

***PESTE DES PETITS RUMINANTS* MANUAL FOR SAMPLES COLLECTION AND
TRANSPORTATION AND BIOSECURITY DURING CLINICAL FIELD
INVESTIGATION IN THE SADC REGION**



PREPARED BY: SADC PPR Working Group

Executive summary

Peste des Petits ruminants (PPR) is an acute highly contagious disease of small ruminants caused by a virus in genus Morbillivirus in the family Paramyxoviridae. Other susceptible species include hedgehogs, armadillos, nutrias, elephants, capybaras, rats, and mice. It can rapidly spread through a region if control and eradication practices are not implemented upon its detection. The disease is characterised by the formation of vesicles (fluid-filled blisters) and erosions in the mouth, nose, teats and feet. Weight loss, poor growth, permanent hoof damage, chronic mastitis and death of young animals are just some of the sequelae of infection. The detection of PPR in a country impacts international trade and embargoes could cause significant economic losses. Transmission primarily occurs by respiratory aerosols and direct or indirect contact with infected animals. Additionally, contaminated objects, such as boots, hands, clothing, vehicles or equipment can spread the virus from animal to animal or farm to farm.

This manual is aimed at guiding field personnel on how to recognize PPR in the field, collect appropriate diagnostic specimens, preserve and dispatch the specimens for laboratory confirmation of the disease with minimum chances of specimen destruction and environmental contamination.

There are three sections of the manual which complement each other. It is advisable that during PPRV field investigations, three teams namely the epidemiology, clinical and laboratory teams are involved. This simplified and concise manual shows how to collect samples from suspected PPR-infected animals, how to preserve these samples in order to reach the laboratory in a good condition while maintaining the biosecurity in the whole process of specimen collection and transportation.

TABLE OF CONTENTS

Executive summary	ii
Introduction	1
SECTION ONE : BIOSECURITY DURING FIELD INVESTIGATIONS FOLLOWING PPR SUSPECTED OUTBREAK IN DOMESTIC RUMINANTS	3
DEFINITIONS OF BIOSECURITY AND BIOSAFETY	3
Biosecurity.....	3
Biosafety	4
Major components of biosecurity	4
Biosecurity principles	5
REQUIRED ITEMS FOR IMPLEMENTATION OF BIOSECURITY IN FIELD INVESTIGATION	6
Disinfection principles:	7
Effective Disinfectants:	7
Vehicle biosecurity for visits	7
SUGGESTED ONSITE INSTRUCTIONS/ PROCEDURE FOR ENTRY ONTO A PREMISES DURING VISITS TO VILLAGES WITH SUSPECT PPR CASES.....	7
Establishing a disinfection point at the farm’s entrance	8
SUGGESTED PROCEDURE FOR EXITING A PREMISES	9
Vehicle biosecurity: Cleaning and disinfecting vehicles.....	10
SUGGESTED INSTRUCTIONS AFTER LEAVING THE INVESTIGATION SITE.....	11
Disposing off PPE	11
Persons involved in the field investigations	11
Biosecurity Protocol	11
REFERENCES.....	11
SECTION TWO: COLLECTION, PRESERVATION AND TRANSPORTATION OF SPECIMENS FOR PPR CONFIRMATION	13
INTRODUCTION	14
EPIDEMIOLOGY	14
Incubation period	14
Persistence of agent.....	14
General properties	14

Environment	14
Live animals	15
Animal products and byproducts	15
Modes of transmission	15
Live animals	15
Animal products and byproducts	15
Equipment and personnel	15
Vectors	16
Semen and embryos	16
IMMUNITY	16
Innate and passive immunity	16
Active immunity	16
Vaccination	16
OBJECTIVES OF THE CLINICAL INVESTIGATION	16
DIAGNOSTIC CRITERIA.....	17
CLINICAL SIGNS.....	17
Goats	17
Sheep	18
PATHOLOGY.....	18
Gross lesions	18
LABORATORY TESTS	19
Specimens required and preservation.....	19
Tissue specimens and swabs	19
Blood specimens and sera specimens	19
Transport of specimens	19
Laboratory diagnosis	20
Laboratory diagnosis of PPR important facts.....	20
Principles of PPR diagnosis.....	20
CVL tests [to be updated]	21
Table 1. Laboratory tests currently available at CVL for the diagnosis of PPR.....	21
Microscopic lesions (histopathology)	21
Sampling Protocol.....	21
Procedure for specimens collection – healthy animals	21
Procedure for specimens collection – clinically sick animals.....	22
PPR case definition.....	22
Collection of diagnostic specimens	22

Swabs specimens collection	22
Collection and preparation of Serum samples:-	23
Collection of whole blood samples:	23
Collection of Tissue specimens	23
Sampling equipment check list	25
General requirements	25
Blood	25
Conjunctival, buccal or nasal swabs.....	25
Tissue specimens.....	25
Biosecurity	25
Transport Planning check list.....	26
The sender	26
The receiver	26

Introduction

Peste des petits ruminants (PPR) is an acute contagious disease of small ruminants caused by a virus in the genus *Morbillivirus* in the family *Paramyxoviridae*. PPRV is a close relative of the Rinderpest virus, a disease of cattle whose earth-wide eradication was celebrated by the World Organisation for Animal Health in June 2011. The natural disease of PPR affects mainly goats and sheep, but it is usually more severe in goats where it causes heavy losses and is only occasionally severe in sheep while cattle can only be infected subclinically. However, in poor conditions it might be possible that cattle develop lesions following PPRV infection, clinical signs of which would be ascribed to rinderpest. Other susceptible species include buffaloes, one-humped camels, gazelles and American white-tailed deer (*Odocoileus virginianus*). Peste des Petits ruminants is an important disease due to the high mortality rates in small ruminants hence threatening food security and has significant economic impact including affecting international trade.

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.

PPRV is transmitted mainly by aerosols between animals living in close contact. The disease is characterised by fever, oculonasal discharges, stomatitis, diarrhoea and pneumonia with foul offensive breath. The natural disease affects mainly goats and sheep, but it is usually more severe in goats where it causes heavy losses and is only occasionally severe in sheep. It is generally admitted that cattle can only be infected subclinically. Occasionally PPR affects small ruminants living in the wild.

Following the incubation period of 4–6 days (range is 3 and 10 days) of an acute clinical disease, pyrexia of up to 41°C that can last for 3–5 days develops; the animals become depressed, anorexic and develop a dry muzzle. The serous oculonasal discharges become progressively mucopurulent and, if death does not occur, persist for around 14 days. Within 4 days of the onset of fever, the gums become hyperaemic, and erosive lesions develop in the oral cavity with excessive salivation. These lesions may become necrotic. A watery blood-stained diarrhoea is common in the later stage. Pneumonia, coughing, pleural rales and abdominal breathing also occur. The morbidity rate can be up to 100% with a mortality rate of up to 100% in severe cases. However, this may not exceed 50% during milder outbreaks. At necropsy, prominent crusty scabs along the outer lips and severe interstitial pneumonia frequently occur with PPR. Erosive lesions may extend from the mouth to the reticulo–rumen junction. Characteristic linear

haemorrhages or zebra stripes occur in the large intestine, commonly at the caeco–colic junction, but they are not a consistent finding; necrotic or haemorrhagic enteritis is usually present. Lymph nodes are enlarged, the spleen may show necrotic lesions, and there is an apical pneumonia. A tentative diagnosis of PPR is made on these clinical signs and post mortem lesions, but laboratory confirmation is required for differential diagnosis with other diseases with similar signs. PPR must be differentiated from rinderpest, bluetongue, foot and mouth disease, contagious caprine pleuropneumonia (CCPP), pasteurellosis, , Nairobi sheep disease, Sheep and goat pox, contagious ecthyma, heartwater, coccidiosis and other exanthemous conditions, hence appropriately collected diagnostic specimens are required for laboratory confirmation of the disease.

SECTION ONE : BIOSECURITY DURING FIELD INVESTIGATIONS FOLLOWING PPR SUSPECTED OUTBREAK IN DOMESTIC RUMINANTS



DEFINITIONS OF BIOSECURITY AND BIOSAFETY

Biosecurity

Biosecurity is a set of practices designed to prevent the spread of disease into an area and is accomplished by maintaining the area in such a way that there is minimal traffic

of disease-causing agents crossing its borders. Biosecurity is the cheapest, most effective means of disease control available as preventing disease is always cheaper than treating or suffering the effects of disease. For most of the viral trans-boundary diseases (VTADs), the principles and practices in applying biosecurity are basically the same with minor differences depending on the epidemiology of the particular TAD.

Biosafety

In case the suspected disease under investigation clinically resembles a zoonotic one (in the differential diagnosis), it is important to consider the biosafety measures. Although when applying biosecurity measures some aspects of biosafety are also taken care of, it is important to make sure that all other measures to biosafety are fully covered. In many cases these are in the personal protective equipment (PPE) aspects which has to consider prevention of human becoming infected through all possible routes of disease transmission as it may involve putting on specific kind of goggles and or mask or even the biosecurity gown.

Major components of biosecurity

Isolation - Keeping animals protected from sources of infection – including unauthorized access and carriers of disease causes – and separating groups of animals to minimize the spread of infection across the population.

Traffic Control - Limiting incoming traffic and traffic within an area, and controlling the movement of equipment, vehicles, people, feed, animals and animal products to prevent exposure to disease.

Sanitation - Regularly cleaning and disinfecting housing, equipment, vehicles, and people to destroy disease agents.

In addition to the three components of biosecurity, understanding disease transmission is another key point to keep in mind.

In PPR, risk for disease transmission may be associated with personnel especially during surveillance and farm visits. It is therefore important to ensure biosecurity is practiced during clinical investigation exercise and vital that disease investigators lead example because, if veterinarians do not observe biosecurity procedures properly, it is very difficult to persuade other staff and farm visitors.

It is recommended that during PPR investigations (like many viral TADs), in order to capture epidemiological information to go with specimens to be collected while maintaining biosecurity, the investigation team should ideally comprise three subteams as indicated below:

1. The clinical team, also known as the "dirty team" whose task is to examine suspect animals and ensure that the most appropriate samples were taken and most accurate epidemiological (infected herd data), aiming at finding the earliest lesion (for sampling) and the oldest (for tracing).
 2. The epidemiology team (Epi team) also called the "clean team" whose task is to try to establish the timelines of events that could have introduced the current infection and which could allow spread. The team leader experienced in participatory epidemiology is an ideal person to spearhead participatory information gathering.
 3. Laboratory team (Lab team). This as the support team, mainly consisting of the laboratory persons to receive and test samples and correlate their findings with Epi and clinical teams. This team should ideally be led by a Local Laboratory staff.
- It is crucial that the investigation group adapts to circumstances achievable level of biosecurity depends on the circumstances.
 - Apply general principles using veterinary judgement and every effort should be made to maximise biosecurity even if local farmers are not observing it.
 - It is vital to avoid any feeling among farmers that the surveillance team may be spreading disease.

Biosecurity principles

Aim at minimising contact between farms

In order to achieve biosecurity, the following are recommended:

- Do not enter or bring anything onto or off a farm unless necessary
- Carry out cleansing and disinfection before and after visiting any farm
- Strict segregation between "dirty" and "clean" areas are essential
- Quarantine period for the persons entering the dirty area should be observed depending on the epidemiological significance of the causative agent.
- Remember, all the containers sent to the dirty area where the clinical team is, should be decontaminated before exiting the dirty area. For this reason, only take the necessary items required for specimen collection..
- Ensure that the containers with specimen are properly closed before being dipped into the disinfectant for decontamination, so as to avoid mixing the disinfectant with the specimen.

REQUIRED ITEMS FOR IMPLEMENTATION OF BIOSECURITY IN FIELD INVESTIGATION

Item	Requirements
Personal protective equipment per person	<p>Waterproof disposable Tyvek suit (legs inside boots)</p> <p>Waterproof outer-suit with hood (legs outside boots) (optional)</p> <p>2 pairs of Latex/nitrile gloves - Two pairs to be worn; the outer pair to be sealed to the Tyvek suit sleeve using tape to protect wrists.</p> <p>gum boots</p> <p>2 pairs of plastic over-boots to reduce dirt on boots and facilitating cleaning</p> <p>Hat/hood</p> <p>Disinfectant</p> <p>Tape about 5cm wide to seal gap at wrist between outer gloves and Tyvek sleeve, and to seal plastic bags.</p> <p>Ear protection if visiting pig farm</p>
	Laminated sheet of Specific instructions on how to dress into and out of the PPE
	Instructions on procedures to follow when leaving farm via disinfection point
At site in the field	<p>Suggested onsite instructions/ procedure for entry onto a premises during visits to villages with suspect PPR cases</p> <p>Disinfectant</p> <p>Brush with long handle</p> <p>Clean water</p> <p>Squeeze bottle</p> <p>At least two Buckets/wide mouth containers</p> <p>Mobile phone, charged, in zip-locked plastic bag</p> <p>Plastic bags, ideally yellow/red biohazard waste bags, for transport/disposal of clothing/equipment.</p> <p>Plastic sheeting about at least 1.5 x 2m to place on ground at disinfection point</p> <p>Equipment required for job on farm depending on the purpose of your visit, but may include sampling equipment, sharps containers, etc.</p>
Vehicles	<p>Plastic sheeting sufficient to place on the boot/where the items used in investigation will be placed</p> <p>Instructions for vehicles</p>

Disinfection principles:

Clean before disinfection: wash thoroughly before disinfection, as dirt and organic matter can protect virus from disinfectant

Disinfect surfaces fully and completely: splashing disinfectant on something is not enough, immerse boots in the disinfectant and wash the overalls thoroughly.

Ensure adequate contact time: disinfectant needs time to work.

Effective Disinfectants:

Virkon 1% (w/v)

Vehicle biosecurity for visits

- Empty all non-essential items from car
- Arrange a "clean" area (e.g. back seat) and a "dirty" area (e.g. boot); line both with plastic sheet/bags
- Do not drive onto farm; park outside premises
- Remove car keys, wallet, watch, jewellery etc and leave in car (e.g. hidden under mat).
- Put all necessary kit into bag to take onto farm; returning to car will require a change of outer-wear

SUGGESTED ONSITE INSTRUCTIONS/ PROCEDURE FOR ENTRY ONTO A PREMISES DURING VISITS TO VILLAGES WITH SUSPECT PPR CASES

- Park the vehicle at a suitable point near the entrance to the premises, but **do not** drive into the yard.
- Take onto the farm only what is required for your visit. Unnecessary items (wallet, keys, etc) should be left in the car. Minimise use of cameras and where necessary, use waterproof video camera.
- Ensure you have all necessary equipment for the visit prior to entering the premises.
- Identify a designated area for putting on PPE. It should ideally be a clean area away from animals or any other potentially contaminated equipment, feed or farm tools. All personnel should use this area to put on their PPE.
- Place mobile phone in zip-locked plastic bag.
- Identify optimal site for disinfection point. This will depend on the circumstances, but an ideal disinfection point should be clean, dry, permit a clear demarcation between "clean" and "dirty" sides, and have a nearby water supply.

- Put on personal protective equipment
 - (a) Put on Tyvek suit. Be certain to zip it up.
 - (b) Put on waterproof outer suit
 - (c) Put on gumboots: Tyvek suit legs **inside** gumboots, waterproofs **outside** gumboots.
 - (d) Put on over-boots.
 - (e) Put on two pairs of gloves.
 - (f) Pull sleeve of Tyvek suit down and tape it to outer glove, sealing the gap at your wrist to ensure that no contaminants come in contact with your skin.
 - (g) Ensure the hoods of the Tyvek and waterproof suits are covering your hair.

Establishing a disinfection point at the farm's entrance

NB Water supply essential: if not available near disinfection point, arrange for supplies to be delivered.

When clearing up disinfection point have all items on dirty side put in plastic bag, sealed and put in safe place (e.g. shed) on farm for subsequent disposal

C&D items (e.g. brush, bucket) on clean side, put in plastic bag, seal, and put in "dirty" area of your vehicle.

- Establish the disinfection point at the site previously identified and best location is at gate/fence where visible division between "clean" and "dirty" sides is important. The procedure for this will vary according to the circumstances and individual judgment will be required, but ideally the disinfection point should consist of:

- (a) Make a clear demarcation between "clean" and "dirty" sides
- (b) Place a bucket filled with disinfectant made up to the appropriate strength and a brush on the clean side
- (c) Place a similar disinfectant bucket and brush on the dirty side

NB – disinfectant must be kept fresh, replenished and at proper concentration

- (d) Lay down a plastic groundsheet (this can be builder's plastic, or a plastic bag) on the ground on the clean side, hold it down with stones.
- (e) Place a plastic bag for contaminated waste on the dirty side
- (f) Place two plastic bags on the clean side: one for the samples and other relevant equipment to be placed in and one for the cleaned and disinfected waterproof overalls and gumboots to be placed in.
- (g) Note: these three plastic bags may require stones or similar to weigh them down while you are on the farm.
- (h) A roll of spare plastic bags should be left at the disinfection point on the clean side.

- (i) A roll of duct tape or similar should be left at the disinfection point on the clean side.

Summary

Dirty side: gumboots, bags containing samples & paper records, mobile phones in bags etc cleaned and disinfected

Clean side: second disinfection of above; C&D of hands, arms, glasses etc

SUGGESTED PROCEDURE FOR EXITING A PREMISES

N.B

Facilities on each premises will vary, and it may be necessary to adapt the guidelines below to the specific circumstances on the farm being inspected.

It is important to minimise the number of potentially infected items taken off the farm.

Therefore discard what you can in the rubbish bag on the dirty side of the disinfection point. Sharps should be placed in a sharps container, which should itself be placed in the rubbish bag.

Take advantage of any hoses/cleaning equipment on the farm to remove as much visible dirt as possible, thus decreasing the potential viral load at the disinfection point.

- At the dirty side of the disinfection point, clean and disinfect any sample containers/bags and equipment and place in the sample/equipment bag on clean side.
- Clean and disinfect ziplock bag containing mobile phone, place on clean side on plastic mat.
- An area for removing PPE should be identified. Ideally, this area will be away from the area that has recently been depopulated and/or decontaminated. All personnel should use this area to remove their PPE.
- Disinfect the outer gloves and if wipes are used, they should be disposed off in the infectious waste bag.
- Remove overboots by holding the top and rolling it off of your foot and place in rubbish bag.
- Disinfect the boots by dipping them in disinfectant then scrub off the dirt from the boots (including soles of boots – all dirt must be removed from the cleats).
- Re-disinfect the cleaned boots
- Clean and disinfect the waterproof suit, taking care to include the shoulders, back and hood as well as front, arms and legs.

- Remove waterproof suit, taking care not to have any part of the outside touch the Tyvek suit. Place it in the boot/waterproof suit bag on clean side.
- Untape and remove the outer glove taking care not to touch the inner gloves while doing this. Place outer gloves in rubbish bag.
- Step onto clean side (onto plastic sheet if applicable).
- Clean and disinfect boots a second time, using bucket on clean side.
- To remove Tyvek suit, unzip and roll it down until it is inside out and place in rubbish bag on dirty side.
- Remove gumboots and place in boot/waterproof suit bag on clean side.
- Remove inner gloves, taking care not to touch bare hands while doing this. Place inner gloves in rubbish bag.
- Clean and disinfect hands and wrists. • Discard boot covers and outer gloves in bag on dirty side; waterproof overalls in bag on clean side
- Clean and disinfect items in to be removed, e.g. mobile phones in ziplock bags, samples, etc, and place in bag in clean area and all the paper work should be placed in plastic folders (one per sheet) and taped up, and disinfected on exit.
- If you wear glasses, these must be submerged in disinfectant when leaving.
- Close the bags by tying the corners of the top of the bag together and seal the two plastic bags on the clean side (containing samples/equipment and boots/waterproof suit) using duct tape.
- Place each of the plastic bags in a second plastic bag (“double-bagging”) and seal these two second bags with duct tape.
- Clean and disinfect hands and wrists again, paying particular attention to fingernails.
- Wipe face with disinfectant wipes, discard in rubbish bag on dirty side.
- Open ziplock bag containing mobile phone, discard bag into rubbish.
- Put on shoes.
- Clean and disinfect outside of bucket on clean side. Pour remainder of disinfectant on plastic mat and place mat in rubbish bag.
- Place the two double-bagged plastic bags in the “dirty” area of your car (e.g. the boot).
- Arrange to hand over samples, equipment, PPE etc for processing at a designated location.

Vehicle biosecurity: Cleaning and disinfecting vehicles

- Apply principles of cleaning and disinfection
- Clean exterior using power-washer or hose and disposable sponge, remove all visible dirt

- NB: wheel arches and tyres
- Spray with disinfectant over exterior
- Interior: dispose of all rubbish, clean all dirt
- Wipe steering wheel, gearstick, pedals, handbrake, footwell, etc with cloth dipped in disinfectant
- Assess risk in rest of vehicle and act accordingly

SUGGESTED INSTRUCTIONS AFTER LEAVING THE INVESTIGATION SITE

Disposing off PPE

- The plastic bags with infectious materials should be sealed and disposed of properly. Follow the instructions of the local officials or person supervising the work on where to place infectious waste bags when they are full.
- Disposal methods (such as burning or burial) may differ by situation or location. Local officials or those supervising the work will likely decide on how best to dispose of used PPE and other items that have come in contact with the virus.

Persons involved in the field investigations

If you do not own susceptible animals, return home directly, change clothes and wash hair and shower.

If you do own susceptible animals, proceed to a suitable lodgings, change clothes, wash hair and shower.

Submerge all personal clothes worn on farms in appropriate strength of disinfectant for 30 minutes in bathtub or bucket before cleaning them. A wash at a hot wash cycle (60 degrees at least) is recommended.

Biosecurity Protocol

Between households:

Wash and scrub boots with FAM. Wipe outside of sampling boxes with FAM.

Wash hands with soap and water.

Change into clean PPE

REFERENCES

Suggested guidelines on use of personal protective equipment and disinfection points when entering/exiting a suspected PPR-infected premises. European Commission for the control of Foot and Mouth disease EU-PPR

Participant Manual, East Africa biosecurity, Surveillance and Outbreak response Training of Trainers BIOSECURITY PRINCIPLES, PROCEDURES and PLANNING, 2008 USAID, STOP AI

Department of Agriculture, Fisheries and Food, State Veterinary Services, Ireland. NDCC April 2009. Protocol for putting on and taking off protective clothing (PPE).

Annex

Department of Agriculture, Fisheries and Food, State Veterinary Services, Ukraine. NDCC April 2009. Protocol for putting on and taking off protective clothing (PPE).

SECTION TWO: COLLECTION, PRESERVATION AND TRANSPORTATION OF SPECIMENS FOR PPR CONFIRMATION



INTRODUCTION

During PPR outbreaks, animals in the infected herd or flock may exhibit different clinical signs and may be in different stages of the disease. It is therefore important to know the stages of the clinical cases and their respective implications. It is also advisable that the age of the infected animal is verified by ageing by dentition during the investigations.

EPIDEMIOLOGY

Our knowledge of the epidemiology of PPR is fragmentary, but some assumptions can be made from the information available for rinderpest.

Incubation period

The incubation period is usually 4–6 days but may range between 3 and 10 days.

The OIE *Terrestrial Animal Health Code* gives a maximum incubation period, for regulatory purposes, of 21 days.

Persistence of agent

General properties

PPR virus is sensitive to a wide range of disinfectants due to its large size, lipid-containing virus envelope and sensitivity to both acid and alkali conditions. In general, the alkalis (sodium carbonate, sodium hydroxide), and the halogens (chloride) are suitable for disinfecting buildings, wooden structures, concrete surfaces, equipment and vehicles. For personal disinfection, citric acid, alcohols and iodophors are suitable

Environment

All members of the *Paramyxoviridae* family are very heat sensitive. Information for PPR virus is not available, but it is assumed that the survival characteristics (eg pH, temperature) of PPR virus are similar to those of rinderpest virus. These are as follows:

- a half-life of 5 minutes in cattle blood, spleen or lymph node at 56°C;
- survival in culture for at least 4 months at –20°C, 8 weeks at 4°C, 1 week at 20–25°C and >2.6 days at 37°C;
- rapid inactivation at temperatures above 70°C (but there is no confirmation that rinderpest virus is destroyed by pasteurisation in milk);
- greatest stability (at 4°C) at a pH of 7.2–7.9, with a half life of 3.7 days; the virus was rapidly inactivated below pH 4.0 or above pH 11.0 (Rossiter 2005); and
- rapid inactivation by ultraviolet light and desiccation within 4 days.

Live animals

Virus is present in all secretions and excretions from infected animals for approximately 10 days after the onset of fever. Animals that have been infected with PPR either die or acquire firm immunity. There appears to be no chronic carrier state.

Animal products and byproducts

Information is not available for PPR virus but it is assumed that, like rinderpest virus, it would be rapidly inactivated by the putrefaction in the carcass of an animal dying from PPR or by a pH of 5.5 in hung meat. Rinderpest virus is reported to remain infectious in salted or frozen meat for several months and may also persist for some time in refrigerated meat (NZMAF 1991ab). In the case of PPR, such persistence would not be important in spreading the disease, because the cycle back to sheep or goats is unlikely to be completed; pigs are not susceptible to infection.

Rinderpest virus can be present in milk from 1–2 days before clinical signs develop and for as long as 45 days after recovery. Goat or sheep milk may be similarly infected with PPR virus.

Modes of transmission

Live animals

Infection spreads to new areas by the movement of infected animals. Transmission between animals is usually by direct contact. Infected animals shed virus in expired air, in all secretions and excretions (including semen and urine) at the onset of the fever, and in the faeces at the onset of diarrhoea. Most infection is through short-range aerosol spread from sneezing and coughing. Infection is primarily acquired via the respiratory system.

At night under cool conditions, infection can be spread via aerosols over a distance of about 10 metres.

Animal products and byproducts

PPR virus may be present in the milk of infected animals. Feeding this milk to kids or lambs may therefore spread the infection.

Equipment and personnel

The virus survives poorly outside the host, making indirect transmission of virus by fomites most unlikely.

Vectors

Insects are not known to spread PPR.

Semen and embryos

The virus is present in semen and embryos and is likely to be transmitted in this manner. Due to insufficient information on the likely transmission of PPR virus, the International Embryo Transfer Society has been unable to make a recommendation regarding the safety of in vivo derived embryos.

IMMUNITY

Innate and passive immunity

Breeds of goats show varying degrees of resistance to PPR (see Section on susceptible animals). Maternal immunity provides protection for 3–4 months.

Active immunity

Infection with PPR provides lifelong immunity in recovered animals.

Vaccination

A homologous attenuated PPR virus vaccine is recommended by the OIE for use in countries following the 'OIE pathway' for rinderpest surveillance in order to avoid confusion. The vaccine is produced at the ----- in, and gives lifelong immunity against virulent PPR virus in goats.

New PPR recombinant marker vaccines are under development that will enable differentiation between infected and vaccinated animals for serosurveillance (Diallo et al 2007). However, two of these are recombinant vaccines using attenuated capripox virus.

OBJECTIVES OF THE CLINICAL INVESTIGATION

- to confirm the presence of clinical signs of PPR
- to collect suitable samples to confirm PPR infection has occurred
- to ensure biosecurity is practiced during clinical investigation exercise.

Following infection with PPRV, the virus can be detected in the different body parts, respectively these organs' affected epithelium or organs. The secretions and excretions

from the affected organs (shown in the principles of PPR diagnosis) can be the choice of specimens to be collected to confirm diagnosis of PPR.

In PPR outbreaks, it is always advisable to collect specimens from healthy and clinically sick animals and where possible, both small ruminants and cattle should be sampled. To avoid further transmission of the disease, specimen collection should start with the healthy looking animals and ending with the sick ones. Where other susceptible domesticated animals such as pigs and water buffaloes are also kept, these should be sampled last.

DIAGNOSTIC CRITERIA

PPR should be suspected when goats or sheep are affected with an acute febrile diarrhoea accompanied by erosions of the mouth lining and high morbidity and mortality. If rapid spread from animal to animal is occurring, and animals of all ages are sick and dying, then the picture is highly suggestive of PPR.

CLINICAL SIGNS

Goats

The clinical disease is acute, with a sudden onset of fever, peaking on the second or third day at 40–42°C, before slowly returning to normal. The fever usually lasts 3–5 days.

With the onset of fever, the animals suffer loss of appetite and become severely depressed. An early watery nasal discharge develops and may become profusely catarrhal, containing mucus and pus. This can lead to encrustation, blocking of the nostrils and respiratory distress. The nasal lining may become necrotic. Conjunctivitis with discharge from the eyes causes matting of the eyelids.

The mouth lining is slightly engorged, with small, red, necrotic mouth lesions appearing within 3–4 days. Small areas of necrosis usually first appear on the lining of the lower gums. In severe cases, these spread rapidly to the dental pad, hard palate, cheeks and buccal papillae and tongue (including the antero-dorsal area). The necrotic tissue sloughs, leaving irregular shallow erosions and remnant tags of necrotic epithelium. In some animals, the mouth lesions may be mild and heal within 48 hours. Such animals are likely to recover.

Most animals develop severe diarrhoea or dysentery about 2–3 days after the development of mouth lesions, resulting in rapid dehydration and loss of weight.

Secondary bacterial infections are common. Pregnant animals may abort. Death usually occurs after a course of 4–12 days.

The morbidity rate in susceptible animals is usually 60–90%. The case mortality rate may be as low as 10%, depending on the PPR strain and the host species and breed, but may range up to 90%.

Peracute cases may be seen in goats. These involve fever and sudden death, with no other signs. At postmortem examination, the only signs may be congestion of the ileocaecal valve and bronchopneumonia.

A subclinical or inapparent form is common in some regions due to the innate resistance of local breeds. The disease lasts 10–15 days with variable signs, often including respiratory distress.

Sheep

The clinical signs in sheep are the same as in goats but generally less severe. The disease may be present in goats without affecting sheep living in close proximity.

PATHOLOGY

Gross lesions

Postmortem findings in acute cases include a dehydrated carcass with faecal soiling. Erosive lesions may extend from the mouth to the reticulo–rumen junction where necrotic or haemorrhagic enteritis is usually present. Other lesions include necrotic lesions in the mouth and nose; congestion of the ileocaecal valve; characteristic linear haemorrhages of folds of the caecum, proximal colon and rectum appearing as engorged and black (zebra striping); enlarged spleen with necrotic lesions; and enlarged oedematous lymph nodes, especially the mesenteric lymph nodes.

The rumen, reticulum and omasum rarely show lesions. Primary bronchopneumonia is a common finding that is specific for the virus and important diagnostically. Pleuritis and hydrothorax may be found. prominent crusty scabs along the outer lips and severe interstitial apical pneumonia.

LABORATORY TESTS

Specimens required and preservation

Virus is present for approximately 10 days after the onset of fever and can be isolated during the acute stage of the disease when clinical signs are still apparent.

Swabs of the conjunctival sac, and from the nasal, buccal and rectal mucosae, as well as clotted blood. Whole blood (with EDTA anticoagulant), should be submitted. Lymph node or spleen biopsies should also be considered. Specimens for virus isolation are best taken from animals with a high temperature and before diarrhoea has started (for example, from the early, less obvious cases).

At postmortem, fresh samples of spleen, lymph nodes and affected sections of alimentary tract mucosa should be collected for virus isolation. Samples of tonsil, tongue, spleen, lung, lymph nodes and affected parts of the alimentary tract should be collected for histopathology. Postmortem samples should be collected only from animals slaughtered for the purpose or very fresh carcasses. Specimens collected for virological tests (including sera) should be preferably not have any preservative media. Tissue specimens for histopathology should be preserved in pathological 10% formalin saline.

Tissue specimens and swabs

PPRV is very fragile and highly susceptible to temperatures, hence the best way to preserve the collected specimens like swabs, biopsies and other tissue organs from dead animals is in liquid nitrogen. If not possible, the unpreserved tissue, blood and swab specimens should be chilled and forwarded with water ice or frozen gel packs. If delays of more than 72 hours are anticipated, specimens should be frozen and forwarded packed in dry ice.

Blood specimens and sera specimens

Blood and sera specimens should be chilled and forwarded with water ice or frozen gel packs. If delays of more than 72 hours are anticipated, sera specimens should be frozen and forwarded packed in dry ice. Blood specimens should never be frozen.

Transport of specimens

Specimens should initially be sent to the district/zonal diagnostic laboratory. From there, they will be forwarded to the Central Veterinary Laboratory (CVL) for laboratory confirmation. This should be done after CVL is notified on the submission of specimens and specimens for testing should be accompanied with the dully filled appropriate specimen submission form (Specimen submission form for diagnosis of viral diseases in appendix 1).

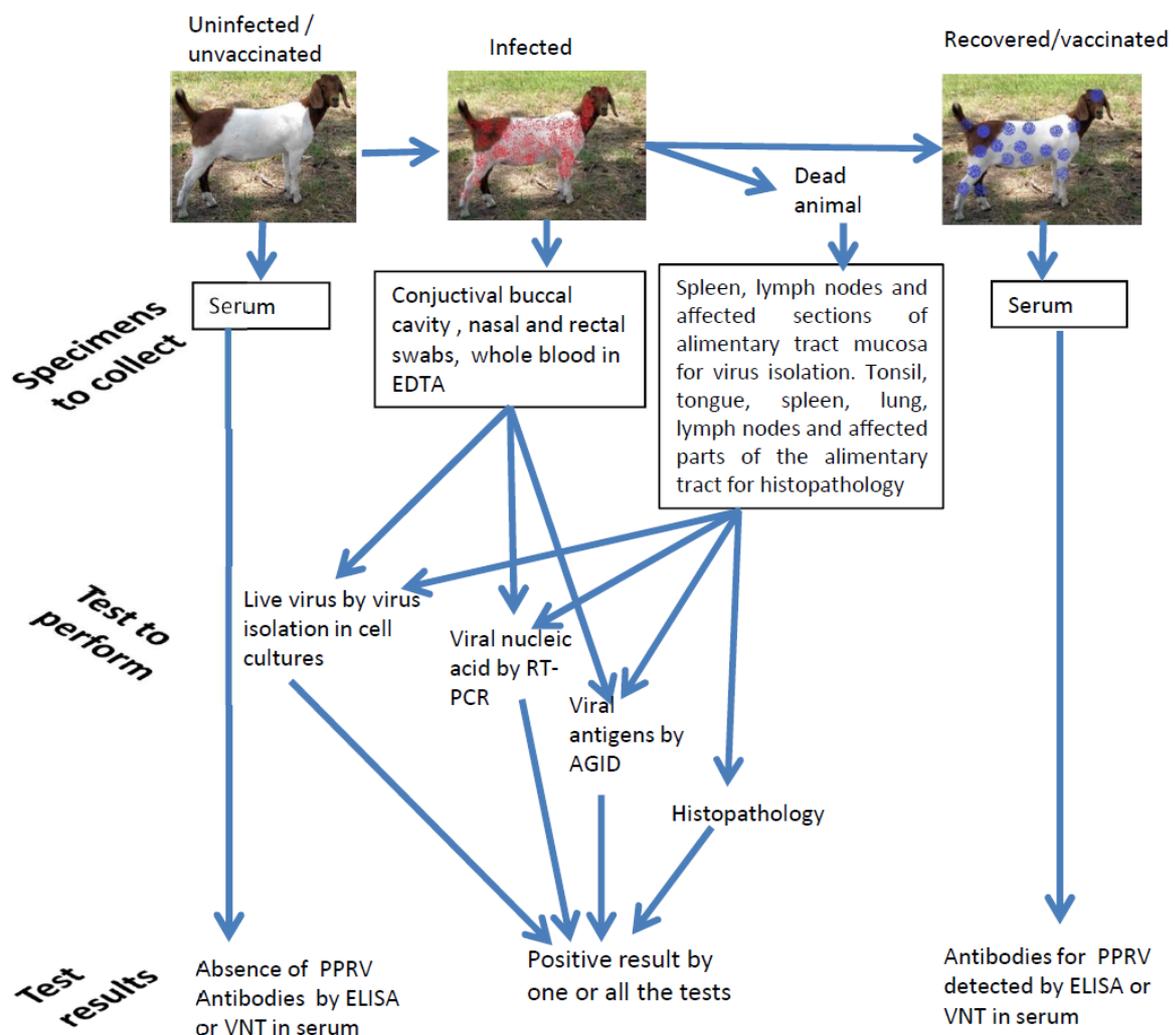
Laboratory diagnosis

Laboratory diagnosis of PPR important facts

- Confirms clinical diagnosis
- Supports but does not replace the need for accurate clinical diagnosis
- The quality of the laboratory diagnosis depends on the selection and quality of the submitted specimens
- Requires full epidemiological information on specimens submitted for rational interpretation

Principles of PPR diagnosis

Specimens to collect, test to be conducted and results



CVL tests

Some CVL laboratories are currently capable of performing a number of tests. Tests available at CVL in Dar es salaam for the laboratory confirmation of diagnosis of PPR for example are shown in Table 1. They include reverse transcriptase polymerase chain reaction (RT-PCR), antibody detection by c-ELISA and histopathology of affected oral mucosa and internal organs.

Table 1. Laboratory tests currently available at CVL for the diagnosis of PPR

Test	Specimen required	Test detects	Time taken to obtain result
RT-PCR	Fresh Tissue/whole EDTA blood or in RNA-later media	Viral genome	2 days
Histopathology	Tissue samples	Microscopic changes	3 days
c-ELISA	Serum	Antibodies for PPRV	2 days

Microscopic lesions (histopathology)

Distinct changes similar to many morbillivirus infections are seen histologically, including multinucleated giant cells, especially in the lungs, and eosinophilic intranuclear and/or intracytoplasmic inclusion bodies.

Sampling Protocol

Put on biosecurity gown and other PPE

Take all the necessary epidemiological information including history

Restrain animal (preferably standing)

Procedure for specimens collection – healthy animals

Start with clinically healthy animals

Check teeth for aging. As randomly as possible, select:

- Sheep and goats: 4 animals aged 6 months – 1 year
- 4 animals aged 1 year – 2 years
- 4 animals aged >2 years
- Cattle: 4 animals aged 6 months – 1 year
- 4 animals aged 1 – 3 years
- 4 animals aged >3years

Procedure for specimens collection – clinically sick animals

Perform Clinical examination - Check eyes, nostrils, mouth and perineal region for signs of PPR, check age. If any sign of PPR lesions, see PPR case definition protocol. Sampling live animals befitting the case definition, specimens from ~ 4 animals with obvious (and early) lesions should be sufficient to confirm a diagnosis in clinically sick animals.

PPR case definition.

PPR should be suspected when a goat and or sheep the following clinical signs are manifested: pyrexia of up to 41°C that can last for 3–5 days, depression, anorexia, oculonasal discharges that become progressively mucopurulent, erosive stomatitis that later becomes necrotic and fibrinous, diarrhoea and pneumonia with foul offensive breath and usually the signs are more severe in goats than sheep. The morbidity rate can be up to 100% with a mortality rate of up to 100% in severe cases.

Post mortem lesions include prominent crusty scabs along the outer lips, severe interstitial apical pneumonia, erosive lesions that may extend from the mouth to the reticulo–rumen junction, characteristic linear haemorrhages or zebra stripes occur in the large intestine, commonly at the caeco–colic junction, necrotic or haemorrhagic enteritis, necrotic lesions on the spleen and enlargement of lymph nodes.

Collection of diagnostic specimens

Swabs specimens collection

Using sterile cotton swabs, the conjunctival sac, nasal and buccal mucosa specimens are collected from suspected PPR-infected animals. To collect the conjunctival sac or the nasal swab, place the swab at the appropriate area and swab the respective mucosa then place the sampled end onto the cryogenic vial. For the buccal cavity specimens, force mouth open with care to avoid bites (fist of hand between incisors and premolars) and systematically check tongue, cheeks and gums for signs of PPR and using the cotton wool swab, swab the tongue, cheeks and the gums and place the end used to swab in the cryovial. The swab stick is then cut releasing the sampled end of the swab into the cryogenic vial. The vial with the specimen is then well secured with the cap, labeled, surface decontaminated and placed in a cool box/dry shipper or liquid nitrogen. If the swabs were placed in cool box at the site of specimen collection, upon reaching the laboratory, they should be preserved at -20°C or lower temperature before being dispatched to the testing laboratory.

Collection and preparation of Serum samples:-

Using the needle, needle holder and vacutainer tubes, collect blood specimen from jugular vein for serum. For serum, approximately volume of 6 ml whole blood for each sample (a minimum of 4 ml is essential). After collection the vacutainer tube should be labeled, surface decontaminated and left at room temperature for several hours or for over-night to separate the serum.

After retraction of the clot, serum can be decanted carefully into a appropriately labeled cryovial or following centrifugation at 3000gm for 10 minutes. The cryovial's cap should be well secured, and vial be surface decontaminated then stored at -20°C or lower temperature before being dispatched to the testing laboratory.

Collection of whole blood samples:

Whole blood specimens should be collected in the same manner as that for serum but the vacutainer tubes coated with EDTA as an anticoagulant are used approximately 2 mls is sufficient. Immediately after collection, the specimen container should be labeled, decontaminated and kept at 4°C until dispatched to the testing laboratory.

Collection of Tissue specimens

Tissues with lesion is the richest source of PPRV and the specimen of choice for diagnosis. At postmortem, fresh samples of spleen, lymph nodes and affected sections of alimentary tract mucosa should be collected for virus isolation. Samples of tonsil, tongue, spleen, lung, lymph nodes and affected parts of the alimentary tract should be collected for histopathology. Postmortem samples should be collected only from animals slaughtered for the purpose or very fresh carcasses. Specimens collected for virological tests (including sera) should be preferably not have any preservative media. Tissue specimens for histopathology should be preserved in pathological 10% formalin saline. At least 2x2 cm² of tissues should be collected. All the primary containers with specimens should be well cap secured, labeled and surface decontaminated. At the field site, the specimens for virological tests should be kept chilled of at -20°C or lower temperature while the histopathological specimens should be kept at room temperature before being dispatched to the testing laboratory.

Mark sampled animal to ensure no repeat sampling of the same animals.

Summary of specimens required for different laboratory tests

Test to be performed	Appropriate diagnostic specimens	Preservative
Virus isolation, PCR and/ histopathology, Antigen detection, AGID	: Whole blood (EDTA), swabs on conjunctival discharges, nasal and buccal mucosae, the buccal scrappings, lung, mesenteric and bronchial lymph nodes, spleen and intestinal mucosae, sections of the ileum and large intestine	Half of each tissue fresh chilled (not frozen) on ice within 12 hours after collection: and half in Fragments of organs collected for histopathology placed in 10% formalin saline
Serology using ELISA and virus neutralization test (VNT), AGID	6 ml clotted blood	Clot enhancer in vacutainer tubes

The basic principles of collection and dispatching of any PPRV specimen to the testing laboratory

The labeled with sufficient information for the identification primary specimen containers are surface decontaminated and if possible, further leaking is prevented by applying the parafilm at the cap junction, then absorbent material is wrapped around it and the container is securely contained in sturdy secondary container in which it fits snugly that is also surface decontaminated. The secondary container should be fluid-tight, preferably with a screw cap and a rubber washer. If such a container is not available, a tin with a tight-fitting lid which can be soldered on should be used. The secondary container is then placed in a cooling facility (Styrofoam box, cool box) into which coolant (dry ice, ice packs) are then placed. The specimen inventory and or specimen submission form should be dispatched in the same package. The package should be labelled with a description of the specimen and its origin.

Correct external package label to identify urgency, address of the target testing laboratory indicating the contents, fragile, the temperature at which it should be kept (keep cool but not frozen, except by prior arrangement, if long delay). The package should be sent to the testing laboratory as soon as possible, by most direct route with a prior notification of the warning to receiver lab. Do not package together with other samples of less urgency. Hazardous (unless inactivated – for PCR). Package and label properly as per dangerous goods standards.

Sampling equipment check list

General requirements

- Animal marking sticks
- Cryogenic, water and alcohol resistant marker pen,
- Liquid nitrogen in an appropriate container / Small dry-shipper (containing liquid nitrogen)/ Cold box containing ice or freezer packs
- Labels
- Sharps bin
- Waste disposal plastic bags
- Plastic bags for samples
- First aid kit including nail brush
- Post mortem kit
- GPS with batteries
- data recording note books, specimen submission forms, protocols, pens

Blood

- Vacutainer tube plain or with clot enhancer for serum
- Vacutainer tube with EDTA
- Needles for blood sampling
- Vacutainer needle holder
- Centrifuge
-

Conjunctival, buccal or nasal swabs

- Cotton swabs
- 2ml cryovial for each separate type of specimen
- Stainless steel artery forceps (medium straight)

Tissue specimens

- Biopsy
- Tissue forceps
- Scissors
- Specimen bottles
- Specimen bottles with 10% formalin saline

Biosecurity

- 3 buckets (stainless steel or plastic)
- Good quality water
- appropriate disinfectant

- Stiff brush
- Gloves
- Each person –biosecurity gown per farm/village and boots,

Transport Planning check list

The sender must to ensure the correct designation, packaging, labeling and documentation of all infectious substances and diagnostic specimens.

Good coordination between the sender, the carrier and the receiver (receiving laboratory), to ensure that the material is transported safely and arrives on time and in good condition.

The sender

- Makes advance arrangements with the receiver of the specimens including investigating the need for necessary permits;
- Makes advance arrangements with the carrier to ensure that:
- The shipment will be accepted for appropriate transport.
- The shipment (direct transport if possible) is undertaken by the most direct routing, avoiding arrival at weekends;
- Prepares necessary documentation including permits, dispatch and shipping documents;
- Notifies the receiver of transportation arrangements once these have been made, well in advance of expected arrival time.

The receiver

- obtains the necessary authorization(s) from national authorities for the importation of the material;
- Provides the sender with the required import permit(s), letter(s) of authorization, or other document(s) required by the national authorities;
- Arranges for the most timely and efficient collection on arrival;
- Immediately acknowledges receipt to the sender.
- Shipments should not be dispatched until:
- Advance arrangements have been made between the sender, carrier and receiver
- The receiver has confirmed with the national authorities that the material may be legally imported
- The receiver has confirmed that there will be no delay incurred in the delivery of the package to its destination.