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Short Communication

Detection of variant infectious bronchitis viruses in broiler flocks in 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

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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