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ARABIC SUMMARY.	



## List of Abbreviation

AB : Antibody.

ADCC : Antibody dependant cellular cytotoxicity.

AEC : Amino-ethyle-carbazone.

AG : Antigen.

AGPT : Agar Gel Precipitation test.
AHSV : African horse sickness virus.

BRV : Bovine Rotavirus.

BEK : Bovine Embryo Kidney Cells.

BEK-1 : Bovine Embryo Kidney Cell Line-1.

BEL : Bovine Embryonic lung cell line.

BFS : Bovine Featal Spleen Cells.

B : Booste dose

BSA : Bovine Serum Albumin

BSE : Bovine Spongiform Encephalopathy.
BTOC : Bovine Tracheal Organ Culture.

BvDV : Bobine viral Diarea Virus

CA Caprylic Acid

C.CH : Column Chromatography

C2Neu 5, 9A : *N-9-0-Acetyl Neuraminic Acid*.

CD : Calf DIarreac

: Conter immune- electrophoresis

CMC : Carboxy Methylcellulose.

CPE : Cytopathic Effect

CTL : Cytotoxic Lymphocytes

 ${\tt DEAE} \qquad : \qquad \textit{Di-Ethyle-AminEthyle-cellulose}$ 

DIF : Direct Immunofluresence.

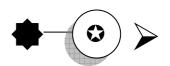
ELISA Enzyme Linked Immunosorbant Assay

EM : Electron microscopy

EPEC : Enteropathogenic Escherichia coli ERMK : Embryonic Rhesus Monky Kidney Cells

ETEC : Enterotoxigenic Escherichia coli FAT : Fluorescent antibody technique

FBK : Fatal Bovine kidney cells.





FCS : Foetal calf serum.

FITC : Fluoresent iso thiocyante.
F-th : Freezing and thawing

FVPT : Fluorecent virus precipitin test

GbpB : Glucan Binding Protein B.

GIT : Gastrointestinal tract.

Gm: Gram.

GOVS : General Organization of veterinary cervices.

gp : Glycoprotein.

HA : *Haemagglotination*.

HE : Haemagglutinin-estrase glycoprtien.

HEHA : Haemagglotination-elution-haemadsorptin assay.

HEV : Haemagglutinating encephalomyelitis virus

HI : Heamagglutination inhibition test HIAC : Human intestinal adnocarcinoma.

HRP : Horse Radish peroxidase.

HRT-18 : Human rectal adenocarcinoma tumer cell line.

: Ileum.

ICTV : International Committee for the Taxonomy of virus

ICV : Influenza virus : Immunodiffusion.

I-E-ch
 Ion Exchanch chromatography.
 IELs
 Intraepithelial lymphocytes.
 Immune electron microscopy.

: Immunofluorescence.

IFAT : Indirect fluorescent antibody technique.

Ig-Igs : Immunoglobulin(s).

 $_{\mathrm{IgY}}$ : Egg yolk immunoglobulin



## 6. Summary

The current thesis aimed to select the best immunization schedule for preparation of anti BRV yolk immunoglobulin; then we started to study the diagnostic potential of the purified immunoglobulin for detection of BRV particles in fecal samples.

Three different schedules of inoculation have been tested. These schedules were different in number and intervals of inoculation dose. It was found that the best schedule to yield high titer of BRV IgY was inoculating hens with the two doses six week apart then we gave the second booster dose after ten weeks from the first inoculation dose, followed by monthly booster for two months.

The pooled IgY of the best inoculation schedule was subjected for different purification protocols either physically by direct freezing and thawing or chemically by the use of Hydroxy-Propyl-methyl-cellulose phthalate (HPMCP) alone or in combination with precipitation of protein using ammonium sulphate. The obtained product were tested by enzyme linked immunosorbent assay (ELISA), neutralization test (NT), estimation of total protein (TP), estimation of total IgY concentration by Zinc sulphate turbidity test (Z.S.T.T.) estimation of the purity and molecular weight of IgYby Sodium-Dodecyl-sulphate-poly- Acrylamide- Gel-Electrophoresis (SDS-PAGE) and Agar Gel precipitation test (AGPT) for precipitation character. The result herby reveled that the combination of the delipidization method (freezing and thawing or HPMCP) with the partial



protein purification procedure (ammonium sulphate precipitation) results in more decrees in the titer of anti BRV antibodies measured by either (ELISA) or (NT) and also decrease the total protein and immunoglobulin concentration. This results is due to the lose of some protein and immunoglobulin which did not recovered by centrifugation after protein precipitation, but on the other hand this results lead to much elevation in the degree of purification as measured by SDS-PAGE analysis. The immuno-precipitation characteristics of IgY are different from IgG presumably because of the different structure of their hinge regions.

Finally the purified anti-BRV IgY was tested for it's potential to diagnosis of BRV infection in calves fecal matter in comparison to commercially available rotavirus check kit. It was found that the using of immune- Dot-Blotting with IgY from the different purification protocols as a primary antibody gave the same sensitivity and specificity as the commercially available rotavirus check kit.

Conclusion of the product of anti BRV IgY, 1<sup>st</sup> inoculation schedule is very suitable and immunoglobulins should be subjected to any of four mentioned purification protocols as they gave to limited difference with the same efficiency. The purified anti BRV IgY could be used in sold phase immune-dot ELISA for detection of rotavirus particles in fecal samples.