led to narrow confidence interval.

Table 17 Intracluster correlation coefficients in the three zones and the whole sample based on the combined sample (RPS and AS)

District	Mean cluster size	Mean square between group	Mean square within group	Intracluster correlation	Inflation factor
East Shewa	22.14	0.0937	0.6073	0.1984	5.1940
N.Wollo	48.67	0.0402	0.0229	0.0229	2.0937
South Wollo	33.5	0.0030	0.0038	-0.0057	-.9085
Overall sample	30.67	0.4573	0.0556	0.1858	6.5132

Table 18 Adjustment for herd effect in the three zones and the total sample

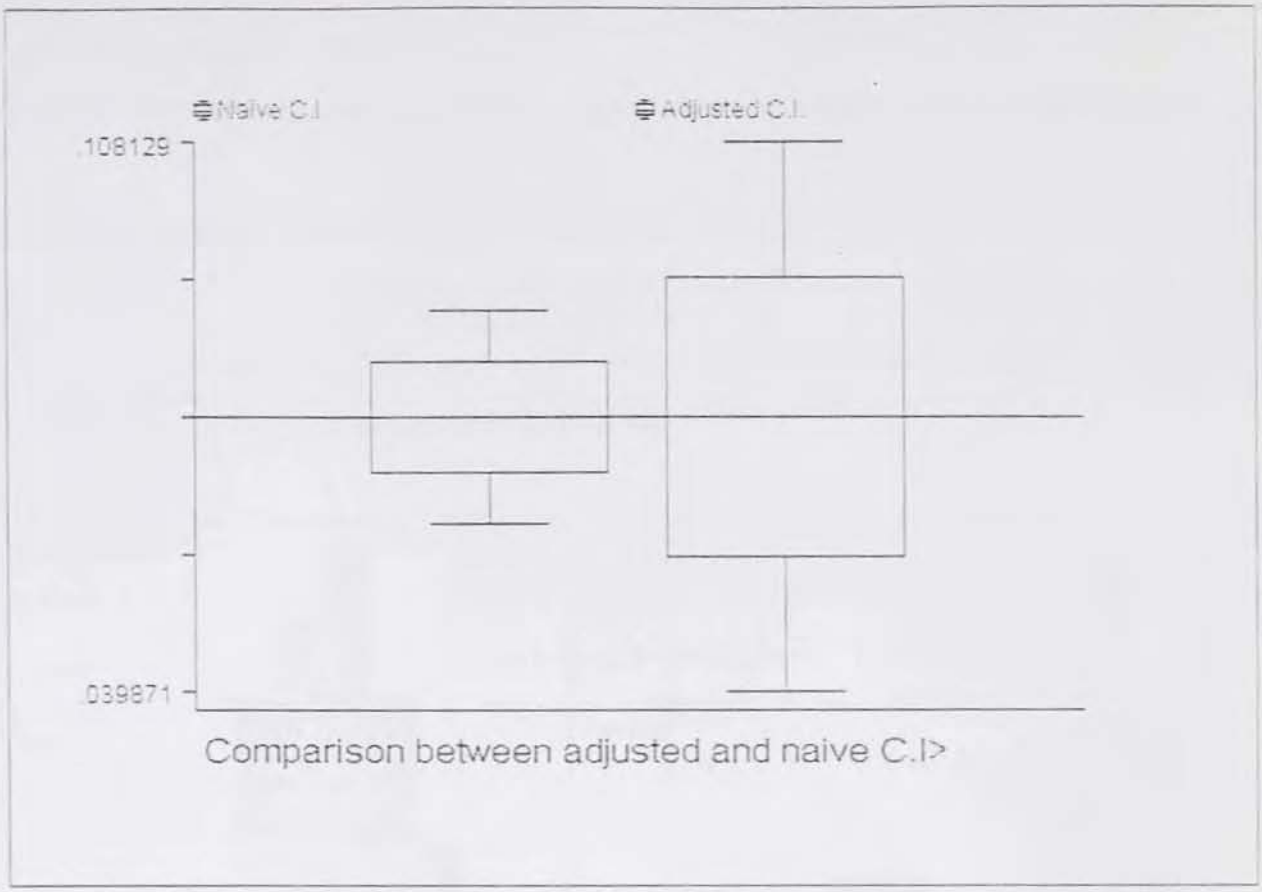
Variable	Prevalence	Naïve Confidence interval	Adjusted Confidence interval
East Shewa	0.134	0.108-0.163	0.195-0.073
North Wollo	0.043	0.028-0.063	0.067-0.019
Total	0.074	0.061-.089	0.04-0.108

Table 19 Inflated confidence interval in the different age groups, sex groups and species in E.Shewa

Variable	Inflated variance	Naïve Confidence interval	Adjusted Confidence interval
Sheep	0.0026	0.082-0.170	0.058-0.002
Goats	0.0016	0.106-0.174	0.218-0.062
Female	0.0014	0.216 -0.150	0.257-0.109
Male	0.0093	0.250-0.084	0.0 (-0.022)-0.356
<=1 year	0.0058	0.046-0.178	0.0 (-0.037)-0.261
1-2 years	0.0024	0.051-0.137	0.0 (-0.003)-0.191
2-3 Year	0.0037	0.114-0.219	0.046-0.286
>3 years	0.0050	0.109-0.231	0.030-0.310
Total	0.0010	0.107-0.1618	0.073-0.195

Table 20 Inflated confidence interval in the different age groups, sex groups and species in N.Wollo

Variable	Inflated variance	Naïve Confidence interval	Adjusted Confidence interval
Sheep	0.0002	0.011-0.049	0.058-0.002
Goats	0.0004	0.088-0.032	0.020-0.100
Female	0.0002	0.03-0.068	0.019-0.073
Male	0.0005	0 (-.007)-.044	0.0 (-0.017)-0.073
<=1 year	np	np	np
1-2 years	0.0009	0 (-0.02)-0.062-	0.0 (-0.038)-0.080
2-3 Year	0.0003	0.017-.065	0.007-0.075
>3 years	0.0004	0.025-0.081	0.012-0.094
Total	0.0001	.027-.059	0.019-0.067



*The mean ± 1 and 2 standard deviation

Figure 7 Comparison between adjusted and naive confidence intervals for the combined sample in the three zones

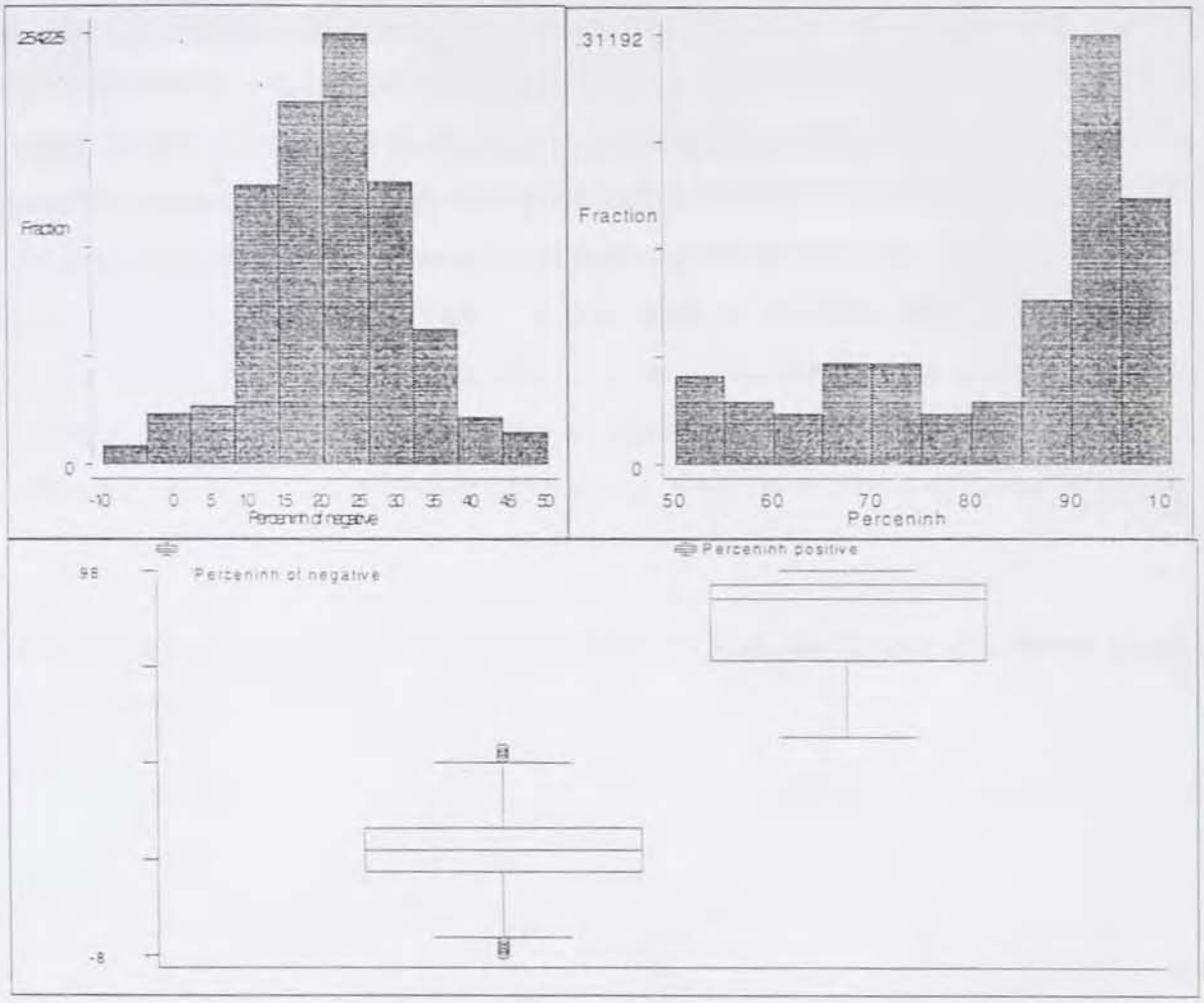
4.1.6. Summary of the percentage inhibition for the positive and negative sera in the three Zones

The mean of percentage inhibition (PI) and the standard deviation for negative test sera were 23.71±10.19 in E.Shewa, 17.136 ±10.96 in N.Wollo and 22.22 ±8.25 in South Wollo. The mean and the standard deviation of percentage inhibition for positive sera (PI>50) were 82.28±14.26 in E.Shewa and 79.32±24.91 in N.Wollo. Comparison between the percentage inhibition of the negative sera in the three zones using Kruskal Wallis test revealed the presence of a significant difference. The frequency of PI for positive and negative sera in the three zones is shown in Table 21 and the distribution of PI of negative sera is shown by the histogram in Figure 8.

Table 21 Summary of percentage inhibition for positive and negative sera in the three zones

Zone	No. of Positive samples tested	PI mean for positive	PI St. Dev. for positive	No. of samples tested	PI mean for negative	PI St. Dev. for negative
E. Shewa	83	82.277108	14.262618	537	23.718808	10.194978
N. Wollo	25	79.32	24.917731	557	17.136445	10.955406
S. Wollo	1	53	0	267	22.220974	8.2452909

Kruskal Wallis test statistics=109.958 P=0.000



*The upper left graph shows the frequency of PI for negative sera and the upper right graph shows PI for positive sera while the box and whisker reflect the dispersion of PI for negative and positive sera.

Figure 8 the percentage inhibition for the ELISA test results in the combined sample (RPS and AS)

4.1.7. Comparison between Sero-positivity in the different strata in N.Wollo and E.Shewa

The null hypothesis that there is no significant difference between sero-positivity in the three zones and the different strata within each zone was tested with either chi-square or Fisher 's exact test. No significant difference (p>. 05) between the sero-positivity in male and female was detected in N.Wollo zone using Fisher's exact test, Table 23 shows the frequency of each category in the sample. A significant difference (p<. 05) in the sero-positivity in male and female was detected in E.Shewa, however adjustment for herd effect and intracluster correlation rendered it insignificant (p>. 05). The frequency of each category is shown in Table 22 and the chi-square adjustment for herd effect is shown in table 24. No significant difference (p>. 05) between sero-positivity in sheep and goats in both E.Shewa and N.Wollo was detected. Tables 25 and 26 were used to calculate the chi-square statistics. Comparison between the sero-positivity in the three zones with chi-square indicated the presence of significant difference (p<. 05) in its occurrence. The difference remained significant even after adjustment for herd effect and intracluster correlation. Table 27 was used to calculate the chi-square statistics for comparison sero-positivity in the three zones. Furthermore the presence of significant difference in the different age groups was explored with Fisher's exact test and no significant difference (p>. 05) was detected in E.Shewa and N.Wollo as shown in Tables 29 and 30.

Table 22 Chi-square frequency of sero-positivity in male and female in E.Shewa based on RPS and AS samples

	Test result		
Sex	+ve	-ve	
Female	79	459	538
Male	4	74	78
Total	83	533	616

Fisher's exact 5.3356 Pr = 0.021

Table 23Chi-square frequency of sero-positivity in male and female in N.Wollo based on RPS and AS samples

	Test result		
Sex	+ve	-ve	
Female	23	448	471
Male	2	106	108
Total	25	554	579

Fisher's exact =1.9541 Pr = 0.198

Table 24 Adjustment of chi-square statistics for herd effect in the sex groups

Zone	Chi-square	Cluster correlation factor	Adjusted chi-square	Chi-square Pr.
E. Shewa	5.3356	5.19398574	1.027265046	>.25

Table 25 Chi-square frequency of sero-positivity in sheep and goats of E.Shewa based on RPS and AS samples

	Test result		
Species	+ve	-ve	
Sheep	28	194	222
Goats	55	341	396
Total	83	535	618

Pearson chi2(1) = 0.1712 Pr = 0.679

Table 26 Chi-square frequency of sero-positivity in sheep and goats of N.Wollo based on RPS and AS samples

	Test result		
Species	+ve	-ve	
Sheep	9	292	301
Goats	16	267	283
Total	25	559	584

Pearson chi2(1) = 2.5257 Pr = 0.112

Table 27 Chi-square frequency of sero-positivity in the three zones based on RPS and AS samples

	Test result		
Zone	+ve	-ve	
E.Shewa	83	537	620
N.Wollo	25	559	584
S. Wollo	1	267	268
Total	109	1363	1472

Fisher's exact (2) =59.9993 Pr = 0.000

Table 28 Adjustment of chi-square statistics for herd effect in the three zones

Variables	Chi-2	Adjusted chi-2	Pr
Zones	59.9993	9.21	.01

Table 29 Chi-square frequency of sero-positivity in the different age groups in E.Shewa

	Test result		
Age group	+ve	-ve	
<1 year	10	79	89
1-2 Years	16	165	181
2-3 Years	33	160	193
> 3Years	120	24	144
Total	83	526	607

Pearson chi2(3) = 7.0773 Pr = 0.069

Table 30 Fisher's exact frequency of sero-positivity in the different age groups in N.Wollo

	Test result		
Age group	+ve	-ve	
<1 year	0	23	23
1-2 Years	1	51	52
2-3 Years	11	258	269
> 3Years	13	227	240
Total	25	559	584

Fisher's exact (3) =2.0712 Pr = 0.749

4.1.8. Risk factor identification

The logistic regression test criteria set by Stata software compiler (2000) was used to fit a model that reflected the variability in the data. Using these criteria the fitting of the model with all assumed risk factor (sex, age, species, source of sample (serum bank or field based) and zonecode) was found poor (p> .9). The model was run without the variable age group with all its dummy variables and checked for goodness of fit (the ability of the model to represent the observed data). No significant improvement in the model was observed. The model was fitted again without the variable species and goodness of fit was assessed. It was found poorly fitted (p>. 5). The final model was fitted with three variables (sex group, source of the sera and the zone). All the three independent variables in the logistic regression model were found to have a significant effect on the sero-positivity. The odds ratio of the sero-positivity for active search (AS) sera was found to be .524 (P-value .09) compared to sera selected from the serum bank. The odds ratio for samples collected from North Wollo was found to be .2688 (P-value 0.00) compared to the reference sample (E.Shewa and S.Wollo). The odds ratio for samples originated from South Wollo were found to be .02031 (P-value 0.000) compared to the reference sample (E.Shewa and N.Wollo). The odds ratio for sero-positivity in male compared to female was found to be .15632 (P-values .019). The independent variables source of the sera (field based samples), North Wollo, South Wollo and the female were found to have a significant effect on the independent variable sero-positivity of PPR and can be assumed as risk factor for the occurrence of the disease. The logistic regression parameters are shown in Tables 31 and 32.

Table 31 Logistic regression parameter estimates for sero-positivity risk factors (odds ratio)

tesresu	Odds Ratio	St.Err	Z	P>z	[95% Conf. Interval]
source	.5246467	.1295558	-2.612	0.009	.3233493 .8512594
Izonec_2	.2688006	.0645842	-5.468	0.000	.167847 .4304739
Izonec_3	.0201377	.0203442	-3.866	0.000	.0027802 .1458615
sex	.1563282	.1563282	-2.353	0.019	.1549659 .8437682

Number of obs = 1455, LR chi2(4) = 86.29, Log likelihood = -344.13131 Pseudo R2 = 0.1114

Table 32 Logistic regression parameter estimates for sero-positivity risk factors (regression coefficient)

tesresu	Coef	St.Err	Z	P>z	[95% Conf. Interval]
source	-.6450302	.2469392	-2.612	0.009	.323349--.8512594
Izonec_2	-1.313786	.2402682	-5.468	0.000	.167847--.4304739
Izonec_3	-3.905162	1.010255	-3.866	0.000	.0027802--.1458615
sex	-1.017214	.4323224	-2.353	0.019	.1549659--.8437682
Constant	-.9062725	.3347167	-2.708	0.007	-1.562305-- -.2502398

Number of obs = 1455, LR chi2(4) = 86.29, Log likelihood = -344.13131 Pseudo R2 = 0.1114

4.2. Active search for the disease

Laboratory examination of specimens collected from suspected cases of PPR yielded negative results for all the specimens shown in Table 2.

4.3. Questionnaire-generated data

4.3.1. Summary of questionnaire results

The variables of the questionnaires in which the owners described two clinical signs or more in East Shewa and three clinical signs in Wollo area were considered eligible for analysis unless there were other indications that could exclude PPRV as a causative agent. This resulted in 34 questionnaires out of 70 (49%) questionnaires in East Shewa and 48 questionnaires out of 100 (48%) questionnaires in Wollo.

The questionnaires in which the owners identified the disease based on the clinical signs were considered in the analysis. However for variables (time of kidding, need and payment for vaccine) that were not affected by recall bias or misclassification bias (poor identification of disease) all the questionnaires filled (100 and 70 questionnaires) were considered. Identification of the time of kidding was used to pinpoint the time when there were so many susceptible animals assuming the disease is endemic. It was compared with the last date of occurrence of the disease and veterinary records, thereafter it was used to identify the optimum vaccination time. Detailed summary of the crude questionnaire before assessment can be found in Annex 7, names and numbers of the villages in each district covered by the questionnaires and its

distribution can be found in Annex 5 and average number of animals owned by the respondents are shown in Annex 6.

4.3.3. Questionnaire output in E.Shewa

In East Shewa the questionnaire covered 35 villages in eight districts (Annex 6). The response of only 34 owners interviewed was considered. 23 out of 34 (68%) owners interviewed were from sedentary production system and 11 out of 34 (32%) were from mixed production system, all of them indicated the need for vaccine and 82% (28/34) of them agreed to pay vaccination cost, while (14%) 5/34 did not. Only 26% (9/34) of the owners interviewed treated their sick animals, half of them said treatment was effective. 32% (11/34) said goats were more susceptible than sheep, 26% (9/34) said sheep were more susceptible (most of them have no goats), while 41% (14/34) said that both species were susceptible. 59% (20/34) said that all age groups of goats were susceptible, while nobody said animals less than 6 months were susceptible. 65% (22/34) said all age groups of sheep were susceptible while nobody said animals less than 6 months were susceptible. 47% (16/34) of the owners said the disease used to occur annually, 18% (6/34) said the disease was always there and 18% (6/34) said disease occurred only once. Concerning season of PPR occurrence 44% (15/34) said the disease used to occur in the dry season, 35% (12/34) said the beginning of the rainy season and 15% (5/34) said the spring (June-April). 18% (6/34) said PPR affected 50% of the herd and 15% (5/34) said 25% of the herd and more than half of the interviewed said the disease affected 10-50% of the herd. Frequency of the aforementioned variables is shown in table 33 and 36. Concerning the last date of occurrence of the disease September 2000 got the highest frequency 18% (6/34) followed by December 2000 and September 1999, 12% (4/34) each. The outbreaks were distributed all over the months of year with higher frequency between September and January (55%=19/34). The frequency of the different months identified by the owners in addition to the local name given to the disease are shown in Table 34. The rate of kidding in East Shewa was higher in September-January with a frequency 33/70 (47%) followed by March-August with 23/70 (33%). A detailed summary for the 34 questionnaires is shown in Table 33, 34, 35 and 36.

Table 33 Summary of the questionnaires found valid for inclusion in the analysis in E.Shewa

Variable\ Code	9	0	1	2	3	4	5	6	7	8
Status	x	x	34	0	x	x	x	x	x	x
Production system	x	0	23	11	x	x	x	x	x	x
Disease with oral lesion	x	26	8	x	x	x	x	x	x	x
Disease with watery dirrhoea	x	0	34	x	x	x	x	x	x	x
Disease with cough and nasal discharge	x	0	34	x	x	x	x	x	x	x
Need for vaccine	0	0	34	x	x	x	x	x	x	x
Vaccine payment	0	5	28	1	x	x	x	x	x	x
Treatment of diseased	0	25	9	x	x	x	x	x	x	x
Treatment effect	24	5	5	0	0	x	x	x	x	x
Species susceptible	0	x	11	9	14	x	x	x	x	x
Source of income	x	x	10	12	0	1	11	x	x	x
Goat age susceptibility	8	x	0	1	2	20	3	x	x	x
Sheep age susceptibility	5	x	0	3	1	22	3	x	x	x
Season of occurrence	1	15	12	5	1	x	x	x	x	x
Frequency of occurrence	4	x	16	1	6	1	6	x	x	x
Disease presence at the time	x	34	0	x	x	x	x	x	x	x

Code definition
Status: 1= Owner, 2= worker
Production system: 0= nomadic, 1= sedentary, 2= mixed, 9=disease not known
Disease with oral lesion: 0= absent, 1= present.
Disease with watery diarrhoea: 0= absent, 1= present.
Disease with cough and nasal discharge: 0= absent, 1= present.
Need for vaccine: 0= No, 1= Yes, 9=disease not known
Vaccine payment: 0= No, 1= Yes, 2= I do not Know, 9=disease not known
Treatment of diseased: 0= No, 1= Yes, 9=disease not known
Treatment effect: 0= No, 1= Yes, 2= somewhat, 9=disease not known
Species susceptible: 1= Goat, 2= Sheep, 3= both, 9=disease not known
Source of income: 1= Selling animal, 2= Cultivation, 3= Other, 4= cultivation and selling animals,
Goat age susceptibility: 1==<6 month Goat, 2=6-18m, 3=>18 month, 4=all, 5= non susceptible, 9=disease not known
Sheep age susceptibility: 1==<6 month, 2=6-18m, 3=>18 month, 4=all, 5= non susceptible, 9=disease not known
Season of occurrence: 0= dry season, 1= rainy season, 2= spring (April-June), 9=disease not known
Frequency of occurrence: 1= yearly, 2= always there, 3= every 2 years, 4= every 3 years, 5= only once, 9=disease not known.
Disease presence at the time: 0= absent, 1= present.
x= not allowed

Table 34 Summary of questionnaire variables "last date of occurrence and local name" in E.Shewa

Last date of the occurrence of the disease		Local name of the disease	
Last date of disease	Frequency	Local name	Frequency
June 2000	2	Albati	3
June 1999	2	Furo	7
March 2000	4	Kofa	1
September 2000	6	Kufa	3
March 2001	3	Lukuche	2
April 2001	2	Saal	2
November 1999	1	Sonba	7
December 2000	4	Tane	1
January 2000	1	Yato	1
January 2001	2	I do not know	7
January 1998	1		
No specific time	1		
September 1999	4		
May 1999	1		

Table 35 Summary of the questionnaire variable "time of kidding" in E.Shewa

Month	Dec-Jan.	June-July	August-Oct	March-June	Sept-Dec	I do not know	No specific
Frequency	3	11	2	10	30	7	7

Table 36 Summary of questionnaire variable "proportion of animals affected in the herd" in E.Shewa

Proportion of herd affected	.1	.01	.25	.3	.4	.5	.6	.75	.9	1
Frequency	6	1	5	3	1	6	3	3	1	3

4.3.4. Questionnaire output in Wollo area

In Wollo region 48 questionnaires were considered after the preliminary assessment. The summary of the crude questionnaire is shown as Annex 7. 35/48 (73%) of the owners were from sedentary production system, 12/48 (25%) were from mixed production system and 1/48 (2%) was from nomadic production system. 44/48 (92%) of them said there was a need for vaccine, while 3/44 (7%) said no need and 1/48 (2%) said they did not know. Of those who indicated the need for vaccine 39/48 (81%) said they would pay for the vaccine, 9/48 (19%)

said would not pay. Concerning the efficiency of treating animals 35/48 (72%) said they treated their sick animals, 24 (63%) of them said the treatment was not effective and 6 (17%) said effective. Based on their previous experience 21/48 (44%) claimed that sheep and goats were both susceptible, 13/48 (27%) said goats and 14/48 (29%) said sheep. 30/48 (63%) said all age groups of goats were susceptible and 1/48 (2%) said goats less than 6 months were susceptible. 32/48 (67%) said all sheep age groups were susceptible, 5/48 (10%) said sheep less than 6 months were susceptible and 6/48 (13%) said sheep were not susceptible. 27/48 (56%) said the disease occurred in the dry season, 10/48 (21%) said beginning of the rainy season and 6/48 (13%) said the spring (April-June). Concerning the frequency of the disease 20/48 (42%) said the disease used to occur every year, 14/48 (29%) said always there and 9/48 (19%) said occurred only once. Frequencies of variables or response to questions are summarised in Tables 37 to 41. 27/48 (56%) said the disease affected 90% of their herd and 13/48 (27%) said 50%. Concerning the variable last date of the disease occurrence the highest frequency was in March 2001 and January 2000 (21% (10/48)). According to the owners most of the outbreaks occurred between November to March (45/48=94%). 20/100 (20%) of the interviewed said the time of kidding was in May-September, 52/100 (52%) said there was no specific time, 20/100 (20%) said June-October and 3/100 (3%) said May-July and January-March each. The peak of kidding and high rate of kidding appeared to be confined to May-September (45%). Frequencies based on the owners' response are shown in Table 37-41.

Table 37 Summary of the questionnaires found valid for inclusion in the analysis in Wollo

Variable\ Code	9	0	1	2	3	4	5	6	7	8
Status	x	x	48	0	x	x	x	x	x	x
Production system	x	1	35	12	x	x	x	x	x	x
Disease with oral lesion	x	0	48	x	x	x	x	x	x	x
Disease with watery diarrhoea	x	0	48	x	x	x	x	x	x	x
Disease with cough and nasal discharge	x	0	48	x	x	x	x	x	x	x
Need for vaccine	0	3	44	1	x	x	x	x	x	x
Vaccine payment	0	9	39	0	x	x	x	x	x	x
Treatment of diseased	0	21	27	x	x	x	x	x	x	x
Treatment effect	13	24	6	5	x	x	x	x	x	x
Species susceptible	0	x	13	14	21	x	x	x	x	x
Source of income	x	x	15	14	2	2	15	x	x	x
Goat age susceptibility	7	x	1	2	0	30	8	x	x	x
Sheep age susceptibility	6	x	5	2	1	32	2	x	x	x
Season of occurrence	3	27	10	2	6	x	x	x	x	x
Frequency of occurrence	1	x	20	14	2	2	9	x	x	x
Disease presence at the time	x	34	14	x	x	x	x	x	x	x

*Codes are shown below Table 32

Table 38 Summary of the questionnaire variable "last date of the disease occurrence and local name" in North and South Wollo

Last date of the occurrence of the disease		Local name of the disease	
Last date of disease	Frequency	Local name	Frequency
July 2000	1	Abdra	2
August 2000	1	Afa mandid	2
March 2000	4	Aklit	1
September 2000	1	Dukuba	3
March 2001	10	Fantatas	2
April 2000	3	Finoita	2
November 1999	1	Gogessa	1
November 2000	7	Intute	2
January 2000	3	Kisan	2
January 2001	10	Kitign	3
January 1999	1	Kufa	5
February 2001	1	Kukiri	1
December 1999	1	Kurufa hatiso	1
		Maaz	4
		Takamt	1
		Gumfar	3
		Nakarsa	1
		Inbikina	1

Table 39 Summary of the questionnaire variable "time of kidding" in Wollo

Month	Jan. –March	May-July	June-Oct.	December	Rainy season	No specific
Frequency	3	3	20	2	20	52

Table 40 Summary of the questionnaire variable "proportion of animals affected in the herd" in N.Wollo

Proportion of herd affected	0	.1	.2	.25	.3	.4	.5	.9
Frequency	13	1	1	1	2	5	13	21

Table 41 Summary of the questionnaire variable "proportion of animals affected in the herd" in S.Wollo

Proportion of herd affected	0	.1	.2	.25	.4	.5	.75	.9
Frequency	6	3	4	1	3	7	1	18

4.3.2. Comparison between the results of the serology and questionnaire outcomes

The frequency of the variable knowledge about PPR disease, which was defined as occurrence of a disease with the clinical signs described in the questionnaire, was summarised as a percentage of the total questionnaires administered in each district in the three zones. The

percentage of the disease occurrence according to the owners was aligned along the result of the serology in each district in Table 41 and Table 42 and plotted in Figure 10. In East Shewa the sera tested were distributed almost all over the districts (warda) covered by the questionnaire (5/8), but in Wollo region only three districts were covered by both the questionnaires and the serology, with small sample size in two districts (Table 5.2). The level of consistency appear reasonable in E.Shewa, however in Wollo the consistency is low; in three districts (89 samples) the owners claimed the presence of the disease while no serological evidence were found in the serum samples. Both the questionnaire outcome and the serology data are shown in Table 42 and 43 and Figure 9.

Table 42 Comparison between the results of the serology and the questionnaire outcome in E.Shewa

Warda*	No. of interviewed know PPR	No. of questionnaire filled in the warda	Percentage of questionnaire	Sample of sera tested	Positive sample	Prevalence estimated
Adama	7	14	.5	121	20	0.1653
Adami tulli	3	8	.375	195	23	0.1179
Arsi Negele	2	8	.25	20	4	0.2
Boset	5	8	.625	58	6	0.1034
Dudgabora	3	8	.375	0	0	0
Adaa Liben	2	8	.25	80	5	0.0625
Fentale	8	8	1	126	24	0.1905
Shashemane	4	8	.5	0	0	0
Total	34	70	.48	600	82	0.1367

Table 43Comparison of the serology results and the questionnaire outcomes in Wollo areas

Warda*	No. of interviewed knows PPR	Number of questionnaire filled	Percentage	Sample of sera tested	Positive sera tested	Prevalence
Bati	10	17	.5882	440	25	0.0568
Chafa Robi	1	5	.2	0	0	0
Dessie Zuri	2	4	.5	0	0	0
Guba lafto	3	9	.33	0	0	0
Habru	7	10	.7	0	0	0
Kobo	8	18	.44	0	0	0
Kutabor	2	7	.2857	11	0	0
Heik	6	12	.5	28	0	0
Waldiya	2	3	.667	41	0	0
Sanbati	7	15	.47	0	0	0
Total	48	100	.48	520	25	0.0481

* Warda in which owners describe at least two of the clinical sign

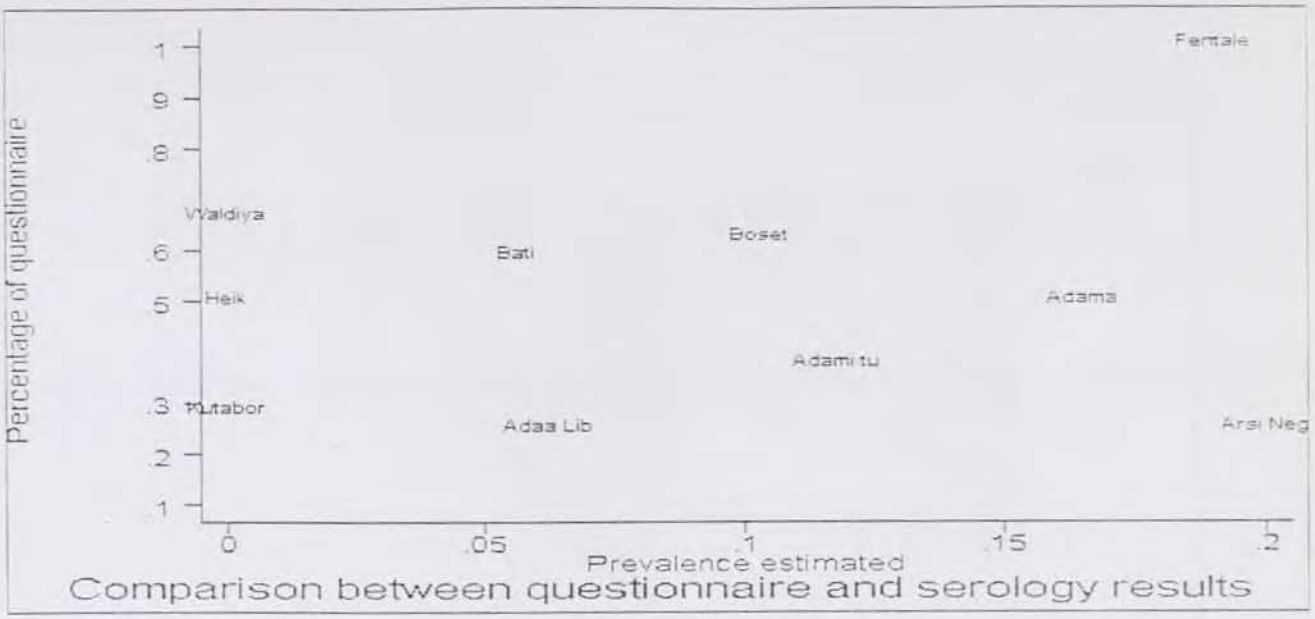


Figure 9 **Comparison between the serology results and questionnaire outcome**

4.4. Veterinary service records

4.4.1. Summary of 18 outbreaks found in the veterinary service records

Based on eighteen outbreaks investigated by the veterinary service during the last three years, PPR affected a fraction of .0325 of goats with a range between .0014-. 826 and a fraction of .0072 of the sheep with a range between .00038-.028. It caused the death of .4465 of the affected sheep with a range between .3-. 94 and .6501 of affected goat with a range between .4-. 75. The average death in the affected sheep herd was found to be .0032 with a range between .000125-. 027, while the average death in the affected herds of goats was .0211 with a range between .00067-. 596. The 18 outbreaks are summarised in Table 44 and Figure 10.

Table 44 Summary of 18 outbreaks reported to the veterinary service

Species	Average proportion of affected animals	Average deaths among affected	Average Deaths in the outbreak
Sheep	0.0072	0.4465	0.0032
Goats	0.0325	0.6501	0.0211

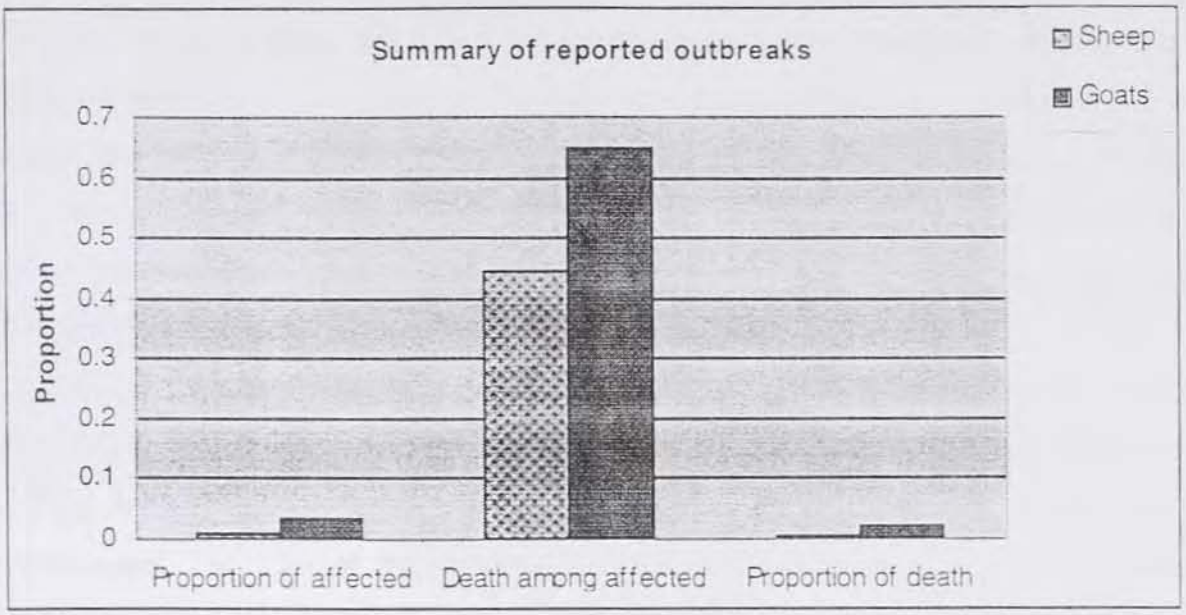


Figure 10 Comparison between veterinary record estimates of affected proportion, death among affected and proportion of deaths in sheep and goats based on reported outbreaks

4.4.2. Comparison between veterinary records estimates in sheep and goats

The average proportion of affected animals, average death among affected animals and average death in the outbreaks involving sheep and goats were compared using chi-square. The result of the comparison is shown in Table 45. A significant difference between the three estimates occurrence in sheep and goats was detected (P-value<. 001).

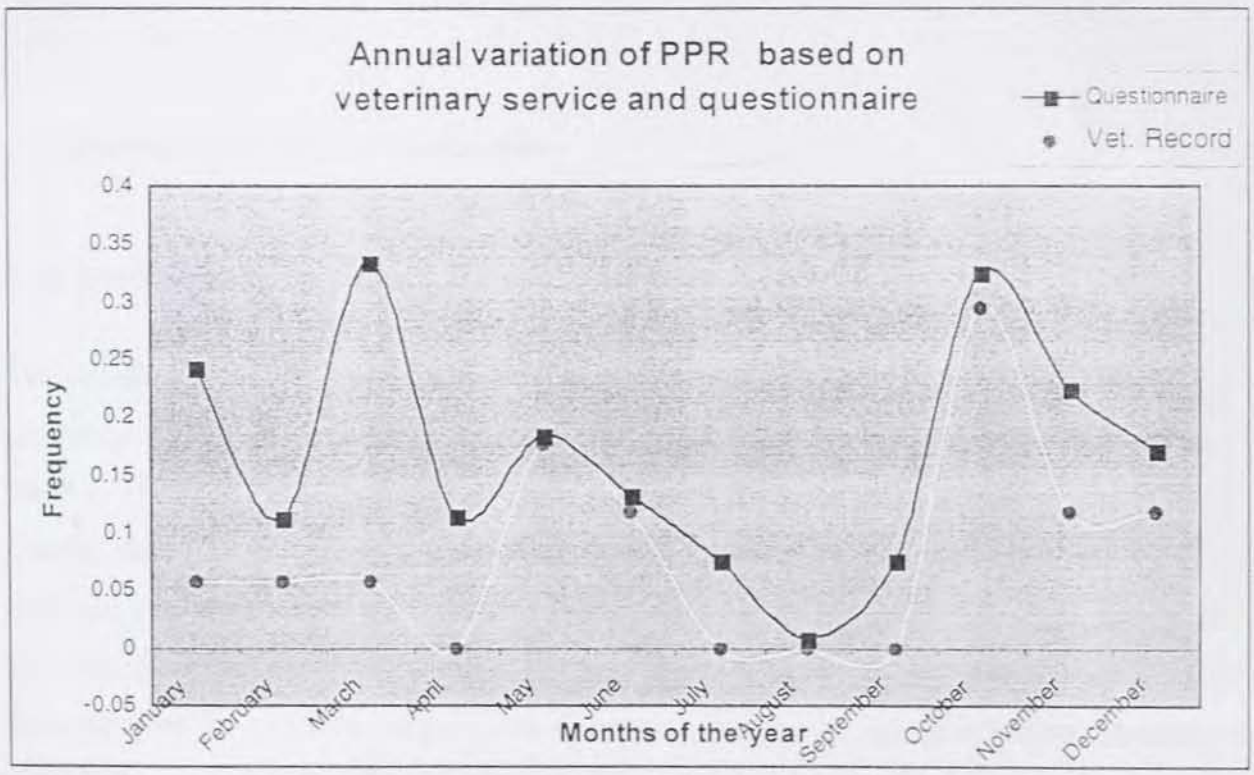
Table 45 Comparison between veterinary service records estimates of affected proportion, death among affected and proportion of deaths in sheep and goats using chi-square

Variable	Chi-square statistics	P-value
Average proportion of affected animals	323.36	0.00
Average death among affected	19.87	0.000
Average death in the outbreak	270.63	0.000

4.4.3. Comparison between the veterinary service records and questionnaire outcomes

The time identified as the last date of outbreak occurrence by the owners interviewed and the outbreaks reported to the veterinary service were plotted (Figure 11). According to both the owners and the veterinary service the disease occurred around the year with high frequency in October to December. Another peak for the disease between January to March was identified by the owners, but not according to the veterinary service records. Based on visual assessment of Figure 11 a high level of agreement could be recognised.

Another questionnaire variable was plotted simultaneously with the time of the last outbreak identified by both the veterinary service and the owner in Figure 12. This variable is the time of kidding. A clear relation between peak of kidding and the occurrence of the disease could by hypothesised. The peak of kidding was found to be in April to August while most of the outbreaks according to both the owners and the veterinary service occur in September to March. This implies that animals born in April-August would become susceptible after waning of the maternal immunity four months later, thus sufficient number of susceptible animals would be



available for the disease to take the form of an outbreak.

Figure 11 Annual variation of PPR based on veterinary service records and questionnaires

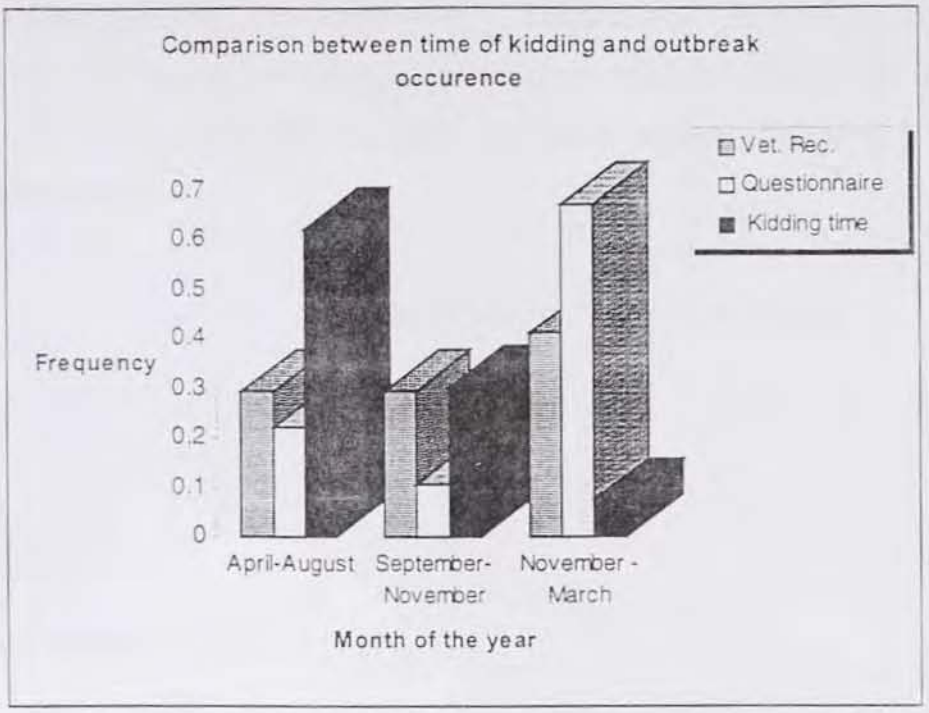


Figure 12 Comparison between kidding time and outbreaks occurrence

4.4. Economic feasibility of vaccination

4.4.1.Decision Tree Analysis

The decision trees for sheep and goats was constructed separately based on estimates of the combined sample (RPS and AS) for the total sample and E.Shewa. Therefore four models were fitted to identify if vaccination of animals against PPR would be profitable or not. All the four models with their outcomes are shown in Figure 13 to 16. Vaccination of goats and sheep in E.Shewa was found to be profitable. The calculated expected return of vaccination was 2.02 birr and 1.14 birr for sheep (Figure 15) and goats (Figure 16), respectively in E.Shewa. A probability of .14 and .126 for goat and sheep was used for the rate of infection. Another model was run using probability based on the total sample, it was run separately for sheep and goats. The calculated expected return of vaccination based on the former model was .23 birr and -. 47 birr for goats and sheep, respectively. According to these models vaccination is profitable on average for all study areas for goats but not for sheep. In all the previous models 2.05 birr was assigned for the cost of the vaccination and 50 birr and 84 birr profit for recovered or non-

infected goats and sheep respectively. The decision tree also presented the financial loss that would be experienced by unvaccinated herds with that rate of infection. Conversely regardless of the animal values, the maximum that can be incurred by vaccinating endlessly is the cost of vaccination.

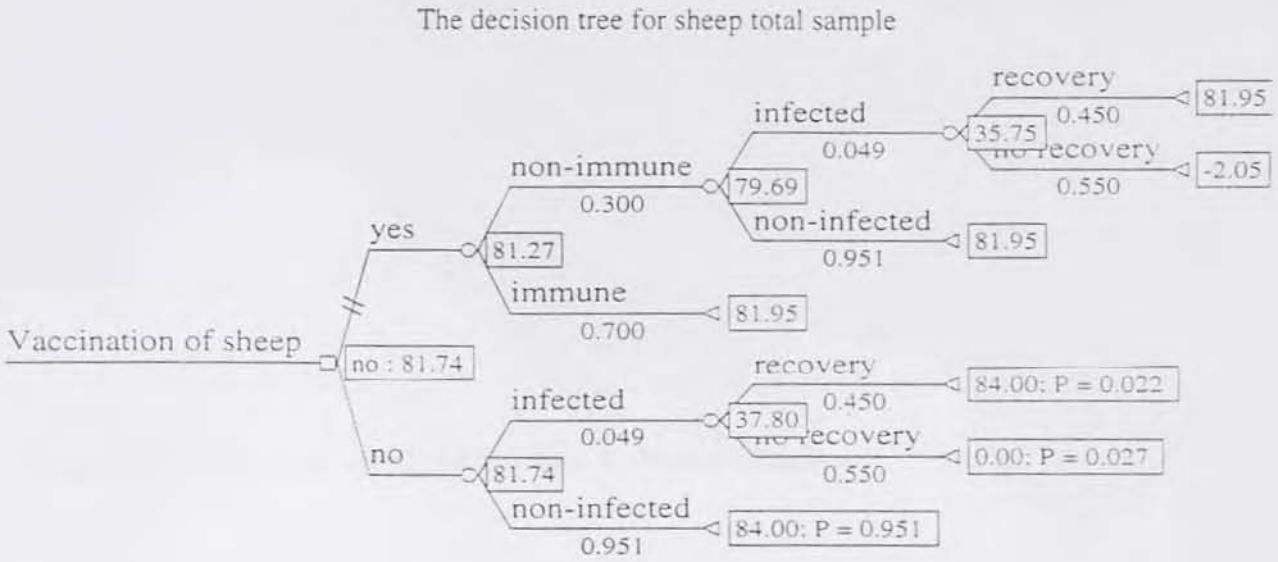


Figure 13 Decision tree for sheep based on sero-positivity found in the combined sample (RPS and AS) for all zones

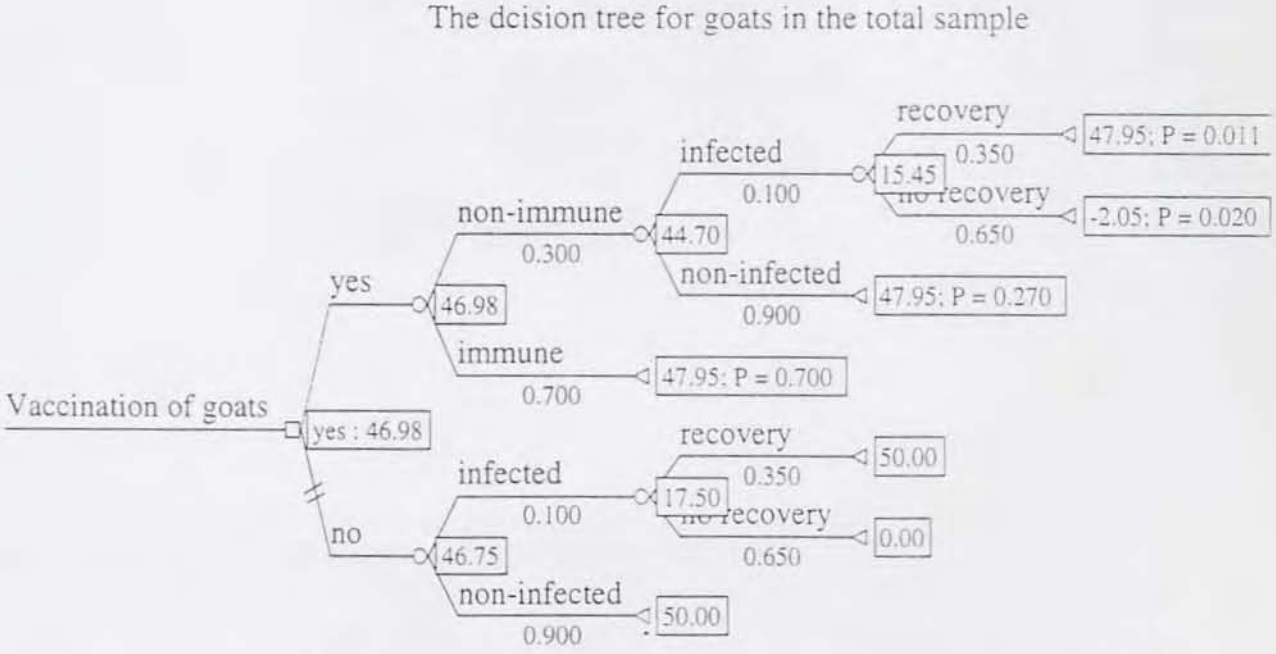


Figure 14 Decision tree for goats based on sero-positivity found in the combined sample (RPS and AS) for all zones.

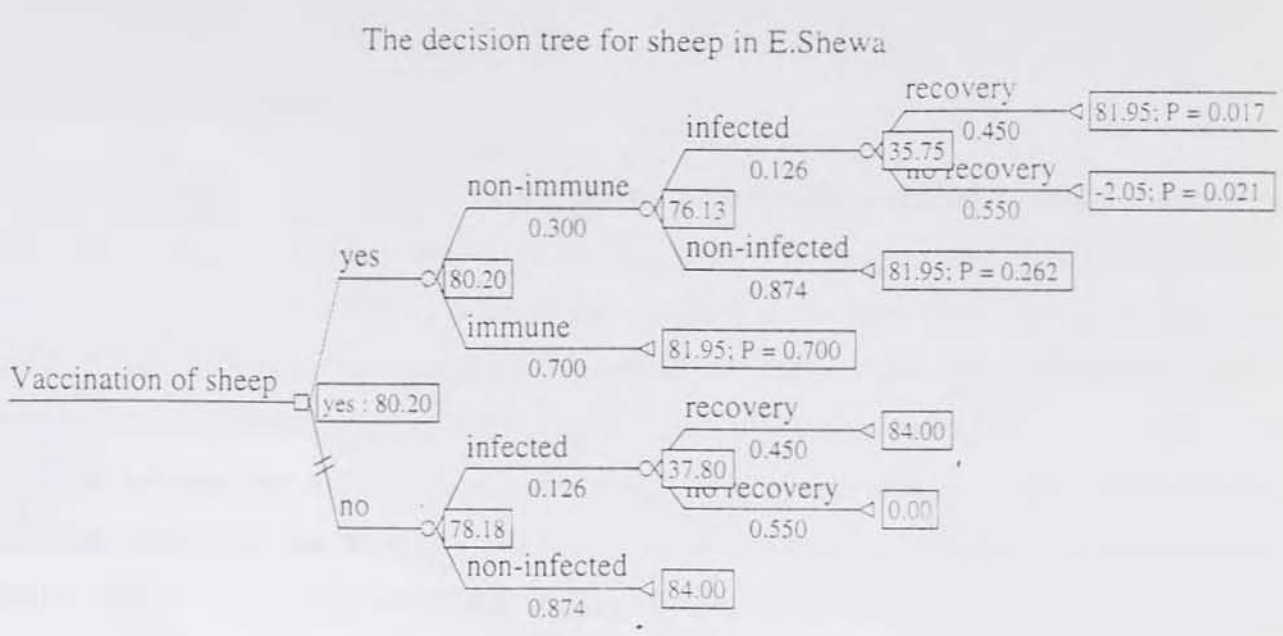


Figure 15 Decision tree for sheep based on E. Shewa estimates.

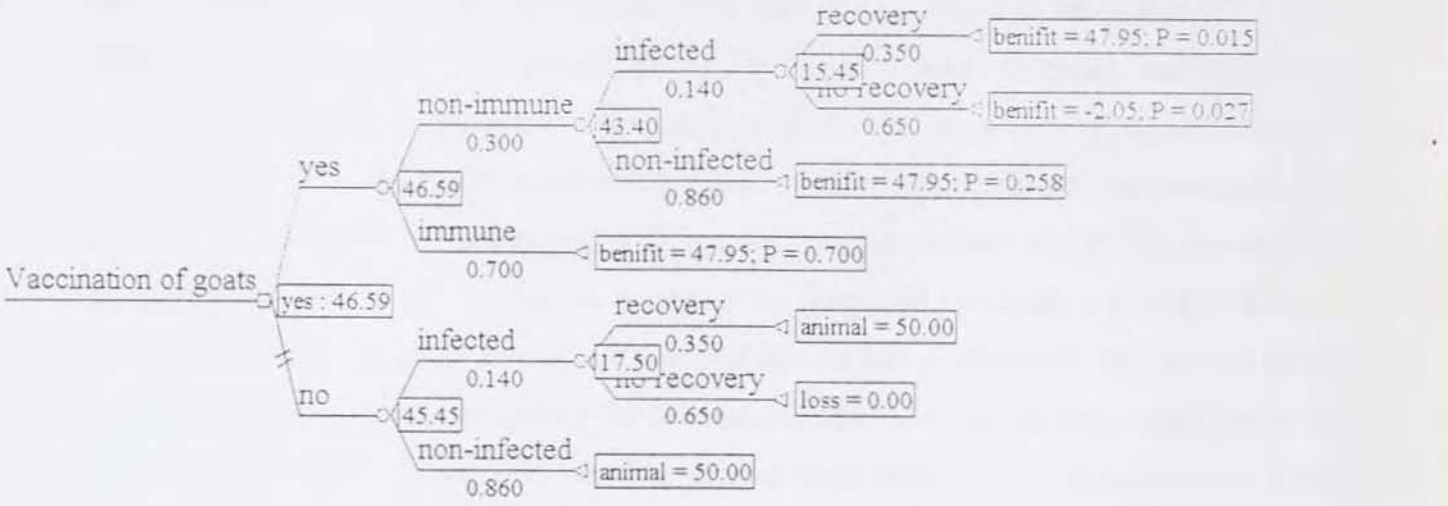


Figure 16 Decision tree for goats based on E.Shewa estimates.

4.4.2. Sensitivity Analysis

Sensitivity analysis was carried out for the rate of infection, the levels of immunity induced by the vaccine, animal price and vaccine price. The sensitivity analysis was run for all the four previous mentioned models using three of the variables at the same time. The outcome of the sensitivity analysis is shown as graphs in Figures 17-24. These figures show the break even for vaccination for specified range of values for the four variables. In figures 19-24 the area above the curve indicates the range of values for which the programme is economic while the area below the curve indicates where vaccination is not economic. The threshold or the break-even point is where the output of vaccination and no vaccination equalised each other. The threshold for the model for goats is shown in Figures 17, 21, 22 and 23. The threshold was identified at a level of sero-positivity of .06 when the immunity was .6 and the animal price was 70 birr and at a level of .03 sero-positivity for .9 level of immunity and 85 birr for the goat price. The price of the vaccine which maintain the balance between profit and loss was found to be 1.6 birr at .13 sero-positivity and 40 birr for the animal price and it was found to be 5 birr at .13 sero-positivity and 100 birr for the animal price. The balance between profit and loss can be maintained at 20 birr for the price of the vaccine when the sero-positivity is .44 and the animal price is 100 birr. For the model based on estimates of sheep the threshold was identified at .12 sero-positivity level for .6 immunity at 52.5 birr for the animal price and at .06 sero-positivity for .9 immunity and for 85 birr for the animal price when the vaccination cost was 2.05 birr. The threshold was identified also at .005 sero-positivity for .6 immunity and at 125 birr for animal price and at .03 sero-positivity for .9 immunity and 150 birr for the animal price when the cost of the vaccination was 2.05 birr. The vaccine price was found to be optimum at 1.8 birr price for .13 sero-positivity at 52.5 birr for the animal price and at 5 birr for .09 sero-positivity when the animal value is 125 birr. The price of 20 birr was found to be suitable at .38 sero-positivity and 125 birr for the price of the animal.

The maximum return of vaccination increases with the increase of the rate of infection in the four models (Figures 19 to 24). It was also found that an increase in the level of immunity induced by the vaccine maximise the profit obtained from vaccination (Figure 19, 21 and 24). The tornado diagrams shown in Figures 17-18 is one way sensitivity analysis brought together in one graph. The length of the bar indicates the importance of the factor. Tables shown below

the tornado diagram show parameters help in the assessment of the tornado diagram. The value of the animal was found to have a significant effect on the overall model outcome, it induced 98% of the change of expected return. It was followed by the level of infection, which contribute with 1-1.5% to the change in the model. The legend on the right side of the diagram indicates the range of variability selected. The risk pct column in the table below the diagram indicates the significance of the effects the variable has on the overall model. Based on the tornado diagram and the three sensitivity analysis it can be conclude that the animal value was found to be the most important factor in determination of the magnitude of the expected return.

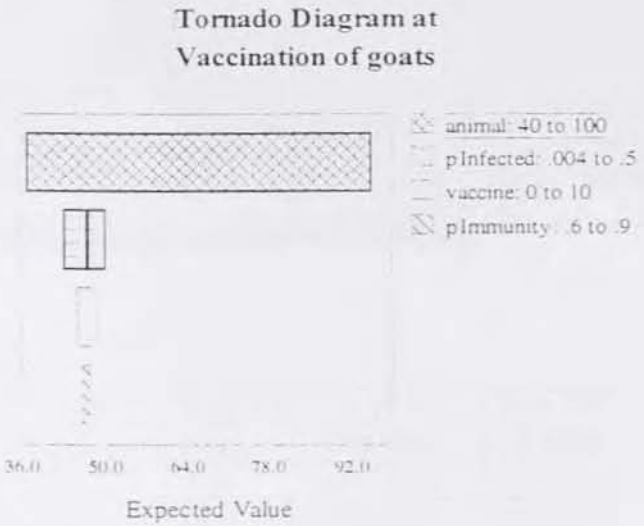


Figure 17 Tornado diagram for vaccination of goats

Variable	Low EV	High EV	Risk Pct	Risk Pct
Animal	37.4	96.0	98.5051	98.5051
Rate of infection	43.1	49.0	1.32447	99.8296
Vaccine	46.8	49.0	.14846	99.9780
Immunity	46.8	47.0	.02196	100

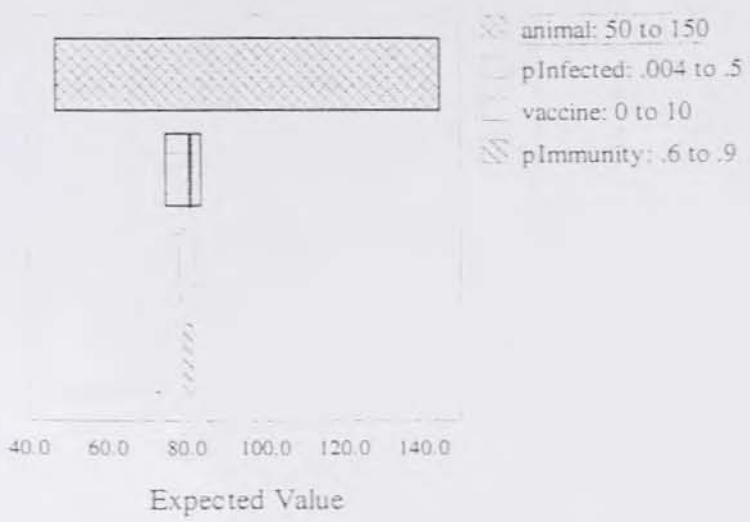


Figure 18 Tornado diagram for vaccination of sheep

Variable	Low EV	High EV	Risk Pct	Risk Pct
Animal	46.9	144.8	98.9984	98.9984
Rate of infection	75.0	83.8	99.7971	99.7971
Vaccine	78.2	82.3	99.9681	99.9681
Immunity	79.6	81.4	100	100

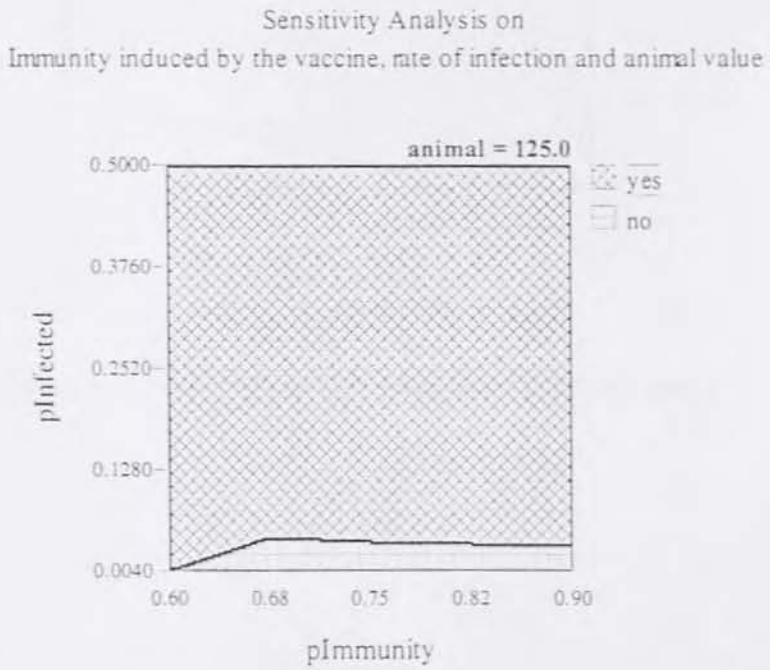


Figure 19 Three-way sensitivity analysis based on estimates of E.Shewa sheep

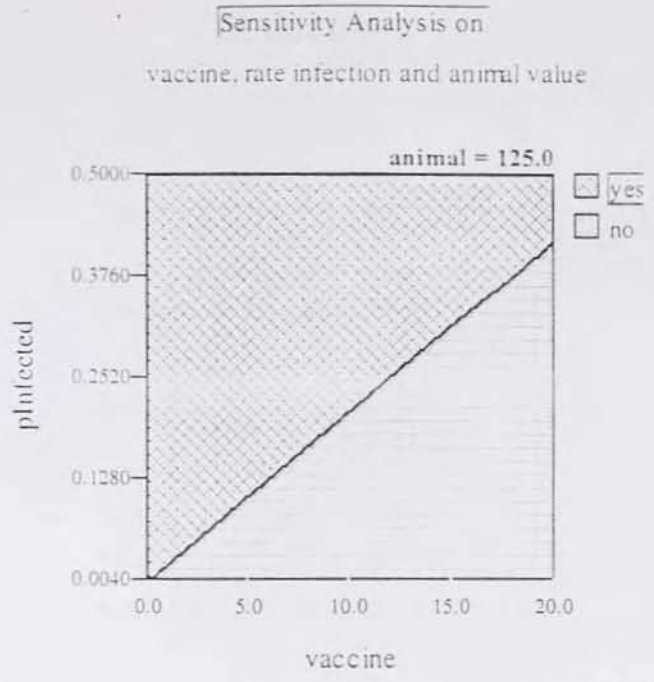


Figure 20 Three-way sensitivity analysis using estimates based on E.Shewa sheep model

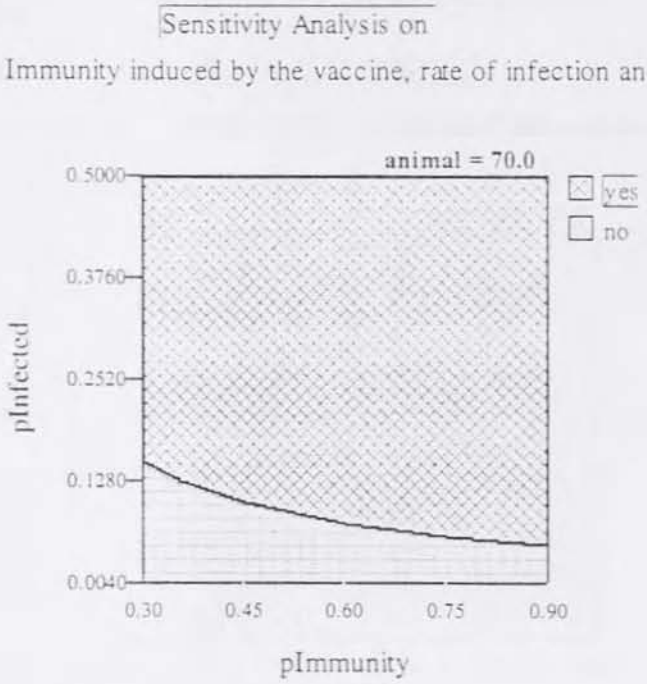


Figure 21 Three-way sensitivity analysis based on E.Shewa goats

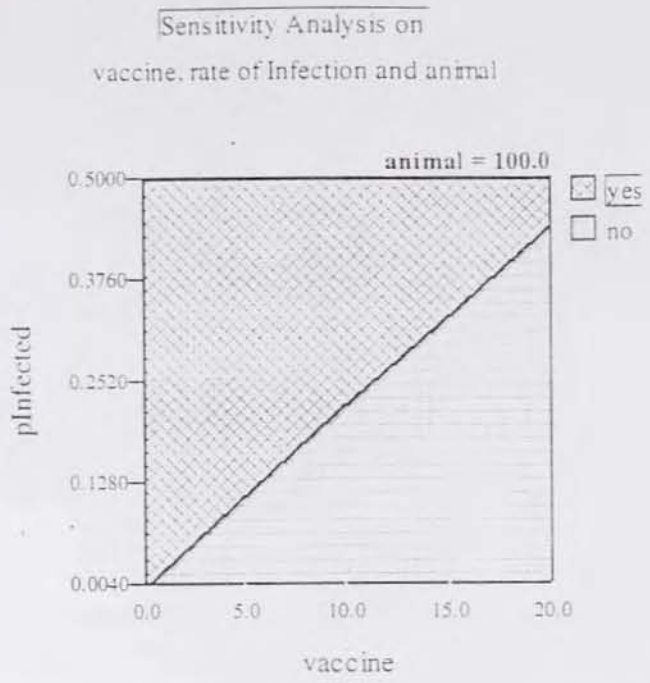


Figure 22 Three-way sensitivity analysis using estimates based on E.Shewa goats' model

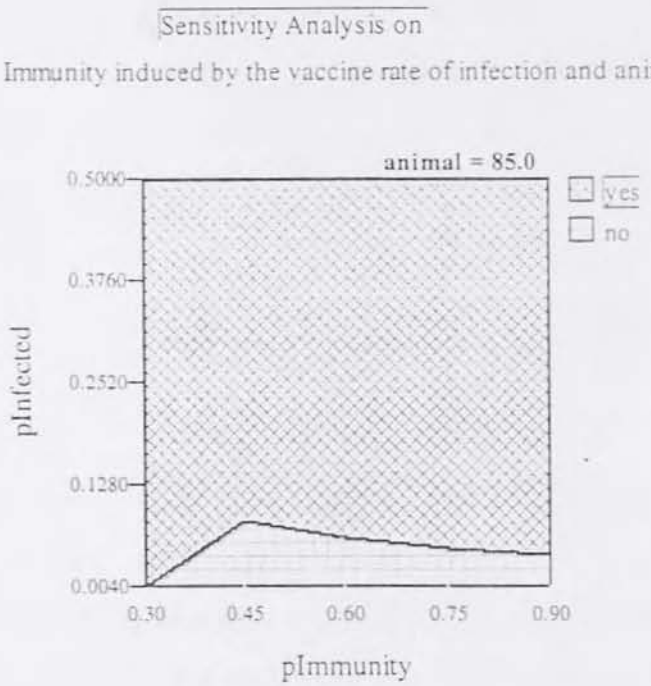


Figure 23 Three-way sensitivity analysis based on the estimates of goats' total samples

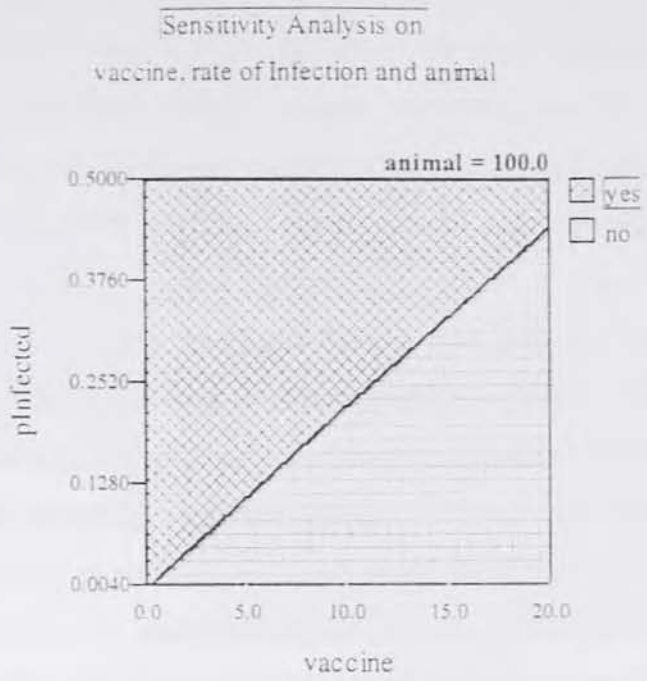


Figure 24 Three-way sensitivity analysis based on the estimates of goats' total samples

5. DISCUSSION AND CONCLUSION

5.1. Discussion

In this study sero-positivity of .134, .043 and .004 was estimated in E.Shewa, N.Wollo and S.Wollo, respectively, based on the result of C-ELISA. The sero-positivity in E.Shewa is in agreement with the finding of a previous study (Yeyherade, 1994), in which a sero-positivity of .167 was reported. However this study was based on samples collected from slaughterhouse and the samples (280) were from only two location in the zone (Metehera and Ziway). The study results are also in agreement with the finding of Roger and Bereket (Gelagy, 1996) who reported 17% and 12% sero-prevalence in North Shewa, but were inconsistent with other findings obtained by the same authors in goats and sheep of Arsi (53% and 33%) and in goats and sheep of Afarland (67% and 0%) in Ethiopia. Furthermore, these estimates are far lower than the estimates obtained by Majiyabe (1994) who reported a sero-prevalence of 35 and 56.5% in Cameroon and Nigeria respectively. These findings also contradict the reports from

Niger (53-63%)(Stem, 193), Nigeria (30-40%) (Taylor, 1984) and Oman (23-24%) (Taylor *et al.*, 1990). Nevertheless the disagreement can be a consequence of the different ecology, production system and study design features. However, examination of the naive confidence interval (.108-. 163) and confidence interval adjusted for herd effect (.073-. 2) indicates that the sero-positivity reported here might be consistent with earlier published results.

The sero-positivity estimates in North Wollo and S.Wollo disagree with the finding of Yeyherade (1994) who reported a sero-positivity of 45% and 30% in N.Wollo and S. Wollo respectively. The disagreement can be attributed to the size of sample from which the estimate was extracted (150 versus 852 in this study) and the source of the sera (slaughterhouse).
Testing serum bank based samples and field based samples yielded different estimates for sero-positivity in both E.Shewa and N.Wollo. The sero-positivity estimates for serum bank were .168421 and .043103 in E.Shewa and N.Wollo respectively while it was .07916 and .0833 for E.Shewa and N.Wollo in the field based sample. Furthermore when the source of the sample was fitted in the logistic regression, the odds of sero-positivity for animals in serum bank sample was found to be tow fold the odds of animals in the field sample. The high risk for animals whose sera were selected from the serum bank and the disagreement between the two estimates may be due to that fact that two districts in E.Shewa (Boset and Arsi Negele) were covered by the field samples but not by the serum bank and another district was covered only by the serum bank (Adaa Liben). Moreover, the district of Delante in N.Wollo and the district of Debrasina in South Wollo were covered by the serum bank samples only, while the district of Waldiya in N.Wollo and districts of Kutabar and Heik in South Wollo were covered by the field based samples only. Furthermore different villages in the different districts were covered by the serum bank and the field samples. Such situation in the presence of strong herd correlation effect can generate different estimates. Beside that the number of samples tested was not the same. 1073 samples were selected from the serum bank and 399 sera were collected during the fieldwork.

The presence of different sero-positivty in E.Shewa and N.Wollo can be attributed to the presence of different patterns of the disease. A situation that was encountered in North and South Nigeria (Taylor, 1984). In North and East Nigeria the disease occurs in waves every three to five years while the disease occurs annually in Southern Nigeria. Evidence to support such hypothesis could be found in the distribution of sero-positvity in the different age groups with a zero sero-positivity in animals less than one year in Wollo. Moreover, PPR is believed to be

endemic in dry areas (Lefevre and Diallo, 1990), a situation that applies to E.Shewa which is predominantly lowland (kolla) with less than 700 meter above sea level (Rift valley) or of a middle altitude with less than 1000 meter above sea level (Nazareth, Modjo) (E.Shewa Dept. Agriculture, 1989). In contrast to E.Shewa most of Wollo (with some exception such as Kobo area) is a high humid land where cyclic outbreaks interrupted by silent periods of 3 to 4 years are more common. The occurrence of endemic PPR in dry areas can be attributed to the great tenacity of paramyxoviruses against dry conditions (Lefevre and Diallo, 1990). However, 42% of the owners in Wollo claimed that the disease used to occur annually. The owners and the veterinarian in the area identified January 2001 as last date of the outbreak. This result is supported by the increasing of the sero-positivity from .04 according to the serum bank to .08 according to the field based samples. True differences between studies are possible although studies are not free of random and systematic error. Significant differences ($p < .05$) between the sero-positivity in sheep and goats in the three zones were established (chi-square) and the zone where the animals originated was found to have a significant effect on the sero-positivity in logistic regression. The odds of sero-positivity in N.Wollo was found to be 1/4 times the odds in the reference sample (E.Shewa and S.Wollo) and the odds of sero-positivity in S.Wollo was found to be 1/10 times the odds in the reference samples (E.Shewa and N.Wollo).

A sero-positivity of .139 and .126 was observed in goats and sheep of E.Shewa and a sero-positivity of .057 and .03 in goats and sheep of N.Wollo. This result is consistent with the finding of Yeyherade (1994) who reported 10% sero-prevalence in E.Shewa caprine and 15% in E.Shewa ovine. It also agrees with the finding of Roger and Bereket (Gelagy, 1996) who reported 17% and 12% sero-prevalence in goats and sheep of North Shewa, respectively. The estimate in N.Wollo contradicted the finding of Yeyherade (1994) who reported a sero-prevalence of 45% in N.Wollo caprine and 30% sero-prevalence in South Wollo ovine. A sero-prevalence of 23% and 24% in sheep and goats was reported from Oman and sero-prevalences of 53% and 63% in sheep and goats, respectively were reported from Niger. The previously mentioned arguments also explain the disagreement here. Generally it is held that goats react more severely to exposure compared to sheep and they exhibit striking clinical signs while sheep undergo a mild form of the disease. However a similar profile of serological status is widely reported (Taylor, 1984, Taylor, 1979a, Taylor *et al.*, 1990). This was supported by the result of chi-square where no significant difference ($p > .05$) was established in this study when the null hypothesis was tested at 95% level of significance. However, significant difference ($p < .05$) in sero-prevalence in sheep and goats of Ethiopia was reported before (Yeyherade, 1994). Moreover the species as a proposed risk factor was removed from logistic regression model

when it was found non significant (Wald test, p> .05) and not required for the fitting of the model.

An increase in sero-positivity with age was observed in E.Shewa and Wollo, this finding is consistent with the finding in Oman (Taylor, 1990) where serological evidence was reported in all age groups from 6 month up to 3 years. A similar finding was also established in Nigeria where antibodies occurred in animals between 4-24 months (Taylor, 1984). Since age determines the intensity and frequency of exposure, an increasing age goes along with exposure. However, no significant age effect was detected (P>. 05). The age group factor, when initially fitted into the logistic regression model, was found not to have a significant effect (Wald test-p> .05) on the dependent variable sero-positivity. A high sero-positivity in animals more than two years in E.Shewa was detected and a zero sero-positivity was detected in animals less than one year in Wollo. The distribution of the antibodies in all the age groups indicated that the virus circulates in year round base maintaining itself in the newly borne animals.

Though the sero-positivity of .04 and .028 was detected in N.Wollo female and male respectively, no significant difference was established (P>. 05). However, a significant difference (p< .05) between the sero-positivity in male and female of E.Shewa turned to be non-significant (p> .05) when adjustment for herd effect (intracluster correlation) was done. The effect of herd on the quality of inference reached when the disease correlate by herd is a well established fact (McDermott and Schukken, 1994, McDermott *et al.*, 1994, Donner, 1993). It causes violation of the assumption of indepence required for chi-square and other significance tests and leads to spurious inference (Donner, 1993). The herd effect problem arises when herds are selected and individual animal responses are assessed (McDermott and Schukken, 1994). The violation occurs because the responses of animals within the same herd are usually more similar than responses of animals from different herds (Donner, 1993). Adjustment for herd effect in this study eliminated the significant effect of sex on sero-positivity detected by chi-square. Furthermore a wider confidence interval was obtained for sero-positivity. The effect of herd correlation was assessed initially visually. A clear indication of correlation by herd was shown in Figures 5 and 6. This is a logical consequence of the contagious nature of the disease and sampling technique used (cluster sampling). An intracluster correlation of .2 and .02 was established in E. Shewa and N.Wollo, respectively. The intracluster correlation can be classified as high in E. Shewa and low in N.Wollo (McDermott and Schukken, 1994). The wide

variation in herd effect and sero-positivity in the three zones rendered producing one estimate for the whole sample invalid, therefore different inflation factor for each zone were used.

The finding of this study is inconsistent with the finding of Yeyherade (1996) who reported a significant difference in sero-prevalence in female and male. Though when the sex was fitted as a risk factor in logistic regression the risk for female was found to be very high compared to the male. This may not be taken as a final conclusion because the effect of the herd was not accounted for in the logistic regression. Ignoring adjustment for herd effect in logistic regression is one of the limitation that reduce the confidence in the validity of the inference obtained. The effect that herd size had on the sero-positivity was explored using scatterplot (Figure 4). Clusters with 20 samples appeared to have high frequency of disease occurrence. Though this does not reflect accurately the real situation, because what was plotted was the sample per cluster, which was assumed to be proportional to the size of cluster sampled. Herds with medium size seemed to have higher frequency of disease occurrence. Correlation of disease with herd size can be explained by the contagious nature of the disease and transmission by direct contact which is enhanced by confinement and close interaction (Braide, 1981). However this is confounded by the fact that most of the herd with 20 animals were in E.Shewa zone where a high sero-positivity was detected. Furthermore, a large cluster is supposed to be accompanied with a high probability that at least one of its many elements is infected, even under conditions of low correlation (Donald, 1993). However the finding of this study does not support this logic, but the nature of the cluster may be the reason. The cluster term in this study imply all the animals in the village where the social distance between the animals varied with time and the availability of pasture.

The apparent sero-positivity or prevalence is affected by the sensitivity and the specificity of the test used (Greiner and Gardner, 2000a). Adjustment for test misclassification and estimation of sero-positivity revealed that PPR c-ELISA underestimated the true prevalence when it is high (above 11%) and overestimated the true prevalence when it is low (11%). The estimates used for sensitivity and specificity were not generated neither by the manufacturer (BDSL) nor by the investigator who validated the test (Anderson *et al.*, 1991). They were produced by another investigator (Saliki *et al.*, 1993), but were found the most relevant estimates. However part of the population used in the validation and deduction of these estimates was not representative for the target population in Africa, a problem encountered in most of the tests issued for developing countries. Since sensitivity and specificity are not invariant from herd to

herd or area to area and are associated with demographic characteristic of the animals tested (Martin, 1984), such violation can affect the accuracy of the estimates generated. Evidence support the variation based on the demographic characteristics can be found in the variation of percentage inhibition of C-ELISA for the negative sera in the three zones as tested by Kruskal Wallis test. The significant difference found between the percentage inhibition of negative sera reflected the reality that diagnostic tests performed differently in different population and subpopulation (Greiner and Gardner, 2000b). Furthermore the estimates used here were obtained by comparison of C-ELISA to virus neutralisation test (Saliki *et al.*, 1993), though it was the most reliable test, it performed poorly (Libeau *et al.*, 1994).

The correlation between the questionnaire outcomes and the test results consolidate the confidence in the questionnaire reliability. The level of agreement between the result of the serology and questionnaire outcomes was explored using scatterplot. A considerable level of agreement was observed. However, the owners' perception of the disease occurrence was high compared to the level of sero-positivity detected, this can be explained by the nature of the disease and the human. The contrast between the questionnaire outcomes and the diagnostic test in some areas may be due to the variation in the size of the sample or the confusion of PPR with other diseases by the owners.

40% of the owners claimed that both sheep and goats were equally susceptible and 26% of them said that sheep were more susceptible. This contradicts most of the previous reports (Opasina and Putt, 1985, Taylor *et al.*, 1990, AbuElZein *et al.*, 1990) and disagrees with the outcome of the veterinary service records analysis in this study. However, some of the owners did not have goats and claimed that PPR affected the goats of their neighbours. Anyway, this can not rule out totally this claim, because there were reports from Nigeria and Senegal where sheep reacted severely compared to goats (Taylor, 1984). The claim of the owners (60%) that all animals age groups are equally susceptible does not agree with field studies which reported that prognosis improved with age (Lefevre and Diallo, 1990, Opasina and Putt, 198). However, the questionnaire outcomes are prone to the subjective judgement of the owners. The claims of the owners in E.Shewa that the disease used to occur annually is consistent with the high sero-positivity and its distribution in all age groups from 6 months to 4 years. However the low sero-positivity and the zero sero-positivity in animals less than one year may not support this claim in Wollo. Nevertheless, there was a recent report of PPR outbreak in the area to the veterinary service in Wollo. Therefore the owners may confused the fresh memories with previously unrelated ones.

Analysis of data extracted from the veterinary service records and data generated through questionnaire revealed that PPR could occur in all months of the year with a peak in October to March. Though the level of agreement in January to March is not as high as October-December, responses to a cross-checking question in the questionnaire about the season of the disease occurrence provided evidence that support this finding. Most of the respondents said that PPR occurred in the dry season (October-Dec) and at the beginning of the short rainy season (January-February). The finding of Gelagy (1996) and Yeyherade (1997) who detected sero-conversion in naïve animals in October and March supports this result. However, this does not agree with Ibadan university records in which the disease is encountered in all months of the year except January and February (Taylor, 1984) with the peak in the wet season. However, it agrees with the finding of Opasina and Putt (1985) who covered outbreaks during the dry season in two different ecological zones in Nigeria. It also agrees with the finding in Southern Nigeria where high incidence of PPR was observed during the dry season compared to the rainy season (Wosu *et al.*, 1992). The inclement dry, cold and dusty weather and the poor nutrition in Southern Nigeria were suggested as possible predisposing factors (Wosu *et al.*, 1992). One limitation of this study that veterinary service records reflected the situation in the whole country while the questionnaires refer to specific zones. Anyway, the circulation of the virus in one locality indicates the possibility of its transmission and occurrence in a neighbouring one. The time of kidding plotted with the annual variation of disease based on both the questionnaire and veterinary records revealed that the peak of kidding is April-August while the disease outbreak peak is confined to October and March. This indicate that the disease is always there but manifests itself in a form of an outbreak after the waning of the maternal immunity at the age of four months (Ata *et al.*, 1989). A similar situation prevailed in Oman where the virus maintained itself in the new borne sheep and goats (Taylor, 1990). The annual variation shown in Figure 13 may explain the pattern of the disease in many places in Ethiopia but not in Wollo area where the zero sero-positivity in animals less than 1 year and the low sero-positvity can not support this. Based on the previous findings September can be suggested as the most suitable time for vaccination. In September a high proportion of the new borne animals would be naïve after the waning of the maternal immunity, therefore immunisation of susceptible animals can prevent the occurrence of outbreaks with tremendous consequences. However Wosu *et al.* (1992) suggested November as optimum time for vaccination in Southern Nigeria.

Analysis of the veterinary service records revealed that there was a significant difference ($p<.001$) in the occurrence of death, proportion of affected and death among affected in sheep and

goats. This agrees with the finding of Opasina and Putt (1985) and Obi *et al.* (1983) who found a significant difference in the occurrence of attack rate, case fatality and mortality rate in sheep and goats.

Vaccination of animals against PPR was found to be economically beneficial compared to no vaccination for all scenarios analysed except for sheep in an average situation of all study area. Though the profit was found to be marginal (.23-2.05 birr for one animal), the sensitivity analysis revealed that profit can be maximised when the level of the immunity induced by the vaccine was increased. However, the animal profit (animal value of slaughter or when sold) is the main factor determining the efficacy of vaccination. The price used in the decision tree was the average for the prices of male, female, young animals and old animals, thus they do not reflect the reality in which most of the animals are sold (either mature male or culled females), thus price assigned in the model underestimated the real value of the animals. The price of the animal was found to be a crucial factor in determination of expected return. The threshold for vaccine price was found to be 5.8 and 8 birr for goat and sheep respectively at .13 sero-positivity if the animal price was 100 birr. Though the profit obtained through vaccination is marginal, the control of PPR is justifiable, not only because of the direct effect of PPR on small ruminants and its production but because of the impact it has on the control of Rinderpest and the role of small ruminant as a potential host of rinderpest. The cost of vaccination used in the model is the cost for cattle, which may be different from that of the small ruminants.

Finally, because this study was based partially on serum bank sera and accurate estimates for test sensitivity and specificity were not available to estimate the true sero-prevalence, only the sero-positivity in the three zones could be estimated. However one of the objectives for this study is to generate an estimate for sero-prevalence in the three zones.

5.2. Conclusion

The detection of sero-conversion in a considerable proportion of animals in E.Shewa and North Wollo highlighted the potential effects induced by PPR on the small ruminants and its production. The presence of different patterns for the disease in Ethiopia can be a possible explanation for disparity in the level of sero-positivity in E.Shewa and N.Wollo. PPR appeared to have become endemic in E.Shewa maintaining itself in the new borne animals while in Wollo the disease has a cyclic pattern with interepizootic periods. Though the location was found to be an important factor in determination of the level of prevailing sero-positivity and

the pattern of disease, more elaborated and extensive studies are needed to confirm that. Strong intracluster correlation of PPR sero-positivity was detected in E.Shewa. Herd effect and intracluster correlation was found to affect the quality of the inference and conclusion reached. Therefore, observational studies based on cluster sampling and dealing with disease transmitted by direct contact such as PPR should account for the herd effect, otherwise spurious inference can be reached. PPR C-ELISA underestimated the true prevalence when the apparent sero-positivity was high and overestimated the true prevalence when the apparent sero-positivity was low. Since no perfect test with 100% sensitivity and specificity existed adjustment for test misclassification should be considered. No significant difference ($p>.05$) in sero-positivity in the different strata of the sample was found. The level of sero-positvity and loss due to mortality and morbidity reported in the veterinary records justify launching of a control programme using vaccination. Moreover vaccination was found to be profitable. Though the profit was marginal (.23-2.02 birr), it eliminates any possible role for small ruminants in complication of Rinderpest eradication programme. September appeared to be the most suitable time for launching annual vaccination campaign.

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Annex 1 Form for specimens collecting in the active search for the disease

Form For Sample Collection

Serial Number			Date of collection	
Region				
District				
Village				
Animal Identifica				
Sex:	Male		Female	
Species	Sheep		Goats	
Production system:				
	Sedentary			
	Nomadic			
Herd Size				
Herd Structure :				
	Sheep :		<4 month	
			4-18 month	
			<18 month	
	Goats		<4 month	
			<18 month	
			4-18 month	

Clinical Sign Observed :

Oral lesion		Lacrimation	
Diarrhoea		Nasal discharge	
Rectal Temperature		Resp. distress	

Specimen Collected :

Nasal Swab		heparinised blood	
Lachrymal swab		Gum Scraping	
Faecal Swab		L.N. Biopsy	

Necropsy Specimen

No. of cases in the herd

Sheep :	<4 month	
	4-18 month	
	4-18 month	
Goats :	<4 month	
	4-18 month	
	4-18 month	

No. of Death in the herd :

Sheep :	<4 month	
	4-18 month	
	4-18 month	
Goats :	<4 month	
	4-18 month	
	4-18 month	

Annex 2 Questionnaire format

Questionnare

Serial number [ ] Date [ ]

Respondent Name [ ]

Status : Worker [ ]

Owner [ ]

Location :

Region [ ]

District [ ]

Village [ ]

Herd Structure:

Sheep: <4month [ ] 4-18month [ ] >18 month [ ]

Goats: <4month [ ] 4-18month [ ] >18 month [ ]

Production System:

Nomadic [ ] Sedentary [ ] Mixed [ ]

Presence of a disease with:

Muzzle & Oral Lesion [ ]

Watery Diarhoea and cough: [ ]

Nasal discharge & lacrimation: [ ]

[ ]

Is it present now:

What is its local Name? [ ]

Last time of its occurrence:month [ ] year [ ] season [ ]

How frequently does it occur:

Every year [ ]

Always there [ ]

Every [ ] Years

When does it occur normally?

In Winter [ ] Summer [ ] Rainy [ ]

Does it affect all the herd? [ ] Proportion [ ]

Which species is highly affected? Sheep [ ] Goats [ ]

Which age groups is severely affected?

Sheep: <4month ☐ 4-18month ☐ >18 month ☐

Goats:<4month ☐ 4-18month ☐ >18 month ☐

Does it cause death ☐

No. of death in sheep:<4month ☐ 4-18month ☐ >18 month ☐

No. of deaths in Goats<4month ☐ 4-18month ☐ >18 month ☐

Effect on milk production milk weight gain ☐

Is it necessary to provide a vaccine? ☐

Will you payfor vaccination? ☐

Peak of kidding in the year: month ☐ season ☐

Average no.of kid per dam ☐

Do you sell milk ☐

Do you buy drugs for your animals? ☐

Where do you get the money?

Milk sell ☐

Selling animls ☐

Cultivation ☐

Other sources ☐

Annex 3 Detailed data about the outbreaks covered by the veterinary service

From 1997-2000

Date of outbreak	Location			No. of suspected cases		No. of death		No.of animal at risk		Vaccination
	Region	Zone	Location	Sheep	Goat	Sheep	Goat	Sheep	Goat	
Jan. 1998	Oromia	Arsi	Dadofana sire		20		12		200	
Feb. 1998	Amhara	Oromia			5		2		3000	
Mar. 1998	Amhara	N. Wollo	Meket	20	180	6	130	714	218	
May 1998	Amhara	N. Wollo	Meket		22		10		2005	
May 1998	SNNP	Gurage	Meskanena	3		2		250		
May 1998	SNNP	Gurage	Meskanena		2		1		250	
June 1998	SNNP	Gurage	Meskanena		2		1		200	
Oct. 1998	SNNP	Gurage	Silit	96		41			1500	
Oct. 1998	SNNP	Gurage	Silit		120		75		1000	
Oct. 1998	Amhara	Oromia	Artumara	3		1		8000		
Oct. 1998	Amhara	Oromia	Artumara		13		6		750	
Nov. 1998	Oromia	E.Shewa	Adama	7		4		9000		
Nov.1998	Somali	Shinile	Aysha	18		17		2000		
Dec. 1998	Oromia	E. Shewa	Adama		5		2		3500	
Dec. 1998	Oromia	E Hararghe	Babile		50		34		390	
Oct.1999	Amhara	Oromia		12		0		700		
Year 1999										
June 2000	Amhara	Oromia	Artumana		4		3		1500	260

Annex 4 List of villages covered during the active search for PPR in sheep and goats

A. List of 18 villages covered during the active search in North Wollo

Adar kebele	Doro Gibire	Mahal Amba	Salmane 2
Ardom	Gadobar (013)	Manantala	Salmane1
Ayub	Genetobar	Mandafara	Sirinka
Bire	Gobiye	Robit	
Burka	Hara	Salmane 1	

B. List of 21 villages covered during the active search for the disease in South Wollo

Ambo	Chito	Gobya	Itacha
Balchi Tikure	Doshi	Goda chala	Jawaha
Bete	Garado	Gode Chale	Tamare
Chafa Dire	Garbi kide	Gode chale	
Chafa Robit	Gobaya	Heik-02	
Tari	Therart-Kaiffar	Towa	

C. List of 34 villages covered during the active search for the disease in East Shewa Zone

Abono Gabziel	Awaat malkasa	Tiyo	Gazzi
Abossa	Awash	Danure Tiyo	Hoit
Alache flarabate	Bosohadha	Dhebiti2	Ilala
Ali wayo	Bosohdha	Dodota dambal	Jogo gudedo
Arba	Buchana	Dungugi	Karsa ilala
Aribona	Bute filicha	Ejersa joro	Karsa korke
Aroge adama	Dangure	Feetoo	Kobo lute
Korme	Melka waba	Tade dildima	Wonji
Laga Banti	Sire robi	Tuchi dambal	
Marmasa	Tututi	Wayo Gabziel	

D. List of 26 village in which the owners identified the three clinical sign in Wollo region

Village	Village	Village	Village
Adar kebele	Chafa Robit	Gobiye	Mahal Amba
Ayub	Chito	Gobya	Manantala
Balchi Tikure	Doro Gibire	Gode Chale	Mandafara
Bete	Garado	Hara	Robit
Bire	Garbi kide	Heik-02	Salmane
Sirinka	Tari	Towa	
Burka	Genetobar	Itacha	

Annex 5 List of warda (districts), number of villages covered by the questionnaires and number of the questionnaires in each warda

A. List of warda (districts), number villages covered by the questionnaires and number of the questionnaires filled in North and South Wollo zone.

Warda	Number of Village	Number of questionnaire filled
Bati	4	17
Chafa Robi	2	5
Desse Zuri	1	4
Guba lafto	3	9
Habru	3	10
Kobo	6	18
Kutabor	5	7
Heik	5	12
Waldiya	1	3
Sanbati	5	15

B. List of warda (districts), number villages covered by the questionnaires and number of the questionnaires filled in East Shewa

Warda	Number of Village	Number of questionnaire filled
Adama	7	14
Adami tulli	4	8
Arsi Negele	4	8
Boset	4	8
Dudgabora	4	8
Lume	4	8
Fentale	4	8
Shashemane	4	8

Annex 6 Average number of animals owned by individuals interviewed

A. Average of animals owned by the interviewed who identify the disease in Wollo area

Variable	Respondent	Mean	St. Deviation	minimum	maximum
Number of Goats kids < 6 month	48	3	6	0	30
Number of Goats .5-2 Years	48	4	8	0	45
Number of Goats >2 Years	48	12	42	0	200
Number of Lamb <6 months	48	3	5	0	20
Number of Sheep .5-2 Years	48	3	5	0	25
Number of Sheep >2Years	48	8	17	0	100
Number of Cattles	48	7	10	1	60
Overall mean	48	6	13		

B. Average of animals owned by the interviewed who identify the disease in East Shewa

Variable	Observation	Mean	St. D.	minimum	maximum
Number of Goats kids < 6 month	34	7	6	0	20
Number of Goats .5-2 Years	34	8	9	0	35
Number of Goats >2 Years	34	14	15	0	60
Number of Lamb <6 months	34	4	5	0	20
Number of Sheep .5-2 Years	34	4	8	0	32
Number of Sheep >2Years	34	8	14	0	60
Number of Cattles	34	18	34	1	200
		9	13		

Annex 7 Summary of questionnaire variables before the adjustment

A. Summary of the questionnaire variables for all the questionnaires filled in North and South Wollo before the primary assessment.

Variable\ Code	9	0	1	2	3	4	5	6	7	8
Status	x	0	100	x	x	x	x	x	x	x
Production system	x	2	76	22	x	x	x	x	x	x
Disease with oral lesion	x	47	53	x	x	x	x	x	x	x
Disease with watery dirrhoea	x	23	77	x	x	x	x	x	x	x
Disease with cough and nasal discharge	x	27	73	x	x	x	x	x	x	x
Need for vaccine	19	13	68	x	x	x	x	x	x	x
Vaccine payment	19	x	x	x	x	x	x	x	x	x
Treatment of diseased	20	31	49	x	x	x	x	x	x	x
Treatment effect	38	39	15	8	x	x	x	x	x	x
Species susceptible	19	34	23	24	0	x	x	x	x	x
Source of income	x	x	20	30	6	5	39	x	x	x
Goat age susceptibility	28	x	1	3	0	57	11	x	x	x
Sheep age susceptibility	30	x	9	3	1	49	8	x	x	x
Season of occurrence	24	42	18	6	10	x	x	x	x	x
Frequency of occurrence	23	x	37	23	5	2	10	x	x	x
Disease presence at the time	x	74	26	x	x	x	x	x	x	x

*Code end of this annex

B. Summary of the questionnaire variables for all the questionnaires filled in North Wollo zone before the primary assessment

Variable\ Code	9	0	1	2	3	4	5	6	7	8
Status	x			x	x	x	x	x	x	x
Production system	x	1	47	9	x	x	x	x	x	x
Disease with oral lesion	x	22	35	x	x	x	x	x	x	x
Disease with watery dirrhoea	x	17	40	x	x	x	x	x	x	x
Disease with cough and nasal discharge	x	17	40	x	x	x	x	x	x	x
Need for vaccine	13	2	42	0	x	x	x	x	x	x
Vaccine payment	13	6	38	0	x	x	x	x	x	x
Treatment of diseased	14	23	20	x	x	x	x	x	x	x
Treatment effect	30	17	5	5	x	x	x	x	x	x
Species susceptible	13	16	6	22	0	x	x	x	x	x
Source of income	x	x	10	21	5	5	16	x	x	x
Goat age susceptibility	16	x	1	3		35	2	x	x	x
Sheep age susceptibility	20	x	4	0	0	27	6	x	x	x
Season of occurrence	16	26	8	1	6	x	x	x	x	x
Frequency of occurrence	14	x	19	11	5	2	6	x	x	x

C. Table summary of the questionnaire variables for all the questionnaires filled in South Wollo zone before the primary assessment

Variable\ Code	9	0	1	2	3	4	5	6	7	8
Status	x	0	100	x	x	x	x	x	x	x
Production system	x	1	29	13	x	x	x	x	x	x
Disease with oral lesion	x	25	18	x	x	x	x	x	x	x
Disease with watery dirrhoea	x	6	37	x	x	x	x	x	x	x
Disease with cough and nasal discharge	x	10	33	x	x	x	x	x	x	x
Need for vaccine	6	1	34	2	x	x	x	x	x	x
Vaccine payment	6	7	30		x	x	x	x	x	x
Treatment of diseased	6	8	29	x	x	x	x	x	x	x
Treatment effect	8	22	10	3	x	x	x	x	x	x
Species susceptible	6	8	17	12		x	x	x	x	x
Source of income	x	x	10	9	1	0	23	x	x	x
Goat age susceptibility	12	x	0	0	22	9		x	x	x
Sheep age susceptibility	10	x	5	3	1	22	2	x	x	x
Season of occurrence	8	16	10	5	4	x	x	x	x	x
Frequency of occurrence	9	x	18	12	0	0	4	x	x	x
Disease presence at the time	x	30	13	x	x	x	x	x	x	x

D. Table Summary of questionnaire variables for all the questionnaires filled in East Shewa before the primary assessment

Variable\ Code	9	0	1	2	3	4	5	6	7	8
Status	x	0	70	x	x	x	x	x	x	x
Production system	x	0	49	21	x	x	x	x	x	x
Disease with oral lesion	x	62	8	x	x	x	x	x	x	x
Disease with watery dirrhoea	x	16	54	x	x	x	x	x	x	x
Disease with cough and nasal discharge	x	29	41	x	x	x	x	x	x	x
Need for vaccine	13	55	2	x	x	x	x	x	x	x
Vaccine payment	15	7	47	1	x	x	x	x	x	x
Treatment of diseased	13	38	19	x	x	x	x	x	x	x
Treatment effect	50	9	10	1	x	x	x	x	x	x
Species susceptible	13	20	15	22	0	x	x	x	x	x
Source of income	x	x	11	27	4	0	28	x	x	x
Goat age susceptibility	26	x	0	2	3	34	5	x	x	x
Sheep age susceptibility	24	x	2	4	3	32	5	x	x	x
Season of occurrence	15	21	24	9	1	x	x	x	x	x
Frequency of occurrence	20	x	29	1	10	1	9	x	x	x
Disease presence at the time	x	66	4	x	x	x	x	x	x	x

Code definition
Status: 1= Owner, 2= worker

Production system: 0= nomadic, 1= sedentary, 2= mixed, 9=disease not known
Disease with oral lesion: 0= absent, 1= present.
Disease with watery dirrhoea: 0= absent, 1= present.
Disease with cough and nasal discharge: 0= absent, 1= present.
Need for vaccine: 0= No, 1= Yes, 9=disease not known
Vaccine payment: 0= No, 1= Yes, 2= I do not Know, 9=disease not known
Treatment of diseased: 0= No, 1= Yes, 9=disease not known
Treatment effect: 0= No, 1= Yes, 2= somewhat, 9=disease not known
Species susceptible: 1= Goat, 2= Sheep, 3= both, 9=disease not known
Source of income: 1= Selling animal, 2= Cultivation, 3= Other, 4= cultivation and selling animals,
Goat age susceptibility: 1==<6 month Goat, 2=6-18m, 3=>18 month, 4=all, 5= non susceptible, 9=disease not known
Sheep age susceptibility: 1==<6 month, 2=6-18m, 3=>18 month, 4=all, 5= non susceptible, 9=disease not known
Season of occurrence: 0= dry season, 1= rainy season, 2= spring (April-June), 9=disease not known
Frequency of occurrence: 1= yearly, 2= always there, 3= every 2 years, 4= every 3 years, 5= only once, 9=disease not known.
Disease presence at the time: 0= absent, 1= present.
x= not allowed

Annex 8 Map of Ethiopia

CURRICULUM VITAE

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Place of Birth Port Sudan-Sudan

Marital status Single

Nationality Sudanese

Religion Muslim

Education:

1. Korea primary school 1977-1983.
2. Amiria intermediate school 1983-1986.
3. Government high secondary school 1986-1989.
4. University of Khartoum-Faculty of Veterinary Medicine-1989-1995.

Working experience:

1. Veterinary officer-Blue Nile province.
2. Department of epidemic disease control
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Contribution to the knowledge of the Epidemiology ofpestedes petits Ruminants in Wollo & East Shewa Zones of Ethiopia

Elzein Bashir

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