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Use of monospecific antisera for differential diagnosis of classic and variant strains of infectious bronchitis virus

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Abstract

This study was carried out to investigate the efficacy of mono-specific antisera prepared in rabbits for the detection and differential diagnosis of classic and variant strains of infectious bronchitis virus (IBV) in comparison with those prepared in specific pathogen free (SPF) chickens in addition to their ability in detection of the degree of cross neutralization and antigenic relationship between these strains. Two serological tests were used; indirect haemagglutination inhibition test (IHI) and serum neutralization test (SNT). Also, two classic (H120, MA5) and two variant (4/91, CR88) IB antigens were used. IHI test gave higher titers not only in case of homologous strains than heterologous but also higher in rabbits than chickens (H120 strain Vs H120 antisera 12 $\log_2$ in rabbits and 9.6 $\log_2$ in chickens. The results of IHI were confirmed by using SNT that classic (H120) and variant (4/91) strains gave high neutralizing index (NI) when using antisera for rabbit (0.5) while in chickens H120 and CR88 gave (3.8). So, rabbit is recommended animal for preparation of commercial monospecific hyperimmune sera which can be successfully used in detection and differentiation of classic and variant strains of IBV and evaluation of antigenic relationship between these strains.

Keywords: Monospecific antisera, classic and variant strains, Infectious Bronchitis virus (IBV).

Introduction

Infectious bronchitis (IB) is a highly contagious and acute disease of chickens, caused by a Corona Virus (**1, 2**). Infectious bronchitis virus (IBV) is the causative agent and it is responsible for causing respiratory and urogenital symptoms. Such disease is characterized by high mortality rates in the affected flocks and severe economic losses due to reduction in weight gain in broilers and drop in egg quality and production in laying poultry birds **(3, 4**). The virus has the ability to mutate or change its genetic makeup very quick, as result numerous serotypes of IBV emerged which complicates the control efforts through vaccination **(5).** IBV genome consists of single – stranded RNA with a high mutation frequency. Molecular studies in IBV have shown that new IBV serotypes and genotypes can emerge as a result of only a very few changes or mutations in the amino acid sequence of the spike gene (**6**). For devising a strong IB control program, in the presence of several classical and variant strains of IBV and the possibility of the emergence of novel ones have to be monitored, the upgrading of conventional and biotechnological techniques is required **(5**).

Serotyping and genotyping are the common methods for classification of IBV strains, including serotyping by virus neutralization (VN), haemagglutination inhibition (HI) and genotyping in which S1- specific RT-PCR followed by sequencing or restriction endonuclease analysis (**7, 8).**

A vaccination program against IB fails when new strains of IBV emerge in geographical region. Therefore, routinely monitoring of the existing IBV

strains in this region has been suggested to choose a suitable virus strain for vaccination (**9**).

All detection techniques of IBV–specific antigen use IBV–specific antibodies, which are either in the form of antisera from a bird was infected or injected with the IBV virus (**10).**

The IB diagnostic serum is not being produced commercially in Egypt and the imported one is very expensive. So, this work aims to standardize a possible method to prepare hyperimmune sera against IBV strains for identification and differentiation of classic and variant strains. Such sera will not serve only as a diagnostic tool but also will save the precious exchange in addition to genotyping of the virus strains.

Materials and Methods

Ethical approval:

Institutional Animal Ethics Committee has permission for conducting this trial.

Biorisk approval:

Biorisk committee in lab has permission for conducting this trial. Personnel who supervised the care of the experimental animals were suitably qualified and trained.

Antigen:

The IBV antigens (classic H120 strain, MA5, variant 4/91 and CR88) were obtained from Merck Sharp and Dom (MSD) Company. Four HA units of this antigen were used in HI test where allantoic fluids were harvested from inoculated eggs, then trypsinized and used in trypsin induced haemagglutination.

Experimental hosts:

Experimental chicks:

250, one day old specific pathogen free (SPF) chicks were obtained from the SPF Production Farm, Koum Osheim, El-Fayoum, Egypt. This farm is apart of the Ministry of Agriculture. All birds were housed in separate negative pressure filtered air isolators and provided with autoclaved water and feed. These chickens were used in preparation of monospecific antisera against IB virus.

Specific pathogen free (SPF) embryonated chicken eggs (ECE):

These eggs were obtained from SPF Production Farm, Koum Osheim, El-Fayoum, Egypt. Eggs were kept in calibrated egg incubator at 37°C with humidity 40-60%. It was used in antigen preparation, virus titration and serum neutralization test.

Rabbits:

A total of 25 healthy adult male New Zealand rabbits with 2.5 kg average weight were used for raising hyperimmune sera. All rabbits were fed on balanced ration and kept under proper hygienic conditions and their serum was checked to be free from antibodies against IBV. These rabbits were used in preparation of monospecific antisera against IB virus.

Vaccines:

Four live attenuated IB vaccines were used in this study and obtained from Central Laboratory for Evaluation of Veterinary Biologics, Abbasia, Cairo (CLEVB); including two classical vaccinal strains (Massachusetts MA5 and H120) and two variant strains (CR88 and 4/91). All vaccines were titrated in Viral Poultry Vaccine Department, CLEVB with titre of $10^{5.5}$ EID_{50}/Dose.

Preparation of hyperimmune serum in rabbits and chickens:

It was done according to **(11**). Both rabbits were divided into five groups (5-rabbits/group) as follows:

Group (1): was inoculated with live attenuated IB vaccine (Classical H120 strain).
Group (2): was inoculated with live attenuated IB vaccine (Classical MA5 strain).
Group (3): was inoculated with live attenuated IB vaccine (Variant 4/91 strain).
Group (4): was inoculated with live attenuated IB vaccine (Variant CR88 strain).
Group (5): was left as control negative

While in case of chickens, 250 chickens were used and divided into 5 groups (50-chickens/group) and then immunized with the same vaccines as in rabbit groups.

Above mentioned vaccines used for raising hyperimmune sera against classic and variant strains of IBV in rabbits and chickens were injected subcutaneously in a dose of 0.2 ml/animal at zero day,

15, 30, 45 and 60 days post inoculation. Each vaccine strain was reconstituted in sterile 2 ml sterile PBS and mixed with equal volume of complete Freund's adjuvant (Sigma Co. Ltd.) (Once in first injection), then incomplete Freund’s adjuvant (Sigma Co. Ltd.) (At 15, 30, 45 and 60 days post inoculation). Two weeks post last inoculation, all animals were bled and blood was collected for serum separation (hyperimmune sera).

Serological tests:

Indirect Haemagglutination Inhibition Test (IHI):

The test was performed for estimation of haemagglutination inhibition antibody titre (Log_2) (**12, 13**).

Serum neutralization test (SNT):

The test was carried out according to **(14)** using IB virus from different strains (H120, MA5, 4/91, CR88) and varying antisera (prepared in chickens and rabbits). The results were expressed as neutralizing index.

Results

Table (1): IB-HI antibody titres against IBV in sera of chickens inoculated with different live IB vaccines using homologous and heterologous IB antigens

Used antigen	Antisera of chickens inoculated with:	HI titers (log_2)
Classical H120	H120	9.6
	MA5	9.4
	4/91	7.0
	CR88	6.6
Classical MA5	H120	9.1
	MA5	9.3
	4/91	7.1
	CR88	6.8
Variant 4/91	H120	8.0
	MA5	7.5
	4/91	9.2
	CR88	9.0
Variant CR88	H120	7.3
	MA5	8.1
	4/91	9.0
	CR88	9.3

Table (2): IB-NI against IBV in sera of chickens inoculated with different live IB vaccines using homologous and heterologous IB antigens

Used antigen	Antisera of chickens inoculated with:	Antigen Titre (log_{10}/ml)	Antigen-Antiserum Titre (log_{10}/ml)	Neutralization Index (NI)
Classical H120	H120	8.5	4.7	3.8
	MA5		5.0	3.5
	4/91		5.9	2.6
	CR88		6.3	2.2
Classical MA5	H120	8.5	5.2	3.3
	MA5		5.1	3.4
	4/91		6.1	2.4
	CR88		6.1	2.4
Variant 4/91	H120	8.1	5.3	2.8
	MA5		5.3	2.8
	4/91		4.5	3.6
	CR88		5.1	3.0
Variant CR88	H120	8.0	5.4	2.6
	MA5		4.6	3.4
	4/91		4.5	3.5
	CR88		4.2	3.8

Table (3): IB-HI antibody titres against IBV in sera of rabbits inoculated with different live IB vaccines using homologous and heterologous IB antigens

Used antigen	Antisera of rabbits inoculated with:	HI titers (Log_2)
H120	H120	**12.0**
	MA5	**11.5**
	4/91	**10.6**
	CR88	**9.5**
MA5	H120	**10.5**
	MA5	**11.0**
	4/91	**9.1**
	CR88	**8.3**
4/91	H120	**10.3**
	MA5	**8.7**
	4/91	**11.3**
	CR88	**11.0**
CR88	H120	**10.1**
	MA5	**8.3**
	4/91	**10.5**
	CR88	**10.7**

Table (4): IB-NI against IBV in sera of rabbits inoculated with different live IB vaccines using homologous and heterologous IB antigens

Used antigen	Antisera of rabbits inoculated with:	Antigen Titre (log_{10}/ml)	Antigen-Atiserum Titre (log_{10}/ml)	Neutralization index (NI)
Classical H120	H 120	8.5	3.5	**5.0**
	MA5		3.8	**4.7**
	4/91		4.5	**4.0**
	CR 88		5.7	**2.8**
Classical MA5	H120	8.5	4.0	**4.5**
	MA5		3.7	**4.8**
	4/91		4.7	**3.8**
	CR88		6.1	**2.4**
Variant strain 4/91	H120	8.1	3.7	**4.4**
	MA5		4.7	**3.4**
	4/91		3.1	**5.0**
	CR 88		3.3	**4.8**
Variant strain CR88	H120	8.0	4.4	**3.6**
	MA5		3.8	**4.2**
	4/91		3.5	**4.5**
	CR 88		**3.4**	**4.6**

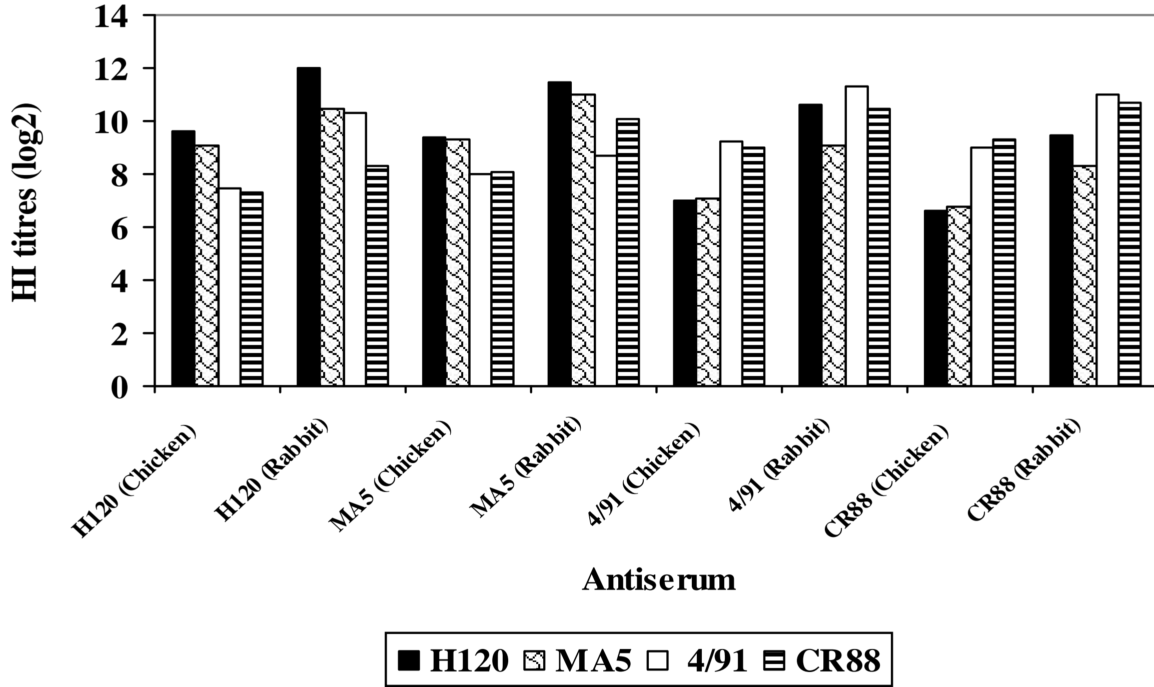
Fig. (1): IB-HI antibody titres against IBV in sera of chickens and rabbits inoculated with different live IB vaccines using homologous and heterologous IB antigens
HI titres (log2)
14
12
10
8
6
4
2
0
H120 (Chicken)
H120 (Rabbit)
MA5 (Chicken)
MA5 (Rabbit)
4/91 (Chicken)
4/91 (Rabbit)
CR88 (Chicken)
CR88 (Rabbit)
Antiserum
H120
MA5
4/91
CR88

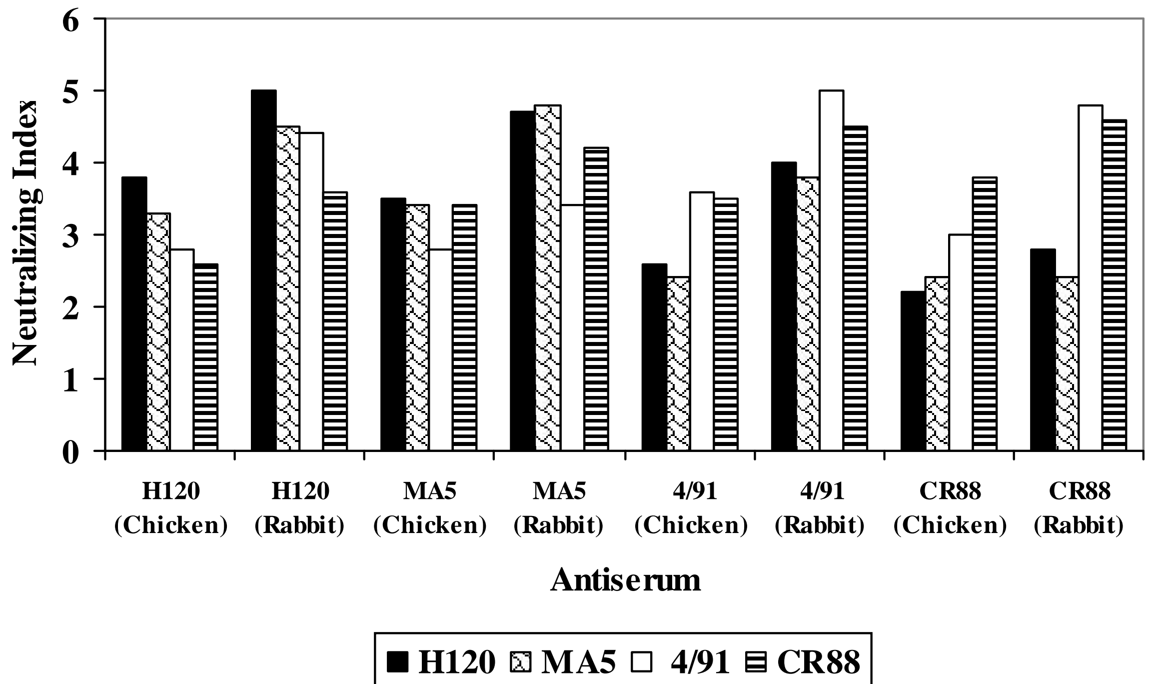
Fig. (2): IB-NI against IBV in sera of chickens and rabbits inoculated with different live IB vaccines using homologous and heterologous IB antigens
Neutralizing Index
6
5
4
3
2
1
0
H120 (Chicken)
H120 (Rabbit)
MA5 (Chicken)
MA5 (Rabbit)
4/91 (Chicken)
4/91 (Rabbit)
CR88 (Chicken)
CR88 (Rabbit)
Antiserum
H120
MA5
4/91
CR88

Discussion

Infectious bronchitis virus (IBV) has an enormous capacity to change by both spontaneous mutation and by genetic recombination which lead to emergence of new variants when they occur in the hypervariable region of the spike gene (**15**).The majority of the currently used diagnostic methods are not able to differentiate the different type of variants IBV, which may lead to diagnostic problems especially when multiple serotypes are co- circulating in a region (**16**) serotyping and genotyping are the most common methods used for classification and identification of IBV strains (**17**) to be able to use with suitable vaccine strains that offer maximum cross protection against the field virus (**5**). Protective immunity, haemagglutination- inhibition (HI) and the virus-neutralizing (VN) antibodies are induced by S1 protein which is considered the main antigenic protein of IBV (**18**). Serotyping is based on virus-neutralization test (VNT) and haemagglutination inhibition test (HI), (**10, and 19**).

In this work, the role of monospecific antisera prepared in different hosts rabbits and chickens in identification and differentiation of classic and variant strains of IBV and the degree of cross reaction between these strains were studied. H120 and MA5 (classic strains), 4/91 and CR88 (variant strains) are the major IBV vaccinal strains used in Egypt. Haemagglutination inhibition antibodies titers (HI) in sera of chickens and rabbits immunized with MA5, H120, 9/41 and CR88 live attenuated strains against different types of IBV antigen and it was found that (HI) titers in the sera of chickens expressed as log2 table (1) and fig (1) was 9.6, 9.4, 7.0 and 6.6; respectively when classic H120 antigen of IBV was used with antisera for H120, MA5, 4/91 and CR88; respectively, while HI titers registered as 9.1, 9.3, 7.1 and 6.8; respectively when classic MA5 was used with antisera for H120, MA5, 4/91 and CR88 ;respectively while, HI titers of variant antigen 4/91 against antisera for H120, MA5, 4/91 and CR88 were 8.0, 7.5, 9.2 and 9.0; respectively at the same time when the variant CR88 was used with antisera for H120, MA5, 4/91 and CR88, the HI antibody titers were 7.3, 8.1, 9.0 and 9.3; respectively.

Table (3) and fig (1) showed results of HI antibody titers for different rabbit antisera as the HI titers were 12.0, 11.5, 10.6 and 9.5; respectively for antisera against H120, MA5, 4/91 and CR88; respectively when H120 antigen is used but were 10.5, 11.0, 9.1 and 8.3 for rabbits antisera against H120, MA5, 4/91 and CR88; respectively when MA5 antigen is used, while HI antibodies titers registered as 10.3, 8.7, 11.3 and 11.0 with rabbits antisera against H120, MA5, 4/91 and CR88; respectively when variant 4/91 antigen is used but recoded as 10.1, 8.3, 10.5 and 10.7 for rabbits antisera against H120, MA5, 4/91 and CR88; respectively when variant CR88 antigen is used, with observation that highest cross reactivity occurred between homologous strains in case of both classic IBV strains (H120 and MA5) and variant strains (4/91 and CR88). This is in agreement with **(10)** who demonstrated that virus strain selection for IBV HI antigen production is important for the specificity and sensitivity of the test as well as for interpretation of the results.

Our results in table (2) and Fig (2) demonstrates the serum neutralizing antibodies titers in antisera of chickens Performed in embryonated chickens eggs (ECE) expressed as neutralization index (NI) when classic H120 antigen was used (NI) were 3.8, 3.5, 2.6 and 2.2 in chickens antisera for H120, MA5, 4/91 and CR88; respectively but classic MA5 antigen resulted in (NI) as 3.3, 3.4, 2.4 and 2.4 in chickens antisera for H120, MA5, 4/91 and CR88; respectively while when variant 4/91 antigen was used the (NI) were 2.8, 2.8, 3.6 and 3.0 for H120, MA5, 4/91 and CR88; respectively. In case of the variant CR88 antigen (NI) with chickens' antisera for H120, MA5, 4/91 and CR88 were 2.6, 3.4, 3.5 and 3.8; respectively.

Table (4) and Fig. (2) illustrated the results of SNT applied on antisera of rabbits prepared against H120, MA5, 4/91 and CR88 (IBV) vaccine strains, when classic H120 antigen was used (NI) were 5.0, 4.7, 4.0 and 2.8 in rabbit antisera for H120, MA5, 4/91 and CR88; respectively but classic MA5 antigen resulted in (NI) as 4.5, 4.8, 3.8 and 2.4 in rabbit antisera for H120, MA5, 4/91 and CR88; respectively while when variant 4/91 antigen was used the (NI) were 4.4, 3.4, 5.0 and 4.8 for H120, MA5, 4/91 and CR88; respectively. In case of the variant CR88 antigen (NI) with rabbit antisera for H120, MA5, 4/91 and CR88 were 3.6, 4.2, 4.5 and 4.6; respectively. Result of (VNT) (Tables 2 and 4) showed high degree of cross reactivity with homologous (IBV) strains antigens and this is in agreement with **(20)** who mentioned that antisera against different homologous genotypes of IBV could neutralizing each other to a high degree.

(21, 22, 23) stated that the major virus neutralizing antibody sites of IBV, which define serotype, reside in the S1 protein. Hence, virus neutralization test (VNT) is the gold standard test for detection of IBV serotype-specific antibodies (**10**).

The results of this study illustrated that sera of rabbits showed better and higher results for serotyping of IBV classic and variant strains than the sera of chickens and this is in agreement with **(11,24)** who mentioned that rabbits are the most used species for production of specific hyperimmune sera because they are naturally free from antibodies against avian viruses, in addition to that they are of convenient size, easy to bleed and handle, have relatively long life span and produce adequate amount of antisera (**25).**

Conclusion

It could be concluded that rabbits are the best laboratory animal of choice for preparation of specific hyperimmune sera for successful monitoring and differentiation of variant and classic strains of IBV which help in preparation of suitable vaccine against co-circulating IBV strains and saves precious expensive costs of imported antisera.

Authors Contributions

All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

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