FIELD TRIAL TO EVALUATE THE EFFICACY OF ALUMINIUM HYDROXIDE GEL (ALV) RABBIT PASTEURELLA VACCINE IN RABBITS

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ABSTRACT

In this study the protective value of aluminium hydroxide gel, a newly adjuvant rabbit pasteurella vaccine (ALV) was evaluated in rabbits under field conditions in comparison to the classical aqueous formalized (AV) and oil adjuvant rabbit pasteurella vaccines. A total of 48 two-month old rabbits were used. They were subdivided into four equal groups each of 12 animals. The first group (group A) was given ALV twice subcutaneously (s/c). The second group (group B) given the same vaccine once. The third group (group C) was administered with AV and boostered with oil adjuvant vaccine. The fourth group (group D) was left as negative control. Vaccinated animals were dosed 0.5 ml per rabbit. Each group was subdivided into two subgroups. One of each subgroup was challenged with virulent *Pasteurella multocida* 6 weeks post boostering. Two doses of ALV and one dose of AV + oil adjuvant bacterins induced high levels of indirect haemagglutination antibody titre (IHA) and good protection levels (83.3 %) in comparison with 33.3 % in vaccinated rabbits with one dose of ALV while it was 0% in unvaccinated controls. Both ALV and AV + oil adjuvant vaccine have a beneficial influence on body weight. No harmful effect was observed in rabbits vaccinated with ALV subcutaneously.

INTRODUCTION

Pasteurella multocida is a pathogen causing severe economical losses in many animals including cattle, sheep, buffalo, pigs, dogs, cats, poultry and rabbit. In rabbits the resulting disease ranged from mild upper respiratory tract infection (snuffles), otitis media, enzootic pneumonia, conjunctivitis to acute septicemia (14). Multiple abscesses and chronic rhinitis were recorded by *Flatt* (15).

Prevention is the most likely means of controlling this disease, thus vaccines would be of great value in the protection of rabbits against pasteurelosis (1). lnactivated vaccines are still in use for controlling such infection (13). Two forms of rabbit pasteurellosis inactivated vaccines, formalized and oil adjuvant one used as a primary and boostering purposes, respectively (10).Inducing effective immunity using the aqueous formalized vaccine (AV) needs several injections (4). Adjuvants, like aluminium hydroxide gel as alhydragel are safe and effective immunopotentiators in human and veterinary vaccines (23).

The aim of this work is to study the protective effect of a newly prepared aliuminium hydroxide gel pasteurella vaccine ALV in comparison to aqueous formalized AV and oil adjuvant vaccines in routine use against pasteurellosis in commercial rabbitries.

MATERIAL AND METHODS

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

2- Vaccine preparation : Inactivated polyvalent rabbit pasteurellosis vaccines were used. Aqueous formalized (AV) represents the commercial local vaccine was prepared according to Borkowska *et al.* (4). The new ALV vaccine was prepared similarly to AV vaccine and an alhydragel adjuvant was added at a rate of 25% as recommended by Blackall and Reid (3). AV and oil adjuvant vaccines were obtained from Vet. Serum and Vaccine Research Institute, Abbasia, Cairo.

3- Rabbits : A total of 48 New Zealand white rabbits of 2 months old were used. They had neither history of pasteurellosis nor previous vaccination to *P. multocida*. Experimental rabbits were kept in separate hatches under observation with the other animals in the same farm which containing 100 breeding does and 500 fattening weaned rabbits in Kalyoubia Province. Rabbits in this farm were routinely vaccinated with the aqueous formalized (AV) and the oil adjuvant vaccines.

Experimental design : The 48 experimental rabbits were divided into 4 equal groups (A, B, C and D). They were treated as shown in table 1. Six rabbits from the first three groups and the group D1 were challenged in our laboratory with 0.2 ml of 10^8 of a virulent 5A (X-73) serogroup of *P. multocida* 6wks post the booster dose of the vaccines used according to Heddleston *et al.* (16)**.** Rabbits in group D2 were kept as unvaccinated unchallenged controls as in table 1**.** Rabbits were observed for 14 days post- challenge where morbidity and mortality were recorded. *P. multocida*- related mortalities were confirmed through gross lesions and reisolation of the challenging bacterium from internal organs of necropsied dead rabbits.

Table 1. Animal grouping and vaccination procedures either with ALV or AV and oil adjuvant *P. multocida* vaccines**.**

Rabbit group	Rabbit sub-group	Number	Primary vaccination at 2 months old	Boostering 3 wks Post primary vaccination	Challenged 6 wks Post boostering
A	1	6	ALV	ALV	Yes
	2	6	ALV	ALV	No
B	1	6	ALV	no	Yes
	2	6	ALV	no	No
C	1	6	AV	oil adjuvant	Yes
	2	6	AV	oil adjuvant	No
D	1	6	no-vaccine	no	Yes
	2	6	no-vaccine	no	No

4- Serology: Serum samples were collected before vaccination and weekly after primary vaccination and every 2 weeks post booster dose during this study and were tested serologically for *P. multocida* antibodies using the indirect hemagglutination test (IHA) described by Carter and Rappy (11)**.**

5-Average body weight (gm) of rabbits in all groups were calculated and presented in table 3 and data were statistically analyzed according to Denenberg (12).

RESULTS

Serological assays for *P. multocida* antibodies are shown in table 2 . Both ALV or AV and oil adjuvant *P. multocida* vaccines gave significant levels of *P. multocida* antibodies in vaccinated rabbits compared to unvaccinated controls. Post primary vaccination of alhydragel vaccine (ALV), vaccinated rabbits in group A and

group B showed higher antibody titers in comparison with those vaccinated with aqueous formalized (AV) and oil vaccine in group C . Boostering of rabbits in group A with ALV as a second dose and with oil adjuvant in group C resulted in higher level of protective serum IgM antibodies in comparison with non boostered rabbits in group B and with unvaccinated rabbits in group D as in table 2. The level of protective serum IgM of either ALV two doses vaccinated and boostered and or AV and oil adjuvant rabbits was almost the same, however by the 2^{nd} and 4^{th} week post boostering it was significant increased in AV and oil adjuvant vaccinated rabbits in group C .

Data from table 3 showed a significant increase in body weight in ALV vaccinated and boostered rabbits in comparison to ALV one dose and AV together with oil adjuvant vaccinated group, while there is no significant differences with those unvaccinated rabbits.

Data from table 4 showed that on challenge 6 wks post boostering the protective level for both of ALV two doses and AV together with oil adjuvant was (83.3 %) while it was reduced to 33.3 % in ALV one dose and to 0% in unvaccinated and challenged controls.

Necropsy of dead challenged rabbits showed severe congestion of parenchymatous organs with petechial hemorrhages. Results of isolation of *P. multocida* from internal organs (Heart blood , lungs and liver) of these dead rabbits are presented in table 4. From the table it is observed that the percentage of isolation was 100 % from dead rabbits of group D1 and from dead rabbit of group C followed by 75 % from group B. While no isolation (0%) was reported in group A.

Table 2 . Monitoring of rabbit *Pasteurella multocida* antibodies in sera of rabbits vaccinated with new vaccine (ALV) and classical rabbit pasteurellosis vaccines (AV and Oil adjuvant) as determined by indirect haemagglutination test (IHA).

Rabbit group	Vaccination treatment	Prevaccination mean of IHA antibody titre	Weeks post primary vaccination mean of IHA antibody titre			Weeks post 2nd vaccination (boostering) mean of IHA antibody titre		
			1st wk	2nd wk	3rd wk	2wks	4 wks	6 wks
A	ALV two doses	5	60	100	120	440	480	707
B	ALV one dose	10	70	100	160	80	45	30
C	Av+ oil adjuvant vaccine	10	27.5	60	70	640	720	880
D	Control no-vaccine	10	7.5	6.25	11.25	8.75	10	8.75

ALV : Aluminium hydroxide gel vaccine
AV + oil : Formalized vaccines + oil adjuvant vaccine
IHA : Indirect haemagglutination

Table 3. Mean body weight of New Zealand white rabbits vaccinated either with ALV or AV and oil adjuvant vaccines of *P. multocida.*

Group	Minimum	Maximum	Mean	± S.E	Remarks
A	1725.00	2075.00	1909.37^{a}	67.35	Two doses of ALV vaccine
B	1575.00	1950.00	1762.50^{ae}	78.06	One dose of ALV vaccine
C	1490.00	1805.00	1648.15^{bc}	66.47	**AV and oil adjuvant vaccine**
D	1805.00	2120.00	1936.25^{a}	70.46	unvaccinated control

* Readings / week/ cases
Values with different letters within the same column are significant at $P< 0.05$
ALV = Alhydragel vaccine
AV + oil = Formalized vaccine + oil adjuvant vaccine

Table 4. Protective effect of different *P. multocida* vaccines in New Zealand white rabbits challenged with virulent 5:A (X-73) serogroup of *P. multocida*

Rabbit group	Vaccination treatments	Challenge results		*P.multocida* isolation	
		D/C	Protection rate	Positive / total dead	%
A	ALV 2 doses	1/6	83.3%	0/1	0.00
B	ALV one dose	4/6	33.3 %	3 /4	75
C	AV + oil adjuvant vaccine	1/6	83.3%	1/1	100
D1	Unvaccinated challenged	6/6	00.0%	6/6	100
D2	Unvaccinated Unchallenged	0/6	100 %	0/0	0.00

***** Challenge performed 6 wks post boostering

D/C = Deaths / No of challenged rabbits

ALV = Alhydragel vaccine

AV + oil = Formalized vaccine + oil adjuvant vaccine

DISCUSSION

The protective efficacy of a newly aluminium hydroxide gel adjuvant rabbit pasteurella vaccine (ALV) in comparison to aqueous formalized (AV) and oil adjuvant rabbit pasteurella vaccines were evaluated in rabbits under field conditions in private rabbit farm. ELISA test is generally used to detect IgG fraction (20), while indirect haemagglutination test (IHA) is used to detect the major immunoglobulin fraction IgM induced in response to such vaccination (21). Vaccinated rabbits in the second group with ALV showed higher level of indirect haemagglutinating antibody titer till the 3rd week post primary vaccination then declined dgradually till the end of the experiment, this indicates that the new vaccine (ALV) is not protective enough in one dose of vaccination and booster dose is necessary especially if rabbits reared for breeding purposes. Both ALV in two doses and AV with oil vaccines showed higher IHA antibodies and 83.3% protection on challenge 6 weeks post boostering while rabbits in group B showed 33.3 % protection , this indicates the necessity of boostering with ALV or oil adjuvant vaccine. The high protection level may be due to the higher IHA antibodies IgM and may be also due to IgG which was not checked in this investigation this in agreement with many authors (2, 5,8,9,20) and this is correlated with long lasting immunity reported with alhydragel as stated by *Stewart-Tull* (23) and *Tariq et al.*, (24). The level of the antibodies in serum of rabbits in group A as well as in group C persisted significantly higher until the end of this experiment, this is due to the depot formed at the site of either ALV or oil adjuvant vaccine injections producing long lasting immunity through continuous slow release

of the vaccinal antigen into circulation (17). Results of isolation of *P. multocida* from internal organs of dead challenged rabbits (table 4) showed that, the recovery rate of *P. multocida* was 0 % from dead rabbit of group A (ALV two doses) in comparison to 75 % from dead rabbits of group B(ALV one dose). While 100 % isolation was reported from dead rabbits of group D1 (unvaccinated and challenged) and from dead rabbit of group C (AV+ oil adjuvant vaccine). These findings are partially in agreement with results of Holt (18). No harmful effect was detected on using the aluminium hydroxide gel (ALV) subcutaneously post vaccination, this agrees with Borkowska – Opaka and Javinen (7,19). Two doses of aluminium hydroxide gel (ALV) had a beneficial influence on body weight, reduced mortality and effective protection on challenge than one dose of the same vaccine this in agree ment with many authors (6,7,19,22).

In conclusion it could be concluded that usage of two doses of the new vaccine (ALV) is at least equal to if not better than usage of formalized AV boostered with oil vaccines. ALV was safe and has a beneficial effect on body weight and no harmful effect on subcutaneous tissue injection. This may lead one to suggest its usage in commercial rabbitries on large scale. Further work is needed to cover the economy of these vaccines as well as to cover the results using ELISA test.

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محاولة حقلية لتقييم لقاح جديد (ALV) للباستيريللا مالتوسيدا في الأرانب

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في هذه الدراسة تم تقييم للقاح جديد محضر ضد باستيريللا ملتوسيدا الأرانب بالألمونيوم هيدروكسيد (الهيدراجيل) كمساعد مناعة تحت الظروف الحقلية بالمقارنة باللقاح التقليدى الفورمالينى وكذلك اللقاح التقليدى الزيتى لباستيرللا الأرانب . وقد استخدم 48 أرنب عند عمر شهرين. وقسمت الأرانب إلي أربعة مجاميع كل منها 12 أرنب تم تحصين المجموعة الأولى باللقاح (ALV) بجرعة عن عمر شهرين والثانية بعدها 3 أسابيع من نفس اللقاح والمجموعة الثانية بجرعة واحدة من نفس اللقاح عند عمر شهرين والمجموعة الثالثة تم تحضينها باللقاح الفورمالينى عند عمر شهرين وبعد 3 أسابيع بجرعة من اللقاح الزيتى وتركت مجموعة غير محصنة كضابطة . وقد أظهرت نتائج التتبع السيرولوجى للأجسام المضادة للباستيريللا ملتوسيدا باستخدام اختيار التلازين الدموى الغير مباشر وكذلك اختيار التحدى ان الأرانب المحصنة مرتين باللقاح الجديد (ALV) وكذلك الأرانب المحصنة باللقاح الفورمالينى مع اللقاح الزيتى قد طورت أجسام مناعية ذات قدرة حماية عالية (83.3%) بينما الأرانب التي تم تحصينها مرة واحدة باللقاح الجديد أظهرت قدرة حماية اقل (33.3%) بينما نجد أن هذه الحماية صفرا في الأرانب الغير محصنة . كما لوحظ أن اللقاح الجديد وكذلك اللقاحين الفورمالييني والزينى لهم تأثير إيجابى علي أوزان الأرانب وأنه لم توجد اى اضرار جانبية من إعطاء هذا اللقاح خاصة تحت الجلد .