Egypt. J. Agric. Res., 78 (4), 2000.

EVALUATION OF INACTIVATED BVD VACCINE IN SHEEP

HUSSIEN METWALLY MOHAMED GHALY

Veterinary Serum and Vaccine Research Institute, Agricultural Research Centre, Ministry of Agriculture, Dokki, Giza, Egypt

(Manuscript received 21 November 1999)

Abstract

To study the efficacy of inactivated BVD virus for protection of the Baladi sheep against pestivirus infection (Border disease), BVD-antibody free twelve lambs were immunized with two doses of the locally prepared BVD, IBR and PI-3 combined inactivated vaccine (Pneumo-3 vaccine) 2 weeks apart. Challenge test was done one month after the first inoculation. The vaccine evoked resistance against the challenge with virulent BVD virus, and a protective level of antibodies persisted up to the 6th month after vaccination.

INTRODUCTION

Sheep are considered one of the most valuable source for wool industry, milk and meat production in Egypt. There are several diseases affecting sheep and goats characterized by respiratory and digestive disorders which cause high economic losses due to high mortality rate , especially, in young lambs. It has been suggested that the BVD virus spread in most instances from cattle to sheep (St. George 1971). Also, French *et al.* (1974) reported that the virus could be transmitted from infected cattle to susceptible sheep. The neonatal lambs become infected with BVDV more than the older sheep in the same environment (Carter *et al.*, 1972 and Terlecki *et al.*, 1980). Also,the surviving hairy lambs were the main reservoir of infection by BVDV with the flock (Westbury *et al.*, 1979 and Terpestra, 1981). The affection of sheep by BVDV called border disease which caused severe losses in USA (Osburn *et al.*, 1972) in California (Clark and Osburn, 1975) and recently in Germany (Liess *et al.*, 1983). In Egypt, Hafez (1973 b) found antibodies to BVD-MD virus in sera of sheep from different localities. The prevention of such problem is achieved principally with the vaccination of lambs. The aim of this study is an attempt to evaluate inactivated BVD virus constituent to the pneumo-3 vaccine.

MATERIALS AND METHODS

1- Animals: Twelve male Baladi lambs aged about 4-6 months, free of detectable neutralizing antibody to BVD virus were used in this study.

2- Vaccine: Combined inactivated vaccine containing bovine virus diarrhoea (BVD), infectious bovine rhinotracheitis (IBR) and parainfluenza-3 viruses were prepared from the local strains of viruses and inactivated by binary ethyleneimine. The antigens were adsorbed by alhydrogel in a bottle (100 ml capacity). This vaccine was produced in Rinderpest Like Diseases Department, Veterinary Serum and Vaccine Research Institute, Agricultural Research Centre.

3- Virus

a. Vaccine strain of BVD virus: This was prepared from bovine virus diarrhoea mucosal disease virus, Egyptian Iman strain, isolated from a Frezian calf with severe pneumoenteritis at Tahrir Province, 2nd plaque purified (Baz, 1975).

b. Challenge virus: This was BVD-MD virus Kena strain (Baz *et al.*, 1982 b). The third passage of this virus was used for the inoculation of lambs as a virulent field strain virus.

4- Cell line culture: Madin Darby Bovine Kidney (MDBK) cell line was obtained from Ames Iowa Laboratories, USA. Cell monolayers were grown in Eagle's MEM supplemented with 10% new born calf serum.

5- Serological test:

a. Serum neutralization test (SNT): It was performed according to Robson *et al.* (1960).

b. Enzyme Linked Immunosorbent Assay (ELISA): This was adopted for antibodies to BVD virus according to Voller *et al.* (1976).

Immunization of lambs:

All experimental lambs were kept under observation for 10 days before vaccination, where general clinical examination was carried out. Six (6) lambs were immunized with combined inactivated vaccine (Pneumo-3 vaccine), 3 ml intramuscularly, by two injections, 2 weeks apart according to Marzoria *et al.* (1979). Serum samples were collected from each lamb every 3 days after the first dose of vaccination, then, every week

after the booster dose for 2 weeks, followed monthly up to 6 months. The other six calves were not vaccinated and kept as contact control. One month after vaccination, three lambs from vaccinated group were challenged with virulent BVD virus by 3 ml inoculated intravenously, and other intramuscularly, according to Zuffa and Feketeova (1980). The other three vaccinated lambs were left for studying the duration of immunity against BVD. Challenged lambs were clinically examined every days for 2 weeks after inoculation with collection of blood samples, nasal, conjunctival discharges and rectal swabs (El-Trabili *et al.*, 1983).

RESULTS

BVD antibody-free lambs aged between 4-6 months vaccinated with two doses of Pneumo-3 vaccine (a combined inactivated BVD, IBR, and PI-3 viruses vaccine) two weeks apart, challenged with virulent BVD virus at the 1 st month post-immunization did not develop clinical signs, except slight viremic reaction exhibition between the 2 nd and 5th days post-challenge. This was specifically confirmed by reisolation of challenged virus from their blood samples and swabs (Table1), while, the unvaccinated lambs challenged with virulent BVD virus showed severe febrile illness 4 to 8 after inoculation. During this time, respiratory distress with ocular and nasal discharges were observed, with excessive salivation, congestion and erosion of buccal and nasal mucosa. Two lambs were recovered fully by the 14 th day post-challenge, and one lamb developed diarrhoea. Challenge virus was isolated from different samples isolated during the febrile reaction between the 2nd and 8th days post-challenge (Table 1).

The pattern of antibody formation in inoculated lambs was studied until the 6th month after vaccination as shown in Table 2 and Fig.1. The results showed the effect of effect of vaccination and challenge to the subsequent antibody response (Table 3).

In vaccinated lambs, BVD-neutralizing antibodies became detectable in serum samples collected on the 14th day post-vaccination (dpv), with a mean titre of 0.35,then, increased to 1.25 one week after boostering and reached the peak one month later (1.75) followed by gradual decline until the 6 th month post-vaccination with a mean titre of 0.9.

In vaccinated lambs, challenge virus inoculation induced high antibody titres. Unvaccinated lambs developed detectable antibodies with mean titres of 0.65 at the 2nd week post-challenge (Table 3). The average of ELISA antibody titres against BVD virus in sera of vaccinated lambs were shown in Table 4. It was protective at 4 weeks post-vaccination, and sufficient to protect susceptible animals from infection with the virulent viruses.

DISCUSSION

The present study was carried out to evaluate efficacy of inactivated BVD virus vaccine constituent in the pneumo-3 vaccine in susceptible Baladi lambs which has been developed by the Veterinary Serum and Vaccine Research Institute. The vaccine containing 6 $\log_{10}$ $TCID_{50}$/ml of BVDV, Iman strain (Egyptian strain isolated from cattle affected with BVDV) (Baz, 1975), inactivated with binary ethyleneimine and adsorbed on alhydrogel adjuvant was sufficient to immunize lambs against challenge BVD virus by the use of two doses of the vaccine, 2 weeks apart according to Koves *et al.*, (1982). The clinical observation indicated that lambs of vaccinated group remained in good condition for 14 days post-vaccination.

The vaccinated challenged group of lambs showed only a slight increase of body temperature which indicated efficacy of the vaccine and protecting lambs against the challenge with the virulent virus, while, the non-vaccinated challenge lambs looked dull and depressed from the 2nd days post-infection with appearance of serous nasal discharge and high increase of body temperature. On the 3rd day post-infection, the observed signs subsided by the end of the 1st week. On the 8th day post-infection, one lamb showed hurried respiration, excessive salivation, congestion and erosions of buccal mucosa. The condition relieved after few days. Two weeks later, the lamb became anorectic and shed watery diarrhoea (Table 1).

The results showed that the first dose was just primed the vaccinated lambs to properly respond to the booster dose, and no adverse effects of vaccination appeared (Salsbury, 1984 and Lehankuhl and Cutlip, 1985).The mean serum neutralizing antibodies titres expressed as $\log_{10}$ developed in vaccinated lambs at 28 days post- vaccination was higher than the minimum accepted titre, and was sufficient to protect susceptible lambs from infection with virulent virus. Bittle (1968) reported on the protective neutralizing antibodies titres of BVD 1:8 or 0.9 $\log_{10}$ (Table 2, Fig. 1). Also, the results of ELISA in Table 4 showed that the average of ELISA titres of vaccinated lambs against BVD antigen was protective at 4 weeks post-vaccination according to Scherrer and Rernard (1977).

From the above studies, it could be concluded that, the prepared local vaccine produced protective level of immune response which protected the lambs from pneumoenteritis syndrome infection safely in Egypt.

Table 1. Reisolation of BVD virus from buffy coat, nasal conjunctival and rectal swabs collected from vaccinated challenged and control infected lambs post-infection.

Animal	Sample	Reisolation of BVD virus post challenge / day
Vaccinated Challenged lambs	B.C.	2 - 4 days
	N.S.	2 - 5 days
	C.S.	-
	R.S.	-
Control infected lambs	B.C.	2 - 8 days
	N.S.	2 - 12 days
	C.S.	2 - 18 days
	R.S.	4 - 11 days

B.C. : Buffy Coat.
N.S. : Nasal Swab
C.S. : Conjunctival Swab
R.S.: Rectal Seab.

+ : BVD virus reisolation.
- : No virus reisolation

P.ch: Post challenge

Table 2. The immune response of lamb vaccinated with combined inactivated vaccine (pneumo-3) constituent to BVD-MD virus (Group 1) and contact control (Grounp 2).

	Log_{10} serum neutralizing antibody titres post vaccination with pneumo-3											
Time →	0 D	3 D	7 D	10 D	14 D	21 D	28 D	2 M	3 M	7 M	5 M	6 M
Animal group ↓												
Vaccinated lambs group (1)	0.00	0.00	0.00	0.00	0.35	1.25	1.75	1.85	1.55	1.3	1.10	0.9
Contact control group (2)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

D : Days M : Month.

Table 3. The immune response of vaccinated lamb by the combined inactivated vaccine (pneumo-3) constituent to BVD-MD post challenge with virulant BVD-MD virus.

Time ➢	Log $_{10}$ serum neutralizing antibody titres post challenge													
Animal group ↓	0 D	1 W	2 W	3 W	28D.P.ch	70D.P.ch	14 D.P.ch	21D.P.ch	1 M.P.ch	2 M.P.ch	3 M.P.ch	4 M.P.ch	5 M.P.ch	6 M.P.ch
Vaccinated challenged group (3)			0.30	1.25	1.75	1.50	1.75	1.75	1.75	1.75	1.55	1.55	1.55	1.55
Control infected group (4)							0.65	1.50	1.75	1.75	1.75	1.75	1.55	1.55

DVP : Days Post Vaccination.
D.P.ch : Days Post Challenge

D.ch : Days of challenge
P.ch: Post challenge.

Table 4. Averge ELISA titre of lambs post-vaccination by pneumo-3 constituent to BVD virus.

Animal	Titre range	Weeks Post Vaccination								
		0	2	4	6	8	12	16	20	24
Vaccinated lambs	200-8000	<1000	2270	2650	3560	4220	5890	5920	3223	2232
Contact control	< 1000	<1000	<1000	<1000	<1000	<1000	<1000	<1000	<1000	<1000

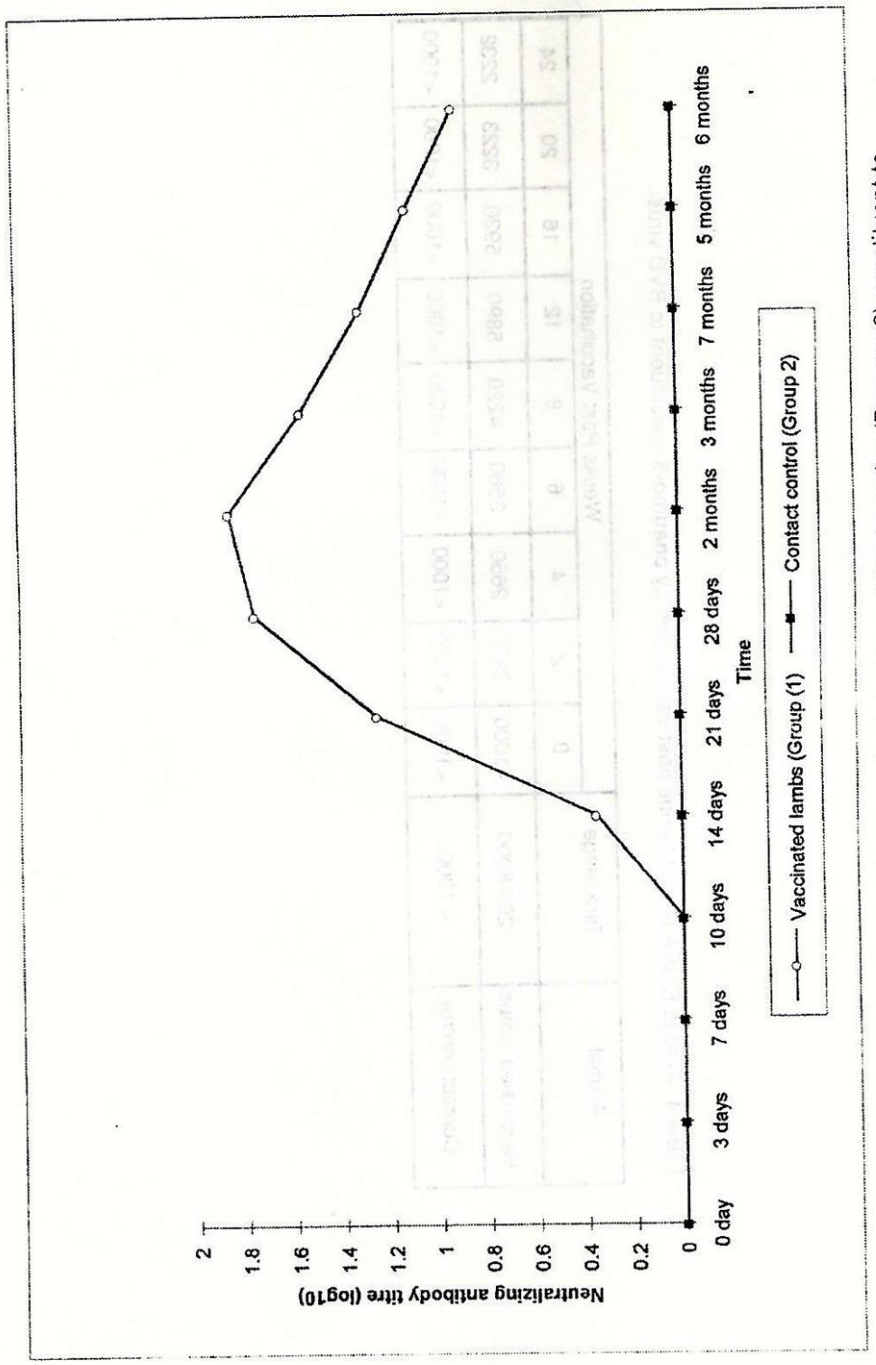

Fig. 1. The immune response of lamb vaccinated with combined vaccine (Pneumo-3) constituent to BVD-MD virus (Group 1) and contact control (Group 2).

REFERENCES

1. Baz, Thanaa, I. 1975. Isolation, characterization and serological studies on bovine viral diarrhoea mucosal disease in Egypt. Thesis, Ph.D., Fac. Vet. Med., Cairo Univ.

2. Baz, Thanaa, I., M.M. Taha, H. Monira Zahran and I. Aida, El-Dobeigy. 1982. Respiratory viral newborn calf diseases in Egypt. 1. Studies on the role of infectious bovine rhinotracheitis (IBR) infection and colostral antibodies. Agric. Mag., 60 (1): 105-122.

3. Bittle, J.L. 1968. Vaccination for bovine viral diarrhoea mucosal disease. J. Amer. Vet. Med. Assoc., 192 (6):861-865.

4. Carter, S. Terlecki and I.G. Shav. 1972. Experimental border disease of sheep. Br. Vet. J., 128:421.

5. Clark, G.L. and B.I. Osburn 1975. Border disease like syndrome in California lambs. Proc. 18th Ann. Meet. Am. Assoc., Vet. Lab. Diagnostician, 303-325.

6. El-Trabili, M.N.A. 1983. Studies on calf viral pneumonia in Egypt. Thesis, Ph.D., Fac. Vet. Med., Assiut Univ.

7. French, E.L., D.E. Hore, W.A. Snowdeon, I.M. Parsonon and June Uren. 1974. Infection of pregnant ewes with BVD-MD virus of ovine origin. Aust. Vet.J., 50:45.

8. Hafez, S.M. 1973. Geographical distribution of bovine viral diarrhoea mucosal disease (BVD-MD) in Egypt as determined by detection of neutralizing antibodies in sheep sera. Proc. 11 th Arab. Vet. Cong., Cairo.

9. Koves, B., S. Belak, M. Rusvai and R. Glavits. 1982. Immunization experiments with inactivated PI-3 virus. Acta Vet. Acad. Sci. Hung., 30 (1-3): 51-58.

10. Lehmkuhl, H.D. and R.C. Cutlip 1985. Vaccinated with a modified live IBR-PI-3 vaccine. Cand. J. Comp. Med., 49 (1): 58-62.

11. Liess, B., S. Orban and H.R. Frey. 1983. Diagnosis of border disease of sheep, exemplified by an outbreak of abortion, wasting and lamb mortality. Tierarztliche Umschau, 38 (8): 547.

12. Morzoria, S.P., M.S. Richards and J.V. Harkness. 1979. A field trial with a multicomponent inactivated respiratory viral vaccine. Vet. Res., 105:410-414.

13. Osburn, B.I., G.L. Crenshaw and T.A. Jackson. 1972. Unthriftiness, hairy fleece and tremors in newborn lambs. J. Am. Vet. Med. Assoc., 160:422-445.

14.Robson, D.S., J.H. Gillespie, and J.A. Baker. 1960. The neutralization test as an indicator of immunity to virus diarrhoea. Cornell Vet., 50: 503-509.

15. Salsbury, D.I. 1984. Control of respiratory disease and border disease in sheep. Experimental use of modified live virus intranasal IBR/PI-3 and modified live virus bovine viral diarrhoea vaccines.

16. Scherrer, R. and S. Rernard. 1977. Application d'un technique immuno enzymologique (ELISA) la detection du Rota virus bovine et des anticorps diriges control lae. Ann. Microbial. (Inst. Pasteur), 128 (A): 499-510.

17. St. George. 1971. Actively and passively acquired antibody to mucosal disease and para-influenza-3 viruses in sheep. Aust. Vet. J., 47 (9) : 428:533.

18. Terpestra, C. 1981. Border disease: virus persistence antibody response and transmission studies. Res. Vet. Sci., 30:185-191.

19. Terlecki, S., C. Richardson, J.T. Done, J.W. Harkness, J.J. Sands, D.S.D. Patterson, and D. Sweasy. 1980. Pathogenicity for the sheep foetus of bovine virus diarrhoea mucosal disease virus of bovine origin. Brit. Vet. J., 136 (6):602-611.

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

21. Westbury, H.A., D.V.H. Naphine and E. Straube.1979. Border disease: persisted infection with the virus. Vet. Rec., 104:406-409.

22. Zuffa, A. and N. Feketoeva. 1980. Protective action of inactivated adjuvant IBR vaccine against experimental infection. Veterinarri Medicina, 25 (1):51-61.

تقييم لقاح الإسهال الفيروسى المعدى الميت فى الأغنام

حسين متولى محمد غالى

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

تم اختبار كفاءة لقاح الإسهال الفيروسى المعدى الميت الموجود باللقاح الثلاثى (النيمو -٣) والذى يضم أيضاً كلا من البارإنفلونزا -٣ والتهاب القصبة الهوائية المعدى . وقد تمت هذه الدراسة على مجموعة من الحملان الصغيرة التى يتراوح عمرها بين ٤ -٦ شهور حيث تم حقن ستة حملان (عدد ٦) بلقاح النيمو-٣ بجرعتين بينهما خمس عشر يوماً وترك ستة أخرون كضابط التجربة . ثم أخذت عينات قبل وبعد الحقن لمدة ٦ شهور متتالية لاختبار وجود أجسام مناعية خاصة بالمر ض. وأثبت بالفحص المصلى قبل إجراء التجربة عن عدم وجود أجسام مناعية بينما وصل أعلى متوسط معيار للأجسام المناعية المضادة للفيروس الى ١,٧٥ بعد شهر من التحصين. وبذلك أثبتت هذه الدراسة ان التحصين باللقاح أعطى مناعة واقية ضد الفيروس بعد ٢١ يوماً من التحصين واستمرت حتى ٦ شهور مما يقلل من حدوث النزلات المعدية الشعبية فى الحملان الصغيرة.