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LABORATORY AND FIELD INVESTIGATIONS OF A LIVE ATTENUATED AND AN INACTIVATED CAMELPOX VACCINE

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ABSTRACT

The aim of this work was to determine the efficacy of two commercially available camelpox (CP) vaccines, namely Ducapox live attenuated (LA) vaccine produced in South Africa and an inactivated adjuvant (IA) vaccine produced in Morocco. Inoculation of the LA vaccine virus into cell culture produced typical growth characteristics of a CP virus (CPV) with high virus titre. Sterility tests proved the safety of the vaccine. Young camels 12 to 18 months of age were divided into groups and vaccinated with both vaccines with single and two doses regimes. Humoral immune response was measured by serum neutralisation test (SNT) and passive haemagglutination test (PHT). Cellular immune response was measured by the delayed type-hypersensitivity (DTH) reaction.

Camels vaccinated with both vaccines and later challenged with a virulent CPV, showed no clinical signs of disease or pox lesions. Development of the humoral antibody response started on the 2nd week post vaccination (PV) for both vaccines and a booster dose increased the antibody titre significantly. The IA vaccine, however, induced a low level of antibodies. In the DTH experiment, all camels vaccinated with both vaccines reacted positively with a remarkable increase in skin thickness as compared to controls indicating a sound cell-mediated immune (CMI) response. The increase in thickness was relatively greater in dromedaries vaccinated with the LA than with the IA vaccine. Field vaccination trials revealed that both vaccines induced a weak immune response in camels less than 6 months of age and also older than 4 years of age. This can be explained by possible existence of maternal antibodies or the immaturity of the immune system in camel calves, and the occurrence of pre-vaccination antibodies in adult camels. Good immune response as measured by serological tests, developed in the age group of 1 to 4 year-old camels.

Both vaccines used in this study were safe, potent and immunogenic since they induced humoral and cellular immune response and protected vaccinated dromedaries against challenge. Camels vaccinated with the IA should be revaccinated 8 weeks post-primary vaccination, while one dose of the LA vaccine is enough to sustain protection for at least one year. The appropriate age for vaccination for both vaccines is 6 months. The Passive Haemagglutination Test was found useful for serology of CP; it is easy to perform, less expensive and also sensitive when compared with the serum neutralisation test.

Keywords : Camelpox vaccines, DTH, field trials, SNT

The ability of the camel to perform efficiently in harsh conditions as well as its productive potentialities are compelling reasons for understanding how to improve its resource. Diseases are considered one of the most important constraints in improving camel health and production. It leads to considerable reduction of productive performance and health status including loss of condition and weight as well as a drop in milk production and death.

Review of the literature reveals that little work has been conducted on viruses of camels. Apart from camelpox (CP) and rabies, most of the viral infections have been demonstrated only serologically (Higgins, 1986). Camelpox is the most widely reported viral disease of camels. It is a contagious skin disease, which affects mostly young camels. The disease is caused by an orthopox virus and is characterised by generalised pox lesions that cause severe outbreaks with mortality rates up to 10%.

In Sudan, the presence of CP was first reported in 1953 (Anon, 1954). The disease is known to exist for years under the name "Al Geddari". In eastern Sudan, Khalafalla and Mohamed (1996) reported that the disease

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has considerable economic significance with epizootic occurring every 2 to 5 years. Detection of antibodies against CP using enzyme linked immuno-sorbent assay (ELISA) indicated that the disease is widespread in all parts of Sudan where camels are raised (Khalafalla *et al*, 1998a).

The first availability of a camel CP was reported in the 1973 in the Soviet Union. However, this report lacks data concerning the nature of the vaccine (Borisovisch, 1974). Recent reports of vaccines produced against CP have come from Saudi Arabia, UAE and Morocco (Wernery and Kaaden, 2002). A preliminary study on the safety of a new attenuated vaccine of CP in France was reported (Nguyen *et al*, 1996).

Despite these data on vaccine development and production, little work has been published on the efficacy as well as on the assessment and duration of the immune response after vaccination.

The aim of this work therefore was to determine the efficacy of a life attenuated (LA) and an inactivated (IA) CP vaccine in Sudan, namely Ducapox vaccine produced in South Africa and inactivated adjuvant vaccine produced in Morocco.

Materials and Methods

Camelpox viruses and antiserum

VD45 reference strain **:** The VD45 strain of camelpox virus was originally isolated in Niger (Nguyen *et al*, 1989). This strain was supplied as freeze-dried ampoules by CIRAD- EMVT, France.

CP/NW/92/2 local strain : This virus is a local strain isolated in 1992 from a 2 year old male dromedary from central Butana, eastern Sudan (Khalafalla *et al*, 1998b; Khalafalla and Mohamed, 1998).

Hyperimmune serum **:** This was previously produced in rabbits against the VD_{45} strain of CPV (Khalafalla *et al*, 1998b).

Camelpox Vaccines

Ducapox (LA) vaccine : This is a live attenuated vaccine commercially produced by Onderstepoort Biological Product, South Africa. The virus was originally isolated in the United Arab Emirates (UAE) and attenuated through serial passages in Vero cell culture (Kaaden *et al*, 1992). The UAE attenuated CP vaccine (Ducapox =Dubai CP vaccine) has been used since 1994 with success (Wernery and Kaaden, 2002). The vaccine was presented as bulk pack of 5 doses per freeze- dried vial, supplied with sterile water for reconstitution, packed as 25x5 doses vaccine per box plus 25x10 ml diluents in a separate box.

Inactivated adjuvant (IA) camelpox vaccine : It is an inactivated adjuvant vaccine produced by Biological and Pharmaceutical Veterinary Product Company, Rabat Morocco. The virus was originally isolated from a severe outbreak of CP which had occurred in Morocco (El-Harrak *et al*, 1991). It was first adapted on chick embryo fibroblast and on Vero cell line. The inactivation was carried out by formalin and then the virus absorbed on aluminium hydroxide (El- Harrak, 1998). The vaccine was presented in a thermal box containing 10 one-dose bottles and used according to the recommen-dations of the manufacturer. This vaccine has been used in Morocco since 1991 in prophylactic campaigns against CP.

Primary lamb testes cell (LTC) cultures : For the preparation of a primary lamb testes cell culture the procedure of Plowright and Ferris (1958) was followed. The growth medium consisted of Glasgow minimum essential medium (GMEM) supple-mented with 5% tryptose phosphate broth, 0.125% lactalbumine hydrolysate, 0.025% yeast extract, penicillin (200 IU/ml), streptomycin (100 ug/ml), mycostatin (50 IU/ml) and 10% foetal calf serum. The maintenance medium possessed 5% foetal calf serum.

Virus infectivity of the LA vaccine : Virus infectivity was carried out in a 75 ml plastic tissue culture flask, containing semi-confluent monolayer of the second passage of LTC. One ampoule of the LA vaccine was reconstituted and 0.2 ml ($10^{6.7}$ $TCID_{50}$/ml) of the virus was inoculated and incubated for virus adsorption at 37°C for 1 hour. The inoculum was then removed. The monolayer was washed twice with phosphate diluent (PD) and maintenance media added. The flasks were kept at 37°C. The inoculated culture flask and a control were examined daily with an inverted microscope for the presence of a cytopathic effects (cpe).

The determination of the tissue culture infective dose 50/ ml ($TCID_{50}$/ml) for the LA

vaccine, the VD_{45} reference strain and the CP/NW/92/2 local strain was performed in 96 well flat-bottomed microtitre tissue culture plates following the procedure described by Villegas and Purchase (1980).

Virus identification of the LA vaccine : The alpha neutralisation procedure (constant-serum, diluted-virus) as described by Beard (1980) was followed. The Ducapox vaccine virus was simultaneously tested against negative and hyperimmune positive serum (HIS).

Sterility test of the LA vaccine : Samples were taken with a Pasteur pipette from a reconstituted vial of vaccine and cultured on thioglycolate media, mycoplasma broth and Sabouraud broth.

Vaccination trials

Experimental dromedaries : Twenty camels aged 12 to 18 months, with no history of CP were chosen as experimental animals. They were purchased from the Showak camel market in eastern Sudan or borrowed from local herders. The camels were taken to the Showak Camel Research Station where the experiments were performed. As soon as the animal reached the station, they were treated against ticks and internal parasites using Ivermectin.

Vaccination protocol : The experimental dromedaries were divided into three groups. Group 1 and 2 consisted of 8 animals each while group 3 consisted of 4 animals. Each animal was shaved on the right side of the neck and inoculated subcutaneously with the recommended dose of the vaccine. The first group received the LA vaccine. The second group received the IA CP vaccine. The third group of 4 camels was kept as control animals. Dromedaries were observed daily for signs of disease, and rectal temperatures were recorded during early morning. Any swelling at the site of inoculation was measured using callipers. After 12 weeks post vaccination (PV), 2 camels of each vaccinated group and 2 controls were challenged with 1 ml of a virulent local CP strain (CP/NW/92/2) having a titre of $10^{5.3}$ $TCID_{50}$/ ml. The rest of the vaccinated animals received a booster dose 14 weeks PV, and were challenged 8 weeks later with the same local CP strain.

Field vaccination trials : Field vaccination trials were done on three different herds of different age and sex including a day-old calf. The camels were vaccinated with both CP vaccines applying the same protocol described above.

The effect of vaccination on pregnant and newborn camels : Four pregnant camels were included in the trials. Serum samples were collected at 4 and 8 months PV from a newborn calf born from a vaccinated dam. Additionally, a day-old calf was vaccinated with a single dose of the LA vaccine and serum samples were collected 3 and 5 months PV.

Serum collection

Blood was withdrawn from the jugular vein of camels into vacutainer tubes, which were kept overnight at room temperature. The separated serum was then collected after light centrifugation at 1000 rpm for 10 minutes, and heat inactivated at 56°C for 30 minutes. Serum samples were collected once before vaccination and 1, 2, 4, 6, 8, 12, 14, 22, 24, 28 days and 54 weeks PV as well as on a monthly basis.

Serum samples were collected from dromedaries in the field before vaccination and then 3 and 5 months PV.

Challenge : The challenge experiment was done by the subcutaneous and intradermal route. The subcutaneous challenge was performed by inoculation of $10^{5.3}$ $TCID_{50}$/ml of CP/NW/92/2 strain of CP into a shaved area on the right side of the neck. The intradermal challenge was performed according to the intradermal titration method described by Davies and Mbugwa (1985).

The challenged dromedaries were kept under observation for 15 days, and any reaction at the site of injection as well as rise in temperature were recorded.

Delayed type hypersensitivity test (DTH): The antigen was prepared by inoculation of LTC grown in tissue culture Roux bottles with CPV-VD_{45} strain. When the cpe involved 80% or more of the monolayer, the contents of the bottles were harvested and centrifuged at 1500 rpm for 10 minutes. The pelleted cells were washed twice with phosphate buffered saline (PBS). Non-infected control cultures were treated similarly

to serve as control. The cell suspensions of the infected and control cultures were subjected to 3 freeze-thawing cycles. The supernatants were collected and clarified by centrifugation, dispensed in 1 ml aliquots and kept at 4°C. Prior to use, the antigen was inactivated by heating at 56°C for 1 hour.

All vaccinated camels and the controls were intradermally inoculated with 0.2 ml of the heat-inactivated CPV-VD_{45} antigen to a shaved area on the left side of the neck. The thickness of the skin at the inoculation site was measured every other day for one week as an index for hypersensitivity reaction by using callipers.

Serum neutralisation test (SNT)

The micro-serum neutralisation test (β procedure) was used for the quantitative determination of humoral antibodies to CP. The test was carried out in flat-bottomed tissue culture microtitre plates following the method of Hedger and Hamblin (1978).

Passive haemagglutination test (PHT)

The test was performed simultaneously on all sera according to the method described by Whitman and Hetrick (1964).

Results

Virus identity of Ducapox vaccine: Growth in lamb testes cell culture : The onset of the cpe was observed on the 5th day post inoculation (PI). The cpe started as focal areas of rounded, swollen and refractive cells. Later on, multinucleated giant cells formed which increased in size and number to form giant cells and occasionally cytoplasmic elongation or strand formation. Detachment of cells from the monolayer formed a plaque type cpe. The sheet was completely destroyed 9 to11 days PI.

Virus titration

The virus titre of Ducapox was $10^{6.7}$ $TCID_{50}$/ml compared to $10^{5.3}$ of the VD_{45} reference strain and $10^{5.6}$ of the local CP/NW/92/2 strain (Table 1).

Virus identification using neutralisation test : The result of the virus neutralisation test using rabbit normal and HIS showed that the vaccine virus was completely neutralised by the HIS at a dilution of 10^{-2} (the lowest virus dilution used) with a 1000 fold reduction in infectivity titre. This gave a neutralisation index of 3.

Table 1. The experimental design of the copper supplementation study in dromedaries.

Camelpox Virus	Titre of the virus ($TCID_{50}$/ml)
Ducapox vaccine virus	$10^{6.7}$
VD_{45}	$10^{5.3}$
CP/NW/92/2	$10^{5.6}$

Sterility test : Freedom from bacterial, mycoplasma and fungi contamination was certified by the absence of any growth on selective media.

Experimental vaccination trials

Post-vaccine reaction : Camels vaccinated with both the LA and the IA vaccines and the contact controls showed no clinical signs of disease. A swelling at site of inoculation was observed in dromedaries vaccinated with the IA vaccine that disappeared in two days post vaccination. Vaccinated and contact control animals appeared healthy and behaved normal.

Response to challenge : All dromedaries vaccinated with both vaccines showed no post challenge pox lesions or clinical signs after subcutaneous or intradermal inoculation with a virulent local CPV. On the other hand the control non-vaccinated animals developed fever of up to 40°C 2 days post challenge. Skin lesions started to appear as small papules at the side of the subcutaneous inoculation 5 days post inoculation with the challenge virus. Papules then developed into vesicles and at 8 days post challenge all 4 inoculated control camels developed typical CP lesions. Similar lesions also appeared around the nose, eyes, and limbs, around the genital and anal area. A few days later, brown crusts formed over the lesions and complete healing took several weeks.

Intradermal titration : The intradermal titration of the challenge virus resulted in a localised pox lesion reaction at the lower dilutions of the virus (10^{0} to 10^{-3}). The reaction started as swelling at the side of inoculation which appeared 3 days PI and disappeared 5 days later followed by a typical pox lesion at the side of inoculation on day 6 PI.

Delayed type hypersensitivity test (DTH) : The results of the DTH reaction are shown in Table

2. All vaccinated camels reacted positively to the hypersensitivity test as measured by increase in skin thickness, whereas the contact control camels inoculated with non-infected cell culture homogenate showed no increase in skin thickness at the site of inoculation. Twenty four hours post inoculation of the heated inactivated VD_{45} strain of CPV, two to three fold increase in the skin thickness was detected in vaccinated animals that regressed on the 5^{th} day post inoculation.

Table 2. Delayed type hypersensitivity reaction (DTH) in camels vaccinated with Ducapox (LA) and an inactivated (IA) vaccine.

Vaccine type	Camel identi-fication	Skin thickness (mm) days after inoculation					
		0	1	2	3	4	5
LA	647	4	4.5	9.5	11.1	11	8
	279	2.5	4	8	8.5	9.1	6.1
	155	5.5	7.5	8.5	9.1	9	6
	031	3.5	5.5	6.9	8.1	8	7
Control	010	3	3	3	3	3	3
IA	164	2.5	3	7	7.5	7	7
	606	4	7	7.5	7	7	6
	246	7.9	9	9.5	9.5	9.5	9
	030	4	4	4.5	5.1	5.1	5
Control	011	3.5	3.5	3.5	3.5	3.5	3.5

Serum neutralisation response: All serum samples collected from experimental animals before vaccination were negative for antibodies against CP as measured by SNT and PHT.

SNT Response to a single vaccination : Figure 1a illustrate the immune response of two groups of camels vaccinated with a single dose of LA or IA vaccines. Camels vaccinated with the IA vaccine developed a low antibody titre of 2 ($\log_2$) one week PV that slightly increased to 3 ($\log_2$) up to week 28 PV. This group of camels showed no marked increase of immune response when challenged with virulent strain. Camels vaccinated with the LA vaccine developed a relatively high titre of 7 ($\log_2$), that remained high for two weeks before it declined. However, the titre increased again to 6 ($\log_2$) two weeks post challenge.

SNT response to a booster vaccination and challenge : Figure 1b illustrates the immune response of two groups of camels given a booster dose of LA and IA vaccine 14 weeks PV. Both groups were also challenged with a virulent CP strain. The booster dose resulted in an increase of antibody titre that reached it's peak (titre of 6 $\log_2$, for LA and 5 $\log_2$ for IA vaccines) by week 24 PV.

Table 3. Comparison of antibody titres obtained with SHT and PNT in camels vaccinated with a live attenuated (LA) and an inactivated (IA) CP vaccine.

Ducapox vaccine (LA)					Inactivated vaccine (IA)				
Weeks	Single dose		Booster dose		Weeks	Single dose		Booster dose	
	SNT	PHT	SNT	PHT		SNT	PHT	SNT	PHT
0	0	0	0	0	0	0	0	0	0
1	2	2	2	2	1	4	4	4	4
2	128	256	128	256	2	8	8	8	8
4	128	256	128	256	4	8	8	8	8
6	64	128	64	128	6	8	8	8	8
8	32	64	32	64	8	4	4	4	4
12	16	32	16	32	12	4	4	4	4
14	64	64	16	16	14	8	32	4	4
22	16	16	32	64	22	4	4	16	32
24	ND	ND	64	128	24	ND	ND	32	64
28	8	8	16	32	28	2	2	8	8
54	8	8	16	16	54	2	2	8	8

ND= not done
SNT= Serum Neutralisation tests
PHT= Passive Haemagglutination test

PHT response to a single vaccination and challenge : Figure 2a shows the immune response of two groups of camels vaccinated with a single dose of the LA and the IA vaccine. Both groups were also challenged with a virulent CP strain.

PHT response to a booster vaccination and challenge : Figure 2b shows the immune response of 2 groups of camels given a booster dose of LA and IA vaccine. Both groups were also challenged with a virulent CP strain.

Comparison between SNT and PHT results

Table 3 shows a comparison between the antibody titres obtained with PHT and SNT in camels vaccinated with a single or a booster dose of LA or IA vaccine. The results revealed that the titres obtained by PHT were equal or two folds higher than those obtained with the SNT.

Field vaccination trials

The results of the field vaccination trial are shown in table 4. Adult camels between 4 and 10 years with pre-vaccination antibodies against

CP failed to develop an increase in immune response after vaccination with the LA or IA vaccine. One to three year old camels with no pre-vaccination antibodies developed a higher immune response to the LA vaccine after 3 and 5 months PV when compared with the IA vaccine. Camels 0-6 months old failed to develop immune response as measured by SNT and PHT when vaccinated with IA vaccine. However, in 2 out of 8 camels low levels of antibodies of 1-3 $\log_2$ were detected (Table 4). On the other hand camels 6-12 months old when vaccinated with both vaccines developed fair immune response (Table 4).

The effect of vaccination on pregnant and newborn camels : Field vaccination trial of pregnant she camels resulted in no abortion or death of the foetuses. A calf born from a vaccinated dam with the inactivated vaccine failed to show passively acquired antibodies 4 and 8 months after birth. A day-old calf which received a dose of the LA vaccine developed poor immune response at 3 months post vaccination and the antibodies completely disappeared 5 months post vaccination.

Discussions

Camelpox is one of the most economically important diseases of camels. Since quarantine measures and restriction of camel movement appear to be of limited success vaccination seems to be the only option to control this viral disease.

The first availability of a CP vaccine was reported in 1973 in the Soviet Union. However, this report lacks data concerning the nature of the vaccine (Borisovisch, 1974). Recent reports of vaccines produced against CP emerged from Saudi Arabia, United Arab Emirates and Morocco (Wernery and Kaaden, 2002). A preliminary study on the safety of a new attenuated vaccine of CP in France was reported by Nguyen *et al* (1996).

Despite these data on vaccine development and production, little work has been published on the efficacy as well on the assessment and duration of immune response.

In the present study we examined the safety and potency of two commercially available vaccines against CP. The LA vaccine was produced from a cell culture attenuated virus. We conducted cell culture assays to determine the growth characteristics of the virus. Inoculation of the virus in cell culture produced typical cpe of CPV similar to that described by Ramyer and Hessami (1974), Nguyen *et al* (1989), Munz *et al* (1997) and Khalafalla and Mohamed (1998). The infectivity titre of the reconstituted vaccine was $10^{6.7}$ $TCID_{50}$/ml, which is similar to the recommen-ded dose that would allow good multiplication of the virus *in vivo* when inoculated into camels. The vaccine was also found safe since no bacteria, fungi or mycoplasma were isolated.

In the vaccination trial, young camels 12 to 18 months old were divided into groups and vaccinated by both vaccines with single and double doses using the methods described by the manufacturers. Humoral immune response was measured by SNT and PHT. Cellular immune response was measured by DTH reaction. Vaccinated and control animals were challenged with a virulent isolate of CPV. The vaccinated animals showed no clinical symptoms of CP while control animals showed generalised pox lesions. This indicated the safety and potency of both vaccines. Consistent with these findings, Wernery and Kaaden (2002), Hafez *et al* (1992) and EL-Harrak *et al* (1998) previously reported similar results when

Table 4. Field vaccination trial: Immune response of camels of different age and sex as measured by SNT and PHT after vaccination with a live attenuated (LA) and an inactivated (IA) CP vaccine.

Age group	Type of vaccine	Mean titre ($\log_2$)						Sero-converted camels/total vaccinated
		Pre-vaccination		3 months post vaccination		5 months post vaccination		
		SNT	PHT	SNT	PHT	SNT	PHT	
Adult 4-10 years old	LA IA	3.3 3.3	4.8 2	3.4 3.3	6.3 2	3.4 2	6.6 2	14/14 6/6
Young 1-4 Years old	LA IA	0 0	0 0	4 1.5	4 2.5	4 1	4 1	4/4 6/6
Calves 7-12 Months	LA IA	0 2	0 3	4 4	5 5	4 3	4 3.5	4/4 4/4
Calves 0-6 Months	LA IA	0 1	0 2	2.5 0	2 0	0 0	1 0	2/8 0/6

SNT= Serum Neutralisation test; PHT= Passive Haemagglutination test

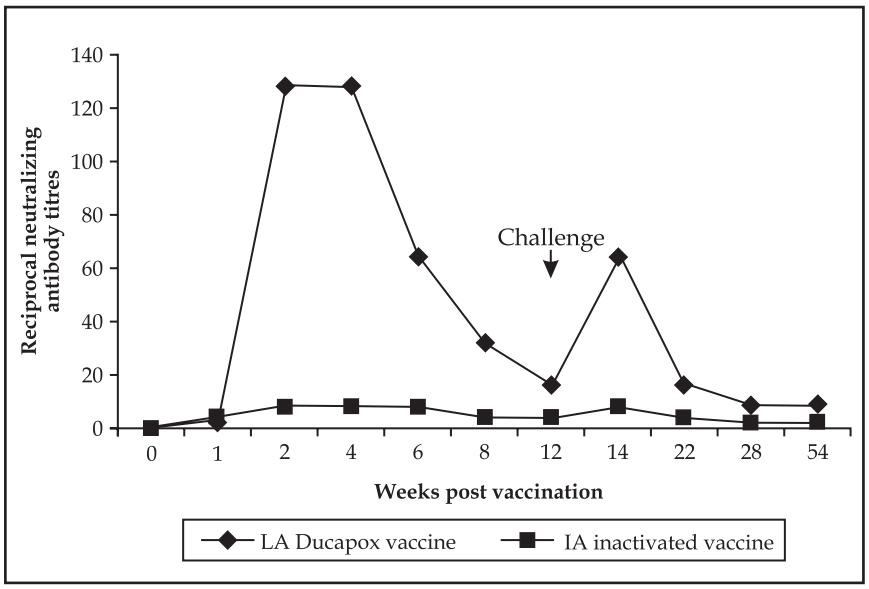

Fig 1a. Results of SNT-antibodies of 8 dromedaries vaccinated with a single dose of LA and IA CP vaccine and after challenge.

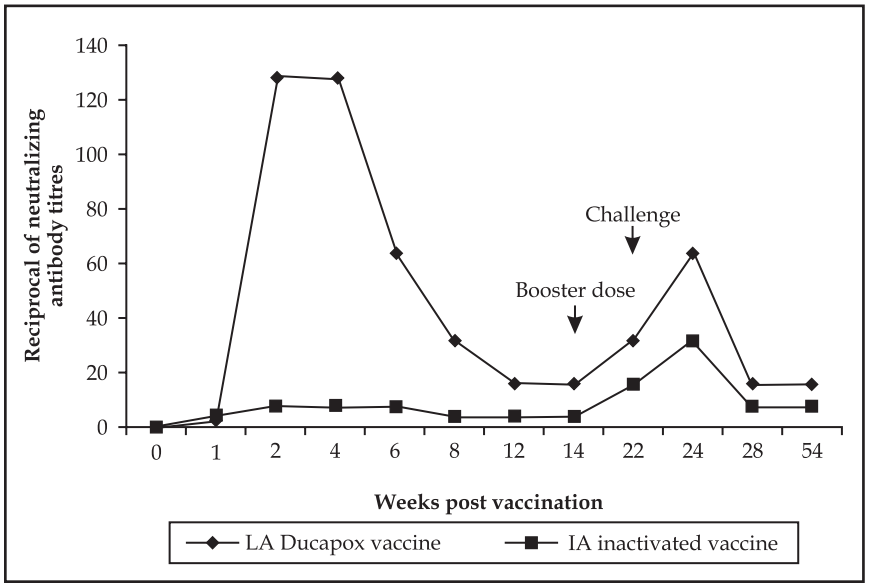

Fig 1b. Results of SNT-antibodies of 8 dromedaries after 2 vaccinations with a LA and IA CP vaccine and after challenge.

camels were vaccinated with Ducapox vaccine; Saudi attenuated vaccine and inactivated adjuvant vaccine, respectively. Specific antibodies against CP were detected in the second week PV for both vaccines. A significant variation in the level of antibody between the two vaccines was observed. The IA vaccine induced a low level humoral immune response whereas the live attenuated Ducapox vaccine induced a high level. This is not unexpected since inactivated vaccines are unable to multiply in the host. However, camels vaccinated with this vaccine, which has been used in prophylactic campaigns in Morocco since 1991, successfully withstood a challenge with a virulent CPV. Accordingly, the protection conferred is mainly of the cell -mediated type. This observation, however, disagrees with the findings of Buchnev and Sadykov (1967), who immunised camels with an aluminium hydroxide inactivated vaccine and reported that this vaccine did not protect camels from CP infection. In consistent, Mayr (1999) concluded that inactivated vaccines do not possess a protective efficacy against any poxvirus.s

In the DTH test all camels vaccinated with both vaccines reacted positively with a remarkable increase in skin thickness as compared with controls. This emphasises the role played by the cell mediated immunity (CMI). The increase in thickness was relatively greater in animals vaccinated with the LA vaccine. It seems that the LA Ducapox vaccine is a more potent cell mediated immune-inducer than the IA vaccine since it multiplied in the host tissues. Live attenuated vaccines are known to induce high CMI in comparison with inacti-vated vaccines. The rapid regression of DTH reaction in control camels observed in this study explains the synergistic action between the CMI and the humoral immunity in the removal of CPV from the host tissue.

Our results agree with the findings of Wernery and Kaaden (2002) who reported, that camels vaccinated with the LA Ducapox vaccine, reach a protective level 2 weeks post vaccination. A booster vaccination is expected to provide immunity against CP for several years. In another study, Wernery and Zachariah (1999) showed

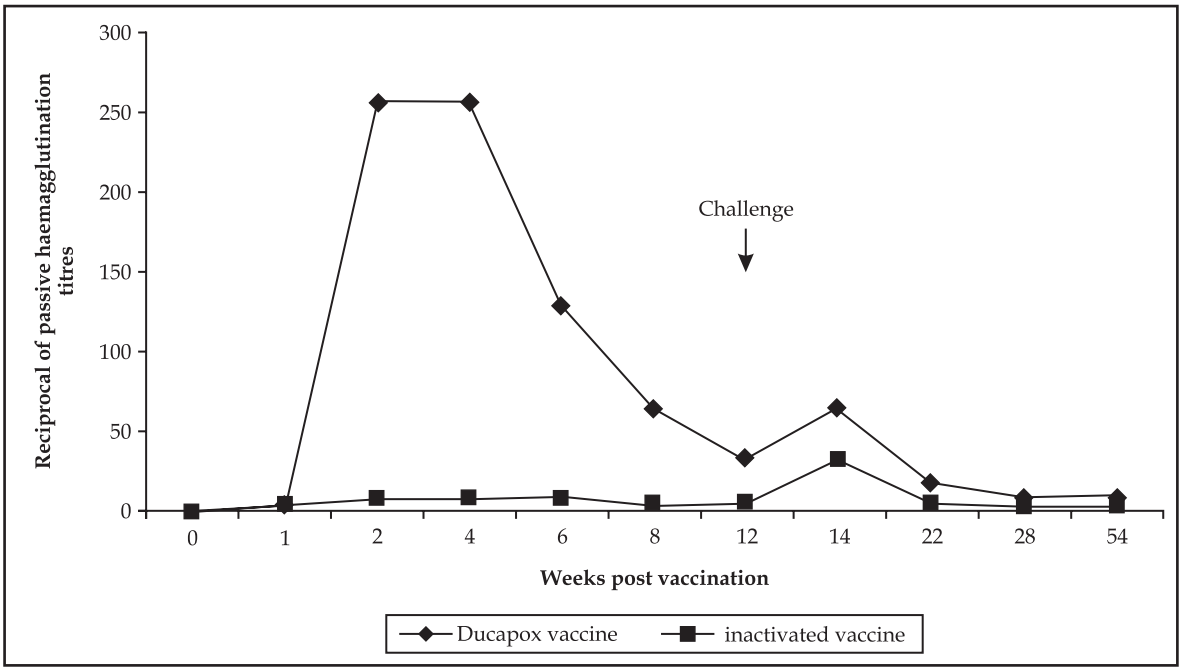

Fig 2a. Results of PHT-antibodies of 8 dromedaries vaccinated with a single dose of LA and IA CP vaccine and after challenge.

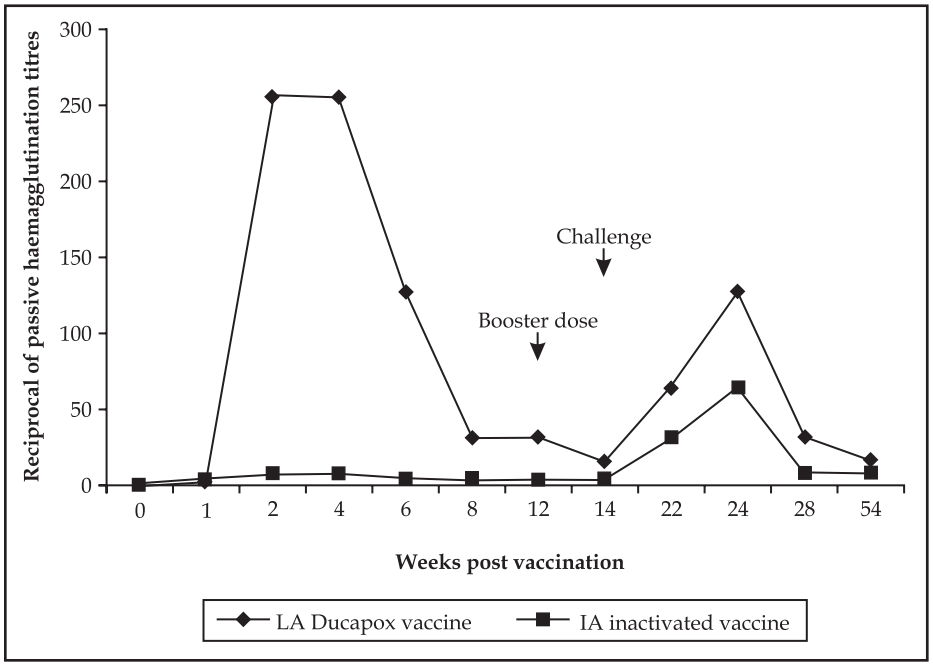

Fig 2b. Results of PHT-antibodies of 8 dromedaries after 2 vaccinations with a LA and IA CP vaccine and after challenge.

that a single dose of the life attenuated Ducapox vaccine given at the age of 12 months can protect dromedaries from CP infection for at least 6 years.

According to the present work, a marked reduction in antibody titre was observed in dromedaries vaccinated with a single dose of the inactivated vaccine, 8 weeks post vaccination. It is therefore recommended to revaccinate camels to induce a protective immune response for more than one year. This agree in agreement with El-Harrak (1998) who recommend that the vaccinated camels must receive a booster dose 4 weeks after the first vaccination and then annually. Even after one year following a single dose vaccination with the LA Ducapox vaccine, the antibody titre was still relatively high, which indicate the high immuogenicity of the vaccine.

For the first time we used the PHT to monitor the humoral immune response produced by CP vaccines. The findings presented here, indicate that the test has a high sensitivity when compared with the standard neutralisation test. The test was found specific since all sera tested using PHT gave titres comparable to those

detected by the SNT. The test is easy to perform and less time consuming.

In the field vaccination trials all camels over 4 years old having a pre- vaccination antibodies, failed to develop any increase in immune response after vaccination with both vaccines. This is due to the neutralisation of the vaccine virus by circulating antibodies. Khalafalla *et al* (1998a) found that the prevalence of antibodies against CP was high (87%) in camels more than 4 years old, compared to young camels (40%). Six to 12 month-old camels developed a sound immunity 3 and 5 months PV with the LA vaccine, whereas camels of the same age vaccinated with the IA vaccine showed a weak immune response 3 months PV and no response 5 months PV. This result could be explained as due to the poor immune response acquired by the single dose of inactivated vaccine. Camels less than 6 months old when vaccinated with the IA vaccine showed poor immune response, while camels with the same age vaccinated with the LA vaccine developed a low titre of antibodies that completely disappeared 5 months post vaccination. Reasons behind this finding could be the immaturity of the immune system of the young camel calves or due to neutralisation of the vaccine virus by the maternal antibodies.

The field vaccination trial showed that both vaccines were safe for pregnant camels. A calf born from a vaccinated dam did not show the presence of serum antibodies when tested at 8 months after birth. This could be explained as due to the short live of the passively acquired antibodies. However, this subject needs further study since only one serum sample of this kind was collected during the period of study.

From the present study it appeared that there is a close relationship between the immune response and age of vaccinated camels, pre-vaccination antibody titre and the type of vaccine. According to this study the appropriate age for vaccination is 6 months for both vaccines. This is in agreement with El-Harrak (1998) who stated that pregnant camels and 6 month-old camels can be vaccinated with the inactivated vaccine. Wernery and Kaaden (2002) suggested that young camels between 6 and 9 months can be vaccinated and should receive a booster injection 4 weeks later.

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