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 ï 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)

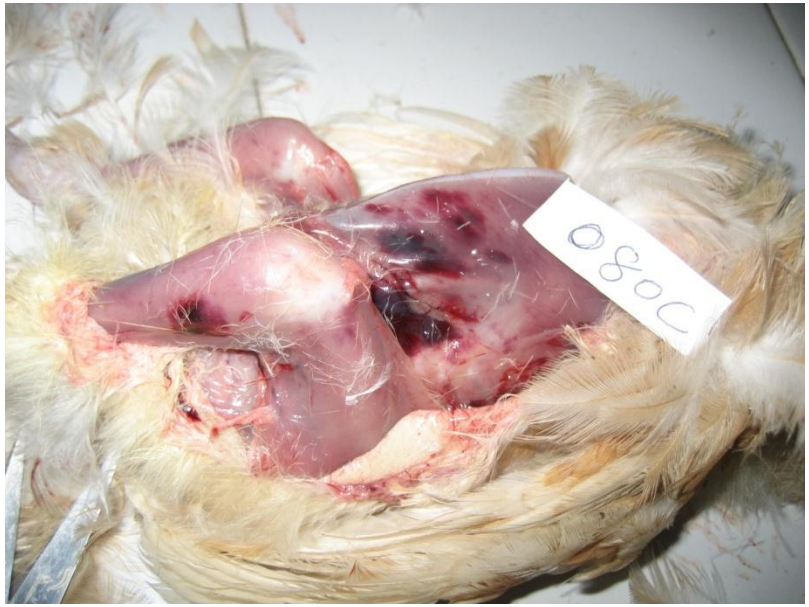
(van den Berg, 2000)

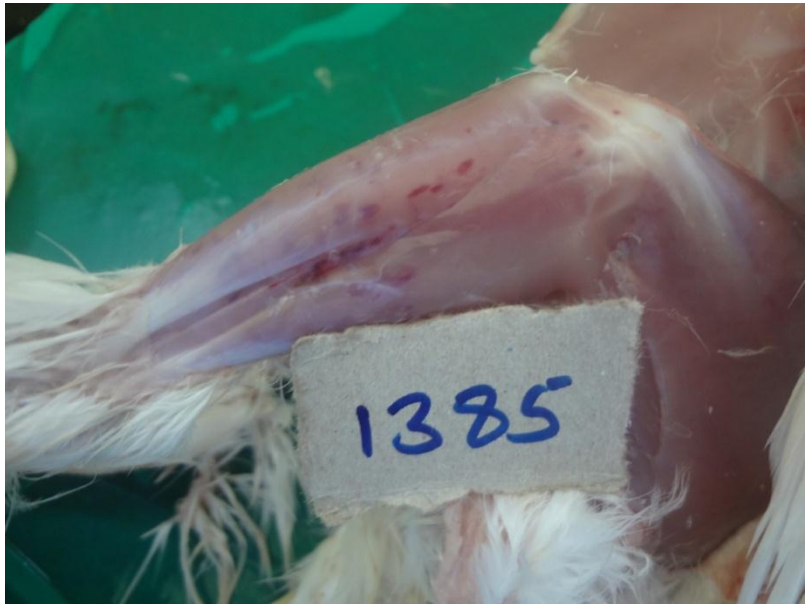
Gumboro Disease in Kenya

- 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)

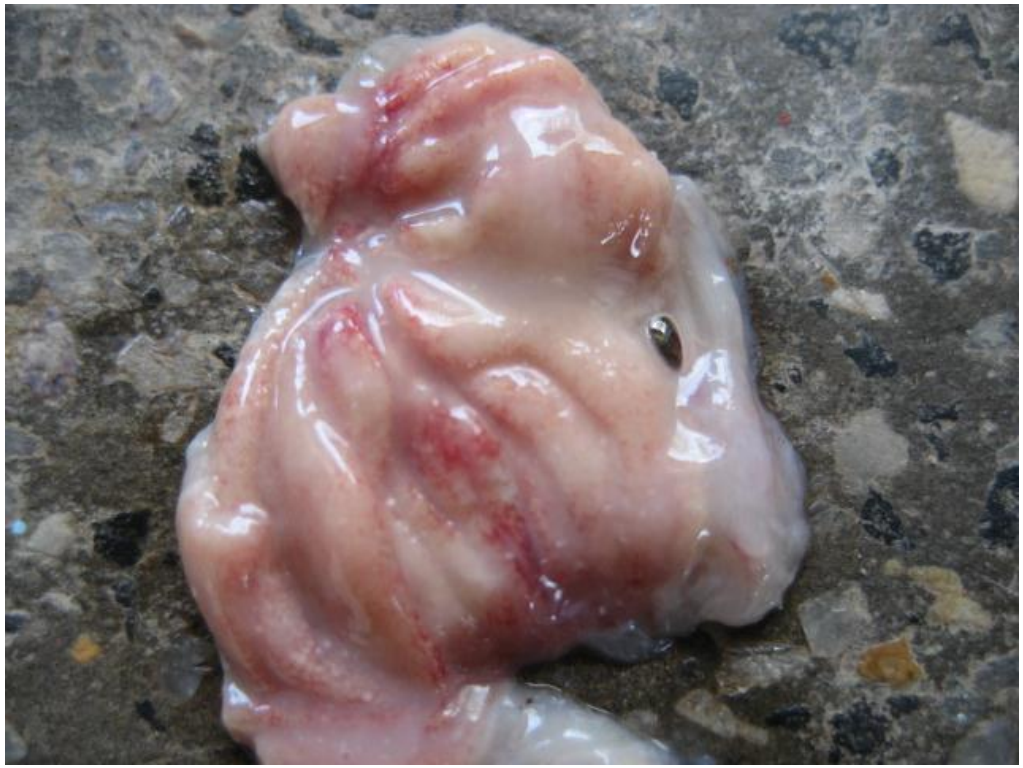
Gumboro Disease cont'd

Gumboro Disease cont'd

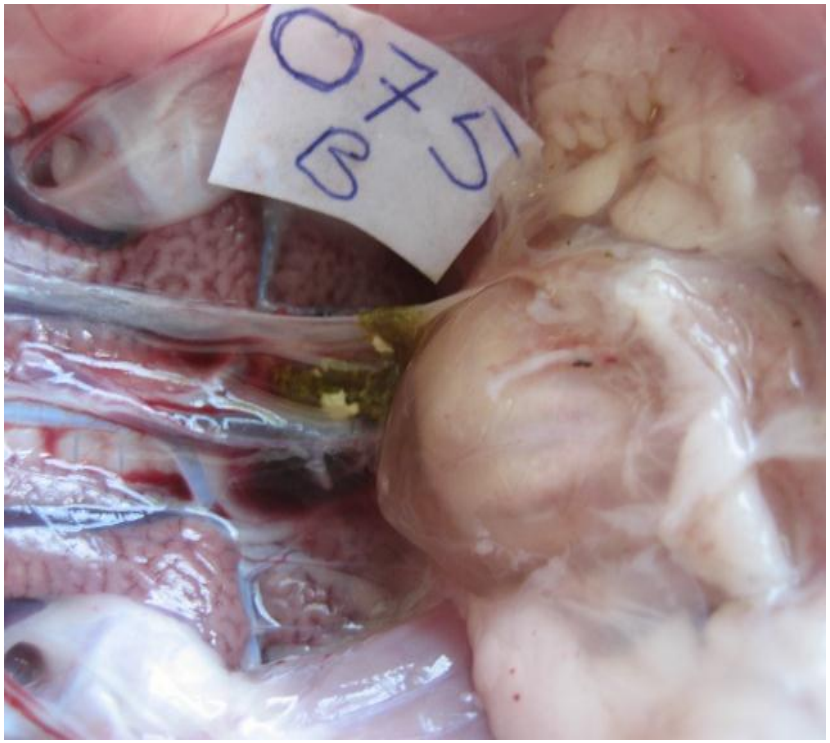




Gumboro Disease cont'd



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).

Materials and Methods

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 ï 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 $10^{4.8}$ 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:-

Primer	*Primer Design*	**Position**
VP2 upstream	5`GCGATGACAAACCTGCAAGAT3`	93-114 bp (CU-1 Strain)
VP2 downstream	5` AGGTGGGAACATGTGGAGAC 3`	1470-1490bp (CU ï 1 Strain)
HVR upstream	5` TCACCGTCCTCAGCTTAC 3`	587-604 bp (STC Strain)
HVR downstream	5` TCAGGATTTGGGATCAGC 3`	1212-1229 bp (STC Strain)

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 ($EID_{50}=10^{3.48}$) 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 generated
- chi-square will be used to analyse difference in mortality in different challenge groups.
- Analysis 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			
	1st qtr	2nd qtr	3rd qtr	4th qtr	1st qtr	2nd qtr	3rd qtr	4th qtr	1st qtr	2nd qtr	3rd qtr	4th qtr
Proposal writing												
Sample collection												
Virus isolation												
Animal inoculation and cross protection tests												
Serological and molecular characterization												
Data analysis												
Thesis writing and submission												

BUDGET

ITEM DESCRIPTION	TOTAL COST IN KSH.
Cost of sampling and virus isolation	**561,000**
• Traveling cost	180,000
• Purchase of eggs (45 @Ksh 200)	200,000
• Purchase of sampling implements	100,000
○ Universal bottles (200 @ Ksh 100)	
○ Needles (200 @ Ksh20)	81,000
○ Bleeding tubes (200 @ Ksh 60)	
○ Serum bottles (200 @ Ksh 50)	
○ Gloves (10 boxes @ Ksh 500)	
○ Agarose @ Ksh 30,000	
Purchase of laboratory reagents	**521,000**
• RT-PCR kit	221,800
• ELISA plates and kits	200,000
• Formalin and media	100,000
Writing and stationery	**20,000**
Contingency	**7,500**
GRAND TOTAL	**KSH1,109,500**

Thanks for listening