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Isolation and Characterization of Infectious Bronchitis Virus Strain 4/91 from Commercial Layer Chickens in the Sudan

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Abstract: Outbreaks of suspected IB disease occurring in a number of commercial layer flocks at Khartoum and El Gazira states were studied during years (2000- 2001). Affected birds were of different breeds (Lohmann, Bovans Hisex). Their ages were from $5^1/_2$ –7 months. The main clinical signs varied from mild respiratory signs to diarrhea sometimes yellowish in colour. Drop in egg production (up to 60%) was recorded in some flocks. IB virus was isolated from these outbreaks. Using virus neutralization (VN) and haemagglutination inhibition (HI) tests, three of these IB field isolates (M114/2000, K170/2000 and K158/2001) were antigenically similar to IB virus strain 4/91 (Neutralization index 4.4 to 4.8 and VN titer $\geq$11($\log_2$) were recorded). Isolate (K110/2000) on the other hand, was antigenically similar to IB virus Mass. (M41) strain (HI titer $\geq$11 $\log_2$ was recorded). This is the first time to identify IB virus strain antigenically similar to IB virus strain 4/91 in Sudan.

Key words: Avian, infectious, bronchitis, strain, 4/91

INTRODUCTION

Infectious bronchitis (IB) is highly contagious viral disease that causes significant economic losses in chickens. Earlier reports indicated that IB was primarily a disease of young chicks. However, it was later observed to be common in semi-immature and laying flocks[1]. Many times, the IB virus may spread through the flock without producing obvious clinical signs of disease except a mild cough. However, the virus may trigger a serious, long-lasting respiratory disease if complicated by mycoplasma and *E. coli*. Some strains of the virus infect the kidneys and cause permanent renal damage[2]. Egg production and quality problems were first attributed to the IB virus in 1951[3]. Prior to 1956, avian IB was considered to be caused by a single antigenic type of virus. However, it was later recognized that IB virus isolates exhibit extensive variations[4]. In UK, the IB virus strain 4/91 (also known as 793/B or CR88) had been associated with deaths in adult birds and a drop in egg productions up to 50% [5,6]. The objective of this work deals with outbreaks of avian IB that occurred among commercial layer flocks in Sudan during 2000-2001and identifying the common serotype(s).

MATERIALS AND METHODS

Investigation of suspected IB field outbreaks

History: Outbreaks of suspected IB disease occurred in a number of layer flocks at Khartoum and ElGazira States (Central Sudan) during 2000-2001. Affected birds were of different breeds (Lohmann, Bovans and Hisex). Their ages were $5^1/_2$ to 7 months. All flocks were previously vaccinated against Newcastle disease (ND) and infectious bursal disease (IBD). Some flocks were vaccinated against IB (IB vaccine strain H_{120} and H_{52} were used). In some flocks, a drop in egg productions up to 60% was recorded. Diarrhea sometimes yellowish in colour, mild respiratory signs and deformed eggs with wrinkled shells were observed from some flocks.

Laboratory testing: Some of the affected birds were killed, necropsies and examined for gross PM lesions and tissue specimens were collected under aseptic condition for virus and bacterial isolations.

For virus isolation, 10% homogenates (W/V) were prepared in PBS containing antibiotics and fungizone. Then the labeled homogenates were inoculated into allantoic cavity of 10 day embryonated chicken eggs and in tissue culture tubes containing individual tracheal ring and incubated at 37°C. Inoculated eggs were candled daily and inoculated culture tubes were examined daily for evidence of ciliostasis. The clarified allantoic fluid collected from inoculated embryos was passage further into embryos (up to 5 passages) and morphological changes in the embryos were recorded. For virus identification haemagglutination (HA), HI and AGP tests were used. Isolated viruses were serotyping using VN[7] and HI[8] (Reference IB antigens and antiserum to strains 4/91 M41, intervet-Holland were used).

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Tissues for bacterial isolation were plated out onto blood and MaConkey agar and identified according to Cowan and Steel[9].

RESULTS

The gross PM lesions of the affected layer flocks are shown in Table 1. Changes such as roughed shell, internal laying and cystic right oviduct are shown in Fig. 1, 2 and 3. Four virus strains (3 from Khartoum and one from Madani) were isolated and identified as IB virus by means of HA, HI and AGPT. All produced the morphological changes (Stunted, Curled and dwarfing) of the inoculated embryos and ciliostasis of inoculated tracheal rings as expected.

On serotyping, 3 isolates (M114/2000, K170/2000 and K158/2001) were antigenically similar to IB virus strain

Fig. 1: Rough-shelled egg laid by hen during an outbreak of infectious bron chitis

Fig. 2: Internal laying in bronchitis infected hen

4/91 with neutralization index ranging from 4.4-4.8 and VN titer ≥11 (log_2). Isolate (K110/2000) was antigenically similar to Massachusetts serotype with HI titer of ≥11(log_2).

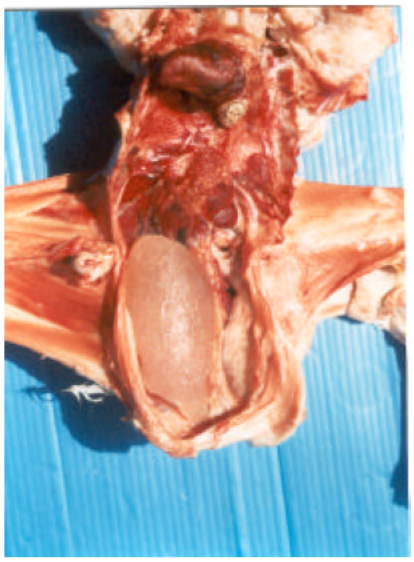

Fig. 3: Cystic right oviduct in bronchitis infected hen

DISCUSSIONS

In the present study, two virus isolates from layer flocks at Khartoum state and one from Madani were confirmed using VN and HI tests as IB and were found to be antigenically similar to IBV strain 4/91. Another isolate from Khartoum was antigenically similar to Massachusetts serotype. Symptoms in the affected flocks varied from mild respiratory signs to gasping. Severe signs were observed among flocks infected with *E. coli* and or mycoplasma. The adverse effect on egg production in the affected farms included increased egg quality problems, deformed and soft egg shells. Rudimentary compact or undeveloped oviducts were recorded (Table 1). On IB infections, varying effect on eggs production, permanent damage to the reproductive tract and kidneys were reported[2]. Also, permanent damage to the oviducts might occur when pullets were infected with the virus earlier in their life-resulting in misshapen eggs being produced through their life. Moreover, early infection could result in the re-excretion of the virus at sexual maturity[2,10]. If hens have low antibody titers, production drop could be severe. On the other hand, in birds that have high antibody levels the only manifestation would be mild to severe effect on both egg shell and internal quality without affecting the overall egg production[2].

The appearance of these outbreaks among the layer flocks at different regions in Sudan might be due to the periodic introduction of pullets and the recycling of the

Table 1:The Gross lesions and laboratory results of some layer chicken flocks affected with IB during year (2000- 2001)

Lab. no.	Location	Breed	Age (months)	IB vaccination	No. of birds at risk	No. dead	Main clinical signs	Main gross lesions	Laboratory results: Virus	Laboratory results: Other microorganisms
114/2000	Madani (Gazira State	Bovans	6	H120, H52	3900	NR	Drop in production (78-50%) mis-shapen eggs and diarrhoea	Flaccid ova, reduced or undeveloped oviduct.	IB Mass	E. coli
110/2000	Soba (Khartoum State)	Hisex	7	No	5000	NR	Gasping, diarrhoea, drop in production, wrinkled shell.	Ruptured yolk and egg-perit onitis.	IB 4/91	*E. coli* and pseudomonas
170/2000	Jabal Awlia (Khartoum State)	Lohmann	$5^1/_2$	H120	2200	NR	Respiratory signs, drop in production.	Compact or undeveloped oviduct, internal laying, cystic right oviduct.	IB 4/91	*E. coli* and Mycoplasma(Ab)
158/2001	Kalakla Khartoum State	Lohmann	6	No	9000 150		Drops in production (81-48%) respiratory signs	Compact or undeveloped oviduct, internal laying, cystic right oviduct.	IB 4/91	*Enterobacter ia sp.*

M.g.=Mycoplasma gallisepticum/ Ab=antibody NR= Not recorded

virus in flocks resulting in greater opportunity for infections and spreading of the disease. Also, the longer life span of the layer flocks is suitable for a new variant serotype to develop especially in multi-aged flocks where there are different levels of immunity.

In Sudan, IB was recorded and the causative virus was isolated since 1981[11]. However, this is the first record of an IB virus antigenically similar to strain 4/91 in Sudan.

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