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IMMUNOLOGICAL RESPONSE OF CAMEL VACCINATED WITH CAMELPOX AND RABIES VACCINES

By

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ABSTRACT

Camelpox and rabies are important zoonotic viral diseases with public health hazard in addition to its great economic lose. Vaccinations with pox lead to nonspecific paramunity in a wide variety of animals; the present work investigates the effect of mutual vaccination of camels with rabies and camel pox vaccines on their immune response. Such investigation was carried out through vaccination of camel groups with single rabies and camel pox vaccines; administration of camel pox vaccine 2 weeks before vaccination with rabies vaccine in another camel group while simultaneous vaccination with the 2 vaccines was applied on another camel group. Cellular and humeral immune responses of such camels were evaluated using lymphocyte plastogenesis assay (LPA) and serum neutralization test (SNT) respectively. The obtained results indicated the possibility of camel vaccination with rabies and camel pox vaccines in singly or simultaneous manner where there was no antagonizing effect between them on the vaccinated camel's immune response. However there was clear rise in the levels of rabies antibodies in case of administration of camel pox before or simultaneously with rabies vaccine reflecting the immune modulatory effect of camel pox to rabies vaccine.

Keywords:

Camel, pox, rabies, vaccine, immune response.

INTRODUCTION

*C*amelpox is an acute contagious Orthopoxvirus (OPV) disease of camelids (*Camelus bactrianus* and *Camelus dromedarius*), the disease was first reported in between 1893 and 1902 from Rajaputana and Punjab parts of India and later from different parts of the world. It is endemic in camel-rearing countries of Africa and Middle East Asia **(Prabhu *et al.,* 2015**). The disease commonly seen in younger camels, between 2 to 3 years of age and outbreak of disease is allied with poor nutrition and weaning. Camelpox infections have three clinical forms: (i) severe, (ii) generalized milder and (iii) localized forms. Generalized and localized

forms of infections mainly observed in immune suppressed camels especially young and older animals. Camelpox morbidity, mortality and case fatality rates may ranges from 30 to 90%, 1 to 15% and 25% respectively. The recovered camels acquire solid and life-long protective immunity to reinfection of homologues virus **(Prabhu *et al.,* 2015**). Camelpox virus was not identified and isolated till 1969, the camelpox infection is endemic in the Middle East countries including Egypt **(Prabhu *et al.,*2015)**.The camel pox virus causes a proliferative skin disease that primarily affects younger animals **(Richard, 1979; Mahnel and Munz,1987 and Dioli and Stimmelmayer,1992)**.The vaccination program and improved management strategies were recorded to diminish the prevalence of Camelpox **(Mona *et al.,* 2012)**. Rabies is an acute viral encephalomyelitis caused by a virus belonging to the family (Rhabdoviridae) gens (lyssa) **(Hummeler *et al.* 1968).** Rabies is a highly fatal infectious disease affects the central nervous system and always ends by death. It is usually transmitted by biting of rabid animal to healthy one. Rabid camels are clinically showing unusual behavior, aggression, pica, ptyalism and terminal paralysis **(Lonati,1995)**.Rabies virus vaccination is widely accepted where neutralizing Antibodies are essential for protection**(Singh *et al.* 2018)** but experimental infection in mice suggested that cell mediated immune responses are required for efficient viral clearance **(Johnson *et al.*, 2010).** Vaccination with poxvirus vaccine leads to non-specific paramunity in a wide variety of animals; Pox virus's genomes encode multiple classes of immunomodulatory proteins **(Johnston and Mc Fadden, 2003)**. So the aim of the, this work was evaluation of improvement of the immune response of camels against rabies by using Camelpox vaccine as immuno-modulator with camel rabies vaccine.

MATERIAL AND METHODS

1-Vaccines:

1.1-Camel pox vaccine:

Live attenuated camel pox vaccine was supplied by the Department of Pox Vaccines Research (DPVR); Veterinary Serum and Vaccine Research Institute (VSVRI) and used for vaccination of experimental camels.

1.2-Rabies vaccine:

Locally prepared inactivated cell culture rabies vaccine prepared according to **Edris (1994)** was supplied by the Department of Pet Animal Vaccine Research (DPAVR), (VSVRI), Abbasia, and Cairo, Egypt.

2-Viruses:

2.1-Camelpox virus (CPV):

Camelpox virus Saudi Jouf strain with a titter $10^{5.5}$ $TCID_{50}$ /ml was supplied by DPVR and used in serum neutralization test and for preparation of virus antigen for ELISA.

2.2- Rabies virus:

BHK-21 cell culture adapted Evelyn Rokintniki Abelseth (ERA) strain of rabies virus with a titter 10^7 $TCID_{50}$ /ml **(Edris, 1994)** was supplied by the same department and used in serological tests.

3-Cell cultures:

African green monkey kidney (VERO) and baby hamster kidney (BHK21) cell cultures were used to monitor camel pox and rabies serum neutralizing antibodies respectively using serum neutralization test (SNT). These cell cultures were supplied by VSVRI and propagated using minimum essential medium.

4-Camels:

Fourteen camels of 6 to 12 months old, with no history of camel pox were kept at the animal house till experiment was performed. They were classified into 4 groups, each consisted of 3 camels in addition to two camels were kept as a control.

Group-1 was inoculated with camel pox vaccine.

Group-2 was inoculated with Camelpox and Rabies vaccines at the same time.

Group-3 was inoculated with camel pox vaccine then Rabies after two weeks.

Group-4 was inoculated with rabies vaccine.

Six ml of inactivated rabies vaccine were injected S/C in camels according to **Khodier (1999) and Khodier and Daoud (2008).**

The dose of Camelpox vaccine was one ml and was injected S/C in camels according to **(Amira, 2001).**

5-Kits:

XTT Cell Viability Assay Kit was supplied by (Applichem) and was used in the lymphocyte blastogenesis assay.

6-Conjugate:

Rabbit Anti-Camel IgG (H+L) Antibody (HRP), abbexa, UK. Used in ELISA.

7-Samples:

7.1-Serum samples:

Serum samples were collected from camels just before and weekly after vaccination on week intervals for twenty weeks. Samples were stored at -20°C until examined by serological test.

7.2-Whole blood:

Whole blood samples were collected on heparin (heparin sodium) containing syringe then directly tested for estimation of the cellular immunity at day 0, 1, 3, 5, 7, 10, 15, 21, 28 and 35 post vaccinations.

8-Evaluation of the cell mediated immune response:

Assay of lymphocyte blastogenesis (XTT) was applied according to **Scudiero *et al*., (1988),** that method adopted and modified by **El -Watany *et al*. (1999**).

9-Evaluation of humeral immune response:

9.1-Serum Neutralization Test (SNT):

SNT was applied according to the method described by **House *et al.* (1990)** the neutralizing index (NI) for camel pox was calculated according to **Reed and Muench (1938)** while rabies antibody titer was calculated as the reciprocal of the final serum dilution which neutralized and inhibited the CPE of 100$TCID_{50}$ of rabies virus according to **Singh *et al* (1976).**

9.2-Indirect ELISA:

It was applied on the collected sera from the experimental camels according to **Babiuk *et al.* (2009) and Bhanuprakash *et al.* (2010).**

RESULTS

Evaluation of the cell mediated immune response of camels:

Assay of lymphocyte blastogenesis (XTT):

The results were illustrated in (Table 1), Fig. (1) disclosed that all vaccinated camels with different vaccination strategies had variable cellular immune responses depending on the vaccination strategy used and they reached to the maximum nearly on the 10^{th} - 15^{th} day post vaccination, then decreased after that.

Table (1): Cell mediated immune response of camels vaccinated with different vaccines (recorded as absorbance).

Vac / DPV	G1	G2	G3	G4
	Mean of Absorbance value of lymphocyte proliferation			
0	0.175	0.123	0.176	0.097
1	0.313	0.245	0.303	0.089
3	0.398	0.309	0.551	0.099
5	0.680	0.581	0.818	0.149
7	1.428	0.801	1.241	0.465
10	1.861*	1.581*	1.910*	0.608
15	1.408	1.322	1.318	0.981*
21	1.211	1.000	1.041	0.830
28	0.805	0.858	1.128	0.586
35	0.462	0.688	0.883	0.328

Vac = Vaccine. **DPV = Day post vaccination.**

G1=camel pox vaccine. **G2=camel pox vaccine with Rabies vaccine.**

G3=camel pox vaccine then Rabies after two weeks. **G4=rabies vaccine.**

*** = Highest results in each group.**

N.B.1: Cell mediates immune response in the contact and isolated camels (absorbance) not exceeded 0.089-0.094 allover the time of study.

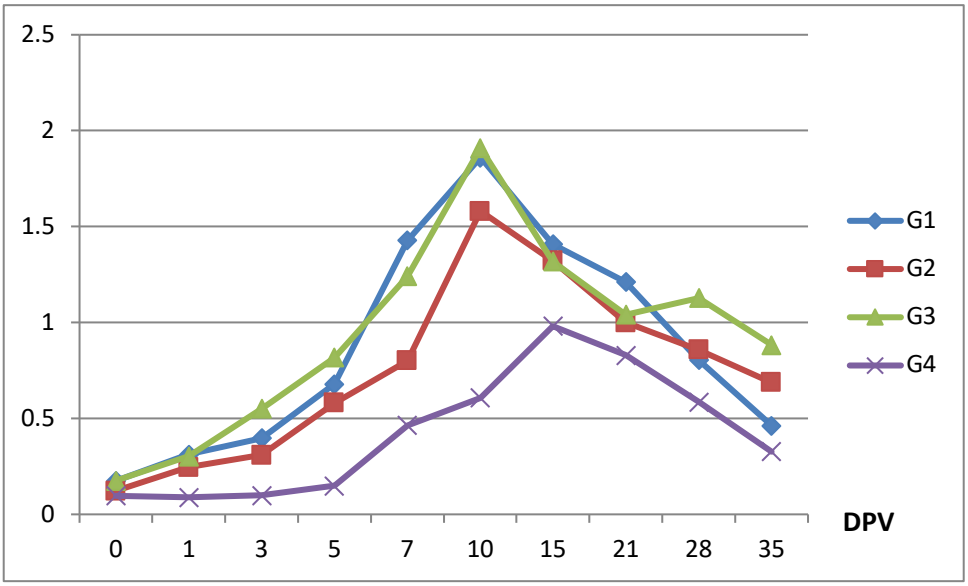

Fig. (1): Cell mediated immune response of camels vaccinated with different vaccines.

Evaluation of humeral immune response:

Serological assays (Serum Neutralization test (SNT) and ELISA):

Serum samples were weekly collected from camels before and after vaccination with different vaccines on week intervals for 20 weeks, and then were tested; the results were illustrated in (Tables 2-5), Fig. (2-5).

Table (2): Comparative S/P of ELISA for Camelpox vaccinated groups in camels**.**

VAC / WPV	G1	G2	G3
0	0.37	0.41	0.28
1	0.89	0.97	1.03
2	1.06	0.99	1.21
3	1.35	1.21	1.35
4	1.38	1.35	1.56
5	1.66	1.31	1.81
6	1.65	1.58	1.76
7	1.97	1.71	1.91
8	1.92	1.62	2.36*
9	1.96	1.88*	2.25
10	2.20*	1.66	2.16
12	1.89	1.68	2.05
14	1.85	1.79	2.12
16	1.92	1.51	1.86
18	1.87	1.48	1.95
20	1.76	1.38	1.77

G1=camel pox vaccine.

G2=camel pox vaccine with rabies vaccine.

G3=camel pox vaccine then Rabies after two weeks.

N.B.1: Isolate and contact control Camels persisting negative S/P till (20 weeks post vaccination).

N.B.2: S/P >1.0 is considered protective **Babiuk *et al.* (2009).**

WPV = Week post vaccination. **VAC** = Vaccine.

S/P= Sample to positive ratio.

*= highest S/P ratio

Table (3): Comparative NI of SNT for camelpox vaccinated groups in camels.

VAC / WPV	G1	G2	G3
0	0.50	0.25	0.25
1	1.00	1.25	1.25
2	1.25	1.50	1.25
3	1.50	1.50	1.75
4	1.75	1.75	2.00
5	2.00	2.00	2.50
6	2.50	2.50	2.75
7	2.75	2.50	3.00
8	2.75	3.00*	3.25*
9	2.75	3.00	3.00
10	3.00*	2.75	2.75
12	3.00	2.50	2.75
14	3.00	2.50	2.50
16	2.75	2.50	2.25
18	2.50	2.25	2 .25
20	2.50	2.25	2.25

G1=camel pox vaccine.

G2=camel pox vaccine then rabies vaccine.

G3=camel pox vaccine then Rabies after two weeks.

N.B.1: Isolate and contact control Camels persist negative NI till (20 weeks post vaccination).

N.B.2: Neutralizing Index (NI) ≥ 1.5 is considered protective **(Cottral, 1978)**.

WPV = Week post vaccination. **VAC**= Vaccine. **NI =** Neutralizing index.

*= highest NI.

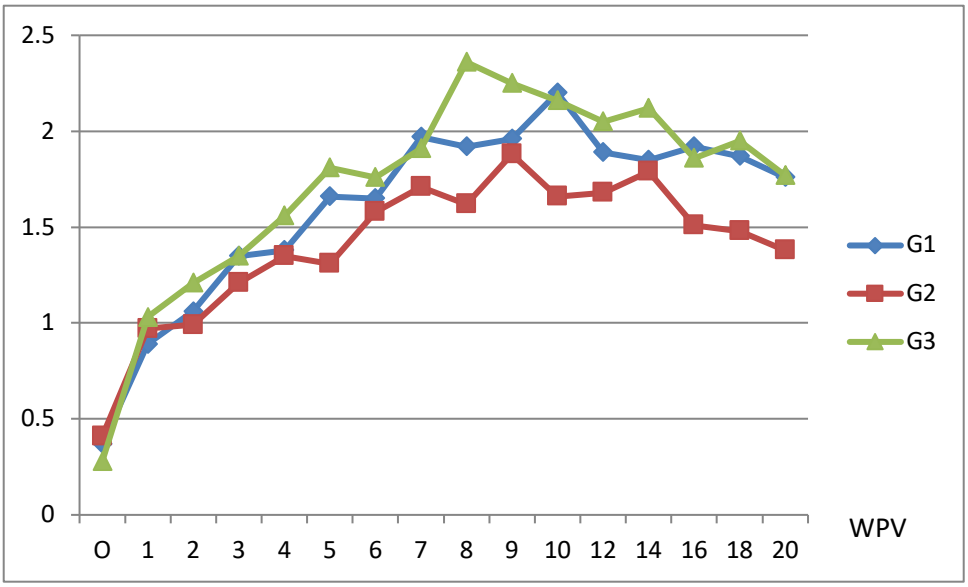

Fig. (2): Comparative S/P of camelpox vaccinated groups in camels.

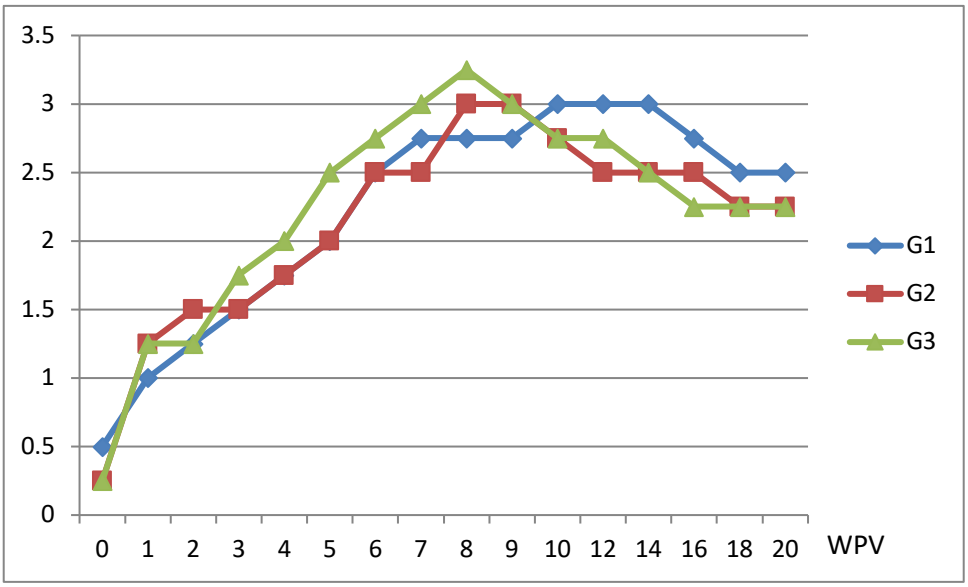

Fig. (3): Comparative NI of camelpox vaccinated groups in camels.

Table (4): Mean rabies serum neutralizing antibody titers in vaccinated camels.

Animal Group	Tested vaccine Formulae	Mean rabies serum neutralizing antibody titer							
		WPV							
		1	2	3	4	8	12	16	20
1	Rabies	2	8	16	32	64	64	64	64
2	Camelpox and rabies simultaneously	4	8	16	32	64	128	128	128
3	Camelpox then rabies	4	8	16	64	128	128	128	128

WPV= week post vaccination.

Table (5): Mean rabies ELISA antibody titers in vaccinated camels.

Animal Group	Tested vaccine Formulae	Mean rabies ELISA antibody titer							
		WPV							
		1	2	3	4	8	12	16	20
1	Rabies	0.5	0.7	1.1	1.6	2.1	2.1	2.1	2.1
2	Camelpox and rabies simultaneously	0.7	1	1.5	2.	2.4	2.5	2.5	2.5
3	Camelpox then rabies	0.7	1.5	1.7	2.4	2.5	2.6	2.6	2.6

DISCUSSION

The results in (Table 1) show that, the groups 1, 2 and 3 reach the peak on the day 10th post vaccination and the group 4 reach the peak on the day 15th post vaccination agree with **Johnson *et al.* (2010)** who mentioned that Some studies suggest the virus can suppress cell-mediated immunity early during the infection although there is little mechanistic evidence to support this beyond suppression of intracellular interferon production by the viral phosphoprotein.

The obtained results from ELISA test in (Table 2) proved that, the Ab titers in vaccinated animals were detectable from the 1st week post vaccination and reach the peak was on week 8 for GP 3 and it was 2.36, on week 9 for GP 2 and it was 1.88 and on week 10 for GP 1 and it was 2.20; and the obtained results in (Table 3) releaved that pox neutralizing Ab indices in all vaccinated animals were not affected by the presence of rabies vaccine showing the same level of antibodies titers detected from the 2nd week post vaccination and increased gradually , its peak was on week 8 for GP 2 and it was 3.00 and GP3 and it was 3.25 and on week 10 for GP 1 and it was 3.00 and come to confirm the results of ELISA and agree with those of **Carn, (1995) and Morgado *et al.* (2002)**.

In all groups antibodies against rabies began to appear in all vaccinated animals from 1stWPV (week post vaccination) and reached the peak titer by the 4th to 5th WPV.

It was noticed that, the Antibodies titers in simultaneously vaccinated group with pox and rabies were highest followed by the group of a vaccinated with pox 2 weeks before rabies vaccination. These result agree with those recorded by **Sambyal and Singh (1980) and Mayer (1981)** who mentioned that pox virus induce para immunity by activation of phagocytosis stimulating the lymphocytes in vitro and in vivo also the present finding agree with those obtained by **(Abdelsamea *et al.*, 1994**, **Hussein *et al.* (1996) and Samir *et al.*,1999)** who used sheep pox vaccine with other viral disease vaccines and obtained similar results indicating that pox vaccine enhanced the immune response of vaccinated animals to other vaccines , Also agree with **Naglaa *et al.* (2008),** who concluded that sheep pox vaccine initiated the immune response of sheep and goat to rabies vaccine.

The obtained results indicated the possibility of camel vaccination with rabies and camel pox vaccines in singly or simultaneous manner where there was no antagonizing effect between them on the vaccinated camel's immune response. However there was clear rise in the levels of rabies antibodies in case of administration of camel pox before or simultaneously with rabies vaccine reflecting the immune modulatory effect of camel pox to rabies vaccine. In conclusion, the present work results showed that, the combination of Camelpox vaccine with camel rabies vaccine is advisable and preferable. However, filed and further studies are required to confirm our results.

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