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Research paper

Infectious bursal disease virus in Algeria: Detection of highly pathogenic reassortant viruses

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ABSTRACT

Infectious bursal disease (IBD) is an immunosuppressive viral disease, present worldwide, which causes mortality and immunosuppression in young chickens. The causative agent, the *Avibirnavirus* IBDV, is a non-enveloped virus whose genome consists of two segments (A and B) of double-stranded RNA. Different pathotypes of IBDV exist, ranging from attenuated vaccine strains to very virulent viruses (vvIBDV). In Algeria, despite the prophylactic measures implemented, cases of IBD are still often diagnosed clinically and the current molecular epidemiology of IBDV remains unknown. The presence of the virus and especially of strains genetically close to vvIBDV was confirmed in 2000 by an unpublished OIE report. In this study, nineteen IBDV isolates were collected in Algeria between September 2014 and September 2015 during clinical outbreaks. These isolates were analyzed at the genetic, antigenic and pathogenic levels. Our results reveal a broad genetic and phenotypic diversity of pathogenic IBDV strains in Algeria, with, i) the circulation of viruses with both genome segments related to European vvIBDV, which proved as pathogenic for specific pathogen-free chickens as vvIBDV reference strain, ii) the circulation of viruses closely related - yet with a specific segment B - to European vvIBDV, their pathogenicity being lower than reference vvIBDV, iii) the detection of reassortant viruses whose segment A was related to vvIBDV whereas their segment B did not appear closely related to any reference sequence. Interestingly, the pathogenicity of these potentially reassortant strains was comparable to that of reference vvIBDV. All strains characterized in this study exhibited an antigenicity similar to the cognate reference IBDV strains. These data reveal the continuous genetic evolution of IBDV strains in Algerian poultry through reassortment and acquisition of genetic material of unidentified origin. Continuous surveillance of the situation as well as good vaccination practice associated with appropriate biosecurity measures are necessary for disease control.

Abbreviations: aa, amino acid; AC-ELISA, antigene-capture enzyme-linked immunosorbent assay; b/B, bursa to body-weight ratio; BLAST, basic local alignment search tool; CAM, chorio-allantoic membrane; cDNA, complementary DNA; DPI, days post-inoculation; EID_{50}, median embryo infectious dose; IBDV, infectious bursal disease virus; mAb, monoclonal antibody; NGS, next generation sequencing; OIE, office international des épizooties = world organization for animal health; PCR, polymerase chain reaction; RT, reverse transcription; SPF, specific pathogens-free; vvIBDV, very virulent IBDV

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1. Introduction

Infectious bursal disease (IBD) is an acute and highly contagious viral disease of young chickens. It is one of the most important avian viral diseases, due to the economic losses it causes to the poultry industry as a result of high mortality rates in its acute clinical form and of immunosuppression induced by subclinical infection (Eterradossi and Saif, 2013). The etiological agent of IBD, infectious bursal disease virus (IBDV), is a non-enveloped virus belonging to the *Avibirnavirus* genus within the *Birnaviridae* family (Delmas et al., 2004). Two serotypes of IBDV have been recognized and designated as serotype 1 and 2 (Ismail and Saif, 1990; Jackwood et al., 1985; McFerran et al., 1980). All viruses that cause disease in chickens belong to serotype 1, whereas serotype 2 viruses are non-pathogenic for both chickens and turkeys (Eterradossi and Saif, 2013). IBDV genome consists of two segments of double-stranded RNA of approximately 3.2 and 2.8 kbp, named segment A and B, respectively (Müller et al., 1979). The larger segment A contains two open reading frames (ORF). The smaller ORF encodes a non-structural polypeptide of 17 kDa known as VP5, a pro-apoptotic factor thought to facilitate viral release (Méndez et al., 2017). The larger ORF encodes a polyprotein that is autoproteolytically cleaved into three polypeptides: pVP2 (48 kDa), the precursor of the capsid protein (Da Costa et al., 2002; Letzel et al., 2007), VP3 (32 kDa), the ribonucleoprotein (Luque et al., 2009), and VP4 (28 kDa) which is the viral protease responsible for polyprotein cleavage (Birghan et al., 2000). Segment B encodes VP1, the 95 kDa RNA-dependant RNA-polymerase responsible for viral genome replication and synthesis of mRNA (Dobos et al., 1979; Macreadie and Azad, 1993). Both genome segments contribute to the pathogenicity of the most virulent strains of IBDV (very virulent IBDV or vvIBDV) (Escaffre et al., 2013), that emerged in Europe in the late nineties (Eterradossi and Saif, 2013). There is significant antigenic, immunogenic, and pathogenic variation between IBDV strains which contribute to determine disease outcome. Controlling IBD and its associated immune suppression is critical to the broiler industry. This control is achieved by vaccinating breeder hens with conventional live attenuated and/or inactivated IBD vaccines, and by the use of live IBD vaccines in broiler chicks, layers or young pullets to provide active protection against IBDV (Müller et al., 2012). Both strategies are often combined. Different criteria are used to establish vaccination programs, and different vaccine strains are used without a complete knowledge of the characteristics of the IBDV strains prevalent in the field. In Algeria, IBD was first observed in poultry production after the emergence of the virus in Europe in the late 1980s (Allamigeon and Comte, 2001). The presence of vvIBDV in Algeria was first suspected in 2000 when the VP2 gene of seven IBDV isolates was partially sequenced (OIE report to the Algerian OIE delegate, unpublished). In GenBank, there are some other reports of IBDV partial sequences for VP2 genetically related to vvIBDV originating from Algeria (Boudaoud, A., unpublished), but reports describing these strains and especially their phenotype have not been published yet.

Currently, despite some biosecurity and vaccination measures implemented at farm level, IBD cases are still often diagnosed, mostly on a clinical basis (Alloui and Sellaoui, 2015), and the molecular epidemiology of Algerian IBDV strains remains largely unknown. In order to get a better understanding of the current epidemiological situation, this study was designed to characterize the genetic, antigenic and pathotypic diversity of some IBDV isolates collected between September 2014 and September 2015 in comparison with isolates from the 2000 study.

2. Materials and methods

2.1. Ethics statement

All animal experiments were performed in agreement with national regulations (authorization by French Ministry for higher education and research under permit number APAFIS#4945-20 16041316546318 v6) on animal welfare and after approval of the protocols by Anses' ethical committee, registered at the national level under number C2EA-016.

2.2. Samples

Bursal samples (n = 190) were collected between September 2014 and September 2015 from 35 broiler chicken flocks suspected to be infected with IBDV. These flocks were located in the major poultry production provinces from the North and the Center of Algeria.

All bursal samples showed lesions of congestion, edema, haemorrhage, hypertrophy or atrophy. They were stored at −20 °C until further analysis in the laboratory. Background information for the studied samples is documented in Supplementary Table 1.

2.3. Reference viruses

Two reference viral strains were used in this study: strain F52/70 (Bygrave and Faragher, 1970) as reference for classical virulent strains, and strain 89163 (Eterradossi et al., 1992) as reference for vvIBDV.

2.4. Previously collected Algerian IBDV isolates

In 1999, the authors' laboratory (ANSES Ploufragan, acting as one of the OIE reference laboratories of IBD) participated in a world-wide survey of IBDV isolates collected during acute IBD outbreaks (Eterradossi et al., 2004). From Algeria, seven isolates were collected in 2000. These were characterized by antigen-capture ELISA and partial sequencing of their VP2 gene (Report to the OIE delegate of Algeria, unpublished). These strains were included in the phylogenetic analysis in this study (Supplementary Table 2).

2.5. Sample preparation

Each virus suspension was prepared separately starting from Bursae of Fabricius of each flock as previously reported (Eterradossi et al., 1992). The viral suspension was treated with chloroform (volume to volume) by stirring overnight at 4 °C to inactivate any enveloped viral contaminants possibly present in the samples.

2.6. Reverse transcription-PCR and partial segment sequencing

Viral RNA was extracted from 140 µL of viral suspension using the QiAMP Kit RNA Mini Kit (Qiagen) as recommended by the manufacturer. Reverse transcription, PCR amplification using chimeric primers and sequencing of viral segments A and B were performed as previously described (Le Nouën et al., 2006), resulting in 514 base pairs (from position 744 to 1180) and 544 base pairs (from position 297 to 1749) fragments for segments A and B, respectively.

2.7. Full-length sequencing of genomic segments by next-generation sequencing (NGS)

One milliliter of IBDV viral suspension was centrifugated at 12 000 g for 10 min. Supernatant was collected, filtered through a 0.22 µm filter (Millex-GV filter unit, Merck-Millipore) and 250 µL of filtrate were incubated for 1 h at 37 °C with 250 Units DNase I and 10 µg RNase to degrade all non-encapsidated nucleic acids. Encapsidated RNA was then extracted using Trizol LS Reagent (Life Technologies) according to the manufacturer's instructions.

Complementary DNA (cDNA) libraries were prepared using the Ion Total RNA-Seq Kit (Life Technologies, Carlsbad, California, USA) according to the supplier's instructions. The cDNA libraries were sequenced using the Ion Proton Sequencer and an Ion PI Chip v2 (Life Technologies).

Reads were cleaned with the Trimmomatic 0.36 software, then a Bowtie 2 (version 2.2.5) alignment was performed with reads on local