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Avian Coronavirus: Case of Infectious Bronchitis Virus Pathogenesis, Diagnostic Approaches, and Phylogenetic Relationship Among Emerging Strains in Middle East and North Africa Regions

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INTRODUCTION

Avian infectious bronchitis (IB) is a highly contagious viral disease of serious economic importance in the poultry industry worldwide (Colvero et al., 2015; Khataby et al., 2016a). It was first described in North Dakota, the United States, as novel acute respiratory disease

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pathogen in young chickens in the late 1930s by Schalk and Hawn (1931), and since then has been documented in all countries with an intensive poultry industry (Jackwood, 2012).

The etiologic agent of IB is the IB virus (IBV), which belongs to the order Nidovirales, a member of the Coronaviridae family, in the subfamily coronaviridae and genus *Gammacoronavirus*, and it is the type species of the genus *Coronavirus* of the domestic chicken (*Gallus gallus*) (Cook et al., 2012; Ujike and Taguchi, 2015; Khataby et al., 2016a).

IBV is a coronavirus that only causes disease in chickens of all ages; nevertheless, the severity is great in younger ages (Glahn et al., 1989). The IB is primarily a tropism for the epithelial lining 55 of the respiratory tract, characterized by respiratory signs (Cavanagh and Naqi, 2003), after that many IBV vaccines were introduced to tackle this problem [H52, H120, M41, 4/91(793/B), and other strains]. Then, different IBV variants have emerged causing other clinical manifestations named nephropathogenic and reproductive problems that require a dramatic change in vaccination programs (Cavanagh, 1997; Liu et al., 2003). The IBV infections can also be further aggravated by the presence of bacterial infections such as *Escherichia coli*, *Mycoplasma gallisepticum*, *Mycoplasma synoviae*, and 50 *Ornithobacterium rhinotracheale* (Landman and Feberwee, 2004).

The enveloped viral particles are round and pleomorphic in shape. The virions are approximately 120 nm in diameter and contain club-shaped surface projection called spikes that are 20 nm in size (Cavanagh and Gelb, 2008). It is an enveloped positive-stranded RNA virus with a genome of about 27.6 kb in length encompassing 5′ and 3′ untranslated regions with a poly(A) tail. A major part of the genome is composed of two overlapping open reading frames (ORFs), 1a and 1b, which are translated into large polyproteins, 1a and 1ab, respectively, and contribute to the formation of the replication and transcription complex (Cavanagh., 1997; Khataby et al., 2016b). The remaining part of the genome encodes for four structural proteins that are called the phosphorylated nucleocapsid protein (N), the membrane glycoprotein (M), the envelope protein (E), and the spike glycoprotein (S). Two accessory genes have been described, ORF3 and ORF5, which express accessory proteins, 3a and 3b and 5a and 5b, respectively (Lai and Cavanagh, 1997). The spike glycoprotein of all coronaviruses contains four domains that are involved in the anchoring of the S protein into the lipid bilayer of the virion. The spike glycoprotein gene of IBV consists of 1162 amino acids and is cleaved into two subunits, S1 and S2, the N-terminal S1 subunit (535 amino acids) and the C-terminal S2 subunit (627 amino acids) (Cavanagh, 1997).

The S2 protein contains the C-terminal portion of the sequence including the *trans*-membrane anchor and two heptade regions

approximately 100–130 Å in length (771–879 amino acid in IBV) which are involved in oligomerization of the protein and entry into susceptible host cells (Casais et al., 2003). The major immunogen of IBV is the S1 subunit protein, which is anchored to the membrane by association with S2 subunit, which is responsible for the infection of the host cells, involved in virus entry, and also contains epitopes that can induce the production of specific neutralizing antibodies and the hemagglutination of inhibition antibody (Koch et al., 1990; Ignjatovic and Galli, 1994). IBVs from different serotypes usually exhibit poor cross-protection (Li et al., 2012).

Multiple IBV serotypes and genotypes were identified and reported worldwide, without, complete cross protection between all isolated strains caused by the nucleotide sequence variation in the spike gene (Cavanagh and Gelb, 2008). The IBV is identified and characterized by a large genetic and pathogenic variability. More than 50 different serotypes of IBV have been reported, and new variants continue to emerge (Bochkov et al., 2006). The high variation in the nucleotide sequences of spike gene can change the protection ability of a vaccine or immunity (Cavanagh and Naqi, 2003). Some reports have demonstrated that this genetic diversity, due to changed genomic virus by frequent point mutations and recombination events in the S1 gene, has given the emergence of new IBV variants that can be partially or poorly neutralized by existing vaccine serotypes (Khataby et al., 2016b). Wherefore, the phylogenetic 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 vaccine serotypes for IB control in each of the geographic regions (Jackwood, 2012).

The high variability of the S1 gene that is responsible of the emergence of new genotypes and serotypes of IBV is mainly due to the high level of mutations (insertions, deletions, and 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). 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, and HVR3) located at the positions 114–201, 297–423, and 822–1161 nt corresponding respectively, to amino acid residues 38–67, 91–141, and 274–387 have been located within the S1 subunit (Bourogâa et al., 2009; Khataby et al., 2016b).

IB is thought to be considered an epidemic virus and widely spread both in vaccinated and unvaccinated poultry farms with an incidence approaching 100% (Ignjatovic and Sapats, 2000), and it is difficult to control the disease within the regions where vaccination has not been correctly practiced; incidence of IBV infection was reported to be between 80% and 90% (Hitchner et al., 1966). In African regions the Massachusetts

serotype viruses remained the dominant ones in many countries; besides, it is one of the most common viral respiratory diseases of chickens, and it is considered an epidemic virus and widely spread both in African vaccinated and unvaccinated poultry farms (Khataby et al., 2016a). 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 variants emerging in Middle East and North Africa (MENA) regions, hence the objective of the present chapter aims to give an update of IBV pathogenesis, diagnosis approaches, and also to establish genetic relationship between the IBV variants emerging in the study target MENA regions.

PATHOGENESIS

IBV mainly affects the respiratory tract. However, some variants and several field isolates affect the reproductive, renal, and digestive systems of chickens. Disease pathogenesis differs according to the system involved, as well as the strain of the virus.

It is accepted that domestic fowl (*G. gallus*) and pheasant (*Phasianus* spp.) are considered to be the most natural hosts of IBV. Studies based on immunofluorescence (IF) tests, immunoperoxidase, and electron microscopy (Nakamura et al., 1991) showed that IBV multiplies in ciliated epithelial cells and secretory cells of mucus. Another study conducted by Lee et al. in 2002 confirmed the epitheliotropic quality and broad tissue tropism of IBV strains, as the virus infects respiratory cells and spreads to other tissues with the appearance of viremia. However, the upper respiratory tract is still the main multiplication site of the IBV. The infected chicken shows a thickening of the mucus, with a serous catarrhal exudate in the nasal passages, sinus and trachea. The presence of nasal exudate is used to assess the severity of the disease in different inbred chicken lines.

Determinants of the Pathogenicity of Infectious Bronchitis Virus

The pathogenicity determinants of IBV are not yet known. The IBV contains in its genome unstable part, which codes for the S gene (Spike S1), and seems to be indispensable in the determinism of the pathogenic power, as well as important antigenic variations that may appear irregularly under the action of mutations and recombination by escaping vaccine protection. Callison et al. (2001) showed that a difference of one or two amino acids in the composition of the IBV S protein

determined whether or not it was pathogenic. Similarly, Haijema showed that the inversion of the mouse protein hepatitis protein S gene with that of the cat's coronavirus allowed the creation of a replication-capable virus in cat. However, the determining role of the pathogenicity of protein S is not yet fully elucidated, and possessing a protein S of a pathogenic strain does not appear to be sufficient to express pathogenicity.

The role of nonstructural proteins (3a, 3b, 5a, and 5b) is still unclear, but it is possible that they are responsible for circumventing the host's immunity, pathogenicity of IBV. This hypothesis is still pure conjecture.

Host Susceptibility

Domestic poultry (*G. gallus*) and pheasant (*Phasianus* spp.) are considered to be the only natural hosts of the IBV. Other similar IBV coronaviruses have been identified in avian species, including pheasant, peacock, turkey, teal, geese, pigeons, quail penguins, ducks, and Amazonian parrots. The IB disease is an acute, highly contagious viral infection of chickens of any age with adverse effects on egg quality and production and is characterized by high depression during the growth period, particularly in laying hens (Cavanagh, 1997). Birds of all ages are likely to be infected with the virus, but sensitivity is greater in subjects less than 6 weeks old, often in favor of stress or immunosuppression. The morbidity is close to 100%, whereas the mortality is variable according to the tropism of the viral strain, low for strains with respiratory tropism and high for strains with renal tropism. Other factors influencing mortality of breeding are virulence of IBV strain, immune status of birds, presence of maternal or active immunity, age, and bacterial superinfections.

Infection and Transmission

The IBV spreads from one building to another or even from one operation to another by the air that remains the preferred route for the IBV by replicating on the mucosa. Thus coughing and sneezing spread pathogenic particles in the form of an aerosol (inhalation) and ingestion of contaminated food, dead bodies of infected poultry, and rodents.

The primary transmission is horizontal and direct, which introduces germs from infected individuals to other healthy ones, or contaminated equipment and materials are a potential source of indirect transmission over long distances. Clinical signs develop through contact in chicks within 3 hours and in nearby hangars within 1–2 days (Cavanagh, 1997).

DIAGNOSIS APPROACHES

Epidemiological studies do not make it possible to diagnose IB, and confirmation of suspicion requires direct and/or indirect detection techniques of infectious agents. Given a growing interest and a better knowledge of the epidemiology of the IBV, diagnostic tests are currently in full development. In general, these methods are based either on the detection or isolation of the virus itself, or on the detection of anti-IBV antibodies (Ac).

Virological Identification: Isolation and Culture

The first researchers who developed viral isolation technique of IBV on embryonated eggs originated in the United States, in fact, Cunningham (1970) of the University of Lansing and Fabricant (1998) of the University of Cornel, have shown that IBV grows rapidly on embryonated eggs, so the isolation of the virus from the allantoic fluid of embryonated eggs has become the method of choice for IBV isolation (Cook et al., 2012).

Suspensions of diluted tissue samples (10−20/100 mL or 10%−20%) are prepared with phosphate-buffered saline or a nutrient medium for inoculation with the egg or culture medium used for the culture agent tracheal (CAT) rings (Cook et al., 2012). The suspensions are clarified by slow centrifugation and filtration on bacterial filters (0.2 μm) before inoculation on egg or on CAT. Embryonic chicken eggs and CATs are widely used to perform primary isolates of the virus.

Cell cultures are not recommended for primary isolation because it is often necessary to adapt IBV isolates to embryonated eggs before a cytopathic effect of viral infection on embryonic stem cells chicken kidney. Embryonic eggs used for the isolation of the virus should preferably come from exempt organism pathogen specific chickens or from breeder who have never been infected or vaccinated. Most often 0.1−0.2 mL of the sample supernatant is inoculated into the allantoic cavity of embryos aged 9−11 days. The eggs are then screened every day for 7 days, and any mortality occurring within 24 hours should be considered nonspecific and the eggs removed. Usually, the initial inoculum has no visible macroscopic effects on the embryo unless it is a vaccine strain already adapted to the egg. Three days after inoculation, the allantoic fluids of all eggs were harvested and mixed. This mixture is diluted to 1/5 or 1/10 in a medium containing antibiotics for a new passage on other eggs to a maximum of 3−4 passages. Generally, a wild strain induces visible lesions on the embryo (stunted embryos with feather dystrophy) from the second to the fourth passage. At the following

passages, embryonic mortality can be observed as the strain adapts to the egg culture.

Serological Diagnosis

The multiplicity of IBV serotypes and the antigenic variations of the latter complicate the selection of appropriate serological techniques and their interpretation. All IBV serotypes have common epitopes, which is mainly explained by the antigenic conservation of the N, M, or S2 proteins of the S protein. But there are also antibodies specific to an IBV serotype, determined by the epitopes of the S1 protein. However, conventional ELISA, IF, or immunoassay tests bind an antibody to antigens generally nonspecific to a viral strain. There are cross-reactions between these viral strains, which makes it generally difficult to distinguish them by serology. In addition, a method of serological diagnosis by hemagglutination has been developed.

Initially, the IBV does not possess agglutinating properties, but after treatment virus with the neuraminidase, the latter becomes able to bind to the erythrocytes. This method makes it possible to titrate the virus by dilution of the sample to be tested, without, however, estimating the pathogenicity of the virus. This is why serology will mostly be carried out to monitor vaccination in a herd to screen for IB, but it will not be precise enough to type the circulating IBV variant. enzyme linked immunosorbent assay (ELISA) commercial tests can detect a viral passage within 1 week postinfection. In general, two serological tests are performed; one at the first signs of infection and the second 10−14 days later. The low cost, simplicity, and rapidity of serological tests make them widely used as a routine diagnosis.

Molecular Diagnosis

Laboratory diagnosis includes serological (antibody) and virological methods (virus isolation, RT-PCR, and nested PCR). Confirmation of the diagnosis is obtained by the demonstration of the viral antigen, sometimes associated with the serological examination.

The widespread use of attenuated or inactivated vaccines can complicate diagnosis using serological methods, since antibodies of vaccine and wild origin cannot always be distinguished. The persistence of a live virus vaccine may also complicate the isolation tests of the causative agent.

In Morocco the disease has been known for a long time and continues to cause significant damage to poultry production. IBV viral strains in circulation are not or partially identified, resulting in vaccination

failures in the field. The emergence of variant antigenic strains in the field may explain the ineffectiveness of immunity induced by conventional vaccines. The RT-PCR genotyping largely replaced serotyping by inhibition dehemagglutination (IHA) and seroneutralisation (SN) to determine the identity of wild-type strains. The molecular bases of the antigenic variation were examined, usually by sequencing the nucleotide of the gene encoding the spicule protein (S) or, more specifically, the gene encoding the S1 subunit of the S protein, where the largest number of epitopes identified by neutralizing antibodies is observed. An exact correlation with the results of IHA or SN is not observed if the different genotypes generally exhibit large differences (20%−50%) in the amino acid sequences of the subunit S1, of the other viruses that are clearly differentiable by serum neutralization present only 2%−3% of differences in the amino acid sequences (Alvarado et al., 2003).

However, the results obtained with the S1 sequence compared to the serotype identified by sero-neutralization make it possible to select the vaccine strains on the basis of the data provided by the sequencing. The first advantage of molecular techniques is their speed and ability to detect a wide variety of genotypes according to the tests used. The restriction fragment polymorphism (RFLP)-coupled reverse-transcription polymerase chain reaction (RT-PCR) distinguishes the different IBV serotypes on the basis of the unique band profiles obtained by the electrophoresis of the restriction fragments obtained by enzymatic digestion of S1 after RT-PCR amplification of the gene. The RFLP RT-PCR method can be used in combination with a biotin-labeled probe to first detect IBV in liquids harvested from eggs inoculated with clinical specimens S1 genotype-specific RT-PCR can identify all IBV serotypes. S1 gene primers specific for the Massachusetts (Mass), Connecticut, and Arkansas serotypes are used in combination with a pair of universal primers that amplify all IBV serotypes.

The other variant serotypes can be recognized as IBV using the conventional primers but the serotype cannot be determined. Multiple infections due to several IBV serotypes can be diagnosed).

Nucleotide sequencing of a diagnostically significant fragment of the S1 gene is the most useful technique for differentiating IBV strains and has become the method of choice in many laboratories Sequencing also observed that recombination often occurred between IBV strains. It is possible to use the sequencing of the RT-PCR product (hypervariable terminal part of S1) to identify isolates or wild-type variants, previously recognized as IBV. Analysis and comparison of sequences of variants and unknown wild isolates with reference strains to verify their degree of kinship are important advantages of sequencing.

PHYLOGENETIC RELATIONSHIP OF AFRICAN VARIANT AND THE REFERENCE STRAINS OF INFECTIOUS BRONCHITIS VIRUS

S1 Gene Sequence Analysis

In this study, we select all the sequences of IBV variants emerging in MENA regions, especially in Morocco, Egypt, Tunisia, Algeria, Libya, Iran, Iraq, Amman, and Jordan. 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 33.1). Their accession numbers are as follows: GenBank: M21969, GenBank: M21883, GenBank: AY561713, GenBank: AF093794, GenBank: AJ457137, and GenBank: AF193423. The percentages of nucleotide and deduced amino acid similarities were calculated for a selection of representative variants of each country using EMBOSS Matcher, a pairwise local alignment tool available in www.ebi.ac.uk.

The phylogenetic analysis was performed using sequences generated from the GenBank database. The set of sequence data was 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 this phylogenetic tools are available in the phylogeny.frn platform (Dereeper et al., 2008). Another tool of the computational phylogenetic parameters, were calculated using the packages of R language.

We note that the S1 gene sequences of IBV variants do not overlap together when aligned, because the studies have targeted different regions from the S1 gene (as shown in Table 33.1). Missing data are often considered to be a significant obstacle in phylogenetic reconstruction. However, to avoid noncomparative analysis, we separate the taxa into two parts, a part containing HVR1 and HVR2, the other part for HVR3. 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 33.1 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

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

(*Continued*)

TABLE 33.1 (Continued)

	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**		
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 boxes indicate sequences that do not match. The targeted regions of S1 gene sequencing are nonhomogenous for all studied IBV variants.

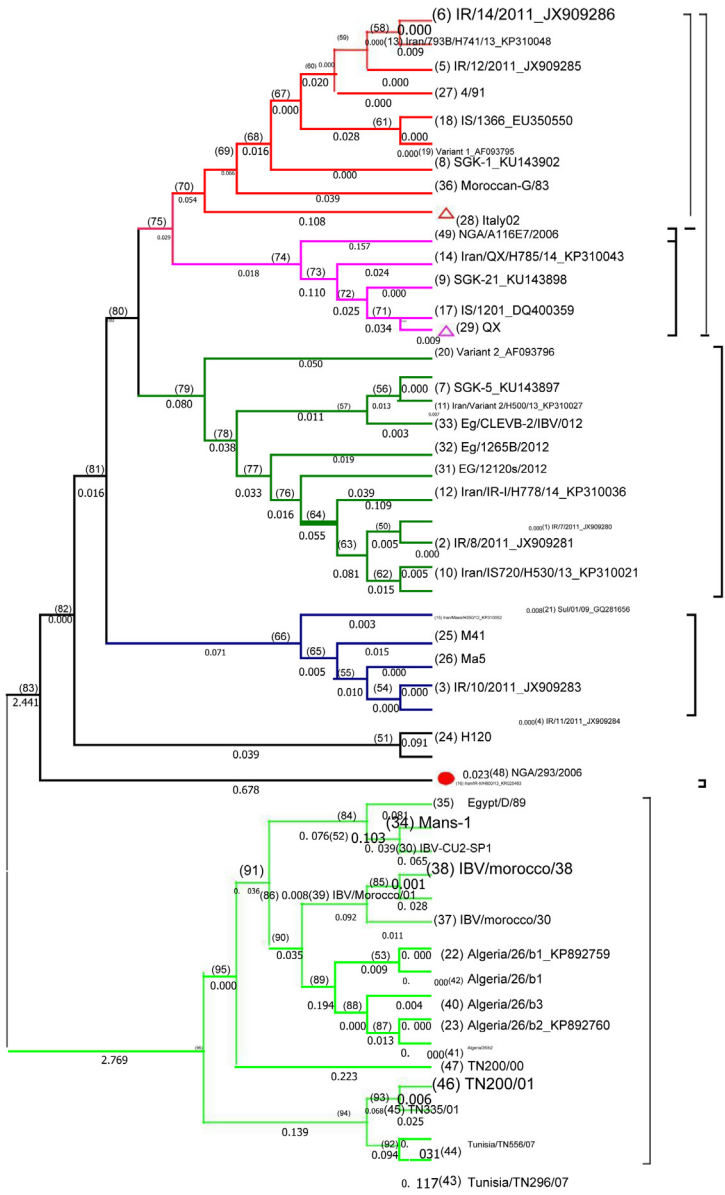

FIGURE 33.1 Phylogenetic trees of HVR regions demonstrate the diversity of different IBV strains. At least two clusters are shown: one lest evolve in Morocco and Algeria; however, the second characterized Egypt, Morocco, and Tunisia. The figure on the right shows the phylogeny of HRV1 and HVR2 regions, then left is the tree of HVR3. In both figures the presence of two lines is observed. The tree was constructed by NJ algorithm basing on Phytool package, and MEGA7 with a 1000 bootstrap. *HVR*, Hypervariable region; *IBV*, infectious bronchitis virus; *NJ*, Neighbor-joining.

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

Phylogenic Construction

Global alignment of the both nucleic and amino acid sequences demonstrated the presence of conserved regions, although the sequences belong to the HVRs. Conserved amino acids in the following positions of HVR3 have a percentage of 100% of conservatism, mentioning thereof in positions V 121, 126/L, 130/L, 142 L, 165/C, 181/N, 196 C, 205 K. Having conserved regions shows that the region is amino acids essential for the proper functioning of virus and that these amino acids require immunological and biochemical studies to study their website hoping to develop antibody capable of having links with the given sites.

In addition, other areas are semiconserved 108 F/D, 112 D/Y, Y 127/*, 137 Y/N, 208 S/P 212 F/L 218 C/R, 222 R/W, 225 F/L, 233 L/-, Y 236/-?, 244 L/-M, while HVR1 for the amino acid at position 555 is retained hyper.

The HVR1 and HVR2 are characterized by multiple nonsynonymous 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, particular in North Africa (Morocco) and Qx-like genotype south part of the African continent (Zimbabwe). In addition, 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 cases of variants from Libya and Egypt that show a high relatedness (Fig. 33.1).

The phylogenetic tree is generated via MEGA 6/7 using the NJ and ML methods whose phylogenetic signal and bootstrap are favorable for tree NJ shown in the image earlier.

In order to avoid phylogenetic tree noise, we studied the phylogenetic signal, which is of the order of 0.4 by the Blomberg's K method. This means that there is an independence of the studied species on the scale of tree. The thing that corresponds to the null hypothesis, that the HVB1/2 region are HVRs and therefore represents an independence. Simulating phylogenetic data is a powerful tool for evolutionists, but it can be a complicated task to use. In this chapter, we used the APE

FIGURE 33.2 Sequence alignment of amino acid of S1 gene showing highly conserved regions (in white) and nonconserved regions (in black) in the HVR1 and HVR2. *HVR*, Hypervariable region.

package of R to do a simulation of our phylogenic tree by the "coalescent" method (Fig. 33.2).

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 HVRs, HVR1, HVR2, and HVR3, and how to explain the no efficiency of vaccination used to stop the spread of IBV disease.

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