

EFFECT OF COCCIDIOSIS AND ITS TREATMENT ON THE IMMUNE RESPONSE OF RABBITS TO INACTIVATED RABBIT HAEMORRHAGIC VIRUS VACCINE

By

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SUMMARY

The present work is a trial to answer an important question about rabbit vaccination against rabbit haemorrhagic disease virus when such rabbits are infested with or treated received a treatment against rabbit coccidiosis which caused by Eimeria Stiedae. The experimental results showed that rabbits infested with Eimeria Stiedae and did not receive any treatment had the lowest antibody titers to rabbit haemorrhagic disease virus as estimated by haemagglutination inhibition (HI) and serum neutralization (SNT) tests which lead to the lowest protection percent (10%) followed by that of unvaccinated challenged rabbits (0%). On the other hand infested rabbits which treated with 33.3% sulphaquinoxaline showed better immune response than the untreated animals with higher protection percent (70%) while healthy rabbits were the superior one showing the highest antibody titers ($10\log_2$ by HI and 128 by SNT) with the highest protection percent (100%). These findings reflect the importance of rabbit treatment against coccidiosis to obtain better immune response to RHD vaccine and high levels of protection.

INTRODUCTION

Rabbits are raised for a variety of purposes including meat and fur production. They also used as laboratory animals. Moreover, pure bred show animals are raised as hobby and rabbits of all shapes and colors are kept as pets (Lieve et al., 1988). Any disease either bacterial or viral or parasitic could affect dramatically rabbit industry causing great economic losses. One of the most familiar parasitic diseases of rabbits is the hepatic coccidiosis which causes high mortalities especially in young rabbits of 4-8 weeks old. It is caused by Eimeria Stiedae which occurs in the liver which is the vital organ for protein synthesis and storage of the most nutritive material for the animal body. The disease prevalence may reach 95-100% in young rabbits. Adult female rabbits are usually act as carriers within the farm and transmit the parasite to younger rabbits causing sever clinical signs end mainly with death (Wang and Tsai, 1991). Deaths due to rabbit coccidiosis may result in economic losses of 75-

80% (Haiba et al., 1955). The clinical signs of rabbit coccidiosis are represented by loss of appetite, emaciation, diarrhoea and distension of the abdomen (Rosimini and Simonj, 1979). The post mortem findings reveal enlargement of the liver which appears with pale greenish color while its surface demonstrates various sized whitish yellow nodules of necrotic tissues. Histopathological sections of the affected liver showed degenerative changes of all cytoplasmic structures of the biliary epithelium. In addition rabbit coccidiosis causes sever interruption of feeding and digestive processes and increased animal susceptibility to other disease agents (Calenk et al., 1997).

Regarding the treatment of coccidiosis using sulfaimethsxine; sulfamonmethsxine and sulfaquinoxiline; Huang and Lee (1975) found that these drugs enhanced the animal resistance to the disease. Jungherr and Winn (1949) stated that continuous administration of 0.01% sulfaquinoxaline lowered mortality due to coccidiosis, improved food utilization and favorable weight gain while Herman et al. (1994) showed that sulfaquinoxaline and sulfadiazine have an immune suppressive activity on T. cells and immune globulin fractions in CDK mice.

One of the most fatal viral diseases of rabbits is the viral haemorrhagic disease (VHD) which is an acute or subacute fatal disease affecting all ages of wild and domestic rabbits and characterized by sudden death and frequently with bloody nasal discharge, cries, (Arguello, et al. 1988a; Cao, et al, 1986; Ciprian, et al. 1985 & 1989; CPA 1989 a&b and Contera 1989a). The disease was first described in China in the Spring and Summer of 1984 by Liu et al. (1984) then spread all over the world recording high morbidity and mortality rates which may reach up to 100%.

The present work was aimed to study the effect of rabbit coccidiosis and its treatment on the immune response of rabbits to the locally produced inactivated rabbit haemorrhagic viral vaccine where some rabbits failed to response well for such vaccination and attract the diseases either in experimental or natural infection. Some of these rabbits were found to be infested rabbit coccidiosis.

MATERIAL AND METHODS

1. Viruses:

1.1. Virulent RHD virus:

The local strain (RHDV-Egypt-96) of RHD virus (Daoud et al., 1998) was supplied by the Department of Newcastle, Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo. It had a titer of 2^{13} HA (haemagglutination) unit/ml and used for challenge of vaccinated rabbits and haemagglutination inhibition test (HI).

1.2. Cell culture adapted RHD virus strain:

The local cell culture adapted RHD virus strain (Khodeir and Daoud, 2002) was supplied kindly by Veterinary Serum and Vaccine Research Institute. It had a titer of 10^7 TCID₅₀/ml in Vero cells and used in serum neutralization test (SNT).

2-Inactivated RVHD vaccine:

Locally produced RVHD inactivated vaccine prepared in rabbit livers was supplied by Veterinary Serum and Vaccine Research Institute. It was used for vaccination of experimental rabbits where each rabbit was inoculated subcutaneous with 0.5ml.

3-Isolation and sporulation of Eimeria stiedae oocysts:

This step was carried out according to Abd El-Rahman (1988) where a field strain of Eimeria stiedae was isolated from the gall bladder of naturally infected rabbits. The obtained oocysts were washed with normal saline to remove the bile then transferred to a clean sterile Petri dish containing 2.5% potassium dichromate solution for a depth of 3-5mm and incubated at 26°C and a relative humidity 76-80%. Sporulation of the oocysts was flowed up daily until completed. When complete sporulation was obtained, the contents of the Petri dish were centrifuged at 3000rpm for 15 minutes where the supernatant was discarded while the sediment was washed several times with distilled water and recentrifuged as before until the supernatant became clear. The final sediment was resuspended in 2.5% potassium dichromate solution and stored at 4°C until used.

4-Propagation of Eimeria stiedae:

It was carried out according to Zhang et.al. (1996) where 10 one month old rabbits free from Eimeria were infected with 30000 freshly sporulated oocysts / rabbit inoculated per os then the oocysts were collected from the gall bladder (after animal slaughtering) and treated as mentioned above.

5-Storage of sporulated oocysts:

Sporulated Eimeria stiedae oocysts was carried out in 2.5% potassium dichromate solution at 4°C as mentioned by Abu El-Ezz (1994).

6. Rabbits:

A total of 40 local breed rabbits weighing 1.5-2.0 kg of 2-3 months age were used in this study. They were found to be free from antibodies against RHDV as tested by HI and SNT and had no history of infection with Eimeria stiedae or vaccination against the two diseases. The rabbits were divided into 4 groups (10 rabbits/ group) as follows:

*Group-1 and 2 were infected experimentally with Eimeria stiedae then vaccinated with the locally produced inactivated RVHD vaccine. Group-1 was treated sulphaquinoxaline (33.3%) on the time of vaccination (each rabbit was injected subcutaneous with 1ml of the drug for 3 days and repeated after 3 days). Group-2 was kept vaccinated without treatment.

*Group-3 was vaccinated as healthy rabbit control.

*Group-4 was kept as unvaccinated healthy rabbits.

Each rabbit group was housed separately under hygienic measures receiving balanced ration and adequate water and subjected to daily clinical observation. Serum samples were obtained weekly from all animal groups post

vaccination up to 3 weeks then all of them were challenged with the virulent RHD virus where each animal inoculated through the intranasal rout with 0.5 ml of 10% liver homogenate in saline having a virus titer of 2^{13} HA unit/ml.

In addition 10 rabbits of one month age were used for propagation of *Eimeria stiedae*.

7-Treatment of experimentally infested rabbits:

Treatment of the experimentally infested rabbits in group-1 was carried out according to Joyner et.al. (1983) using 33.3% sulphaquinoxaline where each rabbit was injected subcutaneous with 1ml for 3 days and such treatment was repeated after 3 days.

8-Serum neutralization test (SNT):

It was carried out for estimating the neutralizing antibodies against RHD virus vaccines according to the method of Villagas (1990).

9- Haemagglutination inhibition test (IHA):

This test was performed according to the technique of Carter and Rappay (1962):

RESULTS AND DISCUSION

Group (1): Vaccinated while they were infested with *Eimeria stiedae* and did not received treatment.

Group (2): Vaccinated while they were infested with *Eimeria stiedae* and treated with sulphaquinoxaline

Group(3):Healthy vaccinated rabbits. Group (4): Healthy unvaccinated rabbits

*Antibody titer= the reciprocal of the final serum dilution which neutralized the CPE of 100:200 TCID₅₀ of RHD virus.

**DPV= Days post vaccination

***WPV=week post vaccination

Table (3): Mortality and protection percent in different rabbit groups after 3 weeks of vaccination to the virulent RHD virus.

Rabbit groups	No. of challenged rabbits	No. of survived rabbits	Protection %
1	10	1	10
2	10	7	70
3	10	10	100
4	10	0	0

Group (1): Vaccinated while they were infested with *Eimeria stiedae* and did not received treatment.

Group (2): Vaccinated while they were infested with *Eimeria stiedae* and treated with sulphaquinoxaline

Group (3): Healthy vaccinated rabbits.

Group (4): Healthy unvaccinated rabbits.

parasitic diseases affect dramatically the general health condition of infested hosts and accordingly affect badly their immune response to the applied vaccine; the thing which may lead to failure of vaccination and increase animal susceptibility to different infections (Calenk et.al., 1997).

Rabbit hemorrhagic viral disease and rabbit coccidiosis represents two major problems facing rabbit industry and may lead to complete destruction of a rabbit farm. So, the present work investigates the effect of rabbit coccidiosis and its treatment on the immune response of rabbits to the inactivated rabbit hemorrhagic viral disease vaccine in a trial to answer the question about the administration of such vaccine to rabbits on the time of vaccination when such rabbits found to be infested with *Eimeria stiedae* or received a specific treatment as sulphaquinoxaline.

The experimental infection of rabbits with *Eimeria stiedae* revealed that such animals showed signs of illness characterized by loss of appetite, diarrhea and distension of the abdomen in agreement with what recorded by Rosimini and Simoni (1979).

It was found that vaccinated healthy rabbits were exhibited good levels of RHD antibodies as detected by the HI (4log₂) and SNT (8) from the 3rd day post vaccination and the infested treated rabbits showed lower titers of such antibodies (2 log₂) and 2 respectively at the same period while infested untreated rabbits were the poorest animals in the immune response showing no detectable antibody titers even by the 3rd week post vaccination on which the other groups showed the peaks of HI (6 and 10 log₂) and SNT (24 and 128) for treated and healthy vaccinated rabbits as shown in tables (1&2). Similar results were obtained in vaccinated healthy rabbits by Kim et.al. (1991); Peters et.al. (1992); Daoud et.al. (1998); Khodeir and Daoud (2002) and Safia et.al. (2005).

On the other hand it was noticed that treated rabbits had a better immune response than those which were not treated where Huang and Lee (1975) found that sulfamethoxazole, sulfamonomethoxazole and sulfaquinoxaline enhanced the animal resistance to the disease and Jungherr and Winn (1949) stated that continuous administration of 0.01% sulfaquinoxaline lowered mortality due to coccidiosis, improved food utilization and accordingly improved the health condition and immune response of vaccinated animals.

Challenge test of all rabbit groups with the virulent RHD virus revealed a protection percent of 10; 70; 100 and 0 in untreated; treated and healthy vaccinated and unvaccinated rabbits respectively (table-3). These findings reflect the immune status of the experimental rabbits showing that the infested rabbits with *Eimeria stiedae* could not respond effectively to RHD vaccine and accordingly they could not withstand the virulent virus as concluded by Calenk et.al. (1997) who reported that rabbit coccidiosis causes severe interruption of feeding and digestive processes and increased animal susceptibility to other disease agents.

From the obtained results it could be concluded that infested rabbits with *Eimeria stiedae* (rabbit coccidiosis) must be treated with the suitable specific drug when introduced to vaccination against infectious diseases even on time of vaccination in order to avoid failure of vaccination.

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الملخص العربي

تأثير الكوكسيديا وعلاجها على استجابة الأرانب المناعية للقاح النزف الفيروسي المثبط

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معهد بحوث الأمصال واللقاحات البيطرية- العباسية

تم تحصين مجموعتين من الأرانب معديتين بطفيل الكوكسيديا (ايميريا سيتيدى) بلقاح النزف الفيروسي المثبط حيث عولجت المجموعة الثانية بمستحضر السلفا كوينكسالين وتركت الأولى دون علاج كما تم تحصين مجموعة ثالثة من الأرانب السليمة بنفس اللقاح وتركت مجموعة رابعة دون علاج ودون تحصين كضوابط للتجربة 0 أوضحت النتائج التجريبية أن المجموعة الأولى هي أفقر المجموعات من حيث استجابتها المناعية للقاح وأقل نسبة حماية ضد اختبار التحدى بالعترة الضارية من الفيروس (10%) بينما كانت هذه النسبة أعلى في المجموعة التي تلقت العلاج بالسلفا (70%) فى حين كانت 100% فى الأرانب السليمة المحصنة وصفر فى الأرانب الضوابط الأمر الذى يوضح أهمية علاج الأرانب ضد الكوكسيديا قبل أو مع التحصين للوصول إلى أفضل المستويات المناعية وبالتالي أعلى نسب من الحماية ضد الأمراض المعدية وتجنباً لفشل التحصين وبالتالي حفاظاً على الثروة الحيوانية وتوفيراً للتكلفة