First report and genomic characterization of a bovine-like coronavirus causing enteric infection in an odd-toed non ruminant species (Indonesian tapir, *Acrocodia indica*) during an outbreak of winter dysentery in a zoo

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Abstract

Bovine Coronavirus (BCoV) is associated with three distinct clinical syndromes in cattle i.e. neonatal diarrhea, hemorrhagic diarrhea in adults (the so-called winter dysentery syndrome, WD) and respiratory infections in cattle of different ages. In addition, bovine-like CoVs have been detected in various species including domestic and wild ruminants. However, bovine-like CoVs have not been reported so far in odd-toed ungulates. We describe an outbreak of WD associated with a bovine-like CoV affecting several captive wild ungulates, including Indonesian tapirs (*Acrocodia indica*) an odd-toed ungulate species (Perissodactyla) which, with even-toed ungulates species (Artiodactyla) form the clade Euungulata (Graur et al, 1997). Genomic characterization of the CoV revealed that it was closely related to BCoVs previously reported in America. This case illustrates the adaptability of bovine-like CoVs to new species and the necessity of continued surveillance of bovine-like CoVs in various species.

KEYWORDS

bovine-like CoV, diarrhea, tapir, genomic characterization

1. INTRODUCTION

Coronaviruses (CoVs) form a large group of viruses that infect a wide diversity of mammalian and avian species causing respiratory, enteric, neurologic and hepatic disorders. CoVs taxonomically belong to the subfamily *Orthocoronavirinae*, in the family *Coronaviridae*, order *Nidovirales* (https://talk.ictvonline.org/taxonomy/). Currently, CoVs are classified into four different genetic genera: *Alphacoronavirus* (group 1), *Betacoronavirus* (group 2), *Gammacoronavirus* (group 3), and *Deltacoronavirus* (group 4). They are enveloped viruses with a positive-sense single-stranded RNA genome. CoVs have the larger genome among all RNA viruses (26 to 32 kb) (Masters, 1999). CoVs can quickly adapt to new hosts and ecological niches. This is attributed to the high mutation rate, their large genomes, and the high frequency of recombination events during RNA replication (Drake and Holland, 1999; Woo et al, 2006).

Bovine Coronavirus (BCoV) was first reported in cattle in the late 1970s (Kaye, Yarbrough, & Reed, 1975; Khamassi Khbou, Daaloul Jedidi, Bouaicha Zaafouri, & Benzarti, 2021). It belongs to the species *Betacoronavirus 1* (subgenus *Embecovirus*) of the *Betacoronavirus* genus along with bovine-like CoVs from sheep, goat, llama, and alpaca (Amer, 2018), human HCoV-OC43 (St-Jean et al., 2004), porcine hemagglutinating encephalomyelitis virus (PHEV) (Lorbach et al., 2017), equine coronavirus (ECoV) (Pusterla, Vin, Leutenegger, Mittel, & Divers, 2016), canine respiratory coronavirus (CRCoV) (Erles & Brownlie, 2008), camel coronavirus (Woo et al., 2014; Wunschmann, Frank, Pomeroy, & Kapil, 2002), bubaline coronavirus (BuCoV) (Decaro et al., 2010), and Yak coronavirus (He, Guo, Zhang, Yue, & Tang, 2019). *Betacoronavirus 1* species members appear to be host-range variants of a parental virus through interspecies transmission events and genome recombination (Alekseev et al., 2008; Cui, Li, & Shi, 2019; Decaro & Lorusso, 2020; Lau et al., 2011; Salem et al., 2020).

BCoV possess 5 major structural proteins: the nucleocapsid protein (N, 50 kDa), the integral membrane (M, 25 kDa), the small membrane/envelope protein (E, 8 kDa), the haemagglutinin-esterase (HE, 120–140 kDa) and the spike (S, 190 kDa). The S protein consists of an S1 subunit that contains the dominant neutralizing epitopes (Schultze, Gross, Brossmer, & Herrler, 1991; Yoo & Deregt, 2001) and an S2 subunit that mediates viral membrane fusion. The S2 subunit of the spike glycoprotein of bovine coronavirus mediates membrane fusion in insect cells. The N protein lies internal to the virus envelope and is associated with the viral RNA, the M spans the viral envelope while the S and HE project from the envelope. In addition to these structural proteins, there are 16 non-structural proteins (nsp, 1-16) produced in the ORF1ab polyprotein and 4 nsp (32 kDa, 4.9 kDa, 4.8 kDa, and 12.7 kDa) in the 3' end (Alekseev et al., 2008).

BCoVs cause respiratory and enteric diseases in cattle and other ruminants (Amer, 2018). It is shed in feces and nasal secretions, and is associated with 3 distinct clinical syndromes in cattle: neonatal diarrhea, winter dysentery (WD) characterized by hemorrhagic diarrhea in adults and respiratory infections in cattle of different ages (Vlasova & Saif, 2021). Of interest, no distinct genetic or antigenic markers have been identified in BCoVs associated with these distinct clinical syndromes (Suzuki, Otake, Uchimoto, Hasebe, & Goto, 2020; X. Zhang et al., 2007).

Besides cattle, bovine-like CoVs were identified in numerous domesticated ruminants such as water buffalo, sheep, goat, dromedary camel, llama and alpaca as well as wild ruminants including wild goats, sitatunga, waterbuck, musk oxen, white-tailed deer, giraffes, Sambar deer, and sable antelope, dogs and even humans (Amer, 2018; Decaro & Lorusso, 2020; Hasoksuz et al., 2007; He et al., 2019; Majhdi, Minocha, & Kapil, 1997; Tsunemitsu, el-Kanawati, Smith, Reed, & Saif, 1995; Vlasova & Saif, 2021; Wunschmann et al., 2002; X.

M. Zhang, Herbst, Kousoulas, & Storz, 1994). Bovine-like CoVs cannot be reliably distinguished from BCoVs using comparative genomics.

In this report, we describe an outbreak of WD in several captive wild ungulates, including a couple of Indonesian tapirs (*Acrocodia indica*), associated with a bovine-like CoV. Interestingly, to our knowledge, it is the first time that a bovine-like CoV is identified in the Indonesian tapir (*Acrocodia indica*), an ungulate but a non-ruminant species. The sequence and phylogenetic analyses of the virus are described.

2. MATERIALS AND METHODS

2.1 Clinical signs and laboratory examination

The outbreak occurred in a zoo located in the Montreal area (Quebec, Canada). No cattle farms were present in the vicinity of the outbreak. Except for one person which has occasional and limited contact with a cattle farm, the zoo personals have no contact with domestic ruminants outside the zoo. No clinical signs of WD have been reported on this farm before or after the WD outbreak in the zoo. Clinical signs began in mid January 2021 with severe haemorrhagic diarrhea (dysentery) in a female Watusi (*Bos taurus primigenius*). Diarrhea spreads within a few days to three other Watusi cattle, a male Gaur (*Bos frontalis*), a male common eland (*Taurotragus oryx*), two Roan antelopes (*Hippotragus equinus*) as well as ten waterbucks (*Kobus ellipsiprymnus*). Interestingly a male and a female of Indonesian tapirs were also affected. The attack rate varied depending on the species: 7% for the common elands, 25% for the gaurs, 50% for the Watusis, 80% for the waterbucks, and 100% for the Indian tapirs and the Roan antelopes. The animals were hosted in two close buildings. Diseased animals presented soft and sometimes blood tinted feces. Respiratory symptoms were not observed. No other species present in the same facilities including Addax (*Addax nasomaculatus*) (n = 16), Dromedaries (*Camelus dromedarius*) (n = 17), Oryx (*Oryx*

dammah) (n = 10), wapitis (*Cervus canadensis*) (n = 17), water buffalos (n = 10), and wildebeests (*Connochaetes gnou*) (n = 6) were affected. Supportive care was given to the more severely affected animals. Clinical signs disappeared after 5-7 days in each affected group. All diseased animals recovered completely.

Fecal samples were collected from several diarrheic animals including the female Watusi, a common eland, three Waterbucks, one Roan Antilope, and the couple of Indonesian tapirs. First cases (the female Watusi, 3 waterbucks and the common eland) were examined for Rotavirus A (RVA), Bovine-like Coronavirus (BCoV), Bovine Viral Diarrhea Virus (BVDV), Bovine Torovirus (BToV), *Salmonella spp*, *Escherichia coli* K99(F5), *Cryptosprodium spp* and *Giardia duodenalis* using commercial RT-PCR kits described below. As only BCoV was detected, subsequent cases (tapirs and roan antelopes) were examined only for RVA, BCoV, BVDV and BToV.

Fecal suspension was prepared by diluting two grams of stool in ten ml PBS. Then, suspensions were homogenized by vigorous vortexing and decanted. Nucleic acids were extracted directly from fecal suspension, using QIAamp DNA Mini Kit (QIAGEN, Hilden, Germany) according to manufacturer's instructions and eluted in 200 µl of nuclease-free water. Samples were examined for the major viral (BCoV, BToV, BVDV and RVA), bacterial (*Salmonella spp* and *E. coli* K99/F5), and parasite (*Cryptosporidium spp* and *Giardia duodenalis*) ruminant enteric pathogens using Bovichek® Calves Intestinal Multiplex (CIM)-Virus qPCR and Bovichek® Calves Intestinal Multiplex (CIM)-DNA qPCR (Biovet). Testing was performed according to manufacturer's instructions. Results were interpreted as "positive" when the Ct value was ≤ 36, and "negative" when the Ct value was > 36.

Seven BCoV RT-qPCR positive samples were also examined for BCoV by antigen capture ELISA (Pathasure® Enteritis 3, Biovet, Saint-Hyacinthe, QC, Canada) and

immunochromatography (Enterichek®, Biovet) (Table 1). Testing was carried out according to the manufacturer's instructions.

2.2 Next generation sequencing and sequence analysis

Fecal samples from the tapir female (1044512-1) and one Watusi (1042952-4) were further examined by next generation sequencing. Extracted positive RNA samples were subjected to a sequence-independent, single-primer amplification (SISPA), then Nextera XT kit was used for library preparation, and the library was sequenced on iSeq 100 using iSeq 100 i1 Reagent v1 kit (300 cycle) (Chrzatek et al, 2017; Wang, Stuber, Camp, Robbe-Austerman, & Zhang, 2016). Raw FastQ files were assembled using SPAdes (Bankevich et al., 2012). Local nucleotide blast of assembled contigs was performed to determine if the complete genome was obtained. Then a blast search of assembled sequences was performed using NCBI basic local alignment search tool (https://blast.ncbi.nlm.nih.gov/Blast.cgi) (Altschul, Gish, Miller, Myers, & Lipman, 1990). Tapir CoV 1044512-1 strain was compared with other selected BCoV strains downloaded at GenBank (www.ncbi.nlm.nih.gov/genbank/) (Table S1). Sequence alignment of complete genome, HE, S, N, and 4.8 kDa nsp genes were performed using MEGA version 7.0.26 (Kumar, Stecher, & Tamura, 2016), and phylogenetic tree was constructed using maximum likelihood method and Tamura-Nei model. The 3D protein structure of spike and HE of Tapir CoV was predicted using the Iterative Threading ASSEmbly Refinement (I-TASSER) protein-modelling online platform (http://zhanglab.ccmb.med.umich.edu/I-TASSER/) (Yang & Zhang, 2015; C. Zhang, Freddolino, & Zhang, 2017).

3. RESULTS AND DISCUSSION

The origin of the diarrhea outbreak remained undetermined. Maybe some unaffected animals were carriers and shedders of BoCV and contaminated susceptible animals. Bovine-like CoV

was the only pathogen detected in the fecal samples from the Watusi female, the waterbuck and Roan antelopes as well as in the male and the female Indonesian Tapirs (Table 1). The Ct values for the fecal samples examined ranged from 16.1 to 29.0. The samples with Ct values below 20 were also positive in the antigen capture ELISA and by immunochromatography. The samples from the two tapirs had Ct values of 18.4 and 29.0. Some affected animals (male common eland and gaur) were negative to BCoV (data not shown).

Bioinformatic analysis of assembled sequences of tapir 1044512-1 and Watusi 1042952-4 indicated that complete genome of tapir CoV 1044512-1 and partial genome of Watusi CoV 1042952-4 (4.8 kDa nsp, E, and N genes have complete genome and 4.9 kDa nsp, 12.7 kDa nsp and M genes have partial genome) were obtained. The accession numbers of complete tapir CoV 1044512-1 and partial Watusi CoV are MZ100070 and MZ144606, respectively. Tapir CoV 1044512-1 has a genome size of 31,016 bases in length. Online blast searches on the tapir CoV complete sequence revealed that this sequence is more related to BCoV 4-17-08 strain (GenBank No. MH043954) isolated in US Pennsylvania dairy calves with nucleotide identity of 99.53% and then to another BCoV Pennsylvania dairy calves strain 7-16-23 (99.38% identity) (Byukusenge et al., 2018). Tapir CoV had a similar genome organization to BCoVs (Figure 1A). In comparison with the BCoV 4-17-08, Tapir CoV 1044512-1 has 145 nucleotide variations throughout the genome with 67 differences in ORF1a, 21 in ORF 1b, 4 in 32kDa protein, 6 in HE, 38 in S, 1 each in 4.9 kDa protein and 4.8 kDa protein, and 3 in M. Interestingly, there are 4 nucleotide differences located in the intergenic region between 4.9 kDa protein and 4.8 kDa protein (Figure 1B). Blast search of the partial Watusi CoV 1042952-4 genome showed that it also was closely related to BCoV 4-17-08 strain with an identity of 99.79%. In addition, there was a 99.9% nucleotide identity between tapir CoV and Watusi CoV in the region from 4.9 kDa nsp to N gene. On the other hand, blast search of spike gene of tapir CoV 1044512-1 showed it had the highest identity

(99.19%) with another BCoV strain VB 7/09/MAYABEQUE/2009 (GenBank No. HE616738) from Cuba. There is 33 nucleotide differences in S gene between tapir CoV and VB 7/09/MAYABEQUE/2009 which is less than 38 nucleotide difference between tapir CoV and BCoV 4-17-08. Since the complete genomes of this BCoV strain are not available, it remains unknown about the identities of them at other genome regions. Overall, these data indicated that the same bovine-like CoV strain derived from a BCoV strain could cause infections in different animal species.

In addition to analysis of nucleotide, amino acid sequence comparison of Tapir CoV with BCoV 4-17-08 showed that there were 3 amino acid variations (V52A, N87K, D98H) in 32 kDa nsp, 1 change (V386L) in HE, 10 changes (G492D, N499S, P501S, Y507S, T509N, Y525H, S718L, V744F, N785K, S927A) in S, 1 change (G38A) in 4.8 kDa nsp, and 1 change (I159V) in M gene (Figure 1B, 1C, and S1). Eight of 10 changes in the spike were located in the S1 region. Tapir CoV has 8 amino acid differences (G492D, Y507S, S718L, V744F, N785K, S927A, H1260D, and N1285K) with BCoV VB 7/09/MAYABEQUE/2009 in the S protein. When blast search of S protein amino acid sequence of tapir CoV, it showed the highest identity (99.49%) with Sable antelope coronavirus US/OH1/2003 (GenBank No. EF424621), and there were 7 sites with amino acid difference (P174S, H509N, P546S, S718L, V744F, N785K, S927A) between them (Figure 1C). Among these changes, tapir CoV had four sites (718L, 744F, 785K, 927A) with unique changes and two sites (492D and 507S) with the same changes as Sable antelope US/OH1/2003. The predicted 3D structure of S protein showed that most of those changes in S1 region were on the surface of protein (Figure 1C).

Phylogenetic tree analysis of complete genome demonstrated that tapir CoV1044512-1 was closely related to four BCoV strains from Pennsylvania under US wild ruminant genotype previously defined (Suzuki et al., 2020) (marked with blue squares in Figure 2A). A similar

pattern was observed in HE tree (Figure 2B). However, in the phylogenetic tree of S gene, tapir CoV 1044512-1 was more closely related to three other BCoV strains with two from Cuba (VB 7/09/MAYABEQUE/2009 and VB 16/10/CIENFUEGOS/2010 strains) and one (VP200 strain) from Vietnam than four Pennsylvania BCoV strains (Figure 2C). In the tree of N gene, tapir CoV and Watusi CoV cluster together with BCoV 4-17-08 as well as other BCoVs and BCoV like strains (Figure 2D). These data further supported that tapir CoV and Watusi CoV were bovine-like CoV strains.

A previous study (Suzuki et al., 2020) reported that BCoVs had different genome sizes of 4.8 kDa nsp gene: 138 and 129 nucleotides. In our study, analysis of BCoV and BCoV-like strains showed that more different sizes of 4.8 kDa nsp were observed (Figure 3). These data showed that 4.8 kDa nsp contained a variable region with different sizes of deletions. Tapir and Watusi bovine-like CoVs have the same genome size (126 nt) of 4.8 kDa nsp as one of the four Pennsylvania BCoV strains (4-17-08). Other three Pennsylvania BCoV strains have 138 nt (7-16-23, 4-17-25) and 129 nt (4-17-03) in ORF6. It is unknown about the role of 4.8 kDa NSP in virus replication and whether the deletion in 4.8 kDa nsp affect virus replication. Different sizes of deletions were previously reported in the nsp genes of different CoVs, including the ORF3a and ORF3b of porcine respiratory coronavirus and NS2 and NS4.9 of PHEV (Kim et al., 2000; Lorbach et al., 2017).

This study reported detection of coronavirus from an outbreak of WD in zoo animals (Watusi, waterbuck, roan antelope as well as indonesian tapir). The very high genome identities (>99.5%) of tapir and Watusi bovine-like CoVs to one US BCoV strain, indicating that a bovine-like CoV strain can have caused the outbreak in these zoo animals. A previous study from South Korean reported that CoV caused an outbreak in four different zoo ruminant species was also a bovine-like CoV strain, highly correlated to BCoV strains isolated in Korea (Chung, Kim, Bae, Lee, & Oem, 2011). Similarly, CoVs detected in four

species of captive wild ruminants (sambar deer, waterbuck, sable antelope, and a white-tailed deer) in US were also bovine-like CoVs (Alekseev et al., 2008). Till now, bovine-like CoVs have been detected in many domestic and wild ruminants, and their genome identities are very high, indicating that viruses take advantage of variable hosts to produce so many host-range variants. This unique feature of multiple host variants for *Betacoronavirus* 1 might be not common for many other CoV species that produce variants by heavily relying on accumulation of mutations and recombination to evade from host immune response. Bovine-like CoVs were reported in many domestic (goat, sheep, water buffalo, dromedary camel, alpaca, llama) and wild ruminants (reindeer, elk, samber deer, sika deer, musk oxen, wisent, wood bison, water buck, sitatunga, stable antelope, nyala, giraffe, and Himalayan tahr) (Amer, 2018). By contrast they were never reported before in odd-toed ungulates such as horses, donkeys, zebras, rhinoceroses, and tapirs. As a CoV (not a bovine-like CoV) has been identified in horses (ECoV) (Pusterla et al, 2016), it is not really surprising that a CoV was detected in another odd-toed ungulate species. However, it is maybe more surprising that this CoV was more related to BCoV than ECoV, another odd-toed non-ruminant species. In fact, our study is the first to report the detection of bovine-like CoV in an odd-toed non-ruminant species, in the present case an Indonesian tapir. This observation is stressing the need for continuing monitoring of the evolution of bovine-like CoVs, especially in the context of recent recognition of several new coronaviruses in various animal species as well as in human.

There are very few studies about how BCoV cross species barrier to infect other hosts including humans. It was reported that HCoV-OC43 might evolve from BCoV and adapt to humans though progressive loss of HE lectin activity (Bakkers et al., 2017; Vijgen et al., 2005). Since HE protein sequences of tapir bovine-like CoV and BCoV 4-17-08 are exactly the same except one variation V386L located outside of previously defined functional

domains (membrane-proximal domain, esterase domain, and lectin domain) (Figure S1), it is less plausible that a parental BCoV strain would utilize the same approach to adapt infecting tapir as it would to infect humans. Instead, multiple changes in the S protein might contribute to the adaptation of BCoV to infect tapir, since there are 7 to 10 amino acid differences observed between tapir bovine-like CoV and other three strains (BCoV 4-17-08, VB 7/09/MAYABEQUE/2009, and Sable antelope US/OH1/2003). These changes are located in both S1 receptor domain and S2 membrane fusion domain. Future studies are needed to explore the role of these changes in the adaption of BCoV to tapirs and to confirm its pathogenetic role in this species.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

ETHICS STATEMENT

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to. Samples were submitted as part of routine clinical diagnostic testing following institutional and national guidelines.

DATA AVAILABILITY STATEMENT

Data set of this study is available within article and sequences obtained in this study were deposited to GenBank (Tapir coronavirus 1044512-1: MZ100070 and Watusi coronavirus 1042952-4: MZ144606).

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Table 1. Results of fecal testing for BCoV

Species	BCoV rtPCR (Ct)	Pathasure® Enteritis 3 BCoV	Enterichek® BCoV
Watusi	16.1	Strong reaction	Strong reaction
Waterbuck-1	17.3	Mild reaction	Weak reaction
Waterbuck-3	17.2	Weak reaction	Mild reaction
Tapir (female)	18.4	Weak reaction	Weak reaction
Tapir (male)	29.0	No reaction	No reaction
Roan antelope	22.9	No reaction	No reaction
Waterbuck-2	24.5	No reaction	No reaction

Figure Legends

Figure 1. (A) Genomic diagram of Tapir coronavirus 1044512-1 (GenBank accession number MZ100070) with a genome size 31016 bp in length. Its genome consists of 5' and 3' untranslated regions (UTR) at both ends, and ORF1a, ORF1b, 32 kDa non-structural protein (nsp), hemagglutinin–esterase protein (HE), spike glycoprotein (S), 4.9 kDa nsp, 4.8 kDa nsp, 12.7 kDa nsp, envelope protein (E), membrane protein (M), and nucleocapsid protein (N). (B) Diagram of genome difference between Tapir coronavirus 1044512-1 and bovine coronavirus 4-17-08 (GenBank accession number MH043954). A white color line represents a nucleotide difference. These two strains have a 99.53% nucleotide identity with 145 nucleotide differences. There are 67 and 21 nucleotide differences in ORF1a and ORF1b, respectively, 4 in 32 kDa nsp, 6 in HE, 38 in S, 1 in 4.9 kDa, 4 in the intergenic region between 4.9 and 4.8 nsps, 1 in 4.8 kDa nsp, and 3 in M. Comparison of amino acid sequences of tapir CoV 1044512-1 and BCoV 4-17-08 showed that 3 changes (V52A, N87K, D98H) in 32 kDa nsp, 1 change (V386L) in HE, 1 change (G38A) in 4.8 kDa nsp, and 1 change (I159V) in M gene. (C) Structural modelling of S proteins of Tapir coronavirus 1044512-1 carried out by I-TASSER (Iterative Threading ASSembly Refinement). S1 and S2 subunits were shown in orange and cyan, respectively. In comparison with bovine coronavirus 4-17-08, VB 7/09/MAYABEQUE/2009, and Sable antelope coronavirus US/OH1/2003S, 14 amino acid changes in the Tapir coronavirus strain are shown as spheres mode in the structure. Four unique changes in Tapir coronavirus were presented with hot pink color spheres and remaining10 non-unique changes were presented with green and yellow spheres in S1 and S2, respectively. The amino acids of these 14 sites for these four coronaviruses were listed. A blue line was placed in the S1/S2 junction site.

Figure 1

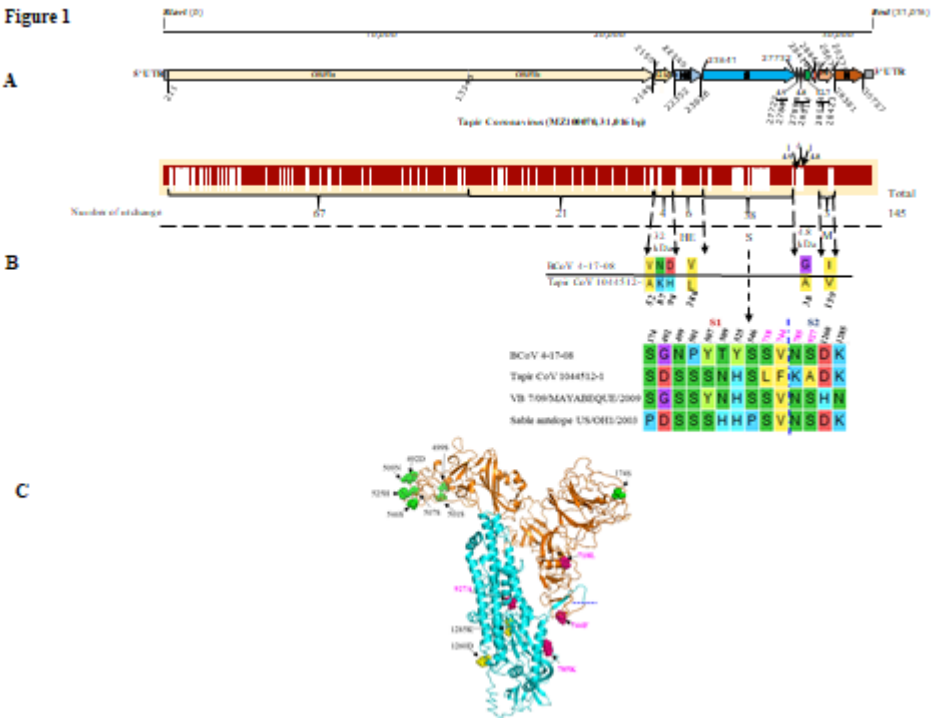

Figure 2. Phylogenetic tree analysis of complete genomes (A), HE (B), S (C), and N (D) genes of *betacoronavirus* 1 including tapir coronavirus 1044512-1. Tapir coronavirus 1044512-1 (GenBank accession number MZ100070) and Watusi coronavirus 1042952-4 (GenBank accession number MZ144606) identified in this study were marked with red square, and four bovine coronavirus strains from US Pennsylvania were marked with blue square. Each sequence used in the trees is labeled with its accession number and strain name. Scale bar indicates nucleotide substitutions per site. Bovine coronavirus (BCoV) and camel coronavirus (Camel CoV) clusters were indicated on trees.

Figure 2B

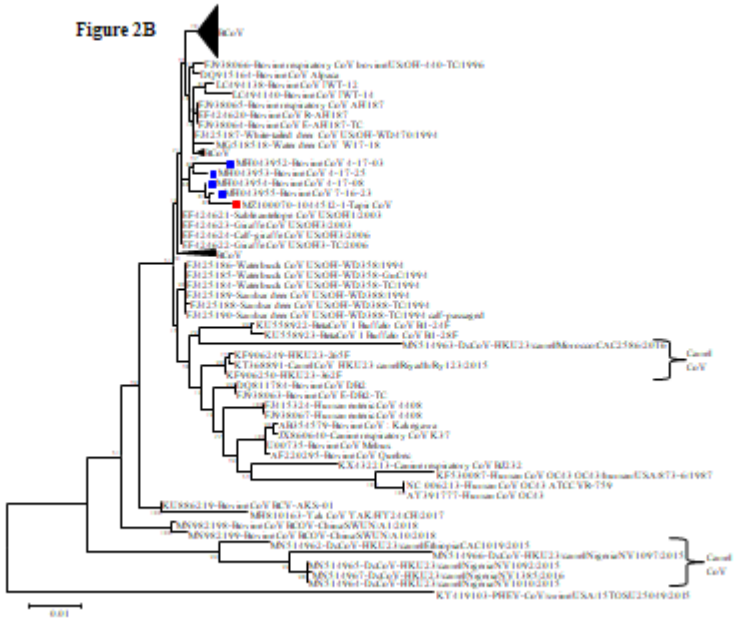

Figure 2C

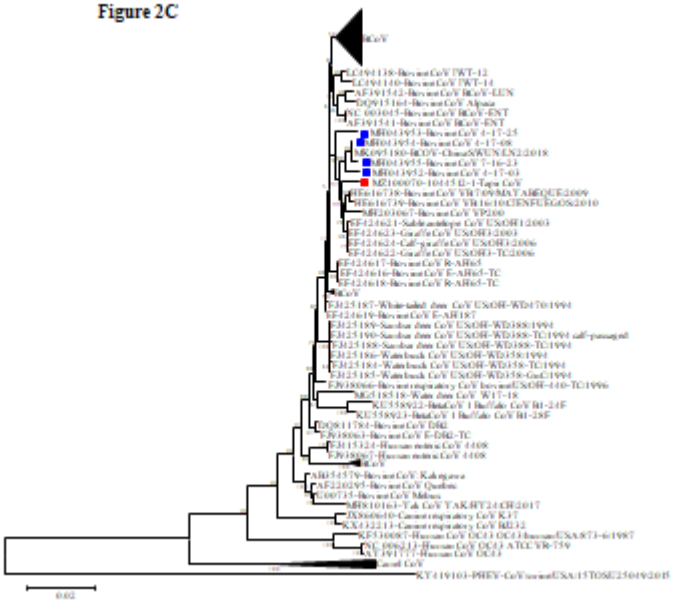

Figure 2C

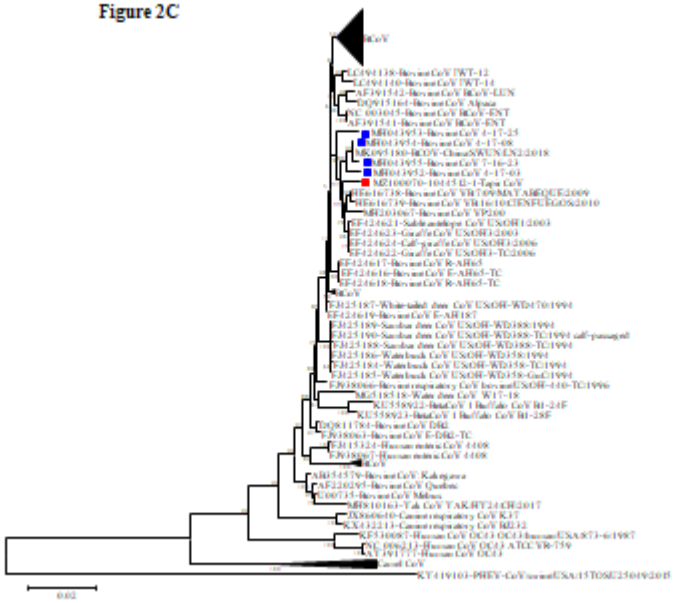

Figure 3. Sequence alignment of 4.8 kDa non-structural protein (nsp) of bovine coronavirus and bovine-like coronavirus strains. Tapir coronavirus 1044512-1 (GenBank accession number MZ100070) and Watusi coronavirus 1042952-4 (GenBank accession number MZ144606) identified in this study were marked with red square, and bovine coronavirus strains from US Pennsylvania were marked with blue square. The regions containing deletion were marked with a red color frame. The AA size of 4.8 kDa nsp for each strain were indicated.