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Genome sequencing and characterization analysis of a Beijing isolate of chicken coronavirus infectious bronchitis virus

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Abstract **Avian infectious bronchitis virus (AIBV) is classified as a member of the genus coronavirus in the family coronaviridae. The enveloped virus has a positive-sense, single-stranded RNA genome of approximately 28 kilo-bases, which has a 5′ cap structure and 3′ polyadenylation tract. The complete genome sequence of infectious bronchitis virus (IBV), *Beijing isolate*, was determined by cloning sequencing and primer walking. The whole genome is 27733 nucleotides in length, has ten open reading frames: 5′-*orf1a-orf1ab-s-3a-3b-e-m- 6a-6b-n*-3′. Alignments of the genome sequence of IBV *Beijing isolate* with those of two AIBV strains and one SARS coronavirus were performed respectively. The genome sequence of IBV *Beijing isolate* compared with that of the IBV strain *LX4* (uncompleted, 19440 bp in size) was 91.2% similarity. However, the full-length genome sequence of IBV *Beijing isolate* was 85.2% identity to that of IBV Strain *Beaudette*, and was only 50.8% homology to that of SARS coronavirus. The results showed that the genome of IBV has remarkable variation. And IBV *Beijing isolate* is not closely related to SARS coronavirus. Phylogenetic analyses based on the whole genome sequence, S protein, M protein and N protein, also showed that AIBV *Beijing isolate* is lone virus in group Ⅲ and is distant from SARS coronavirus. In conclusion, this study will contribute to the studies of diagnosis and diseases control on IBV in China.**

Keywords: avian infectious bronchitis virus (AIBV), severe acute respiratory syndrome coronavirus (SARS-CoV), primer walking, sequence analysis.

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Avian infectious bronchitis virus (AIBV) is a highly infectious, contagious pathogen of chicken and causes disease of the respiration and urogenital tracts, resulting in significant economic losses to poultry industries throughout the world[1]. In China, IBV leads to more than RMB one billion yuan losses every year. IBV, as a member of the family coronaviridae in the new order Nidovirales, has a single-stranded positive-sense RNA genome, which is 27.6 kb in length[2]. The genome of IBV has more than 10 open reading frames, which encode two polyproteins and three major structural proteins and other small proteins. Three major structural proteins are the spike glycoprotein (S), the membrane glycoprotein (M), and the phosphorylated nucleocapsid protein (N). The S protein is cleaved to release S1 and S2 proteins after translation. The S1 protein induces protective immunity[3—5]. And the most antigenic of the virus neutralization antibody-inducing epitopes is formed by a few amino acids that occur in the first and third quarters of the S1 subunit, especially in the hypervariable regions[6,7].

IBV is the lone virus in the antigenic group Ⅲ of coronaviridae[8], however, more than 20 serotypes within IBV have been recognized worldwide on the basis of virus-neutralization test[9]. Therefore, it is very difficult to prevent from the infections of virus. The whole genome of IBV was determined in 1987[2]. And the genome sequences of IBV are very important and useful for disease diagnosis and vaccination. However, remarkable variation was observed in various IBV isolates: the genome of IBV isolate in this region may be very different with that in the other regions. IBV *Beijing isolate*, which caused great economic losses, was obtained from a chicken house in Yukou, Beijing[10].

In additional, an outbreak of atypical pneumonia, named severe acute respiratory syndrome (SARS) by the WHO, was identified in Guangdong Province, China, and spread to several countries. This disease is great threatening to the health of people worldwide. Many reports showed that a novel coronavirus was the pathogen of the SARS[11—13]. And scientists considered that SARS coronavirus could be from some animals. After the complete genome of IBV Beijing isolate was sequenced, we determined the relations between IBV *Beijing isolate* and SARS coronavirus.

1 Materials and methods

(ⅰ) Virus. IBV *Beijing isolate*[10] was propagated in 10-d-old specific-pathogen-free (SPF) embryonated chicken eggs. The allantoic fluid was collected 24—36 h after inoculation, clarified by centrifugation at 5000 r/min for 30 min, 4℃. The pellet was discarded and the supernatant was concentrated by more than 2 h high-speed centrifugation (30000 r/m) at 4℃. The supernatant was discarded and the pellet was resuspended in PBS (pH 7.2). The virus suspension was ultra-centrifuged through a 30%/40%/50% saccharose step gradient at 32000 r/min for 3 h at 4℃. Mixture between 40% and 50% saccharose was transferred and PBS (pH 7.2) was added. Samples were mixed and concentrated by centrifugation at 30000 r/min for 2 h at 4℃. The supernatant was discarded and

the virus pellet was resuspended in PBS. The virus suspension was divided into 250 μL each tube and was stored at –80℃ until use.

(ii) Viral RNA extraction and RT-PCR. Viral genomic RNA was extracted with Trizol LS reagent (Invitrogen, USA). Each 250 μL virus suspension was added with 750 μL Trizol LS reagent and mixed. The homogenized samples was incubated for 5 min at 15℃—30℃ and then added with 200 μL chloroform. The capped sample tubes were shaken vigorously by hand for 15 s and incubated at 15℃—30℃ for 2 min. The samples were centrifuged at 1100 r/min for 15 min at 4℃. The upper aqueous phase was transferred to a clean tube and added with the same volume of isopropyl alcohol. The samples were incubated at 15℃—30℃ for 10 min after being mixed. The mixture was centrifuged at 1100 r/min for 10 min at 4℃. The supernatant was discarded and RNA pellet was washed twice with 75% ethanol. At the end of the procedure, the RNA pellet was air-dried (about 15 min) and dissolved by 10 μL 0.1% DEPC-treated water.

The random hexameric primer (Promega, USA) or a specific reverse primer was used in cDNA first strand synthesis. After cDNA first-strand was synthesized, each specific primer pair was used in the reaction of PCR. A 20-μL reaction volume was used for the first strand cDNA synthesis. Total RNA (10 μL) and random hexameric primer (0.5 μg/μL, Promega, USA) or a specific reverse prime 1 μL were added to a nuclease-free microcentrifuge tube. Mixture was heated to 70℃ for 10 min and was quickly chilled on ice for 1—2 min. After brief centrifugation, the following components: 4 μL 5×first-strand buffer, 2 μL 0.1 mmol/L DTT, 1 μL RNaseOut (40 U/μL) and 1 μL M-MLV reverse transcriptase (200 U/μL) were added. After being mixed gently, the mixture was incubated at 37℃ for 1h. Samples were inactivated at 70℃ for 15 min and subsequently treated with 2 U of Ribonuclease H at 37℃ for 30 min. The cDNA was used as template for amplification in PCR. A 30 μL reaction volume was used in PCR. The components were 1.5 μL cDNA, 3 μL 10×buffer, 2 μL 2.5 mmol/L dNTP each, 1 μL of each primer (25 μmol/L), 0.5 μL Taq DNA polymerase (5 U/μL) and 21 μL ddH_2O. PCR was carried out with the following thermocycling parameters: denaturation at 94℃ for 5 min, 35 or 40 cycles of denaturation at 94℃ for 40 s, annealing at 49℃—53℃ for 1 min, and extension at 72℃ for 1—2 min or 68℃ for 4—5 min, a final extension at 72℃ for 10 min and store at 4℃. Annealing and extension conditions were dependent on primers and the length of amplification fragments. Some primers used in PCR are listed in Table 1.

Rapid amplification of cDNA ends (RACE) was performed to capture the 3′ and 5′ ends of the viral genome. A 20-μL reaction volume was used for the first strand cDNA synthesis in 3′ RACE. The procedure was done as follows: AP (5′-GGCCACGCGTCGACTAGTA-CTTTTTTTTTTTTTTTTT-3′) 1 μL, mixture contained 10 μL total RNA, were heated to 70℃ for 10 min and was quickly chilled on ice for 1—2 min. After brief cen-

Table 1 Primers for PCR

Primer	Position/nt	Size/nt	Forward primer	Reverse primer
gp5t	1—355	355	ACT TAA GAT AGA TAT TAA TAT A	GGT GAC CCC GTA/G TTT C
gp1	1042—1928	887	CAT GGA TGC TT/CT GGG CTC AAA T/C	TCC/T TCA AGA/G TTT/G CGC ACT TTT CC
gp3	2817—6014	3198	G/ACG GTA AAG AC/TT TGG ATT GC/TA T	GCA/G TGT TTA/G TAC AAA TGA AGC
gp3-2	2793—3540	748	AGA ATG TC/TG CAC TTG TTG ATA A	TT/CT TAG CC/TT TCT/C TCT CAC GTT
gp3-3	4050—6014	1965	TGA G/ACC AAG AA/GC AC/TA/G TCG ATT A	AA/GG CG/AT GTT TG/AT ACA AAT GAA G
gp4	6708—10454	3747	TTC TTA AGG AT/AG CAC A/TA/GA AAT G	TAA CA/CA TG/AA CA/TG CCA TTA GA/TA C
gp4-2	6960—9601	2642	ATT ATA AGG CA/GG GCA CAC TTC G	ATT G/ATC A/CC/G CA/TG CCC ACT TAT TA
gp5	10498—14242	3745	TTG TTA CTT TGG A/GTT C/GTA TTG G	CTA TAC GCA ACA AAT TT/AG GCA T
gp5-2	12341—12956	616	TCT GTT CAA TCA GTT GCT GGA/T	CCA ACA TAA CCT/C TTT TCA ACC A
gp5a	14594—17221	2627	AAA AGT TTT ATT CTT ACA/T TG/AT G	CTT CTA CAT CAA AAC CNA C
gp5a-2	14896—16710	1815	GTA GAT GAC/A GTG GAT AAG ACA	CTG G/ATT CAT AGC ATT GTA AGG T
gp6	17612—18097	486	TTA ATT CTC ATA CTC AG/AG A/CTT A	ACA TTG/A CAA TTA/C CAA AAC ATA C
gp8	18442—21319	2878	TAT/C AAT GCA GCT GTT/C ACA	TAG/A AAT TAT AAT AAC CAC TCT
gp9	21706—23182	1477	TTT AG/AA TAA/C G/TTG TGT TGA G/TTA T	GTA TGG TTA A/GG/AA CAT GTC T/GT
gp9-3	22193—22681	489	TTC/T AAC/T TTA/G ACT GTT ACA GAT G	CCA TAG AA/TG CTA CTA G/AAG AA
gp3t-2	25184—25901	718	GAT ACT GGC GAG CTA GAA AG	ACG CGG GAC AAG GCT CTG
gp3t-3	26209—27445	1237	GGT TTA AGC CAG GCA AAG GT	CTG GCG ATA GAC ATG TAG GT

trifugation, 2 μL 10×buffer, 2 μL 0.1 mol/L DTT, 2 μL 25 mmol/L $MgCL_2$, 1 μL 10mmol/L dNTP Mix, 1 μL RNaseOut (40 U/μL) and 1 μL M-MLV reverse transcriptase (200 U/μL) were added. Mixture was mixed gently and incubated at 37℃ for 1 h. Samples were inactivated at 70℃ for 15 min and subsequently treated with 1 μL Ribonuclease H (2 U/μL) at 37℃ for 30 min. 1.5 μL cDNA was used as template in a 25-μL PCR reaction volume. 0.5 μL AP, 0.5 μL GSP (5′-ATTACCTACATGTCTATCGCCA-3′), 2.5 μL 10×buffer, 25 mmol/L $MgCL_2$ 1.5 μL, 0.5 μL 10 mmol/L dNTP Mix, Taq DNA polymerase (5 U/μL) 0.5 μL, 17.5 μL ddH_2O were mixed gently and heated to 94℃ for 3 min to denauture. 35 cycles were carried out for 30 s at 94℃, 1 min at 62℃, 1 min at 72℃, with a final extension at 72℃ for 10 min, then stored at 4℃.

A 25-μL reaction volume was used in 5′RACE for the first strand cDNA synthesis. Mixture consisted of 15.2 μL total RNA, 0.3μL GSP1 (5′-ACTTAAGATAGATATTAATATA-3′, 25 μmol/L) was heated to 70℃ for 10 min and quickly chilled on ice for 1—2 min. After brief centrifugation , 2.5 μL 10×buffer, 2.5 μL 0.1 mol/L DTT, 2.5 μL 25 mmol/L $MgCL_2$, 1 μL 10 mmol/L dNTP Mix, 1 μL SuperScript Ⅱ RNase H^-RT (200 U/μL) were added. Mixture was mixed gently and incubated at 42℃ for 1 h. Samples were inactivated at 70℃ for 15 min and subsequently treated with 1 μL RNase Mix (2 U/μL) at 37℃ for 30 min. cDNA was purified and tailed before PCR. The two steps were done according to the instruction manual. A 30 μL reaction volume contained 3 μL cDNA, 3 μL 10×buffer, 2 μL 2.5 mmol/L dNTP each, GSP2 (10 μmol/L, 5′-CGCTACTTCCACACACCC-3′) 0.5 μL, AAP(10 μmol/L, 5′-GGCCACGCGTCGACTAGTACGGGIIGGGIIGGGIIG-3′) 0.5 μL, 0.4 μL Taq DNA polymerase (5 U/μL) and 21.3 μL ddH_2O was used for amplification in PCR. PCR was carried out with the following thermocycling parameters: denaturation at 94℃ for 3 min, 35 cycles of denaturation at 94℃ for 30 s, annealing at 57℃ for 45 s, and extension at 72℃ for 1 min, a final extension at 72℃ for 7 min and stored at 4℃.

(iii) Sequencing and analysis. PCR products were cut from 1% agarose gels and purified using the GENECLEAN Ⅲ Kit (Qbiogene). The purified PCR products were either sequenced directly or cloned into the PMD 18-T vector (TaKaRa Biotech. Co., ltd., China), and automated sequencing with BigDye Terminator kit (Perkin Elmer) run on an ABI 377 DNA Sequencer. Primer walking is the most common approach for directed sequencing[14], and was used mostly in this study.

Initially, eleven primer pairs to various regions of the genome were designed according to the full-length genome of IBV *strain Beaudette* (Accession：NC_001451.1) and the PCR products were 1.5 kb in size. Many products were obtained. However, none was the target fragments. The PCR products were purified and cloned into the PMD 18-T vector before sequencing. Each clone was sequenced using M13F or M13R. 160 sequences were obtained and used to search the related sequences in the GenBank using the BLAST software (http://www.ncbi.nlm.nih.gov/BLAST). The viral sequences were kept and compared to the complete genome of IBV *strain Beaudette* (Accession: NC_001451.1). Positions of the sequences were determined to have high similarity more than 80%. Twelve contigs and 13 gaps were got after these sequences assembled. According to these sequences, we designed additionally 12 primer pairs for gaps filling. The largest gap was less than 4 kb. Each primer pair was used for PCR and had a target fragment. These fragments were purified and both strands were sequenced. Sequences of gaps were determined by primer walking. Other 77 primers were designed for PCR and sequencing, but not all were used for PCR. Finally, the complete sequence was assembled, by using Phrap (http://www.phrap.org).

2 Results

The complete genome sequence of IBV Beijing isolate was determined using PCR and cloning before sequencing as well as primer walking approaches. After clones (recombinant plasmids) were sequenced, the assembly of 140 sequences were totally 11 kb in length. These sequences comprised 12 contigs, covering these regions more than 4-fold. 101 primers were used for direct sequencing, among which 36 primer pairs were used for PCR and 3 primers for RACE. 136 sequences were obtained by primer walking. The sequences assembled were about 17 kb in size. Totally, 276 sequences resulted in the complete 27733 bp sequence of IBV *Beijing isolate* genome. The average useful length of the component sequences was 350 bases. The IBV *Beijing isolate* genomic sequence has been deposited into GenBank (Accession: AY319651).

(ⅰ) Genome organization. The 3′ and 5′ ends of the viral genome were captured after rapid amplification of cDNA ends (RACE) was performed. A fragment about 300 bp in size from 3′RACE and a band about 350-bp-length from 5′RACE were purified and sequenced directly or cloned into the PMD 18-T vector before sequencing. The sequences of RACE were almost 300 bp in length, with a 17polyA tract in 3′RACE, and 13polyG tail in 5′RACE (Fig. 1).

The IBV genome has five major genes, with a typical gene order: *rep*, *spike*, *envelope*, *membrane*, *nucleocapsid*. The *rep* gene, which comprises approximately two-thirds of the genome, is encoded two polyproteins (encoded by orf1a and orf1b). The four genes downstream of *rep* encoded the structural proteins S, E, M and N. There are also

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  1 ATTACCTACATGTCTATCGCCAGGGAAATGTCTAATCTGTCTACTTAGTA  50
 51 GCCTGGAAACGAACGGTAGACCCTTAGATTTTAATTTAGTTTAATTTTTA 100
101 GTTTAGTTTAAGTTAGTTTAGAGTAGGTATAAAGATGCCAGTGCCGGGGC 150
151 CACGCGGAGTACGATCGAGGGTACAGCACTAGGACGCCCACTAGGGGAAG 200
201 AGCTAAATTTTAGTTTAAGTTAAGTTTAATTGGCTAAGTATAGTTAAAAT 250
251 TTATAGGCTAGTATAGAGTTAGAGCAAAAAAAAAAAAAAAAA
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(a)

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  1 GCACTCGAACCGACCACGGCGTCCGCAGGACGCTGGCACTAGGTAGCCCA  50
 51 AGCAACGGATGTAATAAAGTGAGACACAACAAAACAAGCAGTGATACTAG 100
101 CAACAATAAAAATTTAATAACTAAAACCTGACAGGTGGCCAGGTGCCATC 150
151 CAGGGCACTAAAGTGCACTGCAATGGAACACCTATAGGGACCAGCTGTAG 200
201 GTATTTAAGTCCGTTTTGTTAAGTTGGAAATCTAGCGCAAGGCTAGTGCA 250
251 ATAGATATATATTAATATCTATCTTAAGTCCCCCCCCCCCCC
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(b)

Fig. 1. Sequences obtained by RACE. (a) Sequence of 3′RACE; (b) sequence of 5′RACE.

genes encoding a number of nonstructural proteins that are located between S and E, and between M and N. The genome organization of IBV *Beijing isolate* was determined and ten open reading frames (ORFs) were predicted, including genes encoded unknown proteins: 3a, 3b, 6a and 6b (Table 2, Fig. 2). Other ORFs encoded unknown small proteins were presumed (data not shown).

(ii) Comparison of sequences. To evaluate how conservative the IBV genome was and how homologous the sequences were between IBV *Beijing isolate* and SARS coronavirus, the sequences of two strains of IBV and one SARS coronavirus were downloaded from GenBank. The complete genome of IBV *Beijing isolate* compared with that of IBV *strain Beaudette* (Accession: NC_001451.1) resulted in a similarity of 85.2%. However, there was 91.2% identity between IBV *Beijing isolate* and IBV *LX4* (Accession：AY223860), whose genome had not been sequenced completely and was only 19940 bp in size. And only 50.8% homology was shown between the full-length genome of IBV *Beijing isolate* and that of SARS coronavirus (NC_004718.1, TOR2).

Table 2 Ten ORF of IBV *Beijing isolate*

ORF	Position/nt	Peptide length/a.a.
RNA polymerase	530—12391 12391—20454	6641
Orf1a	530—12421	3963
S protein	20405—23914	1169
3a protein	23914—24087	57
3b protein	24087—24275	62
E protein	24259—24588	109
M protein	24557—25237	226
6a protein	25601—25798	65
6b protein	25795—26040	81
N protein	25983—27212	409

Sequences encoding five proteins of IBV *Beijing isolate* were also compared with those of IBV *strain Beaudette* and SARS coronavirus, at the levels of nucleotide and amino acid, respectively (Tables 3 and 4). S protein and E protein had low identity at the levels of nucleotide and amino acid between two strains of IBV. While RNA polymerase was of relatively high conversation at the amino acid level between two strains. However, four structural proteins of IBV *Beijing isolate* had fairly low homologies (<50%) to those of SARS coronavirus. On the contrary, the homologies of four structural proteins at the amino acid level were lower than those at the nucleotide level, respectively, between IBV *Beijing isolate* and SARS coronavirus.

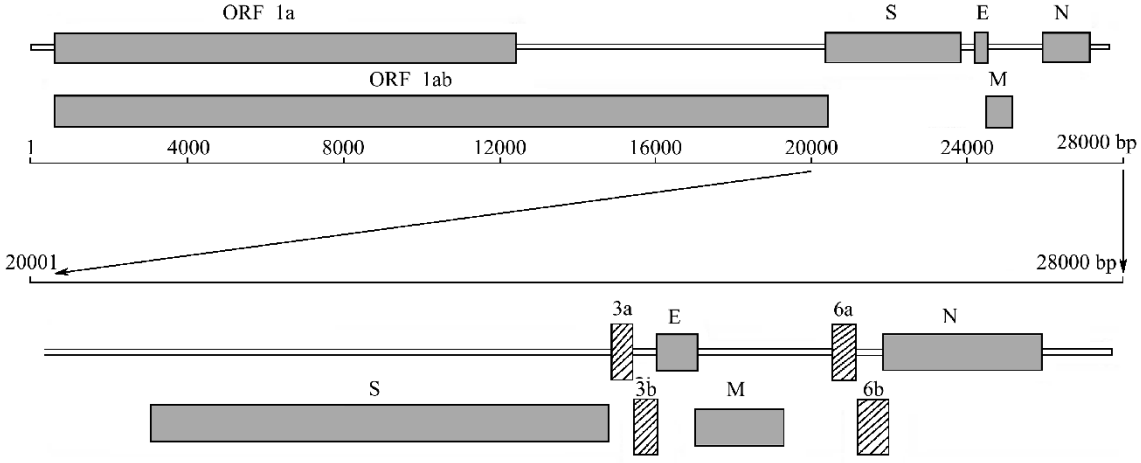

Fig. 2. Genome organization of IBV Beijing isolate. Overall organization of the IBV Beijing isolate is 27733 nt in length. Predicted ORFs 1a and 1ab, encoding the polyproteins, and those in turn encoding the S, E, M and N structural proteins are indicated. ORFs encoding unknown proteins 3a, 3b, 6a, 6b are also shown. The vertical position of the boxes indicates the phase of the reading frame, and the known coding regions (grey boxes) and reading frames encoding potential proteins (oblique boxes) are indicated.

Table 3 nt identity of five proteins (%)

Gene	AIBV[a)]	SARS-CoV[b)]
orf1a	83.5	48.9
orf1b	88.7	59.2
spike	81.5	49.8
envelope	85.5	48.0
membrane	87.5	49.0
nucleocapsid	88.0	48.6

a) AIBV *strain Beaudette* (Accession: NC_001451.1); b) SARS coronavirus (Accession: NC_004718.1/TOR2).

Table 4 a.a identity of five proteins (%)

Protein	AIBV[a)]	SARS-CoV[b)]
Orf1a	91.1	31.5
Orf1b	96.8	67.2
Spike	86.5	32.4
Envelope	86.2	29.1
Membrane	92.1	43.8
Nucleocapsid	94.0	33.3

a) AIBV *strain Beaudette* (Accession: NC_001451.1); b) SARS coronavirus (Accession: NC_004718.1/TOR2).

(iii) Phylogenetic analyses. To determine the relationship among IBV *Beijing isolate*, SARS-CoV and other coronaviruses, we compared the overall genome sequence and three structural proteins of IBV *Beijing isolate* with those of other animal coronavirus and SARS coronavirus for which sequences information was available in the GenBank. The topologies of the resulting phylogenetic trees are remarkably similar (Fig. 3). In all cases, SARS-CoV and IBV *Beijing isolate* are not closely related to other known coronavirus. IBV *Beijing isolate* is a lone virus in group Ⅲ[15] and SARS was segregated into a fourth branch.

3 Discussion

There is very remarkable variation in the genome sequence of IBV. The full-length genome of IBV *Beijing isolate*, which was 27733 bp in length, was 125 bp longer than that of IBV *strain Beaudette* (Accession: NC_001451.1), sharing only 85.2% identity of the nucleotide. And the nucleotide homology (91.2%) between IBV *Beijing isolate* and IBV *LX4* (Accession: AY223860) will decline after the complete genome of IBV *LX4* finished. For there is a gap of more than 6 kb at the 3′end of the genome, which contains three proteins: S, M and E. These sequences have low conservation. The number of amino acid of RNA polymerase and four structural proteins was

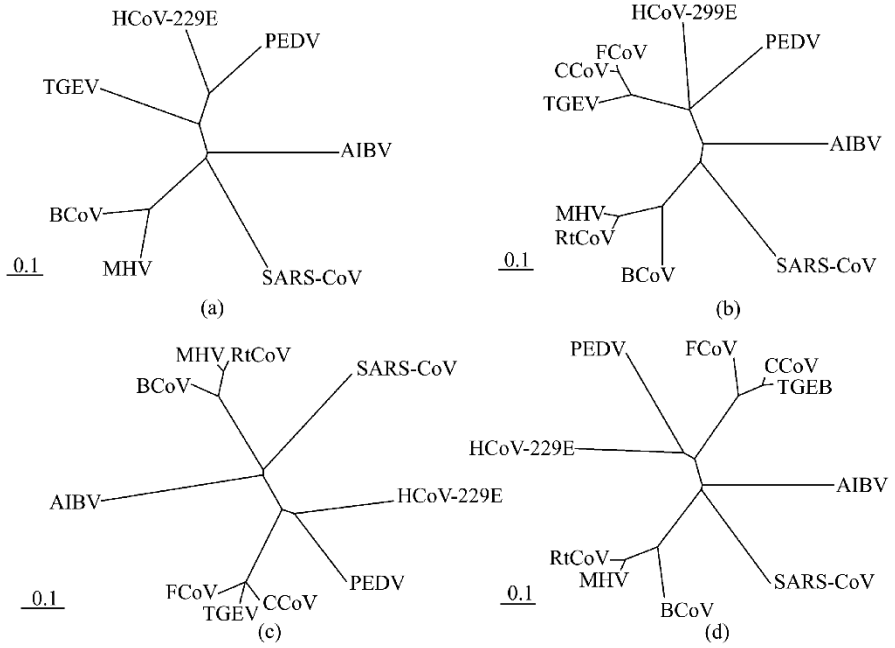

Fig. 3. Phylogenetic analyses of coronavirus complete genome and three proteins. Phylogenetic trees were generated by clustw 1.81 and were drawn with treeview. (a) Complete genome sequence. HCoV-229E (human coronavirus 229E), NC_002645.1; PEDV (porcine epidemic diarrhea virus), NC_003436.1; TGEV (transmissible gastroenteritis virus), NC_002306.2; BcoV (bovine coronavirus), NC_003045.1; MHV (murine hepatitis virus), NC_001846.1; SARS-CoV (SARS coronavirus), NC_004718.1; (b) S protein. HCoV-229 E(human coronavirus 229E), NP_073551.1; PEDV (porcine epidemic diarrhea virus), NP_598310.1; TGEV (transmissible gastroenteritis virus), NP_058424.1; BcoV (bovine coronavirus), NP_150077.1; MHV (murine hepatitis virus), NP_045300.1; SARS-CoV (SARS coronavirus), NP_808851.1; FCoV (feline coronavirus), CAA56778; RtCoV (rat sialodacryoadenitis coronavirus), AAF97738; CcoV (canine coronavirus), CAA543345; (c) M protein. HCoV-229E (human coronavirus 229E), NP_073555.1; PEDV (porcine epidemic diarrhea virus), NP_598313.1; TGEV (transmissible gastroenteritis virus), NP_058427.1; BcoV (bovine coronavirus), NP_150082.1; MHV (murine hepatitis virus), NP_045301.1; SARS-CoV (SARS coronavirus), NP_808855.1; FcoV (feline coronavirus), CAA39851; RtCoV (rat sialodacryoadenitis coronavirus), AAF97742; CcoV (canine coronavirus), AA033711; (d) N protein. HCoV-229E (human coronavirus 229E), NP_073556.1; PEDV (porcine epidemic diarrhea virus), NP_598314.1; TGEV (transmissible gastroenteritis virus), NP_058428.1; BcoV (bovine coronavirus), NP_150083.1; MHV (murine hepatitis virus), NP_045302.1; SARS-CoV (SARS coronavirus), NP_808858.1; FcoV (feline coronavirus), CAA74230; RtCoV (rat sialodacryoadenitis coronavirus), AAF97743; CcoV (canine coronavirus), A44056.

also observed. The *orf1a* of IBV *Beijing isolate* was 36 nt longer than that of IBV *strain Beaudette* (Accession: NC_001451.1), which resulted in 12 amino acids more for RNA polymerase than that of IBV *strain Beaudette*. Among the four structural proteins, S protein, M protein, and N protein of IBV *Beijing isolate* were longer than those of IBV *strain Beaudette* (7a.a, 1a.a, 1a.a, respectively). The genome sequence of IBV *Beijing isolate* was very different from that of IBV *strain Beaudette*, and it would be very important for the studies of diagnosis and diseases control on IBV in China.

Moreover, the full-length genome of IBV *Beijing isolate* showed 50.8% homology to that of SARS coronavirus (Accession: NC_004718.1). The identities of five proteins of IBV *Beijing isolate* were fairly low at the amino acid level, compared to those of SARS coronavirus, respectively. Phylogenetic analyses also showed that *Beijing isolate* was not resembled closely to SARS coronavirus, which was consistent with other reports[11—13].

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