

An updated review on avian infectious bronchitis in North Africa and vaccination strategies - a review

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ABSTRACT

Avian infectious bronchitis (IB) is a highly contagious respiratory disease with worldwide geographic distribution. It is observed systematically in vaccinated and non-vaccinated flocks and is still a major problem with colossal economic repercussions. In this review, the situation regarding (IB) in North Africa is presented, along with a phylogenetic and genetic analysis of the available spike gene. The current situation regarding vaccination is presented, followed by new proposed strategies to control emerging variants in North African countries. In North Africa, infectious bronchitis virus (IBV) genotype I (GI) lineages are the most frequent. Moreover, unique and autochthonous variants have also been detected. Morocco Italy 02 (GI 21), previously absent from the African continent has emerged recently in Morocco, raising the question of how it was introduced. Some Middle East variants have also been detected in Egypt and Libya. Analysis of the S1 gene, showed recombination between strains from non-bordering countries and several point mutations in the hypervariable regions (some are exclusive) compared to commercial vaccines and reference strains, which reflects the continuous evolution of IBV in North Africa. Several vaccination trials with the available and/or new vaccines were studied. The combination of indigenous strains with classic or variants vaccines has shown promising results. Nevertheless, the combination of monovalent heterologous vaccines also conferred good protection. However, other trials to develop effective vaccination programs are required to generalize their use. In the current situation, it seems that updating molecular field data is crucial to understand the evolution of the infectious bronchitis virus to improve the adaptation of vaccine programs and preventive measures to field strains.

Key words: avian infectious bronchitis virus; North Africa; S1 gene; genetic characterization; new variants; vaccination

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Introduction

Avian infectious bronchitis (IB) is an infectious disease causing major economic losses affecting different farming systems ([JACKWOOD, 2012](#)). It was first described in the USA, in young chickens, in 1931 ([BUTCHER et al., 2018](#)), but has become a disease with worldwide distribution ([LI, 2016](#)). This pathogen has multiple tropism (respiratory, renal and genital). The highest mortality rates (60-80%) are associated with nephropathogenic strains ([LEGHARI et al., 2016](#)) or co-infection with other respiratory pathogens ([SID et al., 2015](#); [DE WIT and COOK, 2019](#)).

The IB virus is characterized by great genotypic and serotypic diversity ([DE WIT and COOK, 2019](#)). Its genetic evolution is attributed to point mutations affecting the spike (S1) gene ([CAVANAGH et al., 1992](#)) and genetic recombination in multiple genes between different strains ([NAGUIB et al., 2016](#); [XU et al., 2019](#)). Consequently, new genotypes, variants and serotypes are constantly emerging in the field ([NAGUIB et al., 2016](#); [MA et al., 2019](#)).

In Africa, IBV was detected for the first time in the Mediterranean countries. Various studies showed the co-circulation of different lineages (GI-14, GI-16, GI-19), the emergence of new lineages ([BALI et al., 2022](#)), and the detection of unique, native variants, with exclusive mutations ([LACHEB et al., 2019](#)). Some native types or new variants remain localized to limited geographic regions, without spreading, such as native variants isolated in Tunisia ([BOUROGÂA et al., 2009a](#)) and Algeria ([SID et al., 2015](#)), which have not been detected in other regions. Other genotypes, such as vaccine genotypes (Mass, H120 or IB 4-91) or vaccine-derived strains, are more widespread ([FELLAHI et al., 2015](#); [MARANDINO et al., 2017](#); [ABOZEID and NAGUIB, 2020](#)). In Morocco, the genotype Mass is widely spread in almost all regions. In this country, 25% of the strains are homologous to H120 vaccines. It is also the most frequent compared to other genotypes (Italy 02, 793B) ([FELLAHI et al., 2015](#)). Other genotypes, such as QX, reported in Western Europe ([WORTHINGTON et al., 2008](#)) and Africa (Zimbabwe) ([TOFFAN et](#)

[al., 2011](#)) have not been detected in North African countries so far. Finally, IBV evolution may be complex because IBV in North African countries has been poorly studied. Indeed, [JONES et al. \(2004\)](#), suggest that North African countries, where variants have been less studied, might be a reservoir of the most important IBV variants (793B, 4/91, CR88) found in European countries ([JONES et al., 2004](#)).

Protection against IBV is based on the use of live attenuated or killed vaccines. Vaccine-induced protection is type specific and the vaccine cross protection between different types is low ([COOK et al., 1999](#); [JORDAN, 2017](#)). Differences in the S1 contribute to vaccination failure. Thus, a given serotype may be altered with a difference in the S1 sequence as small as 2-3% (10-15 amino acids 'aa'). In addition, poorer cross protection between vaccines and field strains may be observed with differences as small as 5% (27 aa) ([CAVANAGH et al., 1997](#)). Consequently, outbreaks due to new genotypes/serotypes or variants are continuously being observed despite proper vaccination with the currently used vaccines ([AMMAYAPPAN et al., 2008](#); [LEGHARI et al., 2016](#)). In North Africa, molecular studies based on the partial or complete S1 gene sequence and vaccination trials showed genotypic incompatibility and poor protection of vaccines used against field strains. Vaccination failure is more often observed during the co-circulation of different genotypes ([ABDEL-MONEIM et al., 2002](#); [ABDEL-MONEIM et al., 2006](#); [SABRA et al., 2020](#)). This review focuses on a phylogenetic and S1 gene analysis of the IBV strains circulating in North African countries so far. The vaccination strategies being used are reviewed. In addition, new vaccination trials based on indigenous strains or heterologous vaccines to improve protection against new genotypes and new indigenous variants are discussed. This review is intended to synthesize existing research in Algeria, Egypt, Libya, Morocco, and Tunisia, in the field, based on serological and molecular studies that target S1 gene sequence available in GenBank, and provide a comprehensive overview of the molecular evolution of IBV, and give recent knowledge about the vaccine strategies used to control IBV.

this might be helpful highlight gaps in our knowledge and suggest areas for future research.

Etiology of IBV

Taxonomy. IBV belongs to the order Nidovirales, family *Coronaviridae*, subfamily *Orthocoronavirinae*, genus *Gammacoronavirus*, and subgenus *Igacovirus*. They are enveloped, single-stranded, positive-sense RNA viruses with the largest genome of the RNA viruses. Their name derives from the “corona” which refers to the aureole of the spike protein surrounding the virion ([WOO et al., 2005](#)). The subfamily *Coronavirinae* contains four genera, according to the organization of their genome and phylogenetic relationship: *Alpha-coronavirus* (α CoV), *Betacoronavirus* (β CoV), *Gammacoronavirus* (γ CoV), and *Deltacoronavirus* (δ CoV) ([CUI et al., 2019](#)). Coronaviruses can infect a wide variety of hosts; the first two genera have been identified in human infections, while the latter two include species that infect wild and domestic birds ([MASTERS, 2006](#); [HIDALGO et al., 2021](#)).

The genus *Gammacoronavirus* is classified into three subgenera. Subgenus *Brangacovirus* and subgenus *Igacovirus* have only been identified in birds. The third subgenus, known as *Cegacovirus*, was isolated from marine mammals, and

is known as *Beluga whale coronavirus SW1*. The well-known and widespread species representatives of the genus *Gammacoronavirus* are the Avian coronavirus (AvCoV) and Avian coronavirus 9203 (AvCoV9203) of the subgenus *Igacovirus*. They represent a group that includes all genotypes, as well as other genetically similar viruses ([MARCHENKO et al., 2022](#)).

Organization of the genome. The IBV has a genome of about 27.4 to 27.7 Kb, with slight variations between strains ([FRANZO et al., 2015](#)). The genome consists of six genes. It contains several open reading frames (ORF) and small non-coding regions between them ([LEGHARI et al., 2016](#); [LEGNARDI et al., 2020](#)). Some strains have additional genes (the 6b gene following the N gene) or nucleotide deletions in gene junction region, compared to the old IBV. The 5' end begins with a leader sequence of 65-98 nucleotides (nt), followed by an untranslated region (UTR: untranslated region) of 200-400 nt. A poly (A) tail, of 200-500 nt follows the 3' end. The two overlapping ORFs encode the polyproteins 1a and 1ab, and the regions encoding the main structural proteins - spike (S), envelope (E), membrane (M) and nucleocapsid (N). In addition, two accessory genes, ORF3 and ORF5, coding for proteins 3a, 3b, 5a and 5b, respectively, have been described ([LAI and CAVANAGH, 1997](#)) (Fig. 1).

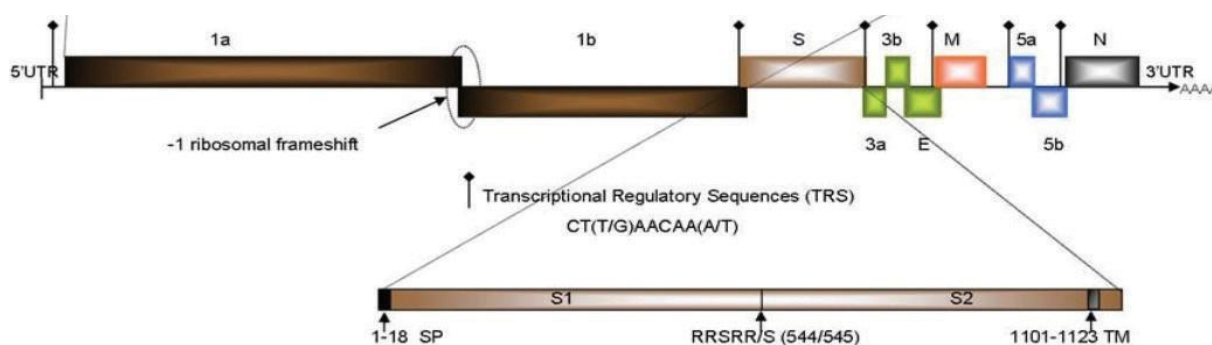


Fig.1. IBV genome and spike glycoprotein

A) The structural organization of the IBV genome includes poly (A) tail and ten open reading frames (ORFs) which code for structural proteins and non-structural protein (nsp). The ORFs are flanked by 5' and 3' untranslated regions (UTRs). B) the spike protein (S) is 1123 aa. The cleavage site (RRSRR/S) is located between 544 and 545 aa ([AMMAYAPPAN et al., 2008](#)).

The virion structure. IBV contains four structural proteins: protein M and E are involved in the assembly of the viral particles and the formation of new virions ([DENG and BAKER, 2021](#)). The S protein (~ 3462 nt) is post-translationally cleaved into the amino-terminal S1 (~ 535 aa) and the carboxyl-terminal S2 (~627 aa) subunits at a multi-basic cleavage site ([LAI and CAVANAGH, 1997](#)). It has a trimer form, with three domains. The first is an external domain containing the S1 and S2 subunits. S1 contains three heads with a receptor-binding domain (RBD), involved in the interaction with cellular receptors. However, S2 contains three stems involved in the membrane fusion and cell entry ([SHANG et al., 2018](#); [DENG and BAKER, 2021](#)). The second is a transmembrane domain (TM) that facilitates the anchoring of the S protein in the membrane. The third domain is composed of a short cytoplasmic tail ([LI, 2016](#); [DENG and BAKER, 2021](#)) (Fig. 2).

The protein S is the most variable of all coronavirus proteins ([LI, 2016](#)). The S1 subunit includes

three-hypervariable regions (HVR1, HVR2, and HVR3), corresponding to aa 38-67, 91-141, and 274-387, respectively ([MOORE et al., 1997](#); [SHAN et al., 2018](#)). These regions are associated with the serotype-specific neutralizing epitopes used to distinguish between different serotypes ([KANT et al., 1992](#); [SHAN et al., 2018](#)). Therefore, mutations in HVRs lead to the emergence of resistant variants ([KANT et al., 1992](#)).

Classification of avian infectious bronchitis virus

IBV classification systems are divided on the basis of two main types of tests: functional or biological tests, and non-functional tests. The first allows viral isolates to be grouped into immunotypes, protectotypes, and antigenic types (serotypes and epitope types). The others are based on sequence variations in the S gene that allow them to be assigned to different genotypes. Immunotype or protectotype classification is the most important because it provides information about the efficacy of vaccine

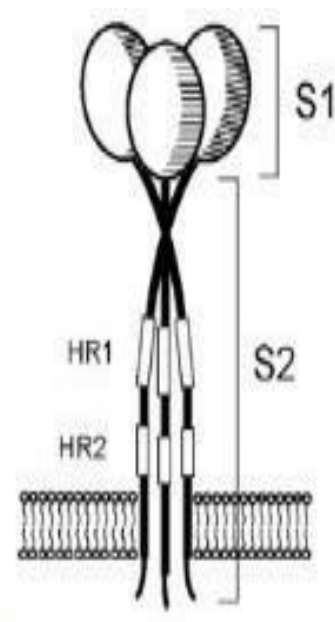
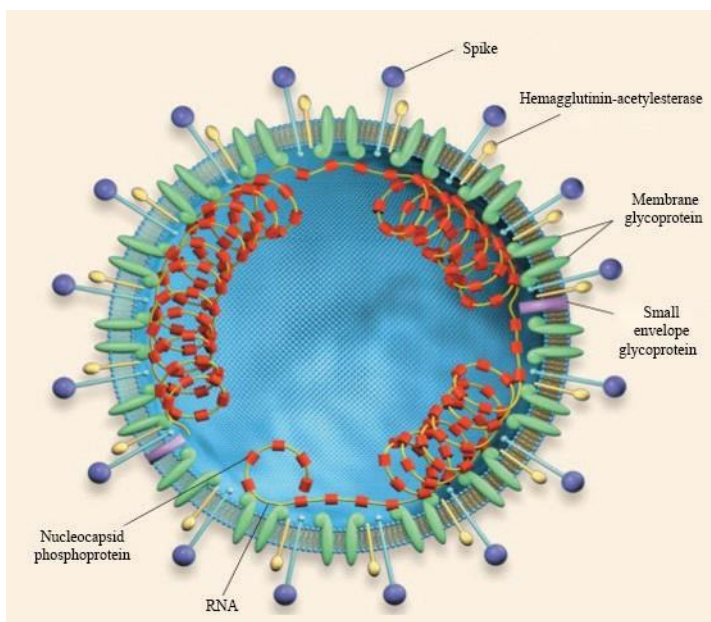


Fig.2. Structural organization of the IBV virion and the spike glycoprotein

A) The coronavirus virion possesses four major structural proteins, including the spike protein (S), envelope protein (E), nucleoprotein (N) and membrane protein (M). However, group 2 of coronaviruses possess a hemagglutinin-acetyltransferase (HE) glycoprotein ([HOLMES, 2003](#)). B) The S spike protein organization is organized as a trimer. Each trimer is composed of two subunits S1 and S2, at the amino-terminal (N) and carboxy-terminal (C) ends, respectively ([MASTERS, 2006](#)).

strains ([DE WIT, 2000](#)). Given the great heterogeneity of the S gene, the phylogenetic classification of IBV was recently harmonized by using the S1 gene full-length sequence, giving rise to the first globally accepted classification method. IBV was divided into eight genotypes (GI–GVIII), with 37 lineages and a number of inter-lineage recombinants. Genotype I (GI) comprises 27 distinct viral lineages (temporarily ordered GI-1 to GI-27), while each of the remaining genotypes comprises a single viral lineage ([VALASTRO et al., 2016](#); [GHETAS, 2021](#)). Since then, new lineages and recombinant variants have emerged: GI-28 and GI-29 in China ([CHEN et al., 2017](#); [JIANG et al., 2017](#)), lineage 1 in genotype VII (GVII-1) ([JIANG et al., 2017](#)) and, very recently, a new GI-30 lineage in Trinidad and Tobago ([BROWN JORDAN et al., 2020](#)).

Distribution of the variants

IBV evolves and spreads easily around the world ([GUZMÁN and HIDALGO, 2020](#)). The above account highlights the large number of IBV variants that exist worldwide, some being unique to a particular area, others having a more general distribution. The lineages GI-1 (Massachusetts), GI-13 (793/B, 4/91, and CR88), GI-19 (LX4 and QX), GI-16 (LDL/97I and Q1), GI-21 (Italy 02) and GI-23 (Var2) are distributed throughout different continents. However, others are localized to specific geographical regions ([VALASTRO et al., 2016](#); [JIANG et al., 2017](#)). A variant of major importance, 4/91, has spread over Asia, Europe and Africa in a short period, but has never been reported in the USA or Australia. On the other hand, the major strain of the USA, the Arkansas strain, has hardly ever been reported outside the USA. A pathogenic strain such as D1466, that has been in some countries in Western Europe for three decades now, has hardly been reported outside Western Europe. It is very difficult to achieve a sufficient level of protection ([COOK et al., 1999](#)) against this strain, which makes its spread to other areas easier.

The exact reason why some strains spread readily over major parts of the world whereas others remain local is unknown. However, it seems likely that geographical isolation, and the control measures and policies employed in some countries may

play a part in preventing the entry and exchange of IBV variants with other parts of the world. An example is Australia, a geographically isolated country that limits the use of live attenuated vaccines, and where all vaccines are manufactured with their own pathogenic strains ([GUZMÁN and HIDALGO, 2020](#)). In the same way, the most widely distributed genotypes and variants are licensed vaccines or vaccine derived strains ([AB-DELMONEIM et al., 2006](#); [FELLAHI et al., 2015](#); [BARBERIS, 2019](#)). The wild bird population can maintain and expose commercial poultry farms to many pathogens when biosecurity measures and the condition of the buildings and equipment are not optimal ([HENNING et al., 2011](#)). This contact can change the field epidemiology of IBV, with a new emergence. The recent discoveries of IBV and IBV-like strains in species of birds other than chickens ([CAVANAGH, 2005](#); [MARCHENKO et al., 2022](#)), such as geese, ducks, and pigeons, might also play a role in the spread of IBV strains around the world. Specific IBV strains that are able to infect another bird species, especially migratory birds, spread more easily over long distances than a strain that cannot replicate in that bird species. An unknown IBV-like virus that might be common in a migratory bird could infect the poultry industry in different parts of the world, leaving us with the mystery of how this new virus spread so fast within the poultry industry. However, the role of wild birds in the dynamics and spread of IBV is largely unknown and speculative. This area deserves more attention and research.

The epidemiological situation of IBV in North Africa

Most molecular studies and the available sequence data of the IBV do not cover the full-length of the S1 gene. They describe a partial S1 gene sequence, including HVR1 and the majority of HVR2 (≈ 392 bp) or HVR3 (≈ 397 pb). Table 1 provides a summary of the IBV strains circulating in North Africa. Phylogenetic and genetic analyses of the S1 gene sequences available in GenBank of North African strains are shown in Fig. 3.

Table 1. Summary of the IBV strains mentioned in the chapter ‘The epidemiological situation of IBV’ in North Africa

GENOTYPES OR LINEAGE	REFERENCE STRAIN	PERIOD OF CIRCULATION	STRAIN NAME	REGION/ COUNTRY
GI-1 MASSACHUSETTS	Be delete	1982-1983	Isolates D, E, F, H and M. Identified by serotyping	Rabat-Temara Morocco
		2004	Group I: Mass serotype	Morocco
		2007-2010	TN1084/08: H120 vaccine strain	North-East Tunisia
		2010-2014	IBV/Morocco/39/2010 IBV/Morocco/02/2014	Morocco
		2017	M41. Tested by HI	Central-West of Algeria
		2019	H-120 vaccine Strains derived from H-120	North East of Algeria
GI-12	D274	2002	Isolate.2: close to Dutch variants D274 and D3898	Egypt
		2009	TN335/01	Tunisia
		2017	IBV D-274. Tested by HI	Central-West of Algeria
GI-13	793B “CR88 or IB 4/91”	1982-198	Moroccan-G/83	Morocco
		2010-2014	IBV/Morocco/32/201	Morocco
		2019	IBV/CK/EG/Fadliah-10/2019	Egypt
		2017	IB 4/91. Tested by HI	Central-West of Algeria
GI-16	IZO 28/86	2017	VSVRI_F3	Egypt
		2017-2018	IBV/CK/EG/QENA-4/2017	Qena province (South of Egypt)
GI-19	QX	2017	QX variant. Tested by HI	Central-West of Algeria
GI-21	Spain/97/314	2010-2014	IBV/Morocco/44/2014	Morocco
GI-23	Variant 2 or 1	2002	Isolate.1: close to Egypt/Beni-Suef/01. Classified later as GI-23	Egypt
		2002	var 1: Egypt/Beni-Suef/01	Governorate of Beni-Suef Egypt
		2012	var 2: Ck/Eg/BSU-2/2011 Ck/Eg/BSU-3/2011	Egypt
		2017-2018	IBV/CK/EG/QENA-48/2017 IBV/CK/EG/QENA-31/2018 IBV/CK/EG/QENA-8/2017 IBV/CK/EG/QENA-14/2017	Qena province (South of Egypt)
NEW GENOTYPES/ VARIANTS		1983	Moroccan-G/83	Morocco
		2006	Egypt/F/03	Egypt
		2009	TN20/00	Tunisia
		2012-2013	Algeria/26/b1 Algeria/26/b2 Algeria/26/b3	Central region of Algeria
		2012	IBV/Libya/9-2012 IBV/Libya/8-2012 IBV/Libya/3-2012 IBV/Libya/7-2012 IBV/Libya/5-2012	East Lybia
		2017	IBDZ13a	Central-West of Algeria
		2007-2010	TN296/07 TN557/07	North-East of Tunisia
		2019	TN1011/16 TN1012/16	Tunisia
		2020	EGY/NR725/2016	Egypt

All the strains were identified by S1 gene sequencing, apart from one exception (tested by HI). For some countries, only some representatives strains are cited

Egypt. In Egypt, IBV has been studied more than in other North African countries, allowing a better understanding the spatiotemporal and genetic evolution of the virus. The different studies have shown the continuous evolution of IBV, the co-circulation of different GI lineages (GI-23, GI-16) and strains closely related or derived from the vaccine strain ([GHETAS et al., 2016](#); [SABRA et al., 2020](#); [AMER et al., 2024](#)).

The first study on IBV dates back to the study by Ahmed in 1954, who isolated IBV on a specific pathogen free (SPF) chicken egg, from chickens showing respiratory signs. IBV was subsequently identified through serological tests, and studied for its pathogenicity. However, no data about serotypes were described ([AHMED, 1954](#)).

Subsequently, several molecular studies were carried out via partial or complete S1 gene sequencing. First, in one study, from samples col-

lected in 1998 from chickens showing respiratory signs and kidney lesions, [ABDEL-MONEIM et al. \(2002\)](#), isolated and partially sequenced the S1 gene (707 bp) of a strain named Egypt/Beni-Suef/01 (AF395531). This strain was considered to be a new genotype (var 1), unique in Egypt, with a nephropathogenic effect. Vaccination and infection trials showed that H 120 (GI-1) vaccine gives weak protection (20%), which explains the IBV outbreaks ([ABDEL-MONEIM et al., 2002](#)). In the same year, [MADBOULY et al. \(2002\)](#), isolated and characterized two strains, by partial sequencing of the S1 gene. The first (named Isolate. 1) was identical (100% aa and nucleotide similarities) to Egypt/ Beni-Seuf/01. The second strain (named Isolate.2) showed 99.4% and 97.8% nucleotide similarity to the Dutch variants D274 (GI-12) and D3898, respectively. Both strains showed a high nucleotide and aa divergence to H120 and M41 vaccine strains ([MADBOULY et al., 2002](#)). Since then, no other reports described the circulation of Dutch variants in Egypt until 2020 ([ABOZEID and NAGUIB, 2020](#)).

Later, in 2003, another nephropathogenic strain Egypt/F/03 (DQ487085), was detected by [ABDEL-MONEIM et al. \(2006\)](#) in unvaccinated flocks, with respiratory signs and severe renal lesions. The complete S1 gene sequence (1600 bp) showed a strong nucleotide homology (98%, 97% and 98%) with the GI-1 vaccine strains, Beau-dette, H120 and M41, respectively. However, several non-silent point mutations and variations in potential glycosylation sites (PGS) were observed as compared to these vaccine strains. Moreover, the Egypt/F/03 is a very distinct from the Egypt/Beni-Suef/01, isolated previously in 1998 (nucleotide and aa identity of 74% and 71% only). Also, the evaluation of the protection offered by the H120 vaccine showed weak protection ([ABDEL-MONEIM et al., 2006](#)), confirming the findings of the previous study by [MADBOULY et al. \(2002\)](#).

In 2012, [ABDEL-MONEIM et al. \(2012\)](#) identified five strains by partial S1 sequencing (418 bp). Two strains, Ck/Eg/BSU-2/2011 (JX174185) and Ck/Eg/BSU-3/2011 (JX174186), were assigned to a new variant – var 2. Var 2 showed several substi-

tutions in the HVR3 region (aa 274-387) compared to the Egypt/Beni-Suef/01, isolated in 1998 ([ABDEL-MONEIM et al., 2012](#)). This suggested the possibility of its introduction to Egypt from Middle Eastern countries ([SABRA et al., 2020](#)). Later, var 1 and var 2 were classified under the GI-23 lineage, which is very widespread in the Middle East countries ([GHE-TAS, 2021](#)).

Later, [GHETAS et al. \(2016\)](#), characterized four strains by partial sequencing of the S1 gene (385 bp) from a flock vaccinated with H120 (GI-1) from various Egyptian governorates (Qaliobia, Sharkia, and Cairo). Two strains, named IBV S40 and IBV S61, were close to the GI-1 reference vaccine strains (M41, H120, Ma5 and M52) and Egypt/F/03, detected in 2006. The other two strains, S78 and S82, were, however close to var 2 (GI-23), Ck/Eg/BSU-2/2011 and Ck/Eg/BSU-3/2011, previously described by [ABDEL-MONEIM et al. \(2012\)](#). This indicates the potential spread of var 2 to these Egyptian governorates ([GHETAS et al., 2016](#)).

[ABDEL-SABOUR et al. \(2017\)](#) by partial sequencing of the S1 gene revealed, for the first time in Egypt, the variant VSVRI_F3 (KP729419), very close (99.1% nucleotide identity) to the Q1 strain (AF286302), of GI-16 lineage, initially isolated in laying hens in China ([YU et al., 2001](#)). Moreover, they described two new variants, VSVRI_G4 (KP729420) and VSVRI_G9 (KP729422), distinct (92.8%-94.3%) from the Eg/12120S/2012 (KC533684) and Eg/12197B/2012 (KC533683) variants of 2012. Phylogenetic analysis of the S1 gene showed that these strains have 29-38 substitutions in serotype-specific regions, compared to the GI 1 (H120, M41, and Ma5) and GI-13 (CR88.4/91) reference vaccine strains, which may influence the success rate of vaccination ([ABDEL-SABOUR et al., 2017](#)).

Between 2017 and 2018, [SABRA et al. \(2020\)](#), isolated nine strains belonging to the GI-16 and GI-23 lineages in the Qena province (South of Egypt). Eight strains were grouped into GI-23 lineage. They were very different from the vaccines (H120, Ma5, Mass 41, 4/91, CR88) (nt identity of 77.4-82.3%) and previous

Egyptian variants (nt identity of 74.6-82.1%). While the GI-16 lineage strain IBV/CK/EG/QENA-4/2017 (MN890126) interestingly showed strong nucleotide similarity with the Chinese (GU938413), Italian (KP780179) and Vietnamese (KY992863) isolates ([SABRA et al., 2020](#)). Consequently, a common ancestor was suggested. The probable route of introduction remains unclear. This study mainly draws attention to the co-circulation of different lineages and the increasing detection in Egyptian poultry farming of isolates from distant countries, which raises the question about the introduction of IBV.

In 2019, [ROHAİM et al. \(2019\)](#), the isolate IBV/CK/EG/Fadllah-10/2019 (KP729419) belonging to the genotype 4/91 (GI-13 lineage), was isolated from flocks vaccinated with Ma5 (GI-1). The complete S1 gene sequencing (1617 bp) revealed several point mutations. The strain was considered as an inter-genotype recombination between the Egy/var. I genotype (GI-23 lineage) and a CR88 vaccine variant (GI-13 lineage). The isolate was also distant from the GI-1 (H120, Ma5, M41) and the GI-13 vaccine variants (4/91 and CR88), with only 75-76% and 90% similarity, respectively ([ROHAİM et al., 2019](#)). These findings reinforce previous studies, suggesting the incompatibility of vaccines with circulating strains. Moreover, they have drawn attention, for the first time in Egypt, to recombination as an evolution mechanism of Egyptian strains. Later, in 2020, [MOHARAM et al. \(2020\)](#), described, by partial S1 gene sequencing and NGS, a new variant EGY/NR725/2016 (MN987230), classified within the GI-23.3.3 subclade. It was distinct from the EGY-var2 (GI-23.2) described previously in Egypt. This study highlighted the continuous circulation of the GI genotype in Egypt, with the continuous emergence of new variants ([MOHARAM et al., 2020](#)).

Morocco. In Morocco, the first study dates back to [EL HOUADFI and JONES \(1985\)](#), with the detection of five isolates (named D, E, F, H and M) close to the Mass serotype, while one distinct isolate (G) showing enterotropism. The isolate G was identified later by S1 gene sequencing, and showed a genetic similarity with the 4/91 (GI-13) variant, suggesting a probable common origin ([JONES et](#)

[al., 2004](#)). In 2004, Alarabi described the circulation of three different groups (I, II and III) in flocks with renal lesions, where Group I was considered a Mass serotype and Group III was closely related to isolate G ([ALARABI, 2004](#)). The Group II serotype was not mentioned.

Later, in 2005, [EL BOUQDAOUI et al. \(2005\)](#) identified five genotypes from different regions of Morocco, by RT-PCR and restriction fragment length polymorphism (RFLP). Three genotypes were different from the GI-1 (Ma5) and GI-13 (4/91) vaccine, which did not provide protection against a challenge with these three new genotypes ([EL BOUQDAOUI et al., 2005](#)).

From samples collected between 2010 and 2014, partial S1 gene sequencing (393 bp) revealed the co-circulation of three GI lineages, represented by wild and vaccine Mass genotypes (66%) (GI-1) and the Italy 02 genotype (32%) (GI-21), for the first time in Morocco and Africa ([FELLAHI et al., 2015](#)). This lineage was one of the most predominant lineages found across many European countries (especially in Spain) and is spreading to other continents ([JONES et al., 2005](#)). The study also showed the temporal distribution change of IBV genotypes in Morocco compared to previous studies, where GI-1 and GI-21 had become more dominant compared to 793B (4/91 and CR88) belonging to GI-13, rather, dominant during the early 1990s ([FELLAHI et al., 2015](#)).

In a retrospective genotyping study conducted on strains collected from 1983 to 2014, [FELLAHI et al. \(2017\)](#) described three lineages in co-circulation (GI-1, GI-13 and GI-21). GI-1 was still the most dominant, due to the frequent use of Mass vaccines (H120, Ma5, etc.).

Tunisia. In Tunisia, IBV was first detected by [BOUZOUAIA\(1986\)](#), by a modified hem-agglutination test. Then, a sero-epidemiological study carried out from 2006 to 2008 by Enzyme-Linked Immunosorbent Assay (ELISA) revealed a seroprevalence of 45% ([BOUROGÂA et al., 2009b](#)).

The first molecular report, by [BOUROGÂA et al. \(2009a\)](#), was based on S1 partial sequencing (HVR1, HVR2 and HVR3; 610 bp) and a cross-viral neutralization test. It revealed the co-circula-

tion of two groups. The first one was identical to the H120 vaccine (GI-1). The second, divided into two clusters, represented by TN20/00 (EF535998), TN200/01 (EF535997), TN335/01 (EF535996), was classified within the CR88121 and D274 (GI-12) genotypes ([BOUROGÂA et al., 2009a](#)). One strain, TN20/00, was considered as a new variant and was recently classified within the GI-14 lineage ([LACHHEB et al., 2019](#)). These strains were distinct from the H120 vaccine (antigenic relationship test) and showed different genetic variations (35- 48 substitutions, deletion and insertion), in the S1 HVRs ([BOUROGÂA et al., 2009a](#)).

In 2012, [BOUROGÂA et al. \(2012\)](#) studied samples collected between 2007 and 2010, in different regions of North-East Tunisia. They described the co-circulation of an H120 vaccine strain (TN1084/08) and four new variants: TN295/07, TN296/07 (FJ716133), TN556/07 (FJ716132) and TN557/07, very different from the IBV reference strains (46% and 55%, with the vaccine strain CR88121). TN296/07 was the most distinct, with only 17% and 18% similarity to D272 (GI-12) and Mass H120 vaccines (GI-1), respectively ([BOUROGÂA et al. 2012](#)). These variants were also distinct (50%-77% similarity) from the Tunisian variants previously isolated by [BOUROGÂA et al. \(2009a\)](#). Similarly, genetic analysis of the S1 gene showed that they shared aa substitutions at the HVR, compared to the H120 vaccine, in positions associated with serotype-specific neutralizing epitopes and this may therefore affect vaccine protection ([BOUROGÂA et al., 2012](#)).

In 2019, [LACHHEB et al. \(2019\)](#) described two new unique variants by S1 partial sequencing (798 bp), TN1011/16 (KX061458) and TN1012/16 (KX061459), classified within the GI-1 genotype. They were very distinct from the vaccine strains H120 (GI-1), 4/91 (GI-13) and the old Tunisian variants isolated in 2009 (only 56.8% similarity with TN20/00). This finding indicates continuous evolution over this period. Surprisingly, they were very closely related ($\geq 92\%$ nt similarity) to the Algerian strains (Algeria/26/b1, Algeria/26/b2, Algeria/26/b3), isolated in 2012-2013, by [SID et al. \(2015\)](#), but grouped into two distinct subgroups on the phylogenetic tree (Fig.3). However, genetic

analysis of the S1 gene showed that the two Tunisian isolates possessed exclusive mutations in the S1 gene, in and near to HVR 1 and 2, at positions 250F, 262W and 281G (TN1011/16), and at positions 262C, 265S and 266I (TN1012/16) which make them unique ([LACHHEB et al., 2019](#)).

Algeria. In Algeria, IBV has been poorly studied and public veterinary services do not recommend any strategies to control the disease ([SID et al., 2015](#)). Only three molecular ([SID et al., 2015](#); [LOUNAS et al., 2018](#); [BARBERIS, 2019](#)) and one serological ([BARBERIS et al., 2018](#)) study were conducted between 2015 and 2022.

They showed a high IBV prevalence, the co-circulation of different serotypes, as well as the emergence of a new native genotype. All the isolated Algerian strains are distinct, as shown by the phylogenetic tree (Fig. 3).

In the North-Eastern region of Algeria, a seroprevalence of 78.25% was detected in broiler chickens, on poultry farms which do not vaccinate their birds against IBV ([BARBERIS et al., 2018](#)). Moreover, [LOUNAS et al. \(2018\)](#), reported co-circulation of different serotypes in one region in the Central Western region of Algeria using the hemagglutination inhibition (HI) test. The isolated strains were QX variant strain (100%) (GI-19), 4/91 '793' (60%) (GI-13), M-41 (50%) (GI-1) and IBV D-274 (7%) (GI-12). This is considered to be the first detection of the QX variant and 4/91 serotypes in Algeria ([LOUNAS et al., 2018](#)).

The first molecular study in Algeria was carried out in 2015, by [SID et al. \(2015\)](#). Samples were collected over the period from 2012 to 2013, in the central region of Algeria, on vaccinated broiler chicken farms, for IBV. These farms showed high mortality rates and a high frequency of viral and bacterial co-infections (avian influenza virus, metapneumovirus, and mycoplasma). They described the circulation of a new nephropathogenic genotype, represented by three strains: Algeria/26/b1 (KP892759), Algeria/26/b2 (KP892760), and Algeria/26/b3 (KP892761). Phylogenetic analysis of the partial S1 gene sequence (HVR1 and most of the HVR2 region), resulting in an amplicon of approximately 700 nt, showed that all the strains were

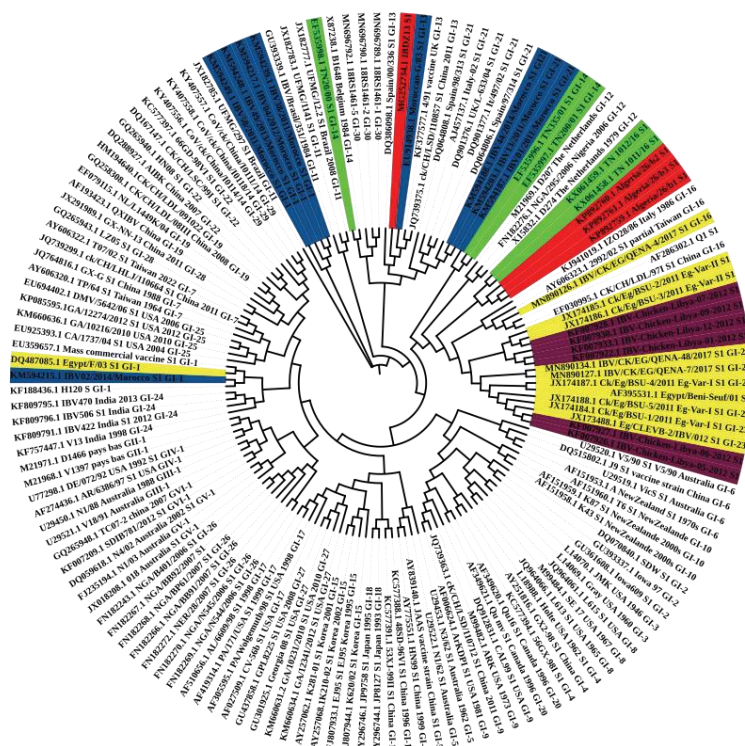


Fig.3. Phylogenetic tree based on the S1 gene sequence of IBV

Representative strains from North African countries, other countries and reference strains were included.

The phylogenetic tree was constructed using the MEGA 11 and the Maximum Likelihood, with a Bootstrap value of 1000. ■ Algerian strains ■ Tunisian strains ■ Moroccan strains ■ Egyptian strains ■ Libyan strains

grouped. They were distinct from strains isolated in bordering countries (Morocco and Tunisia) and IBV representative strains from different genotypes, suggesting that they represent an autochthonous genotype. Nevertheless, they shared a phylogenetic relationship (Bootstrap value: 80) with the BJ1 nephropathogenic Chinese strain (AF347018, GI- 1) and the new African genotype GI-26 identified in Western Africa (represented by NER/28/2007 and NGA/BB92/2007). Thus, the Algerian isolates may be an inter-lineage recombination product of GI-1 and GI-26 (SID et al., 2015). It is noteworthy that this recombination mechanism is well documented and could take place between different lineages. Hence, YAN et al. (2021), described the emergence of a new recombinant variant in China (CK/CH/SCMY/160315), between four lineages, the live attenuated vaccine (GI-1), 4/91 (GI -13), LDT3-A (GI-28 and the field isolate LJL/08-1 (GI-19) (YAN et al., 2021).

Later, LOUANAS et al. (2018), conducted a study on vaccinated chicken farms with kidney injury in the Central-Western region of Algeria. They showed the circulation of a new virulent nephropathogenic genotype, IBDZ13a (MG252734). S1 partial sequencing (HVR1 and the majority of the HVR2 region; 344 bp) showed that the unique sequenced strain shared 93% homology with the IB 4/91 vaccine strain (GI-13) and was therefore considered as a new genotype of IB 4/91 (WORTHINGTON et al., 2008; LOUNAS et al., 2018). IB 4/91 has been detected in several countries around the world and is considered to be the dominant genotype in Africa, Europe and Asia (DE WIT et al., 2011). Moreover, it had 29 nt substitutions and eight aa substitutions, compared to the reference vaccine strain 4/91 (GI-13), which can affect its immunogenicity (LOUNAS et al., 2018). The IBDZ13a was, however, very distant (similarity of 74%) from the autochthonous nephropathogenic genotype described by SID et al. (2015).

More recently, in 2019, in North East of Algeria [BARBERIS \(2019\)](#), in flocks presenting respiratory symptoms and vaccinated against IBV (H120; GI-1 lineage), isolated and sequenced the complete S1 gene of two isolates (2T/17 and 16T/17). The two isolates showed 99.60% and 99.47% nt identity with the H120 vaccine, and a bootstrap value of 100% indicating they were H120 vaccine-derived strains ([WORTHINGTON et al., 2008](#)). However, the two isolates showed two non-synonymous nt substitutions (C56T and T39G), resulting in two aa substitutions (Alv19Val and Cys13Trp respectively) in the HVR1. Concerning the HVR2, the 2T/17 strain showed two nt substitutions at positions 345 and 347, resulting in a Lys117Thr substitution. The 16T/17 isolate showed two non-synonymous nt substitutions (T353G, T380G), giving rise to two aa substitutions, (Val118Gly and Met127Arg, respectively) ([BARBERIS, 2019](#)).

Given the large area of the country and IBV emergence in bordering countries (Italy 2 in Morocco), it is obvious that molecular data on IBV in Algeria remain insufficient. Also, the partial sequencing of the S1 gene in most Algerian studies, is useful for genotyping but still limited in determining the genetic evolution of the circulating strains ([VALASTRO et al., 2016](#)). Strains are also isolated in occasional outbreaks, and a broader approach that includes continuous molecular surveillance across different regions is essential for a complete understanding of IBV dynamics.

Libya. In Libya, the only molecular study carried out so far is that by [AWAD et al. \(2014\)](#). IBV was detected by RT-PCR on broiler farms showing respiratory symptoms and a high mortality rate. Partial sequencing of the S1 gene (380 bp) showed the co-circulation of different and new variants, clustered into two distinct groups, related to Egyptian strains (Fig.3). Most of the isolates were closely related (94-99%) to the Egyptian CK/Eg/BSU-2/2011, CK/Eg/BSU-3/2011 and Eg/1212B. However, two isolates, IBV/Chicken/Libya/05/2012 (KF007926) and IBV/Chicken/Libya/06/2012 (KF007927), formed a distinct cluster, and showed 100% homology to the Egyptian Eg/CLEVB-2/IBV/012 (JX173488) strain.

It has been suggested that the cross-border movement of poultry and poultry products is the cause of IBV spread from Middle Eastern countries to Egypt, and subsequently to Libya. No vaccine trial was performed against these Libyan variants. However, given the weak protection of the H120 vaccine against homologous isolates from other countries, it seems likely that the Libyan variants are also not controlled by the H120 vaccine ([AWAD et al., 2014](#)). A summary of comparison of nt and aa identity (as a percentage: %) of North Africa representative strains, with IBV reference strains is presented in Table 2.

Vaccination strategies against IBV in North Africa

Vaccination strategies against IBV. In poultry farming, the control of IBV is based on the extensive use of live attenuated and inactive vaccines. The use of vaccines has been proven to provide good protection against homologous IBV strains ([ZHAO et al., 2015](#); [JORDAN, 2017](#)). Recent studies have focused on the development of recombinant vaccines expressing IBV genes ([BARBERIS et al., 2015](#)), both alone ([TORO et al., 2015](#)), associated with other pathogens (bivalents vaccines) or multiple IBV genes with adjuvants ([WANG et al., 2009](#); [ZHAO et al., 2017](#); [ABOZEID et al., 2019](#); [TAN et al., 2020](#)). Recombinant IBV vaccines are not licensed for use in the commercial poultry industry ([JORDAN, 2017](#)).

IBV is still a topical problem and difficult to control. As with other coronavirus, it undergoes rapid evolution, due to high rates of mutation, viral recombination, and host selection pressure, producing a growing number of genotypes and serotypes. Also, IBVs have a high genetic and antigenic diversity ([COOK et al., 2012](#); [LI et al., 2023](#)). Consequently, the low level or total absence of cross-protection between different serotypes has been well documented ([REDDY et al., 2015](#); [ZHAO et al., 2016](#)). In addition, the vaccines used in most countries are imported vaccines that do not correspond to field strains ([ELHADY et al., 2018](#)) but remain the only vaccines used. The old vaccine strains of Massachusetts serotypes (H120) are used continuously

against newly emerging IBV all over the world ([JORDAN, 2017](#)). Furthermore, live attenuated vaccines lead to the production of vaccine derived strains by punctual mutations, recombination or virus serotype selection ([GUZMÁN and HIDALGO 2020](#); [BOUDAUD and BARBERIS, 2023](#)). To resolve the problem of vaccine failure, many vaccines from emerging serotypes have been developed ([JACKWOOD, 2012](#)). However, this requires continuous molecular characterization to adapt vaccines to prevalent field strains in each geographic region, and the introduction of new indigenous vaccine genotypes or variants into the vaccine program, to achieve better vaccine protection ([BELKASMI et al., 2017](#); [LACHHEB et al., 2019](#); [YOUSEFI et al., 2019](#)). These have the advantage of meeting the specific needs of local poultry farming for enhanced efficiency, better adaptability to genetic variations, avoiding the problem of vaccine-derived pathogen strains, and a lower production cost. However, continuous monitoring of circulating strains is necessary to ensure that the vaccine remains effective as the virus evolves. Moreover, vaccines need to meet regulatory and safety criteria.

Vaccination strategies in the North African countries

In North African countries, the most frequently used commercial vaccines are imported live attenuated vaccines (H120, Ma5) or variants of the GI genotype (4/91, CR88 '793B', D278) (Table 3). These are the only licensed vaccines available for use. The use of inactivated vaccines based on local strains as candidate vaccines is ongoing ([ALI et al., 2018](#)). As everywhere in the world, the H120 vaccine (based on GI-1) has been used for many decades in North Africa; however, IB remains a persistent problem in many countries ([BIJLENGA et al., 2004](#)). In North Africa, the molecular studies carried out have shown the genetic diversity and phylogenetic distance (GI-1, GI-13, GI-16, GI-23) of circulating isolates compared to classical commercial vaccines. As presented above, the circulating types are represented by autochthonous variants ([SID et al., 2015](#); [ABOZEID et al., 2017](#); [LOUNAS et al., 2018](#); [LACHHEB et al., 2019](#)). Hence, the lack of cross protection between imported vaccines and field strains

in African countries may explain the failure to establish an effective vaccination program against IBV ([TORO et al., 2015](#); [ALI et al., 2018](#)). Consequently, new vaccines should be considered with the emergence of new IBV genotypes. While classical vaccines are still the most used, autochthonous vaccines and vaccines based on heterologous vaccine combinations are gaining importance.

Vaccines based on autochthonous strains

The development of vaccines against indigenous variant strains is encouraged, given the promising results obtained in several studies against challenge with field variants. However, this strategy should be only considered when new variants exhibit strong genetic distance from commercial vaccines or cause a real economic challenge to the poultry sector ([HASSAN et al., 2024](#)). One of the emerging IBV is the GI-23 genotype (including Var. I and Var. II) ([AL-EBSHAHY et al., 2019](#)). In North African countries, most vaccine trials have been conducted in Egypt as this genotype was prevalent in the Middle East.

The only vaccine trial based on local strains was performed by [ELHADY et al. \(2018\)](#), in Egypt. They developed a new vaccine based on the Egyptian variant 2 (Egy/Var2, GI-23) isolated previously by [ABDEL-MONEIM et al. \(2012\)](#). The study showed that the association of the new autochthonous Egy/Var2 vaccine with the H120 vaccine already used, limited infection by this variant to the trachea, conferring a good immune response to infection and a decrease in mortality, compared to the single use of H120. Later, this vaccine was commercialized as ME VAC IB VAR2® (variant 2 IBV) ([EL-HADY et al., 2018](#)).

In contrast, [ENACHE et al. \(2024\)](#) showed that the classical vaccine association H120 (GI-1) + IB 4/91 (GI-13) can control IBV Var 2 (GI-23) infection if the vaccination occurs correctly on day one in the hatchery. Consequently, an immunological study of IBV strain cross protection can allow the development of new control vaccinations for new variants. This example demonstrates that the need to develop a new autochthonous vaccine should be clearly evaluated ([ENACHE et al., 2024](#)). Moreover, the

Table 2. Percentages of nucleotide and amino acid identities of the partial S1 gene of North African variants IBV in comparison to selected references strains

AMINO ACID IDENTITY (%)																							
	Seq->	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1	H120	ID	95,10	70,10	73,00	69,40	99,50	93,30	73,70	72,90	74,10	70,70	68,20	70,50	74,10	70,30	96,40	74,10	77,60	69,20	70,30	71,10	74,60
2	Beaudette	97,00	ID	70,50	72,60	69,80	94,70	92,00	73,70	73,70	72,90	71,60	69,10	69,40	71,70	69,80	95,10	71,70	76,40	68,80	70,30	71,60	74,60
3	It 497 02	72,80	73,40	ID	68,10	72,70	69,60	68,80	78,30	71,40	89,40	69,60	70,50	92,90	83,50	71,80	70,90	83,50	83,50	76,60	77,80	67,00	67,00
4	QX IBV	75,30	74,50	71,60	ID	70,80	73,00	72,60	71,30	69,50	82,30	68,20	70,00	82,30	81,10	70,40	73,90	81,10	78,80	68,50	72,10	68,60	69,10
5	4/91 vaccine	74,90	74,70	74,60	75,10	ID	68,90	68,50	71,60	72,40	89,40	68,10	80,80	87,00	81,10	69,80	70,30	81,10	80,00	70,50	72,90	69,80	68,90
6	Ma5 GI-1	99,60	97,00	72,50	74,90	74,50	ID	92,90	73,30	72,90	72,90	70,70	67,80	69,40	72,90	70,30	96,00	72,90	76,40	68,80	70,30	71,10	74,60
7	M41 GI-1	96,80	96,80	72,80	74,40	74,40	96,80	ID	71,10	71,60	77,10	69,80	68,60	70,50	74,10	68,10	94,20	74,10	77,60	67,90	68,50	70,30	73,30
8	D274 GI-12	76,00	76,40	80,10	73,10	74,00	75,60	75,50	ID	70,30	81,10	68,90	70,40	50,00	81,10	74,10	73,70	80,00	81,10	76,60	80,70	69,80	69,80
9	Algeria/26/b1	78,90	79,10	75,90	74,00	76,20	78,60	77,80	74,20	ID	/	88,10	70,40	/	/	71,10	74,20	/	/	71,40	72,90	68,50	68,50
10	IBDZ13 S1	77,00	76,60	84,90	83,00	89,30	77,00	77,40	81,80	/	ID	/	/	85,80	83,50	/	/	83,50	84,70	/	/	/	/
11	TN 1011/16	78,40	78,90	76,10	74,20	75,30	78,00	77,80	75,60	94,80	/	ID	67,30	/	/	69,80	71,10	/	/	70,10	69,80	68,10	69,40
12	Moroccan-G/83	72,70	72,50	73,00	72,70	89,30	72,30	72,90	73,10	74,70	/	73,60	ID	/	/	68,60	69,10	/	/	67,20	71,60	64,70	63,90
13	IBV44/2014/Morocco	74,30	73,50	96,00	83,00	84,10	74,30	74,30	79,40	/	83,70	/	/	ID	82,30	/	/	82,30	82,30	/	/	/	/
14	Chicken-Libya-05-2012	80,20	79,00	83,00	82,60	82,60	80,20	79,80	83,70	/	84,10	/	/	83,30	ID	/	/	98,80	84,70	/	/	/	/
15	Egy/Beni-Seuf/01	71,60	72,00	71,60	70,50	73,30	71,20	71,40	72,00	72,90	/	72,70	72,90	/	/	ID	69,40	/	/	73,10	79,00	66,80	67,20
16	Egypt/F/03 S1	98,10	97,70	73,40	75,50	75,30	98,10	97,90	76,50	78,60	/	78,20	73,10	/	/	72,00	ID	/	/	69,20	70,30	72,00	73,70
17	Eg/BSU-1/2011 Var 1	80,20	79,00	83,30	83,00	81,80	80,20	79,80	83,00	/	84,90	/	/	83,70	99,20	/	/	ID	84,70	/	/	/	/
18	Eg/BSU-2/2011 Var 2	79,00	78,60	82,60	82,20	80,20	79,00	79,40	81,80	/	82,20	/	/	81,80	85,70	/	/	85,30	ID	/	/	/	/
19	EG/QENA-4/2017	71,40	71,60	77,30	69,40	72,60	71,00	71,60	75,90	73,00	/	73,40	71,70	/	/	71,40	72,10	/	/	ID	80,90	66,20	67,90
20	EG/QENA-7/2017	76,70	76,70	78,10	74,00	76,20	76,40	76,70	83,80	76,40	/	77,30	74,90	/	/	76,40	77,30	/	/	77,20	ID	68,10	68,10
21	NER/28/2007	76,70	76,50	73,40	73,60	74,00	76,40	76,20	73,10	76,00	/	76,40	71,80	/	/	70,90	76,20	/	/	69,20	73,30	ID	90,70
22	NGA/BB92/2007	76,40	76,20	72,70	73,10	73,60	76,00	76,00	72,70	75,60	/	75,80	72,00	/	/	70,30	76,20	/	/	68,70	73,80	93,50	ID
Nucleotide identity (%)																							

ID: identical. The symbol (/): indicates that IBV variants do not overlap when aligned

development of new live attenuated vaccines based on the field strains has other limitations such as the cost and the time required for vaccine production ([GUZMÁN and HIDALGO, 2020](#)). In addition, it is also not possible to make a new vaccine for each emerging strain.

Vaccines based on a heterologous vaccine combination (protectotype concept)

Another approach consists of the combination of heterologous vaccines (polyvalent vaccine). This is based on the “protectotype” concept, using the available commercial vaccines to expand the protection spectrum, and to obtain the additional effect of monovalent vaccination against the newly emerging IBV ([SMIALEK et al., 2016](#); [JORDAN, 2017](#); [SHAO et al., 2020](#)). This approach is based on timing and an appropriate combination of vaccines, to provide improved protection compared to single vaccinations. Most trials based on this concept combine a classical genotype such as Massachusetts (GI 1) and one variant such as IB 4/91 (GI-13) ([COOK et al., 1999](#)).

[BOUROGÂA et al. \(2014\)](#) showed that association of H120 strain at 1 day, and CR88 vaccine ‘793B type’, at 10-14 days of age increased the levels of immunity and protection against nephropathogenic IBV TN20/00 as compared to one vaccine given alone. Notably, this protective effect was achieved even though IBV TN20/00 is not related to the Massachusetts serotype ([BOUROGÂA et al., 2014](#)). [AWAD et al. \(2015\)](#) also reported that simultaneous administration of H120 and CR88 at one day old, followed by a second dose of CR88 at 14 days old, resulted in improved protection against clinical symptoms, tracheal and kidney lesions, and tracheal ciliostasis. This protocol was effective against the highly pathogenic IS/885/00-like (IS/885) and IS/1494/06-like (IS/1494) strains, which are not related to either vaccine strains ([AWAD et al., 2015](#)). The results of these studies highlight the effectiveness of combining commercial vaccines to combat emerging variants, despite their genetic differences. Moreover, [BELKASMI et al. \(2017\)](#) showed the same results under experimental conditions, where H120 ‘GI-1’ given on day 1 and IBV 793/B ‘GI-13’ on day 15 provided protection against the emerging Morocco-Italy-2 ‘GI-21’. More recently, [EID et al.](#)

Table 3. IBV vaccines used in North African countries

COUNTRY	VACCINES	TYPE	REFERENCE
Egypt	H120 (GI-1)	Live attenuated	(ELHADY et al., 2018; SABRA et al., 2020)
	Ma5 (GI-1)	Live attenuated-Inactivated	
	Mass 41 (GI-1)	Live attenuated-Inactivated	
	793B ‘4/91, CR88’ (GI-13)	Live attenuated	
	CR88121 (GI-13)	Live attenuated-Inactivated	
	D278 (GI-12)	Live attenuated-Inactivated	
	ME VAC IB VAR2 ® (variant 2 IBV; GI-23)	Newly developed live attenuated vaccine	
Algeria	H120 (GI-1)	Live attenuated	(LOUNAS et al., 2018)
	Ma5 (GI-1)	Live attenuated-Inactivated	
	Mass41 (GI-1)	Live attenuated-Inactivated	
	IB 4/91 (GI-13)	Live attenuated	
Libya	Data not available	/	/
Morocco	H120 (GI-1)	Live attenuated	(FELLAHI et al., 2015)
	Ma5 (GI-1)	Live attenuated-Inactivated	
	IB 4/91 (GI-13)	Live attenuated	
	Arkansas	Live attenuated	
Tunisia	H120 (GI-1)	Live attenuated	(BOUROGÂA et al., 2014; LACHHEB et al., 2019)
	793-B ‘IB 4/91’ (GI-13)	Live attenuated	

(2024) described the same results. Indeed, the combination of heterologous live vaccines Nobilis® IB MA5 (GI-1) and Nobilis® IB 4/91 (GI-13), at 1 and 14 days of age, via ocular-nasal route, promoted good protection against the recent Egyptian IBV variant II (GI-23) (EID et al., 2024). In this regard, more studies using different commercial vaccine associations should be conducted to choose the best association (BOUROGÂA et al., 2014).

Recombinant vaccines. The plasticity of gene recombination makes recombinant vector vaccines expressing other avian viruses, such as NDV and the IBV S gene, a useful strategy to control dominant circulating genotypes (LI et al., 2023; ABOZEID, 2023). ABOZEID et al. (2019) tested a Newcastle disease virus-vectored vaccine (rNDV) expressing the S protein of the Egyptian IBV variant IBV/Ck/EG/CU/4/2014 (GI-23), and reported that the vaccine promoted good protection against clinical signs and tracheal viral shedding.

Conclusions

There are limited studies of IBV in North African countries. Moreover, they only cover some outbreaks located in limited geographic regions, with a small number of samples especially in Algeria and Libya. In addition, there are no national surveillance programs to monitor IBV field strain evolution and choose IBV vaccine candidates. Consequently, molecular data about IBV are poor.

On the basis of the published research articles, the most common circulating genotype is GI. Most studies drew attention to the continuous emergence and spread of new autochthonous IBV variants and genotypes. The evolution of IBV strains by mutations and recombination between different lineages and strains from bordering and non-bordering countries has been observed. Some European and Asian genotypes, such as Italy 2 and QX, emerged recently in Africa, posing the serious problem of their dissemination to other African countries. The creation of a surveillance network between North African countries may contribute to our understanding of the spatial spread of IBV since the new Tunisian and Libyan variants are closely related to

the Egyptian and Algerian variants, respectively. The number of IBV variants recognized will continue to increase due to mutations and recombinations, together with improved genetic typing methods. The various molecular studies carried out in North Africa have described the genotype and sero-type incompatibility between the field strains and the commercial classical or variant vaccines, since the latter are mainly imported and do not match the relevant field strains. However, only a few, live-attenuated and inactivated vaccines are available, and the development of new vaccines against novel variants is not a viable solution. For that reason, new approaches are being tested. Promising results have been obtained with 'multi-monovalent strategy' against new variants. Therefore, it is recommended that testing should continue to evaluate the available IBV vaccines against heterologous and variant strains, and also to develop vaccines that are safe and effective against new variants, if possible, in the case of insufficient protection. In this regard, there is a lack of knowledge about the genetic, antigenic and immunological background of cross protection. It is known that humoral, local and cell-mediated immune responses are important in determining the outcome of an IBV infection, although cell-mediated responses are still poorly understood.

Declaration of competing interest

The author declares no conflict of interest.

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SAŽETAK

Zarazni bronhitis peradi izrazito je zarazna respiratorna bolest koja je rasprostranjena diljem svijeta. Sustavno se pojavljuje u jatima koja su cijepljena i necijepljena te je i dalje velik problem s golemim gospodarskim posljedicama. U radu je prikazano stanje zaraznog bronhitisa u sjevernoj Africi, uz filogenetsku i gensku analizu dostupnoga gena za protein šiljka. Prikazana je trenutačna situacija u vezi s cijepljenjem, nakon čega slijede nove predložene strategije za suzbijanje novih varijanti u zemljama sjeverne Afrike. U sjevernoj Africi najčešće se pojavljuju linije genotipa I (GI) virusa koji uzrokuje zarazni bronhitis peradi. Osim toga, otkrivene su jedinstvene i autohtone varijante. Varijanta Morocco Italy 02 (GI 21), koje prije nije bilo na afričkom kontinentu, nedavno se pojavila u Maroku, što otvara pitanje načina njezina unosa. Neke varijante s Bliskog istoka također su otkrivene u Egiptu i Libiji. Analiza gena S1 pokazala je rekombinaciju među sojevima iz zemalja koje nemaju međusobnu granicu te niz točkastih mutacija u hipervarijabilnim područjima. U usporedbi s komercijalnim cjepivima i referentnim sojevima neke od točkastih mutacija su ekskluzivne, što upućuje na stalnu evoluciju zaraznog bronhitisa peradi u sjevernoj Africi. Provedeno je nekoliko pokusa cijepljenja dostupnim i/ili novim cjepivima. Kombinacija autohtonih sojeva s klasičnim ili varijantnim cjepivima pokazala je obećavajuće rezultate. No, treba dodati da je kombinacija monovalentnih heterolognih cjepiva također pružila dobru zaštitu. Potrebna su, međutim, dodatna ispitivanja kako bi se razvili učinkoviti programi cijepljenja i omogućila njihova široka primjena. U trenutačnim okolnostima čini se da je ažuriranje podataka na području molekularne dijagnostike ključno za razumijevanje evolucije virusa zaraznog bronhitisa, kako bi se poboljšala prilagodba programa cijepljenja i preventivnih mjera terenskim sojevima.

Ključne riječi: virus zaraznog bronhitisa peradi; sjeverna Afrika; gen S1; genska karakterizacija; nove varijante; cijepljenje
