IMPROVEMENT OF INACTIVATED AVIAN REO VIRUS VACCINE BY USING BINARY ETHYLENEIMINE

ABOU EL-KHAIR[1] M. A. AND M. H. ABDEL-BAKY[2]

1 *Veterinary Serum and Vaccine Research Institute, Agricultural Research Center Ministry of Agriculture , Dokki - Giza-Egypt*
2 *Central Lab for Evaluation of Veterinary Biologics, Agricultural Research Center Ministry of Agriculture , Dokki - Giza-Egypt.*

(Manuscript received 2 November 2003)

Abstract

A comparative study was done between the immunogenic response induced in two groups of one week old chicks vaccinated with formalin-inactivated and binary ethyleneimine (BEI) vaccines of avian Reo virus. Chickens were bled weekly for 8 weeks post-vaccination and tested for neutralizing antibodies. Vaccinated and non-vaccinated control groups of chicks were challenged with virulent virus at the 3rd week post-vaccination. Chicks immunized with binary ethyleneimine inactivated vaccine had a higher level of serum neutralizing antibodies. However, both vaccinated groups were protected when challenged.

INTRODUCTION

Reo virus infections are worldwide in chicken, turkeys and other avian species. They have been concerned with their role in avian arthritis in chicken. They have been associated with a variety of other disease conditions, as malabsorption or runting syndrome (Kouwenhoven *et al.*,1978).

In Egypt, Tantawi *et al.* (1984) and Kheir El-Din and El-Sanousi (1986) isolated and characterized avian reo virus from clinical cases.

Control policy of the disease depends mainly upon vaccination, the available vaccines produced locally are the living attenuated and formalin inactivated vaccines.

The aim of the present study was conducted to determine the role played by binary ethyleneimine as inactivant and its effect in improving the potency of the available inactivated reo virus vaccine.

MATERIALS AND METHODS

- **Embryonated chicken eggs (SPF)**

  These were obtained from Koom Oshim, Fayoum, and used for preparation and titration of reo virus fluid.

- **Chicks**

  One week old chicks originated from SPF eggs reared on special isolators used to test the potency of the prepared vaccines.

- **Cell culture**

  Vero cells (African green monkey kidney cells) were used for serum neutralization test.

- **Viruses**

  Eggs adapted vaccinal and virulent strain of avian reo virus (St. S1133) were kindly supplied by Newcastle Disease Department, Veterinary Serum and Vaccine Research Institute, Abbassia, Cairo.

- **Preparation of the vaccine virus fluid**

  - A batch of living attenuated reo virus strain S1133 was prepared after the method described by Abou El-Khair (1996).
  - Titration of the obtained virus was carried out in SPF embryonated chicken eggs, and the titer was expressed as median embryo lethal dose (EID_{50})/ml and calculated according to Reed and Muench (1938).

- **Kinetics of reo virus strain S1133 inactivation with BEI at final concentration of 0.004 M at 37°C (Abd El-Baky, 1995)**

  - After addition of BEI to the virus fluid, the mixture was left for 24 hours on magnetic stirrer at 37°C.

  - Samples of treated virus fluid were collected at 0 time, 2nd hour, 6, 10, 14, 18, 20 and 22 hours post-treatment, and kept at 4°C after neutralization of their residual BEI with sodium thiosulphate (final concentration of 2%).

- Titration of each sample was carried out by inoculation in SPF embryonated chicken eggs for the detection of EID_{50} which was calculated using the method of Reed and Muench (1938).

- **Virus inactivation**

  - Approximately, one half of virus fluid was inactivated by addition of formalin at a final concentration of 0.2% and incubation for 24 hours at 37°C with stirring.

  - Another half of virus fluid was inactivated by addition of BEI at final concentration of 0.004 M and incubation at 37°C for 18 hours with stirring, followed by addition of sodium thiosulphate at final concentration of 2%, as described by Bahnmann (1975) and Hassanin (1983). Treated virus was kept at +4°C until addition of adjuvant.

- **Residual infectivity**

  Absence of residual infectivity in virus fluids after inactivation and before addition of adjuvant was confirmed by chicken embryo inoculation.

- **Addition of adjuvant**

  Following the method of Stone *et al.* (1978), stable emulsions of both formalin inactivated virus and BEI inactivated virus were prepared with paraffin oil adjuvant made by adding inactivated virus fluids dropwise into the oil during emulsification at 4000 rpm.

- **Serum neutralization (SN) test**

  SNT for detection and titration of avian viral artheritis (AVA), virus neutralizing antibodies in sera of vaccinated chicks was done using Beta method of standard microtechnique as described by Giambrone (1980).

- **Experimental design**

  Seventy-five one-week old chicks were equally divided into three groups. First group was inoculated I/M with formalin-inactivated AVA-virus vaccine (0.5 ml/bird), second group was inoculated with BEI-inactivated AVA-virus

vaccine (0.5 ml/bird), while, the third one was held as a control. Serum samples were collected weekly from each vaccinated and non-vaccinated group for 8 weeks post-vaccination. The samples were examined by SNT to determine their AVA-virus neutralizing antibody- titers.

- **Challenge test**

  Ten chicks from each group were inoculated in foot pad with 5 $\log_{10}$ EID_{50} of virulent strain of avian Reo virus (S1133). Specific morbidities and mortalities post - challenge were scored in each group to determine the protection efficiency for each vaccine.

RESULTS

Inactivation curve of AVA-virus strain S1133 with binary ethyleneimine

The course of inactivation was drawn in which $\log_{10}$ residual virus was plotted against time (Table 1 and Figure 1). Titration of samples collected during the course of inactivation revealed that titer of treated virus was decreased from 7.7 $\log_{10}$ ED_{50}/ml at Zero time of inactivation to 7.7, 7.2, 7.0, 4.6 and < 1 $\log_{10}$ ED_{50}/0.1 ml by the 2^{nd}, 6^{th}, 10^{th}, 14^{th} and 18^{th} hours post -inactivation (HPI), respectively. Samples collected at the 18^{th} and 19^{th} hours were reflecting no virus infectivity.

Testing for residual viruses post - inactivation with 0.2% formalin and 0.004 M BEI

- The virus inactivation was confirmed and completed by inoculation of ECE where there was no specific lesion or deaths observed.
- The emulsions of the prepared vaccines were stable and of moderate viscosity and there were no apparent differences in physical characteristics.
- The prepared vaccines were free from bacterial, fungal and mycoplasmal contamination.

Immuogenicity of oil adjuvant formalin and BEI- inactivated AVA-virus (strain S1133) vaccines

Tables 1 and 2 show results of the evaluation of immunogenicity of each prepared vaccine. The AVA-virus neutralizing (VN) antibody titers of the sera from chickens inoculated with formalin and BEI inactivated vaccines started at the 1st week post-vaccination (wpv) with a titer of (8), gradually increased to their peak value of (128) by the 3rd wpv in group of chickens vaccinated with BEI inactivated vaccine, and by the 4th wpv in group of chickens vaccinated with formalin inactivated one, and recorded a value between (32) and (64) by the end time of experiment (the 8th wpv).

The protection percent in both groups of chicks vaccinated with formalin and BEI-inactivated vaccines was 100% against challenge with the homologous virulent strain (S1133) of AVA-virus three weeks after vaccination, while,the control group showed 20% protection.

DISCUSSION

This work was done to improve inactivated vaccine against avian viral arthritis which is still an important problem of broiler and egg laying chicks. BEI-inactivant was chosen to improve the currently used formalin inactivated vaccine. Inactivation with BEI-inactivated vaccine against AVA might be of higher immunogenicity (Girard *et al.,* 1977) .

The present study has proved the opportunity to compare and evaluate the immunogenic capacity of formalin and BEI inactivated vaccines. It is evident from the results that a single dose (0.5 ml) of either formalin or BEI inactivated vaccines given by intramuscular route conferred a relatively equal good seroconversion from the 1st till the 8th wpv. The significant conversion rate in VN-antibody titers was between 64 and 128 within a period from the 3rd wpv till the end time of the experiment (8th wpv).

Olson (1984) found that AVA-virus neutralizing antibody titre equal 80 in sera of chickens was considered positive. The serological response of chicks to BEI-inactivated vaccine was higher,but , in both groups neutralizing antibodies converted

significantly for 8 weeks and vaccinated chicks resisted challenge with virulent homologous virus.

Table 1. Growth kinetics of inactivation avian reo virus by using BEI .

Hours post inactivation	Titers	
	Treated virus	Untreated
0	$10^{7.7}$	$10^{7.7}$
2	$10^{7.7}$	$10^{7.7}$
6	$10^{7.2}$	$10^{7.7}$
10	$10^{7.0}$	$10^{7.6}$
14	$10^{4.6}$	$10^{7.2}$
18	0	$10^{7.2}$
20	0	$10^{7.2}$
22	0	$10^{7.2}$

Figure 1 Inactivation curve of AVA-virus strain S1133 with binary ethyleimine

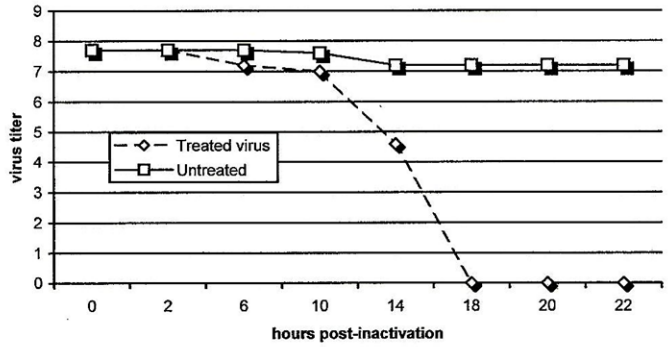

Table 2 . Immunity in chicks vaccinated with a single dose of formalin inactivated and binary ethyleneimine inactivated vaccines.

Type of vaccine	AVA-virus neutralizing antibody titer							
	1 wpv	2 wpv	3 wpv	4 wpv	5 wpv	6 wpv	7 wpv	8 wpv
Formalin inactivated	8 *	32	64	128	128	64	32	32
BEI inactivated	8	64	128	128	128	128	64	64
non vaccinated control	0	0	0	0	0	0	0	0

* = Reciprocal of pooled serum sample dilution which neutralized 100 $TCID_{50}$.

wpv = week post vaccination .

Table 3. Protection percent for chicks vaccinated with formalin and binary ethyleneimine inactivated vaccines of AVA-virus (strain S1133) and challenged at the 3rd week after vaccination.

Type of vaccine	Vaccinated group No. of infected/total	Protection %	Control group No. of infected/total	Protection %
Formalin inactivated	0/10	100	* 8/10	80
BEI inactivated	0/10	100		
control	8/10	20		

- = Chicks showed diffused inflammatory swelling of the tendons and joints of the legs between the 5th and 7th week post inoculation.

REFERENCES

1. Abd El-Baky M.H. 1995. Studies on production and application of African horse sickness polyvalent inactivated vaccine. Ph.D.Thesis (Virology), Fac.Vet.Med., Cairo University.

2. Bahnamam H.G. 1975. Binary ethylinimine as an inactivant for FMD virus and its application for vaccine production. Archive Virology, 47: 47-56.

3. Giambrone J.J. 1980. Microculture neutralization test for serodiagnosis of three avian viral infections . Avian Dis., 24 (1): 284-287.

4. Girard H.C.,O.I Bayramoglu, N. Erol and A. Burgut. 1977. Inactivation of FMD virus by binary ethyleneimine . Bull.Off.Int.Epiz., 82(3-4): 201-207.

5. Hassanin M.M. 1983. Further studies on diagnosis and control of AHS in Egypt . Thesis, Ph.D., Fac.Vet.Med., Cairo University.

6. Kheir El-Din A.M.W. and A. El-Sanousi. 1986. Infectious stunting and runting syndrome . J.Egypt.Vet.Med.Assoc., 46 (3): 283-294.

7. Kouwenhoven B., M.H. Vertmommen and J.H.H. Van Eck. 1978. Runting and leg weakness in broiler: Involvement of infectious factor. Vet.Sci.Comm., 2: 253.

8. Olson N.O. 1984 . Diseases of poultry. Edited by M.S>Hofestad and others. Iowa State University, Ames, Iowa, USA. Chapter No. 27: 561-567.

9. Reed,L.J and H. Muench. 1938 A simple method of estimating fifty percent end point . Am.J.Hyg., 27: 493.

10. Stone D., M. Brugh, S.R. Hophins , H.W. Yoder and C.W. Beard. 1978. Preparation of inactivated oil emulsion vaccines with avian virus or mycoplasma antigen. Avian Dis., 22: 666-674.

11. Tantawi H.A., N. Amina, M.Y.I. Youssef, A.L. Fawzia, J.M. Abdulla, A. El-Batrawi, A., A. El-Chawas, A.A. Nasser and I.M. Reda. 1984. Infectious tenosynovitis in broilers and broiler breeder in Egypt . Vet.Res.Comm., 8: 229.

تطوير لقاح الريو المثبط بإستخدام البينرى إثيلين أمين

محمد عبد الغنى أبو الخير[1] - منصور هاشم عبد الباقى [2]

١ معهد بحوث الأمصال واللقاحات البيطرية- مركز البحوث الزراعية - وزارة الزراعة - الدقى - جيزة - مصر

٢ المعمل المركزى للرقابة على المستحضرات الحيوية البيطرية- مركز البحوث الزراعية - وزارة الزراعة - الدقى - جيزة - مصر

تم إستخدام مادة البينرى إثيلين أمين فى تثبيط فيروس الريو لتحضير لقاح مثبط ومقارنته مناعياً مع اللقاح المثبط بمادة الفورمالين. تم حقن مجموعتين من الكتاكيت باللقاحين كل على حده فى وجود مجموعة ضابطة ، وقد تم أخذ عينات دم أسبوعياً ولمدة ٨ أسابيع وتم إجراء إختبار التعادل المصلى لإستبيان نسبة الأجسام المناعية ، وقد أوضحت النتائج إرتفاعاً ملحوظاً فى نسبة الأجسام المناعية فى المجموعة المحصنة باللقاح المثبط بمادة البينرى إثيلين أمين عنها فى المجموعة الأخرى. وتم إجراء إختبار التحدى المناعى بإستخدام الفيروس الضارى ولم توجد فوارق فى نسبة الصد بين المجموعتين حيث كانت ١٠٠% فى المجموعتين بينما كانت النسبة ٢٠% فقط فى الضوابط .