

## Electronic Supplementary Material

# Phylogenetic analysis of avian infectious bronchitis virus isolates from Morocco: a retrospective study (1983 to 2014)

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Table S1. Data from 62 Moroccan IBV isolates included in this study

Codes	IBV isolates	Breed history reported	Major clinical signs	IBV vaccination	Lineage	GenBank accession numbers
1	Moroccan-G/83	Broiler	Enteric	N/A	GI-13	EU914938
2	IBV/Morocco/97/1986	Broiler	Respiratory	Unvaccinated	GI-13	KM594213
3	IBV/Morocco/91/1986	Broiler	Respiratory	Unvaccinated	GI-13	KM594210
4	IBV/Morocco/17/1988	Broiler	N/A	N/A	GI-13	KM594212
5	IBV/Morocco/11/1989	Broiler	N/A	N/A	GI-13	KM594192
6	IBV/Morocco/86/1996	Broiler	Respiratory	Vaccinated	GI-1	KM594211
7	IBV/Morocco/88/1997	Broiler	Nephritis	N/A	GI-13	KM594202
8	IBV/Morocco/81/1998	Broiler	Nephritis	N/A	GI-13	KM594208
9	IBV/Morocco/85/1998	Layers	Respiratory	Vaccinated	GI-1	KM594209
10	IBV/Morocco/90/2000	Broiler	Nephritis	Vaccinated	GI-13	KM594198
11	IBV/Morocco/92/2000	Broiler	Nephritis	Unvaccinated	GI-13	KM594200
12	IBV/Morocco/93/2001	Broiler	Nephritis	N/A	GI-13	KM594194
13	IBV/Morocco/19/2006	Broiler	Nephritis	Vaccinated	GI-13	KM594191
14	IBV/Morocco/05/2010	Broiler	Respiratory	Vaccinated	GI-1	KM594218
15	IBV/Morocco/06/2010	Broiler	Respiratory	Vaccinated	GI-21	KM594219

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16	IBV/Morocco/31/2010	Broiler	Respiratory	Vaccinated	GI-21	KM594205
17	IBV/Morocco/39/2010	Layer	Respiratory	Vaccinated	GI-1	KM594240
18	IBV/Morocco/13/2011	Broiler	Nephritis	Unvaccinated	GI-21	KM594203
19	IBV/Morocco/18/2011	Broiler	Respiratory	Vaccinated	GI-1	KM594226
20	IBV/Morocco/29/2011	Broiler	Respiratory	Vaccinated	GI-1	KM594232
21	IBV/Morocco/32/2011	Broiler	Nephritis	Vaccinated	GI-13	KM594234
22	IBV/Morocco /33/2011	Broiler	Nephritis	Vaccinated	GI-21	KM594190
23	IBV/Morocco/34/2011	Breeder	Respiratory	Vaccinated	GI-1	KM594235
24	IBV/Morocco/38/2011	Layer	Respiratory	Vaccinated	GI-1	KM594239
25	IBV/Morocco/50/2011	Broiler	Respiratory	Vaccinated	GI-1	KM594249
26	IBV/Morocco/22/2012	Broiler	Respiratory	Vaccinated	GI-1	KM594227
27	IBV/Morocco/14/2012	Broiler	Nephritis	Unvaccinated	GI-21	KM594195
28	IBV/Morocco/24/2012	Broiler	Nephritis	Unvaccinated	GI-21	KM594196
29	IBV/Morocco/27/2012	Broiler	Respiratory	Vaccinated	GI-21	KM594199
30	IBV/Morocco/15/2012	Broiler	Respiratory	Unvaccinated	GI-21	KM594201
31	IBV/Morocco/09/2012	Layer	Respiratory	Vaccinated	GI-21	KM594206
32	IBV/Morocco/19/2012	Broiler	Respiratory	Vaccinated	GI-1	KM594214
33	IBV/Morocco/04/2012	Broiler	Respiratory	Vaccinated	GI-1	KM594217
34	IBV/Morocco/07/2012	Broiler	Respiratory	Vaccinated	GI-1	KM594220
35	IBV/Morocco/08/2012	Broiler	Nephritis	Vaccinated	GI-1	KM594221
36	IBV/Morocco/11/2012	Layer	Respiratory	Vaccinated	GI-1	KM594223
37	IBV/Morocco/26/2012	Broiler	Respiratory	Vaccinated	GI-1	KM594230
38	IBV/Morocco/28/2012	Broiler	Respiratory	Vaccinated	GI-1	KM594231
39	IBV/Morocco/35/2012	Broiler	Respiratory	Vaccinated	GI-1	KM594236
40	IBV/Morocco/36/2012	Layer	Respiratory	Vaccinated	GI-1	KM594237
41	IBV/Morocco/37/2012	Broiler	Respiratory	Vaccinated	GI-1	KM594238
42	IBV/Morocco/40/2012	Broiler	Respiratory	Vaccinated	GI-1	KM594241
43	IBV/Morocco/41/2012	Broiler	Respiratory	Vaccinated	GI-1	KM594242
44	IBV/Morocco/21/2013	Broiler	Respiratory	Unvaccinated	GI-21	KM594193
45	IBV/Morocco/16/2013	Broiler	Respiratory	Vaccinated	GI-21	KM594204
46	IBV/Morocco/20/2013	Broiler	Nephritis	Vaccinated	GI-21	KM594207
47	IBV/Morocco/03/2013	Broiler	Respiratory	Vaccinated	GI-1	KM594216
48	IBV/Morocco/10/2013	Broiler	Respiratory	Vaccinated	GI-1	KM594222
49	IBV/Morocco/12/2013	Broiler	Respiratory	Vaccinated	GI-1	KM594224
50	IBV/Morocco/17/2013	Broiler	Respiratory	Unvaccinated	GI-1	KM594225
51	IBV/Morocco/23/2013	Broiler	Respiratory	Vaccinated	GI-1	KM594228
52	IBV/Morocco/25/2013	Broiler	Respiratory	Vaccinated	GI-1	KM594229
53	IBV/Morocco/30/2013	Broiler	Respiratory	Vaccinated	GI-1	KM594233
54	IBV/Morocco/43/2013	Broiler	Respiratory	Unvaccinated	GI-1	KM594244
55	IBV/Morocco/46/2013	Broiler	Respiratory	Unvaccinated	GI-21	KM594245
56	IBV/Morocco/47/2013	Broiler	Respiratory	Unvaccinated	GI-1	KM594246
57	IBV/Morocco/48/2013	Broiler	Respiratory	Unvaccinated	GI-21	KM594247

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58	IBV/Morocco/49/2013	Broiler	Respiratory	Vaccinated	GI-1	KM594248
59	IBV/Morocco/02/2014	Broiler	Respiratory	Vaccinated	GI-1	KM594215
60	IBV/Morocco/98/2014	Broiler	Nephritis	Vaccinated	GI-13	KM594197
61	IBV/Morocco/42/2014	Broiler	Respiratory	Vaccinated	GI-1	KM594243
62	IBV/Morocco/44/2014	Broiler	Respiratory	Vaccinated	GI-21	KM594188
62	IBV/Morocco/45/2014	Broiler	Nephritis	Vaccinated	GI-1	KM594189

Note: The last four digits of the sequence title represent the year of detection. GI, genotype I; IBV, infectious bronchitis virus; N/A, data not available.

Table S2. Primers used in this study

Primer name	Primer sequence (5'–3')
Forward SX1+	CACCTAGAGGTTTGYTWGCAT
Reverse SX2-	TCCACCTCTATAAACACCYTT
Forward SX3+	TAATACTGGYAATTTTTCAGA
Reverse SX4-	AATACAGATTGCTTACAACCACC

Table S3. Estimates of evolutionary distances over sequence pairs between genotypes

Genotype	Amino acid evolutionary distance										
	1	2	3	4	5	6	7	8	9	10	11
1. GI-13	–	0.236	0.254	0.498	0.219	0.228	0.145	0.245	0.228	0.221	0.176
2. GI-9	0.170		0.227	0.483	0.220	0.194	0.208	0.257	0.231	0.262	0.257
3. GI-1	0.174	0.146		0.449	0.182	0.234	0.230	0.264	0.229	0.239	0.198
4. GII-1	0.356	0.349	0.318		0.481	0.491	0.469	0.483	0.501	0.468	0.496
5. GI-12	0.162	0.169	0.113	0.352		0.232	0.195	0.189	0.215	0.226	0.243
6. GI-3	0.169	0.129	0.137	0.364	0.165		0.205	0.269	0.242	0.269	0.248
7. GI-21	0.106	0.159	0.166	0.339	0.153	0.154		0.234	0.202	0.203	0.181
8. GI-26	0.173	0.181	0.176	0.349	0.150	0.183	0.169		0.266	0.227	0.266
9. GI-16	0.167	0.184	0.163	0.373	0.167	0.180	0.161	0.203		0.250	0.254
10. GI-19	0.171	0.197	0.157	0.365	0.177	0.201	0.158	0.179	0.193		0.267
11. GI-24	0.094	0.192	0.142	0.362	0.177	0.178	0.126	0.192	0.185	0.188	–
	Nucleotide evolutionary distance										

Note: Shown are the base substitutions per site (evolutionary distance) from averaging over all sequence pairs between genetic groups. Analyses were conducted using the Maximum Composite Likelihood model. The rate variation among sites was modeled with a gamma distribution (shape parameter = 1). All positions containing gaps and missing data were eliminated.

Table S4. Estimates of evolutionary distances over sequence pairs between Moroccan lineage GI-13 isolates

Genotype	Amino acid evolutionary distance				
	1	2	3	4	5
Cluster 1	–	<i>0.066</i>	<i>0.052</i>	<i>0.096</i>	<i>0.076</i>
Cluster 2	0.051		<i>0.068</i>	<i>0.116</i>	<i>0.090</i>
Cluster 3	0.030	0.041		<i>0.098</i>	<i>0.072</i>
Cluster 4	0.063	0.084	0.060		<i>0.089</i>
GI-13 reference strains	0.050	0.066	0.044	0.056	–
	Nucleotide evolutionary distance				

Note: Shown are the base substitutions per site (evolutionary distance) from averaging over all sequence pairs between groups. Standard error estimate(s) are shown above the diagonal, in italic font. Analyses were conducted using the Maximum Composite Likelihood model. The rate variation among sites was modeled with a gamma distribution (shape parameter = 1). All positions containing gaps and missing data were eliminated.

Table S5. HVR3 S1 protein mutations of Moroccan IBV isolates from GI-13 and CG-21 lineages compared with reference prototypes

Strain	Deletions or substitutions																	Insertions	
	268	271	285	287	289	293	296	297	299	301	306	326	329	338	340	345	364	291	351
GI-13 prototype <sup>a</sup>	E	I	V	N	A	S	V	D	F	Q	H	K	D	K	N	N	I	-	-
IBV/93/2001	E	V	V	N	A	S	V	D	F	Q	R	K	D	P	N	N	L	-	-
IBV/19/2006	E	V	V	N	A	S	V	D	F	Q	R	K	D	P	N	N	L	-	-
MoroccanG/83	E	T	V	N	S	S	V	D	F	Q	H	I	D	E	T	T	I	-	-
IBV/11/1989	E	T	V	N	S	S	V	D	F	Q	H	I	D	E	T	T	I	-	-
IBV/17/2013	E	T	V	N	S	S	V	D	F	Q	H	I	D	E	T	T	I	-	-
IBV/98/2014	E	T	V	N	S	S	V	D	F	Q	H	K	D	N	N	N	I	-	-
IBV/32/2011	E	I	V	N	A	S	V	D	F	Q	H	K	D	K	N	N	I	-	-
IBV/81/1998	E	I	V	N	V	L	I	E	L	V	H	K	N	P	N	N	I	-	-
IBV/90/2000	E	I	V	N	V	L	I	E	L	V	H	K	N	P	N	N	I	-	-
IBV/88/1997	E	I	V	N	V	L	I	E	L	V	H	K	N	P	N	N	I	-	-
IBV/91/1986	E	I	V	N	V	L	I	E	L	V	H	K	N	P	N	N	I	-	-
IBV/92/2000	E	I	V	N	V	L	I	E	L	V	H	K	N	P	N	N	I	-	-
IBV/97/1986	E	I	V	N	V	L	I	E	L	V	H	K	N	P	N	N	I	-	-
GI-21 prototype <sup>b</sup>	E	V	E	N	P	S	V	N	F	V	H	K	D	K	N	N	I	-	-
IBV/31/2010	E	V	V	D	P	S	V	N	F	V	H	K	D	T	N	N	I	L	W
IBV/13/2011	E	V	V	D	P	S	V	N	F	V	H	K	D	T	N	N	I	L	W
IBV/33/2011	E	V	V	D	P	S	V	N	F	V	H	K	D	T	N	N	I	L	W
IBV/14/2012	E	V	V	D	P	S	V	N	F	V	H	K	D	T	N	N	I	L	W
IBV/24/2012	E	V	V	D	P	S	V	N	F	V	H	K	D	T	N	N	I	L	W
IBV/27/2012	E	V	V	D	P	S	V	N	F	V	H	K	D	T	N	N	I	L	W
IBV/09/2012	E	V	V	D	P	S	V	N	F	V	H	K	D	T	N	N	I	L	W
IBV/21/2013	E	V	V	D	P	S	V	N	F	V	H	K	D	T	N	N	I	L	W
IBV/16/2013	E	V	V	D	P	S	V	N	F	V	H	K	D	T	N	N	I	L	W
IBV/20/2013	E	V	V	D	P	S	V	N	F	V	H	K	D	T	N	N	I	L	W
IBV/44/2014	E	V	V	D	P	S	V	N	F	V	H	K	D	T	N	N	I	L	W
IBV/46/2013	E	V	V	D	P	S	V	N	F	V	H	K	D	T	N	N	I	L	W
IBV/06/2010	E	V	V	D	P	S	V	N	F	V	H	K	D	T	N	N	I	L	W
IBV/15/2012	E	V	E	N	P	S	V	N	F	V	H	K	D	K	N	N	I	-	-
IBV/48/2013	E	V	E	N	P	S	V	N	F	V	H	K	D	K	N	N	I	-	-

Note: <sup>a</sup>GI-13 prototype is gCoV/AvCoV/chicken/Spain/1989Spain/1997. <sup>b</sup>GI-21 prototype is gCoV/AvCoV/chicken/Spain/97/314.