



Review article

Infectious bursal disease: OverviewSanchita Kapoor¹, Harmanpreet Kaur¹, Aqueel Ur Rehman¹, Vijay Kumar², Amit Gupta^{1*}¹Graphic Era Deemed to be University, Dehradun, Uttarakhand, India²Graphic Era Hill University, Dehradun, Uttarakhand, India**ABSTRACT**

In recent years, many infectious veterinary diseases have caused massive losses in India's poultry population. These illnesses have a substantial impact on mortality, morbidity, and case fatality rates, resulting in large economic losses, as well as decreased productivity in the chicken industry. Infectious bursal disease (IBD) is sometimes called Gumboro disease. It is regarded as a highly infectious and epidemic disease that mainly spreads among poultry, turkeys, and wild birds through a virus. IBD is considered in the top 5 infectious diseases affecting chickens and birds which is a huge global threat and has caused many economic losses and welfare concerns. Direct impact on mortality from 3% to 30% depends upon the degree of protection of the birds and the form of the disease. The present review discusses about structure, serotypes, hosts & route and diagnosis of IBD and also collects information regarding surveillance and genomic analysis of IBD.

Keywords: IBD; viral disease; epidemic; birds.

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INTRODUCTION

Infectious bursal disease virus (IBDV; double-stranded RNA; genus Avibirnavirus; family Birnaviridae) is common in birds with age group 3-5 weeks (<17 weeks) and targets the underlying tissues of the birds specially bursa fabricus and causes suppression of immune system making them prone to infections like Salmonella, E. coli, Mycoplasma, coccidia, Marek's disease, and other poultry infections [1-3]. The first case of IBD was described in Cosgrove around Gumboro in Delaware, USA in 1957. In between 1960 and 1964 the disease started spreading to different parts of the USA and reached different parts of Europe from 1962 to 1971. It was first described by Ojo et al., in Southwestern, Nigeria between 1969 and 1971 [1-3]. The disease was seen in 3 to 7 weeks old chickens, seven outbreaks in eleven farms were recorded, mortality was 12.5% and morbidity was recorded to be 60% in that region. Since then, these strains have caused high mortalities in Europe, Asia, and Africa. Chickens inoculated with these isolates showed severe clinical diseases with high mortality rates the symptoms were seen by necrosis of bursa and thymus, anemia, hepatitis with systemic inflammation. Between 1966-1974 the disease had further spread to regions in the Middle East, Southern and Western Africa, India, and Australia. The first case was recorded by Mohantay et al., (1971) in Uttar Pradesh, India, and is considered now an epidemic in many parts of the country [4, 5]. The first recognized 'variant' strain of IBDV

was detected in 1960 and 1964 in vaccinated broiler flocks in the USA, largely by two epidemiological episodes. The second epidemiological event was the emergence of the IBDV in 1962 in the Netherlands, Belgium, and France since then the hypervirulent strains have increased their virulence and have caused a huge spike in mortality rates worldwide (up to 90%). In between 1986 and 1987 many vaccination failures occurred because until 1987 the strains of the virus were very less virulent and caused up to 2% mortality and were controlled by vaccination.

Structure of Virus

IBDV virus has been found to be a small, non-enveloped virus belonging to the family Birnaviridae, which is described by its dsRNA structure of size 65-70 nm. The resemblance of the virus is a prototype of infectious pancreatic necrosis (IPNV) of fish; other members of the family also include Drosophila X Virus (DXV), oyster, and tellina virus. IBDV genome is a bi-segmented structure consisting of two segments i.e. A and B of dsRNA virus which is encapsulated between virions consisting of a single shelled icosahedral particle with 32 capsomeres and a diameter of 60-70 nm [6-9]. The large segment consists of 3400 base pairs polyproteins of size 110 kDa and two opening frames (ORFs) which are processed by VP4 to form proteins like VP2, VP3, and VP4. The smaller segment B is of size 2800 bp and encodes for VP1 and the second reading

frame partially overlaps the polyprotein gene which encodes for VP5. Viral proteins have been identified and characterized into 5 types in IBDV as, VP1 (90Kd), VP2(40Kd), VP3(35Kd), VP4(28Kd), and VP5(21Kd) ^[8, 9]. This virus has an affinity towards immature B-lymphocyte cells of Bursa Fabricius and shows selective tropism for lymphoid tissues which caused lymphoid depletion in Bursa Fabricius in chickens and free-living birds which accounts for the clinical symptoms.

Serotypes of infectious bursal disease

IBDV strains are categorized into two categories, serotype 1 and serotype 2, based on virus neutralization tests. The first categories, i.e. serotype 1 virus, are extremely harmful to chickens and divide into divisions depending on antigenicity and pathogenicity, the categories include attenuated (at IBD) and classic (cvIBDV). ^[10, 11]. However, reports proclaim that serotype 2 is avirulent and does not generate or produce any type of harm or clinical disease in chickens and turkeys. The trends in mortality rates show huge variation in case serotype 1 viruses ranging from no mortality to 20% in classical strains and 50-90% in vvIBDV strains. Wild and free birds play a huge role in epidemiology for serotype 1 as they act as host reservoirs for the virus whereas, IBDV is said to be more prevalent in the serotype 2 category in many species of free-living birds especially turkeys, but it does not affect poultry health and cause production losses as compared to other strains of IBDV ^[10,11].

Hosts and routes of IBDV

IBDV is said to be host-specific especially chicken and turkeys acting as their natural host. Ingestion of contaminated food and water can lead to this illness especially in these birds but it has been also being in free-living wild birds like ostriches, pigeons, Baltic ducks, Herring gulls, and sparrows ^[12]. IBD infection is said to be indirect to free-living birds i.e. exposure to infected poultry dust, scavenging on infected birds, or by contaminated water resources. Furthermore, IBDV infection has also been found in dog feces two days after oral inoculation, concluding that dogs can also be potential carriers of the virus ^[13]. Other examples of birds detected with IBDV include village weavers (*Ploceus cucullatus*), speckled pigeons (*C. guinea*), and various raptors and passerines in Japan ^[14].

Diagnosis

IBDV detection can be done by various methods embryonic eggs, cell cultures, ELISA, RT PCR, serology, and many other virological assays. Phenotypic and genotypic analysis can be done by molecular detection techniques and sequence analysis ^[15]. However, in younger chicks or fetuses in the body, physical and histological bursal lesions are used to diagnose IBD. After incubation of the virus, birds show clinical signs in 3-4 days which are characterized by ruffled feathers, anorexia, depression, diarrhea, intake of feed, and

water content which further leads to death in some cases. Classical strain viruses show up to 10-20% mortality rates whereas vvIBDV shows 50-100% mortality. Studies have shown a rapid recovery rate after 3-4 days of clinical disease. As we know, the bursa is the main objective of the virus, it gets turgid, oedema, hemorrhagic and becomes atrophic within the next 7-10 days, occasionally with Marek's illness or anaemia. Other differential diagnoses and secondary IBD infections include avian coccidiosis, Newcastle disease, chicken anaemia, stunting syndrome, mycotoxicosis and infectious neuropathic bronchitis. ^[16, 17].

Medicinal plants against IBDV

In the current scenario, there are no effective treatments for IBDV yet. Prophylactic or precautionary vaccination is the only restorative method that has been affected in the poultry industry. Hence, it is very urgent to study medicinal plants and research the different anti-viral properties of different species. The utilization of medicinal plants has a prolonged history in our civilization especially as they showed very effective results in treating infectious diseases since ancient times. The increase of new viral diseases and increased incidences of anti-viral resistances have led researchers to explore new areas of study. The plants discovered efficient for the IBDV include *Moringa oleifera* Lam (MOL), *Sonajna*, *Phyllanthus emblicus* Linn (PEL), known in the common languages as Amla, *Glycyrrhiza glabra* Linn liquorice (GGL) and *Eugenia jambolana* Lam (EJL) known as Jamun ^[18].

PEL has proven to show anti-viral against Cocksackie virus B3 and acts as an immunomodulator whereas MOL is used as an anti-inflammatory and a diuretic agent. It is also used as an anti-spasmodic and anti-tumour agent, to help improve vision, inhibit gastric ulcers, improve immune health, and is also effective against simple herpes virus type 1. GGL, on the other hand, is traditionally used to treat Kaposi Sarcoma-associated herpes virus, HIV1, Vaccinia virus and herpes as expectorant, laxative, diuretic and bacterial agent, and extracts thereof. Extracts suppress in vitro proliferation of goat viruses and have antibacterial, antimicrobial, anti-inflammatory, antioxidant, hepatoprotective, antidiarrheal and hypoglycemic activities. These plants are extremely important to the treatment and cure of DNA and RNA virus infections ^[19-21].

Surveillance data

As per the survey report of IBD samples across the country with history and symptoms of the infectious bursal disease. In addition, surveillance data of various states like Haryana, Kerala, Assam, Uttar Pradesh, Maharashtra, Bihar, Himachal Pradesh, and Gujarat have been collected. Different information like the number of birds, total birds in a flock, birds affected, vaccinated and unvaccinated birds, mortality rates, and morbidity was collected from the literature (**Table 1**). The disease occurred all around the year but

the highest number of cases in winter and rainy seasons following summer, to study seasonal variations months were also divided into sub-groups based on three-season mainly summer (March-June), rainy (July-October), and winter (November -February). The diagnosis was done based on history, clinical signs, and post mortem

examinations of dead birds. Lesions and hemorrhages obtained were then further sent for PCR and histopathological studies. Various virus isolation techniques like RT-PCR, FAT, APGT, CIEP, and VNT were used to obtain the epidemiological data (Table 1) [22, 23].

Table 1: Data collected from different states across India reported with IBD outbreaks from both broiler and local chickens

Location	Year	Broiler	Local	Vaccinated	Unvaccinated	Total
Gujrat and Nagpur	2018-2020	33	21	11	21	54
Kerala	2019	11	23	23	18	41
Mizoram	2013-2014	-	-	-	-	476
Ranchi	2012	237	232		469	469
Haryana	2008-2016	1368	-	918	450	1368
Himanchal Pradesh	2004	-	-	110	336	446
Haryana	1994-2003	795		472	323	795

Genomic analysis of IBDV sequence using python (Figure1 and 2)

Bioinformatics is a branch of biological data storage, analysis and recuperation, primarily DNA and Amino acid sequences, in the biotechnology and computer sciences. For a range of applications, Bioinformatics uses computer programs [24].

Python is a language of high-level programming that promotes bioinformatics' vision in our modern environment. It stresses the readability of code and its syntax enables users to express concepts in fewer code lines, which in other programming languages would not be possible. The language provides all libraries that make bioinformatics friendly. From this section, the code and results of the IBDV sequence analysis using python will be introduced. [25].

Methodology and code

The National Center for Biotechnology Information (NCBI) is a collection of series of databases related to important tools of bioinformatics sources. It is an open-access website which provides all information consisting of, BLAST, FASTA-files, nucleotide bases, sequences, proteins etc. [26].

The genomic analysis of IBDV (accession ID: MT179723) was conducted on the sequence taken from NCBI

The code given below consists analysis of:

- IBDV gene data from NCBI.
- Analysis of DNA
- Analysis of RNA
- Length of DNA
- GC percentage of sequence
- Analysis and sorting of proteins in the sequence
- Properties of amino acids and generation of graph.
- Code: -

#IMPORT LIBRARIES

From Bio.Seq import Seq

From Bio import SeqIO

From Bio.SeqUtils import GC

Import pandas as pd

Import matplotlib.pyplot as plt

#Attatch sequence in the code to be use for analysis

```
ibd_data=SeqIO.read(genebank_ibd.gb , gb )
#GETTING THE ncbi DATA
Print (ibd_data) result_file=open (ibd_analysis_result. txt , w )
result_file. Write (Ibd_data-----\n\n )
result_file. Write (str(ibd_data))
#GETTING DNA
ibd_dna=ibd_data.seq Print (ibd_dna)
result_file. write (\n\nIBDV_DNA-----\n\n )
result_file. Write (str(ibd_dna))
#GETTING RNA
ibd_rna=ibd_dna.transcribe () Print (ibd_rna)
result_file. Write (\n\nIBDV_DNA-----\n\n )
result_file. Write (str(ibd_rna))
#LENGTH OF DNA
length_dna=len(ibd_dna) Print (length_dna)
result_file. write (\n\nIBD DNA lenth-----\n\n )
result_file. Write (str(length_dna))
#GC PERCENTAGE OF SEQUENCE
gc_percentage=GC (ibd_dna) result_file. Write
(\n\nGC percentage of sequence-----\n\n ) result_file. Write
(str(gc_percentage))
#PROTEIN ANALYSIS OF IBD
aa_ibd=str (ibd_rna.transcribe (). Split (*) Print (aa_ibd)
df=pd. Data Frame ({proteins: aa_ibd}) df [ length ] =df[ proteins ].
str.len () print (df) result_file. Write (\n\nprotein analysis of IBD is ---
-----\n\n ) result_file. Write (str(df))
plt. Plot (df[ length ]) plt. savefig (plot -length of amino acids )
#DATA CLEANING
#we will remove all the sequences having length <20 Clean
data=df[df[ length ]>20] Print (clean_data) result_file. write (
\n\nsequences having length <20-----\n\n ) result_file. Write
(str(clean_data)) sorted_Proteins=clean_data.sort_values(by='length',
ascending='false') print (sorted_Proteins [0:10]) result_file. write (
\n\nsorted proteins-----\n\n ) result_file. Write
(str(sorted_Proteins))
#PROPERTIES OF AMINO ACIDS POI_dict = {}
MW_list= [] from Bio.SeqUtils import ProtParam
for record in clean_data[ proteins ]: print (\n\n
protein_obj = ProtParam.ProteinAnalysis(str(record))
POI = protein_obj. count_amino_acids () print (POI)
result_file. Write (\nresult-----)
result_file. write (str (POI)) for amino acid in POI:
if amino_acid in POI_dict. keys (): POI_dict[amino_acid] +=POI
[amino_acid]
```

```

else: POI_dict[amino_acid] = POI [amino_acid]
print (POI_dict. keys (), POI_dict. values ())
plt. Show Result: -
IBDV_data-----
ID: MT179723.1
Name: MT179723
Description: Infectious bursal disease virus isolate SHG358 segment
B, complete sequence
Number of features: 5
molecule_type=RNA
/topology=linear /data_file_division=VRL
/date=10-JUN-2020 /accessions=['MT179723']
/sequence_version=1 /keywords= []
/source=Infectious bursal disease virus (Gumboro virus)
/organism=Infectious bursal disease virus
/taxonomy=
['Viruses', 'Riboviria', 'Orthornavirae', 'Birnnaviridae', 'Avibirnavirus']
/references=[Reference(title='Naturally occurring homologous recombination between novel variant infectious bursal disease virus and intermediate vaccine strain'), Reference(title='Direct Submission', ...)]
/structured_comment=OrderedDict ([('Assembly-Data', OrderedDict([('Sequencing Technology', 'Sanger dideoxy sequencing')]))])
Seq
('GGATACGATGGGTCTGACCCTCTGGGAGTCACGAATTAA
CGTGGCTACTAGGGG...CCC')
IBDV_DNA-----
GGATACGATGGGTCTGACCCTCTGGGAGTCACGAATTAA
GTGGCTACTAGGGG-----
IBDV_RNA-----
GGAUACGAUGGGUCUGACCCUCUGGGAGUCACGAAUUA
CGUGGCUACUAGGGGCGAU-----
IBD DNA lenth-----
2827
GC percentage of sequence-----
53.02440749911567
Protein analysis of IBd is -----
Proteins length
0          GYDGSDDLGVNTN      12

```

```

1          RGY      3
2 GRYPPLAATLVAPLLDDSATMSDIFNSPQARSKISAAFGIKPT
VGQ... 899
3          P      1
4          WEPLKKRTLIPDPVSPAFAACGGP      23
Sequences having length <20-----
Proteins          length
2 GRYPPLAATLVAPLLDDSATMSDIFNSPQARSKISAAFGIKP
TVGQ... 899
4          WEPLKKRTLIPDPVSPAFAACGGP      23
Sorted proteins-----
Proteins          length
4          WEPLKKRTLIPDPVSPAFAACGGP      23
2 GRYPPLAATLVAPLLDDSATMSDIFNSPQARSKISAAFGIKP
TVGQ... 899
result-----
{'A': 74, 'C': 6, 'D': 43, 'E': 60, 'F': 23, 'G': 57, 'H': 9, 'I': 40, 'K': 66, 'L': 102, 'M': 20, 'N': 45, 'P': 68, 'Q': 31, 'R': 41, 'S': 66, 'T': 46, 'V': 53, 'W': 16, 'Y': 33}
result-----
{'A': 2, 'C': 1, 'D': 1, 'E': 1, 'F': 1, 'G': 2, 'H': 0, 'I': 1, 'K': 2, 'L': 2, 'M': 0, 'N': 0, 'P': 5, 'Q': 0, 'R': 1, 'S': 1, 'T': 1, 'V': 1, 'W': 1, 'Y': 0}

```

Figure 1: represents graph of protein analysis of IBD

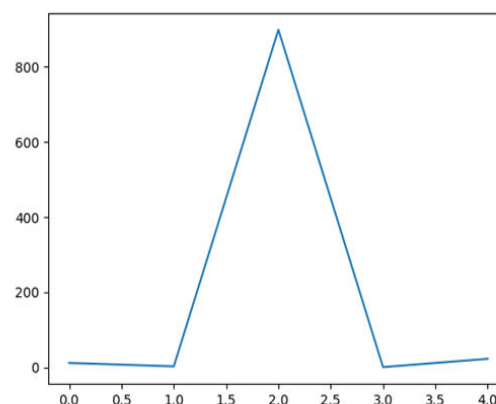
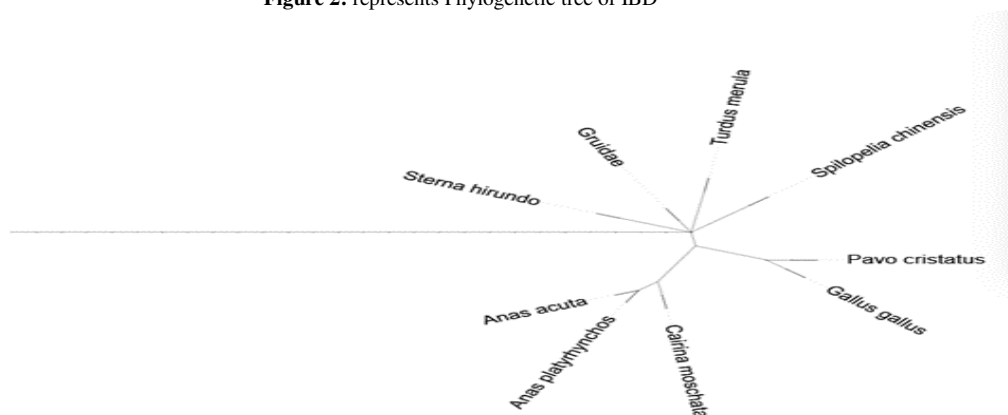


Figure 2: represents Phylogenetic tree of IBD



SUMMARY

Poultry farming has developed rapidly in recent years, resulting in the spread of infectious diseases such as IBD, which is now common in many parts of India. It is an immunosuppressive illness that, despite the fact that it first appeared 60 years ago, continues to pose a serious threat to the poultry industry and has resulted in significant economic losses. Bursal lesions, immune

system suppression, and atrophy are all symptoms of Infectious Bursa Disease Virus (IBDV). The virus is primarily a B-lymphocyte cell killer that is spread by contaminated food and drink. On the basis of virus neutralization assays, it can be divided into two categories: serotype 1 and serotype 2. Serotype 1 is considered to be the most prevalent. After incubation, IBDV causes an increase in death and morbidity rates. Following the onset of clinical signs, the recovery

period lasts 5-7 days. Molecular techniques such as reverse transcription polymerase chain reaction (RT-PCR), agar gel precipitation test (AGPT), and enzyme linked immunosorbent assay are used to diagnose the disease (ELISA). The current research field setting is further complicated by the introduction of antigenic variations and highly virulent strains of IBDV. There is currently no effective treatment for IBD; nevertheless, it can be prevented by the development of new molecular epidemiology, vaccines, and strict biosafety precautions.

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