

**ASSESSMENT OF PESTES DES PETIT
RUMINANTS (PPR)
INFECTION IN CAMELS AND THEIR ROLE IN
EPIDEMIOLOGY OF PPR**

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PRESENTATION FORMAT

- I. Background**
- II. Objectives**
- III. Materials and methods**
- IV. Work plan**
- V. Budget**



BACKGROUND

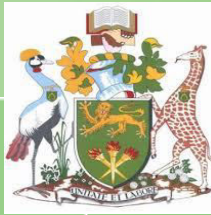
What is PPR?

- Highly contagious viral disease
- High mortality (50-80%)
- Primarily of small ruminants
- Disease resembles rinderpest



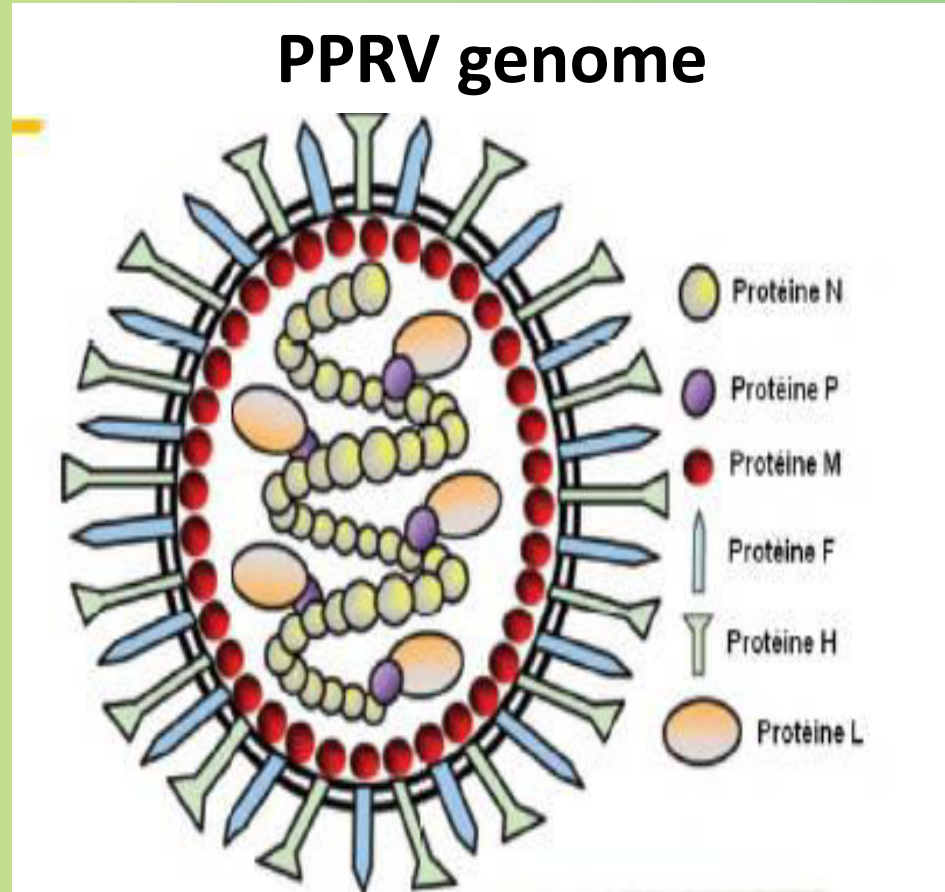
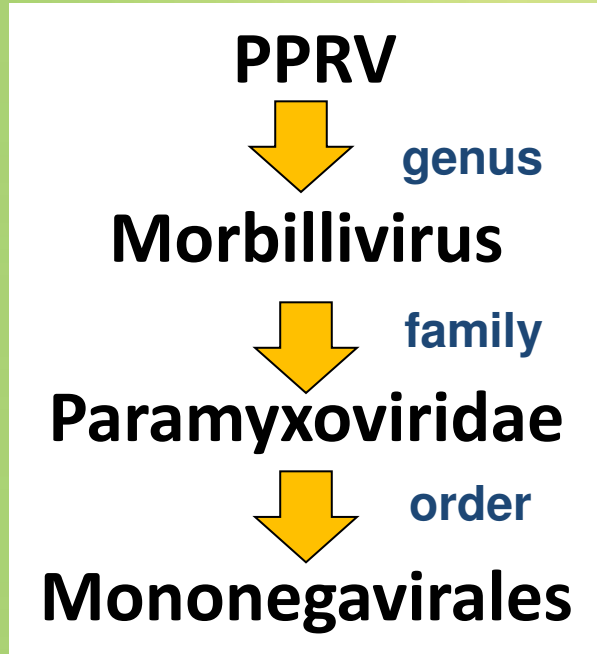
MANIFESTATION

- high fever, ocular and nasal discharge, pneumonia, severe diarrhoea



BACKGROUND

Causative agent



herring bone structure

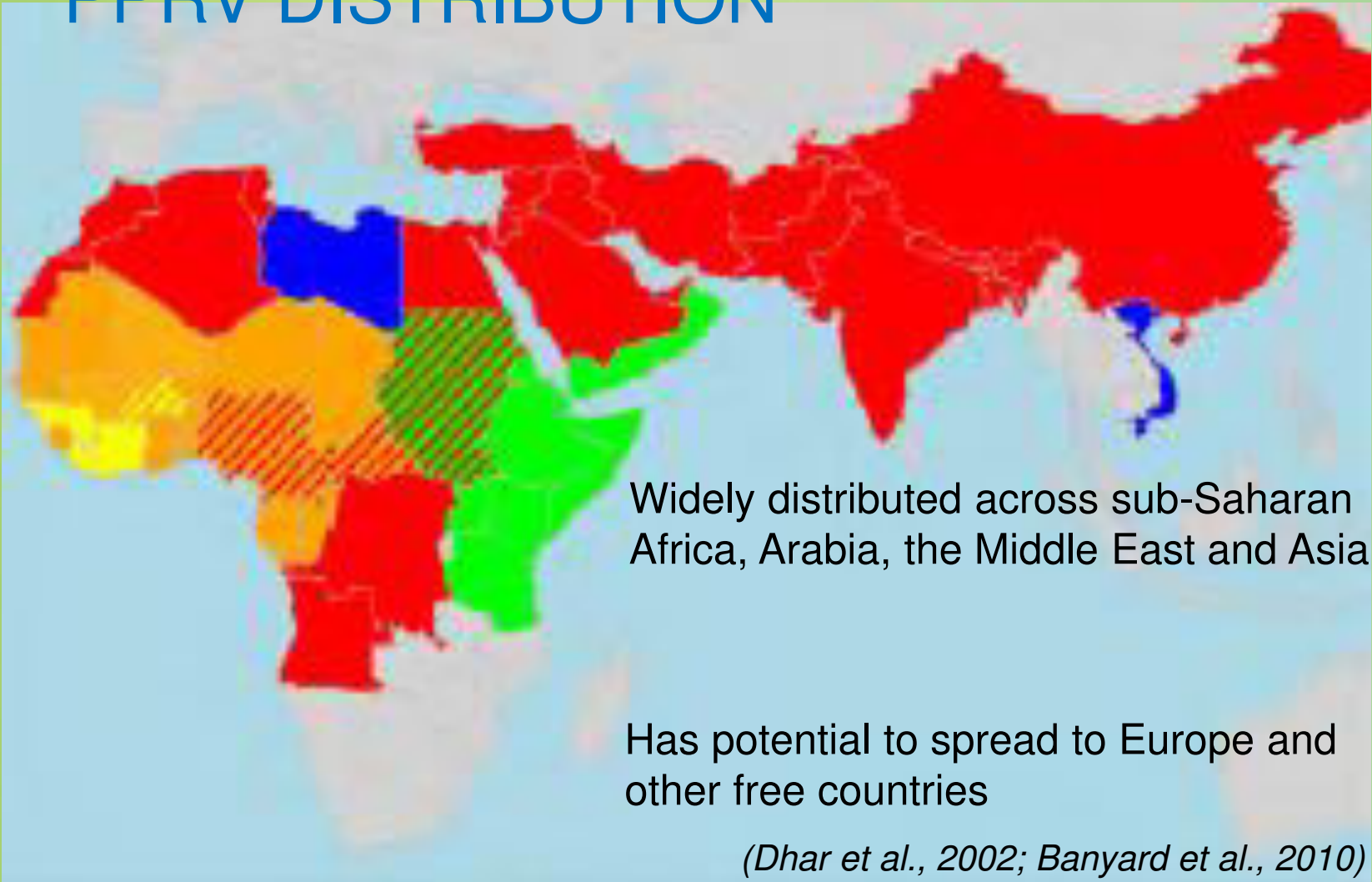
3'-N-P/C/V-M-F-H-L-5'

One serotype; 4 lineages

Lymphotropic and epitheliotropic



PPRV DISTRIBUTION



Widely distributed across sub-Saharan Africa, Arabia, the Middle East and Asia.

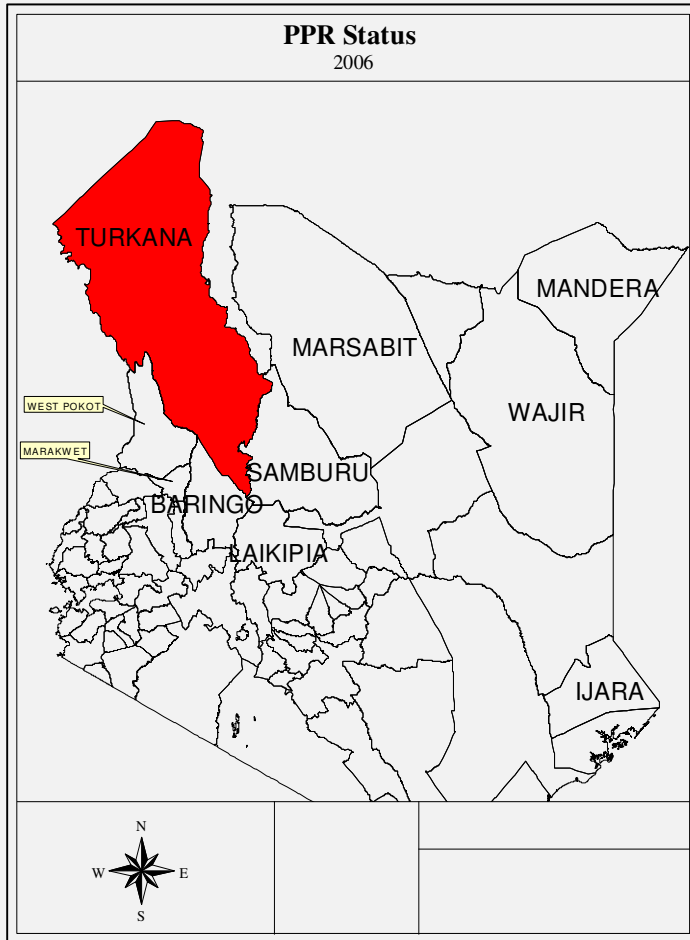
Has potential to spread to Europe and other free countries

(Dhar et al., 2002; Banyard et al., 2010)

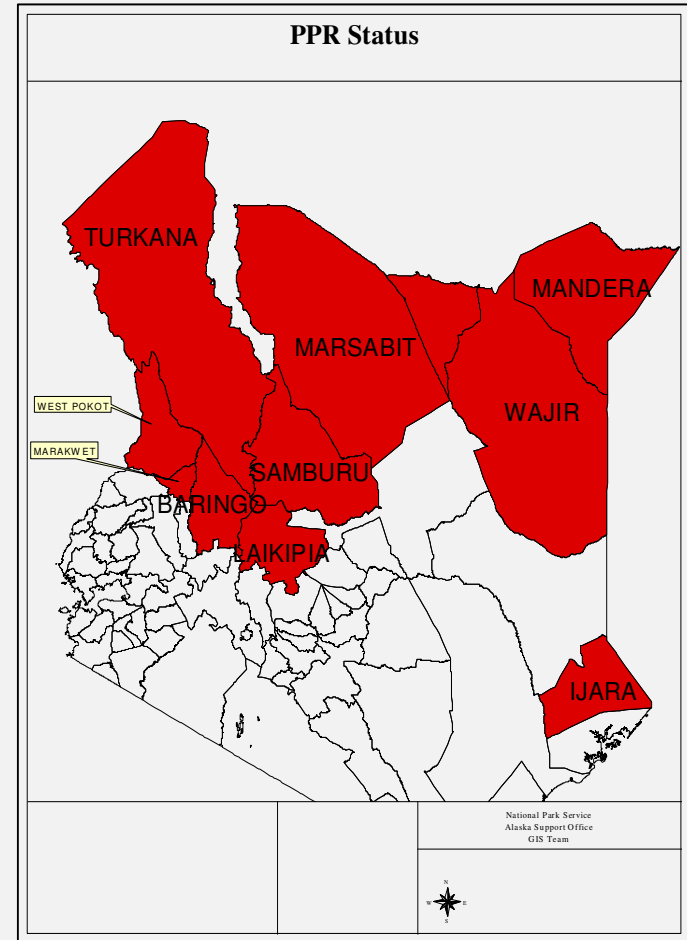
- | | | |
|------------|-------------|-------------------|
| Lineage I | Lineage III | Positive serology |
| Lineage II | Lineage IV | Free of PPR |



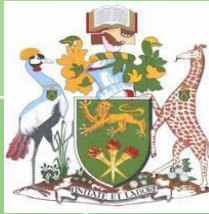
PPRV DISTRIBUTION



Kenya-status 2006



Kenya status-2008

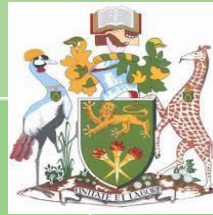


PPRV DISTRIBUTION

PPR INFECTED AREAS - KENYA



Current status (FAO,2009)



BACKGROUND

PPR CONSEQUENCES

POVERTY

FOOD INSECURITY

PESTES DES PETIT RUMINANTS

Small-ruminant production

POOR PASTORALIST & AGRO-PASTORALIST
COMMUNITIES



BACKGROUND

PPR CONSEQUENCES



PPR= high morbidity & mortality



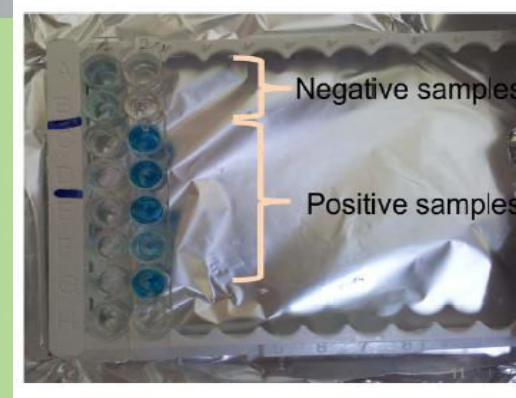
Diagnosis



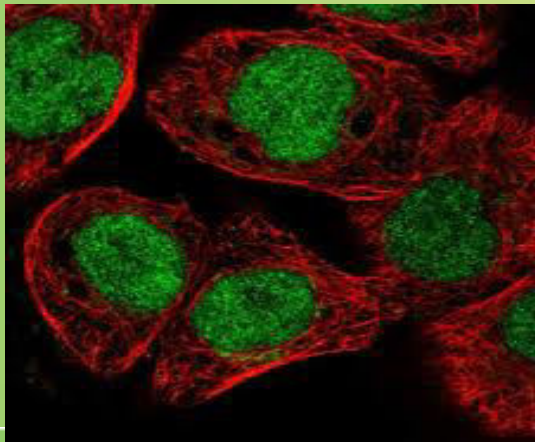
ELISA LAB



Virus isolation



i-ELISA



Immunohistochemistry



C-ELISA

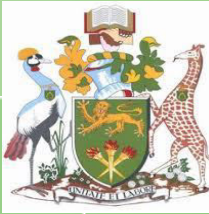


PCR



Transmission

- PPRV is highly labile
- Contact- main mode of transmission
- Discharges from eyes, nose and mouth & loose faeces, contain large amounts of virus



Control

Livestock movement control and immunization of the susceptible flocks

- Rinderpest tissue culture vaccine
- Homologous PPR vaccine is used (Diallo et al., 2007)
- PPR recombinant marker vaccines
- Thermostable vaccine developed & being piloted in various countries (Silva et al 2014)



Immunity

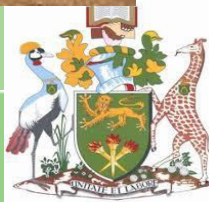
- Humoral and cell-mediated immune responses
(*Sinnathamby et al 2001*)
- Recovered & vaccinated animals develop strong, specific, long-term protective immunity
- Maternal antibodies persist 3-4 months in lambs or kids



Host range



Primarily disease of sheep and goats but has been reported in other wild and domestic species



Host range



- ❑ **Of importance disease of sheep and goats seems to be 'emerging' in camels**



Statement of problem

- PPR is similar to RINDERPEST
=complicates global eradication of rinderpest
- PPR is emerging in new areas, spread fast.
- Control is tantamount
- Proper control rely on proper knowledge of disease



Statement of problem

- Many hosts other than primary hosts susceptible; disease not understood in these hosts
- Of particular interest is the camelid spp in which PPR seems to have ‘emerged’
- Role of camels in the epidemiology of PPRV has not been adequately elaborated.



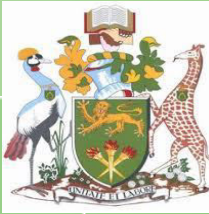
Statement of problem

- Pathogenesis & progression of PPR in camels poorly understood.
- OIE suggests camels may be considered for vaccination in control of PPR; therefore need for development of experimental model/parameters
- In Kenya, PPR is still poorly understood, more so viral circulation in-between outbreaks; other domestic animals may be involved including camels, therefore need to understand role of other animals including camels.



Statement of problem

- A PPRV isolate obtained from Turkana during 2006 outbreak & been successfully used in experimental infection of sheep and goats
- Will be used in trial in camels & needs propagation for future vaccine efficacy trials & host-virus interaction studies.
- Its also important to use an isolate from the Sudan which has reported disease in camels (lineage 4)



Objectives

Main Objective

To assess PPR infection in camels and the possible role of camels in the epidemiology of PPRV

Specific Objective

- To determine the in-vitro growth characteristics and infectivity of the Kenyan vs Sudan PPRV isolate
- To determine and compare the clinico-pathological features of Kenyan and Sudan PPRV isolates infection in the camel
- To quantify viral titres in secretions and investigate the possible role of camels in transmission of PPRV to in contact small ruminants.
- To determine the exposure level of PPR in camels in Kenya.



Materials and Methods

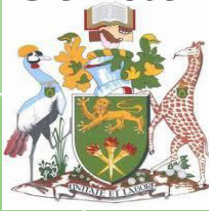
Specific objective 1

Cells and virus

- Kenyan & Sudan isolate of PPRV
- Lamb kidney, BHK and Vero cells

In vitro infection

- Grind animal tissue (kept at -70°C) from previous experiment
- Prepare 10% homogenate in MEM
- Infect cultured cells with supernatant
- 30min adsorption at 37°C , incubated and harvest at different times pi



Materials and Methods

Specific objective 1

Cells and virus

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- Prepare 10% homogenate in MEM
- Infect cultured cells with supernatant containing PPRV
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Materials and Methods

Specific objective 1

Flow cytometry

- Harvest cells
- Immunolabel with anti-N monoclonal & stain with isotype specific mouse antisera (IgG) conjugated with fluorescein isothiocyanate
- Growth of PPRV in cells by cytopathic effect



Materials and Methods

Specific objective 1

Virus infectivity assay

- Cell and supernatant fractions from PPR infected and non-infected cultures will be harvested at different times pi.
- Infected culture will be titrated to determine the viral load per ml



Materials and Methods

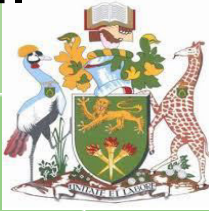
Specific objective 2

Experimental trial

- 19 Camels (8m-1yr) –acclimatize 1 month-Fed on hay, pellets, housed at isolated unit (VRC)
- 3 months

Challenge protocol 5 camels

1. **Camel 1** – different inoculation routes, 10 ml of PPRV inoculum: 2.5 ml SC, 2.5ml I.V & 5 ml intranasally
2. **Camel 2**- Orally 10 ml of PPRV inoculums
3. **Camel 3** - 10ml intranasal spray
4. **Camel 4** - infected I.V with 5ml/Intraocular 5 ml
5. **Camel 5** - uninfected control



Materials and Methods

Specific objective 2

Experimental trial

Challenge protocol 4 camels-

- Infection with different doses 3 camels
- 10^5 - 10^7 TCID₅₀/ml
- 1 control

Clinico-pathological analysis 10 camels

1. Group 1 – Kenyan Isolate (4 camels)
2. Group 2- Sudan Isolate (4 camels)
3. Group 3- Control (2 Camels)



Materials and Methods

Specific objective 2

Experimental trial

- Observe animals twice daily (rectal temp and other C/S-(RP,PR,GIT)
- Collect Oro-pharyngeal (Ph), ocular (Oc) and nasal (Ns) swabs, EDTA whole blood daily.
- Blood for seroconversion studies and blood count studies.



Materials and Methods

Specific objective 2

Experimental trial

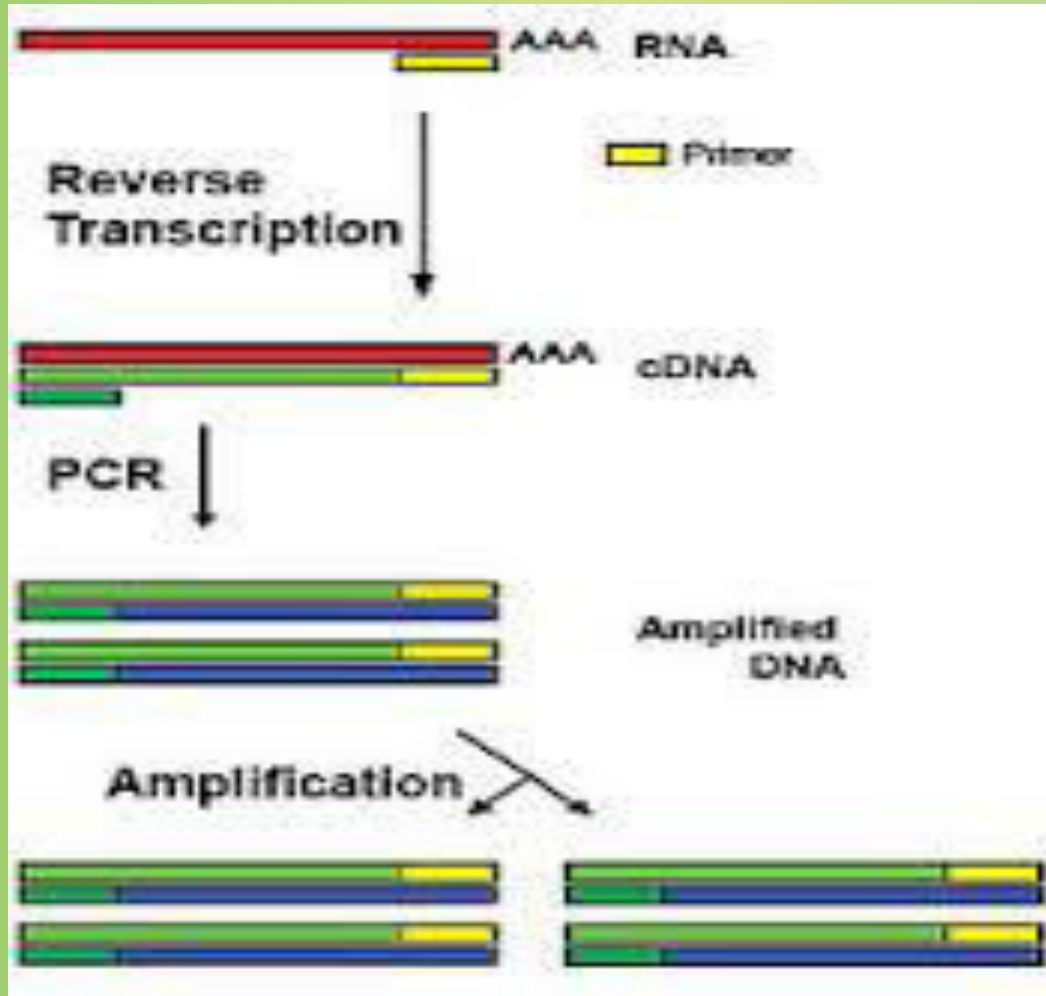
- Camels with severe disease to be euthanized and PM done. Collect lung, lymph node and spleen.
- Seek approval of the Animal welfare committee, KARI-Muguga
- PPRV tissue tropism- histology, immunohistochemistry and RT-PCR



Materials and Methods

Specific objective 3

Quantification of virus in camel discharges by RT-PCR



- Extract RNA from oronasal swabs & other discharges, using RNeasy Mini kit
- Primers specific to PPRV
- Analyse amplicon by electrophoresis
- Quantify RNA by spectrophotometry



Materials and Methods

Specific objective 3

Quantification of virus in camel discharges by RT-PCR

- ❑ One absorbance unit at 260 nm wavelength equals 40 μg RNA per ml
- ❑ Concentration of RNA will be compared in different secretions



Materials and Methods

Specific objective 3

Transmission to in-contact goats

6 small stock : 2 controls(3-goat,3-sheep: 5-6 months) housed with PPR infected camels and monitored daily for clinical signs, pathology for 1 month

Collect Oro-pharyngeal (Ph), ocular (Oc) and nasal (Ns) swabs & blood for virus detection

Post-mortem examination on infected goats.



Materials and Methods

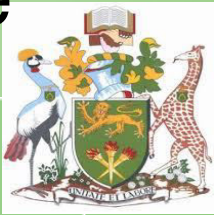
Specific objective 4

Study of exposure level of PPR in camels in Kenya

Obtain 380 camel serum DVS, Kabete and TRC, KARI. The samples have been collected over the years as part of camel disease surveillance.

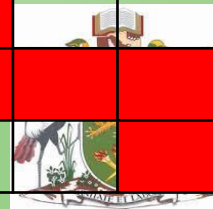
They include samples from North Eastern and Eastern Kenya among others.

Antibody levels against PPRV in camels will be determined using c-ELISA.



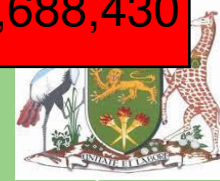
Workpan

| Activity in the yearly quarters | 2014 | | | | 2015 | | | | 2016 | | | |
|--|------|---|---|---|------|---|---|---|------|---|---|---|
| | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 |
| PPR literature review, proposal writing | ■ | ■ | □ | □ | □ | □ | □ | □ | □ | □ | □ | □ |
| Finalize the proposal | □ | ■ | □ | □ | □ | □ | □ | □ | □ | □ | □ | □ |
| Study of PPR exposure levels in camels | □ | □ | □ | □ | ■ | ■ | ■ | ■ | □ | □ | □ | □ |
| PPRV culture and propagation | □ | ■ | ■ | ■ | □ | □ | □ | □ | □ | □ | □ | □ |
| Set up challenge protocol in camel | □ | ■ | □ | □ | □ | □ | □ | □ | □ | □ | □ | □ |
| Experimental trial in camels | □ | □ | ■ | ■ | ■ | □ | □ | □ | □ | □ | □ | □ |
| Quantify PPRV RNA in camel discharges/secretions | □ | □ | ■ | ■ | ■ | ■ | □ | □ | □ | □ | □ | □ |
| Expose goats to infected camels | □ | □ | □ | ■ | ■ | □ | □ | □ | □ | □ | □ | □ |
| Sample analysis, Data entry and analysis | □ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | □ | □ |
| Thesis write up | □ | □ | □ | □ | □ | □ | □ | □ | □ | ■ | ■ | ■ |
| Thesis submission and defense | □ | □ | □ | □ | □ | □ | □ | □ | □ | □ | ■ | ■ |



Budget

| Activity items | | | |
|--|------|------------------|------------------|
| | Unit | Time (months) | Total Costs |
| PPR literature review, proposal writing | 1 | 4 | 38,600 |
| Study of PPR exposure levels in camels | 1 | 5 | 373,950 |
| PPRV culture and propagation | 1 | 5 | 524,000 |
| Set up challenge protocol in camels | 1 | 1 | 357,000 |
| Experimental trial in camels | 1 | 4 | 1,367,000 |
| Quantify PPRV RNA in camel discharges/secretions | 1000 | 5 | 767,880 |
| Expose goats to infected camels | 5 | 2 | 110,000 |
| Sample analysis, Data entry and analysis | 1 | 12 | 150,000 |
| Thesis write up | 1 | 2 | 10,000 |
| Total | | | 3,688,430 |



Thank You

