

Assessment of the Duration of Maternal Antibodies Specific to Live Attenuated *peste des petits Ruminant Virus Vaccine* in Lambs and Kids

Ayman A- Shehata¹, Elshaima M. Fawzi^{1*}, Afaf M. Menaze¹, Mohamed I. Essa¹, Mervat M. Mahmoud².

¹Animal Medicine Department, Division of Infectious Diseases, Faculty of Veterinary Medicine, Zagazig University, 44511, Egypt

²Virology Department, Animal Health Research Institute, Dokki, Giza, Egypt

Abstract

Maternal immunity in lambs and kids born from vaccinated dams with *peste des petits ruminant* (PPR) live attenuated cell culture vaccine was evaluated by using competitive ELISA (c-ELISA). Serum samples were collected from each dam prior to the vaccination to detect the titre of antibody against the infection with PPRV and from lambs and kids on the day of birth, then every 15 days until reach 4.5 months of age to determine the level of maternal antibodies against PPR virus transferred from vaccinated dams to their newly born lambs and kids. Results showed that the antibody titer averages in lambs and kids reach the maximum at one month of age (OD average below 20) then, declined gradually at 105 days in which 80 % of kids still have OD average below 50 while 40% of lambs are still protected. So, we recommended the application of vaccine to newly born lambs and kids from immunized dams at 3 months of age by live attenuated PPR vaccine strain Egypt/87 grown on the Vero cell culture.

Keywords: Small ruminants, PPRV- vaccine, Maternal Immunity, c-ELISA.

Introduction

Peste des petits ruminants (PPR) are highly contagious acute viral disease affecting both domesticated and wild small ruminants [1]. The infection of cattle, pigs and rats is thought to be subclinical [2]. The name PPR (small ruminants plague) was demonstrated by Francophone West Africa and secondly kills a huge number of sheep and goats [3]. The disease is characterized clinically by fever, necrotic stomatitis, anorexia, respiratory distress, oculonasal discharge and diarrhea [4].

PPR is one of fundamental causes hinders the productivity of goats and sheep and associated with high mortality and morbidity rates of affected small ruminants [5]. The disease is caused by *peste des petits ruminants virus* (PPRV) as an enveloped RNA virus belong to Family Paramyxoviridae, Genus Morbilli viruses, is closely related to *measles virus* (MV), *canine distemper* (CDV) virus and *rinderpest virus* (RPV) [6].

The disease is common in developing countries due to depending on small ruminants rearing and trading for food supply [7]. Since 2007, about one billion in Africa and Asia small ruminants had been thought to be at risk

of infection with PPRV [8]. PPR is one of notifiable disease of major economic importance. The PPR characterize by a sudden incidence of illness and associated with restrictions of movements of both animals and its products [9]. Zaher and Ahmed [10] collected Samples from 270 animals (170 Sheep and 100 goats) from Al Daqhaliya, Al Qalyoubiya and Sharkia governorates, Egypt. Rounding and syncytial formation of Vero cells are detected in coincidence with the result of ELISA which revealed that positivity of 56% of examined goats and 35.29% of examined sheep against PPRV.

Libeau *et al.* [11] developed a competitive ELISA as a rapid and sensitive method to detect level of antibodies against infection with PPRV which depends on the recombinant PPRV nucleoprotein. This nucleoprotein is antigenically highly conserved within Morbilli viruses, and is more immunogenic so it was used for the detection of PPRV in spite of its internal location. The competitive ELISA was compared with viral neutralization test (VNT). The VNT is slow and only can differentiate between the cross reactive (RPV) and PPRV while, c-ELISA was more rapid, highly

*Corresponding author email: (elshaimafawzi@yahoo.es), Animal Medicine Department, Division of Infectious Diseases, Faculty of Veterinary Medicine, Zagazig University, 44511, Egypt.

sensitivity (94.5%) and specificity (99.4%) than VNT, in addition to the its used antigen is safe and can be produced in large amount in vitro.

El-Allawy *et al.* [12] reported that a total of 658 sheep and 622 goat sera were collected from Assiut, Sohag and Quena Governorates. Samples were screened for neutralizing antibodies to PPR and rinderpest (RP) viruses. Neutralizing antibodies against both viruses were found in sheep and goat sera. All sera were tested for detection of specific antibodies against PPRV only, using ELISA and an agar gel precipitation test but ELISA was found to be the most sensitive one.

Farmers in areas where the virus is endemic are unable to achieve the strict sanitary control measures. Therefore, the control of PPR requires an effective vaccine and for this purpose several vaccines including both homologous and recombinant vaccines have been developed [13].

The age of newly born lambs and kids to be vaccinated depend widely on the amount of maternal antibodies received so the duration of maternal antibodies varies from several days to weeks be studied by many authors to evaluate more or less exact time of start the vaccination [14-18]. Antibodies of maternal origin were detected in young animals until 180 days of age but the threshold level of protection was assed at 135 and 105days in kids and lambs, respectively. Lambs and kids from naturally infected dams or vaccinated one should be revaccinated at 150 and 120days of age, respectively [14]. Sayem [15] and Ahmed *et al.* [16] demonstrated that Kids of vaccinated dams were advisable to be vaccinated after 4 months of age due to decrease in maternal antibodies. Sanne *et al.* [17] studied the passive immunity transmitted to 112 new born lambs from pregnant ewes vaccinated with homologous Nigeria 75/1 PPR vaccine at 3 and 4 months of pregnancy was analyzed using c-ELISA. Also, it was observed that level of passive immunity of examined lambs from 15 days of birth and every 15 days up to 150 days was declined about 70% at day 75 and 95% at day 90 after birth, so it is recommended to vaccinate lambs against PPR within 2.5 to 3 months of age. The present study was conducted to evaluate the level of

antibodies of maternal origin in both kids and lambs born to vaccinated dams and the efficacy of the passive immunity against PPRV by c-ELISA to determine the preferable time to start the vaccination.

Material and Methods

Animals

Twelve kids and 10 lambs born from susceptible pregnant five goats and five sheep vaccinated with PPR lives attenuated tissue culture virus vaccine. Good healthy five kids and five lambs were selected for assessment of maternal immunity.

Vaccination of dams

Serum samples from each dam were collected prior to the vaccination to determine antibody titer against PPRV. There is only one PPR vaccine commercially available in Egypt which is live attenuated lyophilized-Egypt/87 PPR vaccine grown on the Vero cell culture.

At 3 months of pregnancy, dams were vaccinated with 1 ml, subcutaneously of homologous PPR lyophilized vaccine. Pregnant vaccinated dams were clinical examined during pregnancy period. Serum samples were taken after 15 days after vaccination of pregnant dams to check the seroconversion. An adequate amount of colostrums was fed to young lambs and kids.

Collection and preparation of samples

Serum samples were collected from five kids and five lambs born from vaccinated dams (five goats and five sheep) on the zero days (birthday), within intervals of 15 days until 135 days of age (0, 15, 30, 45, 60, 75 days...etc), to determine the maternal antibodies against PPRV transferred from vaccinated dams to their newly born kids and lambs. Serum samples were stored at -20°C until being examined by c-ELISA.

PPRV antibodies in serum samples were carried out by Competitive

Serum samples from kids and lambs were tested by c-ELISA [ID screen PPR competition ELISA kit (id-vet. CIRAD-EMVT, Montpellier, France)].

The contents of kit are distilled water (30ml), Microplates coated with PPR

nucleoprotein, Negative Control, Positive Control, Dilution Buffer 13, Dilution Buffer 4, Wash concentrate (20X), Wash concentrate (20X), Substrate Solution (TMB), Stop Solution and Concentrated Conjugate (10X) for sheep and goats c-ELISA was outlined as the method developed by Libeau *et al.* [11]. Dilution of the conjugate 10x to 1x in dilution buffer 4. 9 ml dilution buffer 4 was added to 1ml conjugate 10x as each plate needed approximately 10 ml of conjugate 1x.

Washing solution 20x was diluted to 1x using distilled water, 10 ml of washing solution 20x were added to 190 ml of distilled water as each plate needed approximately 200 ml washing solution 1x. To deactivate nonspecific viral inhibitors, serum samples were inactivated for 30 min in a water bath by heat at 56°C. All reagents and serum samples should take the room temperature before use. Twenty-five microliters of dilution buffer 13 were added to each well. Twenty-five microliters of positive control were added to well A1 and B1. Twenty-five microliters of the negative control were added to well C1 and D1. Twenty-five microliters of each serum sample were added to each of the remaining wells. The plate was incubated at 37°C for 45 minutes. Each well was washed 3 times with 300 *ul* of washing solution 1x. 100 microliters of Conjugate 1x were added to each well. The plates were incubated at 21°C for 30 minutes. Each well was washed 3 times with 300 *ul* of washing solution 1x. 100 microliters of

substrate solution were added to each well. The plates were incubated for 15 minutes at 21°C. 100 microliters of stop solution were added to each well. The optical density was read at wavelength 450 nm (OD₄₅₀).

The validity of the test was assessed by calculating the mean value of control negative OD (which must be more than 0.7) and dividing the mean value of control positive OD by the mean value of control negative OD which must be less than 0.3.

In the c-ELISA, the competition percentage (Sample/Negative % [SN %]) of each serum sample was calculated according to the following formula:

$$SN \% = OD \text{ sample} / OD \text{ negative control} \times 100$$

1- Less than or equal to 50 % are considered positive

2- More than 60 % are considered negative

Results

No specific clinical signs of any diseases were showed in kids and lambs during the examination period. Kids and lambs were regularly examined during the period of samples collection.

The collected serum samples from 10 vaccinated dams (5 goats, 5 sheep) were tested using c-ELISA technique. High level of antibodies titre of PPRV was obtained, this mean that all dams exposed to the vaccine were sero-converted.

Table 1: Sample/Negative (SN %) in kids by using c-ELISA.

Animal number	Days after birth									
	0	15	30	45	60	75	90	105	120	135
1	110	9	10	11	29	29	32	41	45	78
2	77	8	7	11	32	38	48	71	89	-
3	87	11	8	12	27	29	35	35	42	80
4	83	24	8	28	34	36	41	44	86	-
5	95	9	6	19	28	30	37	40	47	88
Average SN %	90.4	12.2	7.8	16.2	30	32.4	38.6	46.4	61.8	82

Table 2: Sample/Negative (SN %) in lambs by using c-ELISA.

Animal number	Days after birth									
	0	15	30	45	60	75	90	105	120	
1	70	10	9	20	39	43	54	88	-	
2	95	8	9	27	41	44	53	71	-	
3	72	9	9	19	30	39	44	45	98	
4	102	11	8	10	25	33	33	81	-	
5	89	8	5	15	20	38	41	44	110	

Average SN%	85.6	9.2	8	18.2	31	39.4	45	65.8	104
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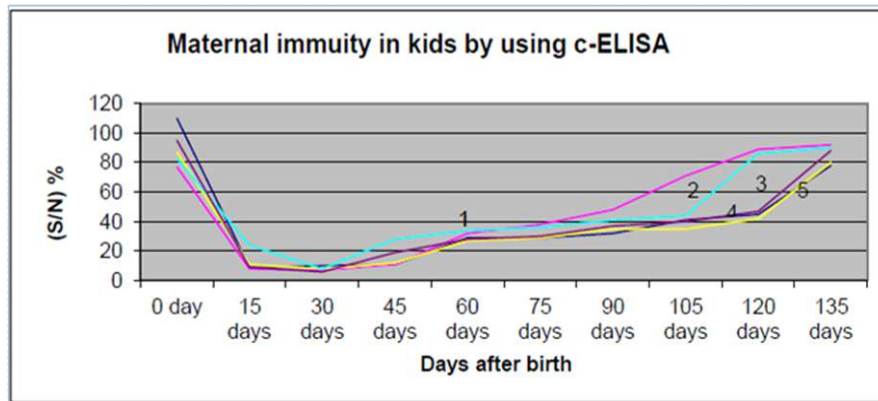


Figure 1: Maternal immunity against PPRV using c-ELISA in kids- Average of Sample/Negative (S/N %) for the five kids was calculated and represented against days after birth.

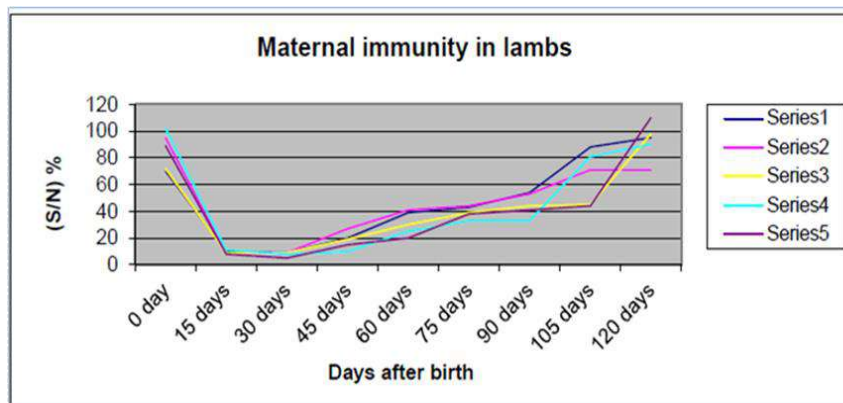


Figure 2: Maternal immunity against PPRV using c-ELISA in lambs- Average of Sample/Negative (S/N %) for the five lambs was calculated and represented against days after birth.

The collection of serum samples collected from kids and lambs were done from the day zero of birth until 135 days post-birth and tested by c-ELISA showed that the highest titer averages in kids and lambs were at one month of age then, decline gradually were shown in Tables (1,2), alternatively.

At 105 days of age, one kid (out of 5 kids) was negative. At 120 days after vaccination, 2 kids became unprotected. All kids showed negative antibody titer at 4.5 months of age as shown in Figure (1).

Out of 5 lambs, 2 lambs were below the protective level at 3 months. At 3.5 months of age 3 lambs became unprotected and were below the protective level (0.5) according to [11], all lambs were negative at 4 months of age as shown in Figure (2).

Discussion

Sheep and goats usually subjected to the PPRV disease at young age and convalescent period after exposed to infection may be extended for 1–2 years [19-21]. Also, Sarker and Islam [22] found that young animals had the highest PPR seroprevalence and they referred that to poor immunity and poor nutrition which predisposed them to PPR. Constable *et al.* [23] discussed that PPR is most potent in young age (less than one year) due to field infection with some virulent strains of PPR hastened by some bacterial infections such as pneumonic pasteurellosis and enterotoxigenic *E. coli*.

The disease is considered as one of most economically important disease of livestock in developing countries based on the high morbidity and mortality rates of the viral infection and the small size of the flock [3].

The study results showed that PPR live attenuated vaccine produce strong immunity in vaccinated dams and also in kids and lambs through colostrum. Neutralizing antibodies against PPR in goats and sheep remain up to 3 years also vaccination provides the immunity for up to 3 years. All this information indicates that PPR can be well controlled and eradicated following mass and well planned vaccination programs [24-25].

All vaccinated pregnant dams (5 goats and 5 sheep) in this study showed 100 % of antibody response to the PPR vaccine. Our results also revealed that all vaccinated dams were seroconverted within 2 weeks after vaccination time. These results agree with findings of the study performed in Pakistan and showed that all vaccinated sheep and goats with PPR vaccine were seroconverted [26].

Our study demonstrated that the vaccine is safe. No post vaccinal reaction was observed in the vaccinated animals that means that the virus failed to revert back to virulence. Our results coincide with [27-28].

Our study cleared the loss of maternal immunity in lambs and kids at 120 and 150 days after birth, respectively, which interpreted the registration of high morbidity and mortality in young kids and lambs due to quick decline of maternal antibodies titer in young animals. Our results less or more similar to Abd El-Rahim *et al.* [29] reported that a relatively high mortality rate among kids and lambs aged 4-9 months.

The study demonstrated that the highest titer averages in kids and lambs were at one month of age then, the titer declined gradually. From 5 lambs, 40% of lambs were below the protective level at 3 months. At 3.5 months of age, 60% of lambs became negative and non-protected; all lambs (100%) were below the protective level at 4 months of age. At 105 days of age, 20% of kid became negative. At 120 days after birth, 40% of kids became unprotected. All kids showed negative non-protective titer of antibodies at 4.5 months of

age. Our result nearly coincide with Bidjeh *et al.* [30] who demonstrated that the duration of passive immunity in both lambs and kids against PPRV was 4-5 months. Also, Sanne *et al.* [17] found that a specific titer of antibodies of PPRV had seen in 92% of totally examined lambs after 15 day of birth. But at 2.5 month of age only 32% still had antibodies titre against PPRV and at 3 months only 5.3% had a positive level of antibody.

Therefore, the results obtained from the actual study suggested that the best age for the vaccination of both kids and lambs with PPR live attenuated tissue culture vaccine was at 3 months of age. These findings agree with results obtained by Awa *et al.*, [14] who recommended vaccination at 135 and 105 days in kids and lambs, respectively. While, Ata *et al.* [31] recommended that the vaccination of lambs against PPR was better to do after 90 days of birth. Balamurugan *et al.* [18] also recommended the kids to be vaccinated against PPR at 120 days of age.

Maternal vaccination is necessary to provide protective passive immunity to the neonate against infections. Live attenuated PPR vaccine is used for sheep and goats and is required to be injected to all animals above the age of 3 month. The vaccine repeated after one year of the primary vaccination before winter season to avoid occurrence of outbreaks especially in endemic regions.

Conclusion

PPR was found to be more prevalent in young aged small ruminants. Lambs and kids should be vaccinated when the maternal antibodies decline. Vaccination of lambs and kids should be applied at 3 months of age.

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Conflict of interest

All the authors have no conflict of interest to declare.

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الملخص العربى

تقييم مده الأجسام المضاده الأموميه الخاصه بمرض طاعون المجترات الصغيره فى الجديان والحملان

أيمن شحاتة^١، الشيماء فوزى^{١*}، عفاف منازع^١، محمد عيسى^١، ميرفت محمود^٢

^١قسم طب الحيوان (الأمراض المعدية)، كلية الطب البيطرى، جامعة الزقازيق، ٤٤٥١١ مصر

^٢قسم علم الفيروسات، معهد بحوث الصحة الحيوانية، الدقى - مصر

تم تقييم المناعه المنتقله من الامهات المحصنه للجديان والحملان حديثى الولاده بواسطه اختبار الاليزا التنافسيه. تم تجميع عينات الدم من الامهات قبل التحصين لتأكد من عدم وجود اجسام مضده ضد مرض طاعون المجترات الصغيره. و ايضا تم تجميع عينات الدم من الجديان والحملان حديثه الولاده من يوم صفرالولاده قبل اعطاء لبن السرسوب ثم بعد ذلك كل ١٥ يوم الى ان يصل اعمارهم الي ٤.٥ شهور لتحديد مستوي الاجسام المضده المنتقله اليهم من الامهات المحصنه ضد مرض طاعون المجترات الصغيره. وأظهرت النتائج النهائيه أن أعلى معدل للأجسام المضاده فى أجسام الجديان والحملان تم الحصول عليه عند عمر الشهر ثم يبدأ فى الأنخفاض التدريجى. لذلك نوصى بتطبيق لقاح طاعون المجترات الصغيره الحي المضعف عترة مصر/ ٨٧ للجديان والحملان عند عمر ٣ اشهور.