Research Note: “Hidden” infectious bursal disease virus infections in Central Europe

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ABSTRACT Infectious bursal disease virus (IBDV) is a major threat to the poultry industry globally, represented by a variety of genetic, pathogenic, and antigenic variants. The recognition of the infection may be challenging due to several factors, as the virulence of the strain, age, and immune status of the birds at infection, to name the most important ones. Here we report about the molecular typing of IBDVs detected over the recent years in Central Europe. The results revealed the diversity of IBDV in the region, that is, very virulent strains being present in all four involved countries, the successive detection of a recently described reassortant variant in the Czech Republic, and the “rediscovery” of a subclinical pathotype virus in Hungary. These findings highlight the need for monitoring the flocks regularly not only by evaluating the production parameters but to look specifically for the occurrence of IBDV and adjust the control measures according to the results.

Key words: infectious bursal disease virus, Central Europe, reassortant

INTRODUCTION Infectious bursal disease virus (IBDV), a highly contagious and resistant pathogen, is the causative agent of an immunosuppressive infection of chickens, first described as avian nephrosis near Gumboro in Delaware, USA, 1962 (Cosgrove, 1962), therefore, also referred to as Gumboro disease. IBDV infections compromise vaccine efficacy in general, and increase susceptibility to other pathogens, accounting for major economic losses to the poultry industry worldwide (Jackwood, 2021). The double stranded RNA IBDV genome has 2 segments, segment A and B, the former coding for the structural proteins and the latter for the viral polymerase. This structure significantly contributes to the complex epidemiology of IBDV infections because it enables IBDV strains to exchange genome segments resulting in newly formed reassortant variants, beyond the mutations and recombination events that shape the genetic composition of IBDV (Alkie and Rautenschlein, 2016).

Three landmarks have been recorded regarding IBDV evolution: the recognition of the classical strains in the fifties of the last century, then the occurrence of antigenic variants in the United States in the eighties, and the emergence of the very virulent IBDVs (vvIBDV) in the late eighties in Europe (Alkie and Rautenschlein, 2016). Over the decades these variants have spread and some of them have a global presence nowadays. Besides these established IBDV types the broader analysis of the recent strains revealed a more complex ecology of these viruses, including the recognition of reassortant or even genetically mosaic variants of differing pathogenicity (Pikula et al., 2021).

As in most parts of World, Central European countries introduced vaccination to control infectious bursal disease as a principal mean. The immunization strategy is based on the breeder and progeny vaccination using different types of live (by virulence intermediate and intermediate plus) or more recently HVT- (Herpesvirus of turkeys) vector IBD and immunocomplex IBD vaccines (Alkie and Rautenschlein, 2016). Thanks to vaccination, acute IBD outbreaks are rare nowadays, which might result in the substantial underestimation of the presence of the pathogen and the undervaluing of its impacts. In line with this assumption, vaccine application lacks accuracy in some places, for example, timing of drinking water vaccination is not adjusted to maternally derived antibody levels; the implementation of drinking water vaccination is not controlled, etc. Further, the newly developed hatchery vaccines, either HVT-vector IBD or immunocomplex IBD, are sometimes omitted in some broiler cycles. This situation allows the spread and emergence of different IBDV variants.
Recent reports described the currently circulating IBDV strains at different parts of Europe (Soubies et al., 2017; Mato et al., 2020; Pikula et al., 2020), but there is a lack of information regarding the Central European situation. Therefore, although through a limited number of strains, we provide a snapshot on the diversity of IBDVs collected from the Czech Republic, Slovakia, Hungary, and Serbia over the recent years.

MATERIALS AND METHODS

Bursa imprints on Whatman FTA cards were received from industrial broiler farms with monitoring purposes from Hungary, Slovakia, Czech Republic, and Serbia over the 2019–2021. Noticeably, only in 2 of 10 submissions was IBD suspected as disease condition. In the rest of the cases the observed deteriorating zootechnical performances substantiated sampling for IBDV detection (Table 1).

The molecular detection and identification of the viruses as well as the phylogenetic analysis of the partial VP2 and VP1 genes was done as described previously (Mato et al., 2020). Nucleotide sequence data obtained largely a decade ago (from 2010 to 2014) were also involved in order to establish their relationships to the newly detected viruses.

RESULTS AND DISCUSSION

Most of the detected viruses were assigned to Genogroup 3 (G3, very virulent, vvIBDV) according to the recently proposed nomenclature (Jackwood et al., 2018), while one virus belonged to G4 (subclinical IBDVs; Table 1.). vvIBDVs were detected from all countries involved in the analysis, supported by the constellation of both genome segments (Figure 1). Such a strain (1/chicken/Czech Republic/D2844/2014) was demonstrated back in 2014 in the Czech Republic. Representatives of the recently described “low pathogenicity” (G3 according to its VP2 hypervariable region sequence and containing “classical-variant-attenuated” segment B) type of vvIBDVs were demonstrated from the samples originated from Slovakia and from the Czech Republic both back in 2010 and in the current sample set. However, different incursions were apparent, some of the more recent viruses located distantly on both relevant phylogenetic trees compared to the viruses detected a decade earlier (Figure 1).

Additionally, a so-called subclinical strain (G4) was identified from Hungary, its genetic composition also having been confirmed by the partial sequencing of VP1 and VP2 of the viral genome. Noticeably, this strain was detected in farm not applying vaccination against IBD.

The aforementioned particular reassortant type of IBDVs, circulating in Northern and Western Europe, and in the Czech Republic, was described recently (Mato et al., 2020). These viruses comprise of a vvIBDV type segment A and an attenuated segment B. Reassortants were detected back in 2010 (e.g., 1/chicken/Czech Republic/D1540-1/2010 and 1/chicken/Slovakia/D1588-3-1/2010), and, interestingly, newer reassortant types could be identified in the more recent samples, one of them showing close relation to contemporary Western European strains (Mato et al., 2020). It is challenging to recognize the presence of this type of reassortant viruses because they cause subclinical infections, though bursa atrophy and increased ELISA antibody titers (measured >30 d of age) may be indicative for the circulating IBDV. Nevertheless, the negative impact of such viruses on poultry husbandry calls for adequate control measures.

The other type of IBDVs could be grouped geographically, and belonged to 2 monophyletic groups, the Slovakian with the Czechs viruses and the Serbian strain with a Hungarian one. Noticeably, the following Czechs and

Table 1. Origin and relevant data of the investigated IBDV strains.

| ID/year          | Country of origin | Type of bird | Vaccination day/ vaccine | Sampling age (days) | Suspect of IBD | HVR of VP2 / genogroup (segment A) | VP1 (segment B) |
|------------------|-------------------|--------------|---------------------------|---------------------|----------------|-----------------------------------|----------------|
| D1588/3/1/10    | Slovakia (SK)     | Industrial broiler | D19 Bar 706              | 29                  | NO             | vvIBDV / G3                       | classical      |
| D1540/1/10      | Czech Republic (EZ)| Industrial broiler | D19 Cevac Gumbo L         | 33                  | NO             | vvIBDV / G3                       | classical      |
| D2844/14        | Czech Republic (EZ)| Industrial broiler | No vaccination            | 28                  | NO             | vvIBDV / G3                       | vvIBDV         |
| D5225/2/20      | Czech Republic (EZ)| Industrial broiler | D18 Cevac Gumbo L         | 30                  | NO             | vvIBDV / G3                       | vvIBDV         |
| D5211/2/20      | Czech Republic (EZ)| Industrial broiler | D15 Cevac Gumbo L         | 35                  | NO             | vvIBDV / G3                       | vvIBDV         |
| D5419/1/20      | Czech Republic (EZ)| Industrial broiler | 15 days                   | 32                  | NO             | vvIBDV / G3                       | classical      |
| D4853/2/19      | Hungary (HU)      | Industrial broiler | No vaccination            | 40                  | YES            | classical / G4                    | classical      |
| D5403/1/20      | Hungary (HU)      | Industrial broiler | D21 Cevac Gumbo L         | 26                  | NO             | vvIBDV / G3                       | vvIBDV         |
| D6092/2/6/21    | Serbia (SRB)      | Industrial broiler | D12 & 15 D78             | 31                  | YES            | vvIBDV / G3                       | vvIBDV         |
| D5035/12/19     | Slovakia (SK)     | Industrial broiler | D21 Cevac Gumbo L         | 45                  | NO             | vvIBDV / G3                       | vvIBDV         |

Abbreviation: HVR, Hypervariable region of the VP2 gene.

The GenBank accession numbers of the obtained VP1 and VP2 sequences are ON375530-39 and ON412153-162, respectively, following the order of the strains presented in the Table.
Slovakian reassortant strains (1/chicken/Czech Republic/D1540-1/2010, 1/chicken/Slovakia/D1588-3-1/2010, and 1/chicken/Czech Republic/D5211-2-2020) did not harbor the established attenuation markers in the hypervariable region of their VP2 gene, indicating that their pathogenicity could be similar (i.e., retained vvIBDV pathogenicity) to that of Bpop/3 Polish reassortant strain described by Pikuša et al. (2018).

A subclinical strain was demonstrated in Hungary, which showed close similarity to some South American viruses (Hernandez et al., 2015). This genetic group (G4) was "rediscovered" during the last decade, with some divergence from their predecessors which had already been circulating in Poland and Hungary in the seventies (Domanska et al., 2004). Their presence also highlights the importance of monitoring to follow the IBDV epidemiological situation.

vvIBDVs have long been considered to cause significant clinical signs and pathological conditions. Apparently, this situation has changed by now, due to several causes, for example, solid vaccination programs in breeder flocks, improved biosecurity and vaccination against Gumboro disease of broilers, genetics of the birds, etc.

In alignment with that, our findings demonstrated that in most cases IBD was out of scope as the potential causative agent to the experienced performance losses. However, not only the presence of G3 (vvIBDV) strains but an unexpectedly wide variety of IBDVs became obvious in Central Europe, based on the presented molecular data, with some established phenotypical associations (Hernandez et al., 2015; Mato et al., 2020). Gumboro disease awareness programs and the related control strategies should benefit from the presented findings. Vaccination programs should be implemented meticulously to prevent the losses and impede the selection of reassortant viruses that could compromise the productivity of poultry industry.

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DISCLOSURES

The authors declare that there is no conflict of interests.

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