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IMMUNOGENICITY OF CAMELPOX VIRUS, JOUF -78 VACCINE - STRAIN IN BOSKAT RABBITS AND GUINEA PIGS

(With 2 Tables)

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دراسة مناعية عترة الجوف ٧٨ لفيروس جدري الإبل في الأرانب والوبر

منصور هاشم عبد الباقي ، عبد الله محمد الصقيران ، كمال صابر مظلوم ، أحمد منصور البقمي ، ودخيل محمد المجلي

لقـد وُجهت الدراسة إلى اختبار كفاءة استخدام حيوانات التجارب المعملية (الأرانب والوبر) في تقييم كفاءة لقاح جدري الإبل المستضعف المحضر بتمرير عترة الجوف ٧٨ في المزارع النسـيجية وفي تحضير الأمصال المناعية وحيدة النوعية لقد أثبتت النتائج أن الأرانب دون الوبـر قد تفاعلت مناعياً وبكفاءة للقاح المحقون بها وأن تحضير الأمصال المناعية المضادة لفيروس جدري الإبل قد نجحت في كل من الأرانب والوبر على السواء.

SUMMARY

Attention was directed to the lack of small laboratory animals (Rabbits and Guinea Pigs) to replace the camels in evaluating the immunogencity of the attenuated tissue culture vaccine of camlepox virus, Jouf-78 strain, and in preparation of monospecific antiserum against camelpox virus. The obtained results proved that rabbits reacted immunologically to the inoculated vaccine, and monospecific antiserum against camelpox virus, Jouf-78 strain was successfully prepared in rabbits and guinea pigs.

Key words: *Vaccination, camelpox virus, rabbits, guinea pigs*

INTRODUCTION

Camelpox is an enzootic contagious viral disease that devastate camels in many countries in Asia and Africa (Borisovich, 1973; Al-Hendi *et al.,* 1994; Gitao 1997 and Warnery *et al.,* 1997). Protection and immunization of camels against camelpox by using live attenated virus vaccine developed from a local isolate (Jouf-78 strain) had been carried out and recommended by Hafez *et al.* (1992). It is difficult and expensive to obtain adequate numbers of susceptible camels for vaccine potency evaluation, and as a result, small laboratory animal model for camelpox virus vaccine potency test virtually disappeared from the research field of this disease. This study was directed to determine the suitability of Boskat rabbits and guinea pigs as an inexpensive laboratory animals of uniform susceptibility in assaying the potency of tissue culture live attended vaccine of camelpox virus, Jouf 78 strain, and preparation of standard monospecfic anti, camelpox virus serum.

MATERIALS and METHODS

Reference Virus:

Camelpox virus, Jouf-78 Strain, isolated from clinical cases of the disease in Al-Jouf, north region of Saudi Arabia, attenuated throughout serial passages in primary cell culture of the kidney of camel by the National Agricultural Research & Animal Resources Center (NARARC), Riyadh, Saudi Arabia, and reviewed by the Institute of Tropical Veterinary Medicine and Pathology, Faculty of Veterinary Medicine, Munich, Germany was used for preparation of the live attenuated vaccine of camelpox virus (Hafez *et al.,* 1992). Stock vials of the vaccine batch No.1 produced by NARARC in 1995 and kept at $-20C^0$ was determined to be efficient by titration in vero cell cultures.

Cell Culture:

Cultures of Vero cell line obtained from Veterinary vaccines Production Center, Riyadh, Saudi Arabia was maintained and grown in Eagle's Minimum Essential Medium (EMEM) supplemented with 2-10% fetal bovine serum plus 100 IU penicillin sodium and 100 µg streptomycin sulphate / ml medium.

Animals:

Groups of adult females and males boskat rabbit weighed 2.5 – 3 kg and Guinea Pigs weighed 300 – 350 gm were obtained from King Faisal Specialist Hospital colonies, Riyadh, Saudi Arabia. Animals groups were kept in special cages .

Preparation of Immunizing Virus Antigens:

One vial of the camelpox virus, Jouf-78 strain vaccine was reconstituted with 1 ml of sterile distilled water and inoculated on confluent sheet of vero cells monolayer cultures, when the cytopathic effect (CPE) was 90 – 100%, the infected cultures fluid was freezed at -20C^0 and thawed, then clarified by centrifugation at 3000 rpm for 30 minutes at 10^{0}C. The clarified supernatant fluid was kept at 4^{0}C, and it was titrated in vero cell cultures. The virus was thereafter precipitated by addition and stirring of 0.4 gm ammonium sulphate per ml of virus fluid at 4^{0}C for 4 hours (Cann, 2000). The precipitate was collected after centrifugation at 4000 rpm for 1 hour at 10^{0}C, and it was reconstituted in 1/20 of its original volume with sterile distilled water then stored at – 70^{0}C. The viability of the concentrated virus was checked by conducting the infectivity test on vero cell cultures.

Virus Infectivity Assay

Virus infectivity was assayed by end point in microititre plate cultures of vero cells, using serial ten fold dilutions (four wells per dilution). Titres were calculated by the method of Reed and Muench (1938) and expressed as Log_{10} $TCID_{50}$ / ml.

Serum Neutralization Test (SNT):

SNT was conducted in guidence of Hafez *et al.* (1992) to determine the titre of camelpox virus – neutralizing antibodies in sera of rabbits and guinea pigs which had inoculated with camelpox virus, Jouf-78 strain vaccine. The test was performed in a microtitre system using monolayer cultures of vero cell, serial double fold dilutions (1/2 – 1/256) of serum samples against 10 and 50 $TCID_{50}$ of clarified virus fluid. The titre was expressed as a reciprocal of the highest dilution of serum that completely almost inhibit CPE in 50% of the inoculated wells.

Experimental Design:

Expriment (1)

Experiment No. 1 was designed to study the susceptibility of Boskat rabbit and guinea pigs to the immunization with a variable doses of camelpox Virus, Jouf-78 Strain Vaccine as follows:

Animal Group	Inoculum Titre	Route of Inoculation	Times of Inoculation	Time of Bleeding
Rabbits 1	5 Log_{10} $TCID_{50}$	I/M in thigh	Two injections with 10 days interval	11 days apart the last injection
Rabbits 2	3 Log_{10} $TCID_{50}$		Two injections with 10 days interval	11 days apart the last injection
Rabbits 3	5 Log_{10} $TCID_{50}$		One injection	21 days post inoculation
G.pigs 1	5 Log_{10} $TCID_{50}$	S/C in axillae	Two injections with 10 days interval	11 days apart the last injection
G.pigs 2	3 Log_{10} $TCID_{50}$		Two injections with 10 days interval	11 days apart the last injection
G.pigs 3	5 Log_{10} $TCID_{50}$		One injection	21 days post inoculation

Experiment 2.

This experiment was conducted to prepare standard monospecific serum against camelpox virus. One group of each rabbits (3 animals) and guinea Pigs (5 animals) was inoculated with initial and booster doses of camelpox virus, Jouf-78 strain vaccine of approximate titre 7 Log_{10} TCD_{50}/ Dose with 10 days interval time between the two injections, then animals once bled 11 days apart from the last injection. Another group of each rabbits and guinea pigs was intramuscularly inoculated with

initial dose of 1 ml. immunizing antigen of camelpox virus, Jouf-78 strain (equivalent to 2 x 10^8 $TCID_{50}$) emulsified with 1ml of Freund's complete adjuvant (FCA), MP, Biomedical, Inc. Lot No. 2231H, followed by booster dose of 1 ml. immunizing antigen emulsified with 1 ml of Freund's incomplete adjuvant (FIA), ICN, biomedical Inc. Lot no. 03667, 28 days later on intitial dose and 14 days before the bleeding time. In experiment 1 and 2, animals were bled by heart puncture, and serum samples were collected and inactivated by heating at 56^0 in water bath for 30 minutes then stored at $-20C^0$.

RESULTS

The results of indirect potency test of the attenuated camelpox virus, Jouf-78 strain vaccine in Boskat rabbit and guinea pigs are summarized in Table (1). The record range and mean values of 10 $TCID_{50}$ Camelpox virus-neutralizing antibody titres were 128-256 (192) & 4-24 (13.2), 32-48 (37.33) & 2-12 (4.4), and 16-64 (42.67) & 2-16 (8.0) respectively in sera of rabbits & guinea pigs of group (1) that was inoculated with an initial and booster doses of the vaccine of titre 5 Log_{10} $TCID_{50}$ with 10 days interval time, group (2) inoculated with initial and booster doses of the vaccine of titre 3 Log_{10} $TCID_{50}$ with 10 days interval time, and group (3) inoculated with a single dose of the vaccine of titre $5Log_{10}$ $TCID_{50}$. Also, 50 $TCID_{50}$ camelpox virus-neutralizing antibody titres of mean values 24, 2 and 2.67 were only detected respectively in groups 1, 2 and 3 of immunized rabbits.

The results of standard monospecific antiserum preparation against camelpox virus, Jouf-78 strain in Boskat rabbits and guinea pigs are shown in Table (2) 50 $TCID_{50}$ camelpox virus neutralizing antibody titres of 96 & 32 and 192 & 128 were recorded respectively in pooled sera of rabbits & guinea pigs of group (1) inoculated with initial and booster doses of the vaccine of titre 7 Log_{10} $TCID_{50}$ with 10 days interval time and group (2) inoculated with initial dose of the concentrated camelpox virus antigen emulsified in Freund's complete adjuvant and booster dose of the antigen emulsified in Freund's incomplete adjuvant with 28 days interval time.

Table 1: Camelpox Virus – neutralizing antibodies in sera of Boskat Rabbit and Guinea Pigs inoculated with variable doses of live attenuated vaccine of Camelpox virus, Jouf-78 strain.

Animal No.	Rabbits Group No.						Animal No.	Guinea pigs Group No.					
	1		2		3			1		2		3	
	SNT1	SNT2	SNT1	SNT2	SNT1	SNT2		SNT1	SNT2	SNT1	SNT2	SNT2	SNT2
1	256*	32	48	2	64	4	1	24	=	12	-	16	-
2	192	16	32	2	48	4	2	16	=	6	-	12	-
3	128	8	32	2	16	-	3	16	=	2	-	6	-
							4	6	=	2	-	4	-
							5	4	=	-	-	2	-
Mean	192	24.o	37.33	2.0	42.67	2.67	Mean	13.2	=	4.4	-	8.0	-

SNT 1 = Serum Neutrlization Test (using 10 $TCID_{50}$ of virus)
SNT 2 = Serum Neutrlization Test (using 50 $TCID_{50}$ of virus)
* titre: Reciprocal of serum dilution.
Group 1 of rabbits and G.pigs received two injections of the virus titre (5 $\log_{10}$ $TCID_{50}$) with 10 days interval time.
Group 2 of rabbits and G.pigs received two injections of (3 log10 $TCID_{50}$) with 10 days interval time.
Group 3 of rabbits and G.pigs received one injection of the virus titre (5 $\log_{10}$ $TCID_{50}$).

Table 2: Camelpox Virus – neutralizing anitbody titres in sera of Boskat rabbits and guinea pigs inoculated with Camelpox virus, Jouf-78 strain vaccine.

Animal / Group	Neutralizing Antibody Titre Against 50 TCID 50 of Camelpox Virus
Boskat rabbit 1	96*
Boskat rabbit 2	192
Guinea pigs 1	32
Guinea pigs 2	128

* = Rciprocal of serum dilution

Group (1) of rabbits and G.pigs was inoculated with two injections of virus titre 7 $\log_{10}$ TCD_{50} with 10 days interval time.

Group (2) of rabbits and G.pigs was inoculated with initial dose of camelpox virus antigen emulsified in FCA and booster dose of the antigen in FIA with 28 days interval time.

DISCUSSION

Currently, ehallenge test of the vaccinated animals with wild – type strain is considered the optimum method for evaluation the degree of protective and immunogenic capacities that induced by the applied vaccine. However indirect evidence of potency for evaluation of vaccine by determining the development of antibodies in the target animals has a benificial value. From a practical point of view, in the present work, Boskat rabbits and guinea pigs were used as a model for conducting the indirect potency of live attenuated camelpox virus vaccine (Jouf 78) and for preparation of a monospecific antiserum against camelpox virus. It has been reported that an Egyptian strain (Fayoum-71) of camelpox virus produces erythematous lesions in rabbits 3-4 days post intradermal inoculation and the virus can be isolated and serially transferred through rabbits without difficulty (Tantawi, 1974). This may candidly refers to the susceptibility of rabbits to camelpox virus infection. The obtained results, cleared that Boskat rabbits were reacted immunologically to primary and booster doses of the vaccine of titre 3 Log_{10} $TCID_{50}$ / dose with 10 days interval time. The inoculated animals showed a satisfactory level of humoral immune. On the other hand, the immunological reaction of guinea pigs that response received the same pattern and bleeding were unsatisfactory. The pattern of dosage and bleeding that choiced in the application of this experiment was dependent upon two considerations, (1) its reliability to safe time, (2) the lack of sharp determination of the required minimal immunizing dose titre for tissue

culture live attenuated vaccine of camelpox virus, Jouf-78 strain, but the common virus titre of 7 Log_{10} $TCID_{50}$ / vial yield in all batches of the vaccine with stability and safety do greatly suggest a titre of at least 3 Log_{10} $TCID_{50}$ as recommended field dose (Hafez *et al,* 1992).

In comparision, serum sampls collected from camels 4 weeks post inoculation with a variable titres (1, 2 and 7 Log_{10} $TCID_{50}$/dose) of tissue culture live attenuated vaccine of camelpox virus, Jouf-78 strain showed camelpox virus neutralizing antibody titres range of 16-32 (Hafez *et al,* 1992).

In view of the relativity in which antibody formation in sera of rabbits and guinea pigs responded accordingly to the increase of the inoculated virus titre, it is clear that inoculation of initial and booster doses of the vaccine titre 7 Log_{10} $TCID_{50}$ / dose was capable to induce formation of a significant neutralizing antibody titres in both of rabbits and guinea pigs. However, a comparatively higher neutralizing antibodies was obtained in sera rabbits and guinea pigs which initiated with camelpox virus antigen emulsified in Freund's complete adjuvant, boostered with the antigen emulsified in Freund's incomplete adjuvant with 28 days interval time and bled 14 days later on.

In conclusion, it is evident from the present study that Boskat rabbit can be used as a small laboratory animal model for assessment of the immunogenic capacity of tissue culture live attenuated vaccine of camelpox virus, Jouf-78 strain, and preparation of camelpox virus monospecific antisera can conducted in Boskat rabbits and guinea pigs.

REFERENCES

Al-Hendi, A.B.; Abuelzein, E.M.E.; Gameel, A.A. and Hassanien, M.M. (1994): A slow spreading mild form of camelpox infection. J. Vet. Med. B.41, 1: 71-73.

Borisovich, Y.F. (1973): Little-known infectious diseases in animals. Kobs, Moscow: 32-42

Cann, A.J. (2000): RNA virus, A practical approach. Published by Oxford University Press.

Gitao, C.G. (1997): An investigation of camelpox outbreak in two principal Camel (Camelus dromedarius) rearing areas of Kenya. Rev. Sci. Tech. Office Int. des Epiz 16 (3): 841-847.

Hafez S.M.; Al-Sukayran, A.; dela Cruz, D.; Mazloum, K.S.; Al-Bokmy, A.M.; Al-Mukayel, A. and Amjad A.M. (1992): Developmet of live cell culture camlepox vaccine. Vaccine, 10 (8): 533-538.

Reed, L.J. and Muench, H. (1938): A simple method of estimating fifty percent end point American Journal of Hygience, 27: 493-497.

Tantawi, H.H. (1974): Comparative studies on camelpox, sheep pox and vaccinia virus. Acta Virologica, 18, 347.

Wernery, U.; Kaaden, O.R. and AU. Ali M. (1997): Orthopox virus infections in dromedary camels in united Arab Emirates during winter season. Journal of Camel practice and research, 4 (1) : 51-53.