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

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I. Background
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III. Materials and methods
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V. Budget
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
Causative agent

PPRV

Morbillivirus

Paramyxoviridae

Mononegavirales

PPRV genome

herring bone structure

One serotype; 4 lineages

3’-N-P/C/V-M-F-H-L-5’

Lymphotropic and epitheliotropic
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)
Kenya-status 2006

Kenya status-2008
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

Healthy herd

PPR= high morbidity & mortality
Diagnosis

ELISA LAB

Virus isolation

Immunohistochemistry

C-ELISA

i-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
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)
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
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
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.
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 tires 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**
- 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 containing PPRV
- 30min adsorption at 37°C, incubated and harvest at different times p.i.
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
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, 2.5 ml I.V & 5 ml intra-nasally

2. Camel 2 - Orally 10 ml of PPRV inoculums

3. Camel 3 - 10 ml intranasal spray

4. Camel 4 - infected I.V with 5 ml/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
  • $10^5$-$10^7$ TCID$_{50}$/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
Quantification of virus in camel discharges by RT-PCR

Materials and Methods

Specific objective 3

- Extract RNA from oro-nasal swabs & other discharges, using RNeasy Mini kit
- Primers specific to PPRV
- Analyse amplicon by electrophoresis
- Quantify RNA by spectrophotometry
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
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.
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.
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<th>2015</th>
<th>2016</th>
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<td>Set up challenge protocol in camel</td>
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<td>Experimental trial in camels</td>
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<td>Quantify PPRV RNA in camel</td>
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<td>discharges/secretions</td>
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<td>Expose goats to infected camels</td>
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## Budget

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<th>Unit</th>
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