



RELATION BETWEEN VACCINATION TIME OF EWES WITH RVF VACCINE AND IMMUNE RESPONSE OF BOTH EWES AND THEIR LAMBS”

H. Aidaros¹, Mona M.A Ashoub¹, Y.F. Metawea¹, Hanaa Abd El Kader ² and Halla Kasem¹

¹Animal Hygiene, Behavior and Management Department, Faculty of Veterinary Medicine, Benha University, Qalyobia, Egypt. ²Senior Researcher, Technical Manager of PCR LAB. Biotechnology Department, Animal Health Research Institute Agriculture Research Center, Doki, Cairo, Egypt.

ABSTRACT

The present study was carried out to identify the effect of vaccination time of pregnant ewes with locally prepared inactivated Rift Valley Fever (RVF) vaccine on seroprevalence of RVF-IgG antibodies in both dams and their lambs. 60 serum samples were collected from 3 groups of both ewes and their lambs (30 samples from each) and RVF IgG antibodies were detected using ELISA test, Also to detect RVF virus by RT-PCR. The 1st group include dams vaccinated one week before pregnancy, 2nd group was vaccinated during the 1st 2 month of gestation, while 3rd group was vaccinated during the last 3 to 6 weeks of gestation. Sera were collected from ewes before vaccination, 2, 8, 12 and 16 weeks after vaccination and from their lambs at age of one month, 2, 2.5, 3 and 3.5 month of age. Our results indicated that RVF IgG antibodies only detected in lambs sera born from ewes vaccinated during the last 3 to 6 weeks of gestation. Not all vaccinated ewes had the RVF virus-IgG antibodies against inactivated RVF vaccine. All sera collected from both ewes and their lambs were negative for RT-PCR. Our results concluded that the best vaccination time for ewes was during the last 3 to 6 weeks of gestation period to achieve the highest immune response for both ewes and their lambs.

(BVMJ-26(2):101-108, 2014)

1. INTRODUCTION:

Rift valley fever is an acute arboviral (insect-borne) disease, mainly affecting ruminants and human, causing abortion in pregnant animals and high mortalities in young animals (AusvetPlan, 1996). Sheep is the highly susceptible animals (20-70% mortality), while cattle is moderately susceptible animals (< 10% mortality) (Paweska 2008). In human, RVF causes a severe influenza-like illness, occasionally with more serious effects, such as hemorrhage complications, hepatitis, encephalitis, blindness and sometimes death (Martin et al. 2008). Egypt has suffered from more than one epidemic of RVF with consequent animal losses and human infection. In 1977, RVF disease invaded

Egypt for the first time causing abortion in about 20% of all infected pregnant domestic animals and deaths in young lambs (Ali, 1979). After that epidemic, the next documented RVF infection was in early 1981 (Imam et al. 1981). After 12 years absence, the RVF was noted in man and domestic animals in Egypt in late May 1993 in Aswan Governorate (Arthur et al, 1993). RVF outbreaks again occurred in Egypt between April to August 1997 in Aswan and Assuit governorates (Abd- el-Rahim et al. 1999). Animal vaccine is the first line of defense against RVF disease. Existing live and inactivated vaccines have been used extensively in Africa but further development of both is warranted. The live virus vaccine may cause abortion in some

cases and its potential for reversion to virulence has not been adequately investigated & it should be used only in enzootic and epizootic areas (FAO 2003). The inactivated vaccines are relatively effective and safe, but they are more expensive and at least 2 doses (with a week interval) must be given. They are currently produced only in Egypt and South Africa. Control of RVF in Egypt depends mainly on periodical vector control and vaccination of susceptible animals with binary inactivated RVF vaccine. Binary ethylenamine inactivated (ZH501 RVF strain) and Alum adjuvanted, which produced by VSVRI (Kamel 2011). The present study was carried out to identify the effect of vaccination time of ewes with inactivated RVF vaccine and the seroprevalence (IgG antibodies) in both dams and their lambs.

2. Material and method:

2.1. Inactivated RVF vaccine:

Tissue culture Binary inactivated RVF vaccine was prepared from ZH501 RVF strain according to (Soliman, 1995) and it supplied by RVF vaccine department, Veterinary Serum and Vaccine Research Institute., Abbassia.

2.2. Designs of experiment:

A total of 6 ewes were divided into three groups each of 2 ewes and later on their lambs. The 1st group of dams was vaccinated during the last week before pregnancy, 2nd group was vaccinated through the 1st 2 month of gestation, while 3rd group was vaccinated through the last 3 to 6 weeks of gestation. A total of 60 blood serum samples were collected (30 samples from ewes and 30 samples from their lambs) for detection of IgG antibodies of RVF by using ELISA test, also to detect RVF virus by Real time RT-PCR. Blood samples collected from ewes before vaccination, 2, 8, 12 and 16 weeks after vaccination, while blood samples collected

from their lambs at age of one month, 2, 2.5, 3 and 3.5 month of age.

2.3. Serum samples:

All blood samples collected from both newly born lambs and their ewes were immediately transported to the laboratory and kept in tightly closed tubes in refrigerator overnight at 4°C, then centrifuged at 1500 rpm for 10 minutes for the separation of sera. The clear sera were obtained by using sterile Pasteur pipettes and placed in eppendorff tubes, labeled and divided into 2 portions then stored at -70°C until tested

2.4. Diagnostic tests:

2.4.1. Real time RT-PCR was carried out on serum samples in the Animal Health Research Institute for RVF virus isolation, Agriculture Research Center, Doki, Cairo, Egypt. Extraction of RNA from serum samples were performed according to Dynabeads SILANE viral RNA Kit. The Primers and probe used in RT-PCR at Sequence (5' - 3') are

RVF(F);AAAGGAACAATGGACTC
TGGTCA,RVF(R);CACTTCTTACTACC
ATGTCCTCCAAT and RVp;
AAAGCTTTGATATCTCTCAGTGCCC
CAA at Amplicon size 94 bp. The one-step RT-PCR system combining superscript reverse transcriptase with platinum taq-polymerase was used in 5'-nuclease and SybrGreen assays. Reference RVF virus was used as a positive control for reverse real time PCR, it was obtained kindly from animal health research institute. The procedures were carried out according to (genesig standard kit handbook HB 10.02.04).

2.4.2. RVFV-specific IgG ELISA was performed on all sera. Indirect method of ELISA technique was done on 60 serum samples from both ewes and their lambs for detection of RVF IgG antibodies according to (Pretorius et al. 1997) , (Paweska and Swanepoel 2005).

3. Results:

The seroprevalence of RVFV-IgG antibodies was detected in one of two lambs sera born from ewes vaccinated during the last 3 to 6 weeks of gestation. On the other hand, RVFV-IgG antibodies not detected in neither lambs sera born from ewe vaccinated during the last 1 week before pregnancy nor ewe vaccinated during the first 2 months of gestation. Moreover, the vaccination of ewes during the last 3 to 6 weeks of gestation period may result in protection of lambs against RVF infection until 2.5 month of lambs' age. The RVFV-IgG antibodies were detected in ewes vaccinated during the last 3 to 6 weeks of gestation and ewes vaccinated during the last week before pregnancy. On the other hand, RVFV-IgG antibodies were detected in one of two ewes vaccinated during the first 2 months of gestation. All serum samples collected from both ewes and their lambs were negative for RT-PCR.

4.4. Discussion:

Animal vaccine is the first line of defense against RVF disease. Control of RVF in Egypt depends mainly on periodical vector control and vaccination of susceptible animals with binary inactivated RVF vaccine. Binary ethylenamine inactivated (ZH501 RVF strain) and Alum adjuvanted, which produced by VSVRI (Kamel 2011). The obtained results in table (1) clarified the effect of vaccination time on the level of RVF-IgG antibodies in both ewes and their lambs. The highest seroprevalence of RVFV-IgG antibodies was detected in lambs sera born from ewes vaccinated during the last 3 to 6 weeks of gestation. On the other hand, RVFV-IgG antibodies not detected in neither lambs sera born from ewe vaccinated during the last 1 week before pregnancy nor ewe vaccinated during the first 2 months of gestation. Moreover, the vaccination of ewes during the last 3 to 6 weeks of gestation period may result in protection of lambs against RVF infection until 2.5 month of lambs'

age. This result agree with (Saad 1985) who found that vaccination of cattle and buffaloes with inactivated RVF vaccine during 2 months before delivery ensures a high antibody titer for vaccinated mothers which transferred to their offspring's as the maternal immunity lasts for 3 months and calves has to be vaccinated at this time and (Sami 1985) who reported that HI antibodies in calves off springs from vaccinated dam with inactivated RVF vaccine reached its maximum titer after one week from suckle then declined gradually reaching low level at the 3rd month and disappeared at the 4th month. Also, (Youssef 1984), (Eissa 1995), (El Sawalhy *et al.* 1997) and (Hassan 1998) reported that lambs born from dams vaccinated at late stage of gestation with inactivated RVF vaccine had antibodies against RVF which still protective till 2.5 months. Not all a vaccinated ewes had the RVFV-IgG antibodies against inactivated RVF vaccine, as the highest seroprevalence of RVFV-IgG antibodies (80%) was detected in serum samples collected from vaccinated ewes during the last 3-6 weeks of gestation as antibodies persisted for 16 week after vaccination then disappear. while for both ewes vaccinated before pregnancy and ewes vaccinated during the 1st 2 month of gestation was 40% of each. Our results suggested that the vaccination of ewes did not prompted the expected immune system. This convection was supported by (WHO/FAO 1983) listed the major advantages of the inactivated virus vaccine as it does not induce abortion, but they must be re-administered annually to ensure adequate protection although animals receiving a single inoculation may have weak protection against the disease and viraemia. An inactivated RVF vaccine available from South Africa in liquid form adsorbed to aluminum hydroxide adjuvant, does not contain live virus. It protected against the disease but a single dose did not prevent viraemia or fetal damage after challenge (Botros *et al.* 1995). Results in table (2) and Figure (1) showed that all

Relation between vaccination time of ewes with RVF vaccine and immune response in ewe

Table (1) Seroprevalence of IgG antibodies in both vaccinated dams and their lambs using ELISA.

		ewes			lamb			
	Time of sampling Before/ after Vaccination	No	samples		Time of sampling (age per month)	Samples		
			No +ve	+ve %		No	No +ve	+ve %
1 st Group	before vaccination	2	0	0	1	2	0	0
	*2Ws.	2	2	100	2	2	0	0
	8Ws.	2	2	100	2.5	2	0	0
	12Ws.	2	0	0	3	2	0	0
	16Ws.	2	0	0	3.5	2	0	0
	Total		10	4	40		10	0
2 nd Group	before vaccination	2	0	0	1	2	0	0
	*2Ws.	2	1	50	2	2	0	0
	8Ws.	2	1	50	2.5	2	0	0
	12Ws.	2	1	50	3	2	0	0
	16Ws.	2	1	50	3.5	2	0	0
	Total		10	4	40		10	0
3 rd Group	before vaccination	2	0	0	1	2	1	50
	*2Ws.	2	2	100	2	2	1	50
	8Ws.	2	2	100	2.5	2	1	50
	12Ws.	2	2	100	3	2	0	0
	16Ws.	2	2	100	3.5	2	0	0
	Total		10	8	80		10	3

*Period after vaccination. Ws :weeks.

Table (2) real time RT-PCR analysis in serum blood samples collected from both vaccinated dams and their lambs

		ewes			lamb			
Time of sampling Before/ after Vaccination		Samples/PCR			Time of sampling (age per month)	Samples/PCR		
		No	No +ve	+ve %		No	No +ve	+ve %
1 st Group	before vaccination	2	0	0	1	2	0	0
	*2Ws.	2	0	0	2	2	0	0
	8Ws.	2	0	0	2.5	2	0	0
	12Ws.	2	0	0	3	2	0	0
	16Ws.	2	0	0	3.5	2	0	0
Total		10	0	0		10	0	0
2 nd Group	before vaccination	2	0	0	1	2	0	0
	*2Ws.	2	0	0	2	2	0	0
	8Ws.	2	0	0	2.5	2	0	0
	12Ws.	2	0	0	3	2	0	0
	16Ws.	2	0	0	3.5	2	0	0
Total		10	4	40		10	0	0
3 rd Group	before vaccination	2	0	0	1	2	0	0
	*2Ws.	2	0	0	2	2	0	0
	8Ws.	2	0	0	2.5	2	0	0
	12Ws.	2	0	0	3	2	0	0
	16Ws.	2	0	0	3.5	2	0	0
Total		10	0	0		10	0	0

*Period after vaccination. Ws :weeks.

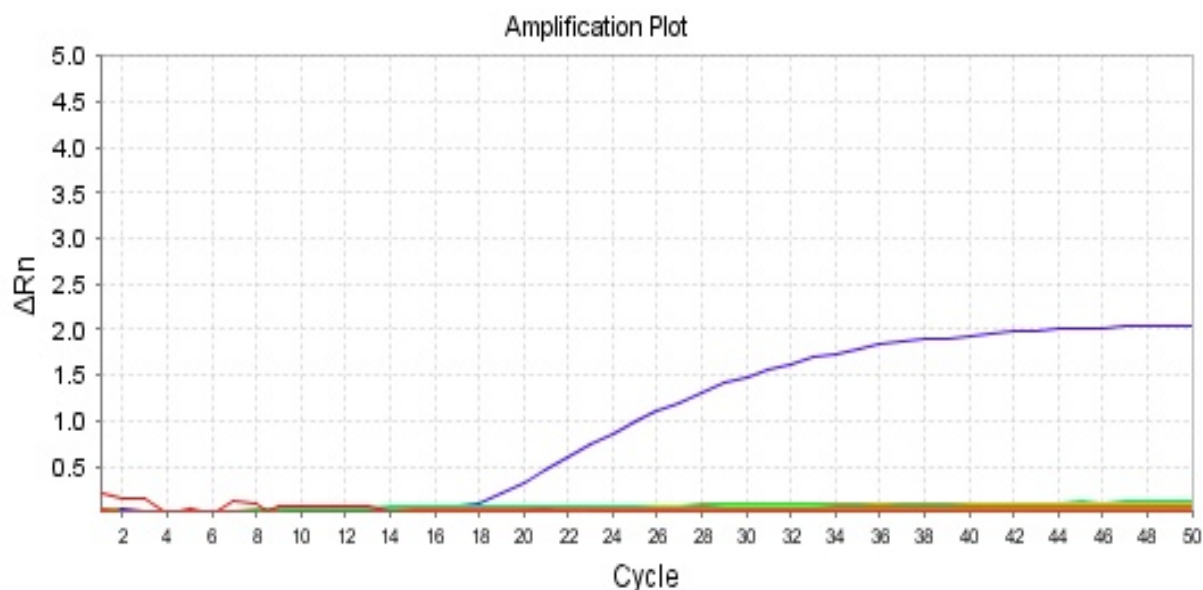


Figure (1): The amplification plot of real time RT-PCR analysis. The blue line indicates the experimental positive control (RVFV- RNA) which was use.

serum samples collected from both ewes and their lambs were negative for RT-PCR. This result indicated that the present RVFV-IgG antibodies in serum of both ewes and their lambs may attributed to immune response of animal body to inactivated RVF vaccine not from the RVFV infection (Sall et al. 2002).

Our results concluded that the inactivated vaccine is quite safe for sheep (including pregnant ewes) but is poorly immunogenic. It is more difficult to produce and is very expensive, moreover, it required at least two or three doses to produce an adequate level of immunity and even then breakdowns may occur during epizootics (FAO 2003). Also, our results concluded that the best vaccination time for ewes was during the last 2 month of gestation period to achieve the highest immune response for both ewes and their lambs.

5. Reference:

- Abd el-Rahim, I.H., Abd el-Hakim, U., Hussein, M. 1999. An epizootic of Rift Valley fever in Egypt in 1997. *Rev. Sci. Tech.*, 18(3): 741-748.
- Ali, R.R., 1979. Rift Valley Fever infections in camels in Egypt *M.V.Sc.*

(Microbiology) Fac.of Vet. Med. Cairo University.

- Arthur, R.R., El-Sharkawy, M.S., Cope, S.E., Botros, B.A., Oun, S., Morrill, J.C., Shope, R.E., Hibbs, R.G., Darwish, M.A., Imam, I.Z. 1993. Recurrence of Rift Valley fever in Egypt. *Lancet*, 342 (8880): 1149-50.
- Ausvetplan. 1996: Disease Strategy, Rift Valley Fever. 2nd Edition.
- Botros, B., Calamaio, Moussa, A., Mohsen, A., Soliman, A., Arthur. 1995. Immunological response of Egyptian Fat -tail sheep to inactivated & live attenuated Smithburn R V F vaccine. *J. Egypt. Vet. Med. Ass.* 55, (4): 895-907.
- Eissa, M.I. 1995. Studies on vaccination against Rift Valley Fever. PhD. V. SC. Fac. OfVet. Med., (Infectious diseases), Zagazig Univ., Zagazig, Egypt.
- El- Sawalhy, A.A., Hamoda, F.K., Elian, K.A. , Gehan, M.K. 1997. clinical and laboratory studies on Rift Valley Fever Vaccines in sheep. *J. Egypt. Vet.Ass;* 57(1): 363-386.
- FAO. 2003. preparation of Rift Valley Fever (RVF) contingency plans 2003. Agriculture and Consumer Protection.

- Hassan, K.E.Z. 1998. comparative studies on inactivated and attenuated Rift Valley Fever vaccines. Ph. D. thesis (Infectious disease) Fac, Vet. Med. Benha branch, Zagazig University, Egypt.
- Imam, I.Z.E., El-Karamany, R., Zaki, R., Omar, F.M. 1981. Isolation of attenuated strains of Rift Valley fever during 1980 and 1981 from man and animals. *Journal of the Egyptian Public Health Association*, LVI (5-6): 435-45.
- Kamel, S.A. 2011. Observations on rift valley fever virus and vaccines in Egypt. *Ahmed Kamal Virology Journal*, 8:532.
- Martin, V., Chevalier, V., Ceccato, P., Anyamba, A., De Simone, L., Lubroth, J., De la Rocque, S., Domenech, J. 2008. The impact of climate change on the epidemiology and control of Rift Valley fever, *Rev. Sci. Tech. Off. Int. Epizoot.*, 27 (2):413–426.
- Paweska, J. 2008. Epidemiology of RVF: Potential risks for introduction into Europe. Workshop on Symposium on Emerging Vector Born Viral Disease Lelystad, Netherlands, 28 November, South Africa.
- Paweska, J.T., Burt, F., Swanepoel, R. 2005. Validation of enzyme-linked immunosorbent assay for the detection of IgG and IgM antibody to Rift Valley fever virus in humans. *Journal of Virological Methods*, (124): 173-181.
- Pretorius, A., Oelofson, M.J., Smith, M.S., Van Dar Ryst, E. 1997. Rift Valley fever, a sero-epidemiological study of small terrestrial vertebrates in South Africa. *American Journal of Tropical Medicine and Hygiene*, (57): 693-698.
- Saad, S.S. 1985. Studies on the immune response of bovine vaccinated with inactivated RVF vaccine. M. V. Sc. Thesis, Micro. Fac. Vet. Med. Cairo Univ.
- Sall, A.A., Macondo, E.A., Sene, O.K., Diagne, M., Sylla, R., Mondo, M., Girault, L., Marrama, L., Spiegel, A., Diallo, M., Bouloy, M., Mathiot, C. 2002. Use of reverse transcriptase PCR in early diagnosis of Rift Valley fever. *Clin. Diagn. Lab. Immunol.*, (9): 713-5.
- Sami, S.M. A., 1985. Studies on the immune response of bovines vaccinated with inactivated RVF vaccine. M.V. Sc. Thesis (Microbiology) Fac. Vet. Med. Cairo Univ. Egypt.
- Soliman, E.M., 1995. Studies on Rift Valley Fever vaccine inactivated with Binary. Ph. D. Thesis, Microbiology, Fac. of Vet. Med., Cairo Univ.
- WHO / FAO Meeting, 1983. The use of vet. Vaccines for prevention and control of RVF. *Bulletin of the World Health Org.*, 61(2): 261-8 .
- Youssef, N.M.A., 1984. the antibody response of pregnant ewes to RVFV vaccine and the resulting maternal immunity. M.V.Sc. Thesis Fac. Of Vet. Med, Cairo University, Giza



كلية معتمدة 2013



العلاقة بين وقت تحصين أمهات الأغنام بمصل حمى الوادي المتصدع الميت والاستجابة المناعية لنعاج وحملاتها

حسن عبد العزيز عيداروس¹، منى عبد الرحمن عشوب¹، ياسر فؤاد مطاوع¹، هناء عبد القادر²، هالة السيد قاسم¹

¹ قسم الصحة والسلوكيات ورعاية الحيوان. ² مساعد باحث قسم البيوتكنولوجيا، معهد صحة الحيوان بالدقي.

الملخص العربي

هذه الدراسة تهدف لمعرفة تأثير وقت التحصين لنعاج بمصل حمى الوادي المتصدع الميت والمسح السيرولوجي للأجسام المضادة (IgG) في كل من النعاج وحملاتهم. تم تجميع 60 عينة دم من 3 مجموعات من كل من النعاج وحملاتهم (30 نعجة و 30 حمل) كل مجموعة تشمل 2 نعجة و 2 حمل لاكتشاف الاجسام المضادة RVF- IgG باستخدام اختبار ELISA. تم تحصين أول مجموعة من النعاج خلال آخر اسبوع قبل العشار، ثانی مجموعة من النعاج خلال اول شهرين من العشار وثالث مجموعة من النعاج خلال آخر 3 أو 6 أسابيع من العشار. تم تجميع عينات دم من النعاج قبل التحصين وبعد التحصين بأسبوعين، 8 أسابيع، 12 أسبوع و 16 أسبوع، أما بالنسبة للحملات تم تجميع عينات دم حسب العمر عند عمر شهر، شهرين، 2.5 شهر، 3.5 شهر. كانت أعلى نسبة للأجسام المضادة RVF- IgG في الحملات التي ولدت من الأمهات التي حصنت خلال آخر 3-6 أسابيع من العشر، بينما لا توجد الأجسام المضادة RVF-IgG في الحملات التي ولدت من الأمهات التي حصنت قبل العشار أو خلال أول شهرين من العشار. ليست كل النعاج التي حصنت بالتحصين الميت لحمى الوادي المتصدع تملك أجسام مضادة IgG. وأشارت النتائج أن أفضل وقت لتحصين النعاج كان خلال آخر 3-6 أسابيع من العشر لتحقق أعلى مناعة لنعاج وحملاتها.

(مجلة بنها للعلوم الطبية البيطرية: عدد 26(2):101-108، يونيو 2014)