PREPARATION AND EVALUATION OF LIVE ATTENUATED INFECTIOUS BOVINE RHINOTRACHEITIS (IBR) VACCINE

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Abstract

Preparation of freeze-dried live attenuated IBR vaccine was used in emergency during outbreaks by nasal vaccination. The evaluation of attenuated IBR vaccine in laboratory was conducted through studying purity, safety in laboratory animals and calves, potency in calves and duration of immunity. Twelve susceptible 6-9 months old calves free from antibodies against IBR virus were vaccinated intranasally with a 2ml dose containing 6.5 $\log_{10}$ $TCID_{50}$/dose. The immunogenic value of the vaccine was determined on the basis of the level of neutralizing antibodies in serum using serum neutralization (SNT). Rise in the level of specific antibody was clearly seen 2 or 3 weeks of vaccination and the ELISA was used to measure the level of antibodies, which came in harmony with those of SNT. Effectiveness of the vaccine was tested 8 weeks post-vaccination by challenging the calves with virulent strain of IBR virus. The results indicate that the live attenuated vaccine was sufficiently immunogenic to stimulate the production of antibodies against IBR infection.

INTRODUCTION

Infectious bovine rhinotracheitis (IBR) is a highly infectious disease caused by bovine herpes virus-I which is capable of producing the respiratory disease rhinotracheitis, conjunctivitis, fever and a short course with a high recovery rate is the most commonly observed disease, abortion of pregnant animals, encephalitis, the systemic form of the disease in newborn calves (Straub and Bohm, 1965).

The virus of IBR is identical with that of infectious pustular vulvovaginitis (IPV) of cow and balanoposthitis of bulls (Studdent *et al.*, 1964), but only rarely do the respiratory and genital forms of the disease occur together.

Researches on preventing viral respiratory diseases in cattle with the use of specific vaccines were undertaken from many years ago. Many countries applied research on attenuated, inactivated monovalent, polyvalent and mixed vaccines (Kita *et al.*, 1983 and Donkersgoed *et al.*, 1995). During this time, new problems developed concerning control of the degree of attenuation of vaccine strains and methods of vaccination.

Despite of certain achievements in the immunoprophylaxis of viral respiratory diseases, there is still problem in heavy intensive herds and research continues to perfect vaccines by modification of attenuation, methods of vaccination, as well as the control of humoral and cellular immunity (Donkersgoed *et al.*, 1995 and Drunen *et al.*, 2001).

The Egyptian authorities succeeded in preparing a safe and potent inactivated IBR vaccine (El-Sabbagh , 1993), pneumo-3 and pneumo-4 which contain IBR, BVD, PI-3 and BRSV (El-Sabbagh *et al.*, 1995 and Samira *et al.*, 2001) which have been used in protecting calves from bovine respiratory pathogen.

The purpose of our research is to determine the immunogenic value of live attenuated IBR vaccine produced for the first time in Egypt on a laboratory scale.

MATERIALS AND METHODS

1- **Virus:** infectious bovine rhinotracheitis (IBR) virus Abou-Hammad strain (Hafez *et al.*, 1976). This virus Egyptian vaccinal strain was used for preparation of pneumo-3 and pneumo-4 vaccine in Rinderpest like diseases Department, Veterinary serum and vaccine Research Institute, Abbbasia, Cairo.

2- **Cell culture:** monolayer MDBK cell culture was tested to be free from the non-cytopathic (NCP) BVD-MD virus (Marcus and Moll, 1968).

3- **Animals**

a- Twelve susceptible calves (Friesian and local) breed 6-9 months of age were used in these studies.

b- Swiss-Albino mice (20-25 g body weight) and guinea pigs were used for vaccine evaluation and safety.

Methods

Attenuation and preparation of IBR vaccine

- The attenuated IBR virus was titrated in MDBK cell line and reached 8 $\log_{10}$ $TCID_{50}$/ml.
- Freeze dried live attenuated IBR vaccine contain 10% sucrose and 5% lactose albumin, equal amount of each to the stock virus (Soad, 1986). The vaccinated dose contains 6.5 $\log_{10}$ $TCID_{50}$.

Sero-conversion

1- **Serum neutralization test** was performed using tissue culture system according to Kita *et al.* (1983).

2- **Enzyme linked immunosorbent assay (ELISA),** according to Voller *et al.* (1976).

3- **Sample for virus isolation:** Buffy coat, nasal and conjunctival swabs were collected from all calves (vaccinated challenged, control infected and contact control) at 3, 7, 10, 14, 21 days post-challenge and infection.

Evaluation of locally prepared attenuated IBR vaccine

1- **Purity:** It was performed in accordance with USA Code of Federal Regulation, **9CFR (1987)** testing 113.2, 113.26, 113.27 and 113.30 to be free from bacteria mycoplasma, fungi and extraneous viruses as non-cytopathic BVD virus.

2- **Safety test:** According To **9 CFR (1987)** testing 113.41 in calves and 113.38 in mice and guinea pigs.

Experimental design

Twelve cross breed calves (Frisian and local) about 6-9 months of age were kept under observation for 7 days before vaccination. General clinical examination was carried out and serum samples were collected for detection of IBR antibodies and all proposed to be free from IBR antibodies. Twelve calves were randomly divided into 2 groups each consists of six calves.

Group (1): Each calf was vaccinated with 2ml intranasally containing 6.5 $\log_{10}$ $TCID_{50}$/dose as described by Kita *et al.* (1983). This group was divided into two subgroups 3 calves each as follows:

Subgroup (A): This group used for studying the duration of immunity. Serum samples were collected from each calf on 0,7,14,21,28,45,60 days post-vaccination and monthly up to one year.

Subgroup (B): This group used for testing the effectiveness of the vaccine, calves of this group were challenged at 8 weeks after vaccination with pathogenic strain of IBR virus. Calves were infected with 2.5ml intratracheally and 2.5ml intravenously containing 10^5 $TCID_{50}$/0.2ml according to Kita *et al.* (1983).

Group (2): This group also consisted of 6 calves and divided also into 2 subgroups:

Subgroup (A): Positive infected control calves: each calf was infected with 5ml of virulent virus containing 5 $\log_{10}$ $TCID_{50}$/0.2ml as mentioned before.

Subgroup (B): Negative control calves: the other calves were left non-infected and non-vaccinated control calves.

RESULTS AND DISCUSSION

Attenuated virus titration: The titre of attenuated IBR virus reached 8 $\log_{10}$ $TCID_{50}$/ml.

Purity or sterility test: Revealed that the local live attenuated IBR vaccine was free from bacteria, fungi and mycoplasmal contamination.

Safety tests: Safety in mice and guinea pigs: revealed that no clinical abnormalities were observed through the observation time.

The effectiveness of the vaccine was evaluated on the basis of experimental challenge and clinical observation of illness in vaccinated and non-vaccinated calves. Three days after challenge, only the control-infected calves became ill. The following clinical signs were seen, fever of 40.50°C-40.7°C, increased pulse and rapid breathing, serous nasal discharge, loss of appetite and depression. No clinical signs were observed in calves vaccinated with live attenuated IBR vaccine.

The estimation of humoral immune response

In vaccinated calves, was achieved by using serum neutralization test (SNT), enzyme linked immunosorbent assay (ELISA) against IBR virus.

A: as presented in Table 1, there was significant increase in IBR SN-Ab titre (32) on the 21 days post-vaccination, and reached its peak on the 28 day (128) and it

remained with the protective level till one year post-vaccination (protective titre 1:4 for IBR virus according to Zuffa and Feketeova (1980).

B: the level of antibodies as measured by ELISA, the titre ranged as shown in Table 2, the average ELISA mean titre in live attenuated IBR vaccine reached its maximum from 2 till 5 months post-vaccination and went in harmony with those of SNT.

Virus isolation

As result shown in Table 3, no IBR virus was isolated from vaccinated and negative control calves post-vaccination. The challenge virus was isolated from different samples collected during febrile reaction between 1 and 14 days post-infection from positive control calves. In vaccinated challenged calves, viruses were recovered from 7 days post challenge only.

The results indicated that live attenuated IBR vaccine is efficacious. In addition to serologic evidence, the efficacy of the prepared vaccine has been substantiated further by protection of vaccinated calves against challenge of immunity with a virulent virus as Kita *et al.* (1983) and Donkersgoed *et al.* (1995).

The results of these studies were important in as much as correlation of challenge of immunity results with serologic finding and indicated that calves with a detectable IBR SN antibody titre are protected as Antonov and Genova (1999) and Drunen *et al.* (2001).

Adverse reactions to the vaccination are not observed. Additionally, none of the control calves developed an SN antibody titre to IBR virus following vaccination. Our results agreed with those of Kita *et al.* (1983), Donkersgoed *et al.* (1995) and Drunen *et al.* (2001) indicating that IBR attenuated vaccine strain has no adverse reaction and safe for vaccination.

The results of our studies showed that 2ml (6.5 $\log_{10}$ $TCID_{50}$/dose) of the vaccine given intranasally are effective for treatment and controlling IBR virus during out-break of IBR infection in Egyptian farm animals.

REFERENCES

1. Antonov, P. and K. Genova. 1999. Efficacy of vaccines against HVB1 and BVD-MD in cattle including various adjuvants. Experimental-Pathology and Parasitology, 2: 34-38.

2. Code of Federal Regulation USA. 1987. Animal products, No. 9 parts 1 to 199. Published by the Office of Federal Register, National Archives and Records Administration.

3. Donkersgoed, J. Van, P. Klassen, and Van Drunen. 1995. Serological study of a modified live virus IBR vaccine given to feedlot calves after arrival. Canad. Vet. J., 36 (6): 394.

4. Drunen, Lihel, S. Van-den-Hurk, Van-den, D., Myers, PA. Doig, B., Karvonen, M., Habermehl, L.A., Babiuk, M, Jelinski, J-Van, Donkersgoed, K. Schlesinger and C. Rinehart. 2001. Identification of a mutant bovine herpes virus-1 (BHV-1) in post-arrival out breaks of IBR in feedlot calves and protection with conventional vaccination. Canadian-J. Vet. Res., 65 (2): 81-88.

5. El-Sabbagh, M.M. 1993. Further studies on infectious bovine rhinotracheitis in cattle. Thesis, Ph.D., Fac. Vet. Med., Cairo University.

6. El-Sabbagh, M.M., Samira Said, H.M. Ghaly, and M.S. Saad. 1995. Binary ethyleneamine as an inactivant for infectious bovine rhinotracheitis (IBR), bovine viral diarrhoea (BVD) and para-influenza-3 (PI-3) viruses and its application for vaccine production. Beni-Suef Vet. Med. Res., 5(2): 29-57.

7. Hafez, S.M., Baz, Thanaa, I. A.Y.A. Mohsen, and Zehran Monira, H. 1976. Infectious bovine rhinotracheitis in Egypt. Vet. Med. Assoc., 36(1): 129-139.

8. Kita, J., J. Oyrzanowska and J. Prandota. 1983. Evaluation of the attenuated vaccine para-Ribovac. Zbl. Vet. Med. B., 30: 502-511.

9. Marcus, S.J. and T. Moll. 1968. Adaptation of bovine viral diarrhoea virus to the Madin Darby Bovine Kidney cell line. J. Vet. Res., 24(4): 817-819.

10. Samira, S. Taha M.M.A. El-Sabbagh and H.M. Ghaly. 2001. Preparation of combined inactivated BVD, IBR, PI-3 and respiratory syncytial virus (BRSV). J. Egypt. Vet. Med. Assoc., 61(4): 251-263.

11. Soad, M.S. 1986. Immunological and Virological studies on fowl pox vaccine. Fac. Vet. Med., Cairo University.

12. Straub, O.C. and H.O. Bohm. 1965. Experimental infection of the central nervous system by bovine rhinotracheitis virus. Dt. Tieraerztl. Umschau, 2: 124-128.

13. Studdent, M.J.C., A.V. Baker, and M. Savan. 1964. Infectious pustular vulvovaginitis virus infection of bulls. Amer. J. Vet. Res., 25: 303-314.

14. Zuffa, A. and N. Feketeova. 1980. Protection of cattle by vaccination with inactivated oil adjuvant IBR vaccine against experimental infection. Vet. Med., 27 (9/10): 725-733.

15. Vollar, A., D. Bidwell and A. Bartlett. 1976. Microplate enzyme immunoassays for the immunodiagnosis of virus infections. Pub. Amer. Soc. Micro., 506-512.

Table 1. The arithmetic mean of IBR virus serotitres collected from vaccinated with live attenuate vaccine as well as control calves.

Groups	Number Of animals	Serum neutralizing antibody titres													
		Time post-vaccination													
		Zero day	3 dpv	7dpv	14 dpv	21 dpv	1 mpv	2 mpv	3 mpv	4 mpv	5 mpv	6 mpv	8 mpv	10 mpv	12 mpv	13 mpv
Vaccinated	1	2	2	4	>16	32	64	128	128	128	>64	64	>32	>16	16	>8
	2	2	4	8	>16	32	64	128	128	128	128	64	32	>16	16	>8
	3	0	2	4	16	32	64	128	128	>64	64	64	32	16	16	>8
Arithmetic mean titre		1.33	2.67	5.33	20	32	64	128	128	109.33	88	64	36	20	16	11
Control group	1	2	2	2	2	2	2	0	0	0	0	0	0	0	0	0
	2	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	3	0	2	2	2	2	2	0	0	0	0	0	0	0	0	0
Arithmetic mean titre		1.33	1.33	1.33	1.33	1.33	1.33	0	0	0	0	0	0	0	0	0

Table 2. Mean ELISA optical density of calves vaccinated with live attenuated IBR vaccine as well as control calves.

Groups	Number of animals	Optical density													
		Time post-vaccination													
		Zero day	1st Wpv	2nd wpv	3rd wpv	4th wpv	6th wpv	8th wpv	3 mpv	4 mpv	5 mpv	6 mpv	8 mpv	10 mpv	12 Mpv
Vaccinated group	3	0.01	0.025	0.030	0.039	0.045	0.052	0.060	0.075	0.084	0.072	0.069	0.050	0.038	0.025
Control group	3	0.008	0.007	0.012	0.015	0.013	0.018	0.014	0.015	0.012	0.009	0.01	0.017	0.008	0.009

Cut off = 0.02 read on a plate reader at 570nm wavelength.

wpv: weeks post-vaccination. mpv: month post-vaccination.

Table 3. Recovery rate of IBR virus from buffy coat and rectal swabs collected from vaccinated challenged and control infected calves (numbers of animals in each group = 3).

Group	Vaccinated challenged			Virus recovery rate per day		Control infected			Virus recovery rate per day	
Site of isolation / Time/day	B.C.	N.S.	C.S.	Total No.	%	B.C.	N.S.	C.S.	Total No.	%
1	0/3	2/3	0/3	2/9	22%	0/3	3/3	0/3	3/9	33.3%
3	3/3	3/3	1/3	7/9	78%	3/3	3/3	3/3	9/9	100%
7	2/3	2/3	1/3	5/9	55.5%	3/3	3/3	3/3	9/9	100%
10	0/3	0/3	0/3	0/9	0%	2/3	2/3	3/3	7/9	78%
14	0/3	0/3	0/3	0/9	0%	0/3	2/3	1/3	3/9	33%
21	0/3	0/3	0/3	0/9	0%	0/3	0/3	0/3	0/9	0%
Total number per sample	5/18	7/18	2/18	14/54	26%	8/18	13/18	10/18	31/54	57.4%

B.C. = Buffy coat N.S. = Nasal swab C.S. = Conjuctival swab

No recoveries were made from control and vaccinated calves through out the experiment.

تحضير وتقييم لقاح مستضعف
لإلتهاب القصبة الهوائية البقرى المعدى

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تحضير اللقاح المجفد المستضعف لفيروس التهاب القصبة الهوائية الرغامى المعدى يستخدم أثناء الوباء بتحصينه عن طريق التنقيط بالأنف.

ولتقيـيم اللقـاح المستضـعف لابد أن يكون نقيا وآمنا عند استخدامه فى حيوانات المعمل والعجول.

تـم دراسة كفاءة اللقاح فى العجول ودراسة هذه المناعة المكتسبة باستخدام ١٢ عجلا قابلة للعـدوى يتراوح عمرها من ٦-٩ شهور وخالية من الأجسام المضادة للفيروس المستخدم فى تغيير اللقـاح. وتم تحصينها عن طريق التنقيط بالأنف باستخدام جرعة مقدارها ٢ مل تحتوى على (٦,٥ لوج١٠ قادرة على إحداث أثر مرضى فى ٥٠% من الخلايا المعدية)، وتم تقييم المستوى المناعى عن طـريق اختـبار السـيرم المتعادل واختبار الاليزا، ووجد أن كلا من الاختبارين يتفقان فى مستوى الأجسام المناعية.

وقد أجرى اختبار التحدى باستخدام الفيروس الضارى ووجد أن اللقاح ذو كفاءة عالية قادرة على صد العدوى التجريبية.