

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

**SURVEY AND SEROLOGICAL INVESTIGATIONS ON
PESTE DES PETITS RUMINANTS (PPR) IN
IN THE WHITE NILE STATE, SUDANI**

By
Wifag Abdalla Mohamed Ali
B.V.Sc. (1989)
University of Khartoum

Supervisor
Dr. Abdelwahid Saeed Ali Babiker

A thesis submitted to the University of Khartoum in partial
fulfillment of the requirements for the Degree of Master of
Tropical Animal Health (M.T.A.H)

Department of Preventive Medicine and Veterinary Public Health
Faculty of Veterinary Medicine
University of Khartoum

June 2009

DEDICATION

To the soul of my father

To my lovely children

To my great mother

Rawan

To my dearest sisters,

Sara

Brothers and husband

Ahmed

With warm wide wishes

with keen kind kisses

ACKNOWLEDGEMENTS

First of all my thanks and praise to almighty Allah for the most beneficent, merciful for giving me health, strength and willpower to complete this study.

Sincere gratitude to my supervisor Dr. Abdelwahid Saeed Ali Babiker for his guidance, advice ,attention ,kindness and unlimited help.

I am grateful to Dr. Khitma Elmalik the coordinator of the master program. Department of Preventive Medicine. Faculty of Veterinary Medicine .for her encouragement and kindness during the master course.

My kind regard and thanks to Dr. Yahia Hassan Ali and Dr . Intisar Kamil Saeed, Department of Virology, the Central Veterinary Research Laboratory(CVRL) Soba ,for performing ELISA.

I would like to express my thanks to general directorate of Animal Resources in White Nile State, for giving me this chance and the leave of the study. special thank to Dr. Mirgani for his help during sample collection.

I am grateful to my colleagues in the Master of Tropical Animal Health Program(batch No.7) specially my friends Suad, Hala and Nour.

Lastly, I am grateful to my mother, sisters, brothers , husband, daughters and son with thankfulness ,for their encouragement , patience and tremendous help.

ABSTRACT

The aim of this study was to determine the situation of peste des petits ruminants (PPR) in the White Nile State, Sudan, during 2007-2008.

Three methods were adopted to achieve this goal: Questionnaire survey among the sheep and goats owners, collection of data from veterinary services records and serological examination of sheep and goats in the state using competitive enzyme- linked immunosorbent assay (c-ELISA).

The questionnaire outcome showed that 51.16% of the owners interviewed, (n=86) in the state have good knowledge of PPR signs, 48.84% mentioned it as most important disease and 41.86% confirmed its presence in their herds. Also, the survey showed that 65.12% of the animal owners are nomads, 65.12% hadn't vaccinated their animals against PPR at all.

Data collected from veterinary services records showed that the relatively good infrastructure available for veterinary services in the state, including manpower, transportation and the vaccine, may help in the control of the disease spread, but in spite of that there was high prevalence of the disease as shown by the results of the questionnaire.

Out of 232 serum samples collected from sheep and goats in four localities and tested for PPR virus antibodies using c-ELISA, 178 (76.7%) were positive. Antibodies against PPR virus were detected in 97 (69.8%) samples from Kosti locality, in 54 (88.5%) samples from Elgeteina locality and in 24 (82.8%) samples from Elgabalain locality. Numbers of samples from the fourth locality, Eldoium, was negligible, only three which were positive.

It was concluded that PPR is prevalent in the White Nile State. It is recommended that the General Directorate of Animal Resources in the state should exert more efforts to increase the awareness of animal owners of the seriousness of the disease and to educate them that the disease is untreatable and they should keep vaccinate their animals.

المستخلص

هدفت هذه الدراسة لتحديد وضع مرض طاعون المجترات الصغيرة بولاية النيل الأبيض (السودان) في الفترة من 2007 إلى 2008م. اعتمدت ثلاث طرق لتحقيق هذا الهدف هي: إجراء مسح إستبائي وسط مربى الضأن والماعز وجمع بيانات من الخدمات البيطرية وفحص عينات المصل المأخوذة من الضأن والماعز بالولاية بإختبار التنافس الإنزيمي المناعي (c-ELISA).

أوضحت نتائج المسح الإستبائي أن 51.16% من المربين الذين تم استفتاءهم (العدد=86) بالولاية على إلمام بأعراض المرض و 48.84% على إلمام بوبائيته وأكد 41.86% حدوث المرض في قطعانهم. أيضاً أثبتت نتائج المسح أن 65.12% من الملاك ينتهجون النمط المتنقل في التربية علاوة على 65.12% لم يقوموا بتطعيم قطعانهم من قبل.

أثبتت تقارير الخدمات البيطرية أن البنية التحتية التي تتمتع بها الخدمات البيطرية بالولاية (القوة العاملة، وسائل الترحيل، اللقاح) يمكنها أن تساعد في السيطرة على إنتشار المرض ولكن بالرغم من ذلك فالمرض منتشر بصورة واسعة، كما أوضحت ذلك نتائج الاستبيان.

من بين 232 عينة مصل جمعت من الضأن والماعز في أربع محليات، فحصت لوجود الأجسام المضادة لفيروس طاعون المجترات الصغيرة، 178 (76.7%) كانت موجبة. تم اكتشاف الاجسام المضادة للفيروس في 97 (69.8%) عينة من محلية كوستي، في 54 (88.5%) عينة من محلية القطينة وفي 24 (82.8%) عينة من محلية الجبلين. عدد العينات من محلية الدويم كان قليلاً جداً، فقط ثلاثة وقد كانت موجبة.

اتضح من هذه الدراسة أنّ طاعون المجترات الصغيرة منتشر بصورة واسعة في ولاية النيل الأبيض. أوصت الدراسة بأن تقوم الإدارة العامة للثروة الحيوانية في الولاية ببذل المزيد من الجهد لزيادة توعية مربى الحيوان بخطورة المرض وأن هذا المرض لا يمكن علاجه وعليه لا بد لهم من المحافظة على برنامج التطعيم.

LIST OF CONTENTS

	Pag e
Dedication	i
Acknowledgements	ii
English Abstract	iii
Arabic Abstract	iv
List of Contents	v
List of Tables	x
List of Figures	xi
List of Abbreviations	xii
Introduction	1
CHAPTER ONE: LITERATURE REVIEW	
1.1. Definition	5
1.2. Disease appellations	5
1.3. History of the Disease	5
1.4. Etiology	7
1.4.1. Classification	7
1.4.2. Virus Properties	7
1.4.2.1. Morphology	7
1.4.2.2 Genomic Structure and Gene Expression	8
1.4.3. Ultra structure of PPRV	8
1.4.4 Replication	8
1.4.5. Physiochemical Properties	9
1.5. Epidemiology of PPR	9
1.5.1. Geographical Distribution	9
1.5.1.1. Lineages of PPRV	13
1.5.2. PPR in Sudan	13

1.5.3. Host Range	14
1.5.4. Transmission	15
1.5.4.1. Natural Transmission	15
1.5.4.2. Experimental Transmission	17
1.6. PPR as a Biological Weapon	18
1.7. Clinical Signs	18
1.7.1. Peracute Syndrome	19
1.7.2. Acute Syndrome	19
1.7.3. Subacute Syndrome	20
1.8. Morbidity and Mortality	24
1.9. Pathology	25
1.10. Diagnosis of PPR	30
1.10.1. Clinical Diagnosis	30
1.10.2. Laboratory Diagnosis	30
1.10.2.1. Samples Required for Diagnosis	30
1.10.2.2. Electron Microscopy (E.M)	31
1.10.2.3. Virus Isolation and Identification	31
1.10.2.3.1. Virus Isolation	31
1.10.2.3.2. Cross Neutralisation Test (CNT)	31
1.10.2.4. Serological Techniques	31
1.10.2.4.1 Antigen Detection Methods	31
1.10.2.4.1. 1. Agar Gel Immunodiffusion Test (AGIDT)	31
1.10.2.4.1.2. Counter Immunoelectrophoresis Test (CIEP)	32
1.10.2.4.1.3. Haemagglutination Test (HA)	32
1.10.2.4.1.4. Immunofluorescent Antibody Test (IFAT)	33
1.10.2.4.1.5. Immunoperoxidase Staining (IP)/	33
Immunohistochemistry (IHC)	
1.10.2.4.1.6. ELISA for Antigen Detection	33

1.10.2.4.1.6.1. Immunocapture ELISA (IC-ELISA)	33
1.10.2.4.1.6.2. Sandwich ELISA (S-ELISA)	34
1.10.2.4.2. Antibody Detection Methods	34
1.10.2.4.2.1. Agar Gel Diffusion Test (AGDT)	34
1.10.2.4.2.2. Precipitinogen Inhibition Test (P.I.T)	34
1.10.2.4.2.3. Virus Neutralisation Test (VNT)	34
1.10.2.4.2.4. Haemagglutination Inhibition Test (H I)	35
1.10.2.4.2.5. Counter Immunoelectrophoresis (CIEP)	36
1.10.2.4.2.6. ELISA for Antibody Detection	37
1.10.2.4.2.6.1. Competitive Enzyme-Linked Immunosorbent Assay (C-ELISA)	37
1.10.2.4.2.6.2. Blocking ELISA (B-ELISA)	37
1.10.2.5. Molecular Techniques(Virus RNA Detection)	37
1.10.2.5.1. Reverse Transcriptase-Polymerase Chain Reaction (RT- PCR)	37
1.10.2.5.2. Specific cDNA Hybridization	38
1.10.2.6. Histopathology	38
1.10.3. Differential Diagnosis	38
1.11. Excretion of PPR Virus	40
1.12. Public Health	40
1.13. Treatment	40
1.14. Immunity to PPRV	40
1.15. Control of PPR Outbreaks	40
1.16. Vaccination	41
CHAPTER TWO: MATERIALS AND METHODS	
2.1. Study area	43
2.1.1. Animal Population	43
2.2. Study Design	45

2.2.1. Questionnaire Survey	45
2.2.2 Veterinary Service Reports	45
2.2.3 Study population and sampling method	45
2.2.3.1 Sample collection	46
2.2.3.2 Laboratory test	46
2.2.3.3. PPR ELISA Antigen	47
2.2.3.4. Control Sera	47
2.2.3.5. Monoclonal Antibodies	47
2.2.3.6. Rabbit Anti-Mouse Immunoglobulins	47
2.2.3.7. Procedure of the test	47
CHAPTER THREE: RESULTS	
3.1. Questionnaire survey outcomes	50
3.2 Veterinary service reports outcomes	51
3.2.1 Veterinary service structure	51
3.2.2 Manpower	53
3.2.3. Transportation	53
3.2.4. Previously reported PPR outbreaks	54
3.2.5. Vaccination program	54
3.2.6 Sero-Prevalence of PPR in the State	55

CHAPTER FOUR: DISCUSSION	56
Conclusions and Recommendation	61
References	62
Appendices	78

LIST OF TABLES

	Page
Table (1): distribution of the sheep and goats in different localities of White Nile State	44
Table (2): Summary of the questionnaire survey responses by owners in white Nile State	53
Table (3): Manpower engaged in animal health services in White Nile State	54
Table (4): Vehicles involved in animal health activities	55
Table (5): Vaccination figures in the state during 2003-2008	55
Table (6): Prevalence of PPRV antibodies in sheep and goats sera in different localities when examined by C-ELISA	56

LIST OF FIGURES

	Page
Figure 1: Geographical distribution of PPR	12
Fig 2: PPR in goat: purulent eye and nose discharges	21
Fig3: PPR in a goat: swollen, eroded lips	21
Fig 4: PPR in a goat: signs of diarrhea	22
Fig 5: PPR in a goat: the early lesions of pneumonia	27
Fig 6: PPR in a sheep: advanced pneumonia	27

Fig 7: PPR in a goat "zebra striping" in the large intestine	28
Figure 8:Competitive ELISA Plate Layout	50

LIST OF ABBREVIATIONS

AGDT	Agar gel diffusion test
AGID	Agar gel immunodiffusion test
AGPT	Agar gel precipitation test
BB	Blocking buffer
B-ELISA	Blocking-Enzyme linked Immunosorbent Assay
C	C-protein
C-	Control negative serum
C+	Control weak positive serum
C++	Control strong positive serum
CC	Conjugate control
cDNA	Complementary DNA
CDV	Canine distemper virus
C-ELISA	Competitive-Enzyme Linked Immunosorbent Assay
CIEP	Counter immunoelectrophoresis
Cm	Monoclonal control
DDW	Deionized distilled water
DNA	Deoxyribonucleic acid
EDTA	Ethylene Diamine Tetra acetic acid
ELISA	Enzyme linked immunosorbent assay
EM	Electron microscopy
F	Fusion protein
FAO	Food and Agriculture Organization
GREP	Global Rinderpest Eradication Program
H	Haemagglutinin protein
H & E	Haematoxylin and Eosin
HA	Haemagglutination Test
HI	Haemagglutination inhibition test
HRPO	Horseradish peroxidase
IAEA	International Atomic Energy Agency's

IC-ELISA	Immunocapture-enzyme linked immunosorbent assay
IFAT	Immunofluorescent antibody test
IHC	Immunohistochemistry
IP	Immunoperoxidase test
L	Large protein
M	Matrix-protein
M	Molar
MAB(s)	Monoclonal antibody(s)
MDBK	Madin-Darby bovine kidney cells
ml	Millilitre
mM	Millimolar
MV	Measles virus
N	Nucleoprotein
NT	Neutralization Test
OIE	Office International des Epizootics
OPD	Orthophenylene diamine
P	Phosphoprotein
P.I.T.	Precipitinogen Inhibition Test
PBS	Phosphate buffer saline
PDV	Phocid distemper virus
PFU	Plaque forming unit
pH	Hydrogen ion concentration
PI	Percentage inhibition
PPR(V)	Peste des Petits Ruminants (Virus)
r.p.m	Revolutions per minute
RNA	Ribonucleic acid
RP(V)	Rinderpest (Virus)
TR-PCR	Reverse transcriptase-polymerase chain reaction
S-ELISA	Sandwich-enzyme linked immunosorbent assay
SNT	Serum neutralization test
TCID	Tissue culture infective dose

μl	Microlitre
μm	Micrometer
V	V-protein
v/v	Volume to volume
VNT	Virus neutralization test
w/v	Weight to volume

INTRODUCTION

Livestock are very important for both the subsistence and economic development of the African continent. They provide a flow of essential food products throughout the year. In some countries, like Sudan, they are a major source of government revenue and export earnings. They also sustain the employment and income of millions of people in rural areas. Contribute to energy and manure for crop production and are the only food and cash security available to many Africans (Brumby, 1990). In many African countries, small ruminants (sheep and goats) constitute a substantial proportion of the nation's meat supply.

Many health problems are encountered to put some obstacles and constraints in the front of developing productivity of small ruminants. Peste des petits ruminants (PPR) is considered the most important single cause of morbidity and mortality for sheep and goats, in Africa.

Peste des petits ruminants (PPR) is a highly contagious and infectious viral disease of domestic and wild small ruminants. It was first described in cote d'Ivoire in West Africa in 1942. Gradually, it was realized that several clinically similar diseases occurring in other parts of West Africa shared the same cause. The virus now called Peste des petits ruminants virus (PPRV). Investigators soon confirmed the existence of the disease in Nigeria, Senegal and Ghana. For many years, it was thought that it was restricted to that part of the African continent until a disease of goats in Sudan, which was originally diagnosed as rinderpest in 1972, was confirmed to be PPR.

The true extent of the disease has only become apparent in recent years and is still being clarified. The realization that many of the cases diagnosed as rinderpest among small ruminants in India may

instead , have involved the PPR virus, together with the emergence of the disease in other parts of western and South Asia, points to its ever- increasing importance.

Peste des petits ruminants is a disease of major economic importance. It is regarded as the biggest constrain to large- scale intensive production of sheep and goats in the West African sub-region. It is acknowledged as the most destructive disease and the number one killer disease of small ruminants in West Africa.

The disease is grouped within the list A of the office International des Epizootics (OIE) due to its highly contagious nature and consequent capacity for rapid spread.

Peste des petits ruminants is an important disease in its own right but it has also created problems because of its apparent similarity to rinderpest. The clinical signs of PPR closely resemble those of rinderpest, making differential diagnosis difficult. It should, however, be borne in mind that clinical disease caused by rinderpest in small ruminants is a relatively rare event, even in Asia.

The Peste des petits ruminants virus (PPRV) is closely related to the rinderpest virus of cattle and buffaloes, the measles virus of humans, the distemper virus of dogs and some wild carnivores and the morbilliviruses of aquatic mammals. Four distinct genetic lineages of PPRV were identified three from Africa while the fourth is restricted in Asia.

Clinically, the disease was characterized by sudden onset of depression, fever, ocular and nasal discharges, sores in the mouth, disturbed breathing and cough, foul- smelling diarrhea and death .with incubation period of 4-5 days. It is an immunosuppressive disease hence secondary latent infection may be activated and complicated the clinical picture. It is transmitted by close contact.

In susceptible flocks, morbidity may be 100% and mortality greater than 90 %, especially amongst animals under six months of age. PPR occurs as an acute disease in goats, while in sheep, it is generally, benign. Surviving animals usually develop a dual immunity to PPR and RP viruses. Pregnant animals may abort.

PPR is endemic in the humid zone and to a lesser extent in the sub humid zone, bearing in mind that 21.5% of the nearly 104 million sheep and 25.4 % of the 125 million goats in tropical Africa are located in the humid and sub humid zone.

The diagnosis of PPR is based on clinical, pathologic, epidemiological findings and maybe confirmed by virus isolation and identification.

There is no specific treatment for PPR; however drugs that control bacterial and parasitic complications may decrease mortality.

For prevention, in the past, the rinderpest vaccine has been used, based on the antigenic relationship between PPR and RP viruses. However, this practice is being phased out to avoid confusion during retrospective serologic studies. A homologous PPR vaccine is now available and gives strong immunity.

In Sudan, PPRV was firstly isolated and identified by Elhag Ali in Eastern Sudan in 1971, although PPR occurs in Sudan as early as the 1970'S and several outbreaks of PPR causing high mortalities among small ruminants occur in many parts of Sudan, but still little is known about it.

Objectives:

- 1- To determine the prevalence of PPR in White Nile State.
- 2- To draw attention to the importance of the disease.
- 3- To set some basis so as to help in the eradication programs of the disease.

CHAPTER ONE

LITERATURE REVIEW

1.1. Definition

Peste des petits ruminants (PPR) is an acute or subacute viral disease of goats and sheep characterized by fever, necrotic stomatitis, gastroenteritis, conjunctivitis, and pneumonia. Goats are usually more severely affected than sheep. Cattle are only subclinically infected. Humans are not at risk

1.2. Disease appellations

In the first time, Kata was the appellation of a stomatitis and pneumoenteritis of Nigerian dwarf goat (Radostitis et al., 2007).

Peste des Petits Ruminants was the French name of a similar disease in sheep and goat first described in Ivory Coast in 1942. Both diseases were shown to be very close to each other (Rowland et al., 1971).

Many authors prefer the appellation of “Ovine Rinderpest”. But official organizations like FAO and OIE use the French name “Peste des Petits Ruminants”, “Peste Des Petits Ruminants”, “Peste-des-Petits-Ruminants” or “Peste-des-petits-ruminants”, even in English. Also known as Pest of small Ruminants, stomatitis-pneumoenteritis complex or syndrome pseudorinderpest of small ruminants. Kata [is a Pidgin English for Catarrh].

Also known as goat plague.

1.3. History of the Disease

Peste des petits ruminants was first described in Côte d’Ivoire in West Africa in 1942. Gradually, it was realized that several clinically similar diseases occurring in other parts of West Africa

shared the same cause. Investigators soon confirmed the existence of the disease in Nigeria, Senegal and Ghana.

For many years, it was thought that the disease was restricted to that part of the African continent until a disease of goats in the Sudan, which was originally diagnosed as rinderpest in 1972, was confirmed to be PPR(Roeder and Obi, 1999).

The West African sub region is considered an endemic zone of PPR. PPR has been found in parts of sub-Saharan Africa for several decades and in the Middle East and Southern Asia since 1993. It has been reported in Sudan, Kenya, Uganda, and Ethiopia. It was first reported in southern India in 1987. The Arabian Peninsula, the Middle East, and the rest of the Indian Subcontinent reported PPR incidence during 1993-1995. The disease has remained endemic in these areas. It has also reported in Turkey in 1996, Iran in 1994, Iraq in 2000, Bangladesh in 1993 and 2000, and Nepal in 1995 (Dahar et al., 2002).

In India, PPR was first confirmed in March 1987 in sheep suspected of having rinderpest. It is now believed that many outbreaks in India previously attributed to rinderpest were actually PPR. The virus was isolated four more times by 1992, and major epidemics occurred in the state of Andhra Pradesh in 1994-1995 and 1997-1998 (Taylor et al., 2002).

In Africa and Asia, the disease is particularly devastating, as these countries often use small ruminants as components of agricultural food production (EMPRES, 1999).

Presently, PPR occurs in most African countries situated in a wide belt between the Sahara and Equator, the Middle East (Arabian Peninsula, Israel, Syria, Iraq, Jordan), and the Indian subcontinent (Taylor, 1984).

The disease is present in West Africa, part of Central Africa (Gabon, Central African Republic), East Africa (North of the Equator), Middle East and Indian subcontinent including Nepal and Burman. In North Africa, only Egypt was once hit. But since summer 2008, Morocco is suffering a generalized outbreak with 133 known cases in 29 provinces, mostly affecting sheep. The outbreak has lead to the vaccination of a large amount of the 17 million of sheep and five million goats in the country (FAO, 2008).

1.4. Etiology

Peste des petits ruminants is caused by a paramyxovirus of the Morbillivirus genus. It is closely related to the rinderpest virus (RPV) of cattle and buffaloes, measles virus (MV) of humans, canine distemper virus (CDV) of dogs and some wild carnivores, and phocid distemper virus (PDV) of sea mammals (seals).

For many years, PPRV was considered a variant of RPV, specifically adapted for goats and sheep, that had lost its virulence for cattle .It is now known that the two viruses are distinct though closely related antigenically.

1.4.1. Classification

Peste des petits ruminants virus (PPRV) is a member of the morbillivirus genus within the family Paramyxoviridae (Gibbs et al., 1979).

1.4.2. Virus Properties

1.4.2.1. Morphology

Peste des petits ruminants virus (PPRV), like other viruses in the family Paramyxoviridae, is an enveloped RNA virus with two external glycoproteins, F and H, associated with the envelope. The size of PPRV varies between 150 and 700 nm. The particles have a

lipoprotein membrane covered with large peplomers (8-20 nm in length) and contain a herring-bone shaped helically symmetrical nucleocapsid (Bourdin and Laurent-Vautier, 1967; Durojaiye et al., 1985; Diallo, 1990).

1.4.2.2 Genomic Structure and Gene Expression

The genome is organized in six transcriptional units or genes encoding two non-structural proteins (V and C) and six structural proteins: the surface glycoprotein (The fusion (F) and the haemagglutinin (H) proteins); the nucleocapsid (N); the phosphoprotein(P); the matrix (M) protein; and the polymerase or large (L) protein which forms the polymerase complex in association with the (P) protein (Crowley et al.,1988; Diallo, 1990; Rima, 1993; Sidhu et al., 1993; Sharma and Adlakha, 1994; Diallo et al., 1994; Haffar et al.,1999; Diallo, 2003).

1.4.3. Ultra structure of PPRV

The morphology of PPRV observed by negative staining electron microscopy was typical of Paramyxoviruses and indicated that the genome was ribonucleic acid (Bourdin and Laurent-Vautier, 1967; Gibbs et al., 1979).

Ultra structure studies of PPRV revealed that the intact virus particle is pleomorphic either spherical or ovoid with a diameter varying between 130 and 390 nm (Durojaiye et al., 1985).

1.4.4 Replication

The replication of Paramyxoviruses was described by Murphy et al., (1999). They replicate mainly within the cytoplasm.

Virions maturation occurs through several processes:

- The incorporation of viral glycoprotein into patches on the host cell plasma membrane.

- The association of matrix protein (M) and other non-glycosylated proteins with this altered host cell membrane.
- The alignment of the nucleocapsid beneath the M protein.
- The formation of the mature virions which is released via budding.

1.4.5. Physiochemical Properties

It is assumed that the survival characteristics of PPRV are similar to those for RPV. Since these viruses are enveloped they are fragile and can easily be destroyed by heat, desiccation, light, ultraviolet (UV) radiation, pH extremes and common disinfectants (Rossiter and Taylor, 1994; Diallo, 2003).

- -PPRV may survive at 60°C for 60 minutes (OIE, 2002).
 - Long survival time in chilled and frozen tissues (OIE, 2002).
- Peste des petits ruminants virus is susceptible to sunlight. It is rapidly inactivated by ultraviolet light and desiccation within 4 days (Scott and Brown, 1961; OIE, 2002).
- The virus is stable from pH 4.0 to 10.0 (OIE, 2002).
- PPRV is killed by alcohol, ether, and detergents as well as by most disinfectants (e.g., phenol, sodium hydroxide) (OIE, 2002).
- Antibiotics and sulphonamides have no effect on RP and PPR viruses (Scott and Brown, 1961).

1.5. Epidemiology of PPR

1.5.1. Geographical Distribution

Peste des petits ruminants (PPR) is present in west and central Africa and the Middle East. Generally, outbreaks that affect only a few animals are not reported; epidemics occur when the population of

susceptible animals increases. Such an epidemic may eliminate the goats or sheep in an area. Because of strengthening surveillance and disease monitoring, as well as the establishment of good reporting systems in Africa and Asia as part of the global strategy to eradicate rinderpest, the prevalence of PPR has been better recognized, and reporting to OIE on PPR has increased (Merck's and Co, 2008).

Peste des petits ruminants (PPR) infection has been recognized in many of the African countries that lies between the Atlantic Ocean and the Red Sea. The affected area extends north to Egypt and south to Kenya, in the east, and Gabon, in the west. PPR has not been recognized in most of North and Southern Africa. In some of the countries where the disease has not been confirmed there are serological and /or clinical indications that the infection is, nevertheless, present. A serological survey in the United Republic of Tanzania in 1998 did not detect any antibodies to PPR suggesting that infection has not extended that far south. (Roeder and Obi, 1999).

In recent years, the disease has been seen in the Near East and the Arabian Peninsula, in countries including the Islamic Republic of Iran, Iraq, Israel, Jordan, Kuwait, Lebanon, Oman, Saudi Arabia, the United Arab Emirates and Yemen, and there is serological evidence from the Syrian Arab Republic and Turkey. Outbreaks of PPR are now known to be common in India, Nepal, Bangladesh, Pakistan and Afghanistan (Roeder and Obi, 1999).

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

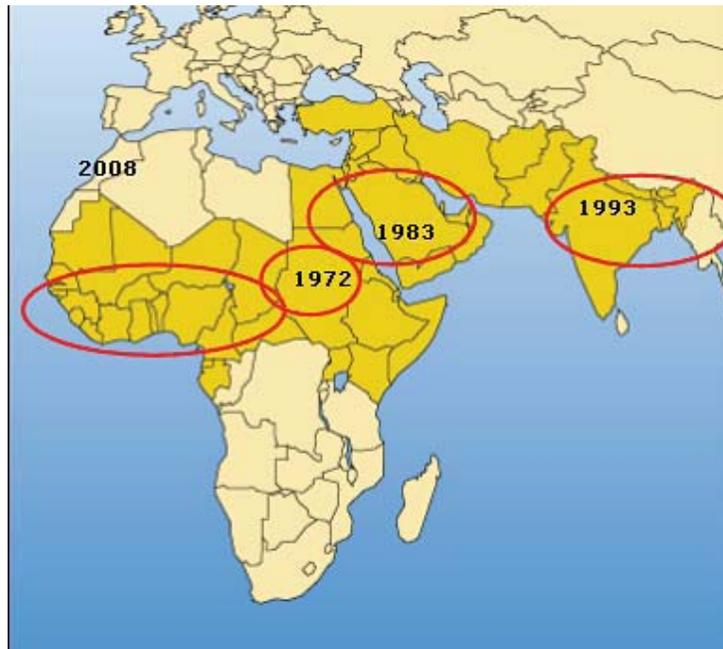
It is still not clear whether the apparent geographical spread of the disease in the last 50 years is real or whether it reflects increased awareness, wider availability of diagnostic tools or even a change in the nature of the virus. It seems most likely that a combination of factors is responsible for the present knowledge of its range and it is known that confusion of PPR with pneumonic pasteurellosis and other pneumonic diseases of small ruminants has delayed its recognition in some countries.

Presently, PPR occurs in most African countries situated in a wide belt between the Sahara and Equator, the Middle East (Arabian Peninsula, Israel, Syria, Iraq, Jordan), and the Indian Subcontinent (Elhag Ali and Taylor, 1984; Taylor, 1984; Lefevre, 1982; Lefevre and Diallo, 1990).

The disease is present in West Africa, part of Central Africa (Gabon, Central African Republic), East Africa (North of the Equator), Middle East and Indian subcontinent including Nepal and Burman.

In North Africa, only Egypt was once hit. But since summer 2008, Morocco is suffering a generalized outbreak.

Figure 1: Geographical distribution of PPR



The dark color indicated that areas experienced PPR and the disease has been reported.

Available from: <ftp://ftp.fao.org/docrep/fao/011/aj120e/aj120e00.pdf>.

1.5.1.1. Lineages of PPRV

Genetic characterization of PPR virus strains has allowed them to be organized into four groups; three from Africa and one from Asia. One of the African groups of PPRV is also found in Asia. The epidemiological significance of these groupings is less clear at present than that of rinderpest virus groupings (Roeder and Obi, 1999).

Lineage (I) have been found in West Africa and include: Senegalese strain, Nigeria 75/1, 75/2, 75/3, 76/1 and Burkina Faso/99. Lineage (II) found also in West Africa and include the isolates: Guinea Bissau/91 and Ivory Coast/89. Lineage (III) includes viruses which have been isolated from East Africa and Asia: Sudan/72, Oman/83, India/TN/92, Ethiopia/96 and Yemen/01. Lineage (IV) found only in Asia and includes viruses whose origins are in the Middle East, Saudi Arabia and South Asia: 15 Indian isolates (India/UP/94, India/MH/94,.....), Bangladesh/ 93 and Bangladesh/ 00, Nepal/95, Turkey/96 and Turkey/00, Israel/94, Pakistan/94 and Pakistan/ 98, Saudi Arabia/ 94, Iran/94, Iraq/00a and Iraq/00 and Kuwait/99 (Dhar et al, 2002).

1.5.2. PPR in Sudan

An outbreak of Rinderpest in sheep and goats was reported in two areas in Southern Gedarif and an area near Dinder River in 1971 by Elhag Ali (1973). The disease was diagnosed as RP (Gedarif RPV/71) according to observed clinical signs.

Another outbreak of RP-like disease occurred during the year 1972 in Sinnar and Mieliq in Central Sudan. Two viruses were isolated (Elhag Ali and Taylor, 1984) and re-examined both serologically, by inoculation of experimental sheep and goats and by cross neutralization with RPV and PPRV. Using differential neutralization, Elhag Ali found close antigenic relationship between

the Sudanese isolates and the Nigerian PPRV and this result was supported by serological tests. Later, these two isolates were considered to represent the PPRV (Elhag Ali and Taylor, 1984) and termed SUD72/1 (Sinnar) and SUD72/2 (Mieliq). Another two PPRV isolates were obtained from Elfashir in Darfur State in Western Sudan by El Sheikh (1992). An extensive outbreak of PPR occurred in Sudan during 1989-1990 with morbidity and mortality rates ranging from 10-66% and 3-37% respectively (Awad El Karim et al., 1994). Awad El Karim and co-workers also isolated a PPRV (PPR/VHL) from Hilalia Area in Gezira State (Awad El karim et al., 1994).

Seroprevalence of PPRV in Khartoum State during 1991-1993 was reported by Zeidan (1994). Serosurveillance using ELISA was also reported by El Amin and Hassan(1999) from Eastern and Western States, by Haroun et al,(2002) from Darfur, Khartoum and Eastern States and by Intisar(2002) from Kordofan and Khartoum States.

Virus isolation during 2000-2002 from Khartoum, Gezira, White Nile, River Nile and Kordofan States was made by Intisar (Intisar, 2002; Intisar et al., 2004).

1.5.3. Host Range

Clinical disease is seen in sheep and goats and has been described in zoological garden collections of wild small ruminants including Laristan sheep, Dorcas-type gazelles, gemsbok and the Nubian ibex. Cattle, buffaloes, camels and pigs can become infected but there is little or no evidence of disease associated with their infection (Roeder and Obi, 1999).

Peste des petits ruminants is primarily a disease of goats and sheep. However, there is one report of naturally occurring PPR in captive wild ungulates from three families: Gazellinae (dorcac

gazelle), Caprinae (Nubian ibex and Laristan sheep), and Hippotraginae (gemsbok). Experimentally, the American white-tailed deer (*Odocoileus virginianus*) is fully susceptible (Saliki, 1998). The role of wildlife on the epizootiology of PPR in Africa remains to be investigated. Cattle, buffaloes, camels and pigs are susceptible to infection with PPRV, but they do not exhibit clinical signs (EMPRES, 1999). Such sub clinical infections result in seroconversion, and cattle are protected from challenge with virulent RPV. Cattle and pigs do not, however, play a role in the epizootiology of PPR because they are apparently unable to transmit the disease to other animals (Furley et al., 1987).

1.5.4. Transmission

1.5.4.1. Natural Transmission

Peste des petits ruminants is contagious and its transmission requires close contact. Ocular, nasal, and oral secretions and feces are the sources of virus. Contact infection occurs mainly through inhalation of aerosols produce sneezing and coughing. Fomites such as bedding may also contribute to the onset of an outbreak. As in rinderpest (RP) there is no known carrier state. Infected animals may transmit the disease during the incubation period (Merck's and Co, 2008).

The discharges from eyes, nose and mouth, as well as the loose feces, contain large amounts of the virus. Fine infective droplets are released into the air from these secretions and excretions, particularly when affected animals cough and sneeze. Other animals inhale the droplets and are likely to become infected. Although close contact is the most important way of transmitting the disease, it is suspected that infectious materials can also contaminate water and feed troughs and bedding, turning them into additional sources of infection. These

particular hazards are, however, probably fairly short-term since the PPR virus, like its close relative rinderpest, would not be expected to survive for long outside the host (Roeder and Obi, 1999).

Trade in small ruminants, at markets where animals from different sources are brought into close contact with one another, affords increased opportunities for PPR transmission, as does the development of intensive fattening units (Roeder and Obi, 1999).

The appearance of clinical PPR may be associated with any of the following:

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

For PPR to spread, close contact between infected and susceptible animals is needed (Ozkul, 2002). There are several means of transmission between animals (Saliki, 1998):

- Inhalation of aerosols produced by sneezing and coughing of infected animals. Outbreaks are more frequent during the rainy season or the dry, cold season (OIE, 2002).
- Direct contact with ocular, nasal, or oral secretions
- Direct contact with feces.

- Fomites such as bedding, water, and feed troughs.
- No carrier state is known to exist.

The disease is spread from a region to another by sick animals. As the virus is early inactivated outside the body, indirect contamination is generally limited.

In an affected flock, even in pest-free regions, the disease do not progress very rapidly, despite close contact between animals. New clinical cases may be observed daily for a one-month period (Mahin, 2008).

1.5.4.2. Experimental Transmission

Experimentally, the virus has been transmitted through different routes: nasal, subcutaneous, intraocular, intratracheal and intravenous or by contact (Durnell, 1972; Durojaiye, 1980).

Experimentally, the American white-tailed deer (*Odocoileus virginianus*) is fully susceptible (Furley et al., 1987).

Pigs with experimentally induced subclinical infections do not transmit the disease to susceptible pigs or goats; therefore, pigs may have no role in PPR epidemiology.

Animals inoculated with PPRV isolates developed clinical signs and lesions of the disease (Nussieba, 2005).

1.6. PPR as a Biological Weapon

Peste des petits virus (PPRV) virus is considered a potential biological weapon because:

- Morbidity and mortality can be as high as 100% and 90% respectively. When associated with other diseases such as capripox, mortality can be 100% (Dhar et al., 2002).

- Aerosol transmission would enable the disease to spread rapidly in large groups of animals.

1.7. Clinical Signs

The pathogenesis of PPR starts after the entry of the virus through the respiratory system, then it localizes first in the pharyngeal and mandibular lymph nodes and tonsils.

Subsequent viremia results in dissemination to visceral lymph nodes, spleen, bone marrow and the mucosa of the gastrointestinal and the respiratory systems (Scott, 1981; Bundza et al., 1988).

Clinical signs appear an average of two to six days after natural infection with the virus (the incubation period).

Susceptibility to infection rises with age; however, the disease is rapidly fatal in the young animals (Ozkul, 2002). The clinical signs imitate those of rinderpest, but changes can occur faster.

The clinical signs vary following the previous immunitary status of sheep (enzootic or newly infected country). They also vary following sheep breed.

The disease has per acute, acute and sub acute syndromes (Losos, 1986).

1.7.1. Peracute Syndrome

It is frequent in goats. Cases are found dead without previous symptoms. They die with a serous, foamy or hemorrhagic discharge coming out of the nose.

1.7.2. Acute Syndrome

Is the most common form. Animals are recumbent, sometimes in self-auscultation position. Body temperature is high (40.5 -41 °C) in the beginning of the onset in acute cases. The most typical signs are

seen in the digestive tract. When entering an affected flock, one sees many animals with hind limbs stained by sticky feces. Some sheep have an arched back and show pain to defecate. Tenesmus may be noticed when taking rectal temperature. Fluid feces are olive green to brown.

Examination of the mouth shows ulceration of the buccal mucosae, especially on the inner face of the lips, and neighboring gum. There can be periodontitis. There is serous nasal exudates and conjunctivitis.

The sudden high fever remaining high for 5-8 days; will return to normal before recovery or drop below normal before death. Serous nasal discharge, becoming mucopurulent; can crust over and occlude nostrils. Purulent ocular discharge with congested conjunctiva; can encrust, cementing eyelids together. Bronchopneumonia. Necrosis and ulceration of mucous membrane and inflammation of gastrointestinal tract leading to severe Non haemorrhagic diarrhea (Salki, 1998; EMPRES, 1999; DEFRA, 2001; Dhar et al., 2002; OIE, 2002; Ozkul et al., 2002). Respiratory distress, including dyspnea and sneezing in an attempt to clear nose. Excessive salivation but not to point of drooling. Anorexia. Severe dehydration and emaciation followed by hypothermia. Death usually occurs after 5-10 days. Abortion in pregnant animals may occur. Mortality rate can reach 100%. Secondary infections may be activated and complicate clinical signs (Opasina, 1980; Lefevre, 1982; Taylor, 1984; Mornet et al., 1956; Hamdy et al., 1976).

Nasal discharge becomes mucopurulent and may obstruct the nose. A dry, fitful coughing develops. Death occurs from 5-10 days after the onset of the fever. Some animals may recover, but a dry, stertorous coughing often persists for some days (Berrada, 2008).

Besides coughing, there is an intensive labial dermatitis with scab formation, resembling orf (op cit). In its acute form it is characterized by high fever, discharges from the eyes and nose, sores in the mouth, lesions of the mucous membranes, laboured breathing, and diarrhea (FAO, 2008).

The prognosis of acute PPR is usually poor, especially when lesions do not resolve within 2 to 3 days or when extensive necrosis and bacterial infection give the animal's breath an unpleasant, fetid odour. Young animals (4 to 8 months) often have more severe disease. Also, poor nutrition, stress of movement, and concurrent parasitic and bacterial infections worsen clinical signs (Saliki, 1998).

1.7.3. Subacute Syndrome

It develops over 10- 15 days, and characterized by pneumonia and inconsistent symptoms.

Fig: 2 PPR in goat: purulent eye and nose discharges



Discharges from the nose and eyes in advanced PPR infection, the hair below the eyes is wet and there is matting together of the eye lids as well as partial blockage of the nostrils by dried –up purulent discharges.

Available from: <http://www.fao.org/DOCREP/003/X1703E/X1703E00.HTM>.

Fig: 3 PPR in a goat: swollen, eroded lips



The lips tend to swell and crack and become covered with scabs.

Available from: <http://www.fao.org/DOCREP/003/X1703E/X1703E00.HTM>.

Fig 4: PPR in a goat: signs of diarrhoea



The hindquarters are soiled with liquid faeces.

Available from: [http:// www.fao. Org](http://www.fao.org) / DOCREP/ 003/ X1703E/ X1703E00.
HTM.

1.8. Morbidity and Mortality

The incidence of PPR in an enzootic area may be similar to that of rinderpest (RP) in that a low rate of infection exist continuously. When the susceptible population builds up, periodic epizootics (outbreaks) occur, that receive more attention than usual. Such epizootics may be characterized by almost 100 percent mortality among affected goats and sheep populations (Taylor, 1984; Lefevre and Diallo, 1990).

The severity of the disease and outcome in the individual is correlated with the extent of mouth lesions. Prognosis is good in cases where the lesions resolve within 2 to 3 days. It is poor when extensive necrosis and secondary bacterial infections result in an unpleasant, fetid odor from the animal's breath.

Respiratory involvement is also a poor prognostic sign. A morbidity rate of 80-90 percent and a case fatality rate of 5 percent are not uncommon-particularly in goats.

Young animals (4 to 8 months) have more severe disease, and morbidity and mortality are higher. Both field and laboratory observations indicate that PPR is less severe in sheep than in goats. Nevertheless, field outbreaks have been reported in the humid zones of west Africa in which no distinction could be made between the mortality rates in sheep and in goats. Poor nutritional status. Stress of movement, and concurrent parasitic and bacterial infections enhance the severity of clinical signs (Taylor, 1984; Lefevre and Diallo, 1990).

Morbidity and mortality can be as high as 100% and 90% respectively. When associated with other diseases such as capripox, mortality can be 100% (Dhar et al., 2002).

Mortality rates can reach 80 percent in acute cases. In "super acute" cases the mortality rate is 100 percent, with affected animals dying in the first week (FAO, 2008).

1.9. Pathology

The pathology caused by PPR is dominated by inflammatory and necrotic lesions in the mouth and the gastrointestinal tract. Unlike RP, there is also a definite, albeit inconstant, respiratory system component; hence, the synonym stomatitis-pneumoenteritis complex (Rowland et al., 1971; Hamdy et al., 1976; Bundza et al., 1988; Brown et al., 1991).

Emaciation, conjunctivitis, erosive stomatitis involving the inside of the lower lip and adjacent gum, cheeks near the commissures, and the free portion of the tongue are frequent lesions. In severe cases, lesions may also be found on hard palate, pharynx, and upper third of the esophagus. The necrotic lesions do not evolve into ulcers because the basal layer of the squamous epithelium is rarely penetrated.

The rumen, reticulum, and omasum rarely have lesions. Sometimes, there may be erosions on the pillars of the rumen. The abomasum is a common site of regularly outlined erosions and often oozes blood (Saliki, 1998).

Lesions in the small intestine are generally moderate, being limited to small streaks of hemorrhages, and sometimes erosions in the first portion of the duodenum and the terminal ileum. Payer's patches are the site of extensive necrosis which may result in severe ulceration. The large intestine is usually more severely affected with congestion around the ileocecal valve, at the ceco-colic junction, and in the rectum (Defra, 2005). In the posterior part of the colon and the

rectum, discontinuous streaks of congestion ("zebra stripes") form on the crests of the mucosal folds (Saliki, 1998).

In the respiratory system, small erosions and petechiae may be visible on the nasal mucosa, turbinates, larynx, and trachea. Bronchopneumonia may be present, usually confined to the anteroventral areas and is characterized by consolidation and atelectasis. There may be pleuritis, which may become exudative and results in hydrothorax (Saliki, 1998).

The spleen may be slightly enlarged and congested. Most lymph nodes throughout the body are enlarged, congested and edematous. Erosive vulvovaginitis similar to the lesions in the oral mucocutaneous junction may be present (Rowland et al., 1971; Hamdy et al., 1976; Bundza et al., 1988; Brown et al., 1991).

The lesions are usually seen in the digestive and respiratory systems, but can be seen in other systems as well.

In the digestive system, inflammatory and necrotic lesions in mouth and gastrointestinal tract (Defra, 2005). Erosive stomatitis in inside of lower lip and adjacent gum. Lesions on hard palate, pharynx, and upper third of esophagus in severe cases.

"Zebra stripes" (discontinuous streaks of congestion) in posterior part of colon and rectum and on crests of mucosal folds.

The liver was pale and sometimes friable and the cut surface showed tiny, whitish-grey necrotic foci (Toplu, 2004).

Lungs are dark red or purple areas; firm to the touch, mainly in the anterior and cardiac lobes (evidence of pneumonia).

Quick post-mortem examination will lead to the discovery of many hemorrhagic patches on the serous membranes, and intense pneumonia, erosions and inflammation is widespread on buccal mucosa, the same lesions are also present in pharynx, esophagus, and

on mucus-producing epithelia of the gut, from abomasum to rectum, zebra-striped lesions on coecum and colon are said to be typical in some cases, rarely, there are also petechiae on the rumen mucosa (Tligui, 2008).

Fig 5: PPR in a goat: the early lesions of pneumonia



Small, red, solid areas of lung tissue caused by PPR virus infection.

Available from: [http:// www.fao. Org / DOCREP/ 003/ X1703E/ X1703E00. HTM.](http://www.fao.org/DOCREP/003/X1703E/X1703E00.HTM)

Fig 6: PPR in a sheep: advanced pneumonia



Dark red / purple areas, mainly in the anterior and cardiac lobes of the lung. These lesions are typical of pneumonic pasteurellosis.

Available from: [http:// www.fao. Org / DOCREP/ 003/ X1703E/ X1703E00. HTM.](http://www.fao.org/DOCREP/003/X1703E/X1703E00.HTM)

Fig 7: PPR in a goat "zebra striping" in the large intestine



Lines of haemorrhage along the tips of the folds of the lining of the caecum and colon .

Available from: [http:// www.fao. Org / DOCREP/ 003/ X1703E/ X1703E00. HTM.](http://www.fao.org/docrep/003/X1703E/X1703E00.HTM)

1.10. Diagnosis of PPR

1.10.1. Clinical Diagnosis

In the field, a presumptive diagnosis can be made on the basis of clinical, pathological, and epizootiological findings. Laboratory confirmation is an absolute requirement-particularly in areas or countries where PPR has not previously been reported.

1.10.2. Laboratory Diagnosis

1.10.2.1. Samples Required for Diagnosis

The following samples should be submitted for evaluation, shipped fresh (not frozen) on ice within 12 hours after collection (Saliki, 1998):

- Blood in EDTA anticoagulant
- Clotted blood or serum
- Mesenteric lymph nodes
- Spleen
- Lung
- Tonsils
- Sections of the ileum and large intestine
- Swabs of serous, nasal and lacrimal discharges

The above samples should be collected in the acute phase of the disease, when clinical signs are readily apparent. Ideally, samples should be collected from several animals in an outbreak. Epidemiological and clinical details should be provided with the samples, and each sample bottle should be marked carefully with an indelible pen. Details of each sample's origin should be recorded for submission to the laboratory (EMPRES, 1999).

1.10.2.2. Electron Microscopy (E.M)

Electron microscopy technique was used for studying the morphology and ultra structure of PPR virus particle (Bourdin and Laurent-Vautier, 1967; Durojaiye et al., 1985).

1.10.2.3. Virus Isolation and Identification

1.10.2.3.1. Virus Isolation

Detection of the virus is done by isolation of the PPR virus in cultured cells. This method of diagnosis can be very valuable as it provides live virus for biological characterization studies. If facilities are available, it should always be attempted and isolated viruses stored for later studies (Roeder and Obi, 1999). Virus culture and isolation done in lamb kidney or African green monkey cell tissue cultures (OIE, 2000).

1.10.2.3.2. Cross Neutralisation Test (CNT)

Neutralisation of virus infectivity by specific antiserum is considered as an essential step in the identity of PPRV isolates (Scott et al, 1986; Anderson et al, 1996).

1.10.2.4. Serological Techniques

1.10.2.4.1 Antigen Detection Methods

1.10.2.4.1. 1. Agar Gel Immunodiffusion Test (AGIDT)

Detection of virus antigens by the agar gel immunodiffusion test (AGIDT) is a relatively simple, fast and cheap process. It is extremely useful as an initial test, but it does not discriminate between PPR and RP viruses and further tests are needed to do this (FAO, 1999).

Agar gel immunodiffusion, very simple and inexpensive and gives results within 1 day, but not sensitive to mild forms of PPR (OIE, 2000).

Agar gel precipitation (AGPT) test is the most frequently used technique for the detection of PPRV and RPV antigens (Appel et al., 1981)

Adu and Joannis (1985) reported that AGPT is a simple and rapid method for the diagnosis of PPR. This test was considered useful for field diagnosis of PPR as it can be applied in rural laboratories which do not have facilities for tissue culture and more sophisticated techniques (Nussieba et al ., 2008).

PPRV was found to cause agglutination of chicken, goat and pig RBCs (Ezeibe et al., 2004; Nussieba et al., 2008).

1.10.2.4.1.2. Counter Immunoelectrophoresis Test (CIEP)

CIEP is most rapid test for detecting viral antigen (OIE, 2000).

It is important to note that both the AGID and the CIEP are group-specific and may not distinguish between PPR and RP infections (Obi and Patrick, 1984).

1.10.2.4.1.3. Haemagglutination Test (HA)

Haemagglutination test is an easy, cheap and effective method for PPRV diagnosis (Johnson and Ritchie, 1968). PPR virus like measles virus has haemagglutination properties (Wosu, 1985; Wosu, 1991; Ramachandran et al., 1993; Ezeibe et al., 2004).

HA test was more sensitive than AGPT for detection of PPRV antigen. Another advantage of the HA test over AGPT was that it can differentiate PPR from RPV.

HA test represents a quick, easy, simple, cheap and reliable confirmatory test for the diagnosis of PPR and differential diagnosis of PPRV and RPV.

The presence of haemagglutinin was indicated by mat formation and its absence by button formation.

Higher HA titre was obtained with PBS of pH 6.8 than with PBS of pH 7.0.

It was found that the higher agglutination titre occurred at incubation temperature of 4°C than at 32°C (room temperature) (Ezeibe et al., 2004; Nussieba et al., 2008).

1.10.2.4.1.4. Immunofluorescent Antibody Test (IFAT)

The IFAT is simple and relatively quick, and has the advantage that facilities are available in most veterinary laboratories (Last et al., 1994). The IFAT technique detected PPR antigen in conjunctival smears from suspected cases of PPR collected from a field outbreak with 100% specificity (Sumption et al., 1998).

1.10.2.4.1.5. Immunoperoxidase Staining (IP)/ Immunohistochemistry (IHC)

Histopathology combined with immunohistochemical staining (e.g. immunoperoxidase) is a useful procedure because it is performed on formalin –fixed material and can discriminate between PPR and rinderpest when performed with specific monoclonal antibodies (FAO, 1999). Specific IHC reaction was characterized by the presence of light to dark brown, fine to coarse granules area in cells and tissues (Kumar et al, 2004).

1.10.2.4.1.6. ELISA for Antigen Detection

1.10.2.4.1.6.1. Immunocapture ELISA (IC-ELISA)

Virus antigens can also be detected by immunocapture ELISA (ICE) which is rapid and sensitive, and differentiates between PPR and rinderpest (FAO, 1999).

The IC-ELISA allows a rapid differential identification of PPR or RP viruses, and this is of great importance as the two diseases have

a similar geographical distribution and may affect the same animal species (Diallo, 2000; Diallo, 2004).

1.10.2.4.1.6.2. Sandwich ELISA (S-ELISA)

PPR virus-specific neutralizing M Ab was used in a simple and rapid double-antibody Sandwich ELISA for specific detection of PPRV antigen in goat tissues and secretions (Saliki et al., 1994). Singh and co-workers (2004) described a Sandwich ELISA test using PPR specific MAb (clone 4G6) to N protein.

The technique which is simple, convenient, rapid and cost-effective is preferred for intensive clinical surveillance and routine diagnosis of the disease (Singh et al., 2004).

1.10.2.4.2. Antibody Detection Methods

1.10.2.4.2.1. Agar Gel Diffusion Test (AGDT)

AGDT was used for the detection of antibodies against PPR in the sera of the affected goats (Durojaiye, 1982). This test is considered useful for field diagnosis of PPR. It provides a rapid serological diagnostic tool for PPR. Precipitating antibodies were detected in sera obtained in the acute phase of the disease and also in sera obtained at convalescence. Also sera which precipitated PPRV antigen did not precipitate RPV antigen (Durojaiye, 1982).

1.10.2.4.2.2. Precipitinogen Inhibition Test (P.I.T)

The principle of P I T is based on the ability of antibody in serum to inhibit diffusible virus antigen (precipitinogen) from developing a precipitin line against hyper immune serum in AGPT. It was observed that this test is more sensitive (33%) as compared to NT (28%) (Durojaiye, 1987).

1.10.2.4.2.3. Virus Neutralisation Test (VNT)

This test characterized by the following (OIE, 2000):

- Prescribed test for international trade.
- Cross-neutralisation with rinderpest virus must be completed, so the test can be time-consuming.
- Highly sensitive and specific.

Even the serum neutralisation test (SNT) currently being used as the confirmatory diagnosis for PPR and rinderpest also shows cross-reactivity (Mornet et al., 1956; Taylor, 1979; Obi, 1984).

1.10.2.4.2.4. Haemagglutination Inhibition Test (H I)

A simple and rapid serological method for definitive identification for kata virus or peste des petits ruminants (PPR) virus specific antibody. The technique is based on adsorbing out the cross-reacting antibodies to rinderpest antigen from a PPR serum and leaving the specific antibody to PPR which is determined by haemagglutination-inhibition test. Wosu (1985) was the first to demonstrate the haemagglutinin or PPR homogenate antigen, to porcine erythrocytes. This test is used in eliminating the cross-reactivity between PPR virus and rinderpest virus serologically.

The adsorption technique described by Johnson (1967) and Wosu (1977) was used as the basis for the removal of antibodies cross-reacting with rinderpest virus from known PPR serum. Since PPR and RP viruses belong to the same genus, they show a great deal of cross-reactivity. They cross-react in the immunodiffusion and complement fixation tests (Johnson and Ritchie, 1968; Ihemelandu et al., 1976; Nawathe, 1983), immunoosmo-precipitation test (Majiyagbe et al., 1984) and indirect haemagglutination-inhibition test using measles virus and monkey red blood cells (Nawathe, 1983).

This technique appears to have elucidated the problem of cross-reactivity which exists in the diagnosis of PPR and rinderpest.

The HI test, especially in less sophisticated laboratories, is a very useful, quick and accurate method for determining the antibody levels in a given immune serum (Johnson, 1971). Where there are closely related antigens which cross-react, the test cannot readily be a definitive diagnosis of a specific antibody unless it is possible to eliminate the cross-reactivity due to the other related antigens.

It is possible to make a definitive serological diagnosis of PPR specific antibodies by haemagglutination-inhibition test using the adsorption technique.

1.10.2.4.2.5. Counter Immunelectrophoresis (CIEP)

The CIEP is simple to perform, requiring very small quantities of reagents, and highly adaptable for use in titration of serum antibody (Majiyagbe et al., 1984). CIEP can be used for seroepidemiological studies as well as experimental studies on PPR (Durojaiye and Taylor, 1984; Majiyagbe et al., 1984).

CIEP is group –specific test and may not distinguish between PPR and RP infections in small ruminants (Obi and Patrick, 1984; Nussieba et al., 2009).

More positive serum samples were obtained by CIEP test than by C-ELISA when these tests were employed for detection of antibodies against PPRV, due to the different factors that affect the CIEP run compared with the optimized conditions of the C-ELISA (Nussieba et al., 2009).

One of the main advantages of the CIEP is its rapidity in producing results ,as precipitin lines were often visible after the test was run for 30-45 minutes (Nussieba et al., 2009).

CIEP, like HI test, could be a useful screening test where it is not possible to use C-ELISA.

1.10.2.4.2.6. ELISA for Antibody Detection

1.10.2.4.2.6.1. Competitive Enzyme-Linked Immunosorbent Assay (C-ELISA)

The C-ELISA is considered suitable for large scale testing due to its simplicity and availability of the recombinant antigen (Libeau et al., 1995).

C-ELISA, sensitivity is 99.4 % and specificity 94.5% (OIE, 2000).

A competitive ELISA based on a PPRV monoclonal antibodies specific for haemagglutinin (H) protein (Anderson et al, 1991; Saliki et al, 1993; Singh et al, 2004) or nucleoprotein (N) (Libeau et al., 1992; Libeau., 1995; Choi et al., 2003) was developed for detection of antibodies to PPRV in serum samples of sheep and goats. This test may be a useful tool for a standardized and accurate determination of the immune status of animals because of its superior sensitivity to conventional tests (Libeau et al., 1992).

1.10.2.4.2.6.2. Blocking ELISA (B-ELISA)

Blocking ELISA is proved to be simple, more rapid, sensitive and specific method for detection of PPR antibodies (Saliki et al., 1993).

Unlike the VNT, B-ELISA may be less affected by the quality of sera such as cytotoxicity and contamination (Saliki et al., 1993).

1.10.2.5. Molecular Techniques (Virus RNA Detection)

1.10.2.5.1. Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR)

Detection of virus genetic material is performed by the reverse transcriptase polymerase chain reaction (RT- PCR) which requires specialist facilities and expertise. Despite its high cost, it is now one of the tests used most frequently in reference centres, together with

enzyme linked immunosorbent assay (ELISA), because it is rapid, accurate, highly sensitive and can discriminate between PPR and rinderpest (FAO, 1999). This technique is very sensitive compared with other tests and results are obtained in 5 hours, including the RNA extraction (Diallo, 2000; Diallo, 2004).

1.10.2.5.2. Specific cDNA Hybridization

Nucleic acid technology was applied to the detection of RP and PPR viruses by using cDNA probes corresponding to the nucleocapsid gene of each virus and labeled with ³² P nucleotides (Diallo et al., 1989). This hybridization technique can be used to clearly identify the virus involved in an outbreak (Taylor et al., 1990).

1.10.2.6. Histopathology

Tissue samples were fixed in 10% neutral buffered formalin or in Bouins fluid, embedded in paraffin wax, sectioned at 5µm, and stained by routine methods with haematoxylin and eosin (H& E) (Rowland et al., 1969; Rowland and Bourdin., 1970).

1.10.3. Differential Diagnosis

PPR should be differentiated from the following conditions (Appel et al., 1981). :

1. Rinderpest, Clinical RP is rare in goats and sheep in Africa. In India, these species are quite often involved in RP outbreaks. Clinically, RP and PPR are similar, but the former should be the prime suspect if the disease involves both cattle and small ruminants. Confirmation requires virus isolation and cross-neutralization.
2. Pasteurellosis. Enzootic pneumonia or the septicemic form of pasteurellosis is characterized by obvious respiratory system infrequent diarrhea, and a fatality rate rarely exceeding 10 percent.

3. Contagious caprine pleuropneumonia. There is no digestive system involvement, and the clinical signs and lesions are confined to the respiratory system and pericardium.
4. Blue tongue. Swelling of the lips, muzzle, and oral mucosa, together with edema of the head region, should serve to differentiate bluetongue from PPR. Coronitis, common in bluetongue, is not a feature of PPR. Also, sheep are more affected than goats.
5. Heart water. There is often central nervous system involvement, including convulsions. There is no diarrhea.
6. Contagious ecthyma (contagious pustular dermatitis, orf). The orf virus causes proliferative, not necrotic lesions that involve the lips rather than the whole oral cavity. The absence of nasal discharges and diarrhea also distinguish orf from PPR.
7. Foot-and-mouth disease. This condition is comparatively mild, and the most characteristic clinical sign, lameness, is not a feature of PPR.
8. Nairobi sheep disease. Sheep are more severely affected than goats. It is limited geographically to parts of east and central Africa. Diagnosis requires isolation and serologic identification of the virus.
9. Coccidiosis. There is no upper digestive tract and respiratory system involvement.
10. Plant or mineral poisoning. Several plants and minerals may cause severe intestinal lesions. Case history and absence of fever should distinguish poisoning from PPR

Also gastrointestinal helminth infestations should be considered in differential diagnosis of PPR (Saliki, 1998; EMPRES, 1999).

1.11. Excretion of PPR Virus

Infected animals shed the virus in expired air and in ocular and nasal discharges, saliva, urine, milk and semen at the onset of fever and in the feces at the onset of diarrhea (Johnson and Ritchie, 1968; Abegunde and Adu, 1977; Scott, 1981; Sharma and Adlakha, 1994).

1.12. Public Health

Peste des petits ruminants is not infectious for humans.

1.13. Treatment

There is no treatment for PPR. However, mortality rates may be decreased by the use of drugs that control the bacterial and parasitic complications. Specifically, oxytetracycline and chlortetracycline are recommended to prevent secondary pulmonary infections (OIE, 2000). Supportive care including fluid therapy can also decrease deaths loss due to dehydration and subsequent electrolyte imbalance (Wosu, 1989).

1.14. Immunity to PPRV

Sheep and goats that recover from PPR develop an active immunity against the disease and resist infection with PPRV (Sharma and Adlakha, 1994). Young animals from dams with previous history of PPR are protected up to 3-4 months of age by maternal antibodies (Ata et al., 1989; Bidjeh et al., 1999). Clostral immunity protects kids and lambs until they are weaned (Sharma and Adlakha, 1994).

1.15. Control of PPR Outbreaks

Methods applied for rinderpest eradication may be appropriate for PPR. These include the following (Saliki, 1998): Quarantine, slaughter, proper disposal of carcasses and contact fomites,

decontamination of facilities and equipment, restrictions on importation of sheep and goats from infected areas.

Control of PPR outbreaks relies on movement control (quarantine) combined with the use of focused ("ring") vaccination and prophylactic immunization in high-risk populations (Roeder and Obi, 1999).

The only effective way to control PPR in endemic areas is by vaccination of the animals.

1.16. Vaccination

Until recently, the most practical vaccination against PPR made use of tissue culture rinderpest vaccine (Roeder and Obi, 1999). The tissue culture rinderpest vaccine at a dose of 102.5 TCID_{50} protects goats for at least 12 months against PPR.

In the past, the rinderpest vaccine has been used. However, this practice is being phased out to avoid confusion during retrospective serologic studies (OIE, 2002).

The use of rinderpest vaccine to protect small ruminants against PPR is now contraindicated because its use produces antibodies to rinderpest which compromise serosurveillance for rinderpest, and thereby the Global Rinderpest Eradication Program (GREP) (Roeder and Obi, 1999).

A homologous PPR vaccine is now available and gives strong immunity (OIE, 2002).

Recently, a homologous PPR vaccine has been developed, the vaccines can protect small ruminants against PPR for at least three years (Roeder and Obi, 1999).

An effective live vaccine is currently in use, it was attenuated by serial passage of the Nigeria 75/1 strain of PPRV in Vero cells (Diallo et al., 1989). This vaccine is thermolabile, it is necessary to

maintain it in an effective cold chain, condition which is difficult to achieve in many of the endemic countries. Thus a more heat –stable vaccine would be beneficial for use in countries with hot climates.

There are also genetically engineered recombinant vaccines undergoing limited field trials (OIE, 2002).

Goats were protected against a lethal challenge of PPRV following vaccination with a recombinant capripoxvirus containing either the fusion(F)gene of RPV or the haemagglutinin(H)gene of RPV. The H gene recombinant produced high titres of neutralizing antibody to RPV in the vaccinated goats, where as the F gene recombinant failed to stimulate detectable levels of neutralizing antibody.

Attenuated capripoxvirus strain KS-1 was used to develop an effective recombinant rinderpest vaccine expressing the fusion (F) and haemagglutinin (H) proteins of the rinderpest virus (Romero et al., 1993; Romero et al., 1994). This vaccine has now been tested and shown to be effective in long-term trials (Ngichabe et al., 1997; Ngichabe et al., 2002).

A recombinant capripoxvirus vaccine containing a cDNA of the peste des petits ruminants virus fusion protein gene was constructed. A quick and efficient method was used to select a highly purified recombinant virus clone (Berhe et al., 2003). This recombinant was able to protect goats against both PPR and capripox at a dose as low as 0.1 PFU. With the original capripoxvirus-rinderpest virus F recombinant, a dose of 1.5×10^3 PFU was unable to protect cattle against virulent RPV challenge (Romero et al., 1994). apparently it was 10^4 times less effective than the recombinant of (reCaPPR/F).

CHAPTER TWO

MATERIALS AND METHODS

2.1. Study area

The study was conducted in White Nile State which is located in the central region of the Sudan between the longitudes 31:30 – 33:15 and the latitude 12:15 – 15:15. The state covers an estimated area of 39701 Km² (annual report of General directorate of animal resources. White Nile State, 2008). The state is divided into eight localities, kosti, Tandalti, Elsalam, Rabak, Eljabaleen, Eldoium, Umrimta and Elgeteina. White Nile State borders are, Khartoum State to the north, Gezira State, Blue Nile and Sinnar State to the east, North and South of kordofan States to the west and Upper Nile State to the south.

The dominant climate is Savannah. The annual rainfall ranges from 150–700mm (Annual report of General directorate of animal resources White Nile State, 2008).

The human population in the state is estimated about 1.8 million (Annual report of General directorate of animal resources, White Nile State, 2008).

The major activities of the people in the state are the livestock ownership and agriculture.

2.1.1. Animal Population

White Nile State is a rich state of animal resources and different components of the livestock distributed all over the state according to the climate and common tribes in the area.

The general directorate of the animal resources in White Nile State estimated the animal population of about 8,215,252 animal head with the following details:

2,414,697	head of sheep
2,341,120	head of goats
3,430,516	head of cattle
28,919	head of camel

Nomadism is the natural phenomenon for the animal owners, their movement is according to the rainfalls. They usually move to the south in autumn, but sometimes they move towards the grazing area in Gezira State in the dry season.

The geographical distribution of the sheep and goats in different localities as estimated by the general directorate of the animal resources as follows:

Table 1: distribution of the sheep and goats in different localities of White Nile State

Locality	sheep	goats
Kosti	169,029	351,168
Tandalti	603,675	117,055
Elsalam	265,616	234,113
Rabak	362,204	117,056
Elgabaleen	169,029	234,112
Eldoium	193,176	936,248
Umrimta	579,527	327,957
Elgeteina	72,441	23,411
Total	2,414,697	2,341,120

2.2. Study Design

To achieve the objectives of this study, three methods were used to come up with a conclusion on the epidemiological situation of the PPR in the area of the study. The three methods are, data collection based on veterinary service reports, questionnaire survey and serological determination of antibodies against PPRV.

2.2.1. Questionnaire Survey

Data on pastoralist's knowledge about PPR clinical signs, its impact on their herds, their attitude to vaccination and the effect of animal's movement on the spread of the disease were obtained by means of a questionnaire distributed among owners of sheep and goats.

The questionnaire survey was done in six localities of the state, localities of kosti (kosti-Tandalti-Elsalam), localities of Elgabaleen (Elgabaleen and Rabak) and locality of Elgeteina based on the willingness of owners to respond. The questionnaire was distributed to 86 pastoralists in the different localities to come up with information about the situation of PPR in the state and the extent of the pastoralist's knowledge.

2.2.2 Veterinary Service Reports

Depending on the monthly and annual available reports of the General directorate of animal resources of White Nile State, all the data which were necessary for this study had been collected. Detailed information was collected on the personnel, infrastructures and vaccination program.

2.2.3 Study population and sampling method

The study animals that were sampled are traditionally managed sheep and goats Regardless of its health status from different

herds with different sites of White Nile State Data on age, sex and location of sampled animals were recorded.

A total number of 232 serum samples were randomly collected from herds in the State. The number of herds tested was 44 in 15 different sites represent the most popular places for sheep and goats for drinking water within the State.

All the samples collected from different areas with no history of vaccination against PPR. So as to end with the aim of the study which is to determine the presence of PPR antibodies and the prevalence of the disease in the state.

2.2.3.1 Sample collection

The puncture area of the jugular vein was cleaned by 70% ethanol. A plain glass vacutainer with a tube –holder and two way needle was used. Then 5 ml of blood was withdrawn. The vacutainer tubes were labeled indicating location, age and sex of the animal, put on a rack a way from direct sun light, and transferred to the laboratory. The vacutainers were kept over night in the refrigerator (4°C), then centrifuged for 5 minutes at 1500 r.p.m. Each serum sample was decanted in eppendorf tube, labeled indicating location, species, age and sex of the animal then stored at -20°C until used.

2.2.3.2. Laboratory test

The serological test which conducted in the study was C-ELISA to test all the serum samples for the detection of antibodies against PPR virus.

2.2.3.3. PPR ELISA Antigen

The peste des petits ruminants antigen was a vaccine strain of PPR virus cultured on Madin –Darby bovine Kidney cell (MDBK) and supplied as freeze-dried 1 ml aliquots.

2.2.3.4. Control Sera

Control sera were supplied as freeze-dried 1 ml aliquots of strong positive (PI 80-100), weak positive (PI 50-81) and negative (PI -25 to +25) anti-PPR ovine sera.

2.2.3.5. Monoclonal Antibodies

1 ml aliquots of monoclonal antibody (MAb) directed against PPR haemagglutinin were supplied as a freeze-dried hybridoma culture supernatant.

2.2.3.6. Rabbit Anti-Mouse Immunoglobulins

Supplied as freeze-dried rabbit anti-mouse immunoglobulins conjugated to horseradish peroxidase (HRPO). The PI was 95-105.

2.2.3.7. Procedure of the test

An aliquot (50µl) of pretitrated antigen diluted 1:100 in PBS was added to each well of an ELISA plate (Nunc-immuno Maxisorb plates, Copenhagen, Denmark). The plate was incubated at 4°C overnight or at 37°C for one hour on an orbital shaker (Vari-shaker, Dynatech) then washed 3 times. After the adsorption of the antigen, test sera and control were added in duplicate at a dilution of 1:5 in blocking buffer (BB) (10µ of the sera + 40 µl of blocking buffer). Controls with known strong positive, weak positive and negative ovine sera and also a monoclonal antibody control were added. Then followed by addition of 50 µl of a 1:100 dilution of the pre-titrated MAb. M Ab controls wells received 50µl at the same concentration in addition to 50 µl blocking buffer. Then incubated at

37°C for 1 h and washed , after that anti-mouse Horse Radish Peroxidase (HRPO) conjugate diluted 1:1000 in blocking buffer was added. Conjugate control wells received 100 µl blocking buffer followed by 50 µl conjugate. Incubation at 37°C for 1 h and washing. Freshly prepared chromogen (OPD) solution containing 0.004% (v/v) substrate (H₂ O₂) was added. The plates then incubated at room temperature for 10 min, and then the reaction was stopped by the addition of IM H₂ SO₄. Blank were prepared by adding chromogen/substrate solution plus stopping solution. Plates were read on a Titertek Multiscan ELISA reader using 492nm filter.

2.2.3.7. Procedure of the test

1. Coating of PPR Ag 1:100 in PBS. 50µl in each well for 1hr in orbital shaker or overnight at 4°C.
2. Washing 3 times with washing buffer (1:5 PBS+DDW), and blot to dry.
3. Add 40µl of blocking buffer (PBS+0.1% tween 20+0.3% negative serum) in each well.
4. Add 10µl of control sera in columns 1 and 2, 60µ of BB in CC wells.
Add 10µl of tested sera in columns 3 to 12 in duplicate vertically.
5. Add monoclonal antibody (1:100 in BB). 50µl in each well except conjugate wells A1, A2.
6. Incubate for 1hr in orbital shaker.
7. Wash 3 times with washing buffer and blot to dry.
8. Add conjugate (1:1000 in BB) 50µl in each well.
9. Incubate for 1hr in orbital shaker.
10. Wash 3 times.

11. Add chromogen+H₂O₂ (OPD+H₂O₂). 4μl of H₂O₂ for each 1 ml of OPD, incubate without shaking in darkness for 10 minutes.
12. Stop reaction with Sulphoric Acid 50μl for each well.
13. Read in filter 492→ read blank then read plate.

The positive serum samples were indicated by colourless wells while negative samples were indicated by yellow colour wells.

Figure 8. Competitive ELISA Plate Layout

1	2	3	4	5	6	7	8	9	10	11	12
Cc	Cc	1	5	9		17		25		33	
C++	C++	1	5	9		17		25		33	
C++	C++	2					22		30		38
C+	C+	2					22		30		38
C+	C+	3			15			27		35	
Cm	Cm	3			15			27		35	
Cm	Cm	4	8	12		20			32		40
C-	C-	4	8	12		20			32		40

Cc : Conjugate

C++: Strong Positive Serum

C+ : Weak Positive Serum

Cm : Monoclonal Antibody

C- : Negative Serum Control

CHAPTER THREE

RESULTS

3.1. Questionnaire survey outcomes:

Table (2) summarized the questionnaire survey responses among the herd's owners. The results showed that the herd owners were nomads (65.12%, n = 56) and settled (34.88%, n = 30). Forty two owners (48.84%) had their herd mixed with others, while forty (51.16%) were not.

Forty two (48.84%) selected PPR as the most important disease, forty (46%) selected other diseases, where as four (4%) have no ideas about the disease.

41.86% (n = 36) of owners confirmed the presence of PPR in their herds, while 58.14 (n = 50) confirmed absence of it. Forty four (51.16%) know the signs of PPR and mentioned it, whereas forty two (48.84%) didn't know the signs. 6.98% (6), 20.93% (18), 11.63% (10) selected the most age affected as adult, young and both respectively, however 60.47% have no idea about the age.

Eighteen owners (20.93%) stated that the morbidity is higher than mortality, sixteen (18.60%) mentioned that mortality is higher, where as fifty two (60.47%) have no answer 16.28% of owners (n = 14) recorded the presence of abortion in their herd, 23.26% (n = 20) not recorded and 60.47% (n = 52) have no idea.

12 (13.95%) mentioned that the economic impact is due to death, 8 (9.30%) due to loss of production, 14 (16.28%) due to both and 52 (60.47%) have no comments. Thirty owners (34.88%) had vaccinated against PPR while fifty six (65.12%) hadn't.

3.2 Veterinary service reports outcomes

3.2.1 Veterinary service structure

The general directorate of animal resources works under the umbrella of state Ministry of agriculture and animal resources. It consists of six Departments including animal health, extension and training, animal production, fisher production, reports and information and directorate of animal resources in the localities.

Table (2): Summary of the questionnaire survey responses by owners in white Nile State:

Subject	Localities			Total
	El geteina	Kosti	Rabak	
Animal owners respond	36 (41.86)	32 (37.21)	18 (20.93)	86 (100.00)
Herd composition				
a- Sheep	2 (5.56)	4 (12.50)	4 (22.22)	10 (11.63)
b- Goat	0.00	0.00	0.00	0.00
c- Sheep & goats	12 (33.33)	8 (25)	8 (44.44)	28 (32.56)
d- Others	0.00	0.00	0.00	0.00
e- Mixed	22 (61.11)	20 (62.5)	6 (33.33)	48 (55.81)
Production system				
a- Nomads	24 (66.67)	16 (50)	16 (88.89)	56 (65.12)
b- Settled	12 (33.33)	16 (50)	2 (11.11)	30 (34.88)
Migratory route				
a- East, middle, west	18 (50)	10 (31.25)	12 (66.67)	40 (46.51)
b- North, middle, south	6 (16.67)	6 (18.75)	4 (22.22)	16 (18.60)
Herd mixed with others				
a- Yes	10 (27.78)	14 (43.75)	18 (100)	42 (48.84)
b- No	26 (72.22)	18 (56.25)	0.00	44(51.16)
Most important disease				
a- PRR	18 (50)	10 (31.25)	14 (77.78)	42 (48.84)
b- Other disease	18 (50)	18 (56.25)	4 (22.22)	40 (46.51)
c- No idea	0.00	4 (12.50)	0.00	4 (4.65)
Presence of PRR in herd				
a- Yes	24 (66.67)	6 (18.75)	6 (33.33)	36 (41.86)
b- No	12 (33.33)	26 (81.25)	12 (66.67)	50 (58.14)
Knowledge of RR signs				
a- Yes	22 (61.11)	10 (31.25)	12 (66.67)	44 (51.16)
b- No	14 (38.89)	22 (68.75)	6 (33.33)	42 (48.84)
Most age affected				
a- Adult	4 (11.11)	2 (6.25)	0.00	6 (6.98)
b- Young	10 (27.78)	4 (12.50)	4 (22.22)	18 (20.93)
c- Both	8 (22.22)	0.00	2 (11.11)	10 (11.63)
d- No Idea	14 (38.89)	26 (81.25)	12 (66.67)	52 (60.47)
Morbidity and mortality				
a- Morbidity is high	10 (27.78)	4 (12.50)	4 (22.22)	18 (20.93)
b- Mortality is high	12 (33.33)	2 (6.25)	2 (11.11)	16 (18.60)
c- No answer	14 (38.89)	26 (81.25)	12 (66.67)	52 (60.47)
Presence of abortion				
a- Yes	10 (27.78)	2 (6.25)	2 (11.11)	14 (16.28)
b- No	12 (33.33)	4 (12.50)	4 (22.22)	20 (23.26)
c- No answer	14 (38.89)	26 (81.25)	12 (66.66)	52 (60.47)
Economic impact				
a- Death	10 (27.78)	0.00	2 (11.11)	12 (13.95)
b- Loss of production	6 (16.67)	0.00	2 (11.11)	8 (9.30)
c- Both	6 (16.67)	6 (18.75)	2 (11.11)	14 (16.28)
d- No comment	14 (38.89)	26 (81.25)	12 (66.67)	52 (60.47)
Vaccination against PPR				
a- Yes	8 (22.22)	10 (31.25)	12 (66.67)	30 (34.88)
b- No	28 (77.78)	22 (68.75)	6 (33.33)	56 (65.12)

N = Number of owners

(%) = percentage of owner

3.2.2 Manpower

Animal health, animal production, extension and training, and reports are the responsibility of the personnel who work in the general directorate of animal resources.

Table (3) shows the total personnel of general directorate working all over White Nile State.

Table (3): Manpower engaged in animal health services in White Nile State

Locality	Vets	Tech	Assistants	CA H W S	Support staff	Drivers	Total
State Head Quarter	19	1	2	-	53	5	80
Kosti	18	7	30	31	33	5	124
Algableen	12	7	19	-	14	2	54
Al doium	8	-	24	18	2	1	53
El geteina	3	2	8	-	6	-	19
Total	60	17	83	49	108	13	330

Vests = Veterinarians, Tech = Technicians

C A H W S = Community Animal Health Workers

3.2.3. Transportation

The transportation means which are especially important for vaccination regimes and other activities in the veterinary services of the state are described in Table (4).

Table (4): Vehicles involved in animal health activities

Locality	Lorries	Cars	Mobile units	Total
State head Quarter	-	2	3	5
Kosti	3	5	3	11

Elgableen	1	3	3	7
Eldoium	1	1	2	4
EL geteina	-	1	2	3
Total	5	12	13	30

3.2.4. Previously reported PPR outbreaks

Although 41.86 of owners confirmed the presence of PPR in their herds. There are no reported outbreaks in the general directorate of the State.

3.2.5. Vaccination program

The demand for vaccination against PPR increased in recent years in the state. All the vaccine doses distributed were used and no vaccine remained.

Table (5) shows the vaccination figures in the six years ago.

Table (5): Vaccination figures in the state during 2003-2008.

Year	Vaccine distributed (doses)	Vaccine used (doses)	Remain doses
2003	97500	97500	-
2004	300200	300200	-
2005	302000	302000	-
2006	250100	250100	-
2007	106700	106700	-
2008	95380	95380	-

3.2.6 Sero-Prevalence of PPR in the State

The 232 serum samples were tested for detection of antibodies against PPR Antigen using competitive ELISA (C-ELISA) test. Generally 178 samples were positive with prevalence of 76.72% while 54 samples were negative with prevalence of 23.28%.

Table (6) shows the distribution of Ab responses specific to PPRV in the different localities of the State.

Table (6): Prevalence of PPRV antibodies in sheep and goats sera in different localities when examined by C-ELISA

Locality	Total No. of sera	Percentage positive	Percentage negative
Kosti	139	69.78%	30.22%
Elgabaleen	29	82.76%	17.24%
Elgeteina	61	88.52%	11.48%
Eldoium	3	100%	0.00%

CHAPTER FOUR

DISCUSSION

In Sudan, Peste des petits ruminants virus (PPRV) was firstly isolated and identified by Elhag Ali in Eastern Sudan in 1971 and several outbreaks of PPR causing high mortalities among small ruminants occur in many parts of Sudan, but still little is known about it. Severe outbreaks of PPR continued to occur in small ruminants in Sudan causing significant economic losses.

There is also reported isolation of PPRV in the Sudan came in 1984 from disease outbreak at Sinnar and Elmeileg (Elhag Ali and Taylor, 1984).

In Sudan, detection of antibodies against PPRV has also been reported in several species including sheep, goats, cattle and camels (Anderson and Mckay 1994; El Amin and Hassan 1999; Haroun et al., 2002). Rapid detection of infected animals is very important for PPR control to be effective.

Recently, severe outbreaks of PPR among small ruminants were reported in different parts of Sudan. These outbreaks initiate the need for a simple laboratory tests for the rapid diagnosis of the disease and for differentiation of PPR from RP.

Several outbreaks of PPR occurred in White Nile State, but until yet there is no one report to explain the real losses due to these outbreaks or the intervention of the veterinary services towards the investigation and control of the disease. This may be due to that most veterinarians in the field are not familiar with the signs of PPR, weakness of the diagnostic tool available in the field and delay of the owners in informing the veterinary services units. One outbreak

occured in Elgeteina locality in 2000 in sheep, it take long time for diagnosis until lastly confirmed to be PPR. This initiate the need for more studies about this disease .

In an attempt to investigate the prevalence of PPR in sheep and goats in White Nile State, epidemiological study based on three disciplines was conducted in different localities of the state, to draw attention to the importance of the disease and to set some bases so as to help in the eradication programs of the disease.

The result of the questionnaire survey in this study revealed that the majority of owners interviewed in the state are owing sheep and goats alone or mixed herds including sheep and goats, most of them are nomads. Bearing in mind the large number of sheep and goats in the State (4,755,817) and the majority of population depend entirely on small ruminants in addition to the role of sheep in exportation, explain the importance of small ruminants in the State and hence the need for more attention .And as the PPR is transmitted by close contact keeping animals in nomadic system, and as the result revealed large number of herds mixed together explain what size of problem will be if outbreak occur.

The result of the study also showed that most of owners confirmed PPR as the most important disease and most of them observed the presence of PPR in their herds, majority of owners knowing the signs of PPR and described it, this explain the wide spread of the disease and thus PPR will be a great problem in the state if not controlled. Minority of them mentioned that most age affected are adult while many of them reported its presence in young alone or in both ages, this result was proved previously by Taylor (1979), Obi (1982), Lefevre and Diallo (1990), Wosu (1994) and Ozkul et al, (2002) who reported that infection rates in sheep and goats rise with

age and the disease is rapidly fatal in young animals. Ozkul et al., (2002) reported that young animals aged 4-8 months often have more severe disease. Nduaka and Ihemelandu (1973) proved that morbidity and mortality rates are higher in young animals than in adult.

From owners interviewed, 20% mentioned that morbidity is higher than mortality while 18% mentioned that mortality is higher, this nearly agreed with Intisar (2002) who informed in another study that in White Nile State morbidity rate is 22% and mortality is 15%.

The regular reporting system in the State (monthly and annually) facilitates the process of data collection from the general directorate of animal resources. But although many owners confirmed the presence of PPR in their flocks and as the serosurveillance indicated high prevalence of disease, there is no any reported outbreak by the general directorate of animal resources. The structure of the general directorate seem to be reasonable, but actually there were a lot of gaps, as performing of regular investigation system of the disease and disease mapping in the state need to be improved to establish organized works concerning animal health. The manpower engaged in animal health services in White Nile State are able to perform many activities of veterinary services including the control program of infectious diseases. But there is a need for more training and redistribution of personnel within the localities.

The available vehicles and other facilities mentioned in this study are suitable for the on going control program for epidemic diseases in the state, but budgets are not enough to maintain them.

The result of the study also revealed that majority of owners hadn't vaccinated against PPR at all, while minority of them done, this may be due to shortage in the vaccine doses available for the state as the vaccination figures showed that all the vaccine doses distributed

during every year being used completely and no dose remain. Also vaccination program done with difficulty because the understanding of the owners to the seriousness, unavailable treatment and importance of vaccination in the protection against disease is not well developed. The vaccination activities done according to the owner's willing.

Severe cases in which animals show clinical signs in the field can easily be detected through clinical surveillance and the detection of antigen in clinical samples, while the diagnosis of PPRV infection in sub clinically infected animals can be achieved by serological surveillance.

Monoclonal antibody-based C-ELISA was used for the specific determination of antibodies to PPRV in sera of sheep and goats because C-ELISA is simple, rapid, specific, sensitive and preferred over VNT for intensive surveillance as stated by Singh et al (2004) and the test could clearly differentiate infected from uninfected population (Lefevre et al., 1991; Saliki et al., 1993; Libeau et al., 1995; Singh et al., 2004).

The result of the examination of sera samples from different localities by C-ELISA proved that about 76.72% were positive .High prevalence of PPR was detected in the samples from Eldoium locality followed by Elgeteina, ELgabaleen and Kosti. The prevalence rates obtained by this study look too much high. Even kosti locality which represent the lowest prevalence (69.78%) this percentage considered high.

The detection of a high prevalence of antibodies against PPRV in sera collected from field samples of sheep and goats, explained the exposure of these animals to the field virus. No vaccination with PPR vaccine was carried out previously. Also indicated the wide spread of the disease all over the state. This result coincide with the previous

reports(Nussieba, 2005; Nussieba et al., 2009)who reported that PPR is prevalent in Sudan, and high prevalence of PPR antibodies detected by C-ELISA was in Blue Nile State. And as this State one of seven States that share borders with White Nile State, thus this wide spread of the disease and high prevalence in the State may be attributed to the animal movement between neighbouring States.

Conclusions and Recommendations

Conclusions:

In conclusion, the result of the present study revealed that PPR is prevalent in White Nile State.

Recommendations:

Based on the results of the study, and depending on the information about the transmission of the disease. The following recommendation should be considered:

- Improvement of the information system and plan unit in the state for proper control of diseases.
- Increase awareness of owners on the importance of the disease.
- Strict quarantine measures should be carried out around the infected areas.
- Since the state shared borders with many states, check point must be established for security of the state.
- Mass vaccination against PPR to cover all the state.

REFERENCES

- Abegunde, A. A. and Adu, F. D. (1977). Excretion of the virus of peste des petits ruminants by goats. *Bulletin of Animal Health and Production in Africa*, 25(3): 307-311.
- Anderson, J., Barret, T. and Scott, G. R. (1996). Confirmatory Diagnosis, Part III, chapter 7. In: *Manual of the Diagnosis of Rinderpest (FAO Animal Health Manual -1)*. 2nd edition. Food and Agriculture Organization of the United Nations, Rome, Italy, pp. 1-79.
- Anderson, J., McKay, J. A. and Butcher, R. N. (1991). The use of monoclonal antibodies in competitive Elisa for detection of antibodies to rinderpest and peste des petite ruminants viruses. In: *Seromonitoring of rinderpest throughout Africa, Phase one*. Proceedings of final Research Coordinating Meeting of the FAO/IAEA/ SIDA/OAU/PARC coordinated Research Programme, Bingerville, Cote d'Ivoire, 19-23 November 1990. IAEA-TECDOC-623. pp. 43-53.
- Anderson, J. and McKay, J. A. (1994). The detection of antibodies against peste des petits ruminants virus in cattle, sheep and goats and the possible implications to rinderpest control programmes. *Epidemiology and infection*, 112:225-231.
- Appel, M.J.G., Gibbs, E.P.J., Martin, S.J., Ter Meulen, V., Rima, B.K., Stephenson, J.R. and Taylor, W.P. (1981). Morbillivirus Diseases of Animals and Man. In *Comparative Diagnosis of Viral Diseases IV*, E. Kurstak and C. Kurstak, New York: Academic Press, pp. 235-297.

- Ata, F .A., Al Sumry, H . S., King, G. J., Ismaili, S. I. and Ata, A .A. (1989). Duration of maternal immunity to peste des petits ruminants. *Veterinary Record*, 124:590-591.
- Awad El karim, M. A., Ali, Y. O., Hajer, B. S., Fayza, A. O. and Hadya, J . A. (1994). Observation on the epidemiology of peste des petits ruminants in the Sudan. *The Sudan Journal of Veterinary Research*, 13: 29-34.
- Berhe, G., Minet, C., Le Goff, C., Barrett, T., Ngangnou, A., Grillet, C., Libeau, G., Fleming, M., Black, D.N and Diallo, A. (2003). Development of a dual recombinant vaccine to protect small ruminants against peste-des- petits- ruminants virus and capripox virus infections. *Journal of Virology*, 77(2): 1571-1577.
- Berrada, J.(2008). Observations des premiers cas confirmés de peste des petits ruminants au Maroc, oral presentation, El Jadida, 31-07.
- Bidjeh, K ., Diguimbaye, C ., Hendrikx, P ., Dedet, V ., Tchari, D . and Naissingar, S. (1999). Maternal immunity in young goats or sheep whose dams were vaccinated with anti-peste des petits ruminants vaccine. *Cahiers Agricultures*, 8(3):219-222.
- Bourdin, P., and Laurent- vautier, A.(1967). Note Sur La structure du virus de la peste des petits ruminants. *Revue d' Elevage et de Medecine Veterinaire des Pays Tropicaus*, 20(3):383-386.
- Brown, C.C., Mariner, J.C. and Olander, H.J. (1991). An immunohistochemical study of the pneumonia caused by peste des petits ruminants virus. *Veterinary Pathology*. 28(2): 166-170.
- Brumby P.(1990). Systems and livestock development in tropical Africa. In: Dauda K Jawara (ed), *Animal disease as a factor*

limiting economic development in Africa .Comell
Veterinary.80:17-25.

Bundza, A., Afshar, A., Dukes, T .W., Myers, D.J., Dulac, G.C., and
Becker, S.A.W.E.(1988).Experimental peste des petits
ruminants (goat plague) in goats and sheep. Canadian Journal
of Veterinary Research, 52:46-52.

Choi, k. S., Nah, J. J., Choi, C. U., Ko, Y. J., Sohn, H . J., Libeau, G.,
kang, S. Y. and Joo, Y. (2003). Monoclonal antibody-based
competitive ELISA for simultaneous detection of rinderpest
virus and peste des petits ruminants virus antibodies. Veterinary
Microbiology, 96(1): 1-16.

Crowley, J. C., Bowling, P. C., Menonna, J., Silverman, J. I.,
Schuback, D., Cook, S. D. and Blumberg, B . M. (1988).
Sequence variability and function of measles virus 3' and 5'
ends and intercistronic region. Virology, 164: 498-504.

DEFRA (Department for Environment, Food and Rural
Affairs).(2005). Animal health and welfare. Peste des petits
ruminants: pictures. May 24.

DEFRA (Department for Environment, Food, and Rural Affairs)
(2001).Disease surveillance and control, peste des petits
ruminant. Nov 2001.

Dhar, P., Sreenivasa, B.P., Barrett, T., Corteyn, M., Singh, R.P. and
Bandyopadhyay, S.K. (2002).Recent epidemiology of peste des
petits ruminants virus (PPRV). Veterinary Microbiology, 88
(2):153-159.

Diallo, A., Taylor, W.P., Lefevre, P.C. and Provost, A. (1989).
Attenuation d'une souche du virus de la peste petits ruminants:
candidat pour un vaccine homologue vivant. Revue d' Elevage
et de Medecine Veterinaire desPays Tropicaux, 42(3): 311-319.

- Diallo, A. (1990). Morbillivirus group: genome organization and proteins. *Veterinary Microbiology*, 23:155-163.
- Diallo, A. (2000), Peste des petits ruminants. In: OIE Manual of Standards for Diagnostic Tests and Vaccines, 4th edition .Chapter 2. 1. 5. Office international des Epizooties, Paris, pp. 114-122.
- Diallo, A. (2003). Control of peste des petits ruminants: classical and new generation vaccines. *Developmental Biology (Basel)*, 114: 113-119.
- Diallo, A. (2004). Peste des petits ruminants. In: OIE Manual of Standards for Diagnostic Tests and Vaccines for Terrestrial Animals, 5th edition. Chapter 2.1.5. Office international des Epizooties, Paris, pp. 153-162.
- Diallo, A., Barret, T., Barbron, M., Meyer, G. and Lefevre, P. C. Cloning of the nucleocapsid protein gene of peste-des-petits-ruminants virus: relationship to other morbilliviruses. *Journal of General Virology*, 75: 233-237.
- Durojaiye, O. A. (1982). Precipitating antibody in sera of goats naturally affected with peste des petits ruminants. *Tropical Animal Health and Production*, 14:98-100.
- Durojaiye, O. A. (1987). Application of a precipitinogen inhibition test in the detection of antibody to peste des petits ruminants virus . *Revue D'Elevage et de Medecine Veterinaire des Pays Tropicaux*, 40(1):17-20.
- Durojaiye, O. A. and Taylor, W. P. (1984). Application of countercurrent immuno-electro-osmo-phoresis to the serology of peste des petits ruminants. *Revue d'Elevage et de Medecine Veterinaire des Pays Tropicaux*, 37(3):272-276.

- Durojaiye, O. A., Taylor, W. P. and Smale, C. (1985). The ultra structure of peste des petits ruminants virus. *Journal of Veterinary Medicine B, Infectious Diseases Veterinary Public Health*, 32:460-465.
- Durojaiye, O. A. (1980). Brief notes on history, epizootiology and the economic importance of PPR in Nigeria. In: *Proceedings of the International Workshop on Peste des Petits Ruminants*, IITA, Ibadan, Nigeria, 24-26 September, pp.24-27.
- Durtnell, D. R. (1972). A disease of sokoto goats resembling “peste des petits ruminants”. *Tropical Animal Health and Production*, 4:162-164.
- El Amin, M. A. G. and Hassan, A. M. (1999). Rinderpest and PPR surveillance in Sudan (1996-1998). In: *The Seromonitoring and Surveillance of RP Throughout Africa-phase 111. Results for 1998. Proceedings of a research co-ordination meeting of the FAO/IAEA/OAU/IBAR/PARC co-ordinated research project organized by the joint FAO/IAEA division of nuclear techniques in food and agriculture, Machakos, Kenya, April 26-30.*
- El Hag Ali, B. and Taylor, W.P. (1983). The isolation of. Pests des petits ruminants virus (PPRV) from the Sudan. *Research in Veterinary Science*, 36:1-4.
- El Sheikh, E.I. (1992). Some aspects of PPR epidemiology in North Darfur. M.V.Sc, University of Khartoum, Sudan.
- Elhag Ali, B. (1973). A natural outbreak of rinderpest involving sheep, goats and cattle in Sudan. *Bulletin of Epizootic Diseases of Africa*, 21:421-428.

- Elhag Ali, B. and Taylor, W. P. (1984). Isolation of peste des petits ruminants virus from the Sudan. *Research in Veterinary Science*, 36:1-4.
- EMPRES (Emergency Prevention System for Transboundary Animal and Plant Pests and Diseases)(1999). *Recognizing peste des petits ruminants: a field manual*. Rome, Italy: FAO (Food and Agriculture Organization of the United Nation).
- Ezeibe, M. C. O., Wosu, L. O. and Erumaka, I. G. (2004). Standardization of the haemagglutination test for peste des petits ruminants (PPR). *Small Ruminant Research*, 51(3): 269-272.
- FAO,(2008). “Outbreak of ‘peste des petits ruminants’ in Morocco. (<http://www.fao.org/newsroom/en/news/2008/1000919/index.html>)”, FAO Newsroom, FAO (September 9, 2008). Retrieved on 10 September 2008.
- Furley, C. W., Taylor, W.P. and Obi, T.U (1987). An outbreak of peste des petits ruminants in a zoological collection. *Veterinary Record*, 121:443 – 447.
- Gibbs, E.P.J., Taylor, W.P., Lawman, M.J.P., and Bryant,J. (1979).Classification of peste des petits ruminants virus as the fourth member of the genus Morbillivirus. *Intervirology*, 11:268-274.
- Haffar, A., Libeau, G., Moussa, A., Cecile, M. and Diallo, A. (1999). The matrix protein gene sequence analysis reveals close relationship between peste des petits ruminants virus (PPRV) and dolphin morbillivirus. *Virus Research*, 64:69-75.
- Hamdy, F.M., Dardiri, A.H., Nduaka, O., Breese, S.S., and Ihemelandu, E.C. (1976). Etiology of the stomatitis

- pneumoenteritis complex in Nigerian dwarf goats. *Canadian Journal of Comparative Medicine*, 40: 276-284.
- Haroun, M., Hajer, I., Mukhtar, M. M. and Elhag Ali, B. (2002). Detection of antibodies against peste des petits ruminants virus in sera of cattle, camels, sheep and goats in Sudan. *Veterinary Research Communications*, 26(7):537-541.
- Ihemelandu, E. C., Hamdy, F. M., Dardiri, A. H., Nduaka, O. and Breese Jr., S. S. (1976). Etiology of the stomatitis pneumoenteritis complex in Nigerian Dwarf goats. *Canadian Journal of Medicine* 40:276-284.
- Intisar, K. S., Khalafalla, A. I., El Hassan, S. M. and El Amin, M. A. (2004). Peste des petits ruminants (PPR) in the Sudan: investigation of recent outbreaks, virus isolation and cell culture spectrum. *Journal of Animal and Veterinary Advances (JAVA)*, 3(6): 361-365.
- Intisar, K.S. (2002). Studies on peste des petits ruminants disease in Sudan. M.V.Sc, University of Khartoum, Sudan.
- Johnson, A. E. (1967). Comparison of six strains of *Trichomonas foetus*. 11. Gel diffusion precipitation studies of soluble antigens. *Experimental parasitology* 21: 336-343.
- Johnson, R. H. and Ritchie J.S.D. (1968). A virus associated with pseudorinderpest in Nigerian Dwarf goats. *Bulletin of Epizootic Diseases of Africa* 16:411-417.
- Johnson, R.H. (1971). Serological procedures for the study of feline panleukopenia. *Journal of the American Veterinary Medical Association* 158:876-884.
- Kumar, P., Tripathi, B .N. Sharma, A. k., Kumar, R., Sreenivasa, B .P. ,Singh, R. P., Dhar, P . and Bandyopadhyay, S. K . (2004). Pathological and immunohistochemical study of experimental

peste des petits ruminants virus infection in goats. *Journal of Veterinary Medicine B, Infectious Diseases Veterinary Public Health*, 51: 153-195.

- Last, R. D., Jardine, J. E., Smit, M. M. and van der Lugt, J. J. (1994). Application of immunoperoxidase techniques to formalin –fixed brain tissue for the diagnosis of rabies in Southern Africa. *Onderstepoort Journal of Veterinary Research*, 61(2):183-187.
- Lefevre, P.C. and Diallo, A. (1990). Peste des petits ruminants. *Revue Scientifique et Technique de l' Office International des Epizooties*, 9:951-965.
- Lefevre, P.C. (1982). Peste des petits ruminants et infection bovinepestique des ovins et caprins (synthese bibliographique). Institut d'Elevage et de Medecine Veterinaire des Pays Tropicaux, 94704 Maisons-Alfort, France, 95.
- Lefevre, P. C., Diallo, A ., Schenkel, F ., Hussein, S. and Staak, G . (1991). Serological evidence of peste des petits ruminants in Jordan. *Veterinary Record*, 128: 110.
- Libeau, G., Diallo, A., Calvez, D. and Lefevre, P. C. (1992). A competitive ELISA using anti-N monoclonal antibodies for specific detection of rinderpest antibodies in cattle and small ruminants. *Veterinary Microbiology*, 31:147-160.
- Libeau, G ., Prehaud, C ., Lancelot, R ., Colas, F ., Guerre, L ., Bishop, D . H. L. and Diallo, A. (1995). Development of a competitive ELISA for detecting antibodies to the peste des petits ruminants virus using a recombinant nucleoprotein. *Research in Veterinary Science*, 58:50-55.
- Losos, G. J. (1986). Peste des petits ruminants. In: *Infectious Tropical Diseases of Domestic Animals*, 1st edition. Published by Longman Scientific and Technical, England in Association with

International Development Research Centre, Canada, pp. 549-558.

- Mahin, L. (2008). Observations sur un foyer de Peste des petits ruminants, unpublished data.
- Majiyagbe, K. A., Nawathe, D. R. and Abegunde, A.(1984). Rapid Diagnosis of peste des petite ruminants (PPR) infection, application of immunoelectroosmophoresis (IEOP) technique. *Revue d'Elevage et de Médecine Vétérinaire des pays tropicaux* 37(1):11-15.
- Merck's and Co. (2008). Peste Des Petits Ruminants. In: *The Merck Veterinary Manual .Inc, Whitehouse Station, N.J., USA.*
- Mornet, P., Orue, J., Gilbert, Y., Thiery, G., and Sow, M. (1956). La peste des petits ruminants en Afrique occidentale française et Ses rapports avec la peste bovine. *Revue d' Elevage et de Medecine Veterinaire des Pays Tropicaux*, 9(4):313-342.
- Murphy, F. A., Gibbs, E. P. J., Horzinck, M . C . and Studdert, M. J. (1999). Paramyxoviridae. In: *Veterinary Virology*. 3rd edition. . Academic press, U .S.A., pp.411-428.
- Nawathe, D. R. (1983). Some observations in the diagnosis of peste des petits ruminants in Nigeria. In: Hill D H (ed), *Peste de petits ruminants (PPR) in sheep and goats. Proceedings of the international workshop held at IITA (International Institute of Tropical Medicine), Ibadan, Nigeria, 24-26 September 1980. ILCA (International Livestock Centre for Africa), Addis Ababa, Ethiopia .pp.34-39.*
- Nduaka , O . and Ihemelandu, E . C. (1973). Observations on “pneumonia-enteritis complex” in dwarf goats in Eastern States of Nigeria . *Bulletin of Epizootic diseases of Africa*, 21(1):87-98.

- Ngichabe, C. K., Wamwayi, H.M., Barrett., Ndungu,E.K., Black,D.N. and Bostock, C.J. (1997). Trial of a capripoxvirus-rinderpest recombinant vaccine in African cattle. *Epidemiology and Infection*, 118:63-70.
- Ngichabe, C. K., Wamwayi, H.M., Ndungu, E.k., Mirangi, P.K., Bostock,C.J., Black, D.N and Barrett.(2002). Long term immunity in African cattle vaccinated with a recombinant capripox-rinderpest virus vaccine. *Epidemiology and Infection*, 128:343-346.
- Nussieba, A . O ., Mahasin, E . A ., Ali, A . S . and Fadol, M . A . (2008). Rapid detection of Peste des Petits Ruminants (PPR)virus antigen in Sudan by Agar Gel Precipitation (AGPT) and Haemagglutination (HA) Tests. *Tropical Animal Health and Production* . 40(5): 363-368.
- Nussieba, A.O. (2005). Peste des petits ruminants (PPR) in Sudan: Detection, virus isolation and identification, pathogenicity and serosurveillance, M.V.M, University of Khartoum, Sudan..
- Nussieba, A . O ., Ali, A . S ., Mahasin, E . A . and Fadol, M . A . (2009). Antibody seroprevalences against Peste des Petits Ruminants (PPR) virus in sheep and goats in Sudan. *Tropical Animal Health and Production*. Doi:10.1007/s 11250-009-9333-8.
- Obi, T . U . (1982). Peste des petits ruminants in goats in Nigeria; clinical, microbiological and epizootiological aspects. Third International Conference on Goat Production and Disease, Tucson, USA .
- Obi, T . U . (1984). Serological survey of some viral infections in goats in Southern Nigeria. In: Sumberg, J . E . and Cassaday, K . (Eds): sheep and goats in humid West Africa. Proceedings of

Workshop on Small Ruminants Production Systems in The Humid Zone of West Africa, held in Ibadan, Nigeria 23-26 Jan. ILCA, Addis Ababa, pp.17-21.

Obi, T . U . and Patrick, D . (1984). The detection of peste des petits ruminants (PPR) virus antigen by agar gel precipitation test and counter-immunoelectrophoresis. Journal of Hygiene, Cambridge, 93: 579-586.

OIE (Office International des Epizooties /World Organization for Animal Health)(2002). Peste des petits ruminants. Technical disease card database. Apr 22.

OIE (Office International des Epizooties/World Organization for Animal Health)(2000) .Peste des petits ruminants. In: Manual of standards for diagnostic tests and vaccines. 4th edition. Chapter 2.1.5. Paris: OIE.

Opasina, B.A .1980 . Epidemiology of PPR in the Humid Forest and Derived Savanna zones. In: Peste des petits ruminants (PPR) in sheep and goats. Proc. International Workshop, I ITA, Ibadan, Nigeria, 24-26 September1980. D. H Hill, ed., Addis Ababa, Ethiopia: International Livestock Center for Africa, 1983.pp.14-21.

Ozkul, A., Ake, Y., Alkan, F., Barrett,T., karaoglu,T., Dagalp,S.B.,Anderson,J.,Yesilbag,K.,Cokcaliskan,C.,Gencay,A .and Burgu,I.(2002). Prevalence, distribution and host range of peste des petits ruminants virus, Turkey. Emerging Infectious Diseases, 8(7) :708-712.

Radostitis, O . M ., Blood, D . C . and Henderson, J . A . (2007). In Veterinary Medicine, Bailmière Tindal, London, ISBN 0-7020-07-18-8.

- Ramachandran, S ., Hedge, N . R ., Raghavan, R ., Subbarao, M . S . and Shyam, G . (1993). Haemagglutination by PPR virus. In: Proceedings of The 3rd International Sheep Veterinary Conference, Edinburgh, pp.1-2.
- Rima, B .K . (1993). The proteins of morbilliviruses. Journal of General Virology, 64: 1205-1219.
- Roeder, P . I . and Obi , T . U . (1999). Recognizing peste des petits ruminants. In: FAO Emergency Prevention System for Transboundry Animal and Plant Pests and Diseases (EMPRES). A field Manual No. 5 . FAO, Rome, Italy.
- Romero, C. H., Barrett,T., Chamberlain, R.W., Kitching, R.P., Fleming, M. and Black, D.N.(1994). Recombinant capripoxvirus expressing the hemagglutinin protein gene of rinderpest virus: protection of cattle against rinderpest and lumpy skin disease viruses. Virology 204:425-429.
- Romero, C. H., Barrett,T., Evans, S.A., Kitching, R.P., Gershon, P.D., Bostock, C. and Black, D.N.(1993). Single capripoxvirus recombinant vaccine for the protection of cattle against rinderpest and lumpy skin disease. Vaccine 11:737-742.
- Romero, C.H., Barrett, T., Kitching,R.P., Carn, V.M. and Black, D.N.(1984). Protection of cattle against rinderpest and lumpy skin disease with a recombinant capripoxvirus expressing the fusion protein gene of rinderpest virus. Veterinary Record,135:152-154.
- Rossiter, P . B . and Taylor, W . P . (1994). Peste des petits ruminants. In: Infectious Diseases of Livestock . Eds. J .A . W . Coetzer . Volume II . P . 758.
- Rowland, A . C . and Bourdin, P . (1970). The histological relationship between “ peste des petits ruminants” and kata in

West Africa. *Revue d'Elevage et de Medecine Veterinaire des Pays Tropicaux*, 23(3): 301-307.

Rowland, A . C ., Scott, G. R . and Hill, D . H . (1969). The pathology of an erosive stomatitis and enteritis in West-African dwarf goats. *The Journal of Pathology*, 98(1): 83-87.

Rowland, A.C., Scott,G.R., Ramachandran, S. and Hill, D.H. (1971). A comparative study of peste des petits ruminants and Kata in West African dwarf goats. *Tropical Animal Health and Production*, 3,241-245.

Saliki, J .T.(1998). Peste des petits ruminants. In : US Animal Health Association, Committee on Foreign Animal Disease. *Foreign Animal Diseases: The Gray Book*. 6th edition. Part IV. Richmond, VA:US Animal Health Association,pp.344-352.

Saliki, J.T., House, J. A., Mebus, C.A. and Dubovi, E.J.(1994).Comparison of monoclonal antibody –based sandwich enzyme-linked immunosorbent assay and virus isolation for detection of peste des petits ruminants virus in goat tissues and secretions. *Journal of Clinical Microbiology*, 32(5):1349-1353.

Saliki, J.T., Libeau, G., House, J.A., Mebus, C.A. and Dubovi, E.J.(1993). Monoclonal antibody-based blocking enzyme-linked immunosorbent assay for specific detection and titration of peste- des- petits- ruminants virus antibody in caprine and ovine sera. *Journal of Clinical Microbiology*, 31(5):1075-1082.

Scott, G . R . and Brown, R . D . (1961). Rinderpest diagnosis with special reference to the agar gel double diffusion test. *Bulletin of Epizootic Diseases of Africa*, 9: 83-125.

Scott, G . R ., Taylor, W . P. and Rossitter, P. R. (1986). *Manual of the diagnosis of Rinderpest*. FAO Animal Health Manual-1, 1st

edition. Food and Agriculture Organization of the United Nations, Rome, Italy, No.23, pp.99.

Scott, G.R. (1981). Rinderpest and peste des petits ruminants. In Virus Diseases of Food Animals. A world geography of epidemiology and control, ed. Gibbs,E.P.J. Academic Press (London, New York), Disease monographs, volume 11, pp. 401-432.

Sharma, S.N. and Adlakha, S.C. (1994). Rinderpest and peste des petits ruminants. In: Textbook of Veterinary Virology, Part 11: Systematic Virology-RNA Viruses, 1st edition, Vikas Publishing House PVT Ltd, New Delhi, India,pp.296-305

Sidhu, M .S., Husar, W., Cook, S .D., Bowling, P.C. and Udem, S .A .(1993). Canine distemper terminal and intergenic non-protein coding nucleotide sequences: completion of the entire CDV genome sequence. Virology, 193:66-72.

Singh, R .P ., Sreenivasa, B . P ., Dhar, P ., Shah, L . C . and Bandyopadhyay, S . K . (2004). Development of a monoclonal antibody based competitive-ELISA for detection and titration of antibodies to peste des petits ruminants (PPR) virus. Veterinary Microbiology, 98:3-15.

Singh, R .P ., Sreenivasa, B .P ., Dhar, P . and Bandyopadhyay, S .K. (2004). A sandwich-ELISA for the diagnosis of peste des petits ruminants(PPR) infection in small ruminants using anti-nucleocapsid protein monoclonal antibody. Archives of Virology, 149(11):2155-2170.

Sumption, K . J ., Aradom, G ., Libeau, G . and Wilsmore, A . J . (1998). Detection of peste des petits ruminants virus antigen in conjunctival smears of goats by indirect immunofluorescence. Veterinary Record, 142:421-424.

- Taylor, W . P ., Al Busaidy, S . and Barrett, T. (1990). The epidemiology of peste des petits ruminants in the Sultanate of Oman. *Veterinary Microbiology*, 22:341-352.
- Taylor, W .P.(1979).Serological studies with the virus of peste des petits ruminants in Nigeria. *Research in Veterinary Science*, 26:236-242.
- Taylor, W.P. (1984). The distribution and epidemiology of peste des petits ruminants. *Preventive Veterinary Medicine*, 2:157-166.
- Taylor, W.P., Diallo, A., Gopalakrishna ,S., Sreeramalu,P., Wilsmore, A.J., Nanda, Y.P., Libeau, G., Rajasekhar, M. and Mukhopadhyay, A.K. (2002). Peste des petits ruminants has been widely present in southern India since ,if not before, the late1980's.*Preventive Veterinary Medicine*, 52(3-4):305-312.
- Tligui.(2008). Observations nécropsiques sur les premiers cas confirmés de peste des petits ruminants au Maroc, oral presentation, El Jadida, 31-07.
- Toplu, N. (2004). Characteristic and non –characteristic pathological findings in peste des petits ruminants (PPR) of sheep in the egg district of Turkey. *Journal of comparative Pathology*, 131:135-141.
- Wosu, L . O . (1977). Trichomonas infection in a bull-An apparent change in serotype of infecting organisms. *Australian Veterinary Journal* 53:340-341.
- Wosu, L. O.(1985).Agglutination of red blood cells by peste des petite ruminants(PPR)virus. *Nigerian Veterinary Journal* ,14:56-58.
- Wosu, L.O.(1989). Management of clinical cases of peste des petits ruminants (PPR) disease in goats. *Beitrag zür Tropischen Landwirtschaft und Veterinarmedizin*, 27(3):357-361.

- Wosu, L.O.(1991). Haemagglutination test for diagnosis of peste des petits ruminants disease in goats with samples from live animals. *Small Ruminant Research*, 5:169-172.
- Wosu, L . O . (1994). Current status of peste des petits ruminants (PPR) disease in small ruminants-A review article . *Student Research in Veterinary Medicine*, 2:83-90 .
- Zeidan, M .I. (1994). Diagnosis and distribution of peste des petits ruminants in small ruminants in Khartoum State during 1992-1994. M . V . Sc, University of Khartoum, Sudan.

APPENDICES

APPENDEX (1)

GENERAL QUESTIONNAIRE

Date: No.:

Name of owner:

Locality :

Site:

Tribe:

Herd composition: cattle sheep goat mixed

Situation: Nomad Settled

Migratory route: East Middle West
North Middle South

Has the herd mixed with others? Yes No

Would you ranking the most five important disease in your area?

Yes No

If yes mention them 1.....2.....3.....4.....5.....

Do you know the signs of the PPR? Yes No

Can you mention these signs? 1..... 2.....
3..... 4..... 5.....

Have you ever seen these signs within your herd? Yes No

Age category most affected? Adult Young

Morbidity %: Mortality rate %:

Abortion rate within affected pregnant ewes:

The effect of the disease in the production

Comment

Have you ever vaccinated against PPR? Yes No

If no why ? Comment

APPENDIX (2)
SAMPLE COLLECTION FORM

Date:

No.:.....

Name of owner:

Locality:

Site:

Species:

Sex

Age:

Observation:

APPENDIX (3)

C-ELISA Solutions, Reagents and Chemicals

1. Coating buffer:

The contents of one sachets of PS (9.99g) was dissolved in 1 litre of locally produced DDW to obtain 0.01 M phosphate buffered saline, pH 7.4 ± and stored at 4°C for no longer than two weeks.

2. Washing buffer:

Washing buffer was prepared by mixing 200 ml of PBS with 800 ml of locally produced DDW and 0.05% Tween 20 to obtain 0.002 M phosphate buffered saline, pH 7.4 ± 0.20 and stored at room temperature for no longer than two weeks.

3. Blocking buffer (BB):

Blocking buffer was prepared locally by mixing 100 ml phosphate buffered saline (PBS) with 100 µl lamb negative serum and stored at 4°C for no longer than two weeks.

4. Anti-species conjugate:

Rabbit anti-mouse immunoglobulin horseradish peroxidase HRPO conjugate was supplied with the kit, it was kept at 4°C.

5. Chromogen ortho-phenylenediamine (OPD):

One OPD tablet (30 mg) was dissolved in 75 ml of DDW to obtain 2.2 mM OPD and stored in the dark at -20°C.

6. Stock solutions of substrate [3% hydrogen peroxide (H₂O₂D)]:

One tablet of hydrogen peroxidase was placed in a brown bottle and dissolved in 10 ml of locally produced DDW to obtain 3% (w/v) hydrogen peroxide (882 mM) and stored at 4°C).

7. Stopping solution:

Fifty five ml of concentrated sulphuric acid (H_2SO_4) were added slowly to 945 ml of locally produced DDW to obtain 1 M sulphuric acid and stored at room temperature.