Quantification Of Antibodies In Sera Of Vaccinated Chickens Against Infectious Bronchitis Virus By Haemagglutination Inhibition Test

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ABSTRACT

A trypsin-induced haemagglutination inhibition (THI) assay was standardized to achieve a quantification of haemagglutinating-inhibiting antibodies against infectious bronchitis virus (IBV) in the sera of chickens vaccinated with different types of commercial imported IBV vaccines. A total of forty four IBV vaccines representing as twenty two live vaccines containing either classical or variant strains and twenty two inactivated vaccines were examined among (THI) assay using trypsin treated classical IB antigen. In a trial for studying the correlation between the results of THI and the other serological tests (ELISA and serum neutralization test "SNT"). The former tests were performed on the same serum samples used in (THI) test. The results showed that the calculated mean HI titers for different examined serum samples ranged between 7.7 log_2 to 7.9 log_2, against live IB variants vaccines (CR88 and 4/91 respectively), 8.7 log_2 to 9.2 log_2 against classical type (Ma5, H120) vaccines, 9.1 log2 (H120+D274) and 6.8 log_2 against inactivated vaccines. It was observed that quantification of antibodies in sera of chickens against infectious bronchitis virus by HI test had correlation with those obtained by either ELISA or SNT tests.

INTRODUCTION

Infectious bronchitis is a highly contagious disease and constitutes one of the most common and difficult poultry diseases to be controlled. IBV is endemic in probably all countries that raise chickens. It exists as dozens of serotypes/genotypes. Only a few amino acid differences in the S1 protein of vaccine and challenge strains of IBV may result in poor protection. Tropism of IBV includes the respiratory tract tissues, proventriculus and caecal tonsils of the alimentary tract, the oviduct and the kidney *(1)*.

In general, IBV infections can be diagnosed by detection of parts of the virus itself or the specific antibody response. There is a number of assays for diagnosing acute IBV infections. The most common for routine use are virus isolation (VI), immunofluorescence assay (IFA), immunoperoxidase assay (IPA) and reverse transcriptase polymerase chain reaction (RT-PCR). IBV infections are detected serologically by demonstrating a seroconversion, using paired serum sets or the demonstration of IBV-specific immunoglobulin M (IgM). Choosing between tests and subsequent interpretation of the results are considered very difficult and confusing. Depending on the demands, a best choice or best combination of techniques for that situation can be made. Haemagglutination inhibition (HI) test, the agar gel precipitation test (AGPT), the enzyme-linked immunosorbent assay (ELISA) and the virus neutralization test (VNT) are often used for routine diagnosis *(2)*.

In haemagglutination inhibition test, haemagglutination inhibiting antibodies are induced primarily by the S1 spike protein *(3, 4)*. Usually, HI tests first detect antibodies between 1 and 2 weeks post infection *(5, 6)*.

More than for the VNT, the serotype-specificity of the HI test is a complex subject. In general, the correlation between HI and VNT when testing high-titred sera is better than for low-titred sera *(7, 8)*. The HI-test is serotype specific when used to detect antibodies after a single inoculation. Although the correlation between these test systems is high under these circumstances, the specificity of the HI is considered lower than that of the VNT *(9, 6)*.

Many studies have compared the performance of two or more serological tests. Due to the huge variations in experimental conditions, test performance and type of antibodies that are detected by the different tests, many different results are reported. However, generally, antibodies can first be detected by ELISA, followed by AGPT and HI test, and last by VNT *(10, 6, 11)*.

Because IBV exists as multiple different types that do not cross protect, it is very difficult to control. Attenuated live vaccines are used in broilers and pullets and killed vaccines are typically used in layers and breeders. Effective control involves identification of the virus type causing disease followed by vaccination with an appropriate vaccine against that type. However, there are only a countless different types and variants of the virus capable of causing disease can be found throughout the world. In addition, some countries only allow vaccination with one or a few vaccine types *(12)*.

Until now, the usual method of examining sera of commercial chickens for avian infectious bronchitis virus (IBV) antibodies has been to utilize the IB serum neutralization (SN) test *(13)*. Haemagglutination (HA) by an IBV when treated with phospholipase C (type 1) was described before *(14)* and reported that this haemagglutination could be inhibited by specific antisera. Nine strains of IBV were examined and determined four strains with HA activity after treatment with phospholipase C (type 1) *(15)*. The Massachusetts 41 (M41) strain possessed the best properties for use in the haemagglutination inhibition (HI) test and the results compared well with those from SN tests.

The current study aims to perform a haemagglutination inhibition test using trypsin treated IB antigen, and studying the correlation between the THI and other serological tests as SNT and ELISA for evaluation of IBV antibodies in the sera of vaccinated chickens.

MATERIAL AND METHODS

Vaccines

All IB commercial live attenuated and inactivated vaccines used in this study were obtained from different companies.

Live vaccines

Twenty two IB commercial live attenuated vaccines were used:

* Nine batches (variant) Nobilis IB 4/91®:
-A118A1J02, A118A1N02, A118A1J01, A125A1J01, A125B1L01, A125A1J02, A125B1L02, A125A1J03, A125A1J04.
*Three batches (variant) Gallivac IB88® (CR8814):
- L404335, L402486, L400921.
*Three batches (classical Ma5) Nobilis IB H120 Sphereon:
- A157A1J03, A157B1J01, A167B1L01.
*Three batches (classical H120) Gallivac IB H120:
- L402487, L4066777, 37X44.
*Four batches (classical + variant) Poulvac IB Primer® (H120/D274):
-1302485D5, 1303594D3, 1304442D5, 1305387D5.

Inactivated vaccines

Twenty two IB commercial inactivated vaccines were used:

* Seventeen batches from different combined (ND+IB): 1209B3SKE, 0608BGN1C, 0073I, 22056, 13639CJ01, 13639DJ01, 13639LJ01, 13639MJ01, BNK0114, 403040, 22056, 1306443, 0711BGN1A, 1712B3S1B, 0101C351A, 61YJ-1, 13639KJ01.

* One batch (ND+IB+Reo): D110A17.

*Two batches (ND+IB+IBD): 210818, C563A01.
*Two batches (ND+IB+EDS): 0949G, 1309AGN1A.

Antigen

The IBV antigen was obtained from MSD company. Four HI units of this antigen was used in HI test, allantoic fluid harvested from inoculated eggs, then trypsinied and used in trypsin induced haemagglutination.

Chickens and chicken embryos

1370, one day old specific pathogen free (SPF) chicks were purchased from SPF farm, Koum Oshiem, then classified into 45 groups (30 chicks per each), for vaccination with different live and inactivated IB vaccines and 50 chicks were kept as non vaccinated control. SPF embryonated chicken eggs (ECE) from the same source were used in propagation of the obtained IB antigen for HI test.

Experiment Design

1370, one day old, SPF chicks were used in this study and divided into forty four groups (30 chicks per each) as follows:

Groups (1-22)

They were vaccinated on the one day of age via the nasal route with the recommended dose of different tested live attenuated commercial vaccines (Table 1).

Groups (23-44)

They were vaccinated intramuscularly at three weeks of age with the recommended dose of different tested inactivated commercial vaccines (Table 1).

Group (45)

Fifty chicks were kept as non-vaccinated control.

Blood samples

The blood samples were collected from all chicks after 3 weeks post vaccination and further processed to obtain sera for HI test, ELISA and SNT.

Serological tests

Indirect Haemagglutination Inhibition Test (IHI)

The test was performed for estimation of hamagglutination inhibition antibody titre (Log_2) ***(16, 17)***.

Serum neutralization test (SNT)

SN test was carried out in the conventional manner in 9- day-old SPF embryonated chicken eggs (EGE), using IBV Mass type virus and the varying serum, constant virus method on a pooled sample of the test sera. The results were expressed as log_{10} ***(13)***.

Enzyme Linked Immunosorbent Assay (ELISA)

Synbiotic Proflok corporation IBV kit (Serial No. 1300512, Prod. Code 96/6506) was used according to the kit manufacture.

RESULTS AND DISCUSSION

The advantage of serological based diagnostics (HI, SNT and ELISA) is the historical aspect that they bring to the clinical case, the results measure the reaction of the bird to the vaccine causing agent over a period of time ***(2)***. Humoral antibody responses has been measured by SNT and HI ***(7)***.

Vaccination studies have been almost focused on humoral immune responses in relation with protection, that the S1 (spike) glycoprotein of IBV induces SNT and HI antibodies ***(3)***.

In the present work, attempts were done to detect infectious bronchitis antibody levels in forty-four chicken groups vaccinated with different types of IB vaccine, twenty-two groups vaccinated with live attenuated IB vaccines and another twenty-two groups vaccinated with inactivated IB vaccines. Three parameters were used to evaluate the efficacy of different types of IB vaccines including THL, SNT and ELISA.

The results in table (1 and 4) showed GMT (log_2) HI titers for different live attenuated IB variant vaccines under test as 7.1 to 8.6 with mean 7.7 log_2, 7.3 to 8.3 with mean 7.9 log_2 and 8.6 to 9.6 with mean 9.1 log_2, for IB (4/91), IB (CR88) and IB (primer), respectively, while in case of live classical vaccines (Ma5, H120) were 8.6 to 9.5 with mean ranging between 8.7 to 9.2 log_2. The mean log_2 HI titer for inactivated classical IB vaccine under test were 6.0 to 8.0 log_2 with mean 6.8 log_2 and 6.0 to 7.5 with mean 6.8 log_2 for combined and trivalent IB inactivated vaccines, respectively (Table 2 and 4).

The present results for GMT log_2 HI titers for live variant and classical IB vaccines under test were in agreement with previous authors ***(18, 19)*** who reported the mean log_2 HI titres of IB variant vaccines (9.5 to 10.0 log_2).

The obtained findings in this step of experiment disagree with those obtained before ***(20)*** who reported a medium to low humoral response in keeping with that expected following the administration of live attenuated classical and variant IB vaccines.

As expected, the highest titres were obtained using antigens homologous to vaccinal strains. While in this study, classical used antigen which gave high titres for classical or variant IB vaccines in living and inactivated vaccines under test. These results were in agreement with another researcher ***(2)*** who reported the major circulating immunoglobulin detected by HI.

The second parameter used in this study was ELISA. ELISA and HI tests are both rapid to perform and can detect antibody against IBV strain were reported ***(10)***. ELISA for different live attenuated IB variant and classical vaccines under test showed 2061 to 4658 with mean 3125.6; (2915 to 3584 with mean 3179 and 4432 to 5538 with mean 4739.75 for IB 4/91; CR88 and IB primer, respectively, while it was 3384 to 4936 with mean 4154.6; 4351 to 5718 with mean 5048.6 for classical MA5 and H120, respectively. On the other hand, ELISA titres for inactivated IB vaccine in combined formula were 2025 to -5986 with mean 3923.82 and 1600 to 5778 with mean 4055.4 for trivalent inactivated IB vaccine (Tables 1, 2 and 4).

ELISA technique is a sensitive serological method, which detect earlier reactions and higher antibody titre than other tests ***(10)***. Our findings of serum antibody titres which detected by ELISA agree with those obtained before by other authors ***(6, 19)***.

IBV antibody titres in sera of chickens vaccinated with different live and inactivated IB vaccines measured by SNT as shown in Table 3 and 4 ranging between (2.6 to 2.8); (3.6 to 4.0); (3.0); (3.2 to 3.6) and (3.4 to 3.8) for live IB variant, classical, primer, inactivated combined and trivalent vaccines, respectively.

So, the neutralization index of antibody titres induced by living attenuated IB vaccine either for classical or variant was 2.6 to 4.0 and for inactivated combined or trivalent vaccines was 3.2 to 3.8.

The obtained results agree with those obtained before ***(4)*** who reported the evidence exists that S1 glycopeptide is primarily responsible for the induction of VN and HI antibodies and that it plays a major role in the induction of protective immunity, otherwise VN and HI tests for estimation of IBV antibodies were generally considered to be type specific but at the same time VN is rarely used due to the cost and complexity of the test ***(6)***.

For studying the relationship between serological antibodies which detected by the three serological tests (table 4), it can be concluded that HI and ELISA tests were specific, very sensitive accurate, highly reproducible, simple and economical ***(21)***.

Based on the data represented in this study, it could be concluded that quantification of antibodies in sera of chickens against infectious bronchitis virus by HI test had correlation with those obtained either by ELISA or SNT tests. So, HI test can be used for monitoring the immune responses to IB vaccines instead of other tests.

Table 1. Monitoring antibody titres against IBV in sera of chickens vaccinated with different live IB vaccines using HI test and ELISA

Group	Type of vaccinal strain	HI Titre (Log_2)	GMT	ELISA antibody titre	Mean
1	4/91	7.5	7.7	3304	3125.6
2		8.5		3656	
3		7.3		3464	
4		8.0		4658	
5		7.6		2188	
6		8.6		3353	
7		8.0		2061	
8		7.1		3048	
9		7.0		2399	
10	CR88	8.1	7.9	3584	3179
11		8.3		3038	
12		7.3		2915	
13	MA5	9.1	8.7	4144	4154.6
14		8.6		3384	
15		8.5		4936	
16	H120	8.6	9.2	4351	5048.6
17		9.6		5718	
18		9.5		5077	
19	H120+D274	9.6	9.1	5538	4739.75
20		9.5		4432	
21		8.6		4540	
22		9.0		4449	
45	Non-vaccinated control	0.0		120	

GMT: Geometric Mean Titre

Table 2. Monitoring antibody titres against IBV in sera of chickens vaccinated with different inactivated IB vaccines using HI test and ELISA

Group	Type of vaccinal strain	HI Titre (Log_2)	GMT	ELISA antibody titre	Mean Titre
23		7.5		5098	
24		7.0		3484	
25		7.0		4044	
26		7.5		5008	
27		6.6		3530	
28		6.5		4559	
29		7.5		5589	
30		8.0		5986	
31	ND + Mass	6.3	6.8	3762	3923.82
32		6.0		3713	
33		6.3		3826	
34		6.0		3114	
35		7.0		3984	
36		6.8		3056	
37		6.1		2396	
38		6.3		2025	
39		6.6		3531	
40	ND+Mass+Reo	7.5		4532	
41	ND+Mass+IBD	6.7		1600	
42	ND+Mass+IBD	6.0	6.8	3350	4055.4
43	ND+Mass+EDS	7.0		5017	
44	ND+Mass+EDS	7.1		5778	
45	Non-vaccinated control	0.0		120	

GMT: Geometric Mean Titre

Table 3. Monitoring antibody titres against IBV in sera of chickens vaccinated with different live and inactivated IB vaccines using SNT

Type of vaccine	Type of vaccinal strain	Neutralizing Index (NI)
Live IB vaccines	4/91	2.6
	CR88	2.8
	H120+D274	3.0
	MA5	3.6
	H120	4.0
Inactivated IB vaccines	ND+Mass	3.2
	ND+Mass	3.4
	ND+Mass	3.6
	ND+Mass+EDS	3.8
	ND+Mass+Reo	3.4
Non-vaccinated control		0.0

IBV antigen type H120 titre was 7.9 log_{10} EID_{50}/ml

Table 4. Mean IBV antibody titres in sera of chicken vaccinated with different IBV vaccines (classical and variant) as tested by serological tests using classical antigen

Type of vaccine	Test used		
	GMT HI (log_2) Classical Antigen	NI (Classical Antigen)	ELISA Titre
Live IB variants (CR88, 4/91)	7.7 – 7.9	2.6 – 2.8	3125.6 – 3179
Live IB variant (H120+D274)	9.1	3.0	4739.75
Live classical	8.7 – 9.2	3.6 – 4.0	4154.6 – 5048.6
Inactivated combined (ND + Classical)	6.8	3.2 – 3.6	3923.82
Inactivated Trivalent	6.8	3.4 – 3.8	4055.4
Non-vaccinated control	0.0	0.0	120

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الملخص العربى

تقدير كمية الأجسام المناعية فى مصل الدجاج المحصن ضد فيروس الالتهاب الشعبى بواسطة اختبار مانع التلازن الدموى

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** المعمل المركزى للرقابة على المستحضرات الحيوية البيطرية – العباسية – القاهرة

يعتبر اختبار مانع التلازن الدموى باستخدام التريبسين من الاختبارات المستخدمة لتقدير مستوى الأجسام المناعية ضد فيروس الالتهاب الشعبى المعدى فى الدجاج المحصن بأنواع مختلفة من اللقاحات التجارية المستوردة وقد تم فى هذا الدراسة اختبار عدد (٤٥) لقاح ضد فيروس الالتهاب الشعبى المعدى بواقع (٢٢) لقاح حى (ما بين عترات تقليدية وعترات محورة) و(٢٢) لقاح مثبط باستخدام اختبار مانع التلازن الدموى باستخدام التريبسين. وبدراسة العلاقة بين نتائج الاختبار السابق واختبار الاليزا واختبار المصل المتعادل على نفس عينات الامصال، وجد أن معيار الأجسام المناعية المقاسة باستخدام اختبار مانع التلازن الدموى تتراوح بين $\log_2$ 7.9 ,$\log_2$ 7.7 فى حالة اللقاحات الحية لفيروس الالتهاب الشعبى المعدى (CR88, 4/91)، و $\log_2$ 9.2 ,$\log_2$ 8.7 فى حالة اللقاحات التقليدية، بينما كانت 6.8 $\log_2$ فى حالة اللقاحات المثبطة. وبمقارنة نتائج الاختبارات السابقة وجد تقارب فى نتائج اختبار مانع التلازن الدموى باستخدام التريبسين ونتائج كل من اختبارى الاليزا واختبار المصل المتعادل.