Egypt. J. Agric. Res., 81 (4), 2003.

PRODUCTION AND EVALUATION OF A NEWLY ADJUVANTED RABBIT PASTEURELLOSIS VACCINE

DAOUD, A.M., MANAL S. MAHMOUD, HALA A. FADL, ELHAM A. YOUSSEF AND A.H. HASSAN

Veterinary Serum and Vaccine Research Institute, Agricultural Research Center, Ministry of Agriculture, Giza, Egypt.

(Manuscript received 12 July 2003)

Abstract

Rabbit pasteurellosis aluminium hydroxide gel adjuvanted vaccine (ALV) and aqueous formalized one (AV) were evaluated serologically and by 2-stage protection bioassay. Evaluation in mice revealed a good protection level (85%) in either ALV or AV vaccinates, when subjected to homogenous challenge exposure. In rabbits, both vaccines stimulated satisfactory and significantly differed antibody levels. However, level recorded in ALV vaccinates persisted for more prolonged period (16^{th} week post–boostering, PB) than in case of AV. Moreover, sera from rabbits immunized with ALV or AV form passively protected mice at a levels of 70% and 60%, respectively.

The 1^{st}-stage bioassay conducted at 3^{rd} weeks PB revealed no difference in the level of protective immunity (80%) between rabbits vaccinated with either forms of vaccines. However, the 2^{nd}-stage bioassay conducted at 16^{th} week PB revealed a superior protective level (70%) in ALV vaccinates versus 50% only in AV ones.

The ALV-based *Pasteurella* vaccine proved to be safe and effective alternative to the classical aqueous one for controlling *P. multocida* infection in rabbits.

INTRODUCTION

Pasteurellosis is one of the most common diseases affecting rabbits, causing heavy economic losses. The disease manifestation ranges from fatal septicaemia, severe pleuritis and pneumonia to less severe sequellae such as multiple abscesses, chronic rhinitis (Flatt, 1974). The inactivated vaccines still represent a safe and effective means for controlling such infection (Digiacomo and Deeb, 1989). Two forms of rabbit pasteurellosis inactivated vaccines are locally produced. The first form is the aqueous formalized vaccine, while, the second form is the oil adjuvanted one. Effective vaccination programs against rabbit pasteurellosis, should involve usage of both forms for priming and boostering purposes, respectively (Camerson and Smit, 1970). The ma-

jor shortcoming of the aqueous formalized vaccine is the need for injecting rabbits several times to assure effective immunity which naturally is of short lasting duration (Borkowska *et al.* 1995). So, potentiation of such vaccine with an acceptable adjuvant in urgently needed.

Adjuvants like aluminium hydroxide gel (Alhydragel) act as safe and effective immunopotentiator that commonly incorporated several human and veterinary vaccines (Stewart, 1996). It stimulates an earlier, higher and long lasting immunity after primary vaccination. Moreover, the depot formed at the site of its inoculation is very small, less initiating and short lasting than those induced by the oil adjuvant.

This work was planned to study the immunity produced by using prepared aluminium hydroxide gel *Pasteurella* vaccine compared with that of the routinely used aqueous one in rabbits and testing its immunological efficacy for rabbits.

MATERIALS AND METHODS

1. Laboratory Animals

A. Mice

A total of 120 Swiss mice (20-25gm each), was used for vaccination, challenge, as well as, passive protection assays.

B. Rabbits

A total of forty-eight healthy Giza rabbits (1.5-2kg) were proved to be clinically free from pasteurellosis and serologically negative to antibodies of *P. multocida.* They were used to test the prepared vaccines.

2. Strains

Four *P. multocida* lapinized strains were used for vaccine preparation as well as, challenge purposes. Such strains belonged to serogroup A (5:A, 8:A & 9:A) and serogroup D (2:D).

3. Vaccine preparation

Two forms of inactivated polyvalent rabbit pasteurellosis vaccines were used. The 1st aqueous form represents the commercial local vaccine was prepared according to Borkowska *et al.* (1995).

The 2nd form was prepared similarly, and an alhydragel adjuvant at a rate of 25% was added as recommended by Blackall and Reid (1987). Such vaccines were coded AV and ALV, respectively.

Experimental design

Two experiments were conducted in the present study as follows:

Experiment I

It was conducted for preliminary testing of the protective efficacy of the experimentally produced alhydragel adjuvanted vaccine ALV in laboratory mice. For that purpose, three groups of mice were formulated (25 mice in each). Mice in the 1st and 2nd group were intraperitoneally injected with 0.2ml of ALV and AV, respectively; two weeks later, each mouse had received a second similar inoculation. Mice in the 3rd group were kept as unvaccinated controls. Three weeks post the secondary inoculation; all mice including the unvaccinated controls were intraperitoneally challenged with 0.1ml of 10^9 dilution of pooled virulent culture of *P. multocida* strains. All challenged mice were observed for 5 days post challenge where challenge-related mortality was recorded.

Experiment II

This experiment was conducted in rabbits for specific and extensive immunological evaluation of the experimentally produced ALV. Rabbits in two separate groups and a 3rd unvaccinated group (20 rabbits each) were vaccinated subcutaneously and boostered at three weeks later with 0.5ml/rabbit of ALV and AV, respectively. Following primary vaccination, sera were collected at weekly intervals for 3 weeks from vaccinated rabbits as well as, from unvaccinated controls, while, post-boostering, sera were collected every other week till the end of this experiment (16 weeks).

Challenge procedures were done at two stages. In the first stage, 50% of all vaccinated animals, as well as, their corresponding unvaccinated animals were challenged at the 3rd week post-boostering. Thirteen weeks later, the 2nd stage of challenge was done on the remaining 50% of the experimental rabbits. In either stages, challenge procedure was done by using 0.2ml of 10^8 culture dilution of virulent *P. multocida*. Rabbits were observed for 14 days post-challenge where morbidity and mortality were recorded. *P. multocida*-related mortalities were confirmed through gross lesions and re-isolation of the challenging bacterium from internal organs of necropsied dead rabbits.

Passive protection test

Two groups, each of 20 mice, were intraperitoneally injected with 0.1ml per mouse of antisera of rabbits vaccinated with either AV or ALV vaccines. Such sera were collected on the 3rd week post-secondary vaccination with either vaccines. A 3rd group was similarly inoculated with normal sera of unvaccinated control rabbits. Sixteen hours later, all mice in the three groups were challenged with 0.1 ml of 10^9 dilution of virulent *P. multocida* culture as mentioned by Heddteston *et al.* (1965). Mortalities were recorded in all challenged mice for five days post-challenge.

Serology

Serum samples collected during the present study were tested serologically for *P. multocida* antibodies using the indirect hemagglutinin test (IHA) described by Carter and Roppy (1973), as well as, ELISA test described by Borkowska *et al.* (1996).

RESULTS AND DISCUSSION

In the present study, the protective efficacy of the different experimental preparations of *P. multocida* vaccines were initially assayed in mice. It has been recommended that mice act as ideal model for such assays (Okerman and Spanoghe, 1980). The results summarized in Table 1 indicate that either AV or ALV induced the same protection level (85%) in vaccinated mice. Almost, similar findings were reported by Camerson and Smit (1970).

Table 1. Protective effect of different preparations of *P. multocida.* vaccines in mice after challenge exposure.

Mice groups	Type of vaccine	Death/No. challenged	Protection
I	AV	3/20	85%
II	ALV	3/20	85%
III	Unvaccinated controls	20/20	0%

AV = Aqueous vaccine.

ALV = Aluminium hydroxide gel adjuvanted vaccine.

Serological assays for *P. multocida.* antibodies are shown in Table 2, and Fig. 1, 2. Both vaccines gave significant levels of *P. multocida.* antibodies in vaccinated rabbits compared to unvaccinated controls. During the first two weeks post primary vaccination, the aqueous vaccine (AV) induced higher and earlier antibody titres in vaccinated rabbits than those injected by alhydragel vaccine (ALV). Such finding could be attributed to the rapid absorption of AV (Aqueous form) compared to ALV (Adjuvanted form). The major immunoglobulin fraction induced in response to such primary vaccination is the IgM (Solano *et al.* 1983). Expression of such fraction reflects a general immune response rather than specific immunity against pasteurellosis. Indirect hemagglutination test (IHA) has been applied for determining IgM level, while, the immunoglobulin fraction (IgG) responsible for actual protection has been determined in ELISA (Manning *et al.* 1989). The IHT values recorded throughout this experiment were inconsistent and cannot be correlated to the protection level as also documented by several researchers (Alexander and Soltys, 1973). Boostering of all vaccinated rabbits with their respective vaccine resulted in a higher level of protective serum of immunoglobulins (IgG) as determined in ELISA. The level of IgG in sera of either AV or ALV vaccinated groups was almost the same, however, by the 8^{th} week post-boostering, it was reduced in AV vaccinated group. IgG level in sera of ALV vaccinated rabbits persisted at a significantly higher level until the end of this experiment. This is due to the depot formed at the site of ALV injection producing long lasting immunity through continuous slow release of the vaccinal antigen into circulation (Holt, 1950). In this respect, Bunn (1993) mentioned that alhydragel adjuvant potentiated the specific IgG response to a vaccinal antigen compared to the non-adjuvanted one.

Table 2. Monitoring of *P. multocida.* antibodies in sera of rabbits vaccinated with different preparations of *P. multocida* vaccines.

Rabbit group	Vaccinated with	Serological assay	Pre-vacc. Titre	Mean P. *multocida.* antibody titre										
				Weeks post-primary vaccination			Week post 2nd vaccination (boostering)							
				1	2	3	2	4	6	8	10	12	14	16
I	AV	IHA	5	80	150	165	640	320	480	210	320	80	160	210
		ELISA	25	351	534	595	1680	1990	1910	1320	1080	890	685	595
II	ALV	IHA	5	20	80	180	480	320	640	210	640	480	320	160
		ELISA	25	165	345	430	1875	1810	2400	2350	1995	2340	2150	1880
III	Unvaccinated	IHA	5	5	5	5	10	5	5	10	20	10	10	5
	control	ELISA	24	24	33	33	15	20	20	28	30	30	22	20

AV: Aqueous Vaccine.

ALV: Aluminium hydroxide gel vaccine.

IHA: Indirect Haemagglutination.

Regarding challenge assay (Table 3) for the two inoculated vaccines in rabbits, it was clear that a satisfactory protective level (80%) for both vaccines was determined, when the animals were challenged three weeks post-boostering, and it was reduced to 50% in AV vaccine and to 70% in ALV, when the animals were challenged 16 weeks post-boostering. This is due to the long lasting immunity of alhydragel as stated by Stewart-Tull, (1996).

Table 3. challenge exposure results recorded in rabbits vaccinated with different preparations of experimental pasteurellosis vaccines.

Rabbit group	Type of vaccine	1st challenge results*		2nd challenge results**	
		D/C	Protection rate	D/C	Protection rate
I	AV	2/10	80%	5/10	50%
II	ALV	2/10	80%	3/10	70%
III	Un-vaccinated controls	4/4	0%	4/4	0%

AV = Aqueous vaccine.

ALV = Alhydragel vaccine.

* = Performed at the 3rd week post-boostering.

** = Performed at the 16th week post-boostering.

D/C = Deaths / No. challenged

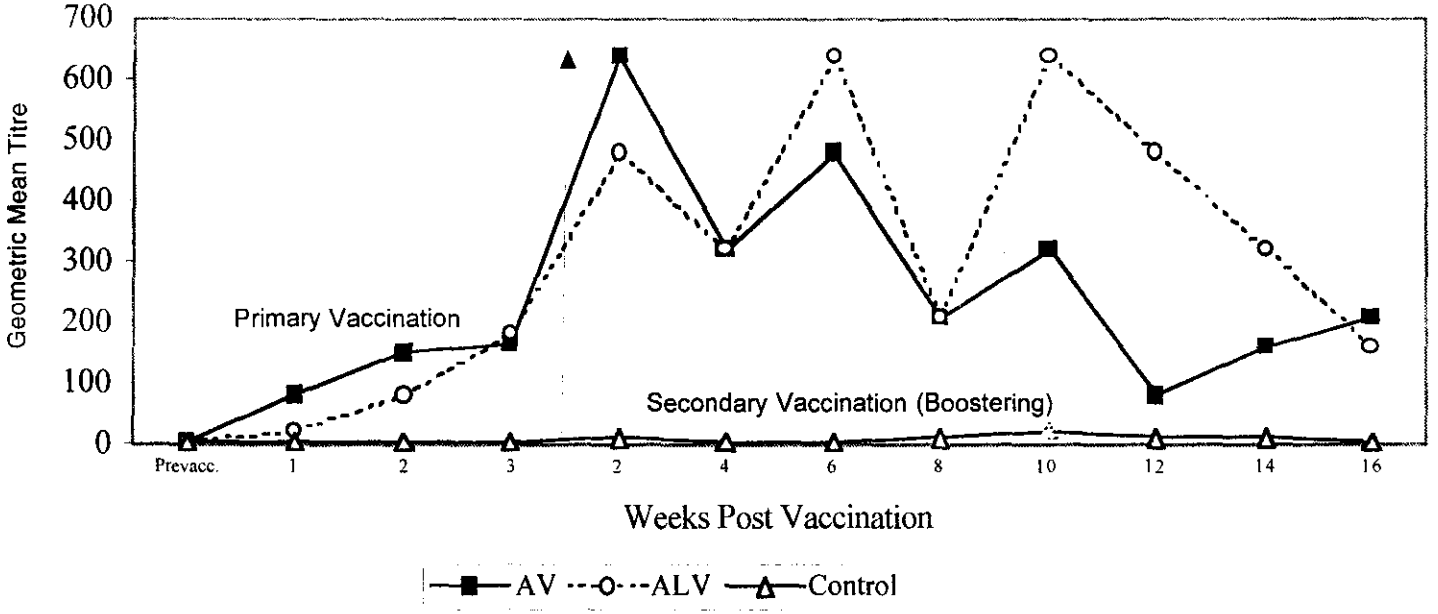
The results of the passive protection test (Table 4) indicated that protection immunity could be transferred to mice with antiserum taken from rabbits vaccinated with either ALV or AV preparation. In this respect, Alexander and Soltys (1973) stated that immunity could be passively established in mice inoculated with antiserum from *P. multocida* vaccinated turkeys.

From results obtained in this experiment, it could be concluded that alhydragel pasteurellosis vaccine proved to be safe and effective alternative to the classical aqueous one for controlling *P. multocida* infection in rabbits.

Table 4. Passive protection in mice injected with antisera from rabbits vaccinated with different preparation of *P. multocida.* vaccines.

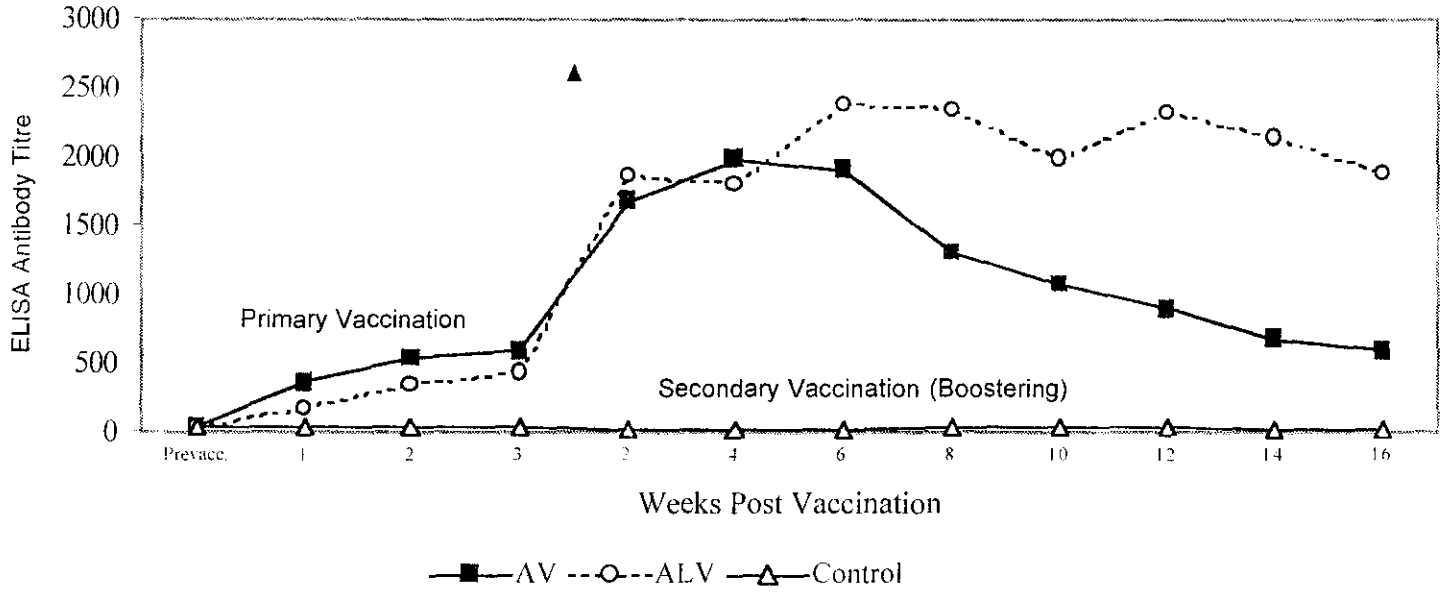
Source of antisera	Death/No. injected	Protection level
AV vaccinated mice	8/20	60%
ALV vaccinated mice	7/20	70%
Unvaccinated controls	20/20	0%

Fig. 1. Monitoring of *P. multocida* antibodies (IgM) in sera of rabbits vaccinated with different forms of pasteurellosis vaccines as determined in indirect haemagglutination test.



AV: Aqueous Vaccine. ALV: Alhydragel Vaccine.

Fig. 2. Monitoring of protective *P. multocida* antibody level (IgG) in sera of rabbits vaccinated with different forms of pasteurellosis vaccines as determined in ELISA.



AV: Aqueous Vaccine. ALV: Alhydragel Vaccine.

REFERENCES

1. Alexander A.M. and M.A. Soltys. 1973. Relationship of serum agglutinins to protective immunity produced in turkey immunized against fowl cholera. J. Comp. Pathol., 83: 191-198.

2. Blackall, P.J. and G.G. Reid. 1987. Further efficacy studies on inactivated aluminum hydroxide adsorbed vaccinated against infectious coryza vaccine. Avian Dis., 31: 527-838.

3. Borkowska-Opacka, B., I.R. Jurga, M. Truszczynski and W. Zymski. 1995. Bull. Vet. Inst. Pulway, 39: 115-119.

4. Borkowska-Opacka B., A. Kedrak and M. Truszezynski. 1996. Bull. Vet. Inst. Pulway, 40: 97.

5. Bunn T.O. 1993. Vaccine adjuvants and carriers. In vaccines for Veterinary application. A.R. Peters ed Butter Worth-Heinemann. T.D., Boston, Mass, PP 295-306.

6. Camerson C.M. and G. Smit. 1970. Immune response of rabbits, mice and sheep to polyvalent *pasteurella* vaccine. Onderstepoort J. Vet. Res., 37 (4): 217-224.

7. Carter, G.R. and D.E. Roppy. 1973. A haemagglutination test employing specific lipopolysaccharides for the detection and measurement as *pasteurella* antibodies to *P. multocida*. Bri. Vet. J., 119: 73-77.

8. Digiacoma, R.F. and B.J. Deeb. 1989. Pasteureilosis (Snuffles) in rabbits-sequelae and vaccination. J. Appl. Rabbit Res., 12 (1): 10-14.

9. Flatt, R.F. 1974. Bacteriological diseases in Weishbrath S.H. The Biology of the laboratory rabbits. New York, Academic Press Inc., 194-232.

10. Heddteston, K.L., P.A. Rebers and A.E. Ritchie. 1965. Immunizing and toxic properties of particulate antigen from two immunogenic types of *pasteurella* multocida of avian origin. J. Immunol., 96 (1): 124.

11. Holt, L.B. 1950. Development in diphtheria prophylaxis. William Heinemann, London.

12. Manning P.J., R.F. DiGi Como and D. Delong. 1989. Pasteurellosis in laboratory animals. In. *pasteurella* and pasteurellosis Acad. Press 1989, P. 283.

13. Okerman L. and L. Spanoghe. 1980. Zbl. Vet. Med. Reihe, B 27, 759.

14. Solano, J.J., V.S. Giambrone and Panangola. 1983. Comparison of enzyme linked immunosorbent assay and indirect hemagglutination test for quantitating antibody response in chickens against *P. multocida.* Avian Dis., 27 (4): 1034-1042.

15. Stewart-Tull D.E. 1996. The use of adjuvants in experimental vaccines. In vaccine Protocol, Humona Press, Totwa, N.J.

إنتاج وتقييم لقاح ضد عدوى الباستريلا في الأرانب بمساعد مناعى مستحدث

أحمد محمود داود ، منال سيد محمود ، هالة أحمد فضل ، إلهام علام يوسف ، حسن عبد المقصود حسن

معهد بحوث الأمصال واللقاحات البيطرية – مركز البحوث الزراعية – وزارة الزراعة – الجيزة – مصر.

فى تلك الدراسة تم تحضير لقاح تجريبى مضاد لعدوى الباستريلا ملتوسيدا فى الأرانب يحتوى الألومنيوم هيدروكسيد (الهيدراجيل) كمساعد مناعى.

وقد تم تقييم الكفاءة المناعية لهذا اللقاح ومقارنتها بمثيلاتها الناجمة عن اللقاح التقليدى المائى (الفورمالينى) حيث أثبتت التجارب الأولية على فئران التجارب قدرة أى من تلك اللقاحات على توفير حماية عالية (٨٥٪) للفئران المحصنة ضد العدوى بالميكروب الضارى.

أما فى الأرانب فأنه وبرغم تماثل مستويات الأجسام المناعية الناجمة عن التحصين بأى من تلك اللقاحات فأن تلك المستويات الجيدة دامت لفترة طويلة (ست عشر أسبوعاً بعد الجرعة التنشيطية) فى الأرانب المحصنة باللقاح التجريبى بينما سجلت هبوطاً ملحوظاً خلال تلك الفترة فى الأرانب المحصنة باللقاح المائى الفورمالينى.

وقد تكرر ثبوت هذا التفوق المناعى للقاح التجريبى عند إجراء اختبار حماية الفئران بالسيرم المجمع من الأرانب المحصنة بهذا اللقاح مقارنة بسيرم الأرانب المحصنة باللقاح الفورمالينى.

أما التقييم العملى للقاح التجريبى فقد تم من خلال إجراء اختبار التحدى للأرانب المحصنة على فترتين (ثلاث وست عشر أسبوعاً بعد الجرعة التنشيطية). وتبين أن القدرة الوقائية للقاح التجريبى أعلى بشكل ملحوظ (٧٠٪) عن مثيلاتها للقاح المائى الفورمالينى ٥٠٪ وذلك عند فترة التقييم الثانية.

مما سبق ذكره يتبين أن اللقاح التجريبى أثبت فاعلية ودرجة أمان عالية مما يجعله بديل مثالى للقاح الباستريلا الفورمالينى المائى للتحكم فى عدوى المرض.