Birnaviridae

Infectious Bursal Disease
'Bi'- two segments of 'rna' genome

Group III - dsRNA viruses (double stranded RNA)
Family *Birnaviridae*
Genus

Aquabirnavirus includes viruses that infect fish, crustaceans and mollusks. Included is the virus that causes **infectious pancreatic necrosis** of salmonoid fish.

Avibirnavirus infects birds

Only one species **Infectious bursal disease** (IBD),
There are two serotypes of IBD virus:
Type 1 strains cause **IBD**; type 2 strains are not pathogenic.

Blosnavirus Blotched snakehead Fish virus

Entomobirnaviruses includes viruses that infect insects.

Infectious bursal disease (**Gumboro** disease)

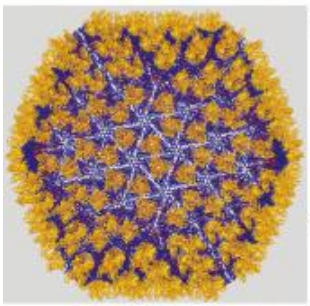
First Recognized in 1962 in an outbreak in **Gumboro**, Delaware.

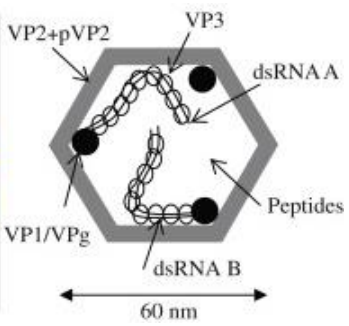
Infectious bursal disease, also known as Gumboro disease, infectious bursitis, and infectious avian nephrosis, is a highly contagious disease of young chickens and turkeys caused by infectious bursal disease virus, characterized by immunosuppression and mortality generally at 3 to 6 weeks of age.

Properties of virus

- Virions are **nonenveloped**, hexagonal in outline, **60 nm** in diameter, **icosahedral symmetry**
- **Genome** -two molecules of linear double stranded RNA, designated A and B, **6 kbp** in overall size (A, 3.2 kbp; B, 2.8 kbp)
- Structural proteins: VP2, VP3, VP4
- one nonstructural protein :

  Seg B- VP1 RNA Dependent RNA polymerase (transcriptase)
  VP5 -Segment A also encodes a 17 kDa non-structural protein VP5, membrane protein plays an important role in pathogenesis, resulting in cell disruption and virion release.
- **Cytoplasmic replication**
- Virions are relatively heat stable, Survives at 60~ for 60 minutes.
- Stable at pH 3 to pH 9
- Resistant o ether and chloroform





Occurrence

- Infectious bursal disease is a frequently occurring, worldwide infection of chickens.
- The disease strikes young chickens at 3–6 weeks of age.
- Layer type chickens are more susceptible to vvIBDV than broiler type.

Transmission

- The virus is sheded in the feces. **Faeco-oral route** and **inhalation** are the major routes of entry of the virus.
- Transmission is by direct contact and indirectly by fomites.
- The primary organ of predilection is **bursa of Fabricius** (BF) where majority of the **B cells are in actively dividing sta**ge in young chicks.

Pathogenesis

The pathogenesis of IBDV can be described as in the following flow chart.

Fecal/ Oral Route/ Inhalation

↓

Virus replicates in gut and associate with microphages and lymphoid cells

↓

Results in primary viremia through portal circulation

↓

Virus spreads to Bursal Fabricusin 11 hours of post inoculation

↓

Active replication of virus in Bursal follicles and B cells

↓

Spreads to bloodstream and causes secondary viremia

↓

Leads to viralinfection in organs like muscles, kidney causing pathognomic clinical signs and death

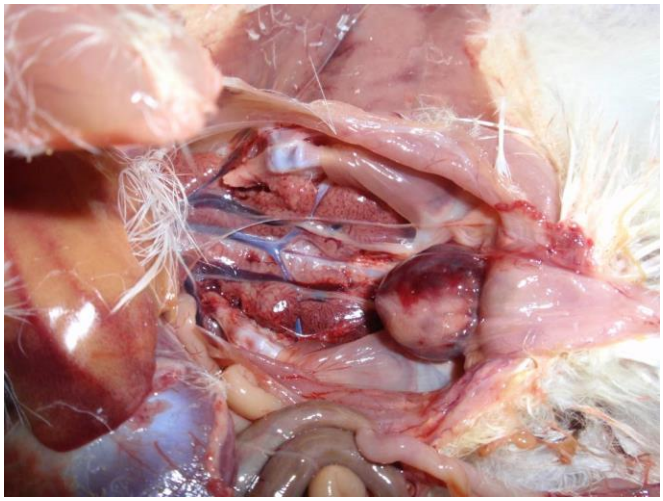
↓

The principal target cells are B lymphocytes

Faeco-oral route and inhalation are the major routes of entry of the virus
Replicates in gut-associate macrophages and lymphoid cells and results in primary viremia through portal circulation.
Following **primary viremia**, the virus reaches BF by 11-hr post inoculation and after active replication in bursal follicles and B cells, the virus enters the bloodstream to cause **secondary viremia**.
This leads to **spread of the virus in other organs like kidneys and muscle tissue** that leads to pathognomonic clinical signs and death. Following infection of the BF, degeneration and **necrosed B-cell follicles** especially IgM+ cells are detected.

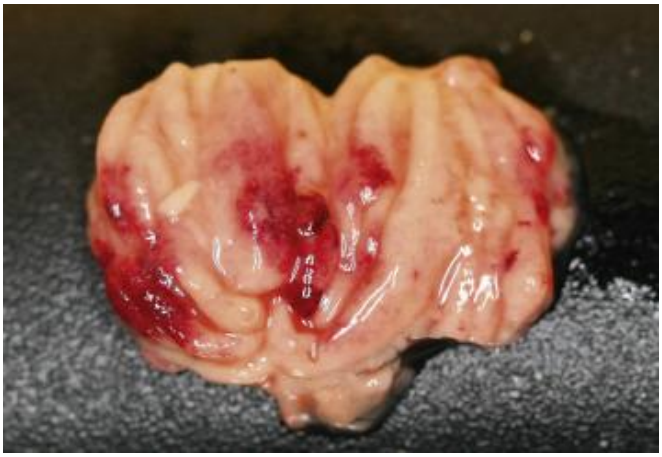
Splenomegaly, **petechial hemorrhages on the mucosa at the juncture of the proventriculus** and **diffused hemorrhages in the thigh and breast muscles**.

Lesions in the caecal tonsils, thymus, spleen and bone marrow confirm infection with vvIBDV, with the **harderian gland** being severely affected following infection in the day-old chicks. Repopulation of B cells in the BF happen in the recovered birds.



Enlarged bursa of Fabricius, infectious bursal disease virus, chicken

Atrophic bursa of Fabricius, infectious bursal disease virus, chicken



Infectious bursal disease. Swollen, edematous, and hemorrhagic cloacal bursa from an infected chicken, with superficial hemorrhage.

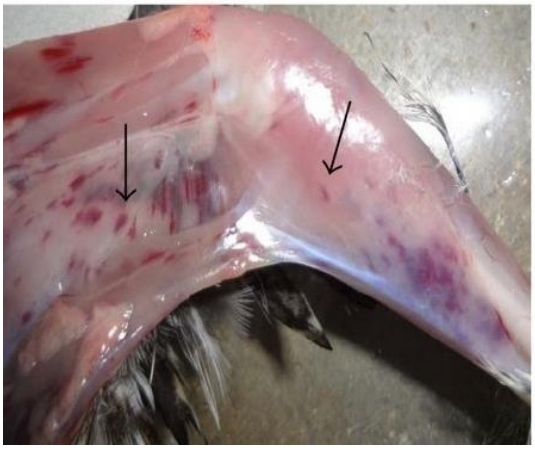


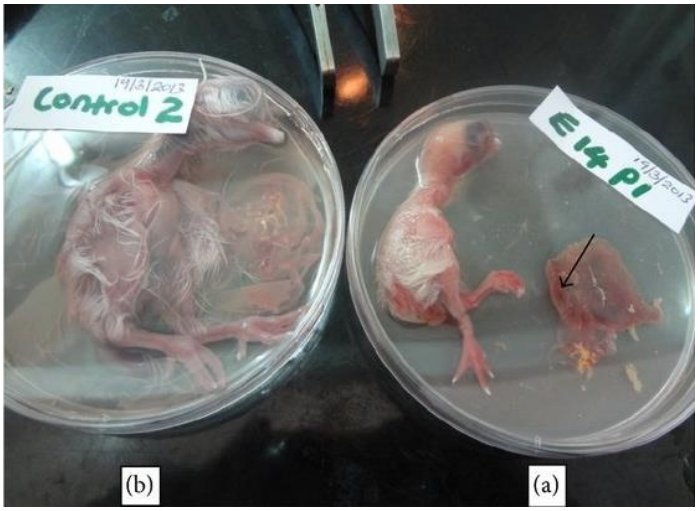
Image showing haemorrhages on the thigh and leg Marked-Haemorhages

Diagnosis of IBD

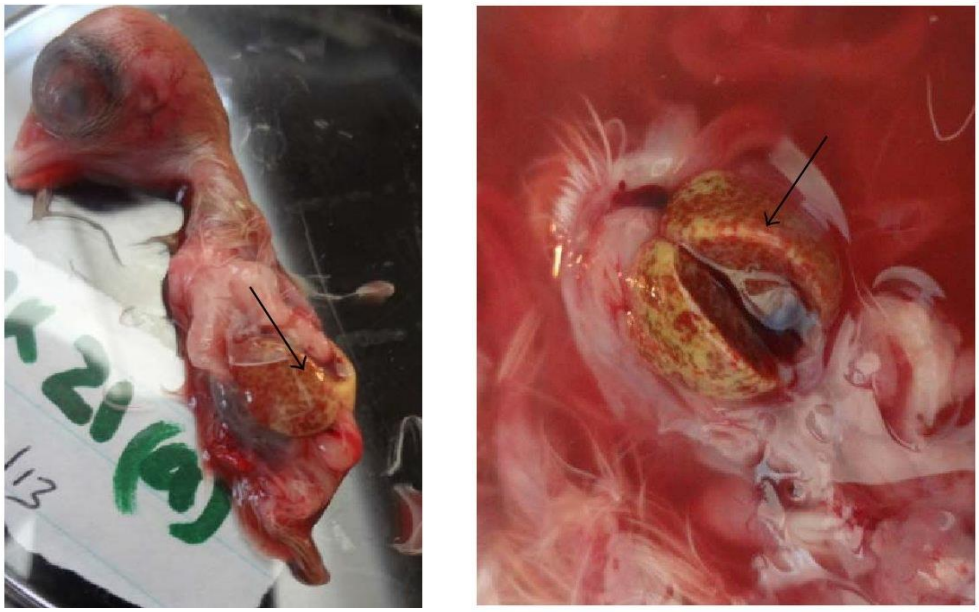
1. Virus Isolation - IBD Virus

9-11 day-old embryonated chicken eggs CAM (SPF)

Dwarfing of the embryo, subcutaneous oedema, congestion and haemorrhages. The liver is usually swollen, with patchy congestion producing a mottled effect. In later deaths, the liver may be swollen and greenish, with areas of necrosis. The spleen is enlarged and the kidneys are swollen and congested, with a mottled effect.



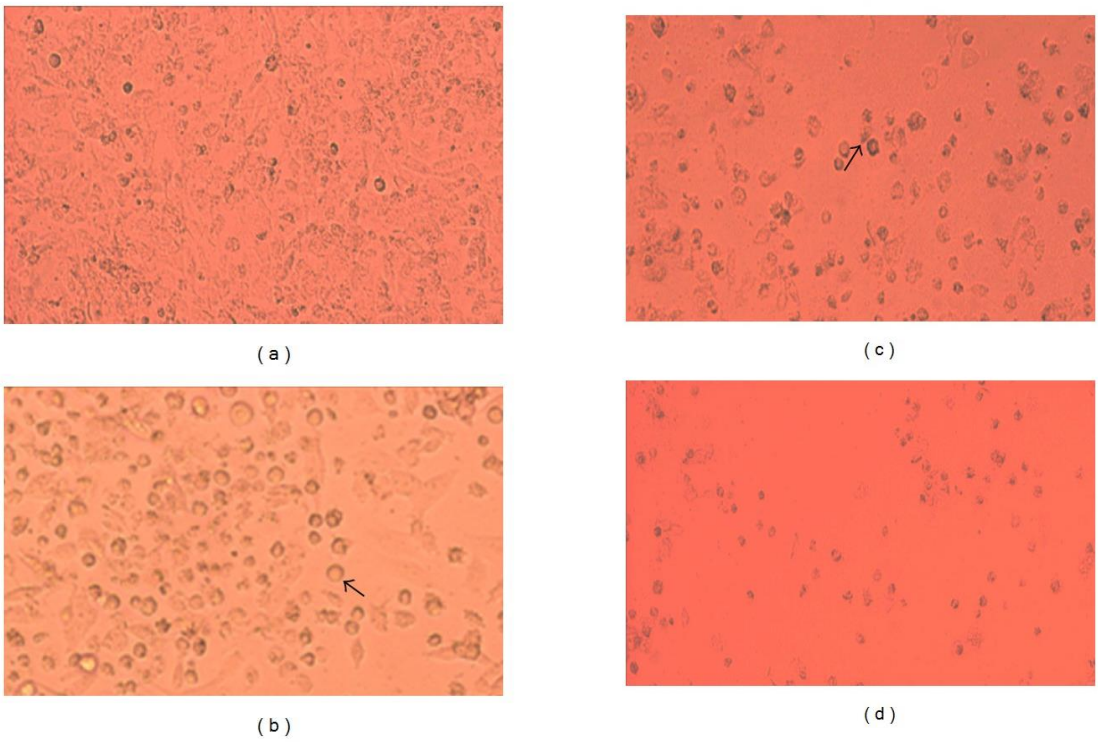
Eighteen-day-old dwarf congested IBDV infected embryo (a) with haemorrhagic chorioallantoic membrane (black arrow in (a)) as compared to the uninfected 18-day-old control (b).



Swollen liver with patchy congestion and pale yellow-green colouration producing a mottled effect (black arrows in (a and b); (b) a closer view of the liver) in an indigenous chicken embryo inoculated with Infectious bursal disease virus.

Cell cultures: Chicken Embryo Fibroblast- CPE

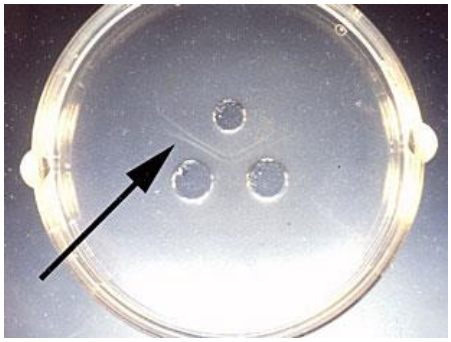
IBDV infection produced a CPE characterized by a marked granulation of cell cytoplasm, particularly around the nucleus, and further resulted in cell rounding, followed by fragmentation of cells into small particles and finally detachment from the substrate, until eventually the entire monolayer was destructed.



Cytopathic effects of infectious bursal disease virus (IBDV) in monolayers of DF-1 cells observed under an inverted microscope (100x) at various hours postinfection (h.p.i.). Monolayer at 24 h (a); granulation around nuclei (arrows) and cell rounding at 48 h (b) and 72 h (c); cell detachment from surface at 96 h.(Rekha *et al.,* 2014)

2. Detection of viral antigens

Agar gel immunodiffusion (AGID) test detects the antigen in the bursa by placing the minced bursa from susceptible chicks in the wells of the AGID plate against known positive serum. Freeze-thaw cycles of the minced tissue release the IBDV antigens from the tissue and the freeze-thaw exudate is used to fill the wells.

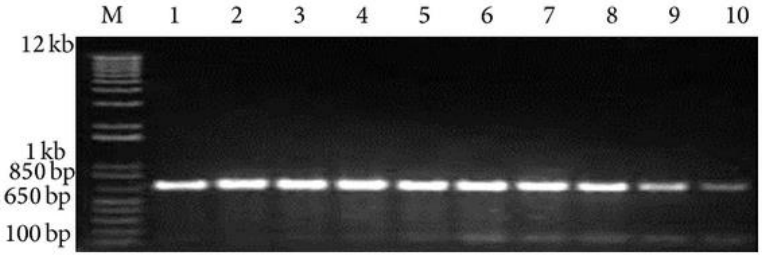


Antigen-capture-ELISA was described for the detection of serotype 1 IBDV in which the ELISA plates were coated with mouse anti-IBDV monoclonal antibodies (Mabs) or chicken anti-IBDV polyclonal

3. Virus Neutralization test: **VNT** has the highest specificity and at the same time, it correlates with protection.

4. Molecular diagnostic tests

Sequencing the hVP2 gene together with pathogenicity testing in chickens is the most accurate and accepted method for identifying the IBDV strains. The amplification of hVP2 gene by reverse transcription PCR (RT-PCR) followed by sequencing and phylogenetic analysis represents the only valuable tool for the classification of IBDV strains.



RT-PCR amplified products of 723 bp fragment of VP2 gene of field isolates from lanes 1–8 of IBDV in 1% agarose gel electrophoresis. M: molecular marker (1 KB plus DNA ladder).

Real-time RT-PCR

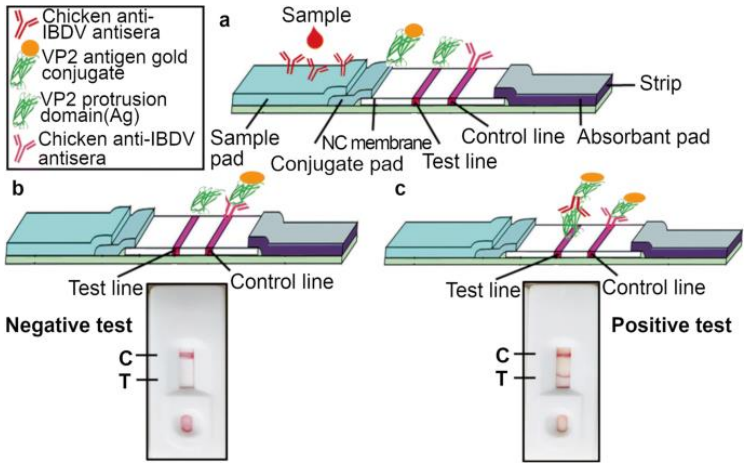
Real-time RT-PCR allows IBDV differentiation based upon time and number of samples that can be tested simultaneously. The RT-PCR SYBR green technology is robust and may serve as a useful tool with high capacity for diagnostics as well as in viral pathogenesis studies..

Loop-mediated isothermal amplification (LAMP)

LAMP compared to RT-PCR is 10 times more sensitive, rapid and specific assay and is the method of choice for field virus detection with no cross-reactivity. The test is specific as 2–3 pairs of primers are used to amplify the more conserved-targeted regions.

5. One-step strip test

One-step strip tests based on colloidal gold labeled monoclonal antibodies were developed to detect the IBDV antigen. The test was found to be highly sensitive, specific and rapid for diagnosis of the infection in the field when compared with AGID test.



IBD - Prevention and Control

- *Birnavirus* is highly stable in nature, practically impossible to eradicate the source of IBDV infection once the rearing site got infected.
- Cleaning and disinfecting the sheds before chick's arrival, **all-in all-out system** and **disinfection with formaldehyde** and **iodophors** have shown effective improvement.
- Along with the general practice of treatment with 3 types of viral disinfectants, the **combination of glutaraldehyde and QAC, phenolic compound and triple salt is the best way of disinfection treatment**.
- Maintaining proper **biosecurity measures** and chicks from a **good hatchery source** will also reduce IBDV infection.
- Despite the hygienic measures, **vaccination** is unavoidable to prevent IBDV infection in poultry farm.

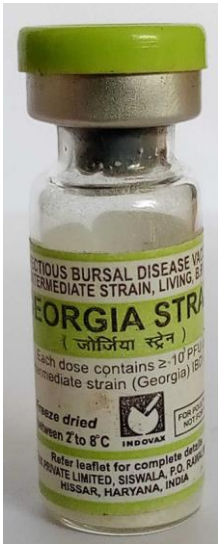
Disinfection

The infectious bursal disease was inactivated by **heating at 56 and 80 C for at least 300 and 120 minutes**, respectively.

Sodium hypochlorite 0.5% was able to inactivate the virus with 60 minutes contact time, while **Virkon** with the concentration of **1:200 and 1:400** was able to inactivate the virus for 30, 60, 120, and 300 minutes exposure. This study showed that due to its resistance, proper use disinfection process and physical treatment are required to inactivate the virus.

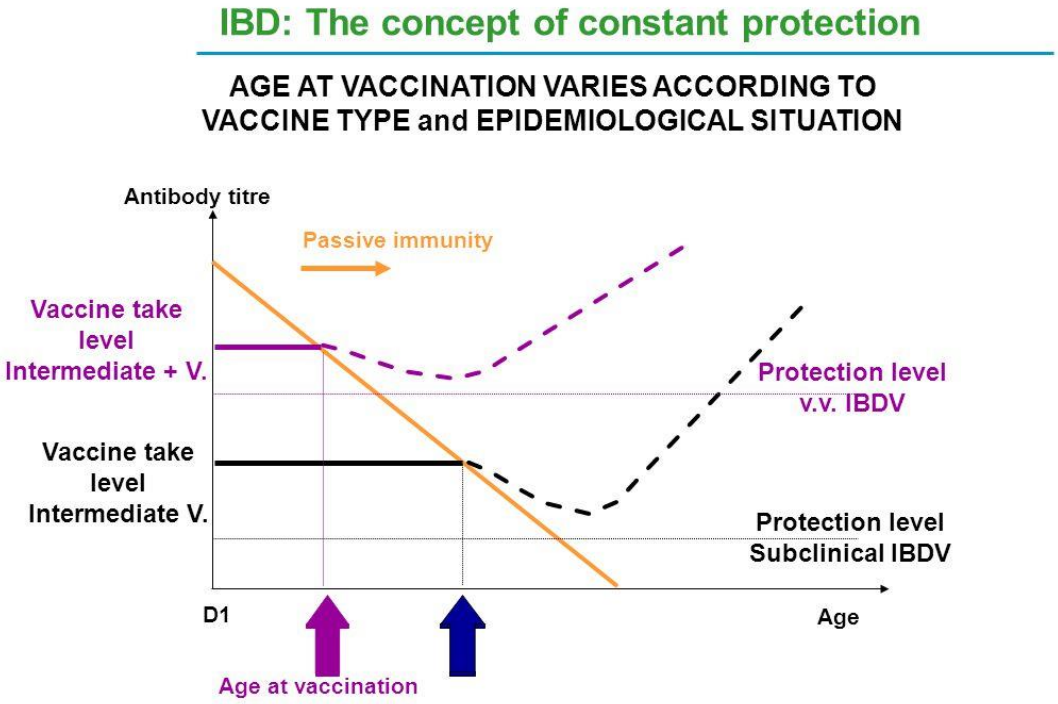
Vaccines and vaccination for the effective control of Gumboro disease

Broiler Layer/ breeders	Infectious bursal disease Vaccine (IBDV-Georgia strain) (oral/ntraocular/drinking water)	**Broilers** 2-3 weeks (Primary Dose) **Layers** 16 weeks (Booster Dose)
Commercial layers/ Breeders	Infectious Bursal disease (Killed vaccine Inj.)	40th week (Booster Dose)



Vaccines and vaccination for the effective control of Gumboro disease

- Exposure can be reduced by thorough cleaning and disinfection of poultry houses.
- Killed virus vaccines are used in breeders.
- Attenuated live virus vaccines of chicken embryo origin are administered by eye instillation or drinking water to chicks during the first 1 - 2 weeks of age, but vaccination may not be effective if passively acquired immunity is high.



Reference

Rekha K, Sivasubramanian C, Chung IM, Thiruvengadam M. Growth and replication of infectious bursal disease virus in the DF-1 cell line and chicken embryo fibroblasts. Biomed Res Int. 2014;2014:494835. doi: 10.1155/2014/494835. Epub 2014 May 14. PMID: 24949455; PMCID: PMC4053150.

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