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Research paper

Full genome sequence analysis of a newly emerged QX-like infectious bronchitis virus from Sudan reveals distinct spots of recombination

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ABSTRACT

Infectious bronchitis virus (IBV) infection continues to cause economically important diseases in poultry while different geno- and serotypes continue to circulate globally. Two infectious bronchitis viruses (IBV) were isolated from chickens with respiratory disease in Sudan. Sequence analysis of the hypervariable regions of the S1 gene revealed a close relation to the QX-like genotype which has not been detected in Sudan before. Whole genome analysis of IBV/Ck/Sudan/AR251–15/2014 isolate by next generation sequencing revealed a genome size of 27,646 nucleotides harbouring 13 open reading frames: 5′-1a-1b-S-3a-3b-E-M-4b-4c-5a-5b-N-6b-3′. Highest nucleotide sequence identity of 93% for the whole genome was found with the Chinese IBV strain Ck/CH/LHLJ/140906, the Italian IBV isolate ITA/90254/2005 and the 4/91 vaccine strain. Phylogenetic analysis of the S1 gene revealed that the IBV/Ck/Sudan/AR251–15/2014 isolate clustered together with viruses of the GI-19 lineage. Recombination analysis gave evidence for distinct patterns of origin of RNA in the Sudanese isolate in multiple genes. Several sites of recombination were scattered throughout the genome suggesting that the Sudan-QX-like strain emerged as a unique recombinant from multiple recombination events of parental viruses from 4/91, H120 and ITA/90254/2005 genotypes. The Sudanese QX-like isolate is plausibly genetically different from IBV strains previously reported in Africa and elsewhere.

1. Introduction

Avian infectious bronchitis virus (IBV) belongs to genus *Gammacoronavirus* in the *Coronaviridae* family (King et al., 2011). Globally distributed IBV induces an acute, highly contagious infectious disease affecting chickens and results in huge annual losses in the poultry industry. IBV was first reported in North Dakota, USA, by Schalk and Hawn 1931 (Schalk & Hawn, 1931) as a novel respiratory disease affecting chickens. Only chickens and pheasants act as the natural hosts for IBV (Ignjatovic & Sapats, 2000). IBV initially infects the respiratory tract; for some IBV strains further virus spread may involve kidneys and oviduct causing reduction of growth rate, decreased performance and reduction of egg quality and quantity (Cavanagh, 2003). Also another shift of tissue tropism causing proventriculitis has been recorded (Yu et al., 2001a). The infection spreads by aerosols, direct contact and indirectly through contaminated fomites (Ignjatovic & Sapats, 2000).

IBV harbors a monopartite RNA genome of positive polarity which is approximately 27.6 kb in size and codes for four structural proteins: the spike (S) glycoprotein, the membrane (M) glycoprotein, the nucleocapsid (N) phosphoprotein, and the envelope (E) protein (Spaan et al., 1988). The N protein is a major structural protein, which is highly conserved among different IBV serotypes. The spike (S) glycoprotein, an integral membrane protein, is another major structural protein of the IBV; it is post translationally cleaved into the S1 (N terminal part) and S2 fragments. In their matured forms the S1 constitutes trimers of the globular head while the S2 forms the trimerized stalk domain of the peplomer spikes in the viral lipid envelop (Cavanagh, 2007; Belouzard et al., 2012). The S1 protein carries the receptors binding site and thus plays an important role in tissue tropism and induction of protective immunity (Belouzard et al., 2012; Wickramasinghe et al., 2011). Along the S1 gene three hypervariable regions (HVRs) are distinguishable that are targets of neutralizing and serotype specific antibodies (Moore et al., 1997; Cavanagh et al., 1988). Variation in these epitopes has been

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implicated in escape from vaccine-induced immunity (Belouzard et al., 2012).

Numerous distinct serotypes have been described which are based on the variability of the S1 protein sequence that differs by 20–25%, and sometimes up to 50% between serotypes (Adzhar et al., 1997). Consequently, cross protection between these different serotypes is limited (Jackwood, 2012; Wickramasinghe et al., 2014; Cavanagh, 2005; Kuo et al., 2010). New S1 genotypes of IBV that are often also showing antigenic variation and, hence, define new serotypes, appear to emerge frequently in different parts of the world (Jackwood, 2012). Frequent mutations have been made accountable for the emergence and evolution of multiple S1 variants (Jackwood, 2012; Cavanagh et al., 1992) including point mutations, insertions, deletions, and also recombination between different strains (Adzhar et al., 1997; Hewson et al., 2014). Recombination tends to be not a rare event during IBV replication and the emergence of chimeric viruses harbouring sequences from two or more viruses were reported previously (Jia et al., 1995; Abro et al., 2012).

The QX IBV genotype, recorded for the first time in China in 1996, is associated with renal infections, proventriculitis, or impaired egg production (Yu et al., 2001b; Liu & Kong, 2004). This genotype has spread from Asia to Europe (Monne et al., 2008), and recently was reported from the southern part of the African continent, namely Zimbabwe and South Africa (Toffan et al., 2011; Abolnik, 2015). QX IBV has become the predominant field strain in many Asian and European countries (Worthington et al., 2008; de Sjaak et al., 2011). In the Middle East, QX-like strains have been detected in Kurdistan-Iraq in 2011 (Amin et al., 2012). QX IBV was shown to be antigenically different from both classical vaccine type and other variant strains. (Ducatez et al., 2009). However, a previous study has reported that vaccination using the Ma5 (Mass type) and 4/91 (793B) can reduce the clinical impact of QX IB virus infections in SPF layers and in commercial broiler chickens (Terregino et al., 2008).

In Sudan, IBV was recorded for the first time in 1981 (Elamin et al., 1986). Serosurveillance studies reported widespread IBV infection in Sudan associated with the 4/91 virus strain in 2000–2001 (Ballal et al., 2005a; Ballal et al., 2005b) and again in 2013. Widespread occurrence in non-vaccinated poultry of different sectors is assumed (Selma & Ballal, 2013).

Little is known to date on IBV from Sudan; in particular, genetic and virological data are missing. In the current study, an IBV isolate from chickens in Sudan is characterized as a QX-like IBV recombinant genotype.

2. Materials and methods

2.1. Virus isolation

Two IBV viruses (Ck/Sudan/AR251–15/2014 and Ck/Sudan/AR252–15/2014) were isolated from field samples obtained at the National Laboratory for Veterinary Quality Control on Poultry Production (NLQP, Ministry of Agriculture) from chickens which showed severe respiratory disease in a holding in Sudan. Viruses were submitted to the Friedrich-Loeffler-Institut, Germany, for further molecular and genetic characterization. Virus passaging was performed in 10-day old SPF chicken eggs (OIE, 2013).

2.2. RNA extraction and molecular diagnosis

Viral RNA was extracted using the QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. RNA was eluted in 70 μL nuclease-free water, and stored at −80 °C until use. Presence of IBV RNA was confirmed using RT-qPCR and conventional RT-PCRs specific for HVRs of the S1 gene of IBV and Sanger sequencing of these regions was performed. Primer sequences, amplification and sequencing conditions are available from the author upon request (Callison et al., 2006).

2.3. Sequence and phylogenetic analyses

Spike gene-specific RT-PCR amplicons were size-separated by agarose gel electrophoresis, excised and purified from gels using the QIAquick Gel Extraction Kit (Qiagen). Purified PCR products were used directly for cycle sequencing reactions (BigDye Terminator v3.1 Cycle Sequencing Kit, Applied Biosystems, Darmstadt, Germany). The reaction products were purified using NucleoSEQ columns (Macherey-Nagel GmbH & Co, Düren, Germany) and sequenced on an ABI PRISM® 3100 Genetic Analyzer (Life Technologies, Darmstadt, Germany). Thereafter, MegaBlast (http://blast.ncbi.nlm.nih.gov/Blast.cgi) analyses using the S1 HVRs sequences were carried out.

The obtained HVRs sequences of the S1 gene were assembled and edited using the Geneious software, version 9.0.5 (Kearse et al., 1647-1649). Alignment and identity matrix analyses were performed using MAFFT (Katoh & Standley, 2013) and BioEdit (Hall, 1999). Sequences generated in this study were deposited in the Genbank database, and assigned accession numbers are shown in Table 1. Sequences of other viruses required for further analyses were retrieved from public databases. For maximum likelihood analysis of phylogenetic relationships, a best fit model was chosen first on which further calculations and an ultrafast bootstrap equivalent analysis was based, using the models and algorithms implemented in the IQ-tree software version 1.1.3 (Minh et al., 2013; Nguyen et al., 2014). Trees were finally viewed and edited using FigTree v1.4.2 software (http://tree.bio.ed.ac.uk/software/figtree/).

2.4. Full-genome sequencing

For full-genome sequencing of Ck/Sudan/AR251–15/2014, RNA was extracted using TRIzol LS reagent (Life Technologies) and an RNeasy minikit (Qiagen) with on-column DNase digestion according to the manufacturer's instructions. RNA conversion into double-stranded DNA was done using a cDNA synthesis system (Roche, Mannheim, Germany) according to the Genome Sequencer Rapid RNA Library preparation manual (Roche, Mannheim, Germany). Library preparation was done as previously described (Juozapaitis et al., 2014). Sequencing was performed with an Illumina MiSeq Instrument using the MiSeq reagent kit version 3 (Illumina, San Diego, CA, USA). The raw sequencing reads were assembled into a single contig representing the complete IBV genome using the Genome Sequencer software suite (v3.0, Roche).

The sequence was analyzed with BLASTn (BLASTn; http://blast.ncbi.nlm.nih.gov/Blast.cgi) and ORFs were detected and the genome annotation was carried out using Geneious software, version 9.0.5. The S-ORF encoding the spike protein was analyzed and compared using programs implemented in Geneious to those of previously reported QX and QX-like strains from Asia, Europe and Africa focusing in particular on the

Table 1
Genes, coding regions, and deduced proteins of the Sudan/AR251–15 IBV strain.

Open reading frame	Frame	Genome location	Nucleotide length (bp)	Number of amino acids
5′ UTR	–	1-452	452	–
1a	3	453-12254	11802	3934
1b	2	12329-20287	7959	2653
S	3	20238-23735	3498	1166
3a	2	23735-23908	174	58
3b	1	23908-24099	192	64
E	2	24080-24412	333	111
M	3	24381-25061	678	226
4b	3	25062-25355	294	98
4c	1	25267-25437	171	57
5a	2	25421-25618	198	66
5b	1	25615-25863	249	83
N	3	25806-27035	1230	410
6b	2	27044-27268	225	75
3′ UTR	–	27269-27646	273	–

GenBank accession: KX272465.

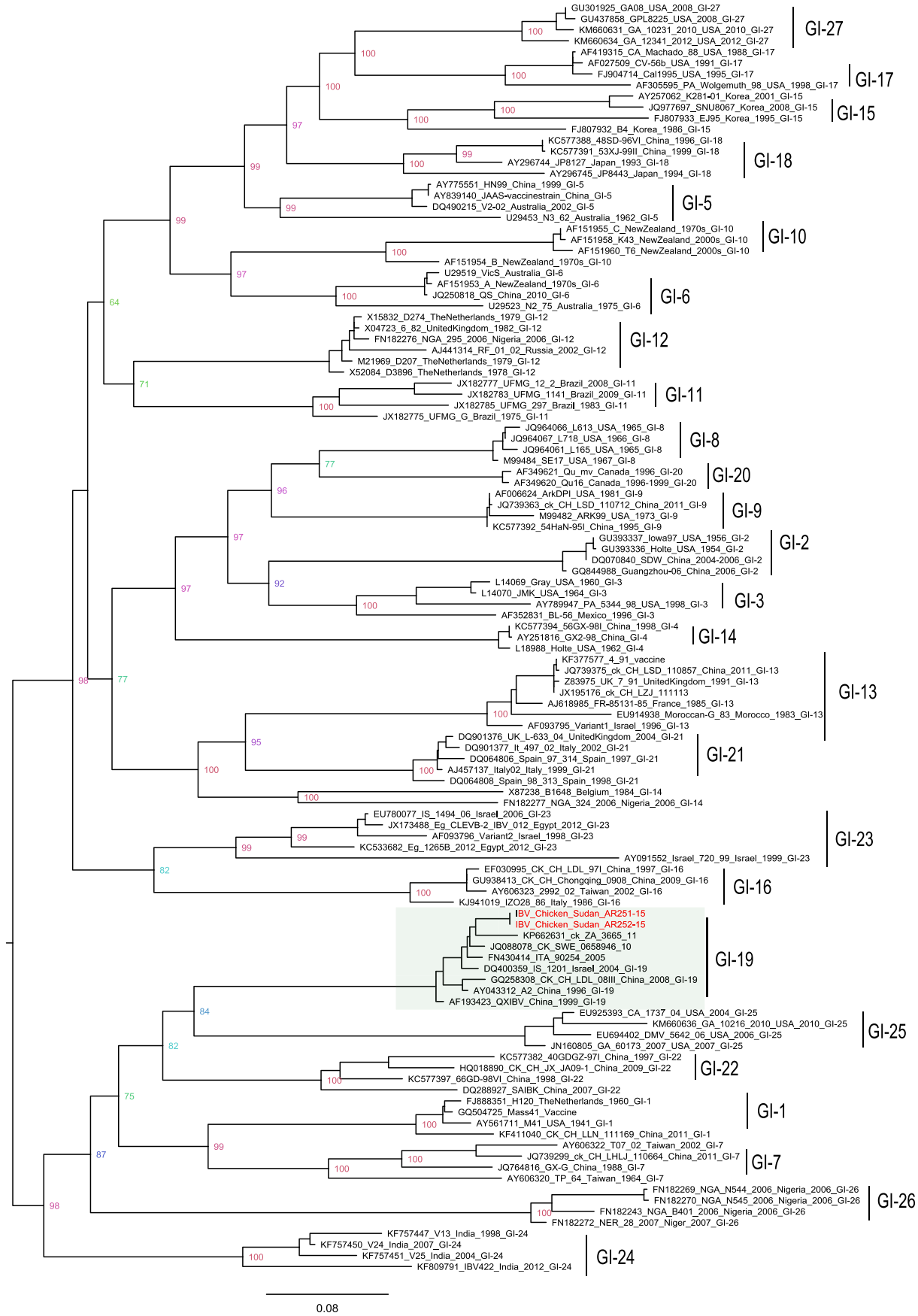

Fig. 1. Phylogenetic tree of concatenated S1 HVR 1, 2 and 3 sequences based on maximum likelihood calculations (IQTree software) under the best fit model according to the Akaike criterion (TVM + I + Γ4). Numbers at nodes represent measures of robustness based on an ultrafast bootstrap approach implemented in IQTree. Viruses of the current study are shown in red.

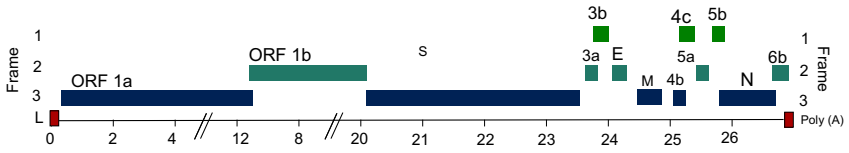

Fig. 2. Genome organizations of Infectious bronchitis virus Ck/Sudan/AR251–15/2014 showing different open reading frames. The positions of the leader sequence (L) and poly (A) tract are indicated.

highly variable regions (HVRs). In addition, N-glycosylation sites in the spike protein were predicted by services available on http://www.cbs.dtu.dk/services/NetNGlyc. Phylogenetic trees were also generated based on the obtained full genome sequence and the S1 gene sequence separately according to methods described in section 2.3.

2.5. Recombination analysis

Programs embedded in the Recombination Detection Program 4 (RDP4) software suite (Martin et al., 2015) were used to identify recombination events in the full-length IBV genome sequence of isolate Ck/Sudan/AR251–15/2014 through detection of breaking points using specific algorithms implemented in RDP4: RDP, Genecov, Bootscan, Maxchi, Chimaera, Siscan and 3Seq with the highest acceptable *P*-value adjusted to 0.05. For this purpose, an alignment was produced, as mentioned in the previous section, featuring other complete IBV genomes from North America (USA), Asia (China, Korea and Taiwan), Africa (Nigeria) and Europe (Sweden, Ukraine and Italy) that are relevant as vaccine strains and/or representatives of major IBV lineages. The same alignment was also used to examine nucleotide and amino acid identity for each ORF between the Ck/Sudan/AR251–15/2014 and other IBV strains.

3. Results

3.1. Identification of IBV

Two virus isolates generated from clinical samples collected from diseased chickens in a holding in Sudan tested strongly positive in IBV specific RT-qPCRs targeting 5′-UTR. Conventional RT-PCR specific for the HVRs of the S1 gene amplified fragments of approximately 450 (HVR1 and 2) and 380 (HVR3) nucleotides, respectively. Sanger sequencing of the amplicons confirmed the identity of these isolates as IBV (GenBank accession number KX272466–7).

3.2. Genetic and phylogenetic characterization

Nucleotide sequences of the S1 HVRs were used to conduct a BLAST search for further characterization. Highest identities for the assembled sequences of HVR1 and −2 (amino acids 52 to 177) were found at the nucleotide level with the IBV isolates SLO/305/08 (Slovenia) and Kr/D42/05 (Korea) (97%). At the amino acid level, the highest similarities were detected with Kr/354/03 (Korea) and RF/28/2011 (Russia) (95%). For the HVR3 amplicon (amino acids 253 to 364), the highest identity (>98%) was found with ITA/90254/2005 (Italy) and AZ-40/05 (Italy), both at the nucleotide and protein levels. These data revealed that the S1 gene of the two Sudanese IBV isolates clusters with QX and QX-like IBV viruses.

In order to confirm these findings, phylogenetic analysis was conducted using a maximum likelihood method (IQTree). The HVR1–2 and HVR3 nucleotide sequences of the S1 gene of IBV Ck/Sudan/AR251-14/2014 and Ck/Sudan/AR252-14/2014 clustered together with QX and QX-like viruses reported previously in Asia, Europe, Middle East and West and South Africa (Fig. 1a, b).

3.3. Full-genome characterization

Since the HVR sequences of the two viruses were very similar to each other (99.2% and 100% identity for HVR1–2 and HVR3, respectively), only one virus, Ck/Sudan/AR251–15/2014, was selected for further analysis by full genome sequencing. The complete genome of the Ck/Sudan/AR251–15/2014 strain as obtained by next generation sequencing was found to be 27,646 nucleotides (nt) in length, including both UTR 5′ and the poly (A) tail. The complete genome sequence of the Ck/Sudan/AR251–15/2014 isolate has been assigned into the GenBank sequence database in the National Center for Biotechnology Information (NCBI) accession number KX272465. The sequence obtained showed a classical IBV genome organization with 13 open reading frames (ORFs) in the order 5′-1a-1b-S-3a-3b-E-M-4b-4c-5a-5b-N-6b-3′ (Table 1, Fig. 2). Across the whole genome the highest identities were seen with the Chinese IBV strain Ck/CH/LHLJ/140906 (93%; accession KP036502), an Italian IBV isolate (ITA/90254/2005; 93%; accession FN430414), and the 4/91 vaccine strain (93%; accession KF377577). Overall nucleotide identities of 92% and 90% across the whole genome were obtained compared to the most commonly used IBV vaccine strains, H120 and M41, respectively. However, the S1 gene revealed identities of only 76.6 and 76.7% to these latter strains.

Table 2
Nucleotide sequence identity (%) of the Sudan/AR251–15/2014 IBV strain (GenBank accession: KX272465) compared to full-length sequences of representative IBV strains.

				IBV/Sudan/AR251–15/2014											
Strain	INSDC Accession	ORF1a	ORF1b	S1	S2	ORF3a	ORF3b	E	M	4b	4c	5a	5b	N	6b
CK/ZA/3665/11(QX-like)	KP662631	92.1	91.7	92.6	**97.3**	**98.9**	**97.9**	96.7	**98.7**	84.0	86.8	91.0	92.0	91.8	70.1
CK/SWE/0658946/10(QX-like)	JQ088078	92.8	**95.9**	95.8	94.3	83.3	91.1	88.9	88.5	83.7	81.6	91.0	91.2	90.7	**99.1**
ITA/90254/2005 (QX-like)	FN430414	89.4	94.4	**96.1**	94.8	84.5	92.7	**97.9**	95.3	**86.1**	87.9	94.5	94.0	93.7	**99.1**
SDZB0808 (Chinese QX)	KF853202	84.2	90.6	93.5	91.1	82.1	82.7	89.8	89.3	82.7	78.7	81.4	91.6	87.5	71.4
YN (Chinese YN genotype)	JF893452	86.7	90.4	76.9	89.0	84.5	75.9	86.8	88.4	84.6	77.7	88.4	97.2	86.7	69.2
Delaware072 (USA 1992)	GU393332	95.3	92.0	56.9	75.2	90.8	90.1	86.8	90.9	84.7	81.6	89.4	96.8	90.0	91.0
Conn46_1991(Connecticut)	FJ904719	89.4	91.4	75.9	84.8	89.7	77.9	85.9	90.9	**86.1**	86.8	90.5	96.8	90.6	82.5
Gray_1960 (USA)	GU393334	89.4	91.8	74.4	85.4	94.3	76.9	85.6	90.0	**86.1**	86.2	90.5	94.8	89.4	91.5
H120 (Vaccine strain)	GU393335	**95.6**	92.3	76.7	84.9	84.5	78.5	87.4	92.5	85.0	81.6	89.9	94.0	91.1	58.8
Mass41 (Vaccine strain)	GQ504725	93.0	91.8	76.6	84.9	85.1	79.0	87.4	93.0	85.0	81.6	89.9	95.4	90.8	–
IBV 4/91 (Vaccine strain)	KF377577	95.4	91.4	77.5	85.1	94.3	80.5	82.0	92.1	85.0	**89.7**	**99.5**	**99.6**	**99.3**	**99.1**
Beaudette_CK	AJ311317	88.4	91.2	76.4	84.7	88.5	80.3	88.6	92.1	72.1	82.8	91.0	96.0	90.0	91.5
NGA/A116E7/2006 (Nigeria)	FN430415	89.8	92.5	75.9	85.4	86.8	80.0	82.0	94.0	82.0	81.0	91.5	92.4	90.7	90.2

Bold face numbers depict the highest percentage identity.

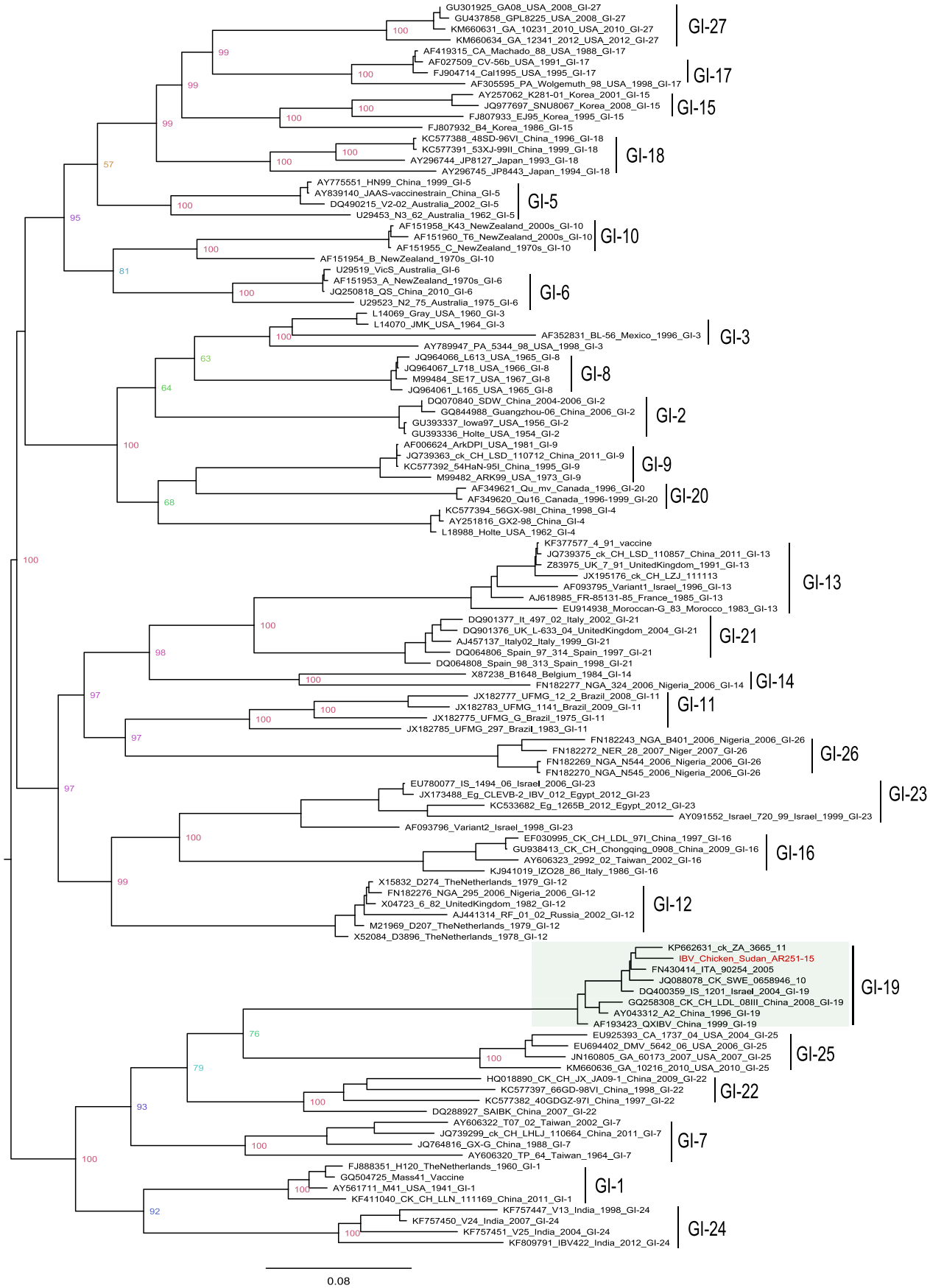

Fig. 3. Phylogenetic tree of the nucleotide sequences of the S1 gene of the IBV/Sudan/AR251–15/-QX viruses. Maximum likelihood calculations were done with the IQTree software using an ultrafast bootstrap approach. Best fit models were calculated in IQTRee as well. Grouping nomenclature according to Valastro et al., 2016.

Similarity searches using each ORF of the IBV/Ck/Sudan/AR251–15/2014 separately produced variable rankings with other IBV reference and vaccine strains: IBV/Ck/Sudan/AR251–15/2014 revealed higher sequence identities of the 1b, S, 3a, 3b, E, M, ORFs with QX and QX-like viruses. On the contrary, the 1a, 4c, 5a, 5b and N ORFs showed higher sequence identities with the H120, 4/91 and IBVUkr27–11 (4/91 like) strains (Table 2). ORF 4b revealed overall low identities with all genotypes as shown in Table 2. ORF 6b showed mixed identity values with either QX-like viruses or with 4/91. These findings suggested a possible recombination between different IBV genotypes shaping the genome of the Sudanese isolate. Phylogenetic analysis based on the full genome sequence revealed a close relation with the QX-like viruses from Italy, Sweden and South Africa (Fig. S1). By phylogenetic analysis based on the whole S1-gene according to (Valastro et al., 2016) it appeared that the IBV/Ck/Sudan/AR251–15/2014 is closely related to ITA/90254/2005 and the previously reported recombinant strains detected in South Africa (Ck/ZA/3665/11) and Sweden (Ck/SWE/0658946/10) which are clustered together within GI-19 lineage (Fig. 3).

The S-ORF of the Sudanese isolate has a length of 3498 nucleotides giving rise to 1165 amino acids. The precursor protein harbors an endoproteolytical cleavage site ^{537}RRRR/S^{541} which divides the S-protein precursor into the S1 and S2 fragments of 540 amino-terminal and 625 amino acids at the carboxyl terminus, respectively. A total of 33 N-linked glycosylation sites was predicted in the S protein of Ck/Sudan/AR251/QX strain (S1 = 20 and S2 = 13) similar to the Chinese QX which are possessing seven additional glycosylation sites than the vaccine stain H120 in positions 141, 200, 279, 457, 545, 982 and 1061, and lost two sites at positions 283 and 714. Compared to the Chinese QX strain, the Sudan isolate AR251 gained an additional glycosylation site at position 267, but lost a glycosylation site at position 5.

3.4. Recombination analysis

The employed recombination detection methods embedded in RDP4 revealed that Ck/Sudan/AR251–15/2014 has undergone genetic recombination. Three long recombined sequence stretches were identified with high reliability by at least five programs embedded in RDP4: The first and second recombination regions were observed at positions 1-6468 and 9988-12498 in the ORF1a and ORF1b genes, and the third recombination region was located in position 18369-23219 involving the OR1b and S gene. Further recombination events in different positions were identified as shown in Fig. 4 albeit with lower reliability according to RDP4. The results showed that the Sudanese isolate was a recombinant virus which probably emerged from at least three different genotypes, including the 4/91 genotype as a major parent and the H120 vaccine strain as well as Italy/90254/2005-like viruses as minor parents (Fig. 4).

4. Discussion

Different IBV variants are in circulation around the world; some of them show a geographic restriction, others are globally distributed (de Sjaak et al., 2011). Different genotypes of IBV are classified based on the genetic variation of the spike protein, in particular its S1 fragment (Cavanagh, 2007; Belouzard et al., 2012; Valastro et al., 2016). The HVR1–2 and HVR3 regions of the S1 gene encode serotype specific determinants of IBV and harbor antigenic epitopes important for induction of protection (Promkuntod et al., 2014).

Little is known about the epidemiology of avian respiratory diseases in poultry in Sudan although respiratory disease continues to threaten commercial poultry in the country. In this study, two IBVs were isolated from commercial broiler farms showing respiratory signs with an increased mortality. Flocks were vaccinated with either Mass type alone (H120 or Ma5) or with Ma5 and 4/91.

Partial S1 gene analysis of the three HVRs has demonstrated that IBV strains Ck/Sudan/AR251–15/2014 and Ck/Sudan/AR252–15/2014 are related to the QX-like serotype. QX-like IBV had never been reported from Sudan. QX variant IBV apparently emerged in China in 1998 and reported thereafter in Europe as QX-like viruses (Monne et al., 2008; Wang et al., 1998).

Recombination in coronaviruses is likely to be occurred by their unique mechanism of RNA synthesis involving polymerase jumping

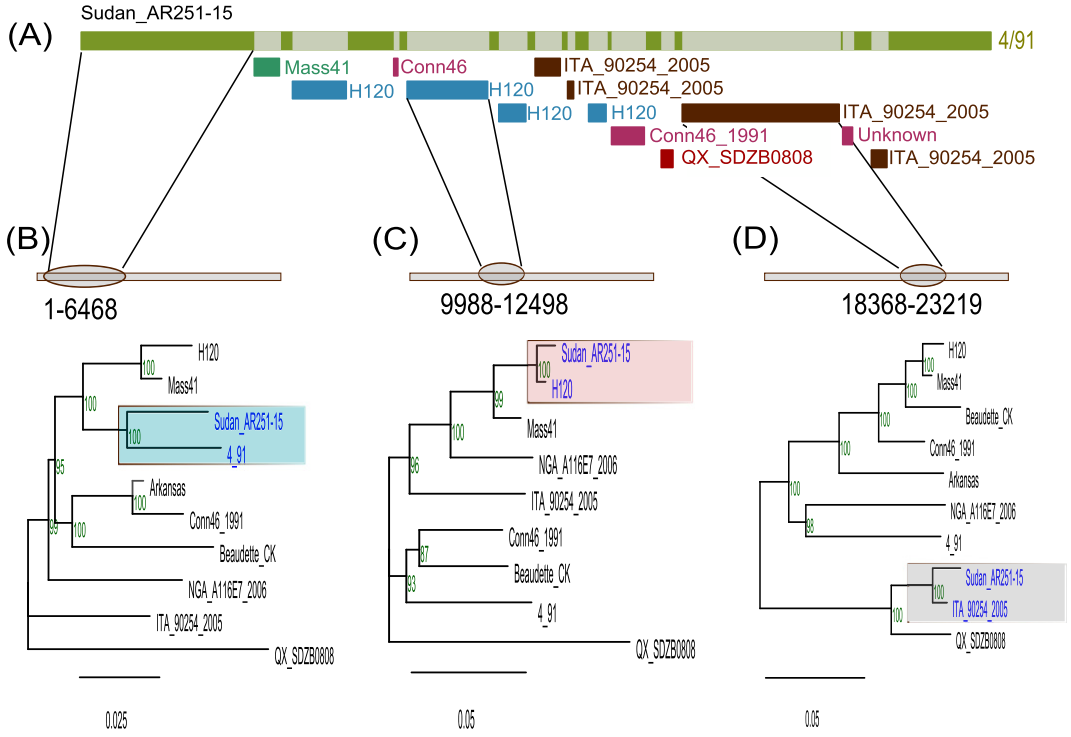

Fig. 4. Examination of putative recombination events in the genome of the IBV isolate Sudan AR251–15/2014 (query sequence) (a). The analysis was conducted using the Recombination Detection program V4. Maximum likelihood trees of the selected recombinant regions were estimated using algorithms embedded in RDP4 (B, C, D).

and discontinuous transcription (Lai, 1996). Different recombination evidences were reported among different IBV field strains through recombination between two or more strains resulting in the emergence of new variants (Abro et al., 2012; Abolnik, 2015; Ammayappan et al., 2008). Further, distinct IBV recombination has been experimentally illustrated in vitro, in ovo and in vivo (Wang et al., 1997).

Here, the full-length genome of the Ck/Sudan/AR251–15/2014 strain determined by next generation sequencing (NGS) revealed thirteen open reading frames ORFs (5′UTR-1a-1b-S-3a-3b-E-M-4b-4c-5a-5b-N-6b-3′UTR) showing different spots of variations and recombination with other genotype located in multiple genes ORF1a, ORF1band S. The start of the ORF1a gene revealed a high frequency of recombination events with 4/91. Other genes exhibited recombination with the H120 and Italy/90254/2005 type IBVs, indicating that recombination might involve more than two strains.

Taken together, phylogenetic and recombination analyses performed on the complete genome of the Sudan/AR251–15/2014 virus showed that this strain is a mosaic of different parental lineages never described so far. In particular, the virus most likely resulted from a natural recombination event involving at least three distinct IBV variants namely the QX-like, 4/91 and H120 strains.

In conclusion, it is noteworthy that there are no records of the presence of a QX-like variant in Sudan prior to this study. Whatever the way in which this virus has reached Sudan, identification of this chimeric QX like virus highlights the need to improve monitoring programs for IBV in Sudan and neighboring countries for a better understanding of its epidemiology. In addition, updating of vaccines may be required with further experimental studies to demonstrate the efficacy of the currently used vaccine.

Supplementary data to this article can be found online at http://dx.doi:10.1016/j.meegid.2016.10.017.

Author contributions

Mahmoud M. Naguib, Timm Harder, Abdel-Satar A. Arafa, conceived the study. Dirk Höper conducted and interpreted the NGS sequencing. Mahmoud M. Naguib and Timm Harder produced, analyzed and interpreted genetic and phylogenetic data. Ahmed Setta provided and analyzed epidemiological data. Timm Harder and Mahmoud M. Naguib drafted the manuscript. All co-authors critically analyzed, revised and finally approved the manuscript.

Conflicts of interest

The authors declare no conflict of interest.

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