WORLD HEALTH ORGANIZATION
ORGANISATION MONDIALE DE LA SANTÉ

WHO/SE/73.54

~~ORIGINAL~~ ENGLISH only

CAMELPOX VIRUS[1]

(Properties, differentiation from related viruses of the pox group)

S. S. Marennikova, L. S. Shenkman, E. M. Akatova-Shelukhina, N. N. Maltseva
Moscow Research Institute of Virus Preparation, Moscow, USSR

SUMMARY

The virus of camelpox isolated by the authors was studied. A number of features of this virus indicate that it should be classified as an independent species of the genus Orthopoxvirus. Its pathogenicity for certain animals other than camels was established. Criteria for differentiating camelpox virus from related viruses of the pox group are suggested. Causes of differences in the pattern of lesions produced by this virus in the chorioallantoic membrane of chick embryo were found.

INTRODUCTION

A lack of knowledge of the characteristics of causative agents of some pox-like diseases of animals creates difficulties in their classification, methods of differentiation, etc. Among these little-studied viruses is the causative agent of camelpox (Zhdanov, 1953; Andrewes, 1964; Mayr et al., 1972). Interest in the further study of this virus is also stimulated by the persistence of outbreaks of this disease, some of which are associated with high case fatality rates in camels (Borisovich & Orekhov, 1966; Buchnev & Sadykov, 1969).

This paper presents the results of comparative studies of the causative agent of camelpox and related viruses belonging to the genus Orthopoxvirus (classification by Mayr et al., 1972).

MATERIALS AND METHODS

Viruses - The camelpox virus (T-72 strain) was isolated from crusts of a sick animal by inoculation of chick embryos according to the conventional method (WHO, 1969). For the present studies, materials of the first to fifth passages were used. For comparison, vaccinia virus (Tashkent and EM-63 strains), monkeypox virus (Copenhagen strain), variola virus (Harvey strain and the Ahmed Beg strain isolated in this laboratory in 1971 from a smallpox patient from Pakistan) were used. The virus-containing materials consisted of suspensions of infected chorioallantoic membranes (CAM) of chick embryos from the first to seventh passages.

Sera - Sera against vaccinia and monkeypox viruses prepared by combined immunization of rabbits according to the method of Gispen (1955) as well as the serum from the camel from which the camelpox virus (T-72 strain) had been isolated were used. Before tests the sera were heated at 60°C for 30 minutes.

---

[1] Paper submitted for publication in Archiv für die Gesamte Virusforschung.

Virus assay - The activity of the virus-containing material was determined on the CAM of chick embryos by the method of pock count of Westwood et al., 1957. The infected chick embryos were incubated for two to seven days, depending on the purpose of the tests, at temperatures of 20°, 34.5°, 37° and 39°C.

Chick embryo LD_{50} - 12-day-old chick embryos were inoculated on CAM with the viruses under study in doses from 10^1 to 10^4 PFU/0.1 ml and incubated for seven days at 34.5°C. Beginning at 48 hours post-infection the death of embryos due to infection was recorded. The LD_{50} was calculated by Kerber's method.

Helbert's test (accumulation of virus in chick embryo liver) - 12-day-old chick embryos were inoculated with 1.7×10^5 PFU/0.1 ml of virus. After 72 hours of incubation at 34.5°C the embryos were opened, the liver was removed aseptically and a 20% suspension (by weight) was prepared in McIlvaine buffer solution. The virus concentration in the liver was assayed in chick embryos by pock counting.

PATHOGENICITY FOR ANIMALS

Chickens - Three-day-old chickens were inoculated into the follicles by rubbing the virus-containing material into the skin over the shin after removal of the down. A suspension of CAM from uninfected chick embryos in an amount equal to that of the virus-containing material (0.1 ml) was rubbed into the skin in the same manner. The animals were observed for seven days.

White mice - 10-day-old mice were inoculated intracerebrally with doses of 10^2-10^5 PFU/0.03 ml and were observed for 10 days. Beginning at 48 hours after infection and for a 10-day period, deaths due to specific infection were recorded. The LD_{50} was calculated by Kerber's method.

Rabbits - Chinchilla rabbits weighing 2.5-3.0 kg were inoculated on the scarified skin and intradermally with a dose of 10^5 to 10^6 PFU/0.1 ml. The reaction was observed daily for five days.

Cell culture - Primary chick embryo fibroblast culture (CEF) was used. The cells were seeded in amounts of 2.5×10^5 per 1 ml of the nutrient medium consisting of medium 199 with 10% bovine serum. After monolayers were formed, the nutrient medium in the tubes was substituted with medium 199 with 2% bovine serum, and the cultures were inoculated with varying doses of the virus (from 10^1 to 10^4 PFU/0.1 ml) in 0.1 ml volumes per tube. From 10 to 15 tubes were used for each dose. The inoculated cultures were incubated at 37°C for seven days and examined daily under the microscope.

For detection of inclusions, the cells were grown on slides in tubes. At various intervals after inoculation the slides were removed, rinsed in distilled water and the cells were fixed and stained with haematoxylin and eosin (Marennikova et al., 1959).

For the study of the types of plaques formed, the cell monolayer was stained with 1% neutral red solution six days after inoculation. The maintenance medium was removed and 1 ml of the stain per tube was added and left in contact with the cells for 5-10 minutes after which time the stain was removed and the cell sheet was washed with physiological saline.

For the haemadsorption test, a 1% suspension of rooster erythrocytes at a temperature of 22°C was used. The results were read 15 minutes after the monolayer was washed twice with physiological saline.

The haemagglutination (UA) and haemagglutination-inhibition (HI) tests were carried out by the conventional methods with a 1% suspension of rooster erythrocytes. Four HA units were used in the HI test.

The test of double diffusion in agar gel was carried out by the conventional method (WHO, 1969) and according to Ouchterloni's method (1949).

The electron microscopy - The preparations were treated by the method of negative staining (Cruickshank et al., 1966) and examined at magnifications of 15 000 and 40 000.

RESULTS

Morphology of the virus - The shape and size of virions detected both in specimens from the sick camel and in infected CAM suspensions of the first passage were identical. The virions had the brick-like shape characteristic of the viruses of the pox group and the internal structure was essentially indistinguishable from that of variola, vaccinia and monkeypox viruses. Their size was 280 x 180 nm.

Morphology of pocks on the CAM - Cultivation of the camelpox virus on CAM of chick embryos at 37°C was accompanied by formation of monomorphic, pinpoint, proliferative dense white pocks of 0.2-0.3 mm in size which resembled those produced by variola and alastrim viruses. Only a very careful examination revealed differences: the pocks produced by camelpox virus were smaller and did not rise above the surface of the membrane. Change of the temperature of incubation of infected chick embryos to 34.5°C caused marked changes in the character of the lesions produced by camelpox virus. They became flatter and haemorrhages appeared in the centres of the pocks. Under these conditions the camelpox virus could be easily differentiated from variola virus. It was more difficult to differentiate it from monkeypox virus which also produces under these conditions small pocks with haemorrhages in the centre. However, the latter virus exhibited some polymorphism of the pocks; small pocks with haemorrhagic centres and large white pocks without haemorrhage both being present.

Pathogenicity for chick embryos - Camelpox virus showed a low pathogenicity for chick embryos. As a rule, at 72 hours after inoculation, the embryos remained viable. The LD_{50} for camelpox virus and other viruses under study are presented in Table 1. It will be seen that the LD_{50} was the lowest for camelpox virus.

Accumulation in the liver of chick embryos - As will be seen in Table 1, camelpox virus accumulated in the liver of chick embryos in lower amounts than other viruses tested.

The ceiling temperature for development of lesions on the CAM - In addition to incubation temperatures of 34.5° and 37°C, the behaviour of camelpox virus in chick embryos was tested at 20° and 39°C. No lesions on the CAM were produced by camelpox virus at either of these temperatures. The failure to produce lesions at 39°C differentiated camelpox virus from monkeypox virus which produced pocks at this temperature.

Pathogenicity for three-day-old chickens inoculated into the skin follicles - All the viruses tested by inoculation of the skin follicles of three-day old chickens in doses of 10^5 and 10^6 PFU/0.1 ml produced pustules at 3-4 days after a dose of 10^6 and at 5-6 days after a dose of 10^5 PFU. Usually, 2-3 days after inoculation the openings of the skin follicles extended and were filled with clear material. The content of the follicles clouded rapidly and typical pox pustules appeared, resembling in their shape halves of peas. At 7-8 days the pustules dried up and dry scales formed.

Pathogenicity for white mice - The results of LD_{50} determinations by the intracerebral inoculation of the viruses under study are presented in Table 1. In its pathogenicity for mice, the virus of camelpox was more pathogenic than variola but less pathogenic than monkeypox virus.

Pathogenicity for rabbits - The virus of camelpox was found practically apathogenic for rabbits when inoculated by scarification or intradermally. In the former case, only a

slight hyperaemia of the inoculated area of the skin was observed at the fourth day while in the latter, infiltrates of 3-5 ml in size and of low density appeared. This feature sharply differentiated camelpox virus from vaccinia and monkeypox viruses which in the same infective dose produced confluent papulous-pustulous eruptions and, after intradermal inoculation, large solid infiltrates with necrosis in the centre (monkeypox virus, Tashkent strain of vaccinia virus).

Behaviour in cell culture - In CEF cultures infected with a dose of 10^1 PFU/0.1 ml, cytopathic changes were first noted at 72 hours. With lower doses of the virus the time of appearance of CPE increased to 5-7 days. In the time of appearance and in the general pattern of cytopathic changes (of the focal type) the camelpox virus more closely resembled variola virus. Initially, the foci of CPE had an irregular elongated form. Cells in such foci retained their fibro-blast-like structure but were more refractile. However, in contrast to the CPE caused by variola virus at this time, the cells were not rounded or increased in size. Later, at 5-7 days, the size of CPE foci increased and the foci became rounded. The cells in the centres of the foci became rounded and slightly increased in size. Hollowness formed between the cells. The process of lysis, however, was much less marked than in vaccinia infection. It should be noted that even seven days after inoculation of the culture with the above dose of virus, complete destruction of the cell sheet did not occur: in addition to "old" foci of lesions, new foci appeared in which changes in the cells were those of the initial period.

Camelpox virus in CEF culture produced lytic plaques of irregular form and up to 3 mm in diameter (examination at 144 hours after infection). Groups of degenerated cells could be seen in the centres of the plaques as stained points. Plaques produced by the camelpox virus appeared by 96 hours after inoculation. They differed markedly from the small plaques of a proliferative type produced by variola virus and from stellate lytic plaques of vaccinia and monkeypox virus.

Inclusions - In cells of camelpox virus-infected CEF culture cytoplasmic inclusions resembling Guarnieri bodies were found. The inclusions were concentrated in the foci of CPE.

Haemadsorption capacity - Like other viruses under study, the camelpox virus produced a definite haemadsorption phenomenon in cell culture.

Haemagglutinating activity - The virus showed poor haemagglutinating activity: the infected CAM suspension had a titre of 2 to 16 in the HA test.

Serologic studies - When the serum from the camel, from which the T-72 virus was isolated, was tested by Ouchterloni's agar precipitation test, precipitin for vaccinia, variola, cowpox and camelpox viruses were found. The same serum had antihaemagglutinins for vaccinia virus in a titre of 32. Ouchterloni's agar precipitation test with antivaccinia serum and the battery of the viruses under study revealed a common antigen. In addition, the camelpox virus produced a spur in the test when placed between the wells with vaccinia virus and a double shoulder when placed next to cowpox virus.

DISCUSSION

On the basis of the biological properties studied, it is apparent that camelpox virus differs significantly from the three members of the genus Orthopoxvirus studied in parallel (monkeypox, vaccinia and variola viruses) and from other viruses of this genus.

Our studies confirm the reported data regarding the morphology of the causative agent of camelpox (Roslyakov, 1972) and its low pathogenicity for animals (Sadykov, 1970; Mayr et al., 1972). At the same time this virus was found to have a certain degree of pathogenicity for chick embryos, white mice (intracerebral inoculation) and chickens (inoculated into the follicles). The latter observation is at variance with data presented by Mayr et al., 1972, who found no reaction after infection by this route. This discrepancy may be due to the use of different infective doses, strain features of the virus, etc.

Our studies would seem to explain the discrepancies reported in the literature with regard to the pattern of pocks on the CAM of chick embryos. According to Sadykov (1970), camelpox virus produces on the CAM pocks with haemorrhages in the centre, whereas according to Mayr et al., 1972, it produces pinpoint proliferative pocks without haemorrhages. We were able to demonstrate that camelpox virus can produce both types of pocks depending on the temperature of incubation of embryos.

The study of the antigenic structure of camelpox virus confirmed the presence in it of the antigenic components common for the other viruses under study. At the same time, the features of localization and the character of precipitation bands formed by it with anti-vaccinia serum in the agar gel suggest some differences in the antigenic structure of this virus as compared with that of vaccinia and cowpox virus.

The results obtained in this comparative study of pox viruses indicate a number of tests which permit differentiation of camelpox virus from related members of the genus Orthopoxvirus (Table 2). The necessity of such differentiation is emphasized by the fact that camels, like many other animals, are susceptible to vaccinia virus (Samartsev, Praksein, 1950) and therefore may be infected with this virus (from vaccinated people, for example). Conceivably, also under certain conditions, camelpox virus may be pathogenic for man, as cowpox and monkeypox viruses.

In recent years, our views of camelpox virus as an independent species have changed considerably. Not long ago it was considered to be a variety of vaccinia or cowpox virus (Herrlich, 1960) and for this reason it was not included as a separate species in published classifications (Fenner, 1962). However, subsequent data on the properties of camelpox virus indicate that it should be considered to be an independent species. This was proposed by Mayr et al. in 1972 even though with some reservations.

The data presented here do show this virus to differ from vaccinia, monkeypox, variola and cowpox viruses, not less than these viruses differ from each other. We believe therefore that there is sufficient reason to consider camelpox virus as an independent species.

CONCLUSIONS

1. The isolate of camelpox virus produced lesions on the CAM of chick embryos the character of which depended upon the temperature of incubation.

2. The lesions produced by this virus on the CAM at the incubation temperature of 34.5°-35°C permit it to be differentiated from the viruses of variola and cowpox, vaccinia and monkeypox viruses. Additionally, this virus differs from monkeypox virus in its inability to produce pocks on the CAM at 39°C.

3. Camelpox virus is pathogenic for white mice when inoculated intracerebrally and for three-day-old chickens by inoculation into follicles. Its pathogenicity for chick embryos was the lowest as compared with other viruses under study.

4. Features of the CPE and plaque production by camelpox virus in tissue culture differentiate it from vaccinia, variola and monkeypox viruses. In the affected cells this virus produces cytoplasmic inclusions.

5. Camelpox virus shows a poor haemagglutinating activity; in cell cultures it gives haemadsorption.

TABLE 1. PATHOGENICITY OF THE VIRUSES UNDER STUDY FOR WHITE MICE AND CHICK EMBRYOS

Virus	LD_{50} for chick embryos (PFU/0.1 ml)	LD_{50} for white mice (PFU/0.03 ml)	Accumulation in the liver (PFU/g) of chick embryos
Camelpox	2.8	4.5	6.8
Monkeypox	<1	2.7	9.08
Variola	2.1	5.6	8.3
Vaccinia	<1	not done	8.0

TABLE 2. PRINCIPAL TESTS FOR DIFFERENTIATION OF CAMELPOX VIRUS FROM SOME OTHER POX VIRUSES

Virus	Pocks on the CAM		Reaction in the skin of rabbits
	Character (34.5°C)	Formation at 39°C	
Camelpox	small, pinpoint with central haemorrhages 0.2-0.3 mm	-	-
Vaccinia	large (2 mm), flat, usually without haemorrhages	+	+
Cowpox	majority haemorrhagic ("red")	+	+
Variola and alastrim	pinpoint, prominent without haemorrhages 0.7-0.9 mm	-	-
Monkeypox	majority is small, pinpoint, with central haemorrhages 0.5-0.7 mm	+	+

REFERENCES

1. Borisovich, Yu. F & Orekhov, M. D. (1966) Camelpox, Veterinary (In Russian), 3, 50-52

2. Buchnev, K. N. & Sadykov, R. G. (1969) On camelpox in Kazakhstan, Trans. Alma-Ata Zootechn. Veterinary Inst. (In Russian), 15, 4, 12-18

3. Zhdanov, V. M. (1953) Guidebook of human and animal viruses, Moscow, (In Russian)

4. Marennikova, S. S., Gurvich, E. B., & Yumasheva, M. A. (1959) Isolation and study of the properties of variola virus. I. Direct isolation of variola virus in tissue culture, Vop. Virusol. (In Russian), 4, 703

5. Roslyakov, A. A. (1972) Comparative ultrastructure of camelpox viruses, virus of pox-like disease of camels ("auzdyk") and contagious enthyma of sheep, Vop. Virusol. (In Russian), 1, 26-30

6. Sadykov, R. G. (1970) Propagation of camelpox virus in chick embryos. In: "Virus Diseases of Agricultural Animals", M., 1, 55

7. Samartsev, A. A. & Praksein, S. T. (1950) Study of camelpox, Transact. Research Veterinary Inst., Alma-Ata, 5, 198-200

8. Andrewes, Ch. (1967) Viruses of vertebrates, London

9. Cruickshank, J. G., Bedson, H. S. & Watson, D. H. (1966) Electron microscopy in the rapid diagnosis of smallpox, Lancet, ii, 527

10. Fenner, F. (1962) Interaction between pox viruses, Proc. roy. Soc. Med., 156, 388

11. Gispen, R. (1955) Analysis of pox virus antigens by means of double diffusion. A method for direct serological differentiation of cowpox, J. Immunol., 74, 2, 134-141

12. Holbert, D. (1957) Smallpox and alastrim. Use of chick embryo to distinguish between the viruses of variola major and variola minor, Lancet, 1, 6977, 1012-1014

13. Herrlich, A. (1960) Die Pocken, Stuttgart

14. Mayr, A., Mahnel, H. & Munz, E. (1972) Systematisierung und Differenzierung der Pockenviren, Zbl. Vet.-Med. B., 19, 69-88

15. Ouchterloni, O. (1949) Antigen-antibody reaction in gels, Acta pathol. microbiol. Scand., 26, 4, 501-515

16. Westwood, J. C. N., Phipps, P. H. & Boulter, E. A. (1957) The titration of vaccinia virus on chorioallantoic membranes of developing chick embryos, J. Hyg. (Lond.), 55, 123

17. World Health Organization (1969) Guide to the laboratory diagnosis of smallpox for smallpox eradication programmes, Geneva