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Phylogenetic analysis of infectious bursal disease virus strains delivered from natural infection in last decade during 2013-2022, Egypt

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ABSTRACT

Infectious bursal disease (IBD) is an acute, highly contagious, and immunosuppressive disease of chickens resulting in global economic losses to the poultry industry despite extensive vaccination. The emergence of very virulent IBDVs in global epizootics, including those from Egypt, demonstrates how occasionally occurring genetic changes lead to simultaneous evolution of distinct IBDV strains across the world. Upon phylogenetic analysis of Egyptian IBDVs, virus strains from chickens and turkey (n= 146) were clustered within genogroup 3, whereas, twenty nine isolates were closely related to vaccinal strains in genogroup 1. Regarding to sequences from wild birds (cattle egret and green winged teal) were clustered within G1a (n=2) and G3a (n=1) subgen groups. The highest IBDV percentages during 2013-2022 was in 2020 (n=71; 20%) and in Behera Province (n= 54; 15.3%) followed by Sharkia Province (n= 51; 14.4%). The data revealed the characteristic molecular traits of IBDVs circulating in Egypt between 2013 and 2022, which will help in development of effective vaccines subsequently disease prevention. In addition to, it offers helpful insights for carrying out further surveys that are required to increase the understanding of IBDV prevalence in wild migratory and free-living birds.

Introduction

Infectious bursal disease (IBD), also known as the Gumboro disease, is an economically significant viral disease that affects young birds and causes acute death or immunosuppression (Müller *et al*., 2013). It is characterized by bursal damage secondary to an invasion of immature lymphocytes. The causative agent is the IBD virus (IBDV), which is a typical non-enveloped, icosahedral, double-stranded RNA virus belonging to the genus *Avibirnavirus* of the *Birnaviridae* family (Brown and Skinner, 1996; Jackwood, 2017). Viral genome is composed of 2 segments (A and B) of double-stranded RNA (Müller *et al*., 1979). Segment A encodes 2 structural proteins (VP2 and VP3), a viral protease (VP4), and a nonstructural protein (VP5) (Raja *et al*., 2016). VP2 is the main structural protein, participating in viral entrance, cell tropism, pathogenicity, and antigenic variation (Jackwood *et al*., 2008; Qi *et al*., 2016; Wu *et al*., 2020). The hypervariable region (HVR) of VP2 (amino acids 206-350) is a fragment representative of the gene features of segment A, which is generally used in analyses of the genetic evolution of IBDV (Letzel *et al*., 2007; Jackwood, 2017). VP3 also contributes in the formation of viral particles, and involved the serotype specificity, apoptotic regulation, and viral assembly (Tacken *et al*., 2002; Ye *et al*., 2014; Ferrero *et al*., 2015). VP4 plays a crucial role in the interdomain proteolytic autoprocessing of the pVP2-VP4-VP3 polyprotein and is accountable in virus-induced immune suppression (Lejal *et al*., 2000). VP5 prevents apoptosis during early course of an infection, but later on, it induces apoptosis (Mendez *et al*., 2015). Segment B encodes an RNA-dependent RNA polymerase (VP1), that participates in genetic evolution and viral replication (von Einem *et al*., 2004; Escaffre *et al*., 2013; Gao *et al*., 2014). Comparable to the HVR of VP2, the B-marker of VP1 (amino acids 110-252) is a fragment representative of the gene features of segment B (Alfonso-Morales *et al*., 2015).

Two distinctive serotypes (1 and 2) of IBDVs have been identified (Jackwood *et al*., 1982). Serotype 1, which has been confirmed to be pathogenic in chickens, can be further divided into four phenotypes, including the classical IBDV (clIBDV), very virulent (vvIBDV), attenuated IBDV (atIBDV) and variant IBDV (varIBDV); however, serotype 2 was non-pathogenic to chickens (Jackwood, 2017). The IBDV was initially documented in Gumboro, USA in 1957, and is now known as the classical IBDV (cIBDV). Since then, IBDV strains of varying virulence have sequentially emerged and spread to almost all poultry producing countries globally, posting new challenges to the disease's prevention and control (Withers *et al*., 2005; Alkie and Rautenschlein, 2016). Thereafter, in the late 1980s, the vvIBDV that was characterized by high mortality, spread from Europe to the rest of the world (Chettle *et al*., 1989), resulting in considerable financial losses for the world's chicken sector. Nonetheless, IBDV was progressively brought under effective control with the widespread use of vaccines and advancements in poultry management and feeding. The novel variant of IBDV (nVarIBDV), which causes an atypical IBD, has however expanded throughout China since 2017, escaping the immuno-protection brought on by the current vaccines against vvIBDV (Fan *et al*., 2019).

In Egypt, IBDV was first detected by El-Sergany *et al.* (1974), with outbreaks continue to infect broiler chickens, resulting in severe economic losses despite mandated vaccination against the disease (Hassan, 2004; Mohamed *et al*., 2014; Mawgod *et al*., 2014; Sedeik *et al*., 2018; Alkhalefa *et al*., 2019; El-Aried *et al*., 2019). Therefore, the aim of this study was

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to investigate the genetic characterization of circulating IBDV strains in Egyptian provinces during the last decade 2013-2022 and the results were interpreted in terms of genogroups according to proposed model for classification against IBDVs (Michel and Jackwood, 2017).

Materials and methods

Viruses

A total of 353 Egyptian IBDV serotype 1 strains (segment A, VP2 gene), were obtained from NCBI (www.ncbi.nlm.nih.gov, accessed on early September 2023) during the last decade 2013-2022 to be included in the study analysis.

Sequence and phylogenetic analysis

Following the principles of sequence selection (Michel and Jackwood, 2017), a ~366 bp fragment (n=175) from segment A (nt 631–1021, aa 211–340) was used for phylogenetic analysis of IBDVs using the MEGA 6.0 software (Tamura *et al*., 2013). The sequences were aligned using the Clustal W method then were cut to equal length. The genetic pattern was assessed by constructing a neighbor-joining phylogenetic tree based on VP2 nucleotide sequences, using the Kimura 2 parameter at 1000 bootstrap replicates.

Results and Discussion

Being, one of the most substantial contagious immunosuppressive diseases, the IBD causing irreversible immune suppression in young chickens, which makes them more vulnerable to other pathogens (Saif, 1991). Despite the disease's mandated immunization against IBD since its first detection by El-Sergany *et al.* (1974), IBDV outbreaks continue to affect broiler chickens, resulting in significant financial losses for the poultry industry. The current study was conducted to investigate the molecular characteristic features of field IBDVs circulating in Egypt during 2013-2022. Intensive poultry farming follows a stringent vaccination schedule and implement strict biosecurity measures. Though, backyard/small-scale farmers rarely immunize their birds. Such a divergence in IBDV control approaches considerably aids in disseminating the virus. In this regard, following the molecular pattern of IBDV in chickens is highly beneficial.

In this study, a total of 353 Egyptian IBDV serotype 1 strains (segment A, VP2 gene), were obtained from the GenBank during the last decade 2013-2022 to be included in the study analysis (348 from chicken, 2 from turkey, 2 from cattle egret, and 1 from green winged teal) as shown in Fig. 1A. It was reported that both IBDV serotypes 1 and 2 can infect turkeys and the virus was successfully isolated from bursa up to 14 days post-inoculation (Abdul *et al*., 2015). Two IBDV sequences from turkey were isolated from naturally infected turkey poults in Dakahlia Province in 2017 (Mosad *et al*., 2020). The molecular IBDV reports started in Egypt as early as 2008 (Metwally *et al*., 2009). However, the NCBI database had other Egyptian NDV strains that are assumingly from 1989 (AF159218.1 IBDV/K406/89/Egypt/vvIBDV). Consistent with the numbers of IBDV sequences during 2013-2022, the maximum detection was in 2020 (n=71; 20%) (Fig. 1B). All recorded sequences belonged to IBDV serotype I. Regarding to the geographical distribution of IBDV strains in different Egyptian provinces (Fig. 2), the highest IBDV percentages was detected in Behera (n= 54; 15.3%) followed by Sharkia (n= 51; 14.4%) compared with South Sinai (n=2; 0.6%). However, 67 sequences were of unknown origin.

There are two main IBD forms, the clinical and subclinical disease forms; either linked to age of infection or virus type. To date all disease conditions in chickens are related to classic and variant strains of serotype 1. Classical clinical form of IBDV infection comprises typical gross lesions in are swollen bursa (from edematous to hemorrhagic), hemorrhages in

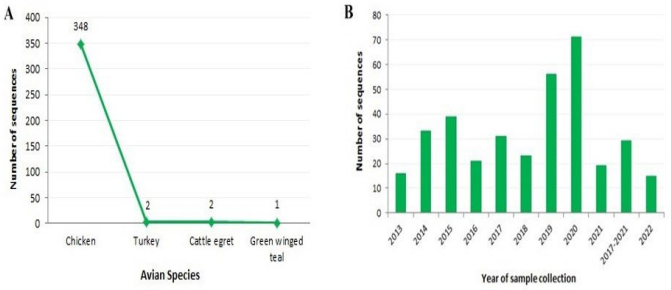

Fig. 1. The distribution of IBDV strains between 2013 and 2022 (VP2 gene sequences; data obtained from GenBank in early September 2023) in Egypt. The grouping of detected IBDV strains according to year of sample collection (A) and species of origin (B).

skeletal muscle (especially on thighs in breast), pale kidneys and signs of dehydration. Edematous bursa (may be slightly enlarged, normal size or reduced in size depending on the stage, may have hemorrhages, rapidly proceeds to atrophy). Swollen kidneys with urates were recorded. The severity of these pathological changes varied comparably to the virulence of the involved virus with the extreme severity in vvIBDV infection. However, the subclinical form of IBD usually appears when birds are early infected under 3 weeks of age, or at older age by some variant" strains of serotype 1, which leads to rapid bursal atrophy without mortality and were capable of evading maternal immunity directed primarily at "classical" strains. These variants or subtypes exhibited different biological properties, compared to classical strains, and could be a consequence of immune pressure due to the extensive application of vaccine plans. Successively, vvIBDV strains, responsible for 90% mortality rates (Eterradossi and Saif 2020). The naturally infected chickens showed a characteristic clinical picture for IBDV infection (Fig. 3), as confirmed previously (Sharma *et al*., 1977; Lukert and Saif, 2003). The bursal hemorrhagic lesions were confirmed in viruses carrying aa 253Q and 284A (Brandt *et al*., 2001) clarifying that these lesions were irrespective of the genotype of the virus. However, the reproducibility, multiplicity, or severities of pathologic lesions in affected chickens are consistent with virulence and antigenic variations of IBDVs. As reported elsewhere (Mwenda *et al*., 2018; El-Aried *et al*., 2019), lesions in the proventriculus and muscles were seen in the examined strains with a vvIBDV genotype.

Fig. 2. The map of Egypt represents the number of IBDV strains circulating in different Egyptian provinces between 2013 and 2022 (VP2 gene sequences; data obtained from GenBank in early September 2023).

Upon phylogenetic analysis, the majority of Egyptian IBDVs (n=146; 83.4%) were grouped in the vvIBDV genogroup (G3) as shown in Fig. 4A, which explains why most IBD outbreaks were found in vaccinated birds. The vvIBDV strains are capable of overcoming protection provided by recent vaccines (Müller *et al*., 2012). The abovementioned result poses a se

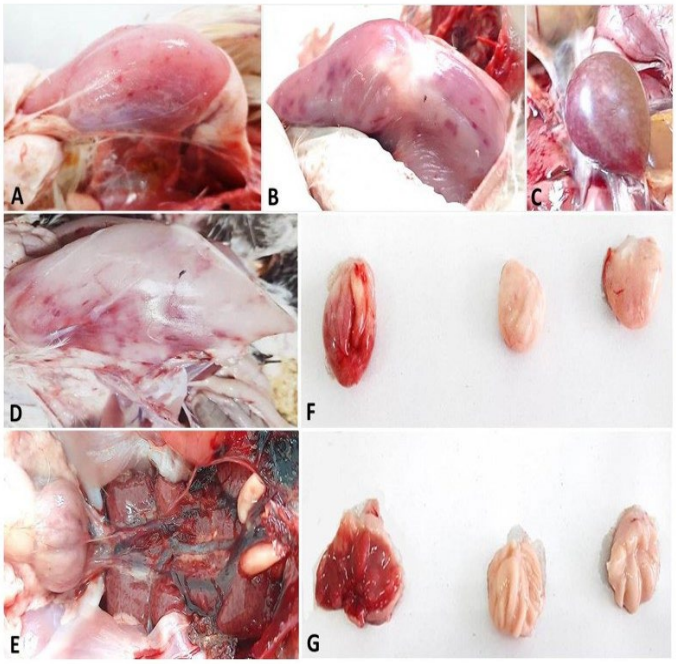

Fig. 3. The pathological changes associated with IBDV in natural infections among chickens aged 26-35 days. (A) Minute hemorrhagic spots on thigh muscle. (B) Severe petechial hemorrhages on thigh muscle. (C) Mottled enlarged spleen. (D) Petechial hemorrhages on pectoral muscle. (E) Severe enlarged bursa with nephritis. (F) Closed enlarged bursa with hemorrhagic one (Left). (G) Opened folded bursa with hemorrhagic one (Left).

rious challenge for controlling the disease in Egypt. Hence, further biosecurity measures should be strictly enforced. Besides, some virus strains (n=29; 16.6%) were genetically closely related to vaccine strains within genogroup 1 (Fig. 4B), suggesting the involvement of vaccinal strains in the IBD epidemiology in Egypt. Considering the continuous detection of diverse strains of IBDV, disentangling the impending role of wild birds in the epidemiology of this virus is crucial (Graziosi *et al*., 2022). According to sequence analyses, cattle egret and green winged teal isolates of IBDVs are closely related to IBDV genogroup 3 strains (n=1) or genogroup 1 strains (n=2) (Naggar *et al*., 2021), suggesting epidemiological links between domestic chicken and wild birds. Along with, the isolation of IBDVs in live, migratory wild birds emphasizes the possibility of a long-distance dissemination of the virus (Naggar *et al*., 2021).

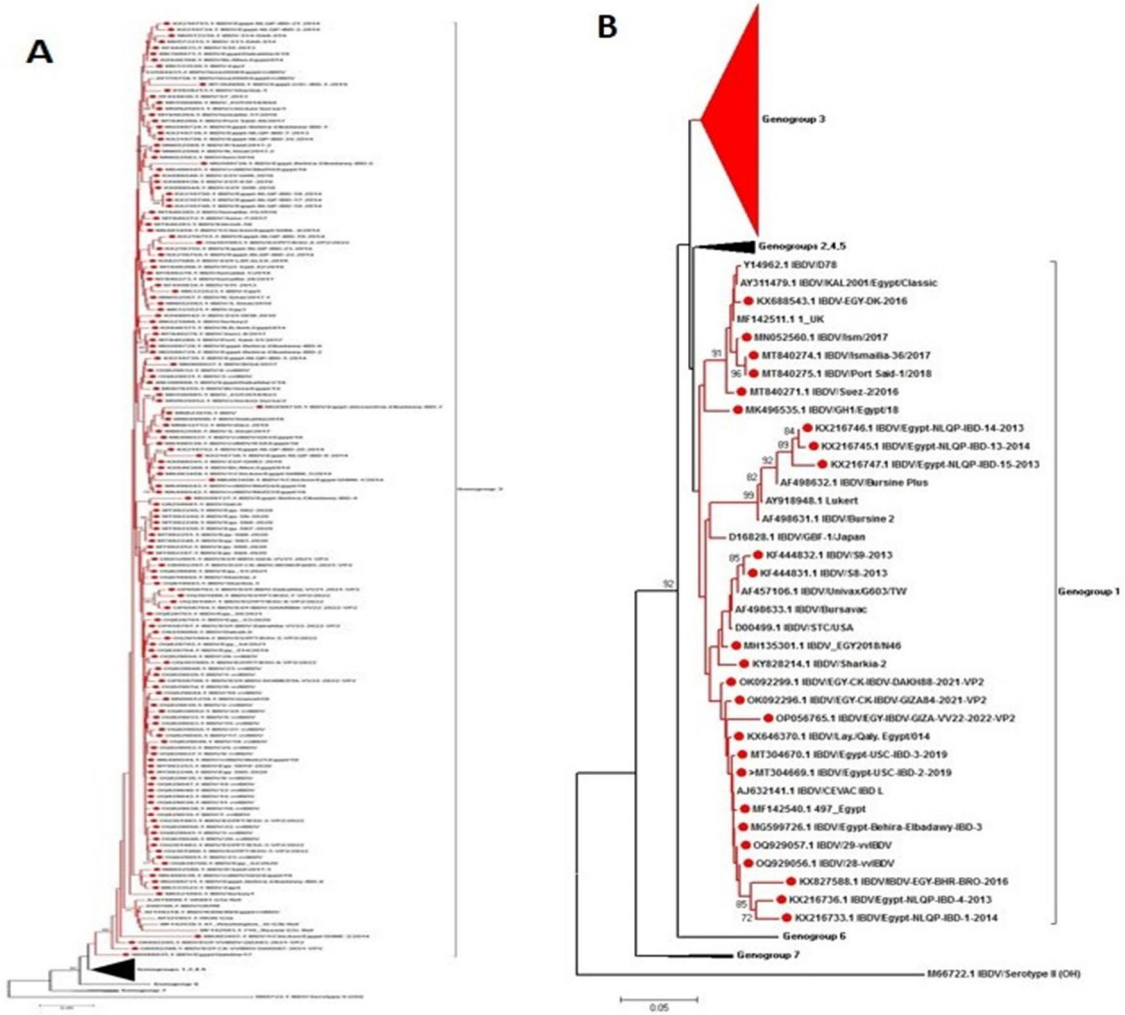

Fig. 4. The neighbor-joining phylogenetic tree of segment A of Egyptian IBDVs based on VP2 hypervariable region in different Egyptian isolates between 2013 and 2022 plus reference genogroup strains. Bootstrap values of 70% are shown above the branches. Sequences from Egypt are labelled with a red circle. The IBDV serotype II (OH) strain was selected as an outgroup sequence (Left). The taxons of VP2 tree were compressed for well presentation (Right). The detailed phylogenetic tree of Egyptian IBDVs within genogroup 3; very virulent IBDVs (A) and genogroup 1; classic IBDVs (B).

Conclusion

In the view of VP2 hypervariable region sequence analysis, the data revealed the continuous circulation of vvIBDV strains in the Egyptian field despite vaccination efforts, suggesting the continuous evolution and mutation of the virus. Indeed, the detection of IBDV in turkeys and wild birds highlights the potential for a long-distance spread of the virus and raises questions about epidemiological connections between domestic chickens and wild birds. National active surveillance with VP1 and VP2 nucleotide sequencing encompassing domestic and migratory birds will be helpful to find out the virulence and antigenic characteristics and evolutionary history of IBDVs.

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Conflict of interest

All authors declare that they have no conflicts of interest.

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