IMPROVEMENT OF PPR DIAGNOSIS IN KENYA THROUGH EXPERIMENTAL INFECTION AND APPLICATION OF IMMUNOHISTOCHEMICAL TEST.

INVESTIGATOR: DR. MAINA SIMON MIGWI
**INTRODUCTION**

- *Peste des Petits Ruminants* is an acute or subacute viral disease of goats and sheep characterized by fever, erosive stomatitis, conjunctivitis, gastroenteritis, and pneumonia. The name is French for "disasterous disease of small ruminants."

- Disease first described in Cote de Voire in 1942

- Disease was thought to be restricted in W. Africa but is known to exist in West, Central and East African countries

- Outside Africa, disease has been diagnosed in India, Western and Southern Asian countries.
Aetiology

- Paramyxovirus of the Morbillivirus genus

Transmission

- Natural transmission: close contact of infected animals in febrile stage with susceptible animals.
- Discharges from mouth, eyes, nose and loose feces from sick animals contain large amount of virus.
- Experimentally: Intra tracheal route, subcutaneous injection, intranasal and by contact
Host range

- PPR is primarily a disease of goats and sheep. Goats are severely affected, sheep undergo mild form.
- Cattle infected asymptotically
- Severe experimental form has been described in sheep and goats.
- PPR affect wildlife both under field condition and experimentally.
Clinical signs

- I.P. 2-4 days
  - Predominant form is acute form
  - Fever 39.5-41 degrees Celsius. Lasting 5-7 days.
  - Nasal discharges: initially clear later becomes grey.
  - Matted eyelids and congested conjunctiva.
  - Erosions in the mouth, and diarrhea appear 2-3 days form onset of fever.
  - Severely affected animals die within 5-10 days
Experimental infection: Fever (41ºc) develop 3-5 days p.i. and lasts up to 5-7 days.

- Depression and anorexia.
- Catarrhal nasal discharges, cough and respiratory distress. Oral lesions are observed infected animals.
- 6 days P.I. feces from infected animals become mucoid and blood tinged.
- Dehydration prior to death on day 9-11 P.I.
- Animals that survive, clinical signs gradually subside.
Post mortem lesions

- Carcass is emaciated. Eyes and nose have white to grey discharges.
- Lips, swollen and erosions, scabs or nodules are seen in later stages.
- Zebra stripes of congestion on the mucosal fold of posterior colon.
- Lymphnodes: generally congested and enlarged.
- Lung: dark red or purple with areas that have firm consistency.
Histopathology

- Hydropic degeneration of epithelial cells at the edge of erosion at the oral mucosa.
- Body lymphnodes: depletion of lymphoid cells, infiltration of macrophages in the germinal centers and sinuses and pyknosis and karyorrhexis of lymphoid cells.
- Spleen: Extensive hemorrhage, necrosis and hemosiderin deposits.
- Lungs: diffuse interstitial pneumonia with occasional eosinophilic inclusions in the nucleus of pneumonocytes.
**Diagnosis**

- Achieved by combination of clinical signs and epidemiology, gross and microscopic pathological lesions, virus isolation and characterization and serological tests designed to detect virus antigen or antibody.

**Diagnostic tests**

- Agar gel immuno diffusion: Simple and inexpensive. Can be performed in the laboratory or field.
- Counter immuno electrophoresis: Most rapid for antigen detection.
- Immunocapture ELISA: Monoclonal antibodies anti N protein and allow rapid identification of PPR virus.
- Sandwich Elisa: Specific, sensitive, and rapid but expensive.
• Immuno peroxidase staining: Localizes viral antigen in cells using enzyme labeled immunoglobulin. Produce permanently stained preparations examinable under light microscope.

• RT PCR: using purified viral RNA has been adopted for direct detection of virus in clinical samples.

• Experimentally infected animals: PPR virus detected by one step multiplex RT PCR in nasal and ocular swabs 7-17 days post infection and in oral swabs 7-15 days post infection.
- Virus neutralization test: Sensitive and specific but time consuming and expensive.
- Competitive ELISA: More sensitive and specific compared to virus neutralization test.

Control and prophylaxis

- No specific treatment for PPR.
- Control in non-infected countries effected through, quarantine, movement control, restriction of importation from affected countries.
- Slaughter, disposal, burning or burying of infected carcasses.
- Ring vaccination.
JUSTIFICATION

- The disease manifestation in Kenya is not clearly defined considering that the disease may have come from other regions. (Karamoja, Ethiopia). Disease is usually confused with CCPP.

- To evaluate the efficacy of the vaccine currently being used in Kenya there is need to clearly describe the disease manifestation.

- Diagnostic tests currently being used in Kenya are expensive and there is need to apply an immunohistochemical test that is cheap and easy to perform.
Hypothesis:

- The experimental form of PPR disease in Kenya does not present the classical clinical disease and pathological changes similar to those observed in naturally infected animals.
- The diagnostic tests currently being used in Kenya are expensive, time consuming and not easy to perform.

Broad objective

- To enhance the diagnosis of PPR in Kenya through experimental infection and application of immunohistochemistry.

Specific objectives

- To determine the clinical course of experimental PPR infection in sheep and goats in Kenya.
- To determine the pathological changes in experimental PPR infection in sheep and goats in Kenya.
- To apply an easy and cheap immunohistochemical test for PPR diagnosis.
Material and Methods

- Ten east African goats and ten red Maasai sheep aged between 3-18 months which will be shown to be free of PPRV antibodies by cELISA will be used.
- The animals will be divided into two groups (the experimental and the control) each group having five animals of each species.
- Each group will be housed separately. With own drinking and feeding tanks.
- Animals will be allowed time to acclimatize. Each animal will be treated with albendazole, an antihelmintic drug.
PPR infectious material will be obtained from tissue samples collected from goats and sheep that are sick and at peak of vireamia. Tissues will include spleen, mesenteric lymphnodes, intestines and lungs and stored in deep freezer.

- Tissues will be ground using a sterile pestle and mortar to prepare the inoculum. Presence of virus demonstrated by polymerase chain reaction.
- Penicillin (100 units/ml), streptomycin (100mg/ml) and fungizone (20 units/ml)
- Each animal in the experimental group will be infected subcutaneously and intranasal using 5 ml of 10% inoculum per route.
- Animals in the control group will be injected with phosphate buffered saline (Placebo).
- Each animal will be identified with a specific number on the tag tied around the limb.
Animals will be examined for clinical signs of PPR as described by Roeder and Obi (1999) and recorded.

Nasal, ocular and saliva swabs will be collected daily using cotton swabs from all animals and stored in sterile universal bottle and placed in deep freezer at -20°C.

Lymphnodes aspirates and scrappings from oral lessions will be obtained at peak of fever from all sick animals.

Blood for serum will be collected from animals that are infected at regular intervals to determine the humoral immune response against PPR infection.

Blood from the control group will also be obtained for comparison.
Post mortem examination

- Animals that die post infection or 3 per species that will be sacrificed after infection will be necropsied. Gross lesions especially in the gastro intestinal and respiratory tract will be recorded.
- Tissue samples from tongue, different parts of large and small intestines, mesenteric lymphnodes, spleen, lungs, liver and kidney will be collected in 10% formalin histopathology and immunohistochemistry.
- Tissue samples from gastrointestinal tract, lungs, mesenteric lymphnodes and spleen will be obtained for PPR virus demonstration
- Tissue samples from healthy goats will also be obtained for comparison.
Detection of PPR antigen by polymerase chain reaction

- Tissue samples will be triturated with PBS to prepare a 10% (w/v) suspension. Swabs will be directly extracted with 0.5 ml of pbs.
- Presence of PPR virus in these samples will be demonstrated using convectional reverse transcriptase polymerase chain reaction (Belamurugan et al., 2006)

Detection of PPR antibodies using competitive ELISA

- Serum samples will be processed and subjected to cELISA test as described in Biological diagnostic supplies and FAO, cElisa for PPR).
Lymphnode biopsies and scrapings from oral lesions collected from sick goats will be prepared by adding PBS in ratio of 1:10 and then centrifuged at 3000 rpm to obtain discrete monolayer cells that will be mounted on the slides and fixed.

Tissues collected for immunohistochemistry will be fixed in 10% neutral buffered formalin, embedded in paraffin and sectioned at 4-5 micro meter and then deparaffinized by washing with PBS and then mounted on poly-l-lysine coated slides.

Tissues from healthy goats will also be prepared for negative control test.

Slides for both tissues and biopsies will be placed in humidity box and flooded with a labeled with anti PPR hyper immune sera at a dilution of 1:10 in PBS Ph 7.0 containing tween 20 and 1% ovalbumin and incubated at 37oc for 2 hours.
• Slides will be rinsed thoroughly with large volume of PBS, then flooded with a pre-titrated dilution of protein A peroxidase enzyme for 1 hour at 37°C.

• After washing the substrate, orthophenyl diamine will be applied on the slides for 10 minutes at room temperature and then washed with PBS and rinsed in running tap water until all crystals have disappeared.

• Slides counter stained by immersing in Harrisâ€™ hematoxylin for 90 seconds. Then blue them in 1% ammonium hydroxide solution for 3-5 seconds, then rinse in water.

• Slides mounted wet under cover slip using Tris buffered glycerol and then observed under light microscope using x4 and x10 objective lenses.

• Positive reaction will be characterized by light to dark brown fine to coarse granules/areas in cells and tissues.
Expected output

- In this study the clinical course and pathological changes of experimental PPR infection in Kenya will be described. Diagnosis of PPR in Kenya using immunohistochemistry will also be described.

Data analysis

- The data collected will be entered Microsoft Excel to be exported to statistical packages.
- Quantitative variables will be analysed using hypothesis t-test.
- Qualitative variables will be analysed using non-parametric methods. e.g. Man Whitney U test.
- Correlation of variables will be determined using Pearson correlation coefficient.
## Work plan

<table>
<thead>
<tr>
<th>ACTIVITY</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
</tr>
</thead>
<tbody>
<tr>
<td>EXP INFXN, SMPL TAKING</td>
<td>XX</td>
<td>XX</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SAMPLE PROCESSING</td>
<td>XX</td>
<td>XX</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VIIRUS ID AND SEROLOGY</td>
<td>XX</td>
<td>XX</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Findings compilation and data analysis</td>
<td>XX</td>
<td>XX</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thesis writing</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>XX</td>
<td>XX</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thesis submission</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
</tbody>
</table>
## BUDGET

<table>
<thead>
<tr>
<th>ITEM</th>
<th>CONTINGENCIES</th>
</tr>
</thead>
<tbody>
<tr>
<td>EXPERIMENT ANIMALS</td>
<td>60000</td>
</tr>
<tr>
<td>ANIMAL INPUTS AND OTHER INPUTS</td>
<td>30000</td>
</tr>
<tr>
<td>LABORATORY CHEMICALS AND REAGENTS</td>
<td>20000</td>
</tr>
<tr>
<td>ANIMAL ATTENDANTS</td>
<td>50000</td>
</tr>
<tr>
<td>DATA ANALYSIS</td>
<td>18000</td>
</tr>
<tr>
<td>PRINTING AND PHOTOCOPYING</td>
<td>15000</td>
</tr>
<tr>
<td>THESIS BINDING</td>
<td>5500</td>
</tr>
<tr>
<td>THESIS BINDING</td>
<td>20000</td>
</tr>
<tr>
<td>MISCELLANEOUS COSS</td>
<td>5000</td>
</tr>
<tr>
<td>CONTINGENCIES</td>
<td>5000</td>
</tr>
<tr>
<td>TOTAL</td>
<td>228500</td>
</tr>
</tbody>
</table>
SUPERVISORS:
DR. GITAO. C, G
PROF. GATHUMBI, P. K.