Vet.Med.J.,Giza. Vol.53, No.2. (2005):329-339.

PREPARATION OF INACTIVATED INFECTIOUS BRONCHITIS VIRUS DISEASE VACCINE USING NEW OIL ADJUVANT PRIVATE

I.M. AMAL*; A.M. DAOUD, ** and FEKRIA EL-BOURDINY**

* Central Laboratory for Evaluation of Veterinary Biologics, Abbasia, Cairo.

** Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo.

Received: 17. 3. 2005

Accepted: 24. 3. 2005

SUMMARY

Montanide ISA 50 was used as an adjuvant for preparation of inactivated IB vaccines and compared with commercially available inactivated IB vaccine adjuvanted with paraffin oil. The vaccine was tested for safety and potency. Serological tests were carried out to follow up the immune status post vaccination by using serum neutralization test and ELISA test for 6 months post vaccination. The results indicated that the vaccine prepared with Montanide ISA-50 oil adjuvant appeared to be more potent and gave protective level reached up to 6 months in comparison with the commercially used vaccine contained paraffin oil gave protection until 4 months only.

INTRODUCTION

Infectious bronchitis virus is a member of the family Coronaviridae which comprises a single genus; Corona virus.

Avian infectious bronchitis (IB) is an acute highly contagious viral respiratory disease of chickens characterized by tracheal rales, coughing and sneezing. In addition, a nasal discharge may occur in young chicks and in laying flocks there is usually a drop in egg production. Mortality may occur in young chicks due to respiratory or kidney lesions of the infection (King and David, 1991). Infectious bronchitis is of economic importance because it is a cause of poor weight gain and feed efficiency, is a component of mixed infections that produce air saculitis that may result in condemnations at the processing of broilers and is a cause of egg production and egg quality declines.

Experience in recent years has shown that natural exposure does not always result in persistent high levels immunity. Killed vaccine containing massive amount of antigen with suitable adjuvants to

promote slow absorption over a long lasting period of time have been widely used to stimulate higher and longer lasting antibody levels in breeder flocks (Colwell, 1984).

Formalin as inactivator and paraffin as an adjuvant vaccine have been used to control the disease which is a commercially available inactivated vaccine for IB.

Montanide ISA oil adjuvant characterized by highly effective generating strong immune response, cheap, easily manufacture, with smooth and abscess free and finally stable and safe when storage (Yamanaka et al., 1993, Mohammadi et al., 1996, Phanthanh et al., 1999, Abd El-Hady, 2001 and Iyer et al., 2001).

In order to enhance the effectiveness of vaccination against infectious bronchitis disease trials of preparation and evaluation of ISA-50 oil adjuvant vaccine against infectious bronchitis disease with formalin inactivator and classical strain (M41) of the Massachusetts serotype of IB virus.

MATERIAL AND METHODS

1. Material:

Virus propagation and antigen preparation:

Ten to eleven day-old susceptible chicken embryos (SPF) were inoculated via the chorioallantoic (CA) cavity with M41 strain of IB containing antibiotics. Titrations were performed by serially diluting (10 fold) CA fluids and inoculated five embryos per dilution. All embryos were candled daily. Deaths occurring within 48 hours after inoculation were considered non-specific deaths. Between 2 and 7 days post inoculation (PI), the embryos were examined for typical signs of (IBV) infection (National Academy of Science, National Research Council, 1971). CA fluids from stunted or dead embryos were examined for bacterial sterility and frozen at -65°C. After initial isolation, CA fluids were harvested 24 hours PI tested and stored. EID_{50} virus end points were calculated according to the method of Reed and Muench (1938).

CA fluid containing the M41 strain of IBV was filtered through a 0.22 u millipore filter. This material was then diluted 1:10 and injected into the CA cavity of 10 day-old SPF fertile embryos and observed for lesions or deaths twenty four hours PI.

The infectivity titre of the virus before inactivation was 1011 mean egg infective doses (EID_{50}) per 0.1 ml.

The virus was inactivated with 0.2% formalin (Yamanaka et al., 1993). The inactivation was carried out, using 0.2% formalin, for 16 hours while liquid being continuously shaken. All necessary measures were undertaken to guarantee that no virus was left active. This was realized by inoculation of inactivated material into embryo-

nated SPF eggs to make sure that no viable virus had remained, which was done by the completion of inactivation test.

Chickens used for experiments:

These originated from the above mentioned SPF eggs. These were used at 21 days old.

Adjuvant:

1. Oil adjuvant ISA-50 (SEPPIC, Cosmetics Pharmacy Division, Paris, France) was used. Ratio of adjuvant to antigen was 50/50 (v/v).

2. Available commercially inactivated IB vaccine using formalin as inactivator and paraffin oil as adjuvant.

Vaccine production:

(According to Seppic, 1994)

A water in oil emulsion was produced mechanically by using a homogenizer. The antigen prepared as mentioned above, was emulsified in the adjuvanted oil. Each dose of the vaccine contained 0.25 ml oil, 0.25 ml antigen and 0.05 mg thiomersal. The whole procedure of vaccine production was carried out in controlled air conditions under a lamin air flow unit. The vaccine was stored at +4°C.

Evaluation of the vaccines:

These comprised sterility, safety and potency tests.

a. Sterility test:

Antigen was tested for bacterial and fungal contaminants. The final product underwent the same checks.

b. Stability test:

The vaccine was tested for one week at 37oC and for different periods of time at +4°C. The emulsion type was determined by the drop test as described by Stone et al. (1978).

c. Safety test:

A group of 10 SPF chickens were inoculated IM (two field dose) with the vaccine and were observed for a period of 14 days for any possible untoward manifestations as local reactions (abscess formation) irritation or systemic reaction.

d. Potency test:

A group of 20 chickens (SPF) were vaccinated, one dose/bird (0.5 ml I/M) and were bled regularly for six months for detection of changes in the serum antibodies, measured by ELISA test and serum neutralization test (SNT). Twenty one days post vaccination, these birds were challenged by a highly virulent field strain of IBV.

Blood samples:

The birds were bled weekly by cardiac puncture

for at least 6 months for both the prepared and imported IB vaccines. Serum samples were inactivated by heating at 56°C for 30 minutes before testing.

ELISA test (Enzyme linked immunosorbent assay):

The antibody titres to IBV for all serum samples were determined by the enzyme linked immunosorbent assay (ELISA) system at a 1:500 dilution with the use of a commercially available IDEXX kits.

Serum neutralization test (SNT):

The antibody titres to IBV for all serum samples were determined by the use of the constant-serum diluted-virus (alpha procedure) VN test. All antisera were initially diluted 1:5 for use in VN test. Reference IBV strain was used in the analysis. Neutralization index (NI) was used to calculate the results (Gelb et al., 1997 and OIE Manual, 1996).

Histopathological examination:

Samples were taken from the injection site (muscle). They were fixed in 10% neutral buffered formalin and embedded in paraffin by standard methods. Sections were stained with hematoxylin and eosin (H and E) and with Masson trichrome (Yamamaka et al., 1993).

Statistical analysis:

Differences in lesions scores, ELISA titres and SNT titres between treated groups were tested for significance by analysis of vaccine (ANOVA test).

RESULTS

The virus yield was very high and titres as high as 10^{11} EID_{50} were achieved. The viscosity test showed that the proportion of the mixed oil and antigen was appropriate. The vaccine was stable at +4°C for a period more than six months and a month at 37°C. Vaccine were safe for both imported and prepared one for chickens and none of the inoculated birds showed noticeable untoward sign.

Pathological changes at the injection site:

The chickens injected with ISA-50 emulsion vaccine containing IBV at 72 hours and 1 week post inoculation (PI) there were a remnants of the inoculum were present in the subcutaneous tissue compared with the reported IBV vaccines there were induration and swelling at the injection site through the experimental period. Table (1) compress the main histopathological changes at the injection site induced by the two different adjuvants.

Table (1): Main histopathological changes at injection sites in chickens injected with prepared vaccine with ISA-50 and imported vaccine with paraffin adjuvant

Time (PI)	Histopathological changes	Severity of changes induced after injection	
		* ISA-50	** Paraffin
72 hours	Acute inflammation	+	++
	Cyst formation	±	+
	Abscess	-	++
1 week	Inflammation	-	+
	Cyst formation	±	+
	Abscess	-	+
	Plasma cells	+	++
	Granulomatous reaction	+	++
2 weeks	Cyst formation	±	+
	Granulomatous reaction	+	++
	Abscess	-	+
	Plasma cells	±	+
4 weeks	Cyst formation	-	+
	Granulomatous reaction	±	+
	Abscess	-	+
	Plasma cells	-	+

PI : Post inoculation.
* ISA-50: Vaccine prepared by ISA-50 adjuvant.
** Paraffin: imported vaccine contain paraffin as adjuvant.

Table (2): Comparative means of serum neutralizing antibody titres (neutralizing index) for chickens vaccinated by IB oil adjuvanted with Montanide ISA-50 and paraffin oil

Weeks post vaccination	Infectious bronchitis ISA-50 adjuvanted vaccine	Infectious bronchitis paraffin adjuvanted vaccine	Control groups (Non-vaccinated)
0	0.0	0.0	0.0
1	2.5	2.4	Non-protected
2	4.0	3.5	Non-protected
3	6.5	4.0	Non-protected
4	7.0	4.5	Non-protected
5	8.0	5.5	Non-protected
6	7.5	6.0	Non-protected
7	7.5	6.0	Non-protected
8	7.0	6.0	Non-protected
12	6.5	5.5	Non-protected
16	6.5	4.5	Non-protected
20	6.0	4.0	Non-protected
24	6.0	3.5	Non-protected

* Values expressed as neutralizing indices (NI) which represents the log10 difference in the titre of the virus alone and that with the antiserum.
** Non-protected level of antibody non-protective less than 4.5.

Table (3): Comparative means of ELISA antibody titres for chickens vaccinated with ISA-50 oil adjuvanted IB vaccine and paraffin oil vaccine

Weeks post vaccination	Means of ELISA titre		
	IB (ISA-50) oil adjuvanted	IB)Paraffin) oil	Control groups (Non-vaccinated)
0	0	0	0.0
1	2800	1800	Non-protected
2	4500	2500	Non-protected
3	6500	4000	Non-protected
4	8500	6500	Non-protected
5	12500	7500	Non-protected
6	12000	7500	Non-protected
7	12000	7000	Non-protected
8	11500	6500	Non-protected
3 months	11000	6000	Non-protected
4 months	10500	5000	Non-protected
5 months	10000	4500	Non-protected
6 months	9500	4000	Non-protected

* Values expressed of ELISA titres by using IDEXX ELISA kits.
** Non-protected (level of antibody non-protective less than 5000).

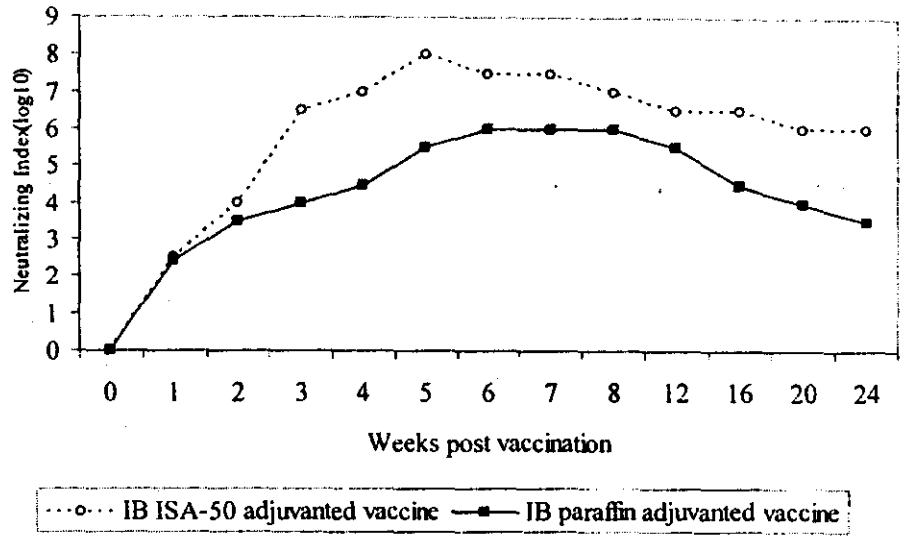

Fig. (1): Comparative means of serum neutralizing antibody titres (neutralizing index) for chickens vaccinated by IB oil adjuvanted with Montanide ISA-50 and paraffin oil

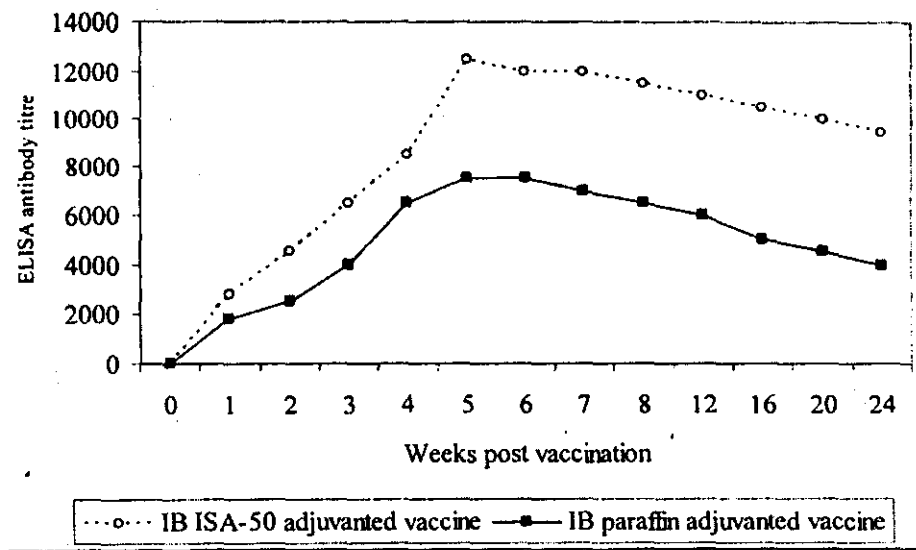

Fig. (2): Comparative means of ELISA antibody titres for chickens vaccinated with ISA-50 oil adjuvanted IB vaccine and paraffin oil vaccine

DISCUSSION

Vaccination against avian infectious bronchitis (IB) has been generally carried out by using paraffin oil in preparation of inactivated vaccine (Gough and Wyeth, 1981 and Bomford, 1997), but when comparing the immune response of different chickens lines many factors should be considered. In this study, we depend on Montanide ISA which is oil adjuvant permitting increased response from the immunity system by antigens. Montanide ISA-50 is designed for the production of oil vaccines by mixing with antigenic solution without the addition of mineral oil, so it produce a good non-specific immunostimulant vaccine adjuvant with smooth and abscess free injection (Phanthanh Pguong et al., 1999).

From table (1) show the use of paraffin as mineral oil for preparation of inactivated IB vaccine when compared with the use oil adjuvant ISA-50 which is a less viscous water in oil type emulsion give the gross and microscopic changes at the injection site after injection of both prepared and imported inactivated IB vaccines, So, we found that the pathological changes induced by ISA-50 was characterized by mild proliferation of epithelioid cells and macrophages around small cysts scattered between muscle fibers in addition, there was no evidence of abscess formation, but the pathological features induced by paraffin oil were well developed with formation of granuloma and abscess (Yamanaka et al., 1992 and Yamanaka et al., 1993).

The serological results obtained in the presence study as shown in table (2), the SNT indices for ISA-50 oil adjuvanted vaccine remained protective till 6 months post vaccination (at the end of the experiment) while paraffin adjuvanted vaccine remained protective for 4 months. The protective cut off of neutralizing index must be not less than 4.5 according to OIE Manual (1996) and Vet. Rec. (1976).

Table (3) shows the ELISA results which were parallel correlation with those obtained with SNT. The protective cut off of ELISA titre for inactivated IB by IDEXX ELISA kits was not less than 5000 which agreed by Sander et al. (1997). Which showed that adjuvanted vaccine with ISA-50 was still protective more than 6 months, but adjuvanted vaccine with paraffin was protective for 4 months only.

The superiority of prepared fluid vaccine with Montanide ISA-50 is due to the maintenance of higher level of antibodies for several months it is phenomena is attributed to several factors, the protective effect played by the water in oil phase which avoids a massive degradation of the antigens by the organisms own defences (Halbert, 1946); the progressive release of the antigens which maintains the immunity defense mecha-

nism for several months (Steward, 1983).

Finally, the injection of fluid vaccine which is easily disperisible in the major organs, is a guarantee of optical efficiency because it releases the antigens from multitude of different location (Seppic, 1994). So, Montanide-ISA improve the production of oil vaccines which are highly efficient, fluid, low viscosity and easily reproducible.

REFERENCES

Abdel Hady, A.I. (2001): Studies on prepared and evaluation of inactivated IBD vaccines by using new oil adjuvants. Ph.D. Thesis, Virology, Fac. Vet. Med., Cairo Univ.

Bomford, R. (1997): The production and quality control of veterinary vaccines for use in developing countries. FAO 1997, p. 277-284.

Colwell, W.M. (1984): Infectious bursal disease booster vaccination of broiler flocks while in production. Vet. Res. Sero. Lab. Hollyfarms, Poultry Ind. Inc. Wilkesboro, North Carolina.

Gelb, J.Jr.; Keeler, C.L., Nix, W.A.; Rosenberger, J.K. and Cloud, S.S. (1997): Antigenic and S-1-genomic characterization of the Delware variant serotype of infectious bronchitis virus. Avian Dis., 41: 661-669.

Gough, R.E. and Wyeth, P.J. (1981): Immune response of breeding chickens to trivalent oil emulsion vaccines: Response to infectious bronchitis. Vet. Rec., 108 (5): 99-101.

Halbert, S.P. (1946): Journal of immunology, 53: 291-301.

Iyer, A.V.; Ghosh, S.; Singh, S.N. and Deshmukh, R.A. (2001): Evluation of three iready to formulatei oil adjuvants for foot and mouth disease vaccine production. Vaccine, 19: 1097-1105.

Jones, R.C. (1976): The isolation and serum biological properties of a variant infectious bronchitis virus. Vet. Rec., 98: 287-279.

King, D.J. and David, C. (1991): Infectious bronchitis. Diseases of Poultry, 9th Ed., 471-484.

Mohammadi, A.A.; Hooshmand-Rad, Ahourai, P.; Momoyiessiakhal, R.; Taleb-Shooshtary, A.; Charkhkar, S.; Ebrahimimi, M. and Ghodsia, N. (1996): Development and Manufacture of inactive oil emulsion Newcastle disease vaccine in Iran. Arch. Inst. Razi,, 46-47.

Montanide and Montanide ISA adjuvants for vaccines (1994): Seppic Technical bulletin. SEPPIC, Paris, 1994.

National Academy of Science, National Research Council (1971): Methods for examination of poultry biology. Natl. Acad. Sci. Natl. Res. Coum. Washington, D.C.

OIE Manual (1996): Avian infectious bronchitis vaccine Chapter, 3.6.6., P. 540-546.

Phanthank, P.; Phancong, H. and Dai Duy, B. (1999): Immune response in hens administered with anti-Gumboro oil adjuvant vaccine VN, 97. Mondial Vet. Meeting in Lyon Sept., 1999.

Reed, L.J. and Muench, H. (1938): A simple method of estimating 50 percent end points. Am. J. Hyg., 27: 493-497.

Sander Jean, E.; Jackwood, W.M. and Rowland, N.G. (1997): Protection by commercial Arkansas type infectious bronchitis virus vaccine against a field isolate of the same serotype. Avian Dis., 41: 964-967.

Steward-Tull (1983): DES in Biololgy of microbacteria.

Vol. 2, Academic Press, London, 1983.

Stone, H.D.; Brugh, M.; Hopkins, S.R.; Yoder, H.W. and Bread, C.W. (1978): Preparation of inactivated oil emulsion vaccines with avian viral or Mycoplasma antigens. Avian Dis., 22: 666-674.

Yamamaka, M.; Okabe, T.; Nakai, M. and Goto, N. (1993): Local pathological reaction and immune response of chickens to ISA-70 and other adjuvants containing Newcastle disease virus antigen. Avian Dis., 37: 459-466.

Yamanaka, M.; Hiramatsu, K.; Hirahara, T.; Okabe, T.; Nakai, M.; Sasaki, N. and Goto, N. (1992): Pathological studies on local tissue reactions in guinea pigs and rats caused by four different adjuvants. J. Vet. Med. Sci., 54: 685-692.