"Relation between vaccination time of ewes with RVF vaccine and immune response of both ewes and their lambs"

H. Aidaros[1], Mona M.A Ashoub[1], Y.F. Metawea[1], Hanaa Abd El Kader [2] and Halla Kasem[1]

1- Animal Hygiene, Behavior and Management Department, Faculty of Veterinary Medicine, Benha University, Qualyobia, Egypt.

2- Senior 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. A total of 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 and16 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.

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 inffected 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 and16 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

RV(F);AAAGGAACAATGGACTCTGGTCA,RV(R);CACTTCTTACTACCATGTCCTCCAAT and RVp; AAAGCTTTGATATCTCTCAGTGCCCCAA at Amplicon size 94 bp. The one-step RT-PCR system combining superscript reverse transcriptase with platinum taq-poymerase was used in 5'- nuclease and SybrGreen assays. 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 RFV IgG antibodies according to (Pretorious et al. 1997) , (Paweska and Swanepoel 2005).

3. Results:

The seroprevelance 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. 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 seroprevelance 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 seropevalence 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 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.

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

	ewes				lamb			
	Time of sampling Before/ after Vaccination	samples No	No +ve	+ve %	Time of sampling (age per month)	Samples No	No +ve	+ve %
1st 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	0
2nd 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	0
3rd 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	30

*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 %
1st 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
2nd 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
3rd 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.

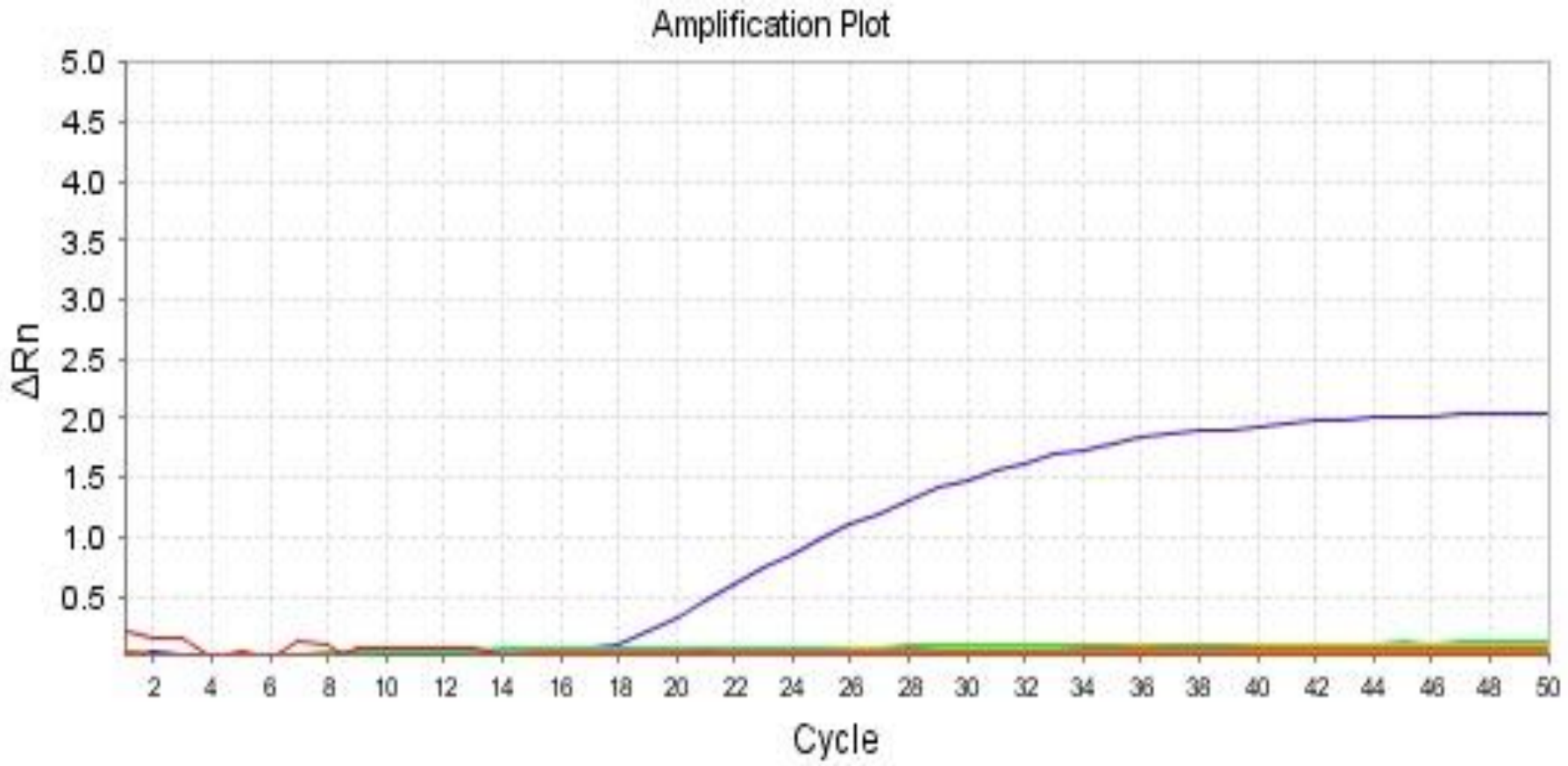

Figure (1): The amplification plot of real time RT-PCR analysis. The blue line indicates the experimental positive control (RVFV- RNA) which was use.

الملخص العربي

العلاقة بين وقت تحصين أمهات الأغنام بمصل حمي الوادي المتصدع الميت والاستجابة المناعية لنعاج وحملانها

أ.د/ حسن عبد العزيز عيداروس[1], أ.د/ منى عبد الرحمن عشوب[1], أ.د/ياسر فؤاد مطاوع[1],
د/هناء عبد القادر[2] ,ط.ب/ هالة السيد قاسم[1]

1- قسم الصحة والسلوكيات ورعاية الحيوان.

2- مساعد باحث قسم البيوتكنولوجي, معهد صحة الحيوان بالدقي.

هذه الدراسة تهدف لمعرفة تأثير وقت التحصين لنعاج بمصل حمى الوادي المتصدع الميت والمسح السيرولوجي للأجسام المضادة (IgG) فى كل من النعاج وحملانهم. تم تجميع 60 عينة دم من 3 مجموعات من كل من النعاج وحملانهم (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 أخر اسبوع قبل العشار, ثانى مجموعة من النعاج خلال اول شهرين من العشار و ثالث مجموعة من النعاج خلال أخر 3أو6 أسابيع من العشار.تم تجميع عينات دم من النعاج قبل التحصين وبعد التحصين بأسبوعين, 8 أسابيع, 12 أسبوع و 16 أسبوع, أما بالنسبة للحملان تم تجميع عينات دم حسب العمر عند عمر شهر, شهرين, 2.5 شهر, 3.5 شهر. كانت أعلى نسبة للأجسام المضادة RVF- IgG فى الحملان التى ولدت من الأمهات التى حصنت خلال أخر 3-6 أسابيع من العشر, بينما لا توجد الأجسام المضادة RVF-IgG فى الحملان التى ولدت من الأمهات التى حصنت قبل العشار أو خلال أول شهرين من العشار. ليست كل النعاج التى حصنت بالتحصين الميت لحمى الوادي المتصدع تملك أجسام مضادة IgG . و أشارت النتائج أن أفضل وقت لتحصين النعاج كان خلال أخر 3-6 أسابيع من العشر لتحقق أعلى مناعة لنعاج وحملانها.