Infection, Genetics and Evolution 13 (2013) 18–26

Contents lists available at SciVerse ScienceDirect

Infection, Genetics and Evolution

journal homepage: www.elsevier.com/locate/meegid

Tracking the molecular epidemiology of Brazilian Infectious bursal disease virus (IBDV) isolates

Fernanda M.F. Silva [a,1], Pedro M.P. Vidigal [a,b,1], Luciana W. Myrrha [a], Juliana L.R. Fietto [a], Abelardo Silva Jr. [a,c], Márcia R. Almeida [a,*]

[a] Molecular Animal Infectology Laboratory, Institute of Applied Biotechnology to Agriculture (BIOAGRO), Federal University of Vicosa, Vicosa 36570-000, Brazil
[b] Bioinformatics Laboratory, Institute of Applied Biotechnology to Agriculture (BIOAGRO), Federal University of Vicosa, Vicosa 36570-000, Brazil
[c] Animal Virology Laboratory, Veterinary Department, Federal University of Vicosa, Vicosa 36570-000, Brazil

ARTICLE INFO

Article history:
Received 22 March 2012
Received in revised form 30 August 2012
Accepted 4 September 2012
Available online 18 September 2012

Keywords:
Infectious bursal disease virus
Epidemiology
Phylogenetics
Avian diseases

ABSTRACT

Infectious bursal disease is a highly contagious disease of young chickens caused by Infectious bursal disease virus (IBDV). Genome segment A encodes the capsid protein (VP2), while segment B encodes the RNA-dependent RNA polymerase (VP1). In the present study, we trace the molecular epidemiology of IBDV in Brazil by analyzing 29 isolates collected in the major regions of poultry production. To genetically characterize the isolates, phylogenetic and population dynamic analyses were conducted using 68 VP1 (2634 nt) and 102 VP2 (1356 nt) coding sequences from IBDV isolates from different regions of the world. Furthermore, the evolution of IBDV was analyzed by characterizing the selective forces that operated during the diversification of viral isolates. We show that IBDV isolates were introduced into Brazil mainly from the Netherlands and the USA. These introductions were associated with all Brazilian poultry production regions analyzed in this work. In addition, we show that the evolution of IBDV has been shaped by a combination of very low recombination rates and relatively high rates of nucleotide substitution (2.988×10^{-4} for VP1 and 3.2937×10^{-4} for VP2), which themselves are a function of purifying selection operating on VP1 and VP2. Furthermore, our extended Bayesian skyline plot suggests that the increase in the effective population size of isolates of IBDV is consistent with its epidemiological history, with a large increase during the emergence of acute outbreaks of IBD in the 1980s.

1. Introduction

Infectious bursal disease virus (IBDV, genus *Avibirnavirus*, family Birnaviridae) is the etiologic agent of infectious bursal disease (IBD), an acute and highly contagious disease affecting young chickens. IBDV is a non-enveloped virus with a capsid containing a genome of two segments (segments A and B) of double-stranded RNA (Dobos et al., 1979). Segment A encodes a precursor polyprotein in a major open reading frame (ORF), which is cleaved by autoproteolysis to yield the mature VP2 (outer capsid), VP4 (protease), and VP3 (inner capsid) proteins (Kibenge et al., 1988). VP2 is also the immunodominant antigen of IBDV (Vakharia et al., 1994). Segment B encodes VP1 (viral RdRp), which performs essential functions in viral replication and transcription (Von Einem et al., 2004).

The potential for genetic variability in RNA viruses has long been considered fundamental to their evolution, adaptation, and escape from host responses (Moya et al., 2000). Since the late 1980s, when IBDV led to high economic losses to the poultry industry, genotyping studies of the VP2 sequences have been widely used as a way to trace changes in the virulence of IBDV isolates (Banda and Villegas, 2004; Hon et al., 2006; Jackwood and Sommer-Wagner, 2007; Sreedevi et al., 2007; Yamaguchi et al., 1997). Most exchanges of amino acid residues in VP2 occur in the four hydrophilic loops of the viral capsid (Coulibaly et al., 2005). These exchanges indicate that selective pressure for the evolution of IBDV is directly focused on the capsid regions that are immediately exposed to the immune system (Durairaj et al., 2011).

Epidemiological research is also critical for understanding viral evolution. Acute severe outbreaks of IBD, due to very virulent IBDV (vvIBDV), were first detected in Europe in 1987 (Chettle et al., 1989; Van den Berg et al., 1991) and since then have been reported worldwide. In Brazil, severe outbreaks of IBD have been reported since July 1997 (Di Fabio et al., 1999). Phylogenetic analyses of the hypervariable regions of VP2 suggested that virulent IBDV isolates in Latin America emerged from virulent strains from Europe

* Corresponding author. Address: LIMA. BIOAGRO, Sala 07, Av. P.H. Rolfs s/n, Campus da UFV, Vicosa, Minas Gerais 36570-000, Brazil. Tel.: +55 31 3899 2911; fax: +55 31 3899 2373.
E-mail address: marcia@ufv.br (M.R. Almeida).
[1] These authors equally contributed to this paper.

1567-1348
http://dx.doi.org/10.1016/j.meegid.2012.09.005

or Asia (Banda and Villegas, 2004). Studies on the molecular characterization of partial sequences of VP2 (Fernandes et al., 2009) using restriction fragment length polymorphisms (RFLPs) (Gomes et al., 2005) suggested these same origins for Brazilian IBDV isolates. On the other hand, further molecular epidemiological studies with a larger number of isolates are still needed to fully understand the origins of IBDV in Brazil.

Prediction of viral genealogies can support an epidemiological framework when the data are correlated in a multidisciplinary way using evolutionary approaches, economic statistics, and information about viral isolation (Vidigal et al., 2012). To expand our understanding of the molecular epidemiology of IBDV in Brazil, the coding sequences of VP1 and VP2 of 29 Brazilian isolates collected in the major regions of poultry production were analyzed by phylogenetic approaches. Additionally, viral isolates of other countries were also analyzed to better understand the molecular epidemiology of Brazilian IBDV isolates. The correlation of the predicted genealogies with the live poultry trade provided important insights about the possible routes of introduction of IBDV in Brazil. A population history of IBDV isolates was reconstructed, and selective forces that operated during the diversification of viral isolates were also characterized, providing further information about the evolution of IBDV.

2. Materials and methods

2.1. Viral samples

Twenty-nine samples of bursa of Fabricius were analyzed in this work. These samples were collected in 1997 and 2000–2009 and are representative of the major regions of poultry production in Brazil: 4 samples from Minas Gerais (MG), 11 samples from São Paulo (SP), 8 samples from Paraná (PR), 1 sample from Mato Grosso (MT), and 5 samples from Santa Catarina (SC) (Table 1).

2.2. RNA extraction, RT-PCR, and sequencing

Total viral RNA in the samples was extracted from 250 μL of homogenized bursa tissue using 750 μL of TRIZOL LS reagent (Invitrogen), according to the manufacturer's protocol. Total RNA was quantified by spectrophotometry (OD_{260}) and stored at −80 °C. Viral cDNA was amplified using the SuperScript™ III First-Strand Synthesis System (Invitrogen) following the manufacturer's instructions.

The PCR was performed using 15 μL of GoTaq Green Master Mix (PROMEGA), 20 pmol of each specific primer, 2 μL of the viral cDNA, and water up to 30 μL. A 625 nt VP2 fragment was amplified using the primer pair VP2-F1 (5′-ATGACAAACCTGCAAGATCAAACC-3′) and VP2-R1 (5′-TTATGGTGTAGACTCTGGGCCTGT-3′). A 752 nt VP2 fragment was amplified using the primer pair VP2-F2 (5′-AGAGTCTACACCATAACTGCAGCC-3′) and VP2-R2 (5′-CCTCCTTATGGCCCGGAT-3′). These two pairs of primers were designed to amplify a sequence of 1356 nt from positions 131–1486 of segment A of the IBDV genome, which corresponds to the coding sequence (CDS) of the VP2 protein (452 aa). The VP1 polymerase was amplified using primer pairs previously described by Yu et al. (2010). The 2634 nt sequence amplified from positions 112–2745 of segment B of the IBDV genome corresponds to the cds of the VP1 protein (878 aa).

The PCR products were purified from agarose gels and sequenced by Macrogen Inc. (Seoul, Korea). The PCR products were successfully sequenced in 6/29 VP1 reactions and 26/29 VP2 reactions. Contigs of the nucleotide sequences of VP1 and VP2 were assembled using the CAP3 program (Huang and Madan, 1999).

Table 1
Sequences of the Brazilian isolates of IBDV.

Target	GenBank ID	Isolate	Area of isolation[a]	Collection year	Phylogenetic group
VP1	JN982245	SP14	SP	2004	VP1-I
VP1	JN982246	MG1	MG	2009	VP1-I
VP1	JN982247	MG8	MG	2009	VP1-I
VP1	JN982248	SC11	PR	2004	VP1-I
VP1	JN982249	SP11	PR	2004	VP1-II
VP1	JN982250	SP6	SP	2004	VP1-II
VP2	JN982251	MG8	MG	2009	VP2-I
VP2	JN982252	MG4	MG	2009	VP2-I
VP2	JN982253	SP9	PR	2004	VP2-I
VP2	JN982257	SP5	SP	2001	VP2-I
VP2	JN982270	SP14	SP	2004	VP2-I
VP2	JN982271	SC1	MT	2004	VP2-I
VP2	JN982272	SC2	SC	2005	VP2-I
VP2	JN982273	SC5	PR	2000	VP2-I
VP2	JN982274	SP3	PR	2004	VP2-I
VP2	JN982275	SP8	PR	2004	VP2-I
VP2	JN982276	SP10	SP	2001	VP2-I
VP2	JN982254	SC9	SC	2002	VP2-II
VP2	JN982255	SP22	SP	2004	VP2-II
VP2	JN982256	SP33	SP	2003	VP2-II
VP2	JN982258	SP17	SP	1997	VP2-II
VP2	JN982259	SP25	SP	2001	VP2-II
VP2	JN982260	SP26	SP	2001	VP2-II
VP2	JN982261	SP28	SP	2001	VP2-II
VP2	JN982262	SP11	PR	2004	VP2-II
VP2	JN982263	SC6	SC	2001	VP2-II
VP2	JN982264	SP20	PR	2003	VP2-II
VP2	JN982265	SP21	PR	2003	VP2-II
VP2	JN982266	SP30	SP	2000	VP2-II
VP2	JN982267	SC8	SC	2005	VP2-II
VP2	JN982268	SC10	SC	2002	VP2-II
VP2	JN982269	SC12	MG	2005	VP2-II

[a] Brazilian states: MG = Minas Gerais; MT = Mato Grosso; PR = Paraná; SC = Santa Catarina; SP = São Paulo.