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Current situation, genetic relationship and control measures of infectious bronchitis virus variants circulating in African regions

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Abstract Infectious bronchitis virus (IBV) is a major viral pathogen of commercial poultry, affecting chickens of all ages and causing major economic losses in poultry industry worldwide. Frequent points of mutations and recombination events in the S1 gene region, result in the emergence of new IBVs variants circulating in the form of several serotypes/genotypes that can be partially or poorly neutralized by current vaccines. IBV is well studied worldwide, nevertheless in African countries epidemiological and scientific data are poor and not updated.

This review aims to give a current overview of IBV situation, to establish evolutionary relationship between the African variants and to list some of the potential measures to control IBV in Africa.

Three S1 gene hypervariable regions were studied and compared to the reference genotypes/serotypes that found emerging in African regions. This comparison was based on phylogenetic trees, nucleotide and amino-acid sequence analysis. It clearly appears that IBV variants reported in Africa, display a low genetic relationship between them and with the majority of the reference strains emerging in neighboring countries, except the case of variants from Libya and Egypt that show a high relatedness. Also the Massachusetts serotypes were the most prevalent co-circulating

Abbreviations: IBV, infectious bronchitis virus; IB, infectious bronchitis; S, spike; M, membrane; N, nucleocapsid; HVR, hyper variable region; nt, nucleotide.

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with both serotypes, Italy02 type in Morocco and Qx-like genotype in South part of the African continent. In order to control the IBV variants in African regions, an efficient vaccination strategy program should be implemented.

Contents

Background

Avian infectious bronchitis (IB) is an acute, highly contagious and infectious disease of serious economic importance in the poultry industry worldwide (Cavanagh and Naqi, 2003). It was firstly reported in North Dakota, USA, as novel respiratory disease pathogen in young chickens in the late 1930s by Schalk and Hawn (Schalk and Hawn 1931).

The disease is caused by infectious bronchitis virus (IBV; *Avian coronavirus*). IBV is the prototype species of the *Coronavirus* family, *Gamma coronavirus* genus, classified in the order *Nidovirales* in the order Nidovirales, and it is the type species of the genus *Coronavirus* of the domestic chicken (*Gallus Gallus*) (Cavanagh and Naqi, 2003; Cook et al., 2012).

Until recently, the chickens of all ages were considered the only natural host of IBV, but the severity is great in younger ages (Glahn et al., 1989). IBV is primarily a respiratory pathogen because it replicates mainly in epithelial cells of the trachea, resulting in respiratory signs (sneezing, cough, tracheal rales, gasping and nasal discharge), which are the most frequent clinical manifestation of this disease (Raj and Jones, 1997). In some cases, field strains of IBV can associate with renal and reproductive infections, producing severe nephritis, and causing severe damage to the oviduct and result in decreased or permanent loss of production and poor egg quality (Balestrin et al., 2014). The virus is transmitted by the airborne route, by direct contact via the respiratory tract from infected chickens, which may travel several kilometers or indirectly through mechanical spread, such as contaminated feed and drinking water, equipment, eggs, working personnel and trucks (Cavanagh et al., 2002).

IBV is an enveloped virus with a single-stranded, positive-sense, RNA genome of approximately 27.6 kilobases (kb) in length, and contains 5 genes encoding the structural proteins, the order of these genes is the following: 5′UTR – polymerase gene (ORF1 a/b)–S-E-M-N – UTR 3′ where the UTR are untranslated regions (Cavanagh, 1997). Two-thirds of the genome consists of two overlapping regions (ORF1a and ORF1b), that encode into large polyproteins 1a and 1ab, and contribute to formation of the replication and transcription complex.

The viral genome encodes for 4 structural proteins, namely the membrane (M), the envelope protein (E), the nucleocapsid protein (N) and the spike protein (S) (Cavanagh, 2007). The spike glycoprotein (S) is translated as a precursor protein (S0) and later cleaved into the two non-covalently bound peptide chains known as S1 and S2. The S1 subunit protein forms the tip of a spike, which is anchored to the membrane by association with S2 subunit, is responsible for the infection of the host cells, involved in virus entry, and also contains epitopes for virus-neutralizing antibodies, protective immunity and serotype specific antibodies (Casais et al., 2003).

Since IBV was first described in 1936, many serotypes were identified, and have complicated immunization efforts, and that do not confer complete cross-protection between all strains isolated in worldwide (Cavanagh and Gelb, 2008). Some studies have indicated that this divergence in serotype, due to the frequent point mutations and recombination events in the S1 gene, results in the emergence of new IBV variants that can be partially or poorly neutralized by existing vaccine serotypes (Liu et al., 2003). For this reason, the analysis of the S1 gene is the most useful strategy to differentiate IBV genotypes and serotypes, because it correlates closely with the serotype and permits the selection of the appropriate vaccinal serotypes for IB control in each geographic region (Jackwood, 2012).

IB has a large geographical distribution and it was found in regions of America, Europe, Asia, Australia and Africa (Liu et al., 2003; Lee et al., 2010). In Africa, IBV is one of the main and the most important viral respiratory diseases of chickens; it is considered an epidemic virus and widely spread both in vaccinated and unvaccinated poultry farms (Ahmed, 1954; Jackwood, 2012; Khataby et al., 2016). It was described and recognized for the first time in North Africa, especially in Egypt since 1950s (Eissa et al., 1963), from birds showing respiratory signs and confirmed by Eissa et al. (1963), in Morocco in 1983 (El Houadfi and Jones, 1985), in Tunisia between 2009

and 2013 (Bourogâa et al., 2009), in Libya in 2012 (Awad et al., 2014), and in Algeria (Sid et al., 2015). Antibodies against the IBV were reported from South Africa with 43% of IB prevalence in QwaQwa (Thekisoe et al., 2003), 86% in Chitungwiza, Zimbabwe (Kelly et al., 1994), and in Botswana (Mushi et al., 2006). Up to now little is known about the distribution and impact of IBV in sub-Saharan African countries including Ivory-Coast. IBV revealed with a seroprevalence of 84% in Nigerian poultry farms (Ducatez et al., 2004; Owoade et al., 2006), and 72% in Ivory-Coast (Kouakou et al., 2015). Besides, two years later, in Burkina Faso, Tarnagda et al. (2011) confirmed during a study on avian diseases [avian influenza virus (AIV), IBV and Newcastle disease (NCDV)] in domestic and wild birds in highly pathogenic avian influenza outbreaks areas that the prevalence of IBV was 3.9% and no co-infection by AIV, IBV, and/or NCDV was found.

Despite the high prevalence of IBV in West Africa, little is known about molecular and serological characteristics of these strains. An increasing number of IB variants has been reported in the later years in African domestic poultry, however till now, no reported information is available about the relationship between the IBV genotypes circulating in Africa, hence the purpose of this present paper to provide a current overview and an update of IBV situation, to establish genetic relationship between the African variants, and also to give some control measures that could be suitable for IBV in Africa.

Case history and current situation of IBV variants in Africa

The diversity of IBV variants that exist in Africa is important and most of the information on IBV comes from North African countries, such as Egypt, Morocco and Tunisia, where some classical IBV variants appear to be present until now (De Wit et al., 2011). Indeed, a large number of IBV variants in Egypt were recognized and strains related to the Massachusetts (Mass) D3128, D274 and 4/91 genotypes have been detected at different poultry farms in Egypt (Abdel-moneim et al., 2002; Sediek, 2010). Subsequently, the Egyptian variants "Egypt/Beni-Seuf/01" isolated from H120 vaccinated broiler flocks, and Israeli variant "Israel/720/99", represent a new IBV genotype that is suggested to be assigned as Egypt/Beni-Seuf (Abdel-Moneim et al., 2006). Around the year 2012, five IBV isolates formed a distinct phylogenetic group with the "Egypt/Beni-Suef/01" variant (Var1), and designate that "Ck/Eg/BSU-2/2011" and "Ck/Eg/BSU-3/2011" can be considered a new IBV variant (Abdel-Moneim et al., 2012). All the IBVs variants circulating in Egypt during 2012 were classified into two variant groups; the first group was clearly variant from "IS/885" strain and the second group was related to as 4/91 and CR/88121 variants vaccine strains (Selim et al., 2013). Other Egyptian variants have also been isolated, named "IBV-CU2-sp1" and "Eg/12120s/2012" (Afifi et al., 2013; Arafa et al., 2013).

Many serotypes and genotypes were identified in Morocco; the first five isolates designated as D, E, F, H and M were related serologically to the Massachusetts (Mass) serotype, although an enterotropic variant called as Moroccan "G" is closely related to 4/91 serotype (El-Houadfi et al., 1986; Jones et al., 2004). Subsequently, several reports confirmed the IBV strains related to the Massachusetts and to 4/91 genotypes have been detected at different poultry farms in Morocco (Alarabi, 2004; El Bouqdaoui et al., 2005). Recently, an epidemiological survey showed the emergence of a novel strain of Italy02 serotype with a prevalence of 32%, co-circulating with two serotypes; Massachusetts and 4/91, with a prevalence of 66% and 2%, respectively, that are isolated from vaccinated and unvaccinated chicken flocks (Fellahi et al., 2015a). In addition, three unique IBV isolates "IBV/Morocco/30", "IBV/Morocco/38", and "IBV/Morocco/01" were characterized using partial S1 sequence analysis, and revealed only a few changes in amino acid composition of S1 protein, thus forming a distinct cluster with the Spanish isolates of the Italy 02 genotype, and the author suggests that a new genotype, represented by the three new IBV isolates described, has been circulating in Morocco since at least 2010 (IBV/Morocco/30) (Fellahi et al., 2015b). This seemed the case for Tunisia, three IBV variants nominated as "TN20/00", "TN200/01", and "TN335/01" isolated from different Tunisian poultry farms, were very different from the Mass H120 vaccinal strain, but closely related to Mass types found in Europe such as D274 and CR88121 serotypes (Bourogâa et al., 2009). In addition, two other new Tunisian IB variants "TN296/07", "TN556/07" isolated during 2007–2010, were also very distant from the reference IBV serotypes, in particular the H120 strain Bourogâa et al., 2009, 2012).

Lately, IBV was detected for the first time in Libya in 2012 by Awad et al. (2014) and in Algeria in 2015 by Sid et al. (2015). S1 gene analysis demonstrated that the IBV variants designated as "IBV/Libya/8-2012", "IBV/Libya/1-2012", "IBV/Libya/7-2012" and "IBV/Libya/3-2012" in Libyan broiler flocks, formed a separate cluster with the Egyptian strains "CK/Eg/BSU-2/2011", "CK/Eg/BSU-3/2011" and "Eg/1212B", and the isolate named "IBV/Libya/5-2012" formed another cluster, with 100% relatedness to "Eg/CLEV B-2/IBV/012" and "IS/1494/06" strains. In the other hand, new IBV variants were recently identified in Algerian poultry farms called "Algeria/26/b1", "Algeria/26/b2"and "Algeria/26/b3", that are different from the strains isolated in neighboring countries, and this could suggest the existence of a new IBV genotype in North Africa. In Western Africa, a novel variant 'IBADAN' has been described between 2002 and 2007 in Nigeria and Niger, and referenced genome is "NGA/A116 E7/2006", which is antigenically and genetically different to other known IBVs (Ducatez et al., 2009). Some serotypes of IBV, such as Qx-like virus which it is highly pathogenic, continues to be a problem in many countries, and there is evidence that it has emerged for the first time in South part of the African continent (Zimbabwe) (Toffan et al., 2011). Meanwhile, two different isolates of IBV, "CkZa/5315/11" and "CkZa/4916/11", were identified in KwaZulu-Natal (KZN) during 2011–2012 (Knoetze et al., 2014).

The high variability of the S1 gene that is responsible of emergence of new genotypes and serotypes of IBV, is mainly due to the high level of mutations (insertions, deletions, substitutions) of the S1 gene, and it has been demonstrated to be a determinant for cell tropism for some other avian Coronaviruses (Alvarado et al., 2003; Cavanagh, 2007). The evolutionary characterization of IBV is essentially based on the analysis of the variable S1 gene or the expressed S1 protein. Moreover, three hypervariable regions (HVR1, HVR2, HVR3) located at the positions 114–201 nt, 297–423 nt and 822–1161 nt corresponding respectively to amino acid residues

38–67, 91–141 and 274–387, have been located within the S1 subunit (Casais et al., 2003; Bourogâa et al., 2009).

Phylogenetic relationship of African variants and the reference strains of IBV

S1 gene sequence data and tools of analysis

The sequences of IBV variants emerging in Africa which were selected for this study, their accession numbers, and countries of their isolation and publication references are listed in Table 1. For the reference sequences of IBV genotypes and serotypes, we selected from those that are found to be emerging in Africa, especially: Mass type H120, M41, Ma5, 4/91, Italy02 and Qx-like genotype and were aligned with the African variants described previously (Table 1). Their accession numbers are: [GenBank: M21969, GenBank: M21883, GenBank: AY561713, GenBank: AF093794, GenBank: AJ457137, GenBank: AF193423]. The percentages of nucleotide and deduced amino acid similarities were calculated for a selection of representative variants of each African country using EMBOSS Matcher, a pair wise local alignment tool available in www.ebi.ac.uk (Table 2).

The set of sequence data were visualized using the BioEdit Software version 5.0.9 (Hall, 1999). Nucleotide sequences were aligned with MUSCLE (v3.8.31) (Edgar, 2004). Phylogenetic tree constructions for partial S1 glycoprotein gene sequences were generated using the neighbor-joining (Kimura two-parameter) method with 1000 bootstrap replicates implemented in Neighbor from the PHYLIP package (v3.66). Distances were calculated using FastDist (Elias and Lagergren, 2007). The K2P substitution model was selected for the analysis. Graphical representation and edition of the phylogenetic tree were performed with TreeDyn (v198.3) (Chevenet et al., 2006). All these phylogenetic tools are available in the phylogeny platform http://phylogeny.lirmm.fr (Dereeper et al., 2008).

We note that the S1 gene sequences of IBV African variants do not overlap together when aligned, because the studies have targeted different regions from the S1 gene (as shown in Table 1). Missing data are often considered to be a significant obstacle in phylogenetic reconstruction. However, to avoid non comparative analysis, we separate the taxa into two parts, a part containing HVR 1 and 2, the other part for HVR 3. S1 gene sequences were then truncated after alignment to form two sets of sequences located from 146 to 546 nt and from 760 to 987 nt respectively.

Table 1 List of the selected African IBV variants used in the present study, their accession numbers, and country of their isolation.

Region	Country	IBV variants	Accession number	S1 gene position	Reference
NorthAfrica	Egypt	Egypt/Beni-Suef/01	JX174183	726–1133	Abdel-Moneim et al. (2002)
		Egypt/SCU-14/2013-1	KF731612	726–1133	Sultan et al. (2013)
		Ck/Eg/BSU-2/2011	JX174185	727–1133	Abdel-Moneim et al. (2012)
		Ck/Eg/BSU-3/2011	JX174186	727–1132	Abdel-Moneim et al. (2012)
		IBV-CU2-SP1	KC985213	104–606	Afifi et al. (2013)
		EG/12120s/2012	KC533684	1–1584	Arafa et al. (2013)
		Eg/1265B/2012	KC533682	5–1583	
		Eg/CLEVB-2/IBV/012	JX173488	1–1598	Mourad et al. (2013)
		Mans-1	KF856872	1–642	Abd El Rahman et al. (2013)
		Egypt/D/89	DQ487086	726–1133	Abdel-Moneim et al. (2006)
	Morroco	Moroccan-G/83	EU914938	1–1620	El-Houadfi et al. (1986)
		IBV/morocco/30	KJ701019	14–605	Fellahi et al. (2015b)
		IBV/morocco/38	KJ701020	14–635	
		IBV/Morocco/01	KM594187	1–710	
		IBV02/2014/MOROCCO	KM594215	743–1088	Fellahi et al. (2015a)
		IBV43/2013/MOROCCO	KM594244	743–1088	
		IBV04/2012/MOROCCO	KM594217	718–1076	
		IBV29/2011/MOROCCO	KM594232	743–1092	
		IBV05/2010/MOROCCO	KM594218	702–1087	
	Algeria	Algeria/26/b3	KP892761	1–685	Sid et al. (2015)
		Algeria/26/b2	KP892760	1–685	
		Algeria/26/b1	KP892759	1–685	
	Tunisia	Tunisia TN296/07	FJ716133	138–726	Bourogâa et al. (2009)
		Tunisia TN556/07	FJ716132	141–546	
		TN335/01	EF535996	151–720	
		TN200/01	EF535997	145–713	
		TN200/00	EF535998	145–713	
	Libya	IBV/Libya/8-2012	KF007929	733–987	Awad et al. (2014)
		IBV/Libya/1-2012	KF007922	733–987	
		IBV/Libya/7-2012	KF007928	733–987	
		IBV/Libya/5-2012	KF007926	733–987	
		IBV/Libya/3-2012	KF007924	733–987	
South Africa	KwaZulu-Natal	CkZa/5315/11	KJ200279	726–1143	Knoetze et al. (2014)
		CkZa/4916/11	KJ200281	726–1143	
West Africa	Nigeria and Niger	NGA/293/2006	FN182275	83–1693	Ducatez et al. (2009)
		NGA/A116E7/2006	FN182257	83–1693	

Table 2 Percentages of nucleotide and amino acid identities of the part-S1 glycoprotein gene of the African variants IBV in comparison to other selected references strains. The boxes with a dash indicates sequences that do not match. The targeted regions of S1 gene sequencing are non-homogenous for all studied IBV variants.

Nucleotide identity (%)

	1	2	3	4	5	6	7	8	9	10	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33		
1		80.4	80.7	78.6	80.4	77.7	77.8	81.2	80.8	59.3	74.9	79.3	79.8	74.8	83.7	83.7	84.2	57.4	90.0	77.2	98.7	84.4	82.9	83.7	84.2	87.6	81.6	80.9	81.7	1	**H120**
2	76.8		97.6	78.5	78.4	78.6	78.8	76.0	76.4	96.4	79.1	71.2	72.4	70.7	80.5	80.9	80.2	63.2	87.0	79.2	77.4	76.5	79.8	79.8	79.0	73.1	81.2	80.9	81.0	2	**M41**
3	77.9	95.9		78.3	78.1	78.7	77.7	76.4	75.9	97.5	79.2	71.7	72.3	72.5	80.1	80.5	81.0	63.6	88.0	78.8	77.6	76.4	79.8	79.8	78.9	72.5	81.0	80.3	81.5	3	**Ma5**
4	78.3	74	74.9		84.1	79.0	93.1	78.2	78.7	77.8	77.2	76.5	75.5	71.1	81.7	80.9	83.1	63.9	77.2	79.1	75.0	75.0	79.0	79.3	78.2	74.3	80.5	80.4	81.7	4	**4/91**
5	80	74.3	75.0	83.0		78.1	83.2	89.0	87.9	76.2	77.4	78.3	79.9	75.8	83.5	83.5	83.9	64.8	78.6	77.5	81.5	79.0	80.4	80.4	80.1	77.1	82.2	81.7	82.7	5	**Italy02**
6	76.7	76.1	76.8	79.1	77.7		77.9	74.8	77.1	78.7	74.6	73.4	71.0	71.4	83.3	83.3	83.4	71.1	77.1	77.4	77.4	74.5	78.5	79.2	78.6	74.2	81.7	81.4	82.2	6	**Qx-like**
7	77.6	74.9	74.4	88.5	80.0	77.9		74.7	76.2	76.6	76.0	74.8	75.0	68.9	80.0	80.0	82.4	63.5	76.9	78.3	74.0	75.3	79.3	79.0	78.1	72.4	79.8	79.6	80.7	7	**Moroccan-G/83**
8	77.9	70.9	71.9	74.4	86.4	69.5	69.5		96.0	-	78.7	78.9	80.3	78.0	-	-	-	-	79.7	72.8	80.9	79.1	78.8	78.6	78.4	79.0	-	-	-	8	**IBV/morocco/30**
9	80.3	74.1	74.1	77.0	85.8	72.5	73.8	92.0		-	78.0	78.0	81.1	78.0	-	-	-	-	79.7	73.4	81.3	79.5	79.2	78.8	78.5	79.1	-	-	-	9	**IBV/Morocco/01**
10	51.1	76.0	76.0	67.8	63.2	75.7	70.0	-	-		-	-	-	-	78.0	78.4	79.8	62.4	94.8	76.5	-	-	80.8	79.7	79.9	-	79.9	79.8	80.5	10	**IBV02/2014/MOROCCO**
15	71.2	71.6	72.9	73.4	74.5	69.6	70.9	76.3	75.8	-		74.7	74.7	73.5	-	-	-	-	75.7	75.7	74.3	76.6	77.5	76.7	74.0	74.4	-	-	-	15	**Algeria/26/b1**
16	80.5	60.1	60.1	70.7	72.0	61.7	62.6	78.2	74.9	-	72.2		85.3	75.6	-	-	-	-	74.0	73.9	79.7	77.8	77.4	78.8	77.5	78.3	-	-	-	16	**Tunisia TN556/07**
17	79.7	64.4	64.4	64.3	71.3	60.0	56.4	73.2	73.2	-	67.3	71.6		76.2	-	-	-	-	73.1	72.0	79.8	78.0	78.3	78.6	76.7	77.2	-	-	-	17	**TN200/01**
18	68.4	59.9	61.8	64.3	71.3	60.0	56.4	73.2	73.2	-	67.3	71.6	67.9		-	-	-	-	71.1	71.2	74.6	74.6	75.0	74.2	75.9	75.6	-	-	-	18	**TN200/00**
19	81.0	76.2	77.4	81.0	83.5	78.6	78.8	-	-	73.2	-	-	-	-		97.6	86.7	66.7	80.2	79.2	-	-	86.7	99.2	98.4	-	98.4	99.1	86.3	19	**IBV/Libya/1-2012**
20	82.1	76.2	77.4	81.0	84.7	79.8	78.8	-	-	71.5	-	-	-	-	96.5		86.3	67.9	81.4	79.6	-	-	86.3	98.4	98.4	-	99.2	98.7	85.9	20	**IBV/Libya/7-2012**
21	82.1	76.2	75.0	82.1	83.5	82.1	80.0	-	-	70.5	-	-	-	-	84.7	84.7		67.2	81.7	80.0	-	-	100.0	87.1	85.9	-	85.9	85.5	99.2	21	**IBV/Libya/5-2012**
22	76.4	75	99.4	78.4	76.4	95.7	77.7	-	-	60.5	-	-	-	-	78.6	78.6	81.0		63.4	64.9	-	-	64.6	64.0	65.5	-	65.5	65.2	66.0	22	**CkZa/4916/11**
23	85.9	86.1	87.4	76.8	76.5	75.7	75.4	74.9	79.1	91.7	70.7	66.7	69.5	63.1	76.5	78.8	75.3	74.9		77.4	86.8	81.9	81.6	81.8	81.0	79.8	80.7	79.8	80.3	23	**NGA/293/2006**
24	76.4	75.9	76.4	77.6	75.0	78.0	76.1	68.3	72.0	74	70.2	64.4	69.0	61.7	72.9	72.9	75.3	77.8	76.4		74.4	71.0	76.9	77.6	77.3	70.7	79.6	79.3	79.9	24	**NGA/A116E7/2006**
25	96.9	71.7	73.5	71.7	79.7	70.9	70.4	76.4	78.9	-	70.4	79.9	78.5	67.7	-	-	-	-	80.5	73.0		84.1	82.9	83.8	87.2	87.8	66.1	-	-	25	**Egypt/D/89**
26	81.8	67.4	68.8	71.6	77.3	69.9	72.6	76.4	76.5	-	73.5	75.5	72.7	70.1	-	-	-	-	76.7	67.0	81.0		98.4	94.3	86.2	86.8	63.9	-	-	26	**Mans-1**
27	82.7	75.4	76.1	79.1	80.7	78.5	78.9	76.4	78.2	75.2	74.2	68.3	72.3	70.7	84.7	84.7	100	78.1	79.9	74.8	80.4	98.6		94.1	91.3	86.7	90.2	90.5	99.5	27	**Eg/CLEVB-2/IBV/012**
28	83.9	76.3	77.3	79.4	80.4	79.2	78.6	75.4	76.6	73.2	71.2	69.7	74.7	68.8	97.6	98.8	85.9	77.7	81.1	75.6	81.9	92.1	92.6		93.7	85.5	98.6	98.9	90.4	28	**Eg/1265B/2012**
29	84.8	76.2	77.3	80.3	80.5	78.6	78.5	75.2	78.1	64.3	70.9	72.4	74.6	68.2	97.6	98.8	84.7	78.2	80.1	75.8	87.0	85.6	90.1	93.8		99.0	99.3	99.0	90.2	29	**EG/12120s/2012**
30	84.7	65	66.1	71.8	75.3	67.8	68.2	75.9	77.1	-	71.8	79.9	74.4	68.2	-	-	-	-	74.0	66.7	85.2	86.4	85.2	84.2	98.8		-	-	-	30	**IBV-CU2-SP1**
31	83.3	80.4	81.2	82.4	86.0	82.5	83.1	-	-	74.3	-	-	-	-	97.6	98.8	84.7	81.0	81.9	77.4	-	-	89.9	98.6	100	-		99.5	89.9	31	**Ck/Eg/BSU-2/2011**
32	83.3	81	81.0	81.7	85.7	83.3	82.5	-	-	74.3	-	-	-	-	98.7	98.7	84.2	81.7	81.7	76.8	-	-	89.7	99.2	100	-	100		90.2	32	**Egypt/SCU-14/2013-1**
33	82.7	79.1	79.9	83.2	85.4	84.8	82.5	-	-	73.2	-	-	-	-	84.7	84.7	97.6	83.3	80.6	78.3	-	-	98.6	90.6	89.9	-	89.9	89.7		33	**Egypt/Beni-Suef/01**
	1	2	3	4	5	6	7	8	9	10	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33		

Amino acid identity (%)

The phylogenetic signal and complementary evaluation study of the sequences were checked by R language using the package picante and phytools (Revell, 2012). MEGA 6 (Tamura et al., 2013) was used to confirm the conservatism in amino-acids sequences of HVR regions.

The data matrix of both set of taxa and phylogenetic analyses were deposited into TreeBase under accession url: http://purl.org/phylo/treebase/phylows/study/TB2:S18513.

Phylogenetic analysis

The phylogenetic trees of the partial S1 gene sequences containing the highly variable regions HVR1, HVR2 (Fig. 1) and HVR3 (Fig. 2) of the selected African viruses of IB were constructed. The nucleotide and deduced amino acid sequences of these African IBVs were blasted and compared with the reference strains emerged in different African countries (Table 2). The pair-wise comparison (Table 2) and the phylogenetic tree (Fig. 1) of highly variable regions HVR1 and HVR2 sequences, revealed that these IBV viruses can be classified into two distinct groups: Group I consisted of six IBV variants of three clusters (cluster of Morocco, Algeria and Nigeria), which included Moroccan-G/83, Algeria/26/b1, Algeria/26/b2, Algeria/26/b3, NGA/293/2006 and NGA/A116E7/2006, these variants belong to 4/91, Mass strains (Ma5 and M41) and Qx-like. IBV variant "Moroccan-G/83" which is closely related to 4/91 (Jones et al., 2004), has a nucleotide sequence identity of 76%, 76.9% and 78.3% with Algeria/26/b1, NGA/293/2006 and NGA/A116E7/2006, respectively. Besides, the nucleotide and amino acid sequences identities of Nigerian isolates with others from the neighboring countries, like Algeria and Morocco, was ranged between 75% and 79%, and between 70% and 77% when compared to Algeria/26/b1 and Moroccan-G/83. Moreover, these low identities found between these variants, revealed that the cited countries don't have commercial exchanges of poultry or contaminated birds migration. Group II consisted of three clusters of the especially variants of Tunisia, Egypt and Morocco, which most of them had a close relationship with H120 and Italy02 strains (Figs. 1–3). The cluster of the Tunisian variants including TN296/07 and TN556/07 genotypes appeared to be very closely related to TN200/01 with highest nucleotide and amino acid TN556/07 identities of 85.3% and 71.6%, but the TN200/00 was branched separately from all Tunisian variants with 75.6% of nucleotide and 71.6% amino acid identities. These variants, when compared to the Egyptian variants, show a nucleotide sequences identities ranged between 74% and 79% and an identity of 68–80% in amino acid level. Furthermore, phylogenetic tree demonstrates that the Egyptian and Tunisian isolates, were closer to Mass

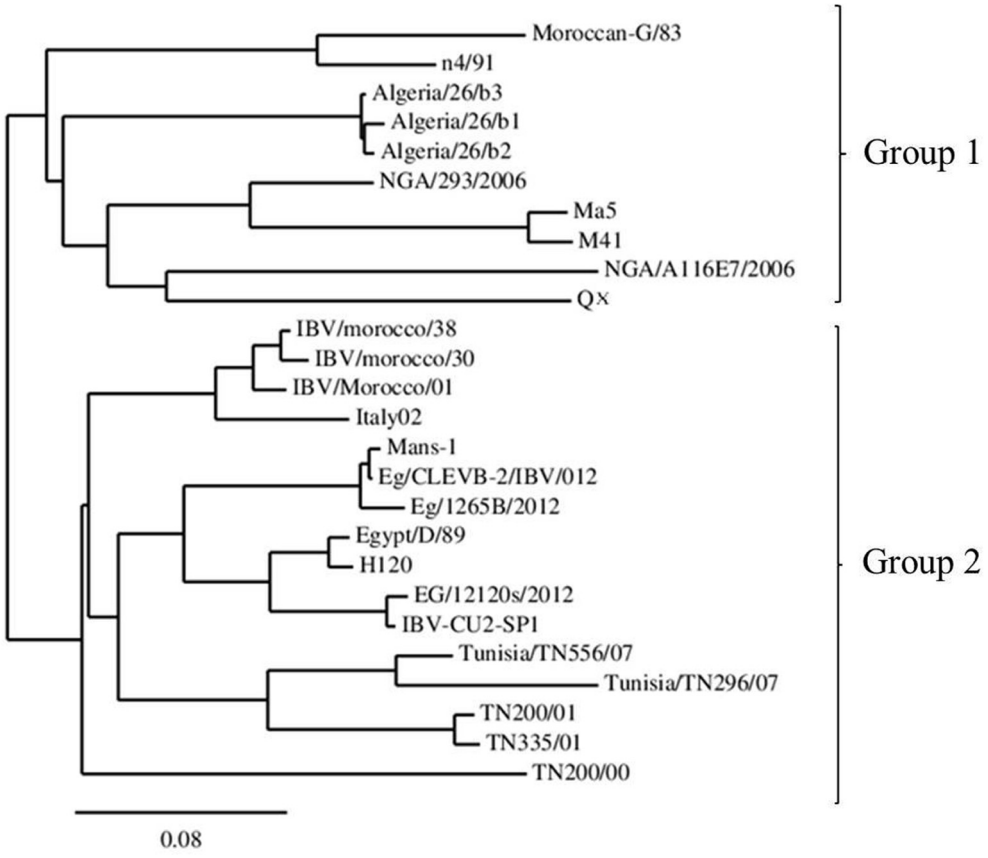

Fig. 1 Phylogenetic tree of all IBV variant isolated from different parts in Africa. The tree analysis is based on comparison of the partial nucleotide sequence of the S1 gene containing the highly variable regions HVR1 and HVR 2 (located between 146 and 546 nucleotide) showing the relationship between the African IBV variants and selected reference strains. The phylogenetic tree analysis was conducted by the neighbor-joining (Kimura two-parameter) method with 1000 bootstrap replicates implemented in Neighbor from the PHYLIP package (v3.66). Numbers along the branches refer to bootstrap values.

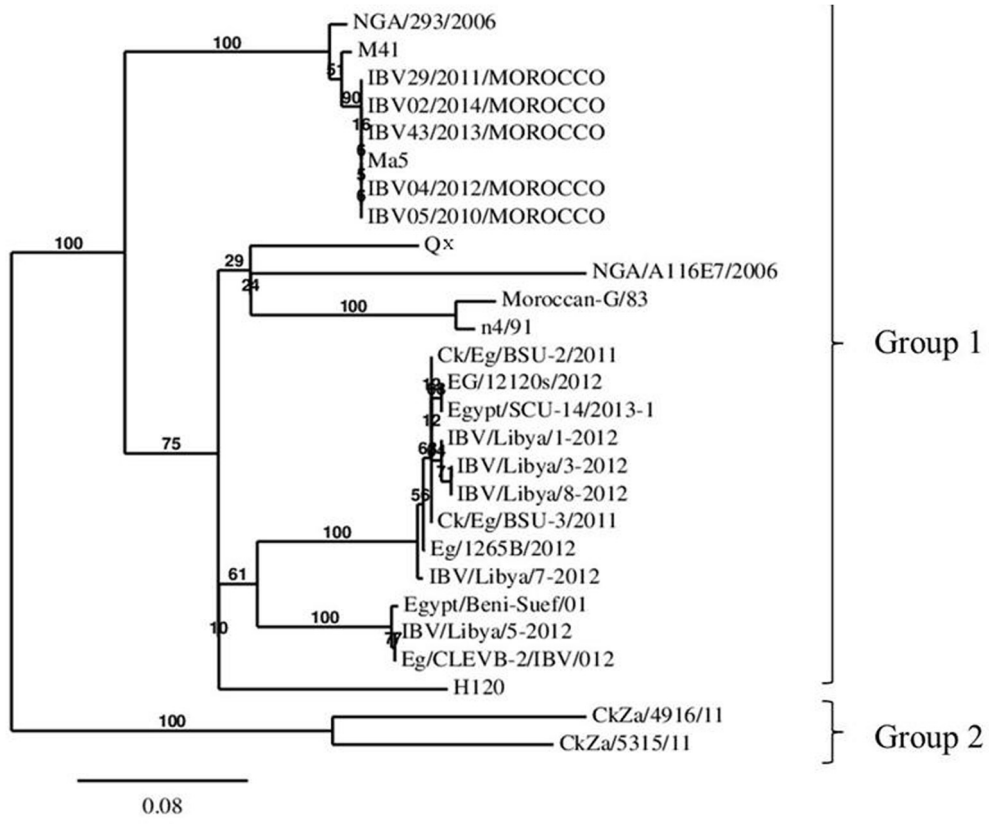

Fig. 2 Phylogenetic tree of all IBV variant isolated from different parts in Africa. The tree analysis is based on comparison of the partial nucleotide sequence of the S1 gene containing the highly variable regions HVR3 (located between 760 and 987 nucleotides) showing the relationship between the African IBV variants and selected reference strains. The phylogenetic tree analysis was conducted by the neighbor-joining (Kimura two-parameter) method with 1000 bootstrap replicates implemented in Neighbor from the PHYLIP package (v3.66). Numbers along the branches refer to bootstrap values.

H120 than Italy02 serotype, this was also confirmed by the pair-wise comparison of the deduced proteins sequences (80.5%) (Fig. 4).

In the other part, the Moroccan IBV isolates (IBV/Morocco/30, IBV/Morocco/38, and IBV/Morocco/01) were found to be different and formed a common branch with Italy02 strain as described previously (Fellahi et al., 2015b). The sequences of IBV/Morocco/30 and IBV/Morocco/01 have a 78–81% of nucleotide, and 75–78% of amino acid sequence identities with the Egyptian isolates which are closely related to H120 strain (Eg/CLEVB-2/IBV/012, Eg/1265B/2012, Mans-1, Egypt/D/89, EG/12120s/2012 and IBV-CU2-SP1).

The present sequence analysis of Moroccan IBV isolates demonstrated that the Moroccan isolates represent a unique genotype compared to the IBV variants of various neighboring countries. These are the only distantly related to African strains, suggesting that their origin may be different and not belong to African countries.

In the other hand, the sequence analysis of highly variable region HVR3 (Fig. 2) revealed that the selected variants emerging in KwaZulu-Natal (KZN) including (CkZa/4916/11 and CkZa/5315/11), were classified in a separate group. The second group contains two clusters, which most of the variants emerging in Libya, Egypt, Morocco and Nigeria, belong to Ma5, M41, H120, 4/91 and Qx. In addition, the Egyptian isolate EG/12120s/2012 has a nucleotide sequence identity of 99.3%, 99% and 90.2% when compared to Ck/Eg/BSU-2/2011, SCU-14/2013-1 and Egypt/Beni-Suef/01, respectively. Also, we note that both trees (Figs. 1 and 2) share the same topology for Moroccan-G/83 and NGA/A116E7/2006 variants. Interestingly, the Libyan variants (IBV/Libya/1-2012, IBV/Libya/7-2012 and IBV/Libya/5-2012) were found in the same subgroup with nucleotide sequence and amino acid identities of 85–99% and 84–98%, respectively to the Egyptian IBV strains Eg/1265B/2012, EG/12120s/2012, Ck/Eg/BSU-2/2011 and Egypt/SCU-14/2013-1. Additionally, sequences of IBV/Libya/5-2012 formed another cluster with 100% relatedness to Eg/CLEVB-2/IBV/012, and it has 99% of nucleotide and 97.6% of amino acid sequence identities to Egypt/Beni-Suef/01. Moreover, the Moroccan and Nigerian variants IBV02/2014/Morocco, IBV05/2010/Morocco, IBV04/2012/Morocco, IBV43/2013/Morocco, IBV29/2011/Morocco and NGA/293/200 are clustered with the Mass serotype Ma5 and M41. Indeed, the variant IBV02/2014/Morocco shares 97.5% and 96.4% identities to Ma5 and M41, respectively. It was shown also that the variants isolated from Moroccan poultry farms are divided into two types of genotypes, Massachusetts and Italy02 as confirmed by a previous study (Fellahi et al., 2015a).

In accordance to this examination, the most recent study of IBVs strains such as in Algeria (Sid et al., 2015), revealed a distinct variant that is different from the previously isolated strains, this confirms the rapid genetic evolution within time due to the high mutation rate of the IBV, this is the case for

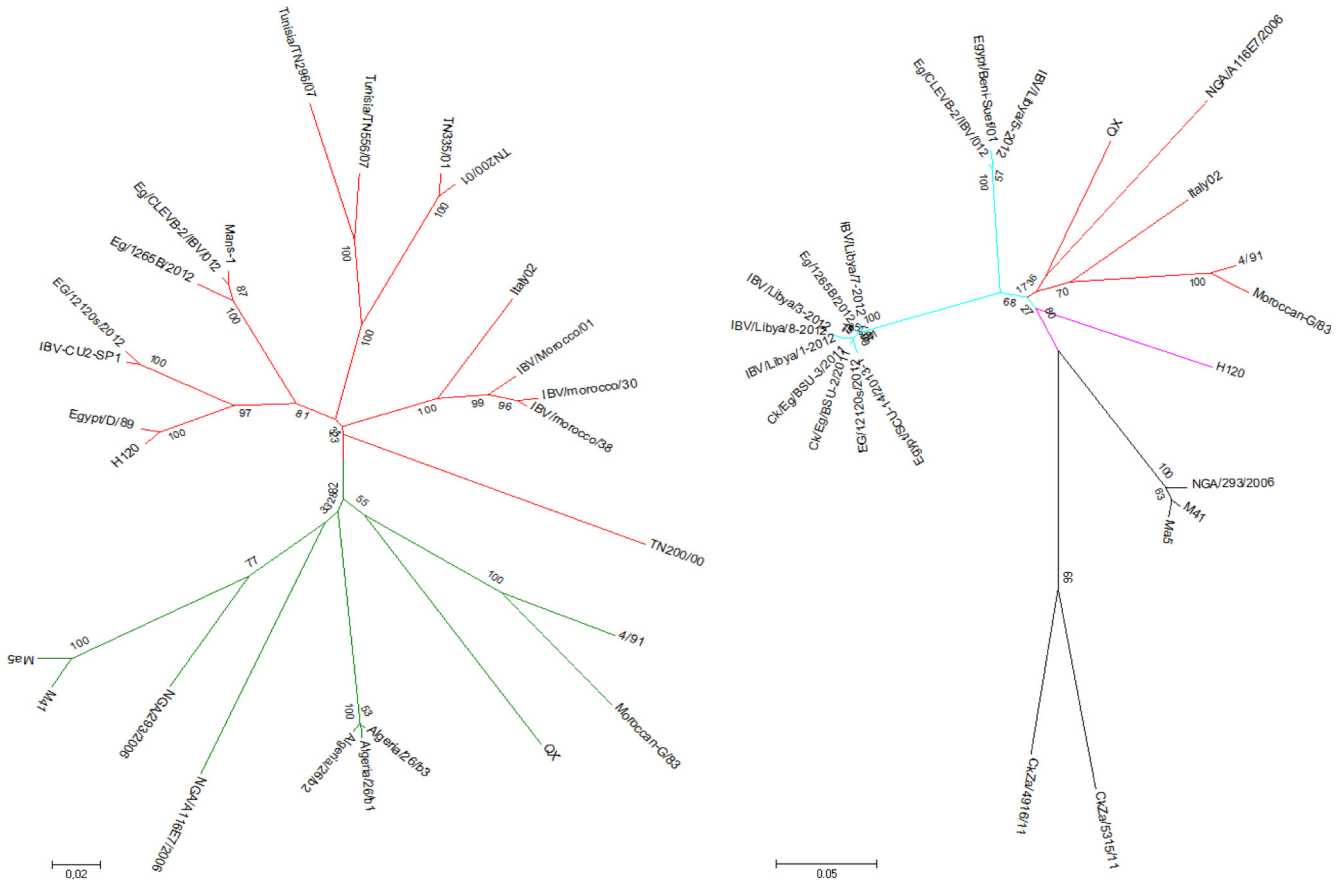

Fig. 3 Phylograms of HVR regions demonstrating the diversity of different IBV strains. The figure on the right shows the phylogeny of HRV1 and HVR 2 region, then left is the phylogram of HVR3. In both figures the presence of two lines is observed.

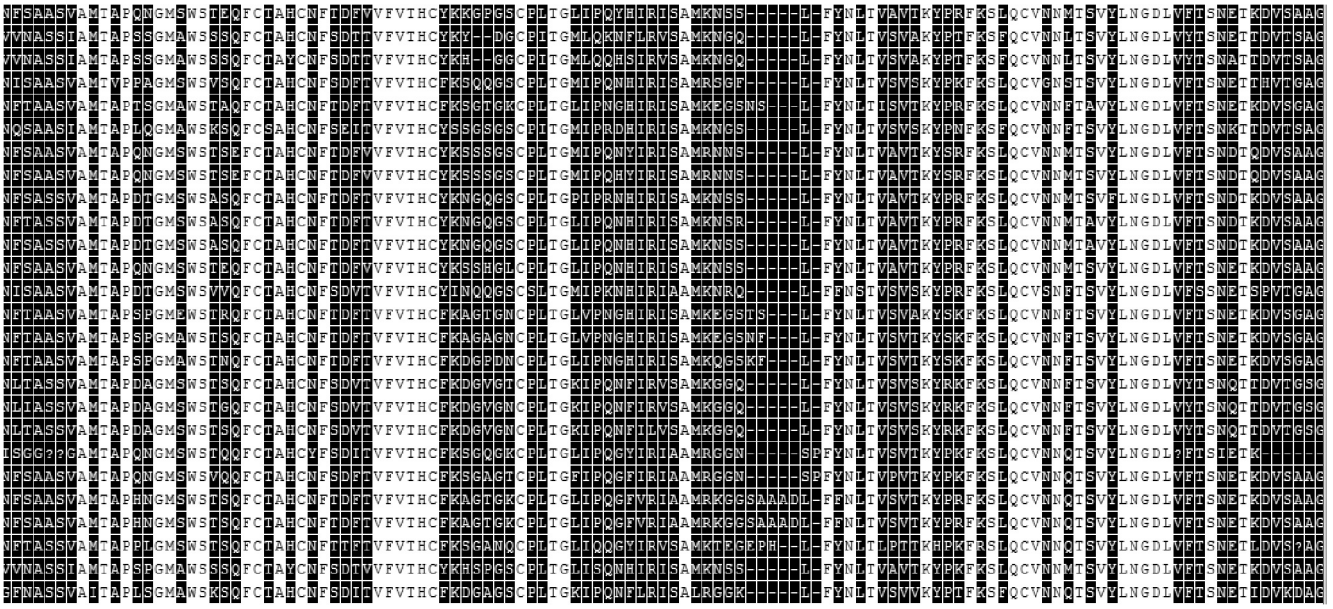

Fig. 4 Sequence alignment of amino acid of S1 gene showing highly conserved regions (in white) and non conserved regions (in black) in the HVR1 and 2.

the rest of the African countries. The present analysis appears to be in agreement with finding results that classified Libyan variants into two distinctive patterns, which one is particular of Libya and the other is highly related to the Egyptian strains cited above (Awad et al., 2014). This high relationship between the Libyan and Egyptian variants seems to be due to the

uncontrolled movement of inhabitants and smuggling through borders of the two neighboring countries.

A second analysis at the evolutionary level based on Fig. 3 reveal genetic diversity with the presence of at least two lines in the Mediterranean region. The first named line (MA) (blue color in Fig. 3) represents a set of genomes found only in Morocco and Algeria. The 2nd cluster (Poly) sequences representing strains Tunisian, Moroccan, Libyan and Egyptian who share the same cluster and have the same ancestor. The MA cluster could represent a reintroduction or dissemination of strains related to those initially introduced to other countries in the region. Such migration may be a question of these results that essentially Poly cluster contains a strain from Morocco which is scalable and this month as compared to other strains. And that take into awareness that the Maghreb countries are importers of eggs allocated to the nursery and chicken Europe and suggested that the possibility of having the same ancestor with virus evolution that different according to a set of environmental requirements and hygiene which vary from one country to the other.

Global alignment of the both nucleic and amino acid sequences, demonstrates the presence of conserved regions, although the sequences belong to the hypervariable regions. The amino acid position style was corrected as a following text: The global alignment of full sequences by CluwterW and Muscule methods shows that the Valine in position 121, Leucine in position 126, 130 and 142, with Cysteine 165 and 196, Asparagine in position 181, and Lysine number 205 are highly conserved with 100% of conservatism. Those conserved regions may be having important roles in the best functioning of the S1 of virus. However there are some areas semi-conserved, the Phenylalanine in position 108 muted to Aspartic acid, the Aspartic acid in position 112 transferred to Tyrosine, however the Tyrosine in position 137 muted to Asparagine, and Serine number 208 to Proline, Phenylalanine number 212 to Leucine, Cysteine 218 to Arginine, another the Arginine 222 transfer to Tryptophan.

The HVR1 and two are characterized by multiple non-synonymous mutations such that it can also be phenotypically distinct, although this requires experimental verification. In general, the obtained data revealed that the most frequently detected IBV variants in Africa were related to Massachusetts serotypes. Whereas, the African IBV variants that are classified distantly to the Massachusetts genotype are related to other strains such as, Italy02 type detected for the first time in Africa (Morocco) and Qx-like genotype South part of the African continent (Zimbabwe). Also the IBV variants reported in Africa, display a low genetic relationship between them and with the majority of the reference strains emerging in neighboring countries, except the case of variants from Libya and Egypt that show a high relatedness. This finding is confirmed by the protein sequence alignment that shows high diversity in the type of the mutations occurring in many residues (data are available but not shown).

Testing the ratio dN/dS = 5.41 using the method of Nei-Gojobori, and 3.344 by Li-Wu Luo's method (Tamura et al., 2013), the ratio dN/dS is for both >1, this predicts that there were more non-synonymous changes as synonyms changes. There was an evolutionary pressure to escape the ancestral state, namely the positive selection pressure. This is explained by the ability of the virus to change the protein to escape the host immune recognition and at the same time retaining the functioning of the protein and the active sites which can locate this region despite its hypervariability.

Control measures for infectious bronchitis virus (IBV) variants

Infectious bronchitis (IB) is one major viral disease affecting the respiratory tracts of birds and whose impact on African poultry is still poorly known (Kouakou et al., 2015).In the recent years, infectious bronchitis knew an extended evolution and became a serious problem in North Africa due to intensive poultry farming practices, and rapidly growing poultry industry (Abdel-Moneim et al., 2012; Fellahi et al., 2015b).

This disease is controlled mainly by vaccination in most African countries, using live attenuated or inactivated vaccines frequently based on Massachusetts strain (Khataby et al., 2016) However, outbreaks of IB are still occurring in vaccinated flocks, indicating that, most probably, emergence of new variants from different serotypes to the vaccine strain used. It is sometimes necessary to develop specific vaccines to control the disease, when the commercial vaccines fail to give an adequate protection against the emerging variants virus. These may be related also to many factors such as the lack of an appropriate vaccination program and application, or lack of bio-security (Jackwood, 2012).

Therefore, studies should be conducted to identify the epidemiological and biological behaviors of IBV variants emerging in Africa, in order to define more effective programs for IB control, taking into consideration that the inefficacy of the current control strategies based only on monovalent vaccination with the Massachusetts serotype vaccine is the main issue to be solved. These variants circulating and evolving in Africa since several years are still not studied enough in the genetic and antigenic levels. Besides, the pathotype variation and dynamics of genotypes within time for a specific region in Africa need as well to be investigated. In brief, all these analyses of partial S1 gene sequences of IBV variants circulating in Africa that are mentioned in this paper, revealed a possible dissemination of IBV between the neighboring countries, but little is known about the mode of IBV spread. However, cross-border movements of poultry and poultry-related products are likely an important factor. With these considerations in mind, the topic of how to type this large number of IB found in various countries, and relate this to the best vaccination strategy to use to protect against them is clearly complex.

Conclusion

In summary, the present paper focused on the relationship between the IBV genotypes circulating in African countries, and incites to reflect on how to implement an efficient control to the increasing number of IBV variants in Africa, which are generated from S1 gene sequence mutations, mainly in hyper variable regions HVR1, HVR2 and HVR3, and how to explain the no efficiency of vaccination used to stop the spread of IBV disease.

Competing interests

The authors declare that they have no conflicts of interest in this study.

Authors' contributions

K.K. performed the design of the study, active contribution to the bibliographic synthesis related to the IBV variants, carried out the data analysis and drafted the manuscript. A.S. carried out bioinformatic analysis, generated phylogenetic trees and helped to draft the manuscript. Y.K. carried out the computational analysis and helped in the bioinformatic analysis. C.L. participated in the design and helped to draft the manuscript. M.M.E. conceived the study, gave the opinion, corrected the final manuscript, and assumed coordination of the project. All authors read and approved the final manuscript.

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