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Short communication

Molecular detection and genomic characteristics of bovine kobuvirus from dairy calves in China

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ABSTRACT

In this study, 96 diarrheic and 77 non-diarrheic fecal samples from dairy calves were collected from 14 dairy farms in 4 provinces to investigate the molecular prevalence and genomic characteristics of Bovine Kobuvirus (BKoV) in China. The results showed that the BKoV positive rate for the diarrheic feces (35.42%) was significantly higher than that for the non-diarrheic feces (11.69%, p < 0.001). Interestingly, three potential novel VP1 lineages were identified from 15 complete VP1 sequences, and a unique triple nucleotide insertion which can result in an aa insertion, was first observed in the 11/12 VP0 fragments with 660 bp long in this study, compared with known BKoV VP0 sequences. Moreover, the first Chinese BKoV genome was successfully obtained from a diarrheic fecal sample, named CHZ/CHINA. The open reading frame (ORF) of the genome from strain CHZ/China shares 87.4%–88.3% nucleotide (nt) and 93.7%–96.4% amino acid (aa) identity, compared with the three known genomes of BKoV. Interestingly, phylogenetic tree based on aa sequences of these genomes showed that CHZ/CHINA was clustered into an independent branch, suggesting the strain may represent a novel BKoV strain. The findings contribute to better understanding the molecular characteristics and evolution of BKoV.

Keywords: BKoV; VP0; VP1; VP0 nucleotide; VP1 amino acid

Abbreviation: BKoV, Bovine kobuvirus; BRV, bovine rotavirus; BCoV, bovine coronavirus; BVDV, bovine viral diarrhea virus; ORF, open reading frame; nt, nucleotide; aa, amino acid

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instructions (TaKaRa Bio Inc.) and then stored at −20 °C. BKoV was detected by an RT-PCR assay targeting a 631 bp fragment of the 3D gene according to previous report (Jeoung et al., 2011). To screen for the presence of co-infections with bovine rotavirus (BRV), bovine coronavirus (BCoV), and bovine viral diarrhea virus (BVDV), all the BKoV-positive diarrheic samples were subjected to specific RT-PCR assays for these viruses (Guo et al., 2019; Zheng et al., 2014).

The complete VP1 (801 nt) sequences were amplified from the BKoV-positive samples according to previous report (Liu et al., 2013). A pair of primers was designed based on known BKoV VP0 sequences, located at positions 1012–1671 in CHZ/China genome sequence. Moreover, 13 pairs of primers (Table S1) were used to amplify the BKoV CHZ/China strain. All PCR products were purified using the Omega Gel kit (Omega), cloned into the pMD19-T simple vector (TaKaRa Bio Inc.), and then sequenced (Sangon Biotech) in both directions. Sequences were assembled using SeqMan software (version 7.0; DNASTAR Inc.), and then sequenced (Sangon Biotech) to answer the question as to whether BKoV causes diarrhea. It may be that calf challenge experiments will be the only way to further to be investigated.

Table 1

| Region     | Number of samples | Positive rate |
|------------|-------------------|---------------|
| Liaoning   | 18                | 83.33% (15/18) |
| Henan      | 27                | 44.44% (12/27) |
| Shandong   | 11                | 63.64% (7/11)  |
| Shanxi     | 40                | 0              |
| Total      | 96                | 35.42% (34/96) |

| The number of non-diarrhea samples and test results |
|-----------------------------------------------|
| Number of samples | Positive rate |
|-------------------|---------------|
| Liaoning           | 0             |
| Henan              | 25            |
| Shandong           | 11            |
| Shanxi             | 40            |
| Total              | 77            |

In this study, we added to a nearly complete BKoV strain (GenBank accession No. MK800265) genome of 7907 nt in length which contains the 7395 bp complete ORF, which is the first BKoV genome from China. Compared with 3 known BKoV, with the exception that the CHZ/CHINA strain's VP0 sequence is 3 nt longer, the lengths of the other CHZ/CHINA genes are identical to those of the other three genomes (Table S3) and shares 87.4%–88.3% nt and 93.7%–96.4% aa identity. Further phylogenetic analysis based on genomic sequences revealed that CHZ/CHINA clusters on an independent branch, with the three VP0, VP3, VP1 protein aa sequences generating the same result (Fig. 3), showing that CHZ/CHINA displays a larger genetic distance from the other three genomes and indicating that CHZ/CHINA may represent a novel BKoV strain. Moreover, the most significant difference between CHZ/CHINA and other BKoV...
Fig. 1. Maximum likelihood phylogenetic tree based on complete VP1 nt sequences. Black circles denote isolates from the present study and hollow circles denote isolates from a previous Chinese study (Liu et al., 2013). Bootstrap values based on 1000 replicates are shown on the nodes.
strains is the VP0 protein. And VP0 from the CHZ/CHINA strain contains 19 unique aa mutations, and a unique triple nt insertion which can result in an aa insertion. The function of BKoV VP0 remains unclear. But, both VP0 and VP1 in AIV may be involved in cellular receptor recognition (Zhu et al., 2016) and viral pathogenesis (Adzhubei et al., 2013). Thus, it is worth studying the functional effects of this unique VP0 aa mutation in BKoV strains.

In conclusion, the results of this study showed that three potential novel VP1 lineages in BKoV were identified and a unique BKoV VP0 sequence type was found in diarrheic feces. The first nearly complete BKoV genome was obtained and phylogenetic analysis shows that this strain may represent a novel BKoV strain. These data contribute to further understanding of the molecular characteristics and genetic evolution of BKoV.

Fig. 2. Maximum likelihood phylogenetic tree based on 220 aa sequence alignment of the VP0 protein from BKoV strains. Black circles denote isolates from the present study. Bootstrap values based on 1000 replicates are shown on the nodes.

Fig. 3. Maximum likelihood phylogenetic tree based on sequence alignments for the ORF, VP0, VP3, and VP1 protein encoding domains from BKoV strains. Black circles denote isolates from this study. Bootstrap values based on 1000 replicates are shown on the nodes.
Depositories

More information about sequences is in the GenBank database: MK080201–MK080265.

Ethical statement

This study did not involve animal experiments besides the fecal sampling of diarrhea calves that visited farm for clinical treatment.

Declaration of Competing Interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.meegid.2019.103939.

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