Dept. of Poultry Diseases,
Fac. Vet. Med., Asiut Universiity.
Head of Dept. Prof. Dr. S.A. Mousa

SOME INVESTIGATIONS ON RABBIT VIRAL HAEMORRHAGIC DISEASE IN UPPER EGYPT

(With 6 Tables)

By

K.EL ZANATY

(Received at 24/10/1993)

استقصاء عن مرض النزف الدموى الفيروسى فى الأرانب فى مصر العليا

كمال الزناتى

اثناء شتاء وربيع عام ١٩٩٣ شوهد الاصابه لعدد كبير من مزارع الأرانب بوباء النزف الدموى الفيروسى فى مناطق مصر العليا (المنيا - أسيوط - سوهاج) بنسب وفيات عاليه تتراوح من ٧ ر ٢٦٪ الى ١٠٠٪ فى الأرانب ذات أعمار من ٤ - ١٦ شهراً تم تسجيل الاعراض الاكلينيكيه والباثولوجيه المختلفه فى القطعان المصابه طبيعياً . تم استبيان الفيروس ذات خاصية تلازن الدم لكرات الدم الحمراء للأنسان (○) فى عدد ١١٧ من ٢٠٦ من الأنسجه المفحوصه من الأرانب الميته والمأخوذه من تسعة عشر مزرعه المصابه حقلياً وكانت قدرتها على تلازن الدم تتراوح من ١ : ١٦٠ إلى ١ : ٥١٢٠ .

هذا وقد تم تشخيص العترات كفيروسات النزف الدموى للأرانب فى المعمل بأستخدام اختبارى تلازن الدم وتلازن الدم المضاد بواسطة المصل المعروف .

أظهرت تجارب تحدى المناعه وتلازن الدم المضاد المقارن وكذلك تلازن الدم للثدييات والطيور المختلفه والعدوى الصناعيه فى الأرانب وحيوانات المعمل المختلفه لعترتين من العترات المعزولة من وبائيات ذات نسب نفوق عاليه ومنخفضه أن هاتين العترتين لهما نفس الخواص ويبدو أنه يوجد نوع واحد من الفيروس المسبب للمرض الفيروسى الدموى للأرانب.

المسح السيرولوجى لمزارع الأرانب المعروفه أنها لم تتعرض من قبل للاصابه بالمرض بأستخدام تلازن الدم المضاد (أعمارها تتراوح من ٧ - ١١ شهراً) وجد أن ٨ من ٢٩ مزرعه أرانب المفحوصه بها أجسام مضاده لفيروس النزف الدموى وأن ٧٩ من ٣٧٤ عينة سيرم كانت موجبه فى أختبار تلازن الدم المضاد .

SUMMARY

Rabbit hamorrhagic disease (RHD) outbreaks were observed during winter and spring, 1993 in most rabbitaries of Upper Egypt (Minia, Assiut and Sohage Provinces) with mortality rate of 26.7 up to 100% in4-16 month-old rabbits. Clinico-pathological manifestations of natural outbreaks are described. Viral hemagglutinins were detected in 117 out of 206 tested tissue homogenate from dead rabbits representing 19 rabbitaries with hemagglutination (HA) titres of 1:160 - 1:5120. The viral hemagglutination inhibition (HI) test against reference RHD-antiserum. Cross protection test, cross HI-test, HA-activity to mammalian and avian erythrocytes and experimental infection to rabbit, rat, mice and guinea pig of two field isolates (from outbreaks with high and low mortalities). Results revealed that both isolates are identical viruses and represent only one serotype of RHD virus. Seroepidemiological study of different rabbitaries revealed that 79 (22.9%) of 374 rabbit sera were positive. These positive sera were From 8 (27. 6%) of 29 examined rabbitaries (7-11 month-old, and without history of RHD) had naturally HI-antibodies.

INTRODUCTION

Nowadays RHD virus has become a serious worldwide disease of rabbits. The disease was first recognized in china (*LIU et al., 1984*). Since then, there are reports about this disease in many countries of Europe (*MORISSE, 1988; Schluter and shirrmeier, 1988; MATTHES and MAESS, 1989; RODAK et al., 1990*), Mexico (*GREGG and HOUSE, 1989*), Korea (*LEE et al., 1990*), Isreal (*KUTTIN et al., 1991*) and in Egypt (*GHANEM and ISMAIL, 1991 and SALEM and EL-BALLAL, 1992*). RHD is peracute or acute highly contagious disease characterized by high fever, hemorrhagic septicaemia, and high mortality within 72 hours among domestic rabbits. Diagnosis of RHD is based on clinical signs, pathological findings (*MORISSE, 1988; GREGG and HOUSE, 1989 and Peeters et al., 1990*) as well as HA-test of tissue homogenate and furthermore the diagnosis can be verified serologically by HI-test and enzyme linked immunosorbent assay (*CAPUCCI et al ., 1989 and OHLINGER et al., 1989*).

Specific RHDV-antibodies in rabbit sera were reported by *KIEPKER, 1990; RODAK et al., 1990; RyLL, 1990; Sodan et al., 1990 and Wawrzkiewicz and MAJOR-DZIEDZIC, 1992.*

The present study was undertaken to study the epidemiology, diagnosis of RHD-outbreaks, susceptibility of experimental animals and comparison between the isolated RHD viral agents as well as serological survey of different rabbitarries without history of RHD for detection of RHD-HI antibodies.

MATERIAL and METHODS

Rabbitaries history:

Heavy losses occured in winter and spring, 1993 in most governmental and private rabbitaries in Upper Egypt (Minia, Assiut and Sohag Provinces). The clinical course of the disease (sudden deaths in adult animals, haemorrhagic diathesis) was indicative for an infection with RHD-virus. Necropsy as well as virological examination (as mentioned below) confirmed the suggestive clinical diagnosis.

Specimens:

Two hundred and sex freshly dead rabbits of both sexes, different breeds and ages, submited to our laboratory from 19 rabbitaries (Table 1) were necropsied. Equal parts of liver, lung, spleen were collected under aseptic conditions for virological examination, loopful from heart blood, liver, lung, spleen as well as intestinal tract for bacteriological examination and also intestinal and liver smears for parasitological examination.

Preparation of specimens for virological examination:

A 1:10 suspension of liver and lung tissue in physiological buffer saline (PBS) pH 7.2 was homogenated and tested in micro HA test. From positive HA-liver and lung cases, 10% suspension of liver, lung, spleen and kidney tissue was made in PBS, homogenised, centrifuged at 3000 rpm for 15 min. The supernatant was collected, antibiotic mixture (penicillin and streptomycin) was added and stored at- 20°C for further studies.

Reference RHD virus and antiserum:

RHD virus (HA-titre 1: 4096) previously identified by *SALEM and EL-BALLAL, 1992* and RHD-hyperimmune serum (HI titre $10X10^2$) prepared in rabbits from our laboratory were used in the experiments.

Normal rabbit serum (NRS):

NRS collected from healthy rabbit, had no inhibitory effect in HI-test was used as negative control serum.

Experimental animals:

Twenty two, 6-month-old weighted about 2 kg. healthy balady rabbits (free from RHD-HI antibodies) from private farm; six, 4-week-old white-swiss mice from the laboratory Assiut Unit, Faculty of Medicine; six guinea pigs and six rats from Faculty of Science, and 9-day-old embryonated chicken eggs (ECE) from Faculty Agriculture Farm, Assiut University were used in experimental studies.

Haemagglutination (HA) test:

A 10% tissue homogenate in PBS (pH 7.2) from dead animals was tested in micro-HA test after *KOLBL et al., 1990* using 0.5% human erythrocytes group 0 (Rh+). After two hours incubation period at room temperature, the test was readed. Tissue homogenate with HA-titre more than 1:100 were considered as RHD-positive cases. Positive HA cases were further identified as RHD virus in HI-test against reference RHD-antiserum.

Haemagglutination inhibition (HI) test:

HI test was performed after *Ohlinger et al., 1989* using 8 HA-units of virus suspension and 0.5% human erythrocytes (group 0, Rh+). Hyperimmune RHD-serum and NRS were used as positive and control serum respectively. Immediately before use sera were inactivated at 58°C for 30 min. (*WAWRZKIEWICZ and MAJER-DZIEDZIC, 1992).*

Virus characterization:

Two RHD-viral agents isolated from field outbreaks with high (100%) and low (26.7%) mortalities were designated R_1 & R_2 respectively and subjected for further characterization.

Antigen preparation:

Three, 6-month-old healthy rabbits per isolate were inoculated I/M with clarified antibiotic treated tissue supernatant of RHD-viral agents R_1 and R_2. All inoculated rabbits died within 48-80 h. postinoculation. liver and lung tissue for each isolate were prepared and identified in micro-HA and HI tests and stored at -20°C till used. (Prior to inoculation, clarified antibiotic treated tissue supernatants of the two RHD-isolates were tested in miro-HA-test and used for animal inoculation).

(1) Virus isolation and propagation in (ECE):

Antibiotic treated tissue supernatant of the two RHD virus isolates were inoculated into twenty 9-day-old embryos/isolate via allantoic sac with 0.1 ml/embryo. Embryos were candled twice daily and observed for 10 days post inoculation (PI). Three blind passages were done.

(2) HA-activity for mammalian and avian erythrocytes:

The two RHD-viral agents (R_1 & R_2) were tested for HA-activity of human (O, A, B and AB groups), sheep, cattle, camel, chickens, quails, pigeons and ducks.

(3) Experimental infection to white mice, rat, guinea pigs:

Two animals of the following species mice, rats, guinea pigs per isolate were inoculated intraperitoneally with 0.2 ml/animal of bacteria free supernatant rabbit passage-RHD viral agents R_1 and R_2 (HA titre of each virus was 1024). Another two animals of each species were injected IP with 0.2 ml/animal of PBS and kept as control. All animals were observed for 10 days PI.

(4) Pathogenicity test:

To reproduce the disease and compare the pathogenicity of the two RHD-viral agents (R_1 & R_2), 10 rabbits/isolate were inoculated I/M with 1 ml of bacteria free tissue supernatant (HA-titre 1024). Another 2 rabbits were I/M inoculated with I ml PBS and kept as control. All animals were observed daily (10 day PI) for clinical signs and deaths. Dead animals were necropsied for PM lesions and detection of viral hemagglutinin in internal organs.

Vaccine preparation:

0.4% formalin oil emulsion inactivated vaccine was prepared from 4^{th} rabbit passage tissue suspension of RHD-viral agents (R_1 and R_2) as previously described by *SALEM and EL-ZANATY, 1992.* The vaccines were designated vac. I and vac. II and used in cross HI and cross protection tests.

(5) Cross HI and cross protection tests:

Twenty, 6-month-old healthy rabbits (free from RHD-HI-antibodies) were divided into four groups (5 rabbits/group), vaccinated S/C with 1 ml of prepared vaccine (Table 2). 21 days post-vaccination, sera were collected and tested in cross HI-test and the animals were challenged with RHDV-isolates (R_1 & R_2) as shown in Table 2. Four rabbits (2 animals/isolate) were inoculated I/M with RHDV-R_1 and RHDV-R_2 as virus control, and another two rabbits were left as nonvaccinated nonchallenged control. 10 days after challange, 2 rabbits from each group were necropsied for PM lesions.

Serological examination of rabbit farms for RHD-HI antibodies:

347 rabbit sera were collected from 29 rabbitaries (7-11 months old, without history of RHDV of both sexes and different breeds). Sera were inactivated at 58°C for 30 min. and tested in micro-HI test using 8-HA units of reference RHDV and human erythrocytes group o (RH+).

RESULTS

Results History, clinical signs and postmortem findings in naturally affected rabbits:

During winter and spring, 1993 (prevalence was highest in March and April) heavy losses among rabbit farms occurred in Minia, Assiut and Sohag Provinces. Sudden deaths without symptoms usually had been seen affecting several groups simultaneously within the affected rabbitaries. The clinical course of the disease was peracute to acute with mortality rates ranged from 26.7% up to 100%. The common age of the disease onset was 4 to 16 months and females particularly those pregnant were more susceptible than males. All rabbit breeds were susceptible to RHDV (table,1). In peracute form, some rabbits showed shortly before death unconscious and most rabbits usually died without any symptoms. In acute form, the rabbits showed severe depression, anorexia, dyspnoea with foamy bleeding from nostrils up to suffocation. Some rabbits developed nervous manifestations in the form of severe convulsions, incoordination and paralysis (in few cases before death). Sometimes, the animals showed a backbended head position and crying before die. Fecal matter tinged with blood was also seen in few affected rabbits. Most affected rabbits die within 36-48 hours and occasionally 72 hours from the onset of the disease.

Postmortem lesions, the most constant pathological lesions were commonly seen in the respiratory tract and liver. Diffuse hemorrhages in the larynx. Trachea were filled with foamy bloody exudate and petechial or diffuse hemorrhages in the edematous lung lobes. Liver was swollen, congested with or without punctate hemorrhages. There was moderate to severe necrotic hepatitis and sometimes fatty degeneration in some carcasses. Other pathological lesions include hyperaemia and hemorrhages in the spleen, thymus and occasionally in the kidneys with rare petechia. Slight to moderate enlargement of the spleen and urinary bladder were present in few carcases.

Bacteriological and parasitological examination revealed no specific bacteria and coccidia (hepatic or intestinal) in some cases.

HA test: 117 (57.8%) of 206 tested tissue (liver and lung) homogenate were positive in micro-HA test with different HA titre 1: 160 to 1: 5120 (Table 1)

HI test: All 117 positive HA viral agents were inhibited by reference RHD-hyperimmune serum in micro HI test. The 117 viral agents were identified as RHD-viruses by HA and HI tests.

Virus characterization:

1-Virus propagation in ECE: After three blind passages, attempts to propagate the two virus isolates (R_1 & R_2) were

unsuccessful, as no lesions or deaths were observed and embryonic fluids were negative in HA-test.

2-HA-activity for avian and mammalian erythrocytes: The results of HA activity are illustrated in Table (3).

3-Experimental infection to white mice, rats and guinea pigs: All inoculated animals were resistant to infection with the two RHD (R_1 & R_2) viral agents.

4-Pathogenicity test: The clinical signs as well as the postmortem lesions produced by the isolated RHDV (R_1 & R_2) in the 1^{st} and 2^{nd} experimentally inoculated rabbit groups were similar to great extent. The incubation period was 24-60 hours. In peracute form, no symptoms can usually be seen since the infected rabbit died several hours after the onset of the disease. In acute form the rabbits developed fever (40.5-41.5°C) and manifested clinical signs which were more or less resembled to those described in natural outbreaks (depression, inappetance, dyspnoea, convulsion, incoordination). Most deaths occurred on the 2^{nd} and 3^{rd} day post inoculation and the mortality rate was 90 and 80% in the 1^{st} and 2^{nd} groups, respectively (Table 4).

Postmortem lesions: Rabbits that dic on the 2^{nd} and 3^{rd} day PI revealed hyperemia and hemorrhages of the lung, tracheal mucus membrane and the liver is slightly swollen with congestion and hemorrhages. Hyperemia and hemorrhages were also seen in the thymus and spleen. Lesions in those rabbits die on 4th and 5^{th} day PI showed bloody mucus in the trachea, punctate diffuse hemorrhages in the larynx and lung lobes, enlarged congestive liver with hemorrhages of variable size scattered through the liver and moderate hyperemic enlargement of the spleen and thymus. Small hemorrhages in the peritoneum was present in few carcasses.

Rabbits necropsied at the end of observation period (10 days) showed emaciated carcasses and darkness, focal hemorrhages in the edematous lung lobes, a tan discoloured enlarged friable liver and hypertrophy and hemorrhages of the thymus and spleen.

RHD-viral hemagglutinin was detected in the tissue homogenate with high titre (1: 512-1:2048) in liver, spleen and lung and with low titre (1: 16-1: 32) in kidney, heart muscle and brain. The HA titres of various tissue homogenates of experimentally infected rabbits were in the following organs: Liver> lung> kidny> heart> muscle> brain.

Cross HI and protection test:

HA activities of the two RHDV isolates (R_1 & R_2) were completely abolished in cross HI test using antisera from rabbits vaccinated with vac. I or vac. II as shown in Table (5).

The vaccines (Vac. I and Vac. II) were complete cross protecting when challenged with the two RHDV-isolates (R_1 & R_2) as indicated by the abscence of clinical signs and postmortem lesions. Unvaccinated challenged rabbits died within 48-72 h. PI and on necropsy revealed typical RHD-lesions. Also, the reference RHD-antiserum completely abolished haemagglutination of the two virus isolates (R_1 R_2) in HI test, whereas normal rabbit serum had no inhibitory effect.

DISCUSSION

Epidemiological, clinico-pathological features and HA & HI tests indicated that the RHD virus is prevalent in rabbitaries in Upper Egypt. Clinical signs, age of the disease onset, susceptibility of females than males, pathological findings and detection of viral hemagglutinin in tissue homogenates reported here were similar to great extent with *LIU et al., 1984; MORISSE, 1988; OHLINGER et al., 1989; ROSELL et al., 1989; KOLBL et al., 1990; LEE et al., NOWOTNY et al., 1990 and DU et al., 1991.* Hyperemia and hemorrhages of spleen and thymus observed in this study and also, by others *(DU, 1990 and LEE et al., 1990)* indicated the adverse effect of RHDV on immune system of rabbits. Spleen and thymus lesions were not reported by *GHANEM and ISMAIL, 1991; SALEM and BALAL, 1992.* Attempts to culture the RHDV in ECE were unsuccessful. Rat, mice and guinea pig were resistant to the experimental infection. Similar results were previously reported by Nowotny *et al., 1990; GHANEM and ISMAIL, 1991 and SMID et al., 1991.*

According to our results (complete cross HI and cross protection test) between the two RHDV-isolates (R_1 & R_2) in addition to HA-activity for avian and mammalian erythrocytes and experimental animals inoculation (rabbits, rat, mice and guinea pig) based on results reported by *DU, 1990,* it was concluded that the two RHD (R_1 & R_2) virus isolates are identical viruses with exists of only one serotype of RHDV.

Although the two RHD viruses (R_1 & R_2) were isolated from field outbreaks with high (100%) and low (26.7%) mortalities, experimentally infected rabbits showed high mortalities, variation in field outbreaks mortalities may be attributed to absence or presence of different RHDV-antibodies levels during the exposure to the natural infection.

Serological survey of rabbitaries without history of exposure to RHD for detection of RHD-HI antibodies revealed wide spread of subclinical infection of RHDV in Upper Egypt. Detection of RHD-HI antibodies was also reported by *KIEPER, 1990; RODAK et al ., 1990; RYLL; SODAN et al., 1990 and WAWRZIEWICZ and MAJER-DZIEDZIC, 1992.*

In conclusion, RHD virus is wide spread among rabbitaries in Upper Egypt causing a serious economic losses in all rabbits breeds and exists only one serotype of RHD virus.

Micro-HA and HI tests owing to their sensitivity, specificity and simplicity are very useful for diagnosis of RHDV. system are in progress.

REFERENCES

Capucci, L; Lavazza, A. and Scicluna, M.T.(1989): Reported at the Meeting of the European Society for Veterinary Virology on Rabbit Viral Haemorrhagic Disease in Lyon, 7th Dec., 1989.

DU, N. (1990): Rabbit haemorrhagic disease (RHD)-A new disease and its viral etiology. Dtsch. Tierarztl. Wschr., 97, 114-116.

DU, N.X.; Xu, W.Y.; Liu, S.J.; Xu.F.N.; YU, Y.R. and Li, R.J. (1991): Studies on rabbit haemorrhagic disease. Scientia Agricultura Sinica, 24 (1) 1-10.

Ghanem, T.A. and Ismail, A. (1991): Occurrence of rabbit haemorrhagic disease in Sharkia Province. Egyptian-German Vet Med. Conf. 12-15 Nov., 1991.

Gregg, D.A. and House, C. (1989): Necrotic hepatitis of rabbits in Mexico: a parvovirus. Vet. Rec., 125, 603.

Kiepker, C. (1990): Investigation of the occurrence of viral haemorrhagic disease of domestic rabbits in Lower Saxony, Germany. Tierarztlich Hochule, Hannover, Germany. p.65.

Kolbi.S.; Settele, J. and Schonbauer, M. (1990): First occurrence of rabbit Haemorrhagic disease (RHD) in Austria. Berl. Munch. Tierarztl. Wsch. 103, 261-266.

Kuttin, E.S.; Nowotny, N.; Nyska, A.; Schilcher, F. and Waner, T. (1991): Rabbit Haemorrhagic Disease-First outbreak in Isreal and review of the literature. Isreal J. Vet. Med., 46 (4) 119-126.

Lee, C.S.; Park, C.K.; Shin, T.K.; Cho, Y.J. and Jyeong, J.S. (1990): An outbreak of rabbit sudden death in Korea suspected of a new viral hepatitis. Jpn. J. Vet. Sci., 52 (5): 1135-1137.

Liu, S.J.; Xue, H.P.; Pu, B.O. and Quin, N.M. (1984): A new viral disease in rabbits-Rabbit viral haemorrhagic disease. Animal Husbandary and Veterinary Medicine, 16, 253.

Matthes, S. und Maess, J. (1989): Infektiose hamorrhagische Erkrankung der kaninchen (Rabbit haemorrhagic disease). Dtsch. Tierarztl. Wschr. 96, 397-432.

Morisse, J.P. (1988): The haemorrhagic septicaemia syndrome in the rabbit: First observation in France. Le Point Veterinaire, 20, 79-83.

Nowotny, N.; Fuchs, A.; Schilcher, F and Loupal, G. (1990): Occurrence of viral haemorrhagic disease of rabbits in Austria I-pathological and virological investigations. Wiener Tierarztliche Monatschrift, 77 (1) 19-23.

Ohlinger, V.F.; Hass, B.; Ahl, R. and Weiland, F. (1989): Die infektiose hamorrhagische krankheit der kaninchen-eine durch ein calcivirus verusachte Tierseuche. Tierarztl. Umschau, 44, 284-294.

Peeters, J.E. Broes, A. et Charlier, G. (1990): Premieres observations de la maladie hemorrhagique du lapin (RHD) en Belgique, Ann Med. Vet. 134, 567-571.

Rodak, L.; Smid, B.; Valicek, L.; Vesely, T.; Stepanek, J.; Hampel, J. and Jurak, E. (1990): Enzyme linked immunosorbent assay of antibodies to rabbit haemorrhagic disease virus and determination of its major structural proteins. J. Gen. Virol. 71, 1075-1080.

Rosell, J.M.; Badiola, J.I and Badiola, J.J. (1989): Viral haemorrhagic disease of rabbits: Epidemiology and control. Cuniculture (Paris) No. 89, 21-26.

Ryll, V.(1990): Occurrence of viral haemorrhagic disease among wild rabbit and hares in lower Saxony, Germany. Tierarztliche Hocschule, Hannover, Germany, P. 84.

Salem, B. and El-Ballal, S.S. (1992): The occurrence of rabbit viral haemorrhagic disease (RVHD) in Egypt. Assiut Veterinary Medical Journal (1992) 27 (53) 295-304.

Schluter, H. and Schirrmeier, H. (1988): Mitteilung des Staatl. Institute F. Epizoot. U. Tierseuchenbekampfung Wusterhausen Dosse und des Fr.-Loefflex Instituts Insel Riems zu einer Virusseuche der kaninchen. Mh. Vet. Med., 43, 813.

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. Mic., 26 (1/2) 77-85.

Sodan, U.; Graeser, K. and Ulbrich, F. (1990): Use of the SUMAL variant of the haemagglutination-inhibition test for the viral haemorrhagic disease of rabbits. Monatshefte fur Veterinarmedizin, 45 (22) 790-791.

Wawrzkiewicz, J. and Majer-Dziedzic, B. (1992): Specific antibodies in rabbit serum samples against rabbit haemorrhagic disease virus. Medycyna Weterynaryjna, 48 (6) 268-269.

Table (1) Shows source, rabbit breed, total number of rabbits/rabbitary, age, deaths number and their percentage, results of HA-test.

Rabbitary number	Source Province	Breed	Age (month)	Total No.	Death No.	Death %	No.of** tested samples	HA-test No.of +ve	HA-test %	No.of +ve HA samples with different HA-titre 1:160	1:320	1:640	1:1280	1:2560	1:5120
1	Assiut	Boskat	12	46	42	91.30	18	11	61.10	-	5	3	2	1	-
2	Assiut	Boskat	4	22	17	77.30	10	7	70.00	2	3	-	2	-	-
3	Assiut	Californian	6-8	63	36	57.10	15	6	40.00	-	3	1	2	-	-
4*	Assiut	Boskat	13	16	16	100.00	7	4	57.10	-	-	2	1	-	1
5	Assiut	Balady	4-6	65	34	52.30	13	7	53.80	3	-	2	2	-	-
6	Minia	Boskat	7	20	13	65.00	5	3	60.00	-	2	1	-	-	-
7	Minia	Boskat	14	42	19	45.20	7	5	71.40	1	3	-	1	-	-
8	Minia	NewZealand*	4-9	57	57	100.00	16	10	62.50	-	2	7	1	-	-
9	Minia	Californian	6-8	52	40	76.90	25	13	52.00	6	1	4	2	-	-
10	Assiut	Cross-breed	14	67	18	26.90	12	7	58.30	-	5	1	-	1	-
11	Assiut	NewZealand*	11	35	17	48.60	11	7	63.60	4	1	2	-	-	-
12	Assiut	Boskat	6	12	11	91.70	7	4	57.10	-	2	1	1	-	-
13	Assiut	Cross-breed	4-5	65	24	36.90	13	8	61.50	-	-	1	5	-	2
14	Sohag	Boskat	12	38	18	47.40	9	6	66.70	3	1	-	2	-	-
15	Sohag	Californian	8	20	7	35.00	4	3	75.00	-	1	2	-	-	-
16	Sohag	NewZealand*	10-11	35	10	28.60	5	2	40.00	1	1	-	-	-	-
17	Sohag	Balady	12	42	32	72.20	13	6	46.10	-	4	-	1	1	-
18*	Sohag	NewZealand*	16	30	8	26.70	5	3	60.00	2	-	-	1	-	-
19	Assiut	Californian	8-9	22	18	81.80	11	5	45.50	1	-	3	1	-	-

* = Two RHD viral agents (R_1&R_2) were isolated from these rabbitaries, respectively.
** = Number of tissue homogenate (liver & lung) from naturally dead rabbits tested in HA-test.

Table (2): Cross HI and cross protection tests between the two RHD viral agents (R_1&R_2).

Rabbit group	Applied vaccine	Challenged virus	Cross HI-test virus (8 HA units)	Antisera*
1	Vac.I	R_1	R_1	Vac.I
2	Vac.I	R_2	R_2	Vac.I
3	Vac.II	R_1	R_1	Vac.II
4	Vac.II	R_2	R_2	Vac.II

The five collected sera of same vaccinated group were tested individually against constat virus-isolate (8 HA-units) and then the mean HI-titre was collected/vaccinated group.

Table (3): HA-activity of RHD viral agents (R_1&R_2) and reference RHDV.

Virus	Human A	Human B	Human AB	Human O	Sheep	Cattle	Camel	Chicken	Quails	Pigions	Ducks
R_1	+++	+++	++++	++++	++	++	++	+++	+-	++	-
R_2	++++	+++	+++	++++	++	+++	++	+++	+++	++	-
Ref.	+++	+++	++++	++++	+++	+++	++	+++	++	++	-

- = Negative HA (Less than 16). ++ = Positive HA (titre 1:64 - 1:256).
+++ = Positive HA (titre 1:512 - 1:1024). ++++ = Positive HA (titre more than 1024).

Table (4): Daily deaths and mortality rate in experimentally infected rabbits.

Rabbit group	Deaths post-inoculation (day) 1	2	3	4	5	6	7	8	9	10	Total	%
1st	-	2	5	-	2	-	-	-	-	-	9	90
2nd	-	3	3	2	-	-	-	-	-	-	8	80
3rd	-	-	-	-	-	-	-	-	-	-	0	0

Table (5):Cross HI test between the two RHDV-isolates (R_1&R_2) and their antisera collected from rabbit vaccinated with Vac.I and Vac.II.

Rabbit group	8 HA units of RHDV-isolate	Tested sera from vaccinated rabbit	HI-titre (Mean Log_2)
1	R_1	Vac.I	10.8
2	R_1	Vac.II	10.6
3	R_2	Vac.II	11.0
4	R_2	Vac.I	10.4

Table (6):HI antibodies titre in 8 positive rabbitaries.

Rabitary number	No.of +ve sera	HI titre 1:32	1:64	1:128	1:256	1:512	1:1024	1:2084
1	13	2	6	4	1	-	-	-
2	8	5	3					
3	15	4	7	2	-	2	-	1
4	7	5	2	-	-	-	-	-
5	10	-	5	2	2	-	1	-
6	8	2	5	1	-	-	-	-
7	6	4	-	2	-	-	-	-
8	12	3	5	4	-	-	-	-