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Comparative Analysis of the Genomes of Orthopoxviruses Isolated from Elephant, Rhinoceros, and Okapi by Restriction Enzymes

Brief Report

By

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With 2 Figures

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Summary

Orthopoxviruses from different zoo-kept mammalian species including *Elephas maximus* (8 isolates), *Ceratotherium simum* (1 isolate), and *Okapia johnstoni* (2 isolates) were characterized by restriction enzyme analysis of the viral genome. The four enzymes BamHI, MluI, NcoI, and SalI were found to be optimal for strain differentiation.

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The identification and classification of orthopoxviruses has been traditionally based on correlating the virus to the host animal from which it had been recovered and on the determination of biological properties like pox morphology, host range of virus, serological and cross protection relationships and finally on a specific ceiling temperature for virus growth (15, 18).

More than 19 outbreaks of pox disease in zoo-kept mammals had been observed in Europe between 1960 and 1984. At least 18 were located within a circle of about 1070 km in diameter. The isolated virus strains showed some similarities in their biological properties. For instance all elephant and rhinoceros isolates produce large inclusion bodies of type A V+ in cells of the chorioallantoic membrane and characteristic skin lesions in laboratory mice (20, 21). Since they resemble cowpox virus in some of their biological properties they were incorporated into a group called cowpoxlike viruses

by BAXBY and GHABOOSI (4). For differentiation of these orthopoxviruses polypeptide analysis had been employed by TURNER and BAXBY (27). However, orthopoxviruses exhibit extensive serologic cross-reactivity and DNA sequence homology of their genomes (2, 3, 5, 6, 12, 17, 28). Analysis of the viral genome using restriction endonucleases is a more precise approach for characterization and provides more definitely the identification of these viruses than phenotypical characterization or polypeptide analysis. It could be shown in aprevious study that cowpoxlike virus strains can be differentiated like other orthopoxviruses by their DNA cleavage patterns using the enzymes XhoI, EcoRI, and HindIII (19, 20).

Recently Esposito and Knight reported about a comparison of restriction profiles of 38 orthopoxvirus DNAs including variola, monkeypox, vaccinia, ectromelia, Tatera, raccoon, cowpox and camelpox viruses (7). Here we report on the genomic characterization of some orthopoxvirus strains isolated from zookept mammals like elephant, rhinoceros, and okapi.

The history of the viruses used in this study is summarized in Table 1, which contains a description of individual isolates. These isolates were

Table 1. *Origin and history of orthopoxvirus strains analyzed by viral DNA*

Virus	Species	Outbreak		Origin of virus strain and (reference)
		Locality	Year	
EP-1	Asian elephant	Augsburg	1971	MAHNEL, Munich (8, 16)
EP-2		Ansbach	1975	MAHNEL, Munich (9)
EP-3		Frankfurt	1977	MAHNEL, Munich
EP-4		Hannover	1980	MAHNEL, Munich (22)
EP-5		Vienna	1974	KUBIN, Vienna (10)
EP-6		Amsterdam	1973	HEKKER, Utrecht (22)
EP-7		Amsterdam	1973	HEKKER, Utrecht (22)
EP-8		Hamburg	1984	PILASKI, Düsseldorf (23)
OP-1	Okapi	Rotterdam	1968	HEKKER, Utrecht (29)
OP-2		Copenhagen	1963	FREUNDT, Aarhus (1)
RP	White Rhinoceros	Münster	1977	PILASKI, Düsseldorf (26)
RP-1				
Cowpox "Brighton"	Cow			BAXBY, Liverpool
Catpox	Anteater, cat	Moscow	1973	MARENNIKOVA, Moscow (13, 14)
Rabbitpox	Rabbit			HEKKER, Utrecht
BP-1	Buffalo			BAXBY, Liverpool
Ectromelia	Mouse			MAHNEL, Munich
CP-1	Camel			RAMYAR, Teheran (24)
Vaccinia "Elstree"				HEKKER, Utrecht (2)

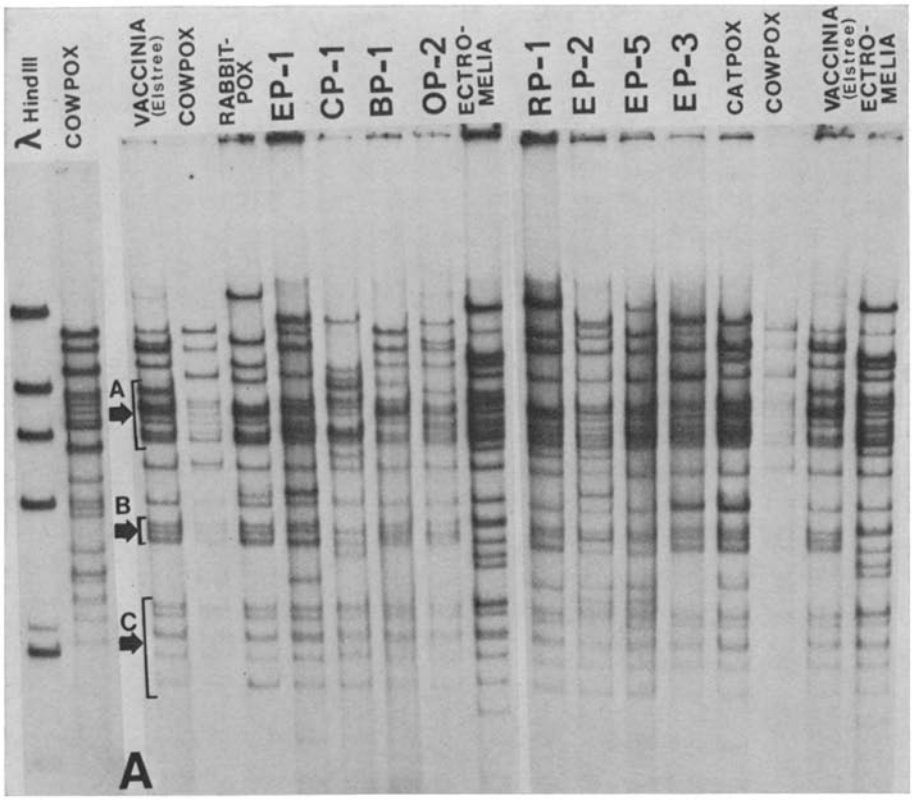

λ HindIII
COWPOX
VACCINIA (Elstree)
COWPOX
RABBIT-POX
EP-1
CP-1
BP-1
OP-2
ECTRO-MELIA
RP-1
EP-2
EP-5
EP-3
CATPOX
COWPOX
VACCINIA (Elstree)
ECTRO-MELIA
A
B
C
A

Fig. 1 A

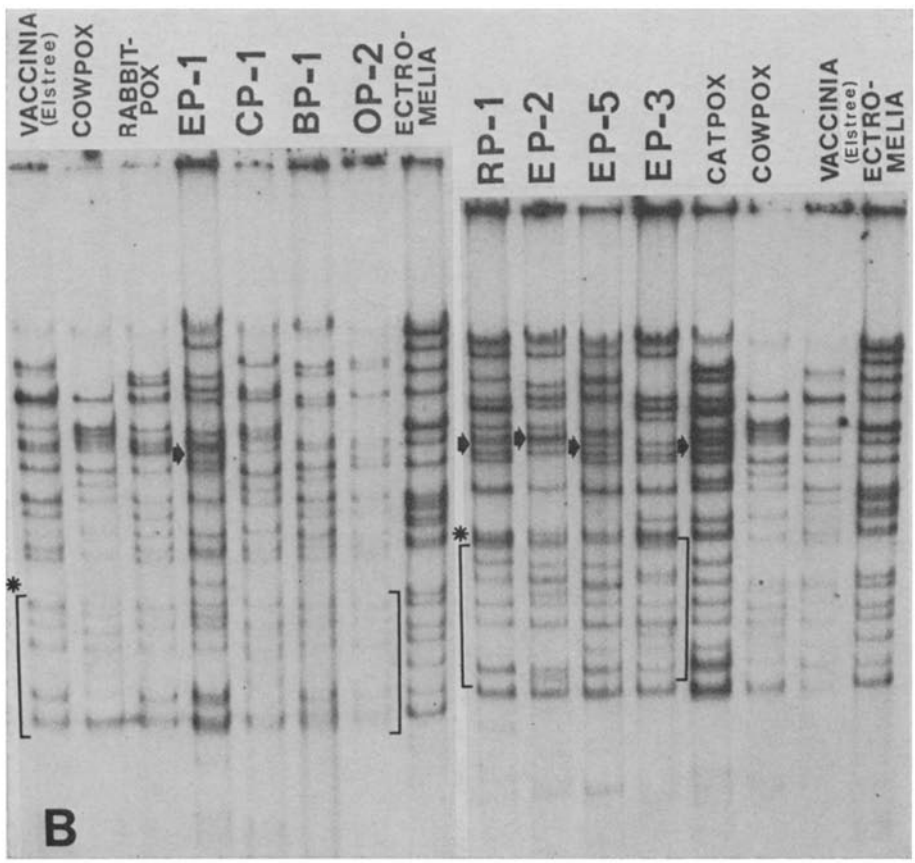
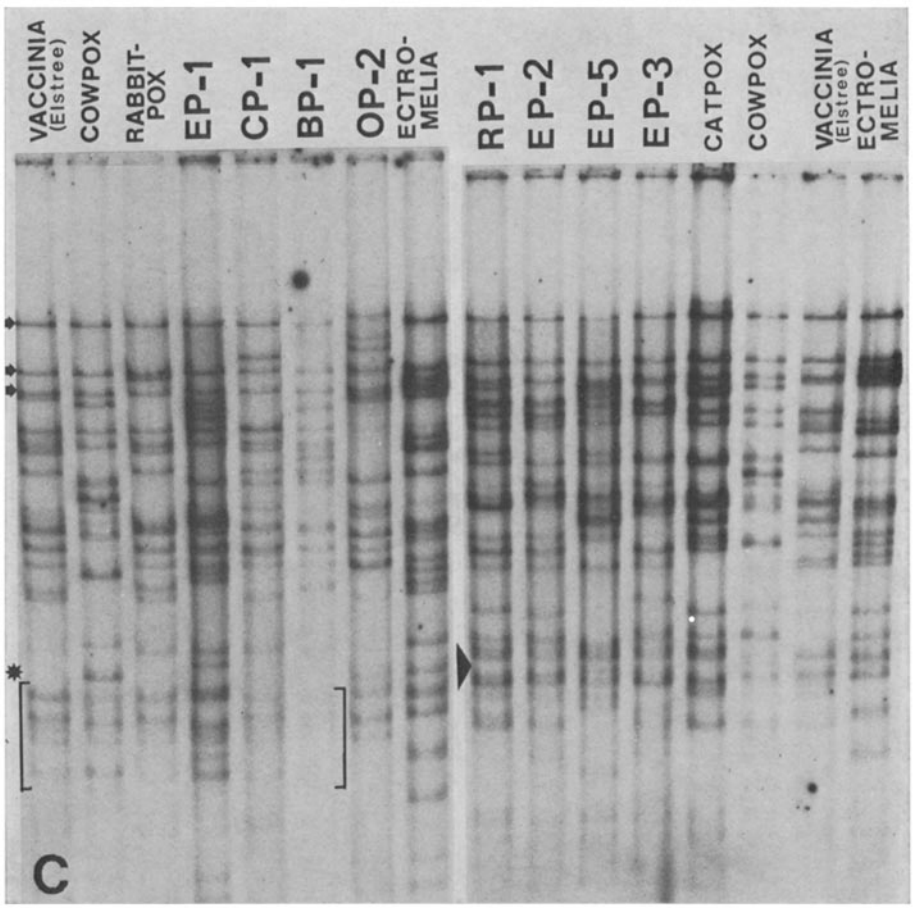

VACCINIA (Elstree)
COWPOX
RABBIT-POX
EP-1
CP-1
BP-1
OP-2
ECTRO-MELIA
RP-1
EP-2
EP-5
EP-3
CATPOX
COWPOX
VACCINIA (Elstree)
ECTRO-MELIA
B
*
*

Fig. 1 B

grown on 12-day-old chick embryo chorioallantoic membranes (CaMs). For ^{32}P labeling of viral genomes the individual isolates were grown once on monkey kidney cells (RC-37), which were grown and propagated as described previously (25). The viral DNA was labeled with ^{32}P orthophosphate (carrier-free, in HCl-free aqueous solution, New England Nuclear) *in vivo* and analyzed as described by Lonsdale (11).

Fig. 1 C



In order to identify and characterize the different isolates of orthopoxvirus species including clinical isolates of eight elephants (EP), one rhinoceros (RP), and two okapis (OP) ^{32}P labeled DNAs of individual isolates were cleaved with restriction endonucleases and the resulting DNA fragments were separated electrophoretically on agarose slab gels. The DNA profiles of these isolates were compared with the DNA cleavage patterns of other orthopoxviruses including vaccinia (Elstree), cowpox, buffalo pox, camel pox, rabbit pox, cat pox, and ectromelia, which were analyzed under the

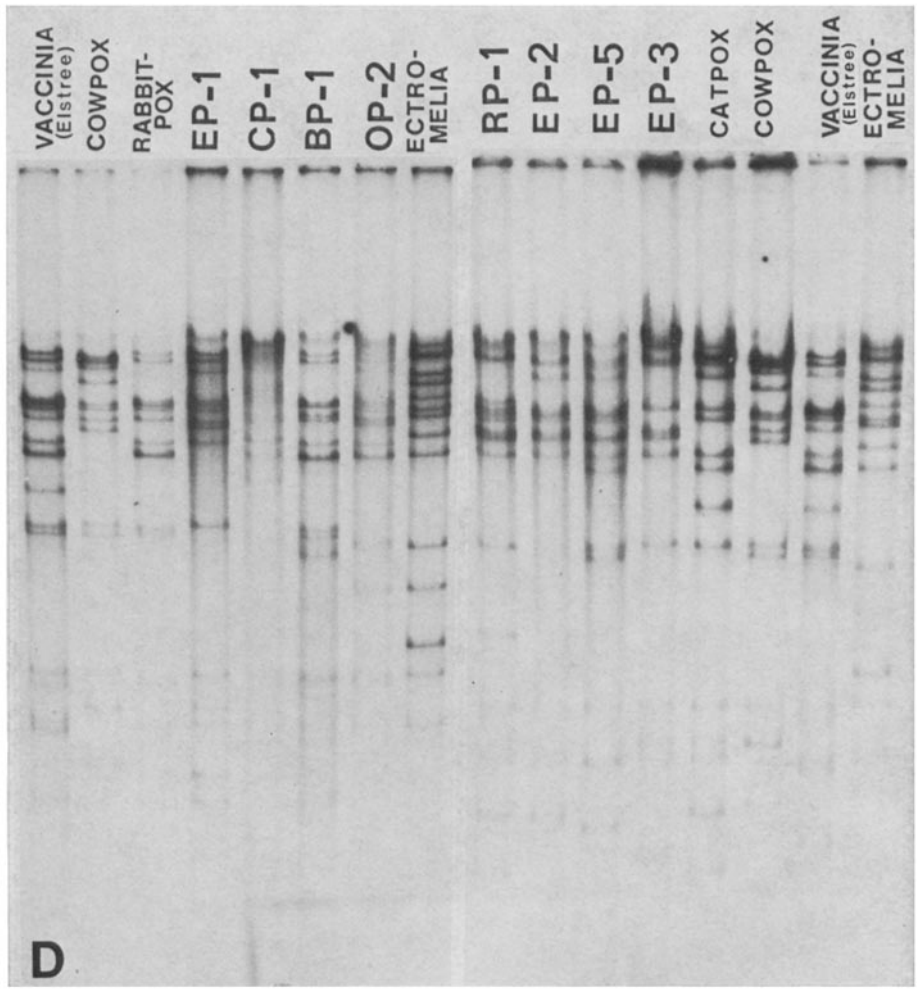


Fig. 1 D

Fig. 1. Autoradiograms of ^{32}P labeled DNAs of poxviruses isolated from elephants (EP-1, -2, -3, and -5), rhinoceros (RP-1), okapi (OP-2), camel (CP-1) and buffalo (BP-1) in comparison to the DNAs of vaccinia, cowpox, rabbitpox, catpox, and ectromelia. The DNAs were cleaved with restriction endonucleases BamHI *(A)*, MluI *(B)*, NcoI *(C)*, and SalI *(D)*. The enzymes were purchased from Biolabs (Beverly, Mass.), BRL (Neu-Isenburg, Federal Republic of Germany), or Boehringer (Mannheim, Federal Republic of Germany). Incubations were carried out according to a standard procedure for each enzyme. The resulting DNA fragments were separated on 0.8 per cent agarose slab gels (Seakem, Biomedical, Rockland, Me.). Electrophoresis was performed at 4° C in vertical gels (35 or 40 by 20 by 0.3 cm) which were subsequently dried for autoradiography. The autoradiograms were exposed to X-ray films (KODAK XAR-5). Arrows (A, B, and C) of Fig. 1A, as well as arrows and boxes with a star of Fig. 1B and 1C mark the DNA fragmentation patterns and/or the DNA fragments, which are common in the virus strains tested

same conditions. The results of this analysis are given in Fig. 1A to D. Among the restriction enzymes tested (BamHI, ClaI, EcoRI, HindIII, HpaI, BglII, MluI, NcoI, PstI, SalI, SmaI, XbaI, and XhoI) it was found that the restriction endonucleases MluI (A/CGCGT) (Fig. 1B), NcoI (C/

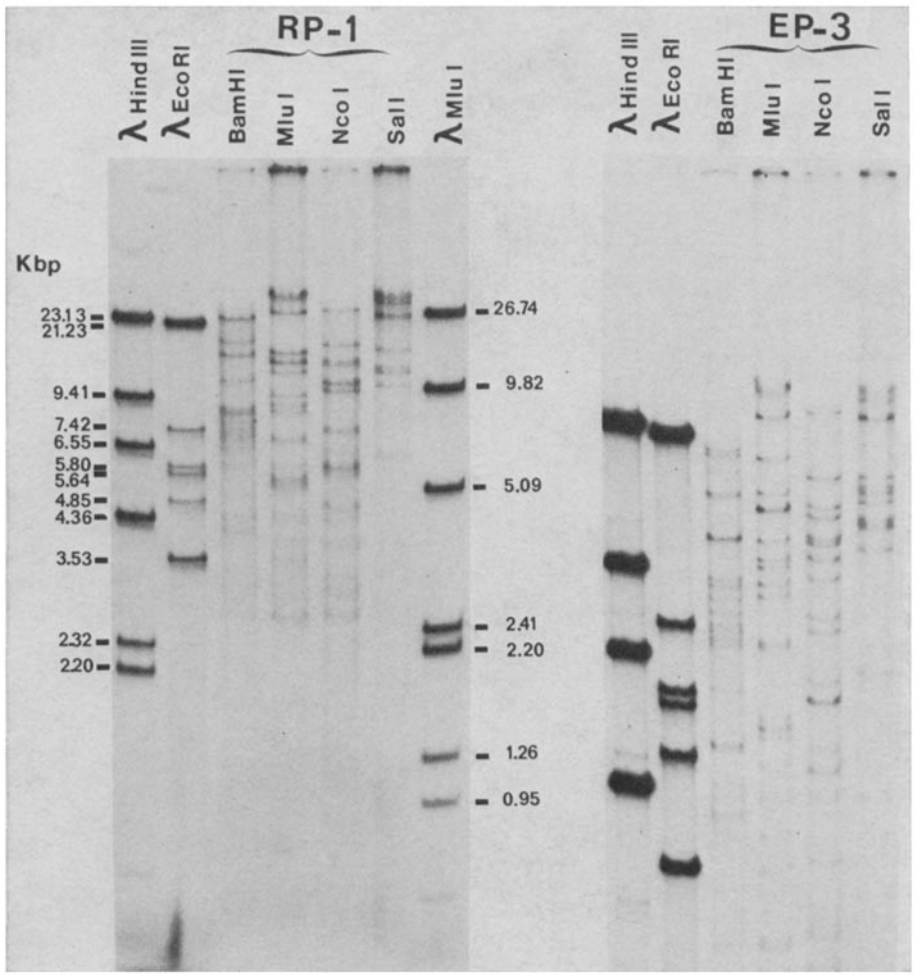


Fig. 2. Autoradiogram of ^{32}P labeled DNAs of elephant poxvirus (EP-3) and rhinoceros poxvirus (RP-1) after cleavage with restriction enzymes BamHI, MluI, NcoI, and SalI. ^{32}P labeled phage lambda DNA cleaved with restriction enzymes HindIII, EcoRI, and MluI served as molecular weight marker

CATGG) (Fig. 1C), and SalI (G/TCGAC) (Fig. 1D) are the enzymes of choice for differentiating these isolates from each other. In contrast, the restriction endonuclease BamHI (G/CATCC) (Fig. 1A) gave characteristic fragmentation patterns (arrows A, B, and C, Fig. 1A) which confirm the evolutionary relationship and conserved region known for orthopoxviruses

(7). Comparative analysis of DNA cleavage patterns of individual isolates obtained by restriction endonucleases MluI (Fig. 1B) and NcoI (Fig. 1C) revealed that some of the DNA fragmentation patterns (marked with arrows or boxes) are similar between the different virus strains tested. It is of interest that the three DNA fragments of high molecular weight (11 to 27 kbp, Fig. 2), marked with small arrows in Fig. 1C are present in the NcoI DNA cleavage patterns of all isolates.

For demonstrating the size of individual DNA fragments obtained after cleavage of the viral genome with these enzymes gel electrophoresis was performed in which the phage lambda DNA cleaved with restriction endonucleases HindIII, EcoRI, and MluI served as molecular weight marker. Fig. 2 shows examples of this study for rhinoceros poxvirus (RP-1) and elephant virus (EP-3).

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