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COMPARATIVE EVALUATION OF EGG AND TISSUE CULTURE ATTENUATED INFECTIOUS BRONCHITIS VACCINES.PRIVATE

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SUMMARY

Egg and tissue culture adapted infectious bronchitis vaccines were used in ovo-vaccination at 18 days embryonation, one-day-old chicks and 66 weeks old hens. It was found that the hatchability was decreased to 54% and 60% on using egg and tissue culture vaccine, respectively. Both vaccines resulted in a good protection percentage 90%, 85% and 85% for egg and tissue culture vaccine, respectively in case of ovo vaccination, 80% and 75% in case of one day old vaccination and 85% for both vaccines in case of hens vaccination with closely similar immune response as measured by Serum Neutralization Test (SNT), Fluorescent Antibody Technique (FAT), Enzyme Linked Immunosorbent Assay (ELISA) and Haemagglutination Inhibition (HI) tests.

INTRODUCTION

Avian infectious bronchitis (IB) is an economically important disease associated with high mortality in young chicks and decrease egg production accompanied by low egg quality in laying flocks (Broadfoot et al., 1954).

Egg production may not recover fully after an outbreak of IB (Sevoian and Levine, 1957).

Infectious bronchitis is an acute highly contagious viral respiratory disease of chicken characterized by tracheal rales, coughing and sneezing. Mortality may occurs in young chicks due to respiratory or kidney manifestations of the infection (King and David, 1991).

Usually, vaccination against infectious bronchitis diseases represents the corner stone of disease control and eradication.

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Among IB, embryo vaccination has been explored as an alternative to conventional methods of vaccination (Wakenell and Sharma, 1986). Under experimental condition, vaccination of specific pathogen free (SPF) 18 day old chicken embryo with tissue culture attenuated IB vaccine protected the hatched chicks against virulent IB virus challenge at 4 weeks of age (Wakenell et al., 1995).

The embryo vaccination with attenuated IB vaccine resulted in at least 80% protection against respiratory tract disease and the virulent IB virus could not reisolated after challenge at 6 weeks of age (Philip et al., 1997).

The present study was planned to compare between the tissue culture and egg adapted Massachusetts vaccines of IB in the following trials:

1. Ovo vaccination in 18 days of embryonation.
2. Vaccination of one-day-old chicks.
3. Vaccination of laying 66 week old hens.
4. Application of some immuno-response evaluating serological tests such as: Serum Neutralization Test (SNT), Fluorescent Antibody Technique (FAT), Enzyme Linked Immunosorbent assay (ELISA) and Haemagglutination Inhibition (HI) tests.

MATERIAL AND METHODS

1. Viruses :

1.1. Vaccinal strains :

1.1.1. Egg adapted infectious bronchitis vaccine (EIBV) :

It was supplied by Intervet International Company (B.V., Boxmeer, Holland). It had a titre of 107.2 EID50/ml (embryo infective dose). The virus titre was estimated according to Anon (1963).

1.1.2. Tissue culture adapted infectious bronchitis vaccine (TCIBV) "

Tissue culture IBV was prepared in Vero cells according to Elham et al. (1996). It had a titre of 108 TCID50/ml (a local strain of avian infectious bronchitis virus "AIBV" was kindly obtained from the Department of Immunology, Animal Health Research Institute, Dokki, Giza).

1.2. Virulent infectious bronchitis virus (IBV-D274):

A local isolate of virulent infectious bronchitis virus was kindly supplied by Dr. M. El-Kadey, Dept. of Poultry Diseases, Faculty of Veterinary Medicine, Cairo University, Beni-Suef Branch. The strains were received in a lyophilized form, then reconstituted in a sterile phosphate buffer saline, pH 7.2 before inoculation in embryonated chicken eggs for titration and use for challenge test. Virus titration was done using the microtitre technique according to Rossiter and Jessett (1982) and calculated according to Reed and Muench (1938). It had a titre of $10^{5.5}$ EID_{50}/ml.

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2. Green African Monkey Kidney cell culture (Vero):

Vero cells established according to Yasumara and Kawatika (1963) were used for SNT in tissue culture.

3. Embryonated eggs and chickens:

3.1.One hundred and fifty SPF, 18-day-old chicken embryonated eggs were supplied by (SPF Department, Central Laboratory for Control of Veterinary Biologics, Abbasia, Cairo). Briefly, the experimental approach involved inoculation of 50 eggs (group 1) with egg adapted IB and another 50 eggs (group 2) with tissue culture IB vaccine. A third group (50 eggs) was kept as (group 3) control for ovo vaccination. Ovo vaccination was carried out according to Wakenll and Sharma (1986) using 0.1ml of virus vaccine containing 107.2 EID50/ml of egg adapted virus and 108 TCID50/ml of tissue culture vaccine.

3.2. One hundred and sixty (160) one day old SPF chicks (obtained from SPF eggs) were assigned randomly as follows in three groups: group 4 (containing 50 chicks) was vaccinated with egg adapted IB vaccine post hatching, group 5 (50 chicken) was vaccinated with tissue culture IB vaccine, while group 6 (60 chicks) was kept as control group. Vaccination at hatching was done by spraying.

3.3. One hundred and fifty (150), 66 weeks old laying hens were assigned as the last three groups containing group 7 (50 hens) which was vaccinated with egg adapted IB vaccine; group 8 (50 hens) vaccinated with tissue culture IB vaccine and group 9 (50 hens) was kept as a control group. Laying hens were vaccinated I/M with 0.1ml of each vaccine.

Control groups (3, 6 and 9) were transferred to separated hygienic isolators under fully observation with good watering and ration. Blood samples were obtained from vaccinated birds as control ones for serological examination at 9, 18 and 27 days post vaccination.

Each bird was challenged intra-tracheally by 10^5 EID_{50} of the virulent virus using a syringe with special tips. It was done 3 weeks after vaccination of birds.

4. Serum Neutralization Test (SNT) :

A beta micro-neutralization procedure was carried out according to Beard (1989). It was used for monitoring of IBV antibodies in chicken sera.

5. Fluorescent antibody technique (FAT) :

FAT was carried out according to Ushijiona et al. (1969).

6. Enzyme linked immunosorbent assay (ELISA) :

It was performed according to Philip et al. (1997).

7. Haemagglutination inhibition test (HI) :

HI test was carried out according to Alexander and Chettle (1977).

8. Statistical analysis :

It was applied using Epi-Info-Computer programme designed by Dean et al. (1994) and produced by World Health Organization (WHO). The calculation was according to Knapp and Miller (1991).

RESULTS

Clinical signs :

Respiratory clinical signs including snacking and rales were observed in 13 out of 20 birds (65%) in group (1) (ovo vaccination) at 1 day post hatching (PH) and lasted for 4 days. Snacking was observed in 2 out of 20 birds (10%) in group (2) (ovo vaccination with TCIBV) (10%) at 2 days PH for duration of 3 days. Respiratory signs were observed to be clinically and statistically greater in group 1 than group (2).

No respiratory clinical signs were observed in chick groups No. 4 and 5 (one day old vaccination).

No clinical signs affecting the urogenital or gastrointestinal systems were observed in groups 7 and 8.

The egg production percentage was affected in vaccinated laying flocks where it decreased in a range of 5-7 % in both types of vaccination and then returned to its normal level after a short time (12-15 days).

Table (1): Effect of IB vaccination on the hatchability of chicks.

Used vaccine	No. of accinated embryo	No. of hatching chicks	Hatchability
EIBV	50	27/50	54%
TCINV	50	30/50	60%
Control	50	44/50	88%

EIBV : Egg Adapted IB Vaccine.
TCIBV : Tissue Culture Adapted IB Vaccine.

Table (2): Mointoring of IB antibodies in embryo vaccinated groups using different serological tests.

Days Post Vaccination	Geometric mean of antibody titre using							
	SNT		FAT		ELISA		HI $\log_2$	
	EV	TCV	EV	TCV	EV	TCV	EV	TCV
9	6	5	9	8	8	7	4.8	5.8
18	20	19.4	32	30.4	38.2	37.4	6.6	6.6
27	38.6	32	42	35.4	64	54.6	7.4	7.6

EIBV : Egg Adapted IB Vaccine.
TCIBV : Tissue Culture Adapted IB Vaccine.
N.B. : Neutralizing antibody titre = the reciprocal of serum dilution which neutralize 100 $TCID_{50}$ of IB virus.
In case of FAT and ELISA , the recorded titre = the reciprocal of serum dilution giving positive results (two fold dilution).

Table (3): Mean IB antibody titre in one day old vaccinated groups

Days Post Vaccination	Geometric mean of antibody titre using							
	SNT		FAT		ELISA		HI log_2	
	EV	TCV	EV	TCV	EV	TCV	EV	TCV
9	7	5	9	5	20	8	6.3	5.8
18	19.5	19.2	30.4	20.8	54.4	30.4	7.5	6.4
27	38.4	35.2	32	32	64	57.6	8.0	7.2

EV : Egg Adapted IB Vaccine.
TCV : Tissue Culture Adapted IB Vaccine.
N.B. : Neutralizing antibody titre = the reciprocal of serum dilution which neutralize 100 $TCID_{50}$ of IB virus.
- In case of FAT and ELISA , the recorded titre = the reciprocal of serum dilution giving positive results.

Table (4): Mean IB antibody titre in 66 week vaccinated hens.

Days Post Vaccination	Geometric mean of antibody titre using							
	SNT		FAT		ELISA		HI log_2	
	EV	TCV	EV	TCV	EV	TCV	EV	TCV
9	19	20	19.7	20	20.8	21.5	6.5	6.6
18	29	32	31.2	38.4	54.2	75.6	7.5	7.6
27	31	38.2	64	64	64	72	7.5	8.0

EV : Egg Adapted IB Vaccine.
TCV : Tissue Culture Adapted IB Vaccine.
N.B. : Neutralizing antibody titre = the reciprocal of serum dilution which neutralize 100 $TCID_{50}$ of IB virus.
- In case of FAT and ELISA , the recorded titre = the reciprocal of serum dilution giving positive results .

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Table (5): Protection percentage of IB vaccinated groups against virulent strain of IBV

Used IBV	Ovo vaccination		One day old vacc.		66 week old vacc.	
	No. of survived	Protection %	No. of survived	Protection %	No. of survived	Protection %
Egg adapted IBV	18/20	90	16/20	80	16/20	85
TC adapted IBV	17/20	85	15/20	75	17/20	85
Control non vaccinated	0/20	0% dead	0/20	0% dead	0/20	0% dead

The level of IB antibodies in laying flock was neglected before vaccination.

The maternal IB antibodies in one day old chicks were detected in (56%).

DISCUSSION

Determination of the immune status of chicken flocks is very important to choose the type of viral vaccinal strain (egg or tissue culture attenuated) to be used and the proper time of vaccination (Wooley et al., 1976). The present study was a comparison between tissue culture and egg adapted vaccinal strains of IBV.

Three factors were put in consideration to study the comparative evaluation of used infectious bronchitis vaccines including :

1. Ovo vaccination.
2. Age of exposure to IB vaccine, and
3. Estimation of induced immunity at 9, 18 and 27 days post vaccination (PV).

The level of induced antibodies was estimated by different comparative serological tests as SNT, FAT, ELISA and HI in addition to the challenge test using a local isolate of IB virus.

It was found that the hatchability of embryos was affected by IB vaccination at 18 day old embryo, indicating that IB vaccine was pathogenic for embryos resulted in the hatchability ratio of 54% and 60% for egg adapted and tissue culture type of IB vaccines, respectively. While, in unvacci-

nated ones. this ratio was 88%. These results agree with those of Wakenell and Sharma (1986) and Wakenell et al. (1995) who reported decreased hatchability of embryos in case of ovo vaccination with IBV.

Using different serological tests, it was found that there was a very little difference between the immune response to tissue culture and egg adapted vaccines of IB.

On applying challenge test in case of ovo vaccination, it was found that the protection percent was 85% and 90% for egg and tissue culture infectious bronchitis vaccine, respectively. Similar findings were reported by Philip et al. (1997).

In case of one day old vaccination with IB vaccine, the results of serological tests indicated that there was an increase in the level of antibodies due to using of egg adapted vaccine than in case of tissue culture IBV. These observations with the results of challenge and protection percentages come in agreement with those of King and David (1991).

The results of serological tests were parallel to protection percentages and come in agreement with what obtained before by Blore and Skeeles (1981) and Philip et al. (1997).

The good protection percent obtained with the two types of IB vaccine with very little difference in serological results could be attributed to the sensitivity of each tests (Philip et al., 1997). The using of more than one test for detection of antibody in the sera of vaccinated birds gave a good mirror for the level of antibodies formed due to vaccination affected by the type of used vaccine either it was a tissue culture or egg adapted as suggested by Khaliel (1999).

The clinical signs and statistical difference were in agreement with Philip et al. (1997).

In conclusion, it could be said that there is no difference between the effect of egg adapted and tissue culture adapted IB vaccines in case of 66 week old laying birds vaccination. While in case of ovo and one day old vaccination, the egg adapted vaccine induced a better protection % than the tissue culture one.

It could be recommended to use the egg adapted vaccine for ovo-vaccination while the cell culture adapted one used for laying birds, however cell culture vaccine is safe, immunologic and free from foreign contamination which may found in eggs.

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