CHARACTERISATION OF INFECTIOUS BURSAL DISEASE VIRUS AND DETERMINATION OF POSSIBLE VACCINE STRAIN(S) IN KENYA

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Definition and Aetiology

- Infectious bursal disease;
  - highly contagious and
  - immunosuppressive
  - disease of growing chickens
    (Jackwood et al., 2009)

Morbidity (10–20%) and mortality (5-50%) vary, depending on the pathogenicity IBDV strain and susceptibility of the flock; both can reach up to 100% (Lukert and Saif, 2003).

- chicken viral disease of economic importance.
Aetiology cont’d

- IBDV is a dsRNA virus
- Genus; Avibirnavirus
- Family; Birnaviridae.
- Replicates in developing B-lymphoid cells
- Results in
  - destruction of B lymphoid cells
  - Immunosuppression
  - vaccination failures
  - susceptibility to other infections and diseases
Serotypes

- Two distinct serotypes of the virus; Serotype 1 and 2.
- Neither cross-neutralize in vitro nor cross protect in vivo.
- Serotype 1 viruses: Pathogenic to chickens.
- Serotype 2 viruses: non-pathogenic to chickens.
- 2 antigenic types among the serotype 1 viruses:
  - classic
  - variant antigenic types.
- Serotype 1 viruses further divided into 6 subtypes by cross neutralization tests (Jackwood and Saif, 1987)
Pathotypes

- Serotype 1 viruses categorized into 4 groups based on pathogenicity:
  - Classical strains (standard)
  - Variants
  - Attenuated strains
  - Very virulent strains (Hypervirulent) (van den Berg, 2000)
Gumboro Disease in Kenya

- First case (1991) reported in Kenyan coast, disease spread to all parts in the country (Mbuthia and Karaba, 2000).

- Both killed and live vaccines available in the country for control of IBD

- All the vaccines are imported

- Vaccination schedules differ, recommended by:- breeders, vaccine companies, millers, agrovets)
Gumboro Disease cont’d
Gumboro Disease cont’d
Gumboro Disease cont’d

Lesions on bursa of Fabricius

Lesions on Bursa of Fabricius and Kidneys
Problem statement

- The three criteria used for the characterization of IBDV strains are:
  - antigenicity,
  - genetic relatedness
  - Pathogenicity

- Hypervirulent pathotypes are circulating in Kenya (Mutinda, 2011)

- However, the serotypes and subtypes in Kenya have not been analyzed.
Problem statement cont’d

- In Kenya, outbreaks occur in vaccinated flocks could be due to:-
  - Mismatch; Antigenicity of the vaccine virus could be different from the field virus
  - Immunosuppression caused by the virus
  - Existence of different antigenic subtypes which need to be pooled to give an effective vaccine
- Imported vaccines in Kenya have not been studied for protection against field strains.
Hypothesis & General objective

Hypothesis

- IBDV strains in Kenya are of diverse antigenicity such that various strains have to be pooled to produce an effective vaccine.

General objective

- To characterize IBDV field isolates and establish a vaccine strain(s) in Kenya.
Specific Objectives

1. Isolate and characterize IBDV from outbreaks in indigenous and commercial chickens.

2. Determine cross neutralization abilities among the isolates to identify a vaccine strain(s) (one that cross-neutralises strongly with others)

3. Do a comparative evaluation of the vaccine strain(s) against available vaccines.

4. Determine the effect of Vitamin A on the immune response to the vaccine strain(s).
Expt 1: Isolation and characterization of IBDV

Sample collection

- Collection of samples will be from various places all over Kenya, as outbreaks are reported.
- n=384, (Martin et al, 1987)
- CVL, RVILs will be involved, on DVSô permission
- Others involved will include: UoN Poultry clinic, Agrovets and private clinics.
- Bursas will be aseptically collected from outbreak cases.
Expt 1: Isolation and characterization of IBDV cont’d

Processing of the samples and viral isolation

- AGID will be done to confirm IBDV outbreaks.

- Virus isolation will be done in 9-10 day old embryonated SPF eggs and 3-7 weeks old susceptible chicken (OIE, 2008).

- Isolated virus will be titrated using Reed and Muench formula (1938).
Expt 1: Isolation and characterization of IBDV cont’d

Characterization of the virus

- Virus characterisation will be done by:
  - Pathogenicity determination in susceptible chickens
  - Antigenic reactivity in cross virus neutralisation assays

- Determination of nucleotide sequence of vVP2 encoding region.
  - RT-PCR/RFLP on VP2 gene as per OIE protocol (2008)
Expt 1: Isolation and characterization of IBDV cont’d

Pathogenicity determination in susceptible chickens

- Six week old SPF chicken inoculated with $10^{4.8} \text{EID}_{50}$ and divided into 2 groups
- Group 1: Necropsied at 0, 1, 4 and 8 days post-inoculation and weighed.
- The bursa (B) and spleen (S) collected, weighed and the bursa/body and spleen/body weight (S/B) ratios calculated.
- Group 2: Observed for c/signs mortality rate determination
**Expt 1: Isolation and characterization of IBDV cont’d**

**Cross Neutralisation test**

- After the characterisation of the viruses, the different isolates will be identified.
- Antiserum will be produced against each isolate.
- The different antisera will be tested for neutralisation against each isolate.
- The strain that strongly cross-neutralises the others will be selected for devpt into a vaccine.
Expt 1: Isolation and characterization of IBDV cont’d

Characterization of the virus cont’d

➢ Restriction enzymes Bst NI (stratagem) and SspI (Roche) will be used.

➢ The primers to be used will be:

<table>
<thead>
<tr>
<th>Primer</th>
<th>Primer Design</th>
<th>Position</th>
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<tbody>
<tr>
<td>VP2 upstream</td>
<td>5’GCGATGACAAACCTGCAAGAT3’</td>
<td>93-114 bp (CU-1 Strain)</td>
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<tr>
<td>VP2 downstream</td>
<td>5’ AGGTGGGAACATGTGGAGAC 3’</td>
<td>1470-1490bp (CU i 1 Strain)</td>
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<tr>
<td>HVR upstream</td>
<td>5’ TCACCGTCTCAGCTTAC 3’</td>
<td>587-604 bp (STC Strain)</td>
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<tr>
<td>HVR downstream</td>
<td>5’ TCAGGATTTGGGATCAGC 3’</td>
<td>1212-1229 bp (STC Strain)</td>
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</table>
Expt 1: Isolation and characterization of IBDV Cont’d

RT-PCR/RFLP cont’d

- The restriction digestion fragments will be analysed on a 1.8% (w/v) agarose gel electrophoresis.

- Ethidium bromide staining will be done to make the bands visible.

- Sizes of the bands will be determined by comparing them with 100 and 50 bp size markers.
Expt 2: Amplification and inactivation of the virus

- Based on expt 1 results (characterization assays), vaccine strain(s) will be identified.

- The identified vaccine strain(s) will further be adapted and amplified in
  - chicken embryos and
  - tissue culture

- 40% formaldehyde added to viral suspension ($\text{EID}_{50}=10^{3.48}$) to make final formaldehyde concentrations of 0.2% (Habib, 2006).

- Protection tests will follow (Expt 3)
**Expt 3: comparative evaluation of the vaccine strain(s) against available vaccines**

**Protection tests**

- Comparative evaluation with 3 other vaccine strains (Hipra ï Murphy, MB - Assia and Hester - India):
  - 20 chicks (per vaccine) inoculated at 14 days of age:
    - The chicks will be screened for antibodies after 2wks and challenged using 100 EID$_{50}$
    - Monitor for c/signs disease and mortality
    - Harvest B/F after 10 days and examine for lesions (grossly and histologically).
Expt 4: Effect of Vitamin A on the immune response to the vaccine strain(s)

The effect of Vitamin A

- For each vaccine 10 chicks will be inoculated with vaccine and vitamin A at 14 days of age:
  - The chicks will be screened for antibodies after 2wks and challenged using 100 EID$_{50}$
  - Monitor for c/signs disease and mortality
  - Harvest B/F after 10 days and examine for lesions (grossly and histologically).
Data management

- Data mortality, P.M lesions, and antibody titers will be collected. Descriptive statistics will be generated.
- Chi-square will be used to analyse the difference in mortality in different challenge groups.
- Analysis of the effect of isolates, vaccines, and vitamin A on B/B S/B weight ratio and antibody titers will be done by ANOVA.
# Workplan

|               | Year 1 | | Year 2 | | Year 3 |
|---------------|--------||--------||--------||--------|
|               | 1<sup>st</sup> qtr | 2<sup>nd</sup> qtr | 3<sup>rd</sup> qtr | 4<sup>th</sup> qtr | 1<sup>st</sup> qtr | 2<sup>nd</sup> qtr | 3<sup>rd</sup> qtr | 4<sup>th</sup> qtr |
| Proposal writing | | | | | | | | |
| Sample collection | | | | | | | | |
| Virus isolation | | | | | | | | |
| Animal inoculation and cross protection tests | | | | | | | | |
| Serological and molecular characterization | | | | | | | | |
| Data analysis | | | | | | | | |
| Thesis writing and submission | | | | | | | | |
## BUDGET

<table>
<thead>
<tr>
<th>ITEM DESCRIPTION</th>
<th>TOTAL COST IN KSH.</th>
</tr>
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<tbody>
<tr>
<td>Cost of sampling and virus isolation</td>
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<tr>
<td>• Traveling cost</td>
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<tr>
<td>• Purchase of eggs (45 @Ksh 200)</td>
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<td>• Purchase of sampling implements</td>
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<td>• Formalin and media</td>
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<td>Contingency</td>
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Thanks for listening