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Short Communication

Detection of variant infectious bronchitis viruses in broiler flocks in Libya

Faez Awad [a,b], **Matthew Baylis** [a], **Kannan Ganapathy** [a,*]

[a] *Institute of Infection and Global Health, University of Liverpool, Leahurst Campus, Neston, South Wirral CH64 7TE, UK*
[b] *University of Omar Al-Mukhtar, Faculty of Veterinary Medicine, Al-Bayda, Libya*

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KEYWORDS

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Abstract A number of broiler flocks with respiratory disease and high mortality in five broiler farms in Libya were sampled for detection of infectious bronchitis virus (IBV). Twelve IBV strains from these farms were detected by reverse transcription polymerase chain reaction (RT-PCR) and differentiated by nucleotide sequencing of the hypervariable region of the S1 gene. A pair-wise comparison of the sequences showed two distinctive patterns. Those from farms 1, 2, 4 and 5, formed a separate cluster with 94–99% relatedness to the Egyptian IBV strains CK/Eg/BSU-2/2011, CK/Eg/BSU-3/2011 and Eg/1212B. Sequences from the farm 3 formed another cluster with 100% relatedness to Eg/CLEVB-2/IBV/012 and IS/1494/06. This appears to be the first report on the co-circulation these variant IBVs in Libya.

1. Introduction

Avian infectious bronchitis virus (IBV) causes a highly contagious disease in chicken. It mainly affects the respiratory tract, and frequently causes damage to the kidneys and reproductive systems [1]. Although vaccination is commonly adopted, outbreaks continue to occur worldwide with significant economic consequences due to a substantial decrease in production performances [1,2]. Different genotypes of IBV have been identified worldwide, and new variants continue to emerge [3]. A number of IBV variant genotypes have been reported in the Middle East, including Iran/793B/19/08, Iraq/Sul/01/09, Israel/720/99, Israel/885/00, IS/1494/06, Egypt/Beni-Seuf/01, Egypt/F/03, Egypt/D/89, CK/CH/LDL/97I, and CK/CH/SCYA/10I [4–9]. Some of these genotypes in particular IS/885/00 and IS/1494/06, have become dominant in the majority of farms in the Middle East countries, causing respiratory and renal diseases [4,10,11]. To date, there is no information available on the circulation of variant IBVs in Libya. In the Middle East, the vaccination against IBV is performed with vaccines that contain live-attenuated or killed

Abbreviations: EXO, Exonuclease; FTA, Flinders Technology Associates; IBD, infectious bursal disease; IBV, infectious bronchitis virus; ND, Newcastle disease; OP, oropharyngeal swab; RNA, ribonucleic acid; RT-PCR, reverse transcription-polymerase chain reaction; SAP, shrimp alkaline phosphatase

* Corresponding author. Tel.: +44 151 794 6019.
E-mail address: gana@liverpool.ac.uk (K. Ganapathy).

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Table 1 Flock details, RT-PCR and genotype results.

Farm	Number of day-old chicks placed	Age (days)	Vaccination programme	Mortality on the day of sampling (%)	RT-PCR (Laboratory sequence No., Genebank Accession No.)				
					OP	Turbinates	Trachea	Lungs	Kidneys
1	6000	32	None	2	–	–	+(01, KF007922)*	+(02, KF007923)	+(03, KF007924)
2	5000	22	IBD (day 14)	3	+(04, KF007924)	–	–	–	–
3	9000	40	NDV (day 7) IBD (day 14)	3.7	–	–	+(05, KF007926)	–	+(06, KF007927)
4	10,000	26	IBD (day 14)	2	–	–	–	–	+(07, KF007928)
5	7500	16	NDV (day 7)	1.4	+(08, KF007929)	+(09, KF007930)	+(10, KF007931)	+(11, KF007932)	+(12, KF007933)

* For details, see Fig. 1.

viruses belonging to the Massachusetts serotype [10]. In the past few years, vaccine strains belonging to 793B and D274 serotypes are also widely used. In spite of this, IBV infection is considered endemic and widely spread both in vaccinated and unvaccinated poultry farms generally associated with kidney damages [4]. The aim of this study is to provide information on the molecular characteristic and the phylogenetic relationship of strains in Libya in comparison to other strains reported in the Middle East.

2. Materials and methods

2.1. Case history and clinical samples

In July 2012, a number of broiler flocks in five different farms with respiratory disease and high mortality at East Libya were visited. The flocks had no vaccination against IBV but were vaccinated against Newcastle disease (ND) and infectious bursal disease (IBD) (Table 1). All flocks showed clinical signs of respiratory distress, manifested by sneezing, tracheal râles, gasping, nasal discharge, head swelling, conjunctival congestion and frothy eyes. Post-mortem examination revealed lesions of inflamed trachea, cheesy exudate in airsacs and swelling of the kidneys. Mortality on the day of sampling ranged from 1.4% to 3.7% (Table 1).

From each of the farms, oropharyngeal swabs (OP) were collected from a total of 40 chicks. These swabs were divided into sets of 10 and were dipped into bijou tubes containing 2 ml of sterile water. After vigorous shaking, 100 µl of the mixture was spotted onto the Flinders Technology Associates (FTA) cards. Ten to twenty diseased birds per farm were killed and tissues of turbinates, trachea, lungs and kidneys were collected. The like-tissues were rubbed gently onto matrix areas of the FTA cards. These cards were air-dried and transported to the poultry virology laboratory at the University of Liverpool for analysis.

2.2. RNA extraction

The FTA cards were processed as described by the manufacturer with some modification. Briefly, the spotted or imprint area of the FTA card were cut using sterile scissors and forceps, each sample was placed into bijou tubes containing 2 ml of guanidinium thiocyanate and stored at −20 °C until required. RNA was extracted using guanidinium thiocyanate–phenol chloroform method as described [12]. Three hundred microliters of the mixtures above were placed in 1.5 ml eppendorf tube containing 300 µl guanidinium thiocyanate and stored at −20 °C for few hours. After thawing, this mixture was transferred into eppendorf tube and 50 µl of 2 M sodium acetate and 650 µl of phenol–chloroform were added. The suspensions were vortexed and centrifuged at 13,000*g* for 5 min. The aqueous phase containing the RNA was mixed with 500 µl isopropanol and stored at −20 °C overnight for precipitation of the RNA. The supernatant was carefully removed, and the precipitated RNA was pelleted at 13,000*g* for 15 min and washed twice with 100% ethanol. The pellet was dried and resuspended in 30 µl of treated water and used for RT-PCR.

2.3. RT-PCR and DNA sequencing

Procedures for the IBV RT-PCR have been described by [13]. Briefly, detection of the IBV genome and molecular

characterization were achieved by identifying (380) base pairs of the S1 region of the S protein gene. The RT-PCR procedure including the primers used were as described [14]. For sequencing, the nested-PCR products corresponding to the spike glycoprotein were purified with 0.15 µl Exonuclease 1 (EXO) and 0.99 µl shrimp alkaline phosphatase (SAP) at 37 °C for 30 min, followed by 80 °C for 10 min to remove any extraneous material. The purified product, together with positive sense primer (forward direction using primer SX3+), were submitted to external laboratory for analysis of the partial S1 gene sequences.

2.4. Phylogenetic analysis and nucleotide comparison

Multiple sequence alignments were carried out with Clustal W [15], and phylogenic tree was constructed with MEGA 5 software [16], using the Neighbor-joining tree method with 1000 bootstrap replicates to assign confidence levels to branches. The IBV sequences were aligned and compared with reference and vaccine strains that were found or used in the Middle East. The sequences were retrieved from GenBank (National Centre of Biotechnology Information) and BLAST search was carried out. The other S1 gene sequences used for comparison or phylogenetic analysis were CK/Eg/BSU-2/2011 (JX174185), CK/Eg/BSU-3/2011 (JX174186), Eg/1212B (JQ839287), Eg/CLEVB-2/IBV/012 (JX173488), IS/885/00 (AY279533) and IS/1494/06 (EU780077), M41 (GQ219712), H120 (GU393335), D274 (X15832), 4/91 (JN600614), CR88 (JN542567) and 793B (Z83979).

3. Results and discussion

The daily mortality on the day sampling is given in the Table 1. At necropsy, the main lesions found were tracheitis, lung congestion, air-sacculitis and enlarged kidneys. IBV was detected in samples obtained from all the farms. The nucleotide sequences of these IBVs were submitted to the GenBank and the assigned accession numbers are shown in the Table 1. Fig. 1 and Table 2 shows the relatedness between the Libyan IBV sequences in comparison to those found in the Middle East and the reference IBVs. A pair-wise comparison of the IBVs, showed two distinctive patterns. The Libyan IBVs, from farm 1, 2, 4, and 5, formed a separate cluster, with 94–99% homology to CK/Eg/BSU-2/2011, CK/Eg/BSU-3/2011 and Eg/1212B. The percent similarity to another regionally important IBV (IS/885/00), which was first detected in Israel ranged from 85–89% (Table 2). The Egyptian IBV strains CK/Eg/BSU-2(also 3)/2011 were associated with high mortality,

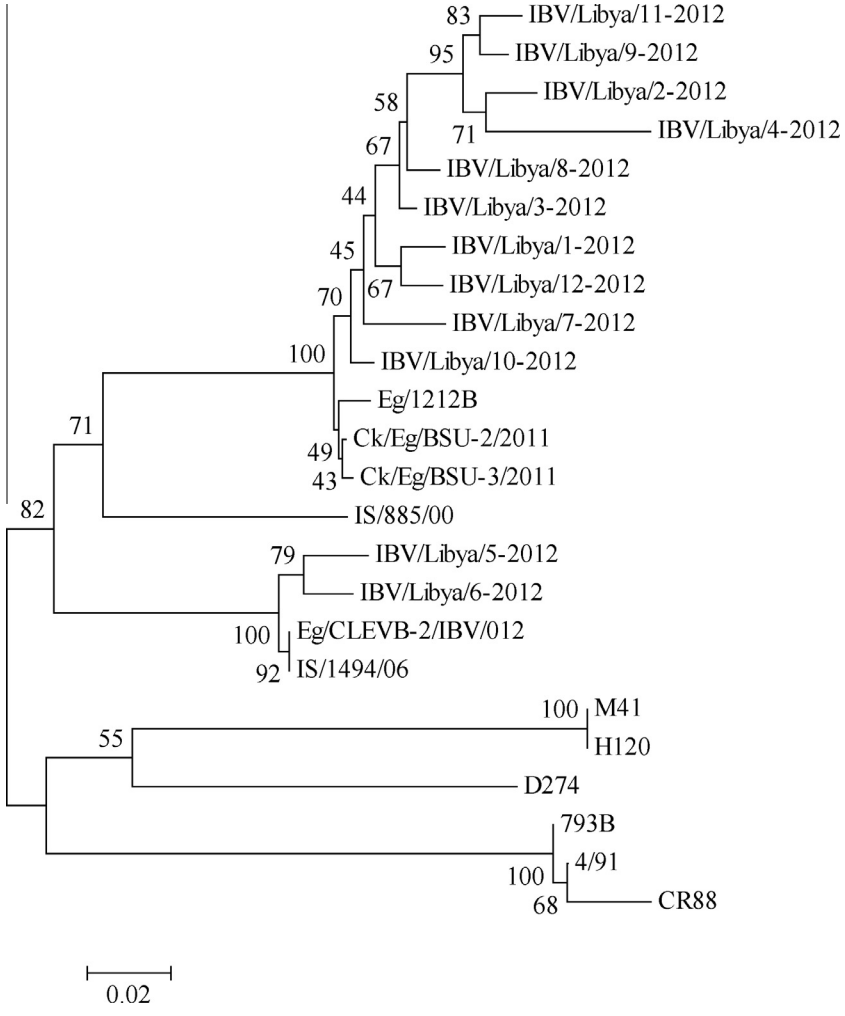

Fig. 1 Phylogenetic tree based on a partial sequence of the S1 gene, showing the relationship between the Libyan and other IBV strains.

Table 2 Nucleotide and amino acid identity of the part-S1 glycoprotein gene of the Libyan in comparison to other IBV strains.

% Amino acid identities																						
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20		
1		96	100	90	83	84	98	100	97	99	97	100	99	98	85	96	88	85	75	79	1	IBV/Chicken/Libya/01/12
2	99		96	94	78	79	94	96	97	95	97	96	95	94	81	91	83	81	73	74	2	IBV/Chicken/Libya/02/12
3	100	99		90	83	84	98	100	97	99	97	100	99	98	85	96	88	85	75	79	3	IBV/Chicken/Libya/03/12
4	99	98	98		74	73	88	90	91	90	91	90	89	88	74	85	77	74	68	67	4	IBV/Chicken/Libya/04/12
5	83	81	82	82		97	84	83	79	82	79	83	84	83	98	82	84	98	70	78	5	IBV/Chicken/Libya/05/12
6	83	81	82	82	100		85	84	81	83	81	84	85	84	99	83	85	99	74	79	6	IBV/Chicken/Libya/06/12
7	99	97	98	98	84	84		98	95	97	95	98	99	98	87	98	89	87	75	79	7	IBV/Chicken/Libya/07/12
8	100	99	100	98	83	83	98		97	99	97	100	99	98	85	96	88	85	75	79	8	IBV/Chicken/Libya/08/12
9	98	98	98	97	80	80	97	98		96	98	97	96	95	82	92	84	82	73	75	9	IBV/Chicken/Libya/09/12
10	100	99	100	99	83	83	99	100	98		96	99	98	97	84	95	87	84	74	78	10	IBV/Chicken/Libya/10/12
11	98	98	98	97	79	79	97	98	99	98		97	96	95	82	92	84	82	73	75	11	IBV/Chicken/Libya/11/12
12	100	99	100	99	83	83	99	100	98	100	98		99	98	85	96	88	85	75	79	12	IBV/Chicken/Libya/12/12
13	100	98	99	99	83	83	99	99	100	100	97	100		99	87	97	88	87	77	79	13	Ck/Eg/BSU-2/2011
14	99	98	99	99	83	83	99	99	99	99	97	99	100		85	97	87	85	75	78	14	Ck/Eg/BSU-3/2011
15	83	81	82	82	100	100	84	82	83	83	79	83	99	83		84	87	100	73	81	15	Eg/CLEVB-2/IBV/012
16	98	97	98	98	82	82	99	98	98	98	96	98	81	99	82		87	84	74	78	16	Eg/1212B
17	85	83	84	84	84	84	86	84	82	85	82	85	85	84	84	84		87	70	78	17	IS/885/00
18	83	81	82	82	100	100	84	82	80	83	79	83	83	83	100	82	84		73	81	18	IS/1494/06
19	70	69	70	96	73	73	71	70	69	70	68	70	73	83	73	69	72	73		68	19	H120
20	74	72	74	71	77	77	72	73	70	74	68	74	76	77	77	73	75	77	63		20	4/91
% Nucleotide identities																						

respiratory and renal pathology [17]. The IS/885/00 strain was isolated in 2000 from broiler chickens in Israel. This strain was reported to cause acute renal disease, severe morbidity and high mortality ranging from 15% to 25% [4].

Those IBVs detected in farm 3 (IBV/Chicken/Libya/05/2012 and IBV/ Chicken/Libya/06/2012) formed another cluster, with 100% relatedness to Eg/CLEVB-2/IBV/012 and IS/1494/06 (Table 2). The IS/1494/06 was first identified in Israel in 2006, was recognised as a nephropathogenic IBV, and later classified as variant 2. It has been reported that birds vaccinated with H120 were poorly protected when challenged with these strains [4,9].

These findings show both, IS/885/00-like and IS/1494/06-like IBVs, are now circulating in Libya. Not much is known about the mode of IBV spread between the countries in the Middle East, however, cross-border movements of poultry and poultry-related products are likely an important factor. These finding shows that high mortality and severe respiratory diseases in Libyan chicken farms is likely contributed by these variant IBVs. The role of other co-infections and other exacerbating factors (e.g. immunosuppression, poor ventilation, stocking density, poor management) need to be examined. In North Africa, both classical and variant IBVs have been reported in Egypt, Tunisia and Morocco [6,17–19]. In this study, even though only small numbers of farms were sampled, our findings have highlighted the circulation of variant IBVs in Libya for the first time. Further studies should include sero-surveillance, isolation and serotyping/genotyping of IBVs in the region.

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References

[1] Cavanagh D. Coronavirus avian infectious bronchitis virus. Vet Res 2007;38:281–97.
[2] Cavanagh D. Coronaviruses in poultry and other birds. Avian Pathol 2005;34:439–48.
[3] Bochkov YA, Batchenko GV, Shcherbakova LO, Borisov AV, Drygin VV. Molecular epizootiology of avian infectious bronchitis in Russia. Avian Pathol 2006;35:379–93.
[4] Meir R, Rosenblut E, Perl S, Kass N, Ayali G, Perk S, et al. Identification of a novel nephropathogenic infectious bronchitis virus in Israel. Avian Dis 2004;48:635–41.
[5] Gelb Jr J, Weisman Y, Ladman BS, Meir R. S1 gene characteristics and efficacy of vaccination against infectious bronchitis virus field isolates from the United States and Israel (1996–2000). Avian Pathol 2005;34:194–203.
[6] Abdel-Moneim AS, El-Kady MF, Ladman BS, Gelb Jr J. S1 gene sequence analysis of a nephropathogenic strain of avian infectious bronchitis virus in Egypt. Virol J 2006;3:78.
[7] Jackwood MW. Review of infectious bronchitis virus around the world. Avian Dis 2012;56:634–41.
[8] Ababneh M, Dalab A, Alsaad S, Al-Zghoul M. Presence of infectious bronchitis virus strain CK/CH/LDL/97I in the Middle East. ISRN Vet Sci 2012, 2012. Article ID 201721, 6 pages. http://dx.doi.org/10.5402/2012/201721.
[9] Kahya S, Coven F, Temelli S, Eyigor A, Carli K. Presence of IS/1494/06 genotype-related infectious bronchitis virus in breeder and broiler flocks in Turkey. Ankara Üniv Vet Fak Derg 2013;60:27–31.
[10] El-Mahdy SS, Ekram S, Ahmed A. Efficacy of some living classical and variant infectious bronchitis vaccines against local variant isolated from Egypt. Nat Sci 2012;10:292–9.
[11] Selim K, Arafa AS, Hussein HA, El-Sanousi AA. Molecular characterization of infectious bronchitis viruses isolated from broiler and layer chicken farms in Egypt during 2012. IJVSM 2013;1:102–8.
[12] Chomczynski P, Sacchi N. The single-step method of RNA isolation by acid guanidinium thiocyanate–phenol–chloroform extraction: twenty-something years on. Nat Protoc 2006;1:581–5.
[13] Worthington KJ, Currie RJW, Jones RC. A reverse transcriptase polymerase chain reaction survey of infectious bronchitis virus genotypes in Western Europe from 2002 to 2006. Avian Pathol 2002;2008(37):247–57.
[14] Jones RC, Worthington KJ, Capua I, Naylor CJ. Efficacy of live infectious bronchitis vaccines against a novel European genotype, Italy 02. Vet Rec 2005;156:646–7.

[15] Thompson JD, Higgins DG, Gibson TJ. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res 1994; 22:4673–80.

[16] Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol 2011;28:2731–9.

[17] Abdel-Moneim, A, Afifi M, El-Kady M. Emergence of a novel genotype of avian infectious bronchitis virus in Egypt. Arch Virol; 20121–20125.

[18] Bourogaa H, Miled K, Gribaa L, El Behi I, Ghram A. Characterization of new variants of avian infectious bronchitis virus in Tunisia. Avian Dis 2009;53:426–33.

[19] El-Houadfi M, Jones RC, CookAG JK, Ambali AG. The isolation and characterisation of six avian infectious bronchitis viruses isolated in Morocco. Avian Pathol 1986;15:93–105.