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THYMIC AND SPLENIC CHANGES DUE TO VIRAL HAEMORRHAGIC DISEASE IN RABBIT AND INFLUENCE OF EMERGENCY VACCINATION

ELHAM F. EL KHASHAB; M. SHAKAL; SAHAR A. ZOU EL FAKAR, and EMAN B.SHAHEED

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SUMMARY

Vaccination against viral haemorrhagic disease in rabbits was tried. The inactivated vaccine was inoculated on different timings; simultaneously with the experimental infection, on appearance of clinical signs and 2 days after the begining of signs. Vaccination during illness with viral haemorrhagic disease was able to stop mortalities and shorten the disease course gaving 70%, 60% & 36% protection rate in simultaneously infected vaccinated , vaccinated on appearance of signs and vaccinated rabbits two days after the begining of signs. Seroconversion of the vaccinated rabbits was estimated and compared with the control group. Histopathological changes in thymus and spleen of rabbits experimentally infected with haemorrhagic disease virus were recorded. Lymphoid organs/body weight ratios on different intervals from vaccination and/or infection of different groups were measured.

INTRODUCTION

Rabbit viral haemorrhagic disease (RVHD) is a contagious highly fatal disease causing high morbidity and mortality (up to 100%) in adult rabbits (Fioretti et al., 1991).

The disease was firstly recorded in China, by Liu et al., (1984) . The disease was then reported in Asia, Europe and other countries world wide (Morisse et al., 1991; Nowotny et al., 1990; and Ohlinger et al., 1989).

In Egypt, rabbit viral haemorrhagic disease has been firstly reported by Ghanem and Ismail (1992), Salem and El-Ballal (1992), Sharawi (1992) and the virus was isolated by El-Zanaty, (1994); and El-Mongy, (1998).

Wei et al. (1987) reported the early onset of protection 3-5 days postvaccination with inactivated

vaccines . Similar observations were described by other authors (Du et al. 1991; and Huang, 1991).

Due to the drastic losses through high mortalities of viral haemorrhagic disease in rabbits and the early protection against the disease by vaccination we planned to study the changes in the lymph organs of rabbit due to vaccination and/or infection and the effect of application of emergency vaccination in case of early and late diagnosis of the disease.

MATERIAL AND METHODS

Rabbits:

One hundred and twenty, two months old New Zealand rabbits were obtained from a healthy private rabbitry with no history of vaccination against rabbit viral haemorrhagic disease.

Challenge virus:

Challenge virus was isolated by Salman (1999) from a field outbreak and identified by Hafez (1999).

3- Inactivated vaccine:

Formalized RVHD virus in 2% aluminium hydroxide gel adjuvant manifactured in Veterinary Serum and Vaccine Institute, Abbasia, Cairo was used in this experiment

4- Serum samples:

Blood samples were collected from the ear vein, and serum samples were separated and tested for seroconversion.

5- Organ samples:

Lymphoid organs (spleen and thymus) were collected and fixed in 10% formol saline for histopathological examination. Livers were homogenised and retested for RVHD virus.

6- Erythrocyte suspension:

Human type "O" erythrocytes were suspended in sterile saline 1% for microtechnique HA and HI tests.

7- Microhaemagglutination test:

RVHD virus was detected and titrated in livers of infected rabbits according to Chasey et al. (1995).

8- Haemagglutination inhibition test:

Seroconversion of the vaccinated rabbits was evaluated by Beta procedure of HI test according to Pu et al. (1985).

9- Histopathological examination:

Sections of the lymphoid organs (spleen and thymus) fixed in formol saline 10% were stained with Hematoxline and Eosin. Parallel sections of thymus and spleen from vaccinated challenged and control rabbits were stained with Methyle green Pyronine (Bancroft et al., 1994) for detecion of the activity of lymphoblast cells in lymphoid organs.

Table: (1): Vaccination and/or challenge against RVHD of the different grops with various investigations.

Group	Infection	Vaccination	Time of vaccination related to challenge	Observation for symptoms and P.M lesions	Histopathology	Serology
G1	+	+	2 days before challenge	+	-	-
G2	+	+	Simultancusly	+	-	-
G3	+	+	On satart of clinical signs	+	-	-
G4	+	+	2 days after satart of sings	+	+	-
G5	+	-	-	+	+	-
G6	-	+	-	+	+	+
G7	-	-	-	+	+	+

+ = Done
- = not done

10- Vaccination:

According to Kim et al. (1989) 0.5 ml of the inactivated vaccine was inoculated subcutaneously into each rabbit.

11- Challenge:

Rabbits were inoculated I/M with 0.5 ml RVHD challenge virus with a titer of 2^{15} HAU.(Salman,1999).

Experimental design.

As shown in Table (1), rabbits were kept under observation for clinical signs and mortalities for two weeks postinfection and/or vaccination. Dead and sacrificed rabbits were examined for postmortem lesions.

Lymphoid organs (spleen and thymus) from the sacrificed rabbits were weighed on 0, 3, 7, 10, 14 and 21 days postchallenge in Group 4 and 5 and postvaccination in Group 6 added to the non-infected control (Group 7). The ratio of the lymphoid organs/body weight were calculated according to Montogomery et al., (1985) as follows:

$$\text{Lymphoid organ/body weight ratio} = \frac{\text{Organ weight}}{\text{Body weight}} \times 10{,}000$$

Speciemen of the thymus and spleen collected from groups 4-7 were fixed and examined for histopathological changes.

Serum samples were collected on 0, 3, 7, 10, 14

and 21 days postvaccination from the vaccinated (Group 6) and the nonvaccinated nonchallenged control (Group 7) and tested for seroconversion.

RESULTS

1- Clinical signs in challenged groups:

The signs were sudden death 2 days postinfection with short course and high mortality especially in G5 (nonvaccinated challenged group) with epistaxis, convulsion and respiratery distress.

2- Gross changes:

a) In vaccinated animals :

Marked increase in size of thymus was seen starting from day 3 and continued for the length of experiment (3 weeks).

b) In challenged groups:

Trachea was filled with bloady foams and pneumonic lungs. Liver was enlarged, congested and dark red in colour. Spleen was enlarged, congested and dark bluish in colour. Intestine shown petcheal haemorrhages on serosal surface. Haemorrhages on thigh muscles and other muscles

3) Lymph organs/body weight ratio:

The lymphoid organs/body weight ratios were calculated according to Montgomery et al., 1985 of the different groups and shown in Table (3). Spleen/body weight ratios and thymus/body weight ratios of the different groups are measured on day 0, 3, 7, 10, 14 and 21 postinfection.

Table: (2): Mortalities and protection rate in different challenged and/or vaccinated rabbits.

Treated groups	No. of Rabbits	Time of vaccination related to challenge								Total deaths	Protection Percent
		0	1	2	3	4	5	6	7		
G1: Vacc. 2 d. before challenge	10	-	-	-	1	1	-	-	-	2	80
G2: Chall. + vacc. Simultanously	10	-	-	1	1	1	-	-	-	3	70
G3: Chall + vacc. On signs beginning	10	-	-	2	1	1	-	-	-	4	60
G4: Chall. + vacc 2d after signs begin	25	-	-	4	5	2	2	2	1	16	36
G5: Chall. Control	25	-	-	4	5	3	3	2	1	18	28
G6: Vacc. Control	20	-	-	-	-	-	-	-	-	0	100
G7: Non chall, non vacc. (Blank Control)	20	-	-	-	-	-	-	-	-	0	-

Table: (3): Lymphoid organs (Spleen, thymus) body weight ratios of different vaccinated and/or challenged rabbits.

Groups	Challenge	Vaccinated	Spleen and thymy body weight ratios at different Days post challeng											
			0 d		3 d		7 d		10 d		14 d		21 d	
			*S/b.w	**Thy/b.w	*S/b.w	**Thy/b.w	*S/b.w	**Thy/b.w	*S/b.w	**Thy/b.w	*S/b.w	**Thy/b.w	*S/b.w	**Thy/b.w
4	Yes	2 day after signs begin	ND	ND	8.445	15.863	11.548	17.66	7.803	23.993	9.138	27.074	11.71	21.211
5	Yes	No.	ND	ND	8.163	15.736	8.41	15.46	3.862	12.782	9.085	21.932	11.43	16.104
6	No	Yes	ND	ND	5.496	20.42	6.813	22.45	7.432	25.23	7.121	25.766	25.766	20.59
7	No	No	ND	9.771	4.964	9.771	1.677	21.02	4.386	21.761	3.988	19.09	19.09	14.06

* S/b.w = Spleen/body weight ratio ND = not done
** Thy/b.w = Thymus/body weight ratio

N. B.: Lymphoid organs ratio = $\frac{\text{Lymphoid organs weight}}{\text{Body weight}}$ x 10.00 (Montgomery et al., 1985).

Table: (4): Geometric mean of Haemagglutinating antibodies of the vaccinated and nonvaccinated rabbits.

Days post vacciantion	Groups	Geometric mean of HI Antibody titre
0 d	Vacc. Non vacc.	0 0
3 d	Vacc. Non vacc.	$2^{2.3}$ 0
7 d	Vacc. Non vacc.	$2^{5.3}$ 0
10 d	Vacc. Non vacc.	$2^{5.8}$ 0
14 d	Vacc. Non vacc.	$2^{6.2}$ 0
21 d	Vacc. Non vacc.	$2^{7.0}$ 0

4- Protection rate and disease course:

Protection percent was calculated after vaccination of the different groups as follows : 80% in rabbits challenged 2 days post vaccination, 70% in simultanseously infected vaccinated rabbits, 60% in vaccinated rabbits on appearance of signs and 36% in rabbits vaccinated 2 days after start of signs. Deaths has stopped by day 4 in groups 1, 2 and 3 as shown in table (2) where mortalities continued to day 7 in groups 4 and 5.

5- Seroconversion:

Geometric mean of haemagglutination inhibiting antibodies against viral haemorrhagic disease in vaccinated and nonvaccinated rabbits were measured on days 0, 3, 7, 10, 14 and 21 postinoculations. (Table 4).

Histopathological changes of lymphoid organs:

Spleen :

In vaccinated rabbits:

Hyperplasia of the lymphoid follicles with lymphoblastic activation were seen (Fig. 1 & 2).

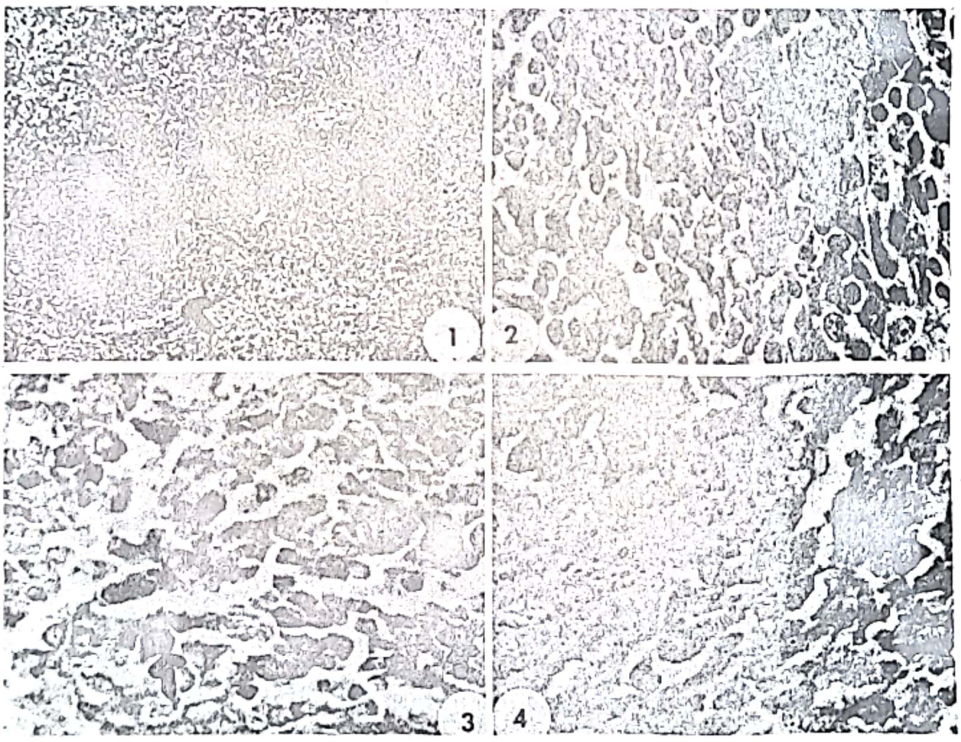

Fig. (1) : Spleen of vaccinated rabbit showing hyperplasia of lymphoid follicles in white pulp (H&Ex33).
Fig. (2): Spleen of challanged rabbit showing depletion of lymphoid follicles (H&Ex132)
Fig. (3): Spleen of vaccinated rabbit showing numerous numbers of lymphoblast cells. (Methyle green pyronine x 330).
Fig. (4): Spleen of challanged rabbit showing few numbers of lymphoblast cells (Methyle green pyronin x 330).

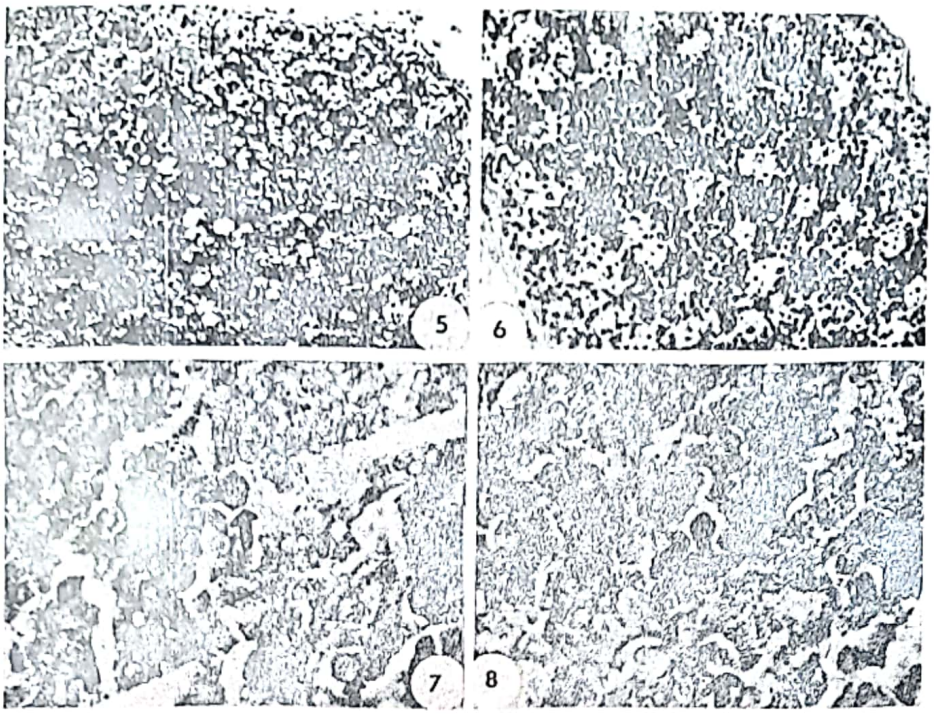

Fig. (5) : Thymus of vaccinated rabbit showing hyperplasia of thymocytes in cortex (H & E x 33).
Fig. (6): Thymus of challanged rabbit showing necrosis of the thymocytes (H&Ex132)
Fig. (7): Thymus of vaccinated rabbit showing increased numbers of lymphoblast cells. (Methyle green pyronine x 330).
Fig. (8): Thymus of challanged rabbit showing numbers of lymphoblast cells (Methyle green pyronin x 330).

In challenged animals:

Lymphocytic depletion began to appear on 3 days and increased till the end of experiment (Figs. 3 & 4).

Thymus:

In vaccinated animals:

In the cortex, hyperplasia of lymphocytes were pronounced forming starry appearance, while in medula lymphoblastic activation was evident (Fig. 5 & 6).

In challenged animals:

The thymic cortex showed necrosis of lymphocytes 3 days post challenge. Later on,congestion and haemorrhages were seen (Fig. 7& 8).

DISCUSSION

Outbreaks of viral haemorrhagic disease in populations of domestic rabbits were recorded in different Governorates in Egypt (Ghanem and Ismail, 1992; Salem and El-Ballal, 1992; El-Mongy, 1998). Owing to the increasing economic losses

(reaching 90%), RVHD is considered the greatest disease problem that threatens rabbit industry.

Vaccination against RVHD using inactivated vaccine have been applied successfully protecting rabbit populations from 6-12 months (Wei et al., 1987; David et al., 1991; Shevchenko, 1994).

Wei et al. (1987) reported the early onset of protection 3-5 days post vaccination with inactivated vaccine. Similar observations were described by other authors (Du et al., 1991 and Huang, 1991).

The drastic losses due to high mortalities urged to search for measures to interfere against the viral haemorrhagic disease during breaks. Based on the rapid and early onset of protection against RVHD as described by Wei et al. (1987), we planned to study the use of vaccination during the disease break to minimize the losses.

A group of rabbits (G 1) were vaccinated 2 days before challenge. The resulting protection due to vaccination as compared to the challenge group (G 5) was found to be 80%. This finding agreed with those reported by Du et al. (1991) and Huang (1991).

Another group (G 2) were vaccinated and challenged simultaneously, a third group (G 3) were vaccinated just on begin of signs and a fourth group (G 4) were vaccinated two days after the onset of signs. The resulting protection rates were 70%, 60% and 36%, respectively showing that vaccination during the disease breaks was able to stop the mortalities successfully.

Haralambiev (1991) studied the interference between the inactivated and virulent RVHD virus. The inactivated suspensions resulted in resistance to infection with virulent virus after 48 hours.

The humoral immune response was evaluated titrating the haemagglutination inhibiting antibodies. Detectable titers were found on the third day (22.3 HIU) , where considerable titers were detected from day 7 and onwards. Similar findings were recorded by Abd El Motalib et al. (1998), Patton (1989) , Popvic (1990) and Arguello et al. (1992).

Although a high protection percent was estimated 2 days postvaccination, indicating that other immune mechanism rather than humoral one would play a principal role for the protection like cellular mechanism.

Changes and consequences of vaccination and/or challenge with RVHD on the lymphoid organs were studied grossly, histopathologically and changes in lymphoid organs/body weight ratio.

As shown in Table (3) the lymphiod organs/body weight ratios increased in the vaccinated animals markedly specially the thymus/body weight ratio as compared to the control nonvaccinated non infected animals starting by day 3 and continued for the length of the experiment (3 weeks). Thymus gland is known to monitor the cellular immune mechanism. The very fast increase in thymus/ body weight ratio on day 3 explain the high protection percentage against challenge in group 1, two days postvaccination before raising a considerable antibody titers. The increase of spleen/ body weight ratio was much slower comparable to the slow rise of humoral antibodies.

The lymphoid organs/body weight ratios of the challenged animals showed lower figures which was proved by the histopathological exmination showing destructive effect of the virulent virus on lymphocytes.

On the contrary the inactivated vaccine was found to activate the lymphocytes in thymus and spleen indicated by hyperplasia and increased lymphoblastic activation as shown in Methyl green Pyronine stained sections (Bancroft, et al., 1994).

Effect of viral infection on lymphoid organs and antibody response was studied by Montgomery et al. (1985).

Vaccination 2 days after the onset of signs resulted in lower protection rate than vaccination just at the onset of signs. This finding emphasis the importance of rapid correct diagnosis of the disease enabling the interference with vaccination to minimize the losses.

It has been concluded that the emergency vaccination of RVHD can minimize the mortalities and shorten the disease course nevertheless late interference in missdiagnosis. The defence mechanism depend on many factors rather than humoral immunity like cellular and local immunity which was obviously clear in lymphoid organs indices.

Repeating of those trials would confirm the results.

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