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Preparation of an inactivated oil emulsified vaccine against Rabbit Viral Hemorrhagic Disease

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SUMMARY

An Experimental batch of an inactivated RHDV oil emulsified vaccine was prepared as water-in-oil emulsion with aqueous to oil ratio 1:2. The efficacy of the prepared vaccine was studied in vaccinated rabbits for one year post vaccination. The efficacy was based on HI antibody response and the resistance to challenge with virulent RHDV. The vaccine induced specific RHDV-antibody titers detected from the 1st week post vaccination in a zigzag like manner through out the year. RHDV-antibody titers measured by HI test in vaccinated rabbits increased and reached the highest level in 6th week post vaccination while the maximum level was attained at 7th month post vaccination. The high level of specific RHDV-antibody titers in the vaccinated rabbits reflected on the challenge test where they could resist the challenge against virulent RHDV up to 12 months post vaccination with 100% protection compared to unvaccinated group (0% protection). This study concluded the need to produce this vaccine locally in a massive production aiming to save the hard currency lost in importing this vaccine.

INTRODUCTION

Rabbit Viral Hemorrhagic Disease (RVHD) is a highly contagious, highly fatal, per-acute and acute viral disease of both wild and domestic rabbits (Peeters et al., 1990) caused by Rabbit Hemorrhagic Disease Virus (RHDV), a member of the family Caliciviridae (Ohlinger et al., 1990). Calicivirus infection is the major cause of the severe decrease in the stocks of wild and farm rabbits that has occurred worldwide during the last two decades (Ferreira et al., 2006). The economic importance of RVHD is due to the high morbidity (70-80%) up to 100% mortality it causes (Chen, 1986). In Egypt,

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RVHD was first reported in 1991 in Sharkia governorate (Ghanem and Ismail, 1992). Sanitary prophylaxis with the rigorous application of vaccination routine can exclude all risk of the RVHD (Thibault, 1990).

Many inactivated RHDV vaccines were prepared locally in Egypt using aluminum hydroxide (Al OH) gel as adjuvant (Daoud et al., 1998; Salman, 1999 and Salman, 2007). Combined bivalent inactivated oil emulsified vaccines against RVHD and rabbit pasteurellosis was prepared too but using montanide oil (Taha et al., 2009). The type of adjuvant determines the duration of the protection that they confer being longer for oil-based vaccines after subcutaneous injection (Pages, 1989), so the present work was planed to prepare an inactivated RHDV oil emulsified vaccine using mineral oil as adjuvant and evaluate its potency in vaccinated rabbits.

MATERIAL AND METHODS

1. Rabbit Haemorrhagic Disease Virus (RHDV):

Local Egyptian strain of RHDV designated as Giza/2006 (Salman, 2007) with a titer of $10^{4.65}$ LD_{50}/ml and of haemagglutination (HA) titer equal to 2^{14} HA unit was used for vaccine preparation, challenge of vaccinated rabbits and in haemagglutination inhibition (HI) test.

2. Experimental rabbits:

Three to four months old, industrial hybrid rabbits with an average body weight of 2 Kg were purchased from a conventional rabbitry. All rabbits were free of detectable RHDV antibodies. The rabbits were used for RHDV vaccine preparation and vaccine evaluation.

3. Serum samples:

Blood samples were collected from the experimental rabbits through the ear vein. The collected blood samples were allowed to coagulate and centrifuged (2500 rpm 10 minutes) in order to separate the serum. Sera of individual rabbits were subjected for inactivation process by heating in a water bath at 56°C for 15 minutes then kept in dry sterile screw capped vials at -20°C till examined serologically to detect the RHDV HI antibodies.

4. Positive control hyper immune serum of RHDV and negative control one:

These sera were kindly supplied by Newcastle disease vaccine research department, Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo and used in HI test. It was supplied in an enzyme immunoassay kit for detection of antibodies to RHDV supplied from Kalon Biological Ltd. UK.

5. Erythrocytes suspension:

Erythrocytes human type "O" were collected using 3.8% sodium citrate solution as

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anticoagulant (9ml blood to 1ml sodium citrate solution) from a healthy volunteer. The packed erythrocytes were suspended in sterile saline in a concentration of 0.75% for micro-technique of HA and HI tests.

6. Oil Adjuvant:

a.Oil base:

Paraffin oil: Light extra white medicinal oil. BATCH No. 25/2007. FDA. USP. BP.UK.FR.

b.Emulcifiers:

1.Aqueous phase emulsifier (Tween 80):

Polyoxyethylene sorbitan supplied by Sigma. BATCH No. 085K0096. (HLB=15)

2.Oil phase emulsifier (span80):

Sorbitan monooleate, supplied by Guagzhou Hanglian chemical co., ltd. China. LOT No. 30807703. (HLB=4.3)

7. Chemicals:

a.Formaldehyde solution (Fluka Riedel-deHaen, Sigma, Germany):

Lot No. 52930. 37% by weight stabilized with approximately 10% methanol. It was used for virus inactivation.

b. Glycine (NH2-CH2-COOH) (El Nasr pharmaceutical company, Egypt):

BATCH No. 2007/1. It was used as emulsifier in a concentration of 4.8 gm/ Liter

c. Sodium thiomersal (PARK scientific limited Northampton, UK):

Lot No. P839F. It was prepared as solution in a concentration of 1/10000 (W/V) and added to the prepared vaccine in a concentration of 1ml/ Liter as a preservative.

8. Haemagglutination (HA) test:

A twofold dilution of the RHDV was incubated with an equal volume of washed human RBCs type "O" (0.75% concentration) in a sealed round-bottom micro-titer plate at 4°C according to Capucci et al., (1996) to determine HAU used in HI test.

9. Haemagglutination inhibition (HI) test:

It was carried out according to Peshev and Christova, (2003a) using 8 HA unit of RHDV and human RBCs type "O" to estimate specific RHDV antibodies in rabbit sera. The antibody titer was the end-point dilution showing inhibition of HA

10. Preparation of inactivated RHDV suspension:

RHDV suspension that incorporated in the vaccine was prepared according to **OIE, (2008).**

The viral inactivated suspension was assayed by HA test and it was found that RHDV titer was 2^{10} HAU after inactivation as it is recorded by Kim et al., (1989), also OIE, (2008) recommended that HA titer of RHDV after inactivation for vaccine preparation should be higher than 2^7. Abolishing viral infectivity was carried out using formaldehyde at 0.4% concentration at 37°C for 48 hours. During inactivation, the fluid was continuously agitated.

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11. Preparation of the oil emulsified inactivated RHDV Vaccine:

The vaccine was prepared as water in oil emulsion (W/O) according to Stone et al., (1983) with aqueous to oil ratio 1:2 (W/W) and the aqueous phase emulsifier (Tween 80) was added to the oil phase. A preservative, thiomersal (Merthiolate), was finally added at a dilution of 1/10,000 (V/V) before distribution into neutral glass of 10 ml capacity vials (each contains 5 ml of the vaccine). The vaccine was stored at 4°C till used.

12. Challenge test:

Virulent RHDV with a titer of $10^{4.65}$ LD_{50}/ml and 2^{14} HAU was used as challenge virus for vaccinated and control rabbits as OIE, (2008) recommended that the challenge made by intramuscular inoculation of a dose of RHDV containing at least 100 LD_{50} or presenting a HA titer higher than 2^8. Each rabbit received 1ml of RHDV I/M.

13. Quality control:

The prepared vaccine was subjected to sterility and safety following standard international protocols of British Pharmacopeia Veterinary (2005)

a. **Sterility test:** The prepared vaccine was tested for the presence of viable bacteria, mycoplasma and fungi.
b. **Safety:** Safety test was carried out by S/C inoculation of 10 sero-negative rabbits with three times the vaccinal dose. The rabbits were observed for 3 weeks post inoculation.

14. Vaccine efficacy:

Vaccine efficacy evaluation was based on HI antibody response and response to challenge.

Experimental design

A total of 105 experimental rabbits were housed in disinfected metal cages in a well ventilated and disinfected room receiving commercial pellet ration and clean water ad libitum. The rabbits proved to be sero-negative for specific RHDV HI antibodies.

The rabbits were grouped into 2 groups:

Group (1): containing 70 rabbits all of them injected S/C with the prepared inactivated RHDV oil emulsified vaccine in a dose of 0.5ml per rabbit.

Group (2): containing 35 rabbits kept as unvaccinated control.

Each rabbit group was housed separately under well hygienic measure and kept under daily observation till the end of experiment.

a. Humoral immune response:

It was followed up for 12 months for both groups starting from the 1st week post vaccination (WPV). Blood samples (individually from ear vein of all rabbits in both groups) were collected weekly till the 4th WPV, every 2weeks till the 8th WPV and then monthly till the 12th month post vaccination

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(MPV). Sera were separated and kept at -20 °C till used to evaluate humoral immune response.

c. Challenge test:

At the 3rd WPV, 3rd MPV, 6th MPV, 9th MPV and 12th MPV, 10 vaccinated rabbits from group 1 and 5 unvaccinated rabbits from group2 were transported to experimental isolators where they were challenged by intramuscular inoculation of 1ml of a suspension of virulent RHDV ($10^{4.65}$ LD_{50}/ml-2^{14} HAU). The challenged rabbits were chosen randomly at each time. The challenged rabbits were kept under daily observation for 2 weeks post challenge.

RESULT AND DISCUSSION

Nowadays, RVHD is endemic in most parts of Europe and Asia, in some African countries, in Australia and New Zealand (Grazioli et al., 2000). In Egypt, the RVHD spread all over most of Egyptian provinces where it was recorded in different localities by many authors (El-Zanaty, 1994; Salman, 1999; Abd El-Ghaffar et al., 2000 and Salman, 2007). RVHD characterized by sever losses with high rate of morbidity and mortality, the latter being close to 90% in adult rabbits (Ohlinger et al., 1989 and Nowotny et al., 1990) leading to high economical losses.

Inactivated tissue vaccine against RHDV was developed by many authors and succeeded in RVHD control (Liu et al., 1984; Kim et al., 1989 and Smid et al., 1991). In Egypt inactivated RHDV vaccine with aluminum hydroxide gel adjuvant was developed by Daoud et al., (1998); Salman, (1999) and Salman, (2007). There is a great intention to have an inactivated vaccine having high adjuvant activity aiming to protect the rabbits for longer duration using one shot, so the objective of this study was to prepare and evaluate an inactivated oil emulsified vaccine from the local RHDV (Giza / 2006).

The prepared vaccine proved to be sterile (no growth of micro-organisms on nutrient agar, blood agar and Sabaroud agar) and proved to be safe (The 10 inoculated rabbits S/C with three times the vaccinal dose did not show notable signs of disease or local reaction and remained healthy during the 3 weeks observation).

The prepared vaccine as the other water in oil emulsion vaccine consisted of an aqueous phase suspended as droplets in mineral oil; the RHDV antigen was contained in aqueous phase and remained dispersed in the oil or suspending phase through the action of emulsifiers as stated by Stone et al., (1983). Inactivated RHDV content in the vaccine was 2^{10} HAU in accordance with Kim et al., (1989) and more than 2^{7} as recommended by OIE, (2008). This inactivated viral mass was certainly high as the ratio between the aqueous and oil phase was 1:2.

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Humoral immune response of the vaccinated rabbits with the oil emulsified vaccine as well as unvaccinated control ones was followed up for 12 MPV using HI test. As shown in table (1) and figure (1), none of the vaccinated and unvaccinated control rabbits had RHDV specific HI antibodies before vaccination then high titers of RHDV specific HI antibodies were detected in the sera of vaccinated rabbits from the 1st WPV where the obtained mean geometric titers (MGTs) was $2^{7.7}$ HI RHDV-antibodies. This result agreed with those obtained by Yu et al., (1992) and Peshev and Christova (2003b) who used RHDV oil adjuvanted vaccine too. Gomes et al., (1980) attributed the higher antibody titer following vaccination with oil emulsified vaccine to low viscosity and high homogenicity.

Our results revealed that the MGTs of HI RHDV-antibodies in the vaccinated rabbits run in a zigzag like manner reaching 2^9 as an initial peak at 6th WPV then the 2nd peak (2^{10}) was attained at 7th MPV. Meanwhile the 3rd peak of HI RHDV-antibodies titer $2^{9.7}$ was recorded at 12th MPV (end of the experiment). The obtained results were satisfactory and comes in agreement with that of Thayer et al., (1983) who stated that oil emulsion vaccines elicited immune response that run in a zigzag like manner.

Challenge with the virulent RHDV ($10^{4.65}$ LD_{50}/ml) (Table 2) killed 100% of unvaccinated control rabbits but resulted in neither morbidity nor mortality in vaccinated rabbits, so the prepared vaccine provided 100% protection against clinical signs and death. This protection detected from the 3rd WPV and lasted for 12 MPV. This is in agreement with Shevchenko, (1994) who found that 100% protection, in the vaccinated rabbits challenged with virulent RHDV, was continued through 12 MPV.

Protection against clinical disease was expected with the HI antibody titer, induced in this study and proved that the prepared inactivated RHDV oil emulsified vaccine containing sufficient amount of antigen that may have the potential to induce higher level of protection against infection than is currently realized, this result comes in contact with those of Stone et al., (1983). Our results also agreed with those of Nowotny et al., (1993) who found that the adult rabbits with RHDV-antibody titers ranging from 2^6 to 2^{13} remained clinically healthy after inoculation with virulent RHDV and Simon et al., (1993) who concluded that a titer > 20 HIU was protective.

From the afford mentioned results, it could be concluded that the locally prepared inactivated RHDV oil emulsified vaccine offered a good protective immunity against RVHD and could be used safely for active immunization of rabbits against this disease that threaten rabbit industry in Egypt.

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Table (1) Geometric mean of RHDV specific HI antibody titers (log2) in sera of vaccinated and unvaccinated rabbits

Time post vaccination	Geometric mean of RHDV specific HI antibody titers (log2)	
	Vaccinated rabbits	Unvaccinated rabbits
0 day	0	0
1st WPV	7.7	0
2nd WPV	7.2	0
3rd WPV	7.6	0
4th WPV	7.75	0
6th WPV	9	0
2nd MPV	8.8	0
3rd MPV	7.7	0
4th MPV	8	0
5th MPV	7.6	0
6th MPV	8.5	0
7th MPV	10	0
8th MPV	9.2	0
9th MPV	9.5	0
10th MPV	9.5	0
11th MPV	8.6	0
12th MPV	9.7	0

RHDV= Rabbit Haemorrhagic Disease Virus
HI= Haemagglutinating inhibiting
WPV= week Post Vaccination
MPV= Month Post Vaccination

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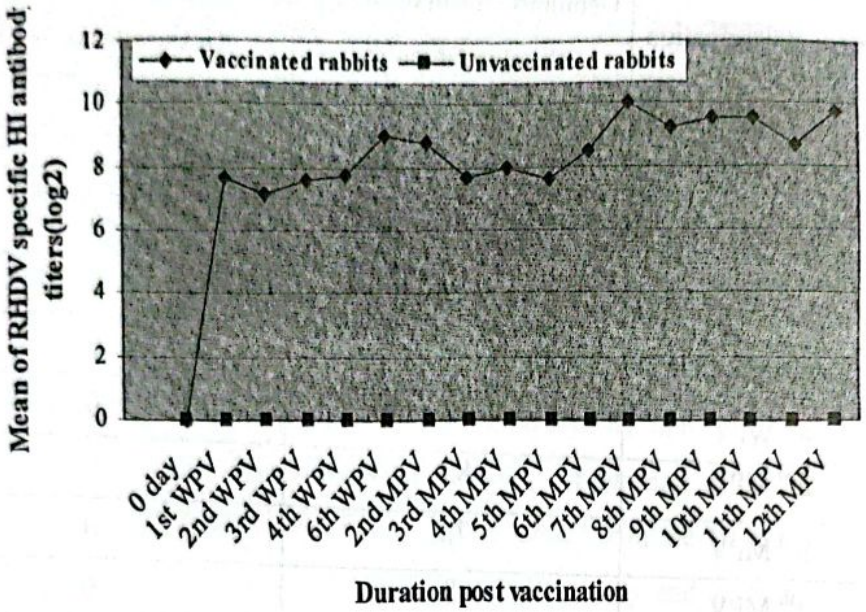
Fig.1: Geometric mean of RHDV specific HI antibody titer(log2) in sera of vaccinated and unvaccinated rabbits



RHDV = Rabbit Haemorrhagic Disease Virus
HI = Haemagglutinating inhibiting

Table (2): Potency of RHDV inactivated oil emulsified vaccine

Time of challenge	Vaccinated rabbits			Unvaccinated rabbits		
	Number of challenged rabbits	Number of dead rabbits	Protection percent	Number of challenged rabbits	Number of dead rabbits	Protection percent
3rd WPV	10	0	100%	5	5	0%
3rd MPV	10	0	100%	5	5	0%
6th MPV	10	0	100%	5	5	0%
9th MPV	10	0	100%	5	5	0%
12th MPV	10	0	100%	5	5	0%

WPV = week post vaccination.
MPV= month post vaccination.

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REFERENCES

Abd El-Ghaffar, S.Kh; Aly, M.; Fatma, A. Moustafa and Mahmoud, A.Z. (2000): Pathological Studies on the Rabbit Viral Hemorrhagic Disease (RVHD) with Special Reference to the USE of Vitamins A, E & C as Prophylaxis. Assiut Vet. Med. J., 43 (85): 251-274.

British Pharmacopoeia Veterinary (2005): Published by the stationary office limited under department of health of the agriculture ministers, London, England.

Capucci, L.; Chasey, D.; Lavazza, A. and Westcott, D. (1996): Preliminary characterization of a non-haemagglutinating strain of rabbit haemorrhagic disease virus from the United Kingdom. J. Vet. Med. [B], 43 (4): 245 - 250.

Chen, K.Y. (1986): Rabbit plaque. Infectious Diseases in Domestic Animals, 3: 53 - 55.

Daoud, A.M.; Khodeir, M.H.; Abbas, A.M. and Ibrahim, S.I. (1998):Preparation of a specific inactivated vaccine against rabbit haemorrhagic disease virus. 4th Vet. Med., Zag. Congress, (26-28 August 1998) in Hurghada (230-234).

El-Zanaty, K. (1994): Some investigations on rabbit viral haemorrhagic disease in Upper Egypt. Assiut Vet. Med. J., 30 (60): 293 - 305.

Ferreira, P.G.; Costa-E-Silva, A.; Oliveira, M.J.; Monteiro, E.; Cunha, E.M. and Aguas, A.P. (2006): Severe leukopenia and liver biochemistry changes in adult rabbits after calicivirus infection. Res Vet Sci., 80(2):218-25.

Ghanem, I.A. and Ismail, A.N. (1992): Occurrence of rabbit haemorrhagic disease in Sharkia province. Zag. Vet. J., 20 (4): 491 - 502.

Gomes, I.; Satmoller, P. and Casaslascouga, R. (1980): Response of cattle to foot and mouth disease (FMD) virus exposure one year after immunization with oil adjuvanted foot and mouth disease vaccine. Pan American Foot and Mouth Disease Center, PAHO/WHO, CP 589, 20000 Rio de Janeiro, Brazil.

Grazioli, S.; Agnoletti, F.; Scicluna, M.T.; Masoero, N.; Guercio, A.; Fallacara, F.; Lavazza, A.; Brocchi, E. and Capucci, L. (2000) : Rabbit haemorrhagic disease virus (RHDV) sub type "A" (RHDVa) is replacing the original strain in some Italian regions. Proc. of the 5th International Congress of the European Society for Veterinary Virology. 202-203.

Kim, B.H.; Lee, J.B.; Song, J.Y.; An, S.H.; Chung, J.S. and Cho, Y.J. (1989): Studies on picorna virus haemorrhagic fever (tentative name) in rabbits. 2. Development of inactivated vaccines. Research Report of the Rural Development Administration, Veterinary, 31 (1):7 - 11.

Liu, S.J.; Xue, H.P.; Pu, B.Q. and Qian, N.H. (1984): New viral disease in rabbits. Animal Husbandry and Veterinary Med., 16 (6): 253 - 255.

Nowotny, N.; Fushs, A.; Schilcher, F. and Loupal, G. (1990): Occurrence of viral haemorrhagic disease of rabbits in Austria. 1. Pathological and virological investigations. Wien Tierarzt Mnsch, 77 (1): 19 - 23.

Nowotny, N.; Leidinger, J.; Fuchs, A.; Vlasak, R.; Schwendenwein, I.; Schilcher, F. and Loupa, G. (1993): Rabbit haemorrhagic disease (RHD): experimental infection of domestic rabbits with regard to clinical, haematological, chemical, virological, serological and pathomorphological features. Wiener – Tierarzt -Mnsch. 80: 3, 65-74.

Ohlinger, V.F.; Haas, B.; Ahl, R. and Weiland, F. (1989): Infectious haemorrhagic disease of rabbits. Contagious disease caused by calicivirus. Tierarzt Umsch, 44 (5): 284 - 294.

Ohlinger, V.F.; Haas, B.; Meyers, G.; Weiland, F. and Thiel, H.J. (1990): Identification and characterization of the virus causing RHD. J. Virol., 64 (7): 3331 - 3336.

الممسوحة ضوئيا بـ CamScanner

OIE Terrestrial Manual (2008): Manual of Diagnostic Tests and Vaccines for Terrestrial Animals, 6th edition, Part 2 Section 2.6. Chapter 2.6.2. - Rabbit haemorrhagic disease.

Pages, M. A. (1989): Consideraciones técnicas de la sueroterapia y de la profilaxis vacunal en la enfermedad hemorrágica vírica del conejo (RHDV). Med. Vet. 6, 285-291.

Peshev, R. and Christova, L. (2003a):A comparison of the immunogenic efficacy of a bivalent vaccine against Pasteurellosis and Rabbit Haemorrhagic Disease with that of three monovalent vaccines against Rabbit Haemorrhagic Disease.Vet Res Commun., 27 (7): 591-594.

Peshev, R. and Christova, L. (2003b): The efficacy of a bivalent vaccine against pasteurellosis and rabbit haemorrhagic disease virus. Vet Res Commun., 27 (6): 433-444.

Peeters, J.E.; Broes, A. and Charlier, G. (1990): First observations of viral haemorrhagic disease of rabbits (RHD) in Belgium. Annales de Med. Vet., 134 (8): 567 - 571.

Salman, O.G.A (1999): Studies on haemorrhagic viral disease in rabbits. M.V.Sc1. Thesis, Cairo Uni.

Salman, O.G.A (2007): Further studies on haemorrhagic viral disease in rabbits in Egypt. Ph.D. Vet. Thesis, Faculty Vet. Medicine, Cairo Uni.

Shevchenko, A.A. (1994): Basic properties of new lyophilized and inactivated vaccine against viral haemorrhagic disease of rabbits. Russian Agri. Sci., 6: 24 - 28.

Simon, M.C.; Girones, O.; Alonso, J.L.; rabbituzquiz, J.L.; Garcia, J.; Ortega, C. and Muguruza, R. (1993): Viral haemorrhagic disease in commercial rabbit farms; efficacy of an inactivated vaccine in protection against experimental inoculation. Medicina Veterinaria, 10 (1): 44 - 48.

Smid, B.; Valicek, L.; Rodak, L.; Stepanek, J. and Jurak, E. (1991): Rabbit haemorrhagic disease; an investigation of some properties of the virus and evaluation of an inactivated vaccine. Vet. Microbiol, 26 (1/2): 77 - 86.

Stone, H.D.; Brugh, M. and Beard, C.W. (1983): Influence of formulation on the efficacy of experimental oil-emulsion Newcastle disease vaccine. Avian diseases, 27 (3) : 688-697.

Taha, M. M.; Amal, M. I.; Hayam, F.; Aboul Soud, S.M.; Amal, E.-S. and Salman, O. G. A. (2009): Comparison on the effect of different adjuvant on the evaluation of immune response of monovalent and bivalent vaccines containing Pasteurella multocida and/or Rabbit Haemorrhagic Disease Virus. 6th Int. Sci. Conf., Mansoura, 59: 893-911.

Thayer, S. G.; Edison, C.S. and Klevev, S.H. (1983): Multivalent inactivated virus oil emulsion vaccines in broiler breeder chickens. I. Newcastle disease virus and infectious bursal disease virus bivalent vaccines. Poultry science, 62: 1978-1983.

Thibault, H. (1990): Sanitary prophylaxis in rabbits. A control plan against viral haemorrhagic disease. Cuniculture Paris, No. 94: 175 - 177.

Yu, Y.R.; Du, N.X.; Zhao, Z.X.; Gou, J.Y. and Hu, G.Z. (1992): Immunological effect of the oil emulsified vaccine to rabbit haemorrhagic disease. Chinese J. Rabbit Farming, 3: 28 - 29.

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تحضير لقاح زيتي مثبط ضد مرض النزف الدموي الفيروسي الأرنبي

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تم تحضير لقاح زيتي مثبط ضد مرض النزف الدموي الفيروسي بنسبة خلط 2:1 مرحلة مائية الى مرحلة زيتية على الترتيب و تم دراسة تأثير كفاءة هذا اللقاح فى الأرانب المحصنة لمدة سنة كاملة بعد التحصين من حيث الاستجابة المناعيه و مقاومة التحدي بفيروس النزف الدموي الأرنبي الضاري حيث تم حقن مجموعة من الأرانب الخالية من الأجسام المضادة الخاصة بهذا المرض باللقاح مع وجود مجموعة اخرى من الأرانب غير محصنة ضابطة. أثبت اللقاح المحضر محليا كفاءة مناعية عالية حيث ارتفع مستوى الأجسام المضادة المقاسة باختبار منع التلازن الدموي في الأرانب المحصنة مسجلا أعلى ارتفاع بداية من الأسبوع السادس ووصل الى اقصى ارتفاع (القمة) في الشهر السابع. استمر مستوى الأجسام المضادة مرتفعا حتي نهاية السنة و قد انعكست هذه النتائج على اختبار التحدي حيث كانت نسبة الحمايه ضد فيروس النزف الدموي الأرنبي الضاري 100% مقارنة بالمجموعة الضابطة الغير محصنة (0%). توصي هذه الدراسة بتحضير هذا اللقاح محليا حيث انه يقدم مستوى مناعي عالى لفترة طويلة مما يوفر العملة الصعبة المهدرة في استيراد هذا اللقاح من الخارج.

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