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BRIEF REPORT

Cattle influenza D virus in Brazil is divergent from established lineages

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Abstract

Influenza D virus (IDV) is endemic in cattle on several continents and can also infect a wide range of hosts. IDV was first detected in a bovine respiratory disease outbreak associated with bovine alphaherpesvirus 1 in Brazil. Sequence analysis of partial segments showed that the virus is phylogenetically divergent from previously described IDVs from other continents. As the first molecular description of IDV in South America, this can be a first step toward investigating IDV infections in cattle in Brazil and surrounding countries in which the beef industry is economically important.

Influenza D virus (IDV), a member of the family *Orthomyxoviridae*, has recently been suggested to be a pathogen involved in bovine respiratory disease. Its presence and clinical severity are dependent on multiple factors, such as viral and bacterial coinfections [1]. Although cattle are the main hosts and reservoir of this virus, its zoonotic potential is not clear, since it can cause respiratory disease in pigs, and antibodies have been detected in several other ruminant species and humans [2]. IDV has already been described in ruminants in North America [3], Europe [4], and Asia [5, 6], and the high serological prevalence in cattle herds in countries with an important cattle industry, such as the USA [7] and many European countries [4], suggesting that IDV is probably a worldwide-distributed cattle virus. Brazil has the largest commercial cattle population and is the largest beef exporter in the world. However, IDV has not been described on the South American continent despite serological evidence [6]. Here, we describe for the first time the presence of IDV in Brazil. IDV was detected in a bovine respiratory disease outbreak associated with bovine alphaherpesvirus 1 (BoHV-1). Phylogenetic analysis showed that the IDV isolate from Brazil is divergent from previously described IDV lineages from North America, Europe, and Asia, revealing that IDV circulates in Brazilian cattle herds (and probably in South America), and its involvement should be considered in respiratory illness outbreaks.

Nasal swab samples from nine European-breed calves aged 4-8 months were collected for diagnostic investigation of a bovine respiratory disease outbreak on a cattle farm in the city of Rio Pardo, Rio Grande do Sul, the southernmost Brazilian state. Several RT-PCR and PCR assays were performed to detect viruses associated with bovine disease, including BoHV-1 [8], bovine viral diarrhea virus [9], parainfluenza virus 3 [10], bovine respiratory syncytial virus [11], bovine coronavirus (BCoV) [12], and IDV [13]. All samples were positive by BoHV-1 PCR and negative for the other pathogens, except two samples, one of which was positive by RT-PCR for BCoV, and the other for IDV [13]. An unsuccessful attempt was made to isolate the virus in embryonated eggs and a sensitive cell culture (ST cell line). Due to the limited amount of sample available, the IDV sample was subjected to high-throughput sequencing (HTS) so that the IDV genome segments could be sequenced and phylogenetically analyzed. Isolated RNA was converted to cDNA, which was enriched, used to prepare a library using a Nextera XT DNA Sample Preparation Kit (Illumina), and sequenced on an Illumina MiSeq platform. High-quality contigs with similarity to IDV sequences were using the BLASTx tool from GenBank, and genome segments were

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determined using the map-to-reference tool in Geneious Prime® software. Partial sequences were obtained from five out of the seven IDV genome segments and were deposited in the GenBank database (accession numbers OK648460-OK648464). Partial sequences were obtained by HTS from segment 2 (PB1), segment 3 (P3), segment 4 (HEF), segment 5 (NP), and segment 6 (p42), whereas no sequence data were obtained for segments 1 (PB2) and 7 (NS2). Maximum-likelihood phylogenetic analysis of sequences from the five segments of this IDV isolate (named D/BR) was performed using MEGA software version X by comparing them to sequences belonging to the two reference IDV lineages D/bovine/Oklahoma/660/2013 (D/660 lineage) and D/swine/Oklahoma/1334/2011 (D/OK lineage) [14] and putative divergent Japanese lineages (Yamma/2016 and Yamma/2019) [15]. The segment that is primarily used for phylogenetic analysis of IDV is segment 4, encoding the hemagglutinin-esterase-fusion (HEF) protein, which is the most variable segment of the IDV genome.

Comparison of the HEF sequences of the Brazilian isolate and representative of the major IDV lineages D/660 and D/OK showed that the nucleotide sequence identity ranged from 98.3 to 99.6% and 98.2 to 100%, respectively. In comparison to the D/BR lineage isolates D/bovine/Oklahoma/660/2013 and D/swine/Oklahoma/1334/2011, it was 97% and 95%, respectively, and even lower (94-95%) when compared with the isolates Yamma/2016 and Yamma/2019. Corroborating this result, the phylogenetic tree topologies revealed a putative new IDV cluster, with the sequences generated in this study clearly clustering into a separate group based on HEF sequences (Fig. 1) and those of the other segments (Fig. 2). Antigenicity experiments could not be performed due to our inability to isolate the virus. Coinfection with BoHV-1 and the limited amount of sample contained in nasal swabs were major limitations in virus isolation, and we believe that these issues, along with the possibility that the samples were collected late in the course of infection, could be the reason for the small amount of IDV obtained and for the fact that only partial segments were sequenced. Nonetheless, all the phylogenetic trees, including the HEF partial segment tree, showed that D/BR diverges from the previously described lineages.

This is the first molecular detection of IDV in South America from a case of bovine respiratory disease. This virus was phylogenetically divergent from known IDVs described in North America, Europe, and Asia. This detection highlights the importance of investigating IDV as a possible causative agent of respiratory disease on the continent and suggests that a divergent lineage of IDV is circulating in Brazilian cattle herds.

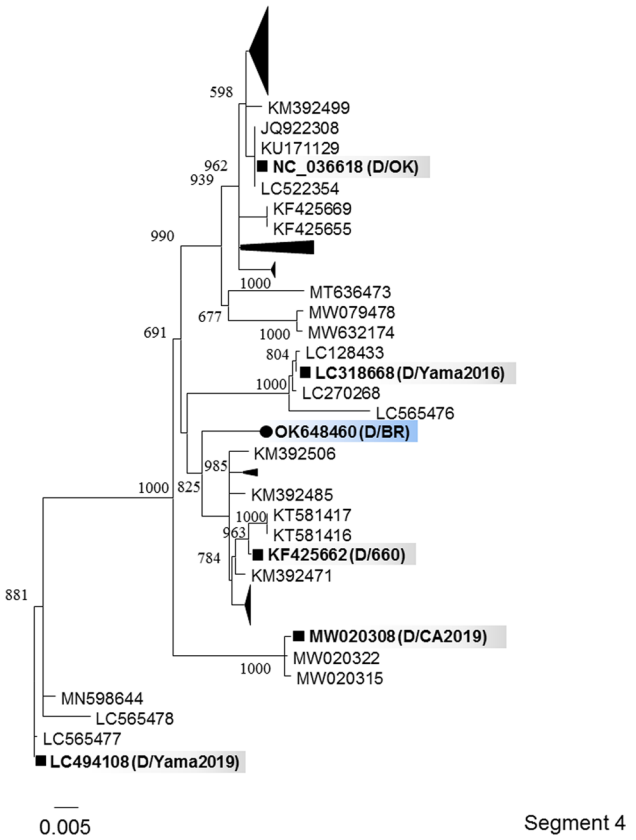

Fig. 1 Phylogenetic tree based on IDV HEF sequences (segment 4; 675 nt). Phylogenetic analysis was performed using the maximum-likelihood method with 1,000 bootstrap replicates. Branches with less than 50% bootstrap support were omitted. Evolutionary distances were computed using SMS (smart model selection) in PhyML 3.0 and AIC (Akaike information criterion). All IDV sequences available in the GenBank database were included in the analysis. Some branches were collapsed due to the large number of sequences. The sequence from this study is indicated in blue with a dot (●), and sequences representing the five established lineages are indicated in gray with a square (■).

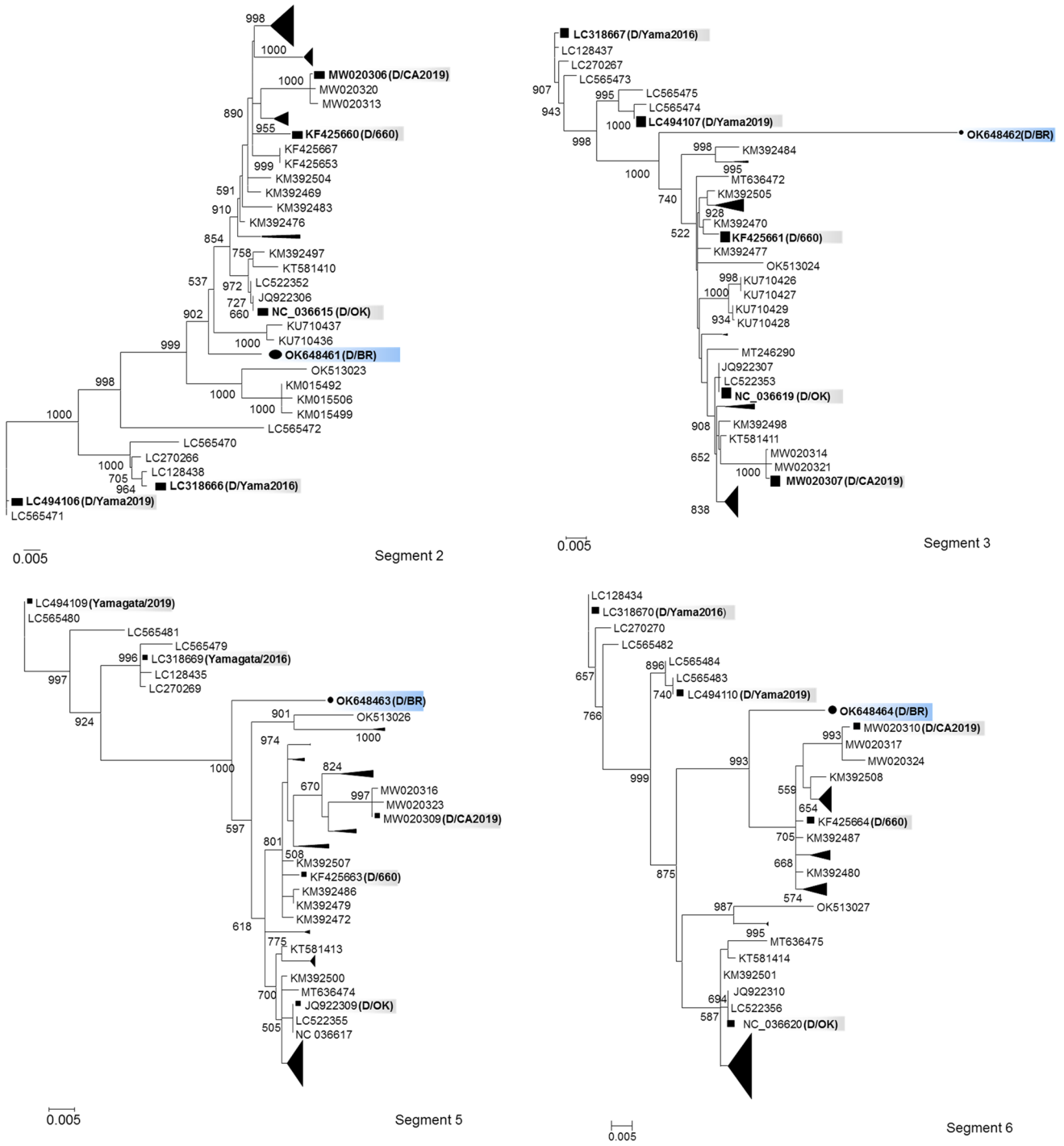

Fig. 2 Phylogenetic trees based on partial nucleotide sequences of four IDV genome segments. (A) Segment 2 polymerase PB1 (PB1) gene (1594 nt). (B) Segment 3 polymerase P3 (P3) gene (1355 nt). (C) Segment 5 nucleocapsid protein (NP) gene (764 nt). (D) Segment 6 p42 (p42) gene (546 nt). Phylogenetic analysis was performed using the maximum-likelihood method with 1000 bootstrap replicates as described above. The sequence from this study is indicated in blue with a dot (●).

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Author contributions Mariana S. da Silva and Ana Cristina S. Mosena were involved with all stages of the experiment. Letícia Baumbach, Saulo P. Pavarini, and David Driemeier contributed with material collection and preparation. Meriane Demoliner and Juliana S. Gularte

performed HTS sequencing. The first draft of the manuscript was written by Mariana S. da Silva and Ana Cristina S. Mosena. Matheus N. Weber, Fernando R. Spilki, and Cláudio W. Canal conceived the study and contributed to the final version of the manuscript. All authors commented on previous versions of the manuscript and approved the final manuscript.

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Data availability The nucleotide sequences generated and used in the current study are available in the GenBank database.

Declarations

Conflict of interest The authors have no competing interests to declare that are relevant to the content of this article.

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