Serological study on camel pox using competitive ELISA, immunoperoxidase and serum neutralization techniques

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SUMMARY

This study estimates the prevalence of antibodies against camel poxvirus disease (CPVD) in three Egyptian governorates (Aswan, Assiut and Fayoum). A total of 1200 blood samples were collected randomly from apparently healthy male camels of 1-3 years old during 4 successive months of various climatic conditions. Sera were separated and investigated using modified competitive Enzyme Linked Immunosorbent Assay (C-ELISA), competitive immunoperoxidase (C-IP), and serum neutralization (SN) techniques. The results revealed that, the number of positive camels sera by C-ELISA and C-IP were 164, 120 and 48 in Aswan, Assiut and Fayoum governorates respectively, and 148, 102 and 40 by SNT in the same governorates. The antibodies level was higher in young camels (one year old) during the winter season in Aswan and Assiut governorates. The highest titre of antibody was 64 by both SNT and C-IP and the percentage of inhibition was 80% by C-ELISA.

INTRODUCTION

Camel pox is the most frequent infectious viral disease of the dromedaries, and man can contract the infection *(Kriz, 1982 and Jerek et al., 1983)*. CPV is belonging to genus orthopoxvirus, which is related to subfamily chordopoxvirinae of family poxviridae *(Pfeffer et al., 1996)*.

In Sudan, CPD appeared in 1996 and the infected camels had clinical findings including fever, oedema of the face and legs, lymph node enlargement and appearance of generalized or localized pox lesions particularly on the head, neck, leg and abdomen *(Khalafalla and Mohamed, 1996)*.

In Egypt CPD appeared in 1971-1972, 1989 and 2000 in Fayoum, Sharkia and Assiut governorates, respectively *(Tantawi et al., 1973; Kenawy et al., 1989 and Zaitoun et al., 2000)*. Many sero-epidemiological virus studies has been performed on camels and have confirmed that the camel produces antibodies to a great number of pathogenic viruses without developing the disease *(Wernery and Kaaden, 1995)*. Sero-diagnosis of CPD up till now was performed using mainly SNT for estimation of the neutralizing antibodies *(Davies et al., 1985; Gabry et al., 1997 and Magda et al., 2003)*. Application

of ELISA and IP techniques on camel sera is difficult as these techniques necessiate some modifications because the patent camel kits are not commercially available *(Munz et al., 1986).*

The aim of the present work is to develop simple, sensitive, accurate, rapid and specific techniques for detection and titration of antibodies to CPV infection such as c-ELISA and IP which are modified to be used for testing of camel sera using rabbit anti-CPV hyperimmune serum in competition with tested camel sera in the same system. In addition, this study estimates the prevalence of antibodies against CPVD in native and imported camel breeds during successive months of various seasons.

MATERIALS AND METHODS

1- Blood and serum samples:

A total of 1200 blood samples were collected randomly from apparently healthy native and imported breeds of male 1-3 years old camels from Aswan, Assiut and Fayoum governorates during the period from January to April 2005 (winter and spring seasons). One hundred blood samples were collected every month from each governorate and sera were separated and used for the serological study.

2- Cell cultures:

African green monkey kidney cell line (Vero) was used in SNT and C-IP against CPV using Eagle's Minimum Essential Medium (EMEM) with 10% newborn calf serum. It was obtained from Pox Department, Veterinary Serum and Vaccine Research Institute (VSVRI), Abbasia, Cairo.

3- virus and antigen:

CPV (Saudi strain) was obtained from Pox Department, Veterinary Serum and Vaccine Research Institute (VSVRI), Abbasia, Cairo. The virus was propagated in Vero cells and had a titre of 5.8 $\log_{10}$ $TCID_{50}$/ml *(Amira, 2001).* It was used for SNT and C-IP. The CPV antigen was prepared according to *Engvall and Perlmann (1971)* and used for C-ELISA.

4- Rabbit hyperimmune serum against CPV:

It was obtained from Pox Department, Veterinary Serum and Vaccine Research Institute (VSVRI), Abbasia, Cairo and prepared according to the method described by *Munz et al., (1986).* It was used for C-ELISA and C-IP techniques. Its neutralizing titre was 128.

5- Anti-rabbit IgG peroxidase conjugate:

It was obtained from Sigma Chemicals Company, USA and used for C-ELISA and C-IP at a dilution of 1/10,000.

6- serum neutralization test (SNT):

It was applied according to the method described by *Davies et al., (1985)* using 96-well flat bottom micro-titration plates.

7- Competitive ELISA (C-ELISA):

It was done according to *Perrin et al., (1996).* In this assay, the coated CPV antigen was reacted with the unknown camel sera (1^{st} antibody) and

positive rabbit antisera of CPV (2nd antibody) in the same system, then anti-rabbit IgG peroxidase conjugate was added. Also, the previous reaction was carried out separately using rabbit hyperimmune sera of CPV only without the tested camel sera. Addition of orthophenylene diamine (OPD) substrate buffer revealed the positive yellowish brown color of rabbit antisera of CPV only. The percentage of competition (blocking) of unknown camel sera was calculated as the difference between rabbit antisera only and the mixture of camel and rabbit sera using the following formula: % competition = 100 – (test OD – background x 100).

8- Competitive immunoperoxidase technique (C-IP):

It was applied according to *Zaghawa (1997).* C-IP was carried out as the same of C-ELISA but CPV was inoculated in Vero cells using microplate and the infected cells were fixed with 20% acetone saline. Addition of investigated camel sera and positive rabbit antisera of CPV was done as mentioned in C-ELISA. The positive reaction of rabbit hyperimmune sera of CPV was evaluated by the appearance of reddish brown color under inverted microscope by using 3,3 diamino-benzidine tetrahydrochloride in 1% sodium acetate as a substrate buffer and anti-rabbit IgG peroxidase conjugate.

RESULTS AND DISCUSSION

The following table showed the number of positive sera samples during winter season by SNT, C-ELISA and C-IP technique. The number of positive sera by SNT was 148, 102 and 40 in Aswan, Assiut and Fayoum governorates respectively, while the number of positive sera by C-ELISA and C-IP in the same governorates was 164, 120 and 48.

The highest titre of neutralizing antibodies was 64 by both SNT, C-IP and the percentage of inhibition was 80% by C-ELISA in Aswan and Assiut governorates during the winter season. No positive samples were detected in the other season. Results of investigated camel sera by C-IP were recorded in photos (1 and 2) which showed a high and diffuse of reddish brown color of positive rabbit antisera of CPV (photo 1) and various levels of reddish brown and no color of both rabbit and camel sera (photo 2).

The highest number of positive animals and titres of tested camel sera against CPV in Aswan and Assiut governorates during the winter season possibly may be due to the presence of high populations of non-vaccinated camels in these governorates as a result of legal and illegal transportation of camels between these regions and Sudan. In addition, these non-vaccinated animals may be exposed to a previous severe infection with avirulent CPV during the rainy season in Sudan and acted as carriers and reservoirs for the CPV. The suitable climatic conditions for CPV replication during winter rainy season in Egypt and the stress factors accompanying camels transportation from Sudan improve the virus replication in these animals. The low number and titre of positive sera samples of native breed camels in Fayoum governorate may be due to that most of these animals were old (3 years old)

and exposed to a recent infection from transported previously infected animals.

These results are in agreement with these obtained by *Kriz (1982); Jerek et al., (1983); Hafez et al., (1986); Gabry et al., (1997); Khalafalla and Mohamed (1996); El-Harrak and Loutfi (2000); Zaitoun et al., (2000); Melaku and Fesha (2001); Tefera and Gebreah (2001) and Magda et al., (2003)* when they reported that the camel pox virus primarily affects younger animals and epidemics occur in regular cycles depending on the rainy season, the density of the insect population and the number of immunized camels in the population.

Concerning results of the serological techniques, the table showed that there is no difference between SNT, C-ELISA and C-IP and a small variation in the number of positive sera samples were due to the degree of sensitivity of each technique for detection the antibodies. Some factors affect on the sensitivity of SNT and play no role in ELISA and IP such as virus strain, dose of the virus, virus pathogenicity, type or age of cell culture and cytotoxic and bacterial contamination of tested sera.

These results agreed with those obtained by *Honda et al., (1991) and Lawrence and Liauw (1995)* who reported that the sensitivity of cell bound immunoassay (immunoperoxidase), ELISA in comparison to the virus neutralization test was 100% for both sera and milk samples. Also, they found 100% sensitivity of a commercial globulin E (gE) blocking ELISA in comparison to the standard virus neutralization test.

In conclusion, this study revealed that most imported Sudanese camels and some native Egyptian camels may be carriers and reservoirs of CPV and considered a source of infection to other camels and man in spite of they are free from clinical signs of the disease. also, C-ELISA and C-IP can be used successfully as rapid diagnostic tests for detecting camel pox antibodies.

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Results of serological investigation of camel sera in Aswan, Assiut and Fayoum governorates

Governorates, breed and age of animals	Investigated camel sera by SNT, C-ELISA and C-IP during Winter season *						
	Total No.	SNT		C-IP		C-ELISA	
		+ve No. and %	Mean Titre	+ve No. and %	Mean Titre	+ve No. and %	PI
Aswan (imported, one year old)	400	148 (37%)	64	164 (41%)	64	164 (41%)	80%
Assiut (imported-native, 1-2 years old)	400	102 (25.5%)	32-64	120 (30%)	64	120 (30%)	70%
Fayoum (native, 3 years old)	400	40 (10%)	16	48 (12%)	32	48 (12%)	45%

* Season of positive samples is Winter.
- SNT = serum neutralization test - C-ELISA = competitive ELISA
- C-IP = competitive immunoperoxidase - PI = Percentage of Inhibition

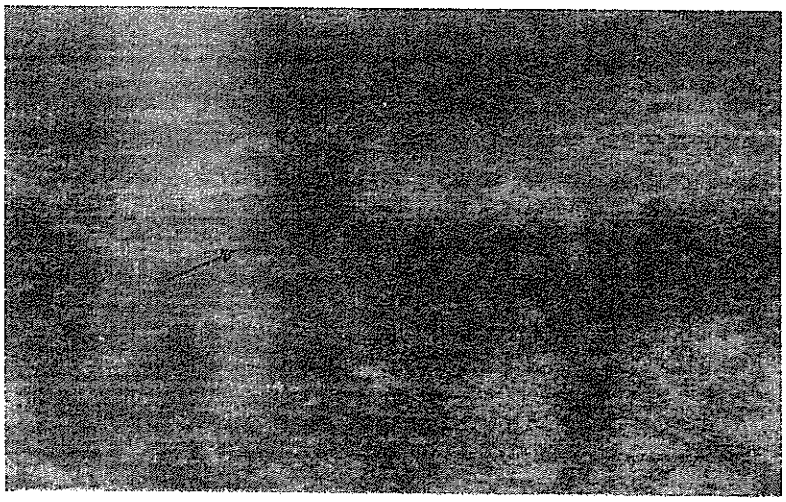

Photo (1): High positive reaction of rabbit antisera for CP and negative for camel sera by competitive immunoperoxidase. The arrow shows high density and diffuse reddish brown color

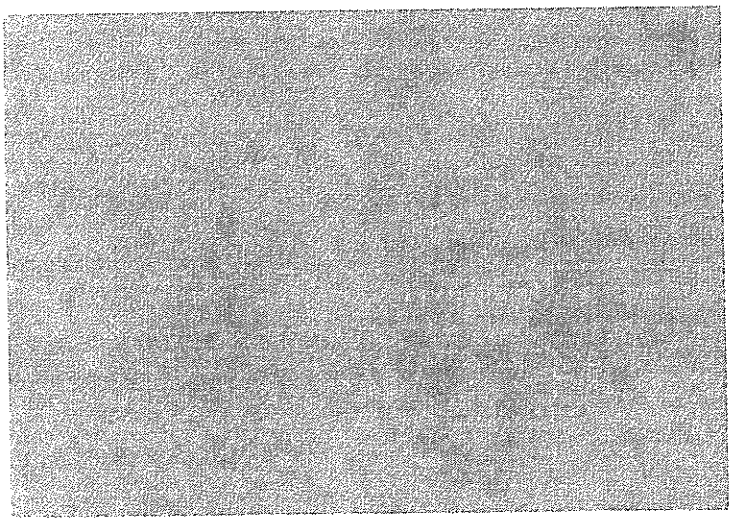

Photo (2): Moderate positive reaction of both tested camel sera (no colour) and rabbit antisera for CPV (reddish brown) by competitive immunoperoxidase

الملخص العربى

دراسة سيرولوجية عن مرض جدرى الجمال باستخدام اختبار الاليزا التنافسى وأنزيم البيروكسيداز المناعى التنافسى واختبار المصل المتعادل

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الهدف من هذه الدراسة هو استبيان الأجسام المضادة ضد مرض جدرى الجمال فى ثلاث محافظات مصرية هى أسوان وأسيوط والفيوم. وتم جمع عدد 1200 عينة دم من جمال سليمة ظاهرياً عند عمر 1-3 سنة خلال أربعة شهور متعاقبة تمثل مواسم مختلفة المناخ. وتم فصل السيرم وفحصه باختبارات المصل المتعادل والاليزا والبيروكسيداز التنافسى. وكان عدد العينات الايجابية فى محافظات أسوان وأسيوط والفيوم هو 164، 120، 48 على التوالى باختبارى الاليزا والبيروكسيداز التنافسى بينما كانت النتائج باستخدام اختبار المصل المتعادل هى 148، 102، 40 فى نفس المحافظات على الترتيب. وكانت أعلى نسبة عيارية هى 64 (المصل المتعادل والبيروكسيداز التنافسى)، 80% (الاليزا التنافسى) فى محافظتى أسوان وأسيوط.