J. Hyg., Camb. (1979), **83**, 267
Printed in Great Britain

Isolation, identification and characterization of camelpox virus in Iraq

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(*Received* 17 *October* 1978)

SUMMARY

A virus was isolated from pox-like lesions in camels during an outbreak of camelpox disease which occurred in December 1977 in an area near the Iraqi–Iranian border. It was identified serologically as a virus of the Orthopoxvirus group. The biological properties of the isolate indicated that it was probably identical with strains of camelpox virus isolated from Iran, Egypt, Kenya and the U.S.S.R.

INTRODUCTION

Camelpox is of economic importance in camel raising areas. Outbreaks are reported every 2–3 years in Iraq. The severe form of the disease appeared mainly among young camels and only a mild form was encountered in older animals. The disease has been reported in many countries. Several strains of poxviruses during the last 10 years were isolated from camels in Iran, Egypt, Yemen, Kenya and the Soviet Union (Ramyar & Hessami, 1972; Baxby, 1972, 1975; Marennikova *et al.* 1974; Tantawi *et al.* 1974; Davies, Mungat & Shaw, 1975).

Some of the isolated camelpox viruses were found to be similar to those of variola strains isolated from East Africa (Bedson, Dumbell & Thomas, 1963; Baxby, 1972). The camelpox virus was identified and classified as a virus of separate identity in the Orthopoxvirus group of the Family Poxviridae (Mahnel & Bartenbach, 1973; Mahnel, 1974; Baxby, 1975). Other outbreaks of 'camelpox' have been caused by vaccinia virus and a virus resembling Orf (Krupenko, 1972; Roslyakov, 1972).

This paper presents some of the biological, physico-chemical and serological properties of true camelpox virus isolated for the first time in Iraq.

MATERIALS AND METHODS

Field material

Four hundred and fifty camels were reported to have camelpox in Al-Etha area near the Iraqi–Iranian border. Selected cases of different ages were examined.

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0022-1724/79/0127-1978 $01.00 © 1979 Cambridge University Press

Skin lesions at different stages of development (vesicles, pustules and dried scabs) were collected and kept in sterile vials in glycerol.

Serum samples were collected from five camels of different ages. Two of these five (Table 2) had generalized pox lesions, while the other three were in contact with the diseased camels but showed no symptoms.

Virus isolation and characterization

The virus was isolated on the chorioallantoic membrane (CAM) of developing chick embryos and was designated Etha 78. The virus was identified and characterized using the conventional methods described by Baxby (1972), Marennikova *et al.* (1974) and Davies *et al.* (1975). The biological criteria used for virus characterization were the following: the pock morphology on the CAM, the ceiling temperature, the histopathology of the infected CAM, the lethality to chicken embryo, the growth in culture of chick embryo fibroblasts (CEF), the plaque morphology in CEF cells, the haemagglutinating activity using RBCs of human type O, chicken and other animal species and the pathogenicity to laboratory animals (rabbit, guinea-pig, mice, 3-day-old chick and monkey).

The physico-chemical properties of the virus were tested using the following criteria: the thermostability at 56, 60 and 70 °C, the effect of different pH (3–8·5) and the sensitivity of the isolate to ether and chloroform.

Virus control

The viruses used as control in this study were the following: Vaccinia virus (strain used for vaccination against smallpox), cowpox virus (strain Brighton), sheep pox virus (strain Romanian and a local isolate) and goat pox virus (strain Iranian and a local isolate). The viruses were used in the form of CAM suspension obtained from infected embryonated eggs with the exception of sheep pox viruses which were used in the form of infected culture fluid.

Antisera

Standard antivaccinia serum was obtained from Professor Marennikova (WHO Cooperative Center for Diagnosis of Smallpox and other Pox Viruses, Moscow). Antiserum against the isolated camelpox virus was prepared in rabbits by the method described by Gispen (1955).

RESULTS

Growth of the isolate Etha 78 *on CAM*

The virus grew easily on the CAM and produced pock lesions on the 5th day after inoculation. The lesions appeared more or less localized to the inoculated area, white, 0·4–0·6 mm in diameter, without haemorrhage or necrosis and not elevated above the surface of the membrane. The virus grew readily at 34, 37, and 39 ± 0·5 °C. It was not lethal to chick embryos even in high doses (10^5–10^6 pk.f.u.).

Growth in tissue culture

The virus replicated in CEF cells and produced CPE on the 4th day after inoculation. The CPE was characterized by cell rounding and detachment from the glass. Small syncytia and cell ballooning were also evident. The virus produced plaques under agar overlay 5 days after infection without the need for adaptation. The plaques had lysed centres and sharp edges with an average diameter of 1·2 mm.

Haemagglutinating activity

The virus agglutinated RBCs of chickens at pH 6, 7 and 8 at room temperature but with a low HA titre (1/8) in comparison with that of vaccinia virus (1/256). It did not agglutinate RBCs from different species of mammals (horse, ox, sheep, goat, human type O, rabbit and dog). The haemagglutination could be completely and partially inhibited by anticamelpox and antivaccinia sera respectively. The virus has a relatively low HA yield in CEF cells 30 h after inoculation.

Sensitivity to ether and chloroform

The Etha 78 virus was resistant to the action of ether and chloroform. No reduction was observed in virus titre after treatment in comparison with the untreated control virus.

Thermostability

The virus was resistant to heat at 56 °C for 1 h. A reduction of 50 % of virus infectivity and complete inactivation occurred at 60 °C after 1 and 2 h respectively. The virus infectivity was greatly reduced at 70 °C after 10 min and completely disappeared after 30 min.

pH resistence

Treatment of the virus with buffer systems of different pH values showed that the virus infectivity remained unaffected at a pH range between 3 and 8·5 for 1 h.

Pathogenicity

Etha 78 (in a dose of 10^3–10^5 pk.f.u./0·5 ml) produced in rabbit skin a mild transient erythyma which disappeared on the 5th day after inoculation and produced in monkeys typical localized pox lesions from which the virus could be reisolated. The multiplication of the virus in the monkey was also evident by serological tests. The virus produced no reaction in chickens and other animal species tested. In contrast vaccinia virus produced hyperaemia and nodular lesions in the rabbit inoculated by scarification, and in 3-day-old chicks typical vesicular pox lesions appeared (Table 1).

Table 1. *Pathogenicity of Etha 78 isolate to laboratory animals*

Animal species	No.	Ages	Dose*	Route	Results
Rabbit	3	2–3 months	0·5 ml (10^3 pk.f.u.)	I/D	Erythema 72 h post inoculation disappeared on the 5th day
	3	2–3 months	0·5 ml (10^5 pk.f.u.)		
	3	2–3 months	0·5 ml	I/T	No reaction
	3	2–3 months	0·5 ml	Skin scarification	No reaction
Guinea-pig	4	3–4 months	0·5 ml	I/D	No reaction
Mice	16	4–6 weeks	0·03 ml	I/C	No reaction
Chickens	20	3 days	0·2 ml	I/F	No reaction
Monkey	2	1 year	0·5 ml	I/D	Typical pox lesions passing all stages. 2–4 mm in diameter. Appeared on the 8th day after infection at the site of inoculation and disappeared by the 18th day after inoculation

I/D, intradermally; I/T, intratesticularly; I/F, intrafolicularly; I/C, intracerebrally.
* 10^3 pk.f.u.

Table 2. *Results of agar gel diffusion and serum neutralization tests carried out on camel sera collected during the outbreak of camelpox*

Animal no.	Age	AGD		SNT	
		Etha 78	Vaccinia	Etha 78	Vaccinia
1	1 year	+	+	1·4*	1·1
2	1 year	−	+	1·45	1·3
3	1 year	+	+	3	1·2
4	15 years	+	+	3·27	1·4
5	15 days	+	+	2·66	1·1

AGD, Agar gel diffusion; SNT, serum neutralization test; * $\log_{10}$ neutralization index.

Serological investigation

The isolated virus was precipitated by antivaccinia serum in the gel diffusion test. The antiserum prepared against Etha 78 gave three precipitating bands with the homologous virus and only two bands with the reference vaccinia antigen. Etha 78 and vaccinia antigen shared a common band.

The serum neutralization test carried out on camel sera (Table 2) showed neutralizing activity against Etha 78, and to a lower degree against vaccinia antigen.

DISCUSSION

The WHO smallpox eradication campaign is on the verge of success. Now it is important to assess the status of related viruses such as camelpox, buffalopox and monkey pox. The pathogenicity of these viruses to man is not well known. It is possible that these viruses may establish themselves in a less immune human population with subsequent re-evolution to cause smallpox or smallpox-like diseases (Baxby, 1975). Only minor differences in the antigenic structure of camelpox virus and smallpox virus were reported by Gispen & Brand-Saathof (1974).

The results introduced in this paper indicated that the biological characteristics of the Iraqi strain of camelpox virus were similar to those described for the Iranian strain. This is not surprising because the Etha 78 virus was in fact isolated near the Iraqi–Iranian border.

It is of interest to point out that the Etha 78 isolate was pathogenic to monkeys. It produced typical localized pox lesions and the virus was reisolated from these lesions. A similar observation was reported by Baxby (1972) using the CM-O_2 camelpox virus isolated in Iran.

The results of this investigation showed that although Etha 78 virus shared a common antigen with vaccinia virus minor differences in the antigenic structures of these two viruses were observed.

Sera collected from camels no. 3 and 4 which had the last stage of generalized pox infection showed a relatively high neutralizing activity against Etha 78 and low activity against vaccinia virus. The low neutralizing activity against vaccinia virus may be attributed to the common antigen shared by the two viruses. Sera collected from camels no. 1, 2 and 5 which had no apparent pox infection showed a lower neutralizing activity against Etha 78. This activity again may be attributed to subclinical infection as these animals were in close contact with diseased camels through the outbreak which lasted 2 months. It is of interest to notice that the young camel no. 5 which was the offspring, born during the outbreak, of camel no. 4, had a relatively high neutralizing activity in its serum against Etha 78 virus which may be due to passively transferred maternal immunity.

It remains of interest from the epidemiological point of view to determine the pathogenicity of camelpox virus to man and the relationships between this virus and smallpox virus.

We are indebted to Dr D. Baxby (Liverpool) for critically reviewing this manuscript.

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