Official Journal Issued by Faculty of Veterinary Medicine

Benha Veterinary Medical Journal

Journal homepage: https://bvmj.journals.ekb.eg/

Since 1990

Original Paper

Comparative genetic sequencing analysis of infectious bursal disease strains in Egypt: Insights into their molecular evolution and diversity based on highly variable region

Norhan W. Khalil[1], Mohamed A. Elshorbagy[1], Ibrahim. M. Elboraay[1], Ahmed . M. Helal[2]

[1] *Avian and Rabbit Diseases Department, Faculty of Veterinary Medicine, Benha University, Benha, Egypt.*

[2] *Central Laboratory for Evaluation of Veterinary Biologics, Cairo, Egypt.*

ARTICLE INFO

Keywords

Genotype 3.
HVR
Phylogenetic analysis,
RT-PCR
vvIBDV

Received 15/05/2023
Accepted 09/06/2023
Available On-Line 01/07/2023

ABSTRACT

Infectious bursal disease virus (IBDV) is an immunosuppressive virus for chicks especially 3-6 weeks of age. It is a double-stranded RNA virus which is characterized by continuous antigenic variation and mutation in its highly variable region (HVR) of viral protein 2. To investigate the genetic relationships among the IBDV strains circulating in Egypt, bursae of six IBDV-positive samples collected from Qalyubia, Gharbia, Giza, and Cairo provinces during 2018-2021 were subjected to partial genomic sequencing of 620 bp HVR of VP2. The results revealed that all the sequenced samples clustered as genotype 3 of very virulent strains based on phylogenetic analysis. Comparing them to the other reference strains isolated from Egypt, they showed 97.9% to 100% nucleotide identity among them although there was 92.8% to 95.1% similarity with other reference genotype 3 strains isolated from other countries. They also recorded high divergence with other isolates from Egypt. In conclusion, this study highlights the endemic circulation of very virulent IBDV strains of genotype 3 which may be the main cause of the ineffectiveness of many used vaccines against IBDV perhaps due to the high antigenic diversity.

1. INTRODUCTION

Infectious bursal disease virus is a highly significant immunosuppressive virus that primarily impacts young chicks by targeting the immature B-lymphocytes located in the bursa of Fabricius, resulting in clinical outbreaks characterized by high mortality rates, especially in light breeds and also subclinical cases of the immunosuppressive chicks and vaccination failure.

Infectious bursal disease virus is non-enveloped with a single protective shell of 65–70 nm in diameter, belonging to the family Birnaviridae, genus Avibirnavirus. The genome of IBDV is double-stranded RNA, bi-segmented into segment A and B (Murphy, 1999; Birghan et al., 2000; Fauquet and Fargette, 2005). Segment A of IBDV contains two partially overlapping open reading frames (ORFs). The larger ORF codes polypeptides that are subsequently cleaved into three crucial proteins: viral protein 2 (VP2) and VP3, which are basic structural proteins, and VP4, a serine protease that plays a significant role in IBDV pathogenicity. The smaller ORF translates a nonstructural protein called VP5. On the other hand, Segment B of IBDV encodes VP1, which is an RNA-dependent RNA polymerase (Xu et al., 2011). Its icosahedral capsid consists of 32 capsomeres of trimeric subunits (Özel and Gelderblom, 1985). The inner surface of the capsid is composed of 200 Y-shaped trimeric VP3 structures, while the outer surface is formed by 260 trimeric VP2 clusters (Böttcher et al., 1997).

There are two distinct serotypes (1 and 2) of the infectious bursal disease virus; serotype 1 produces clinical signs and contains pathogenic strains, while serotype two strains cause no disease. There is only 30% antigenic similarity between two IBDV serotypes as a result of antigenic diversity (McFerran et al., 1980), so there is no cross-protection between them (van den Berg et al., 2004).

Viral protein 2 is recognized as the primary antigenic site which is responsible for antigenic variation and mutation, cell tropism and the main key factor of viral virulence and pathogenicity (van Loon et al., 2002) so, it became the base of most of the phylogenetic classification of IBDV. It has three main domains (the base, shell and projection domains) (Letzel et al., 2007). The conserved N- and C-termini form the base and shell domains, while the projection domain is created by HVR of VP2. (Bayliss et al., 1990) which is a critical region where most amino acid (aa) changes occur (Xu et al., 2011). The mutation of HVR of VP2 resulted in the emergence of new antigenic variants which in turn changes in the viral virulence. Since 1989, IBDV has been categorized into two subtypes, which are the classical and the variant subtypes. Within the classical subtype, further classifications include attenuated, virulent, and vvIBDV pathotypes. (Li et al., 2009). Also, it was classified according to the antigenic variation and virulence into classical strains, variant strains, and very virulent strains (Zierenberg et al., 2000). It is also classified into sub-clinical (scIBDV), classic virulent (cvIBDV), very virulent (vvIBDV), antigenic variant IBDV (avIBDV) and attenuated IBDV (van den Berg et al., 2004).

* Correspondence to: Norhan.walaa@fvtm.bu.edu.eg

A recently modified classification was developed depending on the HVR of VP2 of serotype 1 into eight genogroups which correspond to their pathotypes. There were seven recognized genotypes (A1-A7) (Michel and Jackwood 2017), as well as a new cluster named genotype A8, involved the attenuated IBDV strains (Wang et al., 2021). Genotype A1 involved all the classical strains which are present worldwide. The A2 genogroup comprised of variants that were prevalent in North and South America and were categorized into four clusters, namely A2a, A2b, A2c, and A2d. Recently, a novel variant of IBDVs has emerged and forms a new lineage of A2dB1. These novel variants are genetically distinct from the initially discovered variant IBDV strains in America (Wang et al., 2021). Recently, these novel variant IBDVs (nVarIBDV) were successively reported in China (Fan et al., 2019), Japan (Myint et al., 2021), Korea (Thai et al., 2021) and Malaysia (Aliyu et al., 2021). Genogroup 3 of the very virulent strains became the most highly spread pathotype. In addition, one virulent IBDV named HLJ0504-like strains (A3B3) were endemic in China (Hon et al., 2008), India (Patel et al., 2016) and Pakistan (Hussain et al., 2019). While genogroup 4 isolated from the United Arab Emirates (741_UAE) showed a strong similarity to a specific lineage of IBDV that was commonly found in South America (Hernández et al., 2015). Genogroup 5 strains clustered the IBDV reassortants between the classical and variant strains which were isolated from Mexico (Jackwood, 2012). The strains isolated from Saudi Arabia were similar by 92.26-93.64% to the ITA genotype observed in Italy (Lupini et al., 2016). Genotype 7 included IBDV strains from Australia and Russia (Michel and Jackwood, 2017). Overall, this modified classification classified the IBDVs into nine genogroups providing a more comprehensive understanding of the genetic diversity of IBDV strains and can help in the development of more effective vaccines and treatments for this economically important poultry disease.

As a result of continuous mutation in the IBDV genome which leads to the emergence of variants and the vvIBDV strains consequently, the vaccination failure with the standard IBDV vaccines (Hussein. et al., 2003). Therefore, this study aimed to make a better consideration of the molecular genotyping of IBDV in Egypt and determine the genetic relationships among the strains through partial genomic sequencing of HVR of VP2 for recent circulating field IBDV samples from different governorates in Egypt from 2018 to 2021.

2. MATERIAL AND METHODS

Ethics Declarations.

The authors of this study followed the guidelines established by the animal welfare committee. The protocols used in this research were approved by the Research Ethics Committee at the Faculty of Veterinary Medicine, Benha University (No.: BUFVTM 01-10-21).

2.1. Viral RNA extraction and RT-PCR.

Fifteen bursae of suspected field samples of IBDV were collected in Qalyubia, Gharbia, Giza, and Cairo provinces in 2018-2021. The samples showed caseous and hemorrhagic bursitis, and their full historical data is presented in table (1). They were aseptically well-preserved in phosphate buffer saline (PBS) (pH 7.4) with Penicillin (1000 UI/mL) and Streptomycin (1 mg/mL). The samples were transported in an ice box and stored at -20°C. (OIE, Terrestrial Manual, 2016).

The extraction of RNA was carried out from the bursal homogenate following the instruction of QIAamp Viral RNA Mini Kit (QIAGEN) (Germany) catalogue No. 52904.

Table 1 The historical data of the collected samples.

Location	No. of flock	Type of flock	Age	Previous vaccination
Giza	150	White leghorn SPF chicks	16 D	-
Gharbia	100	Saso breed	31 D	-
Gharbia	100	Saso breed	23 D	-
Qalubia	5000	Cobb broiler breed	26 D	Bursine plus- 12 D
Qalubia	8000	Cobb broiler breed	32 D	D78- 13 D
Cairo	6000	Cobb broiler breed	15 D	E228- 14 D

The suspected IBDV samples were confirmed by RT-PCR using a set of forward and reverse VP2 primers for amplification of 620 bp fragments of HVR of VP2 supplied from Metabion (Germany) (Table 2).

Table 2 The used oligonucleotide sequences of the forward and reverse primers of HVR of VP2.

Gene	Primer sequence (5'-3')	Amplified Segment (bp)	Reference
HVR of VP2	AUS GU: (5´-TCA CCG TCC TCA GCTTAC CCA CAT C-3´)	620	(Bayliss et al., 1990)
	AUS GL: (5´-GGA TTT GGG ATC AGCTCG AAG TTG C-3´)		

The RT-PCR reaction was performed for VP2 according to the QIAamp Viral RNA Mini Kit (QIAGEN) catalogue No. 52904 (one step) at 50 °C for 30 min., then an initial denaturation at 95 °C for 15 min then 35 cycles of secondary denaturation at 94 °C for 30 sec, annealing at 59 °C for 40 sec., extension at 72 °C for 1 min. and final extension at 72 °C for 10 min (Metwally et al., 2009). 5 µl of PCR products were examined by electrophoresis documentation system on a 1.5% agarose gel containing ethidium bromide after amplification (Sambrook et al., 1989).

2.2. Sequencing.

The PCR product obtained from the positive IBDV samples was purified using the QIAquick Gel Extraction Kit from Qiagen Inc., located in Valencia, CA. The purified PCR product was then subjected to sequencing by both the forward and reverse directions by an Applied Biosystems 3130 automated DNA Sequencer (ABI, 3130, USA). The sequencing reaction was performed using the BigDye Terminator V3.1 Cycle Sequencing Kit from Perkinelmer/Applied Biosystems, Foster City, CA, with the catalog number 4336817.

To verify the sequence identity, a BLAST® analysis (Basic Local Alignment Search Tool) was conducted. This analysis involved comparing the obtained sequences to the sequences in the National Center for Biotechnology Information (NCBI) database to obtain GenBank accessions for further analysis and identification (Altschul et al., 1990).

2.3. Sequence analysis.

The sequences of the six samples were comparatively analyzed with reference sequences through their accession numbers from the Gene bank using the CLUSTAL W multiple sequence alignment program, version 12.1 of MegAlign module of Lasergene DNA Star software Pairwise, designed by Thompson et al., (1994) and also, phylogenetically analyzed using maximum likelihood, neighbor joining and maximum parsimony in MEGA6 (Tamura et al., 2013).

3. RESULTS

Out of the fifteen suspected samples, six samples were positive for IBDV through RT-PCR (Figure 1). The six sequenced samples (Egy_S14/2019) - (Egy_S2/2020) - (Egy_S3/2020) - (Egy_S1/2021) - (Egy_S4/2021) - (Egy_S6/2021) of the accession numbers demonstrated in table (3) phylogenetically clustered together and closely related to genotype 3 of IBDV strains which corresponds their very virulent pathotype (Figure 2).

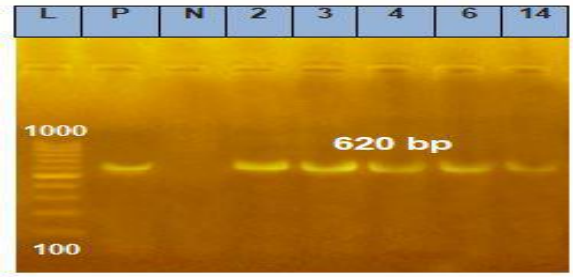

Figure 1 Agarose gel electrophoresis showed the positive amplified PCR product. The size of +ve product (620 bp).

Table 3 The accessions and localities of the six sequenced strains.

Strain	Locality	Accession no.
Egy_S1/2021	Giza	OQ828699
Egy_S2/2020	Gharbia	OQ828700
Egy_S3/2020	Gharbia	OQ828701
Egy_S4/2021	Qalubia	OQ828702
Egy_S6/2021	Qalubia	OQ828703
Egy_S14/2019	Cairo	OQ828704

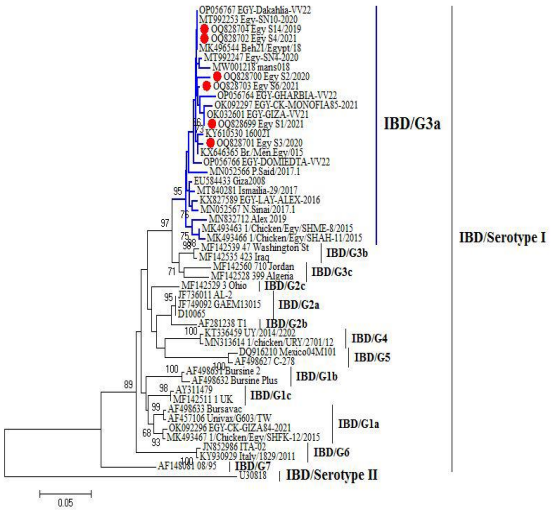

Figure 2 The phylogenetic analysis of the six sequenced strains.

As shown in figure (3), they recorded similarity ranging from 97.9- 100% among them and with reference strains isolated from different provinces in Egypt in different years from 2015 to 2022. For example, they documented (97.9-99.2%) with strain 160021 of accession (KY610530) from Domitta in 2015, (97.4-98.7%) with isolate mans018 (MW001218) from Dakahlia in 2018 – (99.2- 100%) with EGY-SN10-2020 (MT992253) from Giza in 2020 – (97.4-98.2%) with EGY-IBDV-GHARBIA-VV22 (OP056764) from Gharbia in 2022.

They also recorded lower identity (92.1- 95.1 %) with isolate 423 from Iraq of the accession of MF142535, (91.5-94.1%) with isolate 399 from Algeria of the accession of MF142528 which isolated in 2015 which clustered in another lineage of genotype 3.

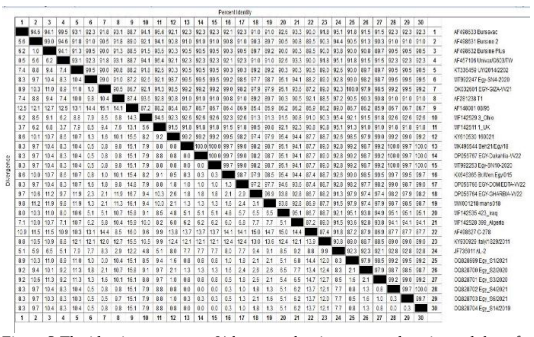

Figure 3 The identity percentage % between the six sequenced strains and the reference ones.

4. DISCUSSION

Infectious bursal disease virus is RNA virus that is characterized by a high mutation rate due to the limited ability of RNA to proofread and correct errors, this poses a significant challenge to control the virus (Durairaj et al., 2013). So, understanding the genetic diversity of IBDV strains is crucial for disease control and prevention measures (Umm-i-Habiba et al., 2020). The sequencing was performed for HVR of the VP2 gene as it is a target for genotyping and strain characterization of IBDV(Bayliss et al., 1990).

The high level of sequence similarity (97.9-100%) among the six samples and the reference strains isolated from Egypt from 2015 to 2022 which were also classified as very virulent strains and clustered to the genotype 3 of IBDV suggests the endemic circulation of this genotype in the Egypt for several years. The reason for the genetic similarity among all the vvIBDV strains can be traced back to the emergence of a single phylogenetic lineage that evolved from a common shared ancestor. (Silva et al., 2013). This finding is in line with preceding studies reporting the predominance of genotype 3 of very virulent pathotypes strains in Egypt since 2012 where 9 isolates collected from Qalyubia, Menofia, Giza and Fayoum provinces showed 96.7–100% amino acid identity with Giza 2008 very virulent strains (Mawgod et al., 2014). Also, Moneim El Sayed et al. (2021) recorded ten strains that were collected from Qalubia, Behera, Alexandria, and Gharbia. They were found to be associated with vvIBDV, exhibiting a nucleotide identity ranging from 95.7% to 96.7% and an amino acid identity ranging from 98.2% to 99.4% when compared to vvIBDV strains isolated in Europe and Asia. Elshall et al. (2018) suggested that four isolates from Behera, Alexandria were grouped with vvIBDV strains such as Giza 2008, and Giza 2000 even it also recorded one classical strain in their isolates with high similarity with CEVAC IBDL vaccine. And they attributed that to the continuous rotation of classical vaccinal strains because of the heavy vaccination programs.

The observed divergence of the sequenced samples from other IBDV strains isolated in Egypt, such as Chicken/Egypt/SHFK-12/2015 of genotype 1 of the classical pathotype suggested the existence of genetically distinct strains from the local IBDV lineage that is currently circulating in Egypt. This is consistent with previous studies that have shown that circulation of both genotype 1 and 3 strains of the classical and vvIBDV strains where 3 strains belonged to the genogroup 3 whereas the other two strains were classified as genotype 1 clustered with the winter field 2512 vaccine. (Zanaty et al., 2022).

The lower identity (92.8-95.1%) of the sequenced samples with strains isolated from Iraq, Algeria, and Jordan, which clustered in another lineage of genotype 3, indicates the existence of geographically distinct sub-lineages within this genotype. This may be due to the presence of distinct clusters and genomic lineages at the same genotype and this was proved in China where there were several changes in the genome of vvIBDV strains isolated there despite the similarity in the antigenic features with previous vvIBDV strains (Umm-i-Habiba et al., 2020).

5. CONCLUSION

To sum up, the findings highlight the predominance of genotype 3 strains with a very virulent pathotype with their endemic circulation and potential transmission among poultry flocks in various provinces. This underscores the importance of ongoing surveillance and implementation of control measures. Furthermore, the co-circulation of multiple genotypes and lineages within and beyond Egypt emphasizes the necessity of global cooperation for effective disease management.

CONFLICT OF INTERESTS.

The authors of the study stated that no conflict of interest was reported.

ACKNOWLEDGEMENTS

The authors wish to thank Animal Health Research Institute, Dokki, Giza, for their kind effective help.

Funding.

The authors stated that no funds, grants, or other support were received during the conducting of this study.

6. REFERENCES

1. Aliyu, H. B., Hair-Bejo, M., Omar, A. R., and Ideris, A. (2021). Genetic Diversity of Recent Infectious Bursal Disease Viruses Isolated From Vaccinated Poultry Flocks in Malaysia. Frontiers in Veterinary Science, 8(April), 1–17.
2. Altschul, S. F., Gish, W., Miller, W., Myers, E. W., and Lipman, D. J. (1990). Basic local alignment search tool. Journal of Molecular Biology, 215(3), 403–410.
3. Bayliss, C. D., Spies, U., Shaw, K., Peters, R. W., Papageorgiou, A., Muller, H., and Boursnell, M. E. G. (1990). A comparison of the sequences of segment A of four infectious bursal disease virus strains and identification of a variable region in VP2. Journal of General Virology, 71(6), 1303–1312
4. Birghan, C., Mundt, E. and Gorbalenya, A. E. (2000). A non-canonical Lon proteinase lacking the ATPase domain employs the Ser–Lys catalytic dyad to exercise broad control over the life cycle of a double-stranded RNA virus. The EMBO journal 19(1): 114-123.
5. Böttcher, B., Kiselev, N. A., Stel'Mashchuk, V. Y., Perevozchikova, N. A., Borisov, A. V, and Crowther, R. A. (1997). Three-dimensional structure of infectious bursal disease virus determined by electron cryomicroscopy. Journal of Virology, 71(1), 325–330.
6. Durairaj, V., Linnemann, E., Icard, A. H., Williams, S. M., Sellers, H. S., and Mundt, E. (2013). An in vivo experimental model to determine antigenic variations among infectious bursal disease viruses. Avian Pathology, 42(4), 309–315.
7. Elshall, N., Sedeek, M., Elbadawy, M., Hussein, E., and Awad, A. (2018). Phylogenetic Characterization of Infectious Bursal Disease (IBD) Viruses Isolated From Field Outbreaks in Chickens From Behera And Alexandria Governorates, Egypt. Alexandria Journal of Veterinary Sciences, 56(1),153-161 https://doi.org/10.5455/ajvs.284794
8. Fan, L., Wu, T., Hussain, A., Gao, Y., Zeng, X., Wang, Y., Gao, L., Li, K., Wang, Y., Liu, C., Cui, H., Pan, Q., Zhang, Y., Liu, Y., He, H., Wang, X., and Qi, X. (2019). Novel variant strains of infectious bursal disease virus isolated in China. Veterinary Microbiology, 230, 212–220.
9. Fauquet, C. M., and Fargette, D. (2005). International Committee on Taxonomy of Viruses and the 3,142 unassigned species. Virology Journal, 2(1), 1–10.
10. Hernández, M., Tomás, G., Marandino, A., Iraola, G., Maya, L., Mattion, N., Hernández, D., Villegas, P., Banda, A., Panzera, Y., and Pérez, R. (2015). Genetic characterization of South American infectious bursal disease virus reveals the existence of a distinct worldwide-spread genetic lineage. Avian Pathology, 44(3), 212–221.
11. Hon, C.-C., Lam, T. T.-Y., Yip, C.-W., Wong, R. T.-Y., Shi, M., Jiang, J., Zeng, F., and Leung, F. C.-C. (2008). Phylogenetic evidence for homologous recombination within the family Birnaviridae. Journal of General Virology, 89(12), 3156–3164.
12. Hussain, A., Wu, T., Li, H., Fan, L., Li, K., Gao, L., Wang, Y., Gao, Y., Liu, C., Cui, H., Pan, Q., Zhang, Y., Aslam, A., Muti-Ur-Rehman, K., Munir, M., Butt, S. L., Wang, X., and Qi, X. (2019). Pathogenic Characterization and Full Length Genome Sequence of a Reassortant Infectious Bursal Disease Virus Newly Isolated in Pakistan. Virologica Sinica, 34(1), 102–105.
13. Hussein, H., Aly, A., Sultan, H. and Al-Safty, M. (2003). Transmissible viral proventriculitis and stunting syndrome in broiler chickens in Egypt: 1. Isolation and characterization of variant infectious bursal disease virus (IBDV). Veterinary Medical Journal Giza 51(3): 445-462.
14. Jackwood, D. J. (2012). Molecular Epidemiologic Evidence of Homologous Recombination in Infectious Bursal Disease Viruses. Avian Diseases, 56(3), 574–577.
15. Letzel, T., Coulibaly, F., Rey, F. A., Delmas, B., Jagt, E., van Loon, A. A. M. W., and Mundt, E. (2007). Molecular and Structural Bases for the Antigenicity of VP2 of Infectious Bursal Disease Virus. Journal of Virology, 81(23), 12827–12835.
16. Li, Y., Wu, T., Cheng, X., and Zhang, C. (2009). Molecular characteristic of VP2 gene of infectious bursal disease viruses isolated from a farm in two decades. Virus Genes, 38(3), 408–413.
17. Lupini, C., Giovanardi, D., Pesente, P., Bonci, M., Felice, V., Rossi, G., Morandini, E., Cecchinato, M., and Catelli, E. (2016). A molecular epidemiology study based on VP2 gene sequences reveals that a new genotype of infectious bursal disease virus is dominantly prevalent in Italy. Avian Pathology, 45(4), 458–464.
18. Mawgod, S. A., Arafa, A. S., and Hussein, H. A. (2014). Molecular genotyping of the infectious bursal disease virus (IBDV) isolated from Broiler Flocks in Egypt. International Journal of Veterinary Science and Medicine, 2(1), 46–52.
19. McFerran, J. B., McNulty, M. S., McKillop, E. R., Connor, T. J., McCracken, R. M., Collins, D. S., and Allan, G. M. (1980). Isolation and serological studies with infectious bursal disease viruses from fowl, turkeys and ducks: Demonstration of a second serotype. Avian Pathology, 9(3), 395–404.
20. Metwally, A.M., Yousif, A.A., Shaheed, I.B., Mohammed, W.A., Samy, A.M. and Reda, I.M. (2009): Re-Emergence of Very Virulent IBDV in Egypt International Journal of Virology 5(1).: 1-17.
21. Michel, L. O., and Jackwood, D. J. (2017). Classification of infectious bursal disease virus into genogroups. Archives of Virology, 162(12), 3661–3670.
22. Moneim El Sayed, W. A. El, Abdelhalim, A., Yehia, N., and Omar, S. E. (2021). Genetic Evolution of Infectious Bursal Disease Virus Isolated from Chicken Poultry Flocks in Egypt. Journal of World's Poultry Research, 11(2), 215–222.
23. Murphy, F. A., Gibbs, E. P. J., Horzinek, M. C. and Studdert, M. J. (1999). Veterinary virology, Academic press.3rd Edition.
24. Myint, O., Suwanruengsri, M., Araki, K., Izzati, U. Z., Pornthummawat, A., Nueangphuet, P., Fuke, N., Hirai, T., Jackwood, D. J., and Yamaguchi, R. (2021). Bursa atrophy

at 28 days old caused by variant infectious bursal disease virus has a negative economic impact on broiler farms in Japan. Avian Pathology, 50(1), 6–17.
25. OIE, Terrestrial Manual (2016): Infectious bursal disease (Gumboro disease). Manual of Diagnostic Tests and Vaccines for Terrestrial Animal. Chapter 2.3.12. (n.d.).
26. Özel, M., and Gelderblom, H. (1985). Capsid symmetry of viruses of the proposed Birnavirus group. Archives of Virology, 84(3–4), 149–161.
27. Patel, A. K., Pandey, V. C., and Pal, J. K. (2016). Evidence of genetic drift and reassortment in infectious bursal disease virus and emergence of outbreaks in poultry farms in India. VirusDisease, 27(2), 161–169.
28. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989). Molecular Cloning a Laboratory Manual Second Edition Vols. 1- 2.
29. Silva, F. M. F., Vidigal, P. M. P., Myrrha, L. W., Fietto, J. L. R., Silva, A., and Almeida, M. R. (2013). Tracking the molecular epidemiology of Brazilian Infectious bursal disease virus (IBDV) isolates. Infection, Genetics and Evolution, 13(1), 18–26.
30. Tamura, K., Stecher, G., Peterson, D., Filipski, A., and Kumar, S. (2013). MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. Molecular Biology and Evolution, 30(12), 2725–2729.
31. Thai, T. N., Jang, I., Kim, H.-A., Kim, H.-S., Kwon, Y.-K., and Kim, H.-R. (2021). Characterization of antigenic variant infectious bursal disease virus strains identified in South Korea. Avian Pathology, 50(2), 174–181.
32. Thompson, J. D., Higgins, D. G., and Gibson, T. J. (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Research, 22(22), 4673–4680.
33. Umm-i-Habiba, U.-H., Maqbool, A., Safdar, M., Zia, N., Mehmood, A., Usman, M., Sharif, M., Khan, A., and Umar, S. (2020). Detection and Phylogeny of Infectious Bursal Disease Virus (IBDV) during Field Outbreaks in Broilers. Pakistan Journal of Zoology, 52(2), 659–667.
34. van den Berg, T. P., Morales, D., Eterradossi, N., Rivallan, G., Toquin, D., Raue, R., Zierenberg, K., Zhang, M. F., Zhu, Y. P., Wang, C. Q., Zheng, H. J., Wang, X., Chen, G. C., Lim, B. L., and Müller, H. (2004). Assessment of genetic, antigenic and pathotypic criteria for the characterization of IBDV strains. Avian Pathology, 33(5), 470–476.
35. Van Loon, A. A. W. M., de Haas, N., Zeyda, I., and Mundt, E. (2002). Alteration of amino acids in VP2 of very virulent infectious bursal disease virus results in tissue culture adaptation and attenuation in chickens. Journal of General Virology, 83(1), 121–129.
36. Wang, Y., Fan, L., Jiang, N., Gao, L., Li, K., Gao, Y., Liu, C., Cui, H., Pan, Q., Zhang, Y., Wang, X., and Qi, X. (2021). An improved scheme for infectious bursal disease virus genotype classification based on both genome-segments A and B. Journal of Integrative Agriculture, 20(5), 1372–1381.
37. Xu, X.-G., Tong, D.-W., Wang, Z. S., Zhang, Q., Li, Z.-C., Zhang, K., Li, W., and Liu, H.-J. (2011). Baculovirus Virions Displaying Infectious Bursal Disease Virus VP2 Protein Protect Chickens Against Infectious Bursal Disease Virus Infection. Avian Diseases, 55(2), 223–229.
38. Zanaty, A., Mossad, Z., Said, M., Samy, M., Rabie, N., Amer, F., and Soliman, M. A. (2022). Genetic Characterization of Co-circulated Classic and Very Virulent Infectious Bursal Disease Viruses in Commercial Broiler Flocks of Egypt. Journal of World's Poultry Research, 12(2), 124–132.
39. Zierenberg, K., Nieper, H., Van Den Berg, T. P., Ezeokoli, C. D., Voß, M., and Müller, H. (2000). The VP2 variable region of African and German isolates of infectious bursal disease virus: comparison with very virulent, "classical" virulent, and attenuated tissue culture-adapted strains. Archives of Virology 2000 145:1, 145(1), 113–125.