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Serotypes of Avian Infectious Bronchitis Virus Isolates From Field Cases in Sudan

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Abstract: Infectious bronchitis (IB) virus field isolates were serotype using virus neutralization (VN) and haemagglutination inhibition (HI) tests. The viruses were isolated from an outbreaks of IB in broiler and layer chicken flocks in Sudan during 2000- 2001. Four isolates (3 from layer and 1 from breeder broiler flocks) were found antigenically similar to IB virus strain 4/91. With neutralization index (NI) 4.4 - 5.0 and VN titer = 11 (log_2). On the other hand, four isolates from commercial broiler flocks and one isolate from layer were found antigenically similar to IB Massachusetts Mass. serotypes with HI titer = 11 (log_2). This is the first report of various IB serotypes that are prevalent in Sudan.

Key words: Avian, infectious, bronchitis, serotypes, Sudan

INTRODUCTION

The IB genome consisted of a single stranded RNA. Like many other RNA viruses, IB virus has a high error rate during the replication of its genomes[1]. This is manly due to the fact that RNA polymerase lacks 3'-5' exonuclease activity (editing function) of DNA polymerase[2]. A new IB virus serotypes and/or genotypes can emerge as a result of only a very few changes or mutations of amino acid sequence of the spike gene or as a result of recombination during mixed infections and this could be promoted by the use of more than one vaccine strain or by mixture of vaccine and wild viruses[3]. Hence, there is usually a multiplicity of serotypes infecting chickens in the field. Different methods were used for serotyping IB were used[4-8].

In Sudan, IB was recorded and the virus was isolated for the first time in 1981[9]. Since then many outbreaks of IB were report and diagnosed[10]. The aim of this study is to identify the serotype(s) of the IB virus field isolates that are prevalent in Sudan.

MATERIALS AND METHODS

Virus: Nine local IB viruses (designated K6/2000, K98/2000, K109/2000, K110/2000, M114/2000, K170/2000, K35/2001, , K112/2001 and K158/2001) previously isolated from an outbreaks that occurred in broiler and layer chicken flocks in Khartoum and Gazira states (during 2000- 2001) were used in this study. Case history, clinical signs and virus isolation were describe[10]. Passage 6th of each IB field isolate and a known IB virus strain 4/91(Intervet) were used.

Antigens and antiserum: Reference IB virus antigen and antiserum to strain M41 (HA and HI) and strain 4/91 (VN Antiserum) Intervet- Holland, kindly provided by Dr. Mohamed Omer (Detasi Company Khartoum) were used.

Preparation of neutralizing antiserum: Antisera were prepared according to Dawson and Gough[11] by ocular-nasal inoculation of 5 weeks Bovans chickens (free from detectable IB antibodies) with 0.2 mL of 10^{-5} EID_{50}/bird (of each field isolates). Two weeks later, birds were re-inoculated with the same isolates and dosage via I/V route. Two weeks later, chickens bled and serum was clarified by centrifugation then inactivated at 56°C for 30 min and stored at -20 until used.

Preparation of HI antiserum: Antiserum against each field isolate was prepared by a single intra-tracheal inoculation of (5 weeks - Bovans) chicks of 0.2 mL of 10^{-3} EID_{50}/bird[7]. Sera were collected 4 weeks post inoculation, clarified , inactivated at 56°C for 30 min and stored at -20 until used.

Serotyping: The IB viruses field isolates were serotype using a virus neutralization and haemaggltination inhibition tests.

Virus neutralization (VN) test: The alpha method of VN was carried out in 10 day old embryonating chicken eggs as described by Dawson and Gough[11]. In brief, equal volume from each 10^{-1} - 10^{-10} dilution of an IB field isolate and an equal volume of undiluted IB virus strain 4/91 antiserum were mixed anused for inoculation of chicken

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eggs (5 embryos for each dilution). A virus control dilutions (virus+ diluents) were similarly inoculated. Deaths were recorded daily and EID_{50} was calculated according to Reed and Muench[12]. The difference between log10 virus control titer log_{10} virus serum titer was expressed as EID_{50} neutralization index(EID_{50} NI).

In the beta method, equal volume of two fold serial dilution of each field IB antiserum, reference strain 4/91 antiserum and reference IB virus strain 4/91 were used. 0.1 mL of each serum virus mixture and virus control were inoculated into 10 day old embryonated chicken eggs. The end point of the serum titers are expressed as reciprocal of the highest dilutions that caused virus neutralization[11]. Isolates were considered to be antigenically similar to a reference strain if the serum titer of the isolates and reference strain were similar.

Haemagglutination inhibition (HI) test: The test was carried out according to Alexander *et al.*[13] brief, 0.025 mL of prepared IB field antiserum, reference IB antiserum, 0.025 mL of 4HA units of virus antigen and 0.025 mL of 1% chicken RBCs were used. Titers were expressed as the reciprocal of the highest dilution of serum causing inhibition to 4HA units of the virus.

RESULTS AND DISCUSSIONS

Isolates M114/2000, K112/2000, K170/2000 and K158/2001 were antigenically similar to IB virus strain 4/91. Neutralization index (NI) ranging from 4.4 to 5.0 and VN titer =11 $l0g_2$ were recorded. On the other hand, field isolates K6/2000, K98/2000, K109/2000, K110/2000 and K35/2001 were found to be antigenically similar to IB Massachusetts serotypes with HI titer =11 log_2 (Table 1).

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 exhibits extensive variations[14]. In the present study, serotyping of IB virus field isolates was accomplished using HI and VN tests in embryonated chicken eggs. Based on VN tests, three of our isolates from layer flocks and one isolate from breeder broiler flock were found to be antigenically similar to strain 4/91. The presence of this strain might explain the existence an occurrence of the disease among layer flocks in spite of the fact that live IB vaccines (H_{120} and H_{52} Mass. strain) were used in some flocks. In such condition, the vaccines strain used might be ineffective for controlling the disease due to the antigenic variations between the vaccines and the field strains.

The HI test has extensively been employed for serotyping IB viruses and had proved useful providing early response sera were used[7,15]. In contrast to VN, HI is a rapid test and any laboratory with a bank of reference IB antisera could make classification of IB viruses based on one-way comparison within 24 hours. In the present study, field isolates of Mass serotypes were identified by HI test. Four(66.7%) out of six isolates made in year 2000 were found to be of Mass while only one (33%) out of three isolates made in year 2001 was of Mass serotype. The prevalence of Mass. strains may indicate improper use of vaccines against these serotypes thus, situation need more investigations.

Table 1: Virus neutralization and haemaggltination inhibition results for serotyping IB viruses field isolates

IB field isolates (virus or anti serum)	*NI (alpha method) antiserum 4/91	VN titer log_2 beta method) virus 4/91	HI titer log_2 antigen IB (M_{41}-HA)	Field Serotype (similar to)
K 6/ 2000	1.4	4	11	[1]Mass
K 98/ 2000	1.2	4	11	Mass
K 109/ 2000	0.8	4	10	Mass
K 110/ 2000	2.2	6	11	Mass
K 35/ 2001	1.2	5	11	Mass
M 114/ 2000	4.8	≥ 11	4	[2]4/91
K 170/ 2000	4.4	≥ 11	5	4/91
K 112/ 2001	5.0	≥ 11	5	4/91
K 158/ 2001	4.4	≥ 11	4	4/91

*: The values represent a difference between log10 virus control and log10 virus-serum mixture, [1]: IB Massachusetts serotype, [2]: IB 4/91 serotype

CONCLUSIONS

The circulation of more than one serotype and the known high genetic variability and mutations of IB viruses the mutation rates during the viral replication have been estimated in the range of 10^{-3} - 10^{-5} substitutions per nucleotide[6] may lead to emer- gence of new genotypes that are capable of escaping vaccination cover hence, situation need to be constantly monitored.

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