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VIRAL HAEMORRHAGIC DISEASE OF RABBITS: COMPARATIVE STUDY BETWEEN THE IMMUNE RESPONSE OF LOCAL AND IMPORTED VACCINE

(With 2 Tables)

By

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مرض النزف الدموى الفيروسى فى الأرانب
: دراسة مقارنة بين الاستجابه المناعيه للقاح المحلى والمستورد

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تم تحضير لقاح فورمالينى ميت محلياً من كبد الأرانب المصابه بفيروس مرض النزف الدموى فى الأرانب. وقد تم تحصين مجموعتان متساويتان من الأرانب (كل منهما ١٦ أرنب) نوع كالفورنيا أحدهما باللقاح المحلى والأخرى باللقاح المستورد تحت الجلد وقد أتضح أن الاستجابه المناعيه للأجسام المناعيه المتكونه فى الأرانب المحصنه باللقاح المحلى أعلى منها فى الأرانب المحصنه باللقاح المستورد عند ٢، ٤، ٦، ٨، ١٠ أسابيع بعد التحصين أما نسبة الحمايه بعد إجراء إختبار تحدى المناعه فكانت كامله ومتساويه فى كلا اللقاحين ٤ أيام، ٣، ٦، ٩ أسابيع بعد التحصين.

SUMMARY

Inactivated formalinized tissue vaccine was prepared locally from liver suspensions of rabbits infected with rabbit haemorrhagic disease virus. Thirty two california rabbits, 2 month old were inoculated subcutaneously with local and imported vaccines. (16 rabbits for each vaccine). Haemagglutination inhibiting antibodies were determined in both vaccinated groups 2, 4, 6, 8 and 10 weeks post vaccination and showed that rabbits vaccinated with local vaccine gave higher antibody

titer than rabbits vaccinated with imported vaccine. Both an inactivated vaccine either locally prepared or imported gave full protection against challenge with rabbit haemorrhagic disease virus at 4 days, 3, 6 and 9 weeks after vaccination in comparison with 100% mortality of unvaccinated control rabbits.

Key Words: *Immune, Response of Local and Imported Rhvv*

INTRODUCTION

Nowadays rabbits has some advantages over other herbivorous and omnivores, together with their importance for meat production in developing countries. Recently one of the most fatal diseases affecting rabbits is viral haemorrhagic disease. It is highly fatal disease causing mortality up to 100% (Fioretti et al. 1991). The causative agent of rabbit haemorrhagic disease (RHD) was discovered and identified in 1984 in china (Liu et al. 1984). The First report of disease in Europe was in Italy (Marcato et al. 1988), Bulgaria (Belemezov et al. 1989). Austria (Kolbl et al. (1990), Belgium (Peeters et al. 1990). In U.K. (Fuller et al., 1993), in scotland (Patterson and Howie, 1995). In Egypt the disease firstly reported by Ghanem and Ismail (1991). Then salem and Ballal (1992), El-Zanaty (1994), Abdel-Aziz et al (1995) and Amina et al. (1996). Rabbit viral haemorrhagic disease affect more frequently adult rabbits and spread rapidly among susceptible flocks with high morbidity and mortality. Recently Jichuanyi et al. (1994) identified the virus isolated from infected suckling rabbits by immunofluorescence and PCR while HA testing could not always detect atypical RHDV infection. No treatment was effective so controlling depend on general preventive measures and vaccination. Inactivated tissue vaccine induce immunity 3-5 days after vaccination and can stop the spread of the disease, induction of rapid immunity coordinated by macrophages and T and B lymphocytes, while humoral immunity plays the main role in long term protection (Wei et al. 1987, Du et al. 1991, Huang. 1991, Haralambiev et al. (1991), Salem and El-Zanaty 1992 and Twigg et al. (1997). The purpose of this study was undertaken to compare the immune response generated following the vaccination with locally prepared and imported vaccines.

MATERIAL and METHODS

Virus: The virus was previously isolated and identified from outbreaks of rabbits in Assiut province by Salem and El-Ballal (1992).

Antigen Preparation:

The isolated virus was injected 1 ml bacteria free suspension of liver subcutaneously into 5 rabbits 2-month-old, within 48-96 hours of infection the rabbits died, livers were collected aseptically, then minced and bacteria free suspension was prepared.

Inactivation for vaccine preparation:

To inactivate the antigen 0.4 formalin was added to the liver suspension, then the treated suspension was left in the refrigerator for 48 hours. The adjuvant (mineral mixture and lanoline) from middle east company was added to the inactivated antigen in equal volume after autoclaving. The prepared formalinized inactivated vaccine contained 1mg of infective tissue (equivalent to 10.000 viral particles) per dose according to (Haralambiev et al 1991).

Sterilily and safety tests for the vaccine were carried out as follows:

1- Sterility:

Subculture from the vaccine on bacteriological media.

2- Safety:

Two healthy rabbits, 2- month-old were subcutaneously injected with 1ml of the vaccine and observed for deaths and symptoms for one week.

Imported vaccine (cunical):

Adjuvanted inactivated vaccine against rabbit viral haemorrhagic disease produced by Rhone Merieux.

Experimental animals: Forty 2-month old california rabbits were obtained from Assiut rabbitary farm with no history of VHD outbreaks and prove free from RHDV and antibodies as well as other bacterial and parasitic diseases. These animals were divided into three main groups.

Group I: Contain 16 animals, each animal inoculated with local vaccine 0.5ml/subcut.

Group II: Contain 16 animals, each animal inoculated with imported vaccine 0.5ml/subcut.

Group III: Contain 8 animals were left as unvaccinated control.

Challenge test:

At periods of 4 day, 3, 6 and 9 weeks from the beginning of the experiment, 2 animals from each of the three groups were challenged by 1ml of virus suspension from infected liver containing the virulent virus for vaccine evaluation. All challenged rabbits were kept under observation for 7 days post challenge. Clinical signs, post mortem and deaths were recorded on both dead and sacrified rabbits.

Haemagglutination inhibition (HI) test. Was performed after Pu et al. (1985). Blood from 8 animals of each of the vaccinated groups was tested at 2, 4, 6, 8 and 10 weeks post vaccination for HI antibodies to RHDV.

Erythrocytes: Citrated blood samples were collected from chicken.

Haemagglutination test (HA test): was detected using the method described by Kolbl et al. (1990).

RESULTS

No animals were distressed or died during vaccination as a result of vaccination with either local or imported vaccines.

The local prepared formalized inactivated vaccine proved to be sterile by no bacterial growth in the bacteriological media and safe by no deaths or clinical signs were observed after vaccination.

HI serum levels in all periods after vaccination are shown in table (1). The highiest HI antibodies were observed after 4 weeks after vaccination and continue till the end of experiment in local vaccine group but only for 8 weeks post vaccination in imported vaccine group. In all challenge periods at 4 days, 3, 6 and 9 weeks post vaccination, no clinical signs or deaths were observed in both local and imported vaccine groups but all challenged unvaccinated control rabbits were died within 48-72 hours after challenge, the result shown in table (2). Depression, off food, incoordination before deaths were the prominent signs, noticed in the challenged unvaccinated group of rabbits. The post mortem lesions were paleness of liver, haemorrhages on lungs and congestion of spleen & kidney in 3 cases.

Table (1): illustrate mean HI titers in rabbits vaccinated with local and imported vaccine.

Group	Type of vaccine	No.of rabbits	Dose and route of vaccination	Mean HI titer				
				Weeks post vaccination				
				2	4	6	8	10
1-	Local vaccine	8	0.5 ml - S/C	1/256-1/512	1/512-1/1024	1/1024-1/2048	1/1024-1/2048	1/512-1/2048
2-	Imported vaccine	8	0.5 ml - S/C	1/128-1/512	1/256-1/1024	1/512-1/1024	1/512-1/1024	1/256-1/512

Table (2): illustrate protection percentage of challenge with RHDV at 4 days .3,6 and 9 weeks post vaccination with local and imported vaccines.

Group	Type of vaccine	Total no. of rabbits	No. of animal per challenge	Breed	Age	Mortality no. %	Protection %
1-	Local vaccine	8	2	California	2month	0/8 00.0	100%
2-	Imported vaccine	8	2	California	2month	0/8 00.0	100%
3-	Non vaccinated	8	2	California	2month	8/8 100.0	00.0

DISCUSSION

Viral haemorrhagic disease of rabbits is one of the greatest catastrophes facing rabbitaries during the last few years of twentieth century. Several outbreaks were reported in Egypt in different localities causing high mortalities in adult rabbits population in our areas, Salem and Ballal (1992) and Aly (1998). In this work immune response of formalinized locally prepared tissue vaccine and imported one were detected. Haemagglutination inhibiting antibodies were detected at 2, 4, 6, 8 and 10 weeks postvaccination, reach top 6-10 weeks and 4-8 weeks post vaccination respectively then gradually declines (Smid et al. 1991). There were a higher HI titer in the immune response of local than imported vaccine.

Rabbits vaccinated with local or imported vaccines were fully protected against challenge with virulent strain of the virus 4 days post vaccination and the full protection continued to 9 weeks post vaccination, Our results nearly in accordance with Wei et al (1987), Haung (1991) and Haralambiev et al (1991) who mentioned that formalinized tissue vaccine can stop the spread of the disease in 3-4 days of vaccination. Finally we can be concluded that formalinized tissue locally prepared and imported vaccines can control and eradicate the

disease in rabbits in the affected areas. We prefer the usage of locally prepared vaccine due to its high immunological response and its low cost in comparsion with the imported one.

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