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Research Article

The Use of Single Dose Dependent Evaluation of Rabbit Pasteurellosis Vaccines in Comparison with Booster Dose Evaluation Assay in Mice

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Abstract

Pasteurellosis is one of the highly contagious bacterial diseases of rabbits caused by *P. multocida* and causes considerable economic loss in large production units throughout the world. Consequently, investigation on the development of effective control and preventive methods are of a vital importance. Therefore it is important to achieve a good vaccine with a good vaccination assay. This work aimed to evaluate the protective effect of a single dose vaccination assay in comparison with booster dose vaccination assay in routine use against pasteurellosis in commercial rabbits. A total of 17 different inactivated formalized used in this study and other 17 oil adjuvent rabbit pasteurellosis vaccine batches, produced by VSVRI were tested by protection mean of vaccination challenge assay method using virulent *P. multocida* strains. The protection mean value of the satisfactory vaccine batches of formalized rabbit pasteurellosis vaccines, was 0.91 log difference between the vaccinated and unvaccinated mice in the single dose assay compared with 2.05 log in the booster dose assay. Meanwhile the unsatisfactory batches it was 0.49 log in the single dose assay compared with 1.08 log in the booster dose assay. Regarding to the satisfactory vaccine batches of oil adjuvant rabbit pasteurellosis vaccines it was 0.93 log in the single dose assay compared with 2.11 log in the booster dose assay. Meanwhile it was 0.52 log in the single dose assay compared with 1.18 log in the booster dose assay in unsatisfactory vaccine batches. Findings of this study proved that the minimum requirement of protection associated with single dose vaccination assay may recommend these finding to be used in the evaluation of rabbit Pasteurellosis vaccine protocols.

Keywords: *Rabbit Pasteurellosis; P Multocida; Dose Vaccine; Booster Dose; Vaccine Evaluation*

Introduction

Respiratory diseases are common in domesticated rabbits. This may vary from a mild, chronic mucopurulent upper respiratory infection (snuffles) to more acute or subacute bronchopneumonia leading to high mortality [1]. Pasteurella multocida is one of the important pathogens that infect rabbits, causing major economic losses in commercial rabbit farming [2]. Pasteurellosis is considered as a common bacterial disease caused by Pasteurella multocida (P. multocida) and has been reported as a constant serious and highly contagious disease of domestic rabbits [3]. Pasteurellosis exhibited 3 forms in rabbits; the first one is snuffles or nasal catarrhal inflammation which is characterized by acute, subacute, and chronic inflammation of the air passages and lungs. This form of the disease often ends with death and the cured animals became carriers. The second form is characterized by abscess formation at any part of the body and the case is terminated with septicemia. The last form is characterized by genital infection, which manifests as acute and subacute inflammation of

uterus and testicles [4]. The elemination of the disease by sanitation and sound management to prevent introduction of the infection and using drugs in prophylactic dose are not sufficient. Therefore the Prevention is the most likely means of controlling this disease, thus vaccines would be of great value in the protection of rabbits against pasteurelosis [5]. lnactivated vaccines are still in use for controlling such infection [6]. Two forms of rabbit pasteurellosis inactivated vaccines, formalized and oil adjuvanted one used as a primary and boostering purposes, respectively [7]. So systemic vaccination of all rabbit 2 months age with local formalized bacterin then boostered after 2-3 weeks is of great value in control of that disease. A reasonable approach to control and eliminate pasteurellosis in rabbits is to develop an improved vaccination assay as the current vaccination assay consumes more time and effort.

The aim of this work is to study and evaluate the protective effect of a single dose vaccination assay in comparison to booster dose vaccination assay to be used as an alternative method for evaluation of rabbit pasteurellosis vaccine.

Materials and Methods

- **Rabbit pasteurellosis vaccines**
    - A total of 17 locally prepared inactivated formalized and other 17 inactivated oil adjuvented rabbit pasteurellosis vaccine batches, produced by VSVRI were selected and tested for relative potency following both single dose and booster dose vaccination by Mouse-vaccination challenge inoculation system using virulent strains of *P. multocida* starting from 2015 up to 2020.
- **Pasteurella multocida strains**
    - Virulent Pasteurella multocida serovars 1, 3, 4 and D_2 were used to perform challenge test. These serovars were supplied from the reference strain bank in CLEVB.
- **Laboratory animals**
- **Mice**
    - A total of 200 Swiss mice weighed about 20-25 gram, were used for evaluation of the efficiency of each of the tested vaccine batches to perform this study which starting from 2015 up to 2020. These mice were obtained from the Laboratory Animals Department, VSVRI, Abbasia, Cairo.
    - Eight Swiss mice weighed about 20-25 gram, two for each P. multocida serovar were inoculated with the stock culture of P. multocida. This was done before every challenge test to rebuild the virulence of P. multocida serovars in a dose of 100- 500 CFU/ mouse intraperitoneally [8].

Mouse-vaccination challenge inoculation system

Immunization of mice

For the vaccine study, This mice were divided into three groups, the first one comprised 50 mice and received only one dose then challenged 3 weeks later to determine the lethal dose fifty (LD50%), the second group comprised also 50 mice and received both primary dose and 3 weeks later received a booster dose then challenged to determine the lethal dose fifty (LD50%) and finally the third group were 100 mice kept as negative unvaccinated control group where 50 mice for each of previous group. All mice were vaccinated with the corresponding R. past. Vaccine batch (0.2ml/dose/mice) subcutaneously [8].

Preparation of challenge strains

Colony count of each of the 4 *Pasteurella multocida* strains was adjusted to 10^6 CFU then mixed together before using as original culture for challenge test.

Challenge test

The vaccinated mice divided into ten groups and challenged with ten serial dilutions from the mixed virulent *P. multocida* strains (5 vaccinated / 5 unvaccinated mice/ each dilution) three weeks post vaccination in case of single dose vaccination assay or two weeks post the second dose in the booster dose vaccination assay. Mortalities were observed and recorded for one week. The Lethal Dose fifty (LD50%) was calculated using the following formula described by the [9].

$$LD50\% = \frac{\text{above50- 50}}{\text{Above50-below 50}}$$

Result

Generally rabbit pasteurellosis vaccines evaluated by sterility, safety and potency tests. Potency testing depends mainly on vaccination- challenge test and determination of lethal dose fifty (LD50).

As shown in (Table 1). A total of 13 out of 17 formalized rabbit pasteurellosis vaccine batches were examined and given satisfactory results for approval to be used in rabbit farms according to the Egyptian standards for evaluation of veterinary biologics [10]. Regarding to protection log values obtained the examined formalized rabbit pasteurellosis vaccine batches were divided into six groups. The protection value in the first group which consists of 3 out of 13 vaccine batches, the vaccinated mice gave protection 0.85 log more than the unvaccinated control mice in the single dose vaccination assay compared to 2.01 log in the booster dose vaccination assay. The second group which consists of 2 out of 13 vaccine batches the protection was 1.03 and 2.12 log, the third group which consists of 3 out of 13 was 0.83 and 2.03 log, the fourth group which consists of only one batch out of 13 was 0.92 and 2.00 log, the fifth group which consists of 2 out of 13 was 0.89 and 2.11 log and finally the sixth group which consists of 2 out of 13 was 1.02 and 2.02 log in the single and booster dose assays for each group respectively.

No of Tested Vaccine Batches	LD 50 of P. Multocida Strains	
	Single Dose Vaccination Assay	Booster Dose Vaccination Assay
3	0.85	2.01
2	1.03	2.12
3	0.83	2.03
1	0.92	2.00
2	0.89	2.11
2	1.02	2.02
total 13	Mean 0.91	Mean 2.05

Table 1: *Protection of Vaccination Challenge Assay in Mice Vaccinated With Either Single or Booster Dose Vaccination Assays of the Satisfactory Tested Formalized Rabbit Pasteurellosis Vaccines.*

By calculating the average of protection value of all 13 vaccine batches, it was found that the protection mean was 0.91 log in the vaccinated mice more than the unvaccinated control mice in the single dose assay compared with 2.05 log in the booster dose assay.

From data available in (Table 2), it can be seen clearly that a total of 4 out of 17 formalized rabbit pasteurellosis vaccine batches were tested and given unsatisfactory results, according to [10] where it got a protection level lower than 2 log which is the minimum requirement for protection. The four vaccine batches gave a protection value 1.42, 1.08, 1, 0.82 log for each in the booster dose vaccination assay while gave a protection value 0.65, 0.52, 0.44, 0.49 log for each, respectively in the single dose vaccination assay.

No of Tested Vaccine Batches	LD50 of P. Multocida Strains	
	Single Dose Vaccination Assay	Booster Dose Vaccination Assay
1	0.65	1.42
1	0.52	1.08
1	0.44	1.00
1	0.36	0.82
total 4	Mean 0.49	Mean 1.08

Table 2: *Protection of Vaccination Challenge Assay in Mice Vaccinated With Either Single or Booster Dose Vaccination Assays of the Unsatisfactory Tested Formalized Rabbit Pasteurellosis Vaccines.*

By calculating the average of protection value of the four unsatisfactory vaccine batches, it was found that the protection mean was 0.49 log difference between the vaccinated and unvaccinated control mice in the single dose assay compared with 1.08 log in the booster dose assay.

The above mentioned results supported by testing also 17 oil adjuvant rabbit pasteurellosis vaccines by vaccination challenge assay and determination of lethal dose fifty (LD50).

From (Table 3) it was noticed that a total of 12 out of 17 of oil adjuvent rabbit pasteurellosis vaccines were tested and recorded satisfactory results for approval to be used in rabbit farms according to [10]. As regards to protection value obtained the tested oil adjuvant rabbit pasteurellosis vaccine batches were categorized into 7 groups. The protection value in the first group which consists of 2 out of 12 vaccine batches, the vaccinated mice gave protection 1.02 log more than the unvaccinated control mice in the single dose vaccination assay compared to 2.09 log in the booster dose vaccination assay. The second group which consists of 3 out of 12 vaccine batches was 0.92 and 2.10 log, the third group which consists of only one batch out of 12 was 0.83 and 2.06 log, the fourth group which consists of 2 out of 12 was 1.03 and 2.18 log, the fifth group which consists of only one batch out of 12 was 0.89 and 2.13 log, the sixth group which consists of only one batch out of 12 was 0.88 and 2.07 log and finally the seventh group which consists of 2 out of 12 was 0.85 and 2.11 log in the single and booster dose vaccination assays for each group respectively.

No of Tested Vaccine Batches	LD 50 of P. Multocida Strains	
	Single Dose Vaccination Assay	Booster Dose Vaccination Assay
2	1.02	2.09
3	0.92	2.10
1	0.83	2.06
2	1.03	2.18
1	0.89	2.13
1	0.88	2.07
2	0.85	2.11
total 12	Mean 0.93	Mean 2.11

Table 3: *Protection of Vaccination Challenge Assay in Mice Vaccinated With Either Single or Booster Dose Vaccination Assays of the Satisfactory Tested Oil Adjuvant Rabbit Pasteurellosis Vaccine.*

By calculating the average of protection value of all 12 vaccine batches, it was found that the protection mean was 0.93 log in the vaccinated mice more than the unvaccinated control mice in the single dose assay compared with 2.11 log in the booster dose assay

From data available in (Table 4), it can be seen clearly that a total of 5 out of 17 oil adjuvent rabbit pasteurellosis vaccine batches were tested and given unsatisfactory results, according to [10] where it got a protection level lower than 2 log which is the minimum requirement for protection. According to protection value obtained the 5 unsatisfactory vaccine batches classified to three groups. The first group comprised of 2 out of 5 vaccine batches gave a protection value of 0.51 log in the single dose vaccination assay compared with 1.12 log in the booster dose vaccination assay. The second group which comprised of 2 out of 5 vaccine batches was 0.48 and 1.31 log. Finally the last third group which comprised of only one batch out of 5 vaccine batches was 0.62and 1.06 log in the single and booster dose vaccination assay, respectively.

No of Tested Vaccine Batches	LD 50 of P. Multocida Strains	
	Single Dose Vaccination Assay	Booster Dose Vaccination Assay
2	0.51	1.12
2	0.48	1.31
1	0.62	1.06
total 5	Mean 0.52	Mean 1.18

Table 4: *Protection of Vaccination Challenge Assay in Mice Vaccinated with Either Single or Booster Dose Vaccination Assays of the Unsatisfactory Tested Oil Adjuvent Rabbit Pasteurellosis Vaccines.*

By calculating the average of protection value of five unsatisfactory vaccine batches, it was found that the protection mean was 0.52 log difference between vaccinated and unvaccinated control mice in the single dose assay compared with 1.18 log in the booster dose assay

Discussion

Pasteurellosis is one of the highly contagious bacterial diseases of rabbits caused by P. multocida and causes considerable economic loss in large production units throughout the world [11,12]. Consequently, investigation on the development of effective control and preventive methods are of a vital importance. Therefore it is important to achieve a good vaccine with a good vaccination assay against all these pathogens that has great effect on this industry like rabbit pasteurellosis. Evaluation of the efficacy of the inactivated rabbit pasteurellosis depends mainly on testing of its potency using vaccination challenge assay prior to sale and distribution [8].

The results of this study evaluate the efficacy of the single dose vaccination assay in comparison to booster dose vaccination assay for the evaluation of inactivated formalized and oil adjuvant rabbit pasteurellosis vaccines using vaccination-challenge test and determination of lethal dose fifty (LD50).

According to the minimum requirement of protection 2 log difference between vaccinated and unvaccinated control mice which should be obtained after booster dose of vaccination of rabbit pasteurellosis vaccine (Egyptian standards for evaluation of veterinary biologics [10] The results of this study reveal that the inactivated formalized rabbit pasteurellosis vaccine giving a satisfactory protective value ranges from 2.00 to 2.12 log with 2.05 log as an average in case of booster dose vaccination assay compared to 0.83log to 1.03 log with 0.91 log as an average in case of the single dose vaccination assay.

These results were confirmed by [13] who evaluated the protective value of aluminium hydroxide gel, a newly adjuvant rabbit pasteurella vaccine (ALV) in rabbits under field conditions in comparison to the classical aqueous formalized (AV) and oil adjuvant rabbit pasteurella vaccines. They found that Two doses of ALV and one dose of AV + oil adjuvant bacterins induced high levels of indirect haemag-

glutination 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.

Also these results agreed with [14] who prepared inactivated formalized *P. multocida* for different serotype of *P. multocida* and the protection percentages ranged from 50 – 100% according to the used serotype, and 100% deaths to the control group.

In the same direction, [15] investigated the protective efficacy of formalized *Pasteurella multocida* vaccine alone or in combination with propolis. They concluded that the use of propolis improved the immune protection of rabbits against pasteurellosis than using the vaccine alone. These findings also proved by [16] who showed that double immunization with formalized killed vaccine of *P.multocida* increased the immune response of the rabbits and the leukocyte phagocytic activity against *P. multocida* and also improved the clinicopathological and histopathological findings.Taken together, they proved that double immunization with formalized killed vaccine of *P.multocida* increased the phagocytic activity of the immune cells and the immune status of rabbits against infection.

Concerning the average protective value of all tested satisfactory batches of inactivated formalized rabbit pasteurellosis, it was 2.05 log with the booster dose compared to 0.91 log with the single dose vaccination assay. On the other hand the average protective value of all tested unsatisfactory batches of inactivated formalized rabbit pasteurellosis was 1.08 log with the booster dose compared to 0.49 log with the single dose vaccination assay.

[17] studied the protective effect of ethanolic extract of propolis given subcutaneously (S/C) either alone or in combination with inactivated formalized *Pasteurella multocida* (*P. multocida*) vaccine in rabbits challenged with virulent *P. multocida* strain. They found that rabbits injected S/C with propolis and *P. multocida* vaccine showed less severe clinical signs, mortality rate, and histopathological changes than control. Meanwhile rabbits injected with vaccine mixed with propolis as adjuvant were apparently healthy with normal histological picture. they concluded that an ethanolic extract of propolis injected alone or combined with formalized inactivated *P. multocida* vaccine improved general health conditions, These finding was confirmed with that obtained by the inactivated oil adjuvant rabbit pasteurellosis vaccine batches where all tested satisfactory batches giving a protective value ranges from 2.06 to 2.18 log difference between vaccinated and unvaccinated control mice with 2.11as an average in case of the booster dose compared to0.83 log to 1.03log with 0.93 log as an average in case of the single dose vaccination assay.

These results supported by [18] produced one shot Lipid A (AV) and Montanide TM ISA 70 (MV) adjuvanted P. multocida vaccines for rabbits and compared between them. The protection rate for both vaccines (A.V. group and M.V. group) was 71.4%, while 100% mortality for control group. Also, [19] when used two doses of Montanide ISA50 as an adjuvant for preparation of inactivated rabbit pasteurellosis vaccine gave a protection percentage 90% and 100% according to the type of antigen. [20] estimated the *P. multocida* vaccines for rabbits by challenge test to compare between Commercial vaccine **(**Oil adjuvanted formalized 0.5% inactivated *P. multocida*) and sonicated *P. multocida* vaccine found that; the sonicated vaccine gave a protection of 100% while the Commercial vaccine gave 80%.

Concerning the average protective value of all tested satisfactory batches of inactivated oil adjuvent rabbit pasteurellosis, it was 2.11 log difference between vaccinated and unvaccinated mice with the booster dose compared to 0.93 log difference between vaccinated and unvaccinated mice with the single dose vaccination assay. On the other hand the average protective value of all tested unsatisfactory batches of inactivated oil adjuvent rabbit pasteurellosis was 1.18 log with the booster dose compared to 0.52 log with the single dose vaccination assay.

These results agreed with that of [21] who prepare a bivalent vaccine against pasteurellosis and the virus of rabbit hemorrhagic disease using Montanide_ ISA70 oil as an adjuvant. They reported that the protection rate was 70% among rabbits vaccinated with monovalent Pasteurella vaccine, commercial Pasteurella vaccine, monovalent RHDV vaccine and 60% among commercial RHDV vaccine. While it was 90% among rabbits vaccinated with bivalent vaccine. They also reported that immunization against both pathogens can be achieved by single vaccination.

Also [22] prepared the alum precipitated and oil based hemorrhagic Septicemia Pasteurella multocida vaccines (APHSV and OBHSV) and evaluated their efficacies. They concluded that both of these vaccines induced high level of indirect hemagglutination (IHA) antibody titer and 100 percent protection to challenge on 90 days post vaccination. (OBHSV) induced higher level of antibodies than that of (APHSV).

Finally from above mentioned results obtained in this study the average protective value with booster dose vaccination 2.05 and 2.11 log difference between vaccinated and unvaccinated control mice when measure LD50 using [8] and [23] for formalized and oil adjuvant rabbit pasteurellosis vaccine respectively compared 0.91and 0.93 log for formalized and oil adjuvant rabbit pasteurellosis vaccine respectively post single dose of vaccination.

Therefore and referring to the minimum requirement in the [10] for veterinary vaccine evaluation which is 2log difference between vaccinated and unvaccinated control mice with the booster dose vaccination assay, we can relatively prove that the minimum requirement of protection associated with single dose vaccination assay is 0.91 log difference between vaccinated and unvaccinated control mice for formalized and 0.93 log for oil adjuvant rabbit pasteurellosis vaccine and we can recommend these findings as alternative method to be used in the evaluation protocols of rabbit pasteurellosis vaccines.

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