REVIEW

The emergence, evolution and spread of infectious bronchitis virus genotype GI-23

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Abstract
Avian infectious bronchitis is a contagious viral disease, caused by avian infectious bronchitis virus (IBV), that leads to severe losses in the poultry industry all over the world. Since the 1950s, IBV has circulated in the Middle East and North Africa, and no tangible evidence has shown any effects of measures taken to control its spread or evolution. Furthermore, new IBV variants are continually discovered. Although several genetic studies on IBV have been conducted, many IBV strains from this region have either been misclassified or remain unclassified. The genotype 23 (GI-23) variant emerged and has prevailed in the Middle East by continuously evolving through inter- and/or intra-genotypic recombination. The GI-23 genotype is currently enzootic throughout Europe and Asia. Although many studies of protection against the circulating strains have been conducted, they have not been standardized according to regulatory requirements. In this review, we provide an overview of the evolution and genetic diversity of IBV genotypes and a genetic classification of IBV strains, with a focus on the GI-23 genotype. The high prevalence of IBV GI-23 strains necessitates the adoption of vaccination schemes using GI-23-based vaccines.

Introduction

Avian infectious bronchitis (IB) is a major viral respiratory disease that occurs in all countries that raise poultry due to its considerable virulence and rapid spread, as well as the existence of several serotypes with poor cross-protection among types. In addition to its severe respiratory effects, IB also produces reproductive and urinary lesions that are associated with increased mortality, depending on the virus strain, age, and immune status of the birds [1].

IB is caused by infectious bronchitis virus (IBV), whose natural hosts include chickens and pheasants [2]. This virus has also been isolated from several types of birds, including peafowl, turkeys, geese, pigeons, quail, ducks, parrots, penguins, Guinea fowl, and other avian species [1, 3, 4]. Chickens of all ages are vulnerable to IBV infection, but younger birds are more susceptible than older ones [5].

IBV is an epitheliotropic virus that attacks organs lined with epithelia, including the respiratory tract (especially the trachea), the alimentary tract, and the urogenital tract [1]. It is believed that the virus replicates primarily in respiratory organs in the ciliated epithelium and mucous-secreting cells of respiratory organs. The virus causes viremia for a short time, followed by systemic dissemination to other organs, where further replication can occur, depending on the virus strain and the immune status of the host [6, 7].

The persistence of the virus and the severity of lesions that form in the trachea depend mainly on the type of IBV strain. [8, 9]. An increased severity of disease occurs mainly

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due to the activation of secondary infections (such as colibacillosis and mycoplasma infection) following tracheal ciliary stasis [10, 11]. The kidneys are the main replication site for nephropathogenic IBV strains after their initial replication in the respiratory tract. They replicate mainly in the collecting tubules and lower parts of the nephron, leading to the precipitation of urate crystals in the kidneys and ureters [12–14]. Some IBV strains disseminate through the bloodstream to the oviduct in young chicks and mature layers [15]. Other affected tissues include the lungs, air sacs, oesophagus, proventriculus, duodenum, jejunum, liver, spleen, bursa of Fabricius, caecal tonsils, liver, ileum, rectum, cloaca, ovaries, and testicles [16–18].

A new classification system based on the diversity of the full S1 sequence has been suggested for IBV. In this system, IBV strains are classified into seven genotypes (GI-GVII) with dozens of genetic lineages. Genotype GI has the largest number of genetic lineages (n: 29). The Massachusetts (Mass) type belongs to the GI-1 lineage, while the Egyptian variants I and II and many Middle East IBV strains belong to the GI-23 lineage, which has spread to many countries in Africa, Asia and Europe [19–23]. Although classification based on the full-length S1 is very efficient, it leaves a gap in which a very large number of IBV strains for which only partial S1 sequences are available, remain unclassified.

Here, we provide an overview of the distribution of IBV strains, with a focus on the GI-23 genotype. The validity of IBV genotyping based on both the full S1 nucleotide sequence and that of hypervariable region 3 (HVR3) of S1 are evaluated, and cross-protection is reviewed.

Virus taxonomy and structure

IBV belongs to the subgenus *Igacovirus*, genus *Gammacoronavirus*, subfamily *Orthocoronavirinae*, family *Coronaviridae*, and order *Nidovirales* [24]. The virus is a single-stranded, non-segmented, linear positive-sense RNA with a large genome of 27.6 kb that encodes several structural and non-structural proteins (NSP), which are essential for viral replication. IBV has the genome organization 5'UTR-ORF1a/b-S-3a-3b-E-M-4b-4c-5a-5b-N-6b-3'UTR. There is a -1 frameshift at the junction of ORF1a/b (the replicase gene), resulting in translation of the 1a and 1b polyproteins. Post-translational cleavage of the polyproteins gives rise to the individual non-structural proteins that are involved in genome replication and transcription [25].

The viral structural proteins include the spike (S) protein, matrix (M) protein, envelope (E) protein, and nucleocapsid (N) protein [15]. The spike protein is the largest structural glycoprotein in avian IBV. Trimers of the S protein form club-shaped or petal-shaped spikes of 128 kDa that are heavily glycosylated, giving them a total molecular weight of about 200 kDa. The S protein is responsible for viral tropism, attachment of the virus to cells, and fusion of cellular and viral membranes. The S protein is cleaved at the highly basic furin consensus motif RRFRR into the subunits S1 and S2. The S1 subunit is derived from the N-terminal portion of the S protein and is responsible for attachment of the virus to the cellular membrane through its interaction with cellular receptors. It contains three hypervariable regions (HVRs) that are responsible for its variation and escape from the immune defence. HVR1, HVR2 and HVR3 are located at amino acid positions 38-67, 91-141 and 274-387, respectively [26]. The S1 protein is also responsible for eliciting neutralizing antibodies, which interfere with the binding of S1 to its receptor. Its amino acid sequence identity varies between serotypes from 5 to 50%. The S2 subunit consists of a narrow stalk ectodomain, a short transmembrane segment, and a C-terminal domain, and is responsible for fusion of the viral membrane with the cell membrane. [26].

Classification of IBV

IBV classification systems are based on two main types of tests: i) functional or biological tests, and ii) non-functional tests. The functional classification allows viral isolates to be grouped into immunotypes, protectotypes, and antigenic types (serotypes and epitope types). Non-functional tests are based on sequence variations in the S gene that allow them to be assigned to different genotypes. In practical terms, immunotype or protectotype classification is the most important because it provides information about the protective efficacy of vaccine strains [27].

Strains that protect against each other are called protectotypes or immunotypes [27]. The number of protectotypes remains unknown. Protectotyping is usually performed experimentally using *in vivo* cross-immunization studies [28]. Protectotype experiments have been replaced more recently by the *in vitro* cross-immunization test (CIT), which uses specific-pathogen-free embryonated chicken eggs (SPF-ECE) or tracheal organ cultures (TOCs) from vaccinated chickens [29]. Both testing procedures may soon be replaced by bioinformatic prediction and structural analysis programs. [30–32].

Antigenic types include serotypes and epitope types. Serotype classification is the classical functional classification of IBV based on a strain's reaction with serotype-specific antibodies raised in chickens. Testing is performed using virus neutralization tests (VNTs) and TOCs or a haemagglutination inhibition (HI) test [29, 33, 34]. Because it is prone to strong and variable cross-reactions, the HI test is considered less trustworthy than VNTs [35]. A VNT is performed using the α or β method, with the α method being

more precise and sensitive. However, serotyping shows a lack of standardization [27].

Epitope typing uses monoclonal antibodies (Mabs) to detect the presence of specific epitopes in viral antigens via antigen-capture enzyme-linked immunosorbent assay (ELISA) [36] and immunofluorescent antibody techniques [37]. Mabs are directed specifically against the HVRs of the IBV S1 protein. The main disadvantage of this technique is that false-negative results may be obtained when the Mabs target conserved epitopes; the presence of a mutation in an epitope does not necessarily indicate a change in the serotype [27].

Genotyping is a genetic-based classification technique based on sequencing, detection of nucleic acids (e.g., by RT-PCR), or determining the position of enzyme cleavage sites (e.g., RFLP, RNase T1 fingerprinting) (Table 1) [27]. S1 gene sequencing is the most widely used approach to classifying IBV isolates into genotypes because it is based on differences in the most variable and antigenic region of the viral genome. Recently, phylogenetic analysis based on IBV S1 gene sequencing has revealed the existence of seven genotypes (GI-GVII) consisting of 35 genetic lineages, as well as inter-lineage recombinants (Table 2). Genotype GI includes 29 genetic lineages, while each of the other genotypes has only one lineage. The GI-1 and GI-13 lineages represent the old Massachusetts (Mass) and 793B lineages, respectively. Both lineages are universally distributed in all endemic countries and are most commonly used as vaccine strains, while other lineages are found only in specific parts of the world [19–23]. The GI-23 lineage includes IBV variants circulating in the Middle East. This lineage continues to spread and poses a major challenge not only in the Middle East but also in many countries in Africa, Asia and Europe [19–23].

The relationship between the different classification systems is complicated and has not been fully clarified. However, amino acid changes in the S1 protein may lead to a different serotype designation, depending on the site of the mutation and its effect on cross-immunity. In general, a high degree of amino acid sequence similarity correlates with cross-protection, but some published data have indicated that very small differences can affect the degree of cross-protection [12, 38].

Epidemiological situation in the Middle East and North Africa (MENA)

The epidemiological situation in the Middle East and North Africa (MENA) is still unclear due to a lack of optimized surveillance programs and the absence of adequate full-genome sequence data for the circulating strains. Egypt was the first country to isolate IBV in the 1950s [39], and the virus was then detected in Morocco in the 1980s [40]. During the 1990s, other countries began surveillance programs for the detection and isolation of IBV [25]. However, the scarcity of epidemiological studies in most MENA countries has made precise monitoring of IBV unfeasible.

Egypt

Serotypes related to the Mass, D3128, D274, D-08880 and 4/91 serotypes have been detected in different chicken flocks [41–43]; however, none of these isolates have been sequenced. The first IBV sequences for two different genotypes (including D274, an archival sample from 1989, and the new Egyptian genotype Egyptian Var I [Egypt/Beni-Suef/01] represented by two strains from 1998 and 2001) were first published in 2002 [44]. Egyptian Var I (Egypt/Beni-Suef/01) was found to be closely related to the Israel/720/99 strain [44]. From 2006 to 2015, many reports of isolation and identification of IBVs circulating in Egypt were published [38, 45–47]. Most of these studies were limited to partial IBV sequences. An IS/1494/06-like strain was detected in Egypt in 2010 [46]. Additionally, a novel IBV genotype was isolated in 2012 and named EGY VAR II. This genotype included both Ck/Eg/BSU-2/2011 and Ck/Eg/BSU-3/2011. The viruses showed 89% amino acid sequence identity in HVR3 to Beni-Suef/01 (EGY VAR I) and IS/885 [45]. The Egyptian VAR II group became the most prevalent group of strains found on chicken farms in Egypt. Egyptian variants I and II were both recently classified as belonging to a wild-type cluster in the GI-23 lineage in the newly established classification system for IBV genotypes [20]. Additionally, a complete genome sequence of the CU/4/2014 strain (EGY VAR II) showed evidence of recombination, with three putative parent strains: the Italian strain 90254/2005 (a QX-like strain) and strains 4/91 and H120 [48].

Lebanon and Syria

National serological surveillance was conducted between 1992 and 1996 in Lebanon for all avian pathogens. The results revealed serological evidence of IBV infection in chicken flocks [49]. No other data about the IBV situation in Lebanon were available until Ganapathy and co-workers identified strains related to Middle East strains (GI-23), 793/B (GI-13) and Mass-like (GI-1) strains that circulated in Lebanon from 2010 to 2012 [50]. The new IBV strain sequences isolated in 2018 from Lebanon were made available in the GenBank database, and our analysis based on these sequences confirmed their clustering with the GI-23 strain. In addition, recently released Syrian IBV strain HVR3 sequences have been determined to belong to the GI-23

Table 1 IBV genotyping and serotyping systems

General classification	Typing method	Test	Description and assessment parameters	Advantages and disadvantages	References
Functional	Immunotyping/ protectotyping	Cross-immunization test	Assesses cross-immunity in chickens following challenge by: 1. Clinical signs 2. Virus re-isolation 3. Histopathological changes 1. Ciliostasis test 2. Detection of viral genome (RT-PCR) 3. Serology	Adv.: 1. Direct information about vaccine efficacy 2. The best protocol to determine cross-protection between IBV strains 3. Whole immune responses are involved in the test Disadv.: 1. Laborious and high cost 2. The vaccine application methods, the challenge virus, assessment parameters, type and age of bird influence the results.	[30, 111, 112]
		Cross-immunization Test	Assesses cross-immunity in TOC by *in vitro* ciliostasis test	Adv.: 1. Useful for comparing tissue tropism 2. More economical than cross-immunization studies 3. The protocol is better standardized than cross-immunization. 4. Less labor-intensive than cross-immunization tests Disadv.: 1. Complex methodology 2. Requires highly trained technicians 3. Some IBV variants show different behavior in TOC and chickens. 4. The immune system is not involved.	[29, 113]
	Antigenic typing/ serotyping	Virus neutralization test	Assesses the neutralizing reaction between specific antisera against the unknown isolates in eggs, TOC, or cell culture	Adv.: 1. More sensitive test than HI Disadv.: 1. Lack of standardization 2. Time-consuming and laborious 3. VNT is less accurate than cross-immunization (lack of internal controls)	[30, 114]
		Hemagglutination inhibition (HI)	Assesses the neutralizing reaction between specifically known antisera against unknown isolates in using HI test	Adv.: 1. Simple test 2. Less expensive than VNT 3. Less time-consuming than VNT Disadv.: 1. Lack of standardization. 2. Higher cross-reactivity between strains than VNT	[35, 115]

Table 1 (continued)

General classification	Typing method	Test	Description and assessment parameters	Advantages and disadvantages	References
	Antigenic typing (epitope-typing)	Monoclonal antibody	Neutralization of IBV samples using specific monoclonal antibodies in eggs, cell culture or TOC	Adv.: 1. Useful in rapid diagnosis and epidemiological studies 2. Useful in dissecting the virion and elucidating functional relationships Disadv.: 1. Higher risk of false-negative results 2. Needs confirmation by other serotyping tests 3. Sophisticated technique that needs epitope identification and mapping	[37, 116–118]
Non-functional genomic		Genotype-specific RT-PCR	Genotype-specific oligonucleotide primers	Adv.: 1. Accurate and fast technique 2. Cost-effective Disadv.: 1. Unable to differentiate between vaccine and field strains	[119–121]
		PCR + restriction enzyme fragment length polymorphism (RFLP)	The S1 gene PCR product is digested with restriction enzymes. The RFLP patterns are compared with the patterns of reference serotypes.	Adv.: 1. Fast typing method for rapid diagnostics 2. Genotyping can be done very quickly compared to serotyping 3. A large number of samples can be tested Disadv.: 1. Mutations with no relevance for the antigenic or biological function of the virus in cleavage sites may impair results. 2. Correlation with biological and functional properties is uncertain 3. Does not give reliable data about antigenicity 4. Subsequent tests needed for identification of field isolates 5. Mixed strains yield difficult-to-read restriction patterns.	[119, 122, 123]

Table 1 (continued)

General classification	Typing method	Test	Description and assessment parameters	Advantages and disadvantages	References
		RNase T1 fingerprinting	IBV genome digestion with RNase T1 to resolve the resulting oligonucleotide in 2D gel electrophoresis to determine the specific fingerprint of the genome in comparison to reference genotypes	Adv.: 1. It gives information about the whole genome. Disadv.: 1. Results cannot be translated into antigenic or biological function. 2. Different serotypes give distinct fingerprints, but within serotypes, different results might be obtained if the genome identity is less than 95%. 3. Complex technique and labor-intensive	[124, 125]
		Sequencing	Partial or full genome sequencing to compare nucleotide and amino acid sequences and to conduct phylogenetic, recombination, and/or phylodynamic analysis	Adv.: Accurate and fast technique Disadv.: 1. Relatively high cost 2. Sophisticated and expensive software is required. 3. Mutations in the RNA may not correlate with biological or functional changes	[20]
Metagenomic			Structural analysis of the most antigenic protein (S protein) to determine changes in ??receptor-binding domain?? or epitopes	Adv.: 1. Better insight into the effect of changes in RNA sequence on the biological or functional characteristics 2. Reduces the usage of laboratory animals. 3. Useful in the development of structure-based vaccines Disadv.: 1. High cost (equipment, sophisticated software and hardware) 2. Requires epitope mapping	[22, 126, 127]

Table 2 IBV genotypes and clusters

GI			GII-1
GI-1	**GI-11**	**GI-21**	D1466_The Netherlands (M21971)
H120_Netherlands (FJ888351) M41_USA (AY561711)	IBV/Brazil/351/1984 (GU393339) UFMG/1141_Brazil (JX182783)	Italy/02 (AJ457137) Spain/98/313 (DQ064808)	V1397_The Netherlands (M21968)
GI-2	**GI-12**	**GI-22**	**GIII-1**
Holte_USA (GU393336) SDW_China (DQ070840)	D3896_Netherlands (X52084) D274_The Netherlands (X15832)	HN08_China (GQ265940) CK/CH/LSC/99I (DQ167147)	N1/88_Australia (U29450) V18/91_Australia (U29521)
GI-3	**GI-13**	**GI-23**	**GIV-1**
Gray_USA (L14069) JMK_USA (L14070)	UK4/91 (Z83975) Moroccan/G/83 (EU914938)	Egyptian Var II (KY805846) Israel Var II (AF093796)	DE/072/92_USA (U77298) AR/6386/97_USA (AF274436)
GI-4	**GI-14**	**GI-24**	**GV-1**
Holte_USA (L18988) GX2-98_China (AY251816)	B1648_Belgium (X87238) NGA3242006 (FN182277)	V13_India (KF757447) IBV506_India (KF809796)	N1/03_Australia (FJ235194) 018_Australia (JX018208)
GI-5	**GI-15**	**GI-25**	**GVI-1**
N1/62_Australia (U29519) V2/02_Australia (DQ490215)	B4_Korea (FJ807932) K620/02_Korea (FJ807944)	GA/10216/2010_USA (KM660636) GA/12274/2012_USA (KP085595)	SDIB781/2012_China (KF007209) TC07-2_China (GQ265948)
GI-6	**GI-16**	**GI-26**	**GVII-1**
Vic S_Australia (U29519) J9_China (DQ515802)	Q1_China (AF286302) IZO 28/86_Italy (KJ941019)	NGA/BP61/2007_Nigeria (FN182268) NGA/N545/2006_Nigeria (FN182270)	10636/26_China (MH924835) GX-NN130021_China (KM365468)
GI-7	**GI-17**	**GI-27**	
TP/64_Taiwan (AY606320) TW2575.98 (DQ646405)	AL/6609/98_USA (AF510656) CV-56b_USA (AF027509)	Georgia 08_USA (GU301925) GA/12341/2012_USA (KM660634)	
GI-8	**GI-18**	**GI-28**	
SE 17_USA (M99484) L165_USA (Q964061)	JP8127_Japan (AY296744) 53XJ-99II_China (KC577391)	GX-NN-13_China (JX291989) ck/CH/LGX/111119 (KX640829)	
GI-9	**GI-19**	**GI-29**	
Ark DPI_USA (AF006624) CAL99_USA (DQ912831)	QXIBV_China (AF193423) LX4_China (AY189157)	10114/14_China (KY407556) 10118/14_China (KY407558)	
GI-10	**GI-20**		
K87_New Zealand (AF151959) T6_New Zealand (AF151960)	Qu_16_Canada (AF349620) Qu_mv_Canada (AF349621)		

genotype. However, no associated publications have been found in either country (Table 3).

Jordan

The first data from Jordan were published in 2007. The presence of the Ark, DE-072, and Mass-like serotypes was demonstrated serologically [51]. Later, IBV strains related to the 4/91 and D274 genotypes were identified in addition to the classical Mass genotype [52]. IBV strains JOA2 and JOA4, which are related to the CK/CH/LDL/97I genotype, were also isolated from chicken flocks in Jordan [53]. Evidence of the circulation of IBV strains related to the GI-23 genotype has been obtained in multiple studies in Jordan since 2009 [50, 54].

The Persian Gulf area

The incidence of IBV in other Gulf countries still remains unclear due to the scarcity of information being published. IBV infection in Saudi Arabia was first detected in 1984 when an IBV isolate was identified using RT-PCR for the N gene without specifying its serotype [55]. IBV infection due to strains related to serotype 793/B (GI-13) was serologically detected in Saudi Arabia in 1997 and 1998 and was further confirmed in 2002 [56]. By 2009 and 2010, another study had characterized two IBV strains (IBV/CHICKEN/KSA/101/2010 and IBV/CHICKEN/KSA/102/2010) related to CH/LDL/011 (GI-16) and IBV/INDIA/TN/92/03 (H120, GI-1), respectively [57]. More recently, evidence of co-circulation of IBV strains related to Mass (GI-1), 4/91 (GI-13), CK/CH/LDL/97I (GI-16),

Table 3 IBV lineages in Middle Eastern and North African countries in the past 10 years (2010–2020)

Country	Number of isolates	Lineage							New unclassified lineage	Sequence availability	References
		GI-1	GI-12	GI-13	GI-16	GI-19	GI-21	GI-23			
Egypt	295	23 (7.8%)	12 (4.1%)	11 (3.7%)	4 (1.4%)	1 (0.3%)	0	244 (82.7%)	0	Yes	[43–45, 93, 128]
Algeria	4	0	0	1 (25%)	0	0	0	0	3 (75%)	Yes	[73, 75]
Libya	12	0	0	0	0	0	0	12 (100 %)	0	Yes	[79]
Morocco	57	35(61.4%)	0	3 (5.3%)	0	1 (1.8%)	18 (31.6 %)	0	0	Yes	[72]
Tunisia	7	0	0	0	5 (71.4%)	0	0	0	2 (28.6%)	Yes	[77, 83]
Jordan	27	9 (33.3%)	0	6 (22.2%)	1 (3.7%)	0	0	11 (40.7%)	0	Yes	[50, 53]
Lebanon	3	0	0	0	0	0	0	3 (100%)	0	Yes	Direct submission (NCBI)
KSA	53	12 (22.6%)	0	17 (32.1%)	12 (22.6%)	0	0	12 (22.6%)	0	Yes	[50, 58]
Oman	34	2 (5.9%)	2 (5.9%)	28 (82.4%)	0	0	0	2 (5.9%)	0	Yes	[59]
Iran	359	33 (9.2%)	2 (0.6%)	78 (21.7%)	2 (0.6%)	39 (10.9%)	0	200 (55.7%)	5 (1.4%)	Yes	[68]
Iraq	46	1 (2.2%)	0	14 (30.4%)	0	3 (6.5%)	0	28 (60.9%)	0	Yes	[54]
Israel	3	0	0	0	0	0	0	3 (100%)	0	Yes	Direct submission (NCBI)
Syria	10	0	0	0	0	0	0	10 (100%)	0	Yes	Direct submission (NCBI)
Turkey	73	9 (12.3%)	0	7 (9.6%)	0	0	0	57 (78.1%)	0	Yes	[81, 82]

and Middle East IBV (GI-23) has been reported in Saudi Arabia [22, 58].

In the United Arab Emirates, the 793/B (GI-13) strain constitutes the major genetic lineage that infects chicken flocks, while the Mass (GI-1) and D-274 strains are less frequently detected [50]. In Oman, Kuwait and Bahrain, the situation was almost unknown until 2014, but studies published in 2015 detected 793/B (GI-13) as the dominant strains with occasional detection of Mass (G1-1), Middle East (G1-23), and Dutch strains (G1-12) [50, 59].

Iraq

The first isolation of IBV in Iraq occurred from 2008 to 2010 with the detection of two novel genotypes, designated as the Sul/01 and 4/91 IBV strains [60]. Recently, the GI-23 and GI-13 genetic lineages were found to be the most prevalent lineages (IS/1494/06, 46.9%; 793/B, 40.6%). In addition, QX (G1-19) (9.4%) and DY12-2-like (3.1%) strains were also detected among the circulating IBVs [54].

Iran

The close relations of the Persian Gulf countries with Iran may suggest that the IBV epidemiology in Iran could be used as an indicator of the current situation in the Gulf region [61]. The first detection of the Mass type of IBV was in 1994 [62]. Ten years later, 4/91-like strains were reported in addition to the continuously circulating Mass type [24, 63–65]. Circulation of diverse genotypes was detected from 2010 to 2014, and partial sequences of the Mass, 793/B, Middle East, Variant 2, QX, IR-I, and IR-II variants were reported [66]. Genotyping of IBV strains between 2015 and 2017 revealed that GI-23 was the dominant genotype in Iran [67, 68].

Arab Maghreb countries

IBV was first isolated in Morocco from 1981 to 1984; the novel IBV strains were related to the Mass serotype and to a unique enterotropic IBV virus designated as Moroccan G [40, 69]. Notably, a potential ancestral relationship between the old North African and the European GI-13 (793/B- and CR88-like strains) was suggested due to the similarity of GI-13 to the Moroccan strains isolated in 1983 [70].

In 2015, two predominant genotypes were detected in Morocco: the Mass type (66%) and the Italy 02 genotype (32%), with only a single detection of the 793B strain [71]. Phylogenetic analysis of all known IBV isolates from 1983 to 2014 in Morocco revealed the circulation of three genetic lineages: GI-1, GI-13 (four genetic clusters), and GI-21 (two genetic clusters). Morocco was the first African country in which the GI-21 lineage was reported (GI-21, cluster 2),

and this lineage is genetically related to the gCoV/AvCoV/chicken/Spain/1997 variant [72]. The second incidence of new IBV genotype introductions in Morocco suggested the probability of the introduction of this lineage through the importation of day-old broiler and layer breeders from Europe [72].

No historical data are available about IBV in Algeria. The first data were published in 2015 and indicated the prevalence of GI-1-lineage IBV strains. The Algerian Mass-like nephropathogenic strain showed no genetic relatedness to the North African variant strains and was different from the H120 (GI-1) and 4/91 (GI-13) genotypes, suggesting the possibility of recombination between the nephropathogenic Mass strain and the novel African genotypes [73]. Recently, seroprevalence has indicated a high incidence of IBV antibodies on unvaccinated farms [74] and the circulation of QX (G1-19) and 4/91 (GI-13) IBV variants. [75].

Similarly, in sero-epidemiological studies in Tunisia, new Tunisian variants genetically related to the CR88 and 793/B (GI-13) strains were isolated and characterized [76, 77]. Meanwhile, the Middle East GI-23 genotype strains were first reported in Tunisia in 2018. The first published report from Libya described the isolation of an IBV strain designated as 3382/06 that was closely related to the GI-16 lineage [78]. In 2013, detection of VAR II-like strains (GI-23) was confirmed by the isolation of strains related to the EG/IB1212B and Beni-Suef/02 lineages [79].

Turkey

There were no published data about the situation of IBV in Turkey until 2013. However, in 2011, Kahya and colleagues reported the detection of Middle East–related isolates (GI-23) from broiler and layer flocks [80]. By 2016, the GI-1, GI-12, GI-13 and GI-23 lineages had been detected [81, 82].

Predominant IBV genetic lineages in MENA countries

IBV has been circulating in MENA countries for decades with the continual appearance of escape mutants. Based on the available data, it seems that the GI-23 (especially the Egyptian VAR II genotype) and GI-19 (QX genotype) strains are expanding their geographic distribution [79, 83]. Alongside these prevalent lineages, vaccine strains belonging to GI-1, GI-12, and GI-13 lineages are still circulating at low or undetected levels (Table 3). The co-circulation of both lineages in addition to other detected lineages suggests the continuous evolution of IBV by either mutation and/or recombination. Recently, an analysis of the complete genome sequences of IBV strains from Egypt revealed two new variants: CU/1/2014, an H120-like strain

that probably evolved due to accumulated multiple point mutations, and the recombinant strain CU/4/2014, which showed evidence of recombination involving three ancestors belonging to QX-like, 4/91, and H120 IB lineages [48].

More information about the dynamics of the spread of IBV is urgently needed to develop better control strategies. Recently, a Bayesian phylodynamic approach to investigate the spread of the QX-like strains (GI-19) revealed that there was a long period of local circulation in China followed by transmission to European, Asian, and Middle East countries in successive waves beginning in 2011 [84]. In addition, the range of the Q1 (GI-16) viruses seems to extend beyond the MENA countries. Despite reports of sporadic detection, the Q1-like strains are likely to have been introduced through the Asian route (from China to Egypt) according to a phylodynamic analysis constructed for Q1 [85].

These strains were first detected in 2011 in Iraq, Saudi Arabia, and Jordan. In Egypt, two sporadic detections were reported in 2010 and 2017 [86, 87], and the same occurred in Iran in 2019 [88]. However, the absence of enough data about IBV in the MENA region makes it difficult to investigate the current state of this lineage.

Novel genetic lineages in MENA countries

The recent classification of IBV based on full S1 nucleotide sequences conducted by Valastro did not take into consideration the available partial S1 gene sequences in the phylogenetic analysis of MENA IBV strains [20]. Although it would be best to use full S gene sequences for IBV classification, this strict criterion would leave many strains without classification. An in-depth analysis that included Middle Eastern IBV isolates with ≥ 600 bp or full S1 sequences revealed a possible new genetic lineage of IBV in North Africa. These IBV strains were isolated in Algeria in 2013 [73] and in Tunisia in 2016 [89] and found to cluster separately, with ≥ 25% amino acid sequence divergence from the closest genetic lineages in the GI-14 strain (Table 4).

Genetic analysis of MENA IBV strains based on HVR3 sequences was used previously to characterize GI-23 IBV strains in Egypt [45]. Additionally, the HVR3 sequences were employed in a recent evolutionary analysis of Egyptian IBV strains, revealing three subclades of historical IBV strains, including GI-23.1, G23.2 (GI-23.2.1 and GI-23.2.2), and GI-23.3 [90]. However, the study focused only on Egyptian strains. In this review, all available full-length S1 sequences from the Middle East were retrieved from GenBank. Their evolutionary history was inferred using the maximum-likelihood method and the Tamura-Nei model [91]. This analysis involved 200 nucleotide sequences with

Table 4 Nucleotide and amino acid sequence identity of the genetically different IBV strains isolated from MENA countries (●) to the most closely related genetic lineage (GI-14)

| Strain	1	2	3	4	5	6	
KP892759 Algeria/26/b1●		98%	93%	93%	77%	78%	sequence
KP892760 Algeria/26/b2●	95%		93%	93%	77%	77%	
KX061458 TN_1011/16●	88%	88%		100%	76%	77%	
KX061459 TN_1012/16●	88%	88%	100%		76%	77%	Nucleotide identity
X87238 Belgium_1984_(GI-14)	74%	73%	73%	73%		80%	
FN182277 NGA/324/2006 _(GI-14)	75%	73%	73%	73%	75%		
Amino acid sequence identity							

1736 total positions in the final dataset and was performed using MEGA X [92]. The analysis revealed that the GI-23 genotype has three sublineages (23.1, 23.2 and 23.3) and that the GI-23.2 sublineage, in turn, has three subclades, GI-23.2.1, GI-23.2.2 and GI-23.2.3 (Supplementary Fig. 1 Fig. 1A and B), with p-distances of 9.8, 13.4, and 15.8%, respectively. The p-distances calculated between the first isolate of sublineage 23.1 and the 23.2 and 23.3 sublineages were 10–14% and 7.7%, respectively (Fig. 1C).

Interestingly, this genetic classification was successfully reproduced using the HVR3 sequences of all IBV genetic lineages (Fig. 2A and B). The only difference was that a few isolates did not cluster with their corresponding genotype as they did with the full sequences. These isolates included the KU238176-D888/2/4/08_IR, DQ386098-Spain/00/336, U29453-Australia/N3/62, and AY296744-AY296746 Japan strains as well as KM660636-GA/10216/2010. Notably, most of these
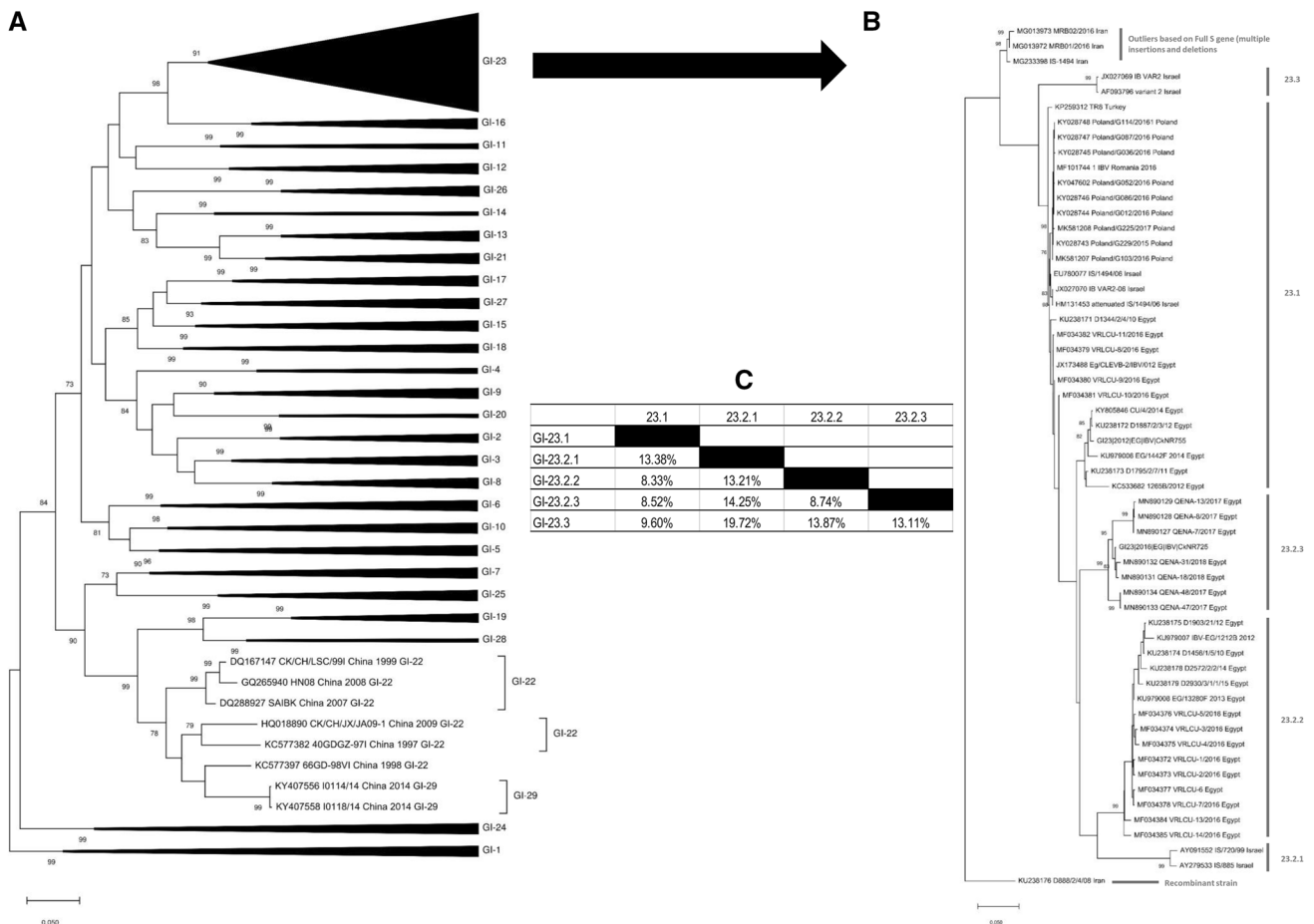
Fig. 1 Phylogenetic analysis of the IBV genetic lineages (200 sequences) (A) and GI-23 sequences (B) from the Middle East based on full-length S1 gene sequences using the maximum-likelihood method and the Tamura-Nei model. There were 1736 positions in the final dataset. Distances between the proposed sublineages and subclades (C) were calculated using MEGA X software.
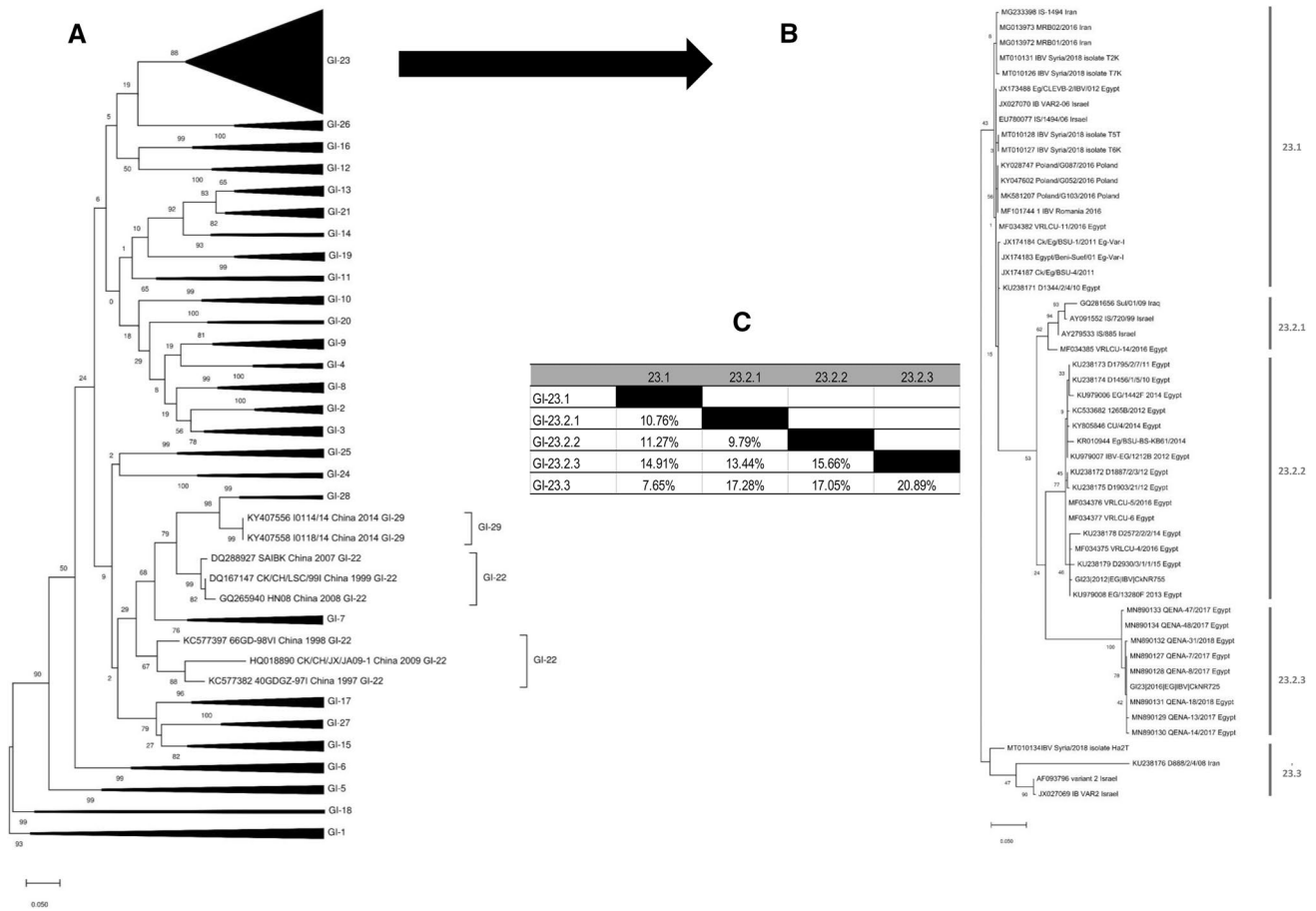
Fig. 2 Phylogenetic analysis of the IBV genetic lineages (194 sequences) (A) and GI-23 sequences (B) from the Middle East based on HVR3 sequences using the maximum-likelihood method and the Tamura-Nei model. There were 349 positions in the final dataset. Distances between the proposed sublineages and subclades (C) were calculated using MEGA X software.

isolates were recombinant IBV strains identified in Iran [93], Spain [94], Australia [95], Japan [96], and the United States [97], respectively. Moreover, the topology of the HVR3 phylogenetic tree was very similar to that of the full S gene except in the case of the GI-22 and GI-29 strains, where their clustering overlapped (Fig. 2A and B). Using the calculated p-distances allowed better discrimination between sublineages (Fig. 2C). GI-29 was recently reported in China [98]. Our analysis showed that this lineage shares 87.5–90.1% nucleotide and 85.6–88.9% amino acid sequence identity with GI-22. This similarity would explain the overlapping clustering of the HVR3 sequences of these two lineages. In addition, the Iranian IS-1494-like strains have multiple deletions and insertions in their HVRs, which led to their separate clustering from the GI-23.1 sublineages (Fig. 2B).

Despite the minor limitation of the misclustering of recombinant IBV strains, for the majority of IBV strains, phylogenetic analysis of HVR3 sequences is reliable as a means of IBV classification for rapid diagnostic and broad surveillance purposes.

The spread of IBV GI-23 and its epidemiological implications for Europe, Africa, and Asia.

The MENA region is in the centre of the Old World and is situated at the conjunction of Asia, Europe, and Africa (Fig. 3). Historically, some IBV lineages that are endemic in Europe have been isolated in North African countries, and vice versa [70, 72]. Recently, the GI-23 lineage was detected in several European countries, including Ukraine, Lithuania, Poland, Armenia, the Russian Federation, the Republic of Belarus, Tajikistan, Kazakhstan and Germany. Additionally, reports from the Middle Asian countries of Kazakhstan, and Afghanistan have described the isolation of GI-23 strains [99, 100]. GI-23 was isolated in West Africa for the first time in 2013 with the isolation

Fig. 3 Geographical distribution of genetic lineage 23 of IBV in the Middle East, North Africa, Europe, and Asia. Countries that have reported the detection of GI-23-like strains are indicated in red.
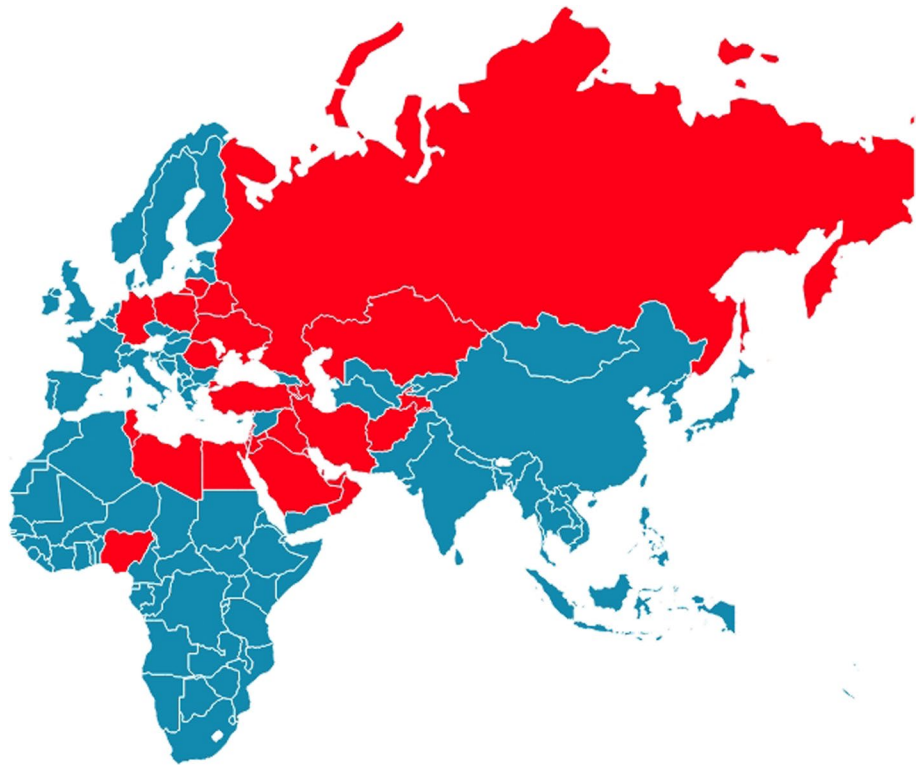
of strains NGA3 (MN082399) and NGA8 (MN082404) in Nigeria. The widespread prevalence of the GI-23 lineage (EGY VAR II-like strains) in the region and recent detections in Asia, Europe, and Africa illustrate the capability of the GI-23 lineage to extend its geographical range and emphasises the need for a comprehensive investigation of the phylodynamic evolution of this lineage, as has been done with other lineages (e.g., GI-19 QX). A recent poster at the XXIst World Veterinary Poultry Association Congress reported the spread of GI-23 in some African and European countries, including Morocco, the Czech Republic, Belgium, Zimbabwe, Zambia, and Uganda; however, there are no published data to confirm the presence of GI-23 in these countries [101]. Therefore, active surveillance is needed to determine the prevalence of the GI-23 IBVs in these nations.

BV is challenging control efforts and will require new approaches

Control of IBV infection is difficult due to the emergence of new variants and the high rate of mutation affecting antigenic on the virion. The subpopulation that remains after replication and any rapid reversion of vaccine strains to pathogenicity also play a role in reducing the ability to control the disease. Additionally, increasing numbers of recombination events further complicate efforts to combat the disease.

Therefore, specific and sophisticated control measures need to be established. In addition, strict biosecurity measures must be applied on farms and in their surrounding environments. However, reliable vaccination programs that take into consideration the causative IBV strains, types of birds, and purpose of production should also be implemented.

To satisfy regulatory requirements, different tests have been applied to evaluate the efficacy of IBV vaccines against homologous challenge. In the USA, the Code of Federal regulations (CFR) requires re-isolation of the challenge virus in SPF-ECE after challenge. [102]. However, in Europe, the European Pharmacopeia (Ph. Eur.) requires the performance of a ciliostasis test after a homologous challenge to assess the degree of protection conferred by the tested vaccine [103]. In most MENA countries, the European guidelines have been adopted, including the evaluation of ciliostasis after challenge with endemic viruses, in addition to protection against clinical signs and associated mortalities. The protection studies that have been performed to evaluate available vaccines against the main circulating genetic lineages with respect to the regulatory requirements of the CFR or Ph. Eur. are summarized in Table 5.

While cross-protection studies have indicated the ability of protectotype vaccine strains (especially those belonging to the GI-1 and GI-13 lineages) and combined vaccines to protect against GI-23 viruses, field observations have indicated that variable vaccines have not provided adequate protection; the viruses continue to circulate. Recent studies

Table 5 Vaccine efficacy studies against circulating IBV viruses in the MENA region

Challenge strain: acc. no., name, year, genetic lineage	Vaccine (strain, time)	Bird type & number / group[1]	Challenge days post last vaccination (dpv), dose, and route	Sampling (dpc)[2]	Protection %[3]		Regulation[4]		Reference
					Tracheal virus shedding	Ciliostasis	Ph.Eur.	CFR	
AF395531, Egypt/ Beni-Seuf-01, 1998, GI-23	H120, WK-3	Com, 15	28 dpv, 10^5, intraocular	4, 7	20%	NT	No[a,b]	No[a,b]	[44]
Unavailable, IS/885/00 like, --, GI-23	H120 & CR88, D-1 & D-14	Com, 30	16 dpv, $10^{4.6}$, oculo-nasal	5	NT	60%	No[a,c]	No[c,e]	[129]
	H120+CR88 &CR88, D-1&D-14				NT	83%			
Unavailable, IS/1494/06, --, GI-23	H120 &CR88, D-1 & D-14		16 dpv, 10^5, oculo-nasal	5	NT	80%			
	H120+CR88 & CR88, D-1 & D-14				NT	94%			
Unavailable, Israel variant 2 like, --, GI-23	IBMM (Mass 1263) & QX-like, D-1 & D-14	SPF, 20	35 dpv, 10^4, oculo-nasal	5	NT	50%	Yes	No[b,e]	[130]
	H120+D274 & QX-like, D-1 & D-14		35 dpv, 10^4, oculo-nasal		NT	70%			
	H120+D274, D-1		21 dpv, 10^4, oculo-nasal		NT	61%			
Unavailable, IS/1494/06 like, --, GI-23	H120, D-1 & D-14	SPF, 20	21 dpv, 10^4, ocular	5	NI	60%	No[e]	No[e]	[131]
	H120 & 1/96, D-1 & D-14				NI	69.2%			
MG334195, EG/ M41-ME01, 2011, GI-1	IB-M41, D-1	SPF, 20	21 dpv, 10^5, nasal	7	100%	100%	Yes	Yes	[110]
	H120, D-1				100%	100%			
JQ839287, Eg/1212B, 2012, GI-23	EGY-VARII, D-1				100%	90%			
KU979007.1, IB/1212B, 2012, GI-23	IB-M41 & EGY-VARII, D-1 & D-14	SPF, 20	14 dpv, 10^5, nasal	7	60%	100%	Yes[c]	Yes[c]	[107]
	EGY-VARII, D-1 & D-14				60%	100%			
	EGY-VARII &M41, D-1 & D-14				40%	80%			
	IB-Ma5 &IB-793B, D-1 & D-14				20%	60%			
	IB-793B, D-1 & D-14				40%	50%			
	IB-793B &IB-Ma5, D-1 & D-14				40%	50%			

[1] Com., commercial; SPF, specific-pathogen-free

[2] dpc, days post-challenge

[3] Protection % as indicated by % of birds not shedding the virus and protection as per ciliostasis test. NT, not tested; NI, not indicated in the study

[4] Ph. Eur., European pharmacopeia regulations; CFR, Code of Federal Regulations.

Superscript letters indicate fulfilled versus unfulfilled regulation as follows: [a]Type of bird (Ph. Eur. SPF bird/CFR susceptible bird); [b] Number of birds per group (20 birds); [c] Challenge date post-vaccination (21 dpv); [d] Sampling date post-challenge (Ph. Eur.:4 to 7 dpc /CFR: 5 dpc); [e] Test method (Ph. Eur.: ciliostasis or virus re-isolation from each bird/CFR; virus re-isolation from each bird)

suggest that the best protection against an IBV challenge can be achieved using a homologous vaccine strain. This has been confirmed with Chinese QX-like IBV strains [104, 105], several Korean nephropathogenic IBV strains [106], and more recently, with GI-23 strains as either live or inactivated vaccines [107–110].

Few studies have followed the international regulations, and there is a lack of standardized methodology (e.g., virus isolation versus virus molecular detection and/or quantification). For instance, the protection levels in vaccinated birds against shedding of the homologous GI-23 strain, as determined by virus isolation, ranged from 60 to 100% [110]; however, lower levels of protection (40–60%) were found using quantitative real-time reverse transcription polymerase chain reaction [107]. Additionally, while protection studies in commercial birds are thought to reflect the field situation, ciliostasis testing does not take into account possible deleterious effects of live IBV vaccines on tracheal cilia under field conditions or cilostasis due to infections with other respiratory pathogens [11]. Hence, the use of SPF birds in ciliostasis tests remains the gold standard for evaluating live IBV vaccines.

Concluding remarks

Avian IBV remains a challenge for the poultry industry due to its rapid spread and the existence of several serotypes that provide poor cross-protection. Although different classification systems of IBV have been proposed, each system has several limitations that compromise its accuracy. Currently, the genotype classification of IBV based on full-length S1 nucleotide sequences is widely used, but genetic analysis of HVR3 could be a reliable tool for rapid diagnosis of IBV and national surveillance purposes. IBV epidemiology in MENA countries is still unclear due to the lack of optimized programs for surveillance of different genotypes. The wide geographical distribution of GI-23 (EGY VAR II-like strains) in the MENA region and in Asia, Europe, and Africa necessitates the study of its phylodynamic evolution and a re-evaluation of different vaccine efficacy rates against this lineage. Cross-protection studies using protectotype vaccine strains and combined vaccines against GI-23 lineages are not in agreement with the field observations of inadequate protection. A standardized vaccine evaluation protocol (e.g., the use of SPF birds and ciliostasis tests) must be followed to allow studies to be compared. However, as has been seen with the QX-like strain, the use of homologous vaccine strains appears to be necessary for maximal protection.

Compliance with ethical standards

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