The capripoxvirus genome

D.N. BLACK*

Summary: The classification of the capripoxviruses according to the disease they cause in either sheep, goats or cattle can no longer be considered adequate. Work by Capstick and, more recently, Kitching and Taylor, has shown that strains of capripoxvirus are not host specific, although many of them do show a clear host preference. It has not been possible to distinguish between strains of capripoxvirus derived from sheep, goats or cattle using the serum neutralisation or agar gel immunodiffusion tests. However, restriction endonucleases have proved very useful in identifying differences between the genome DNAs of closely related orthopoxviruses and parapoxviruses and this paper reports on the use of these enzymes to demonstrate the relatedness of the genomes of strains of capripoxviruses.

KEYWORDS: Antigen structure - Capripoxvirus - Cattle diseases - Diagnostic procedures - Enzymes - Genes - Goat diseases - Nucleotides - Orthopoxvirus - Parapoxvirus - Sheep diseases - Viral diseases.

The DNA of 12 isolates of capripoxvirus were digested with the restriction endonuclease Hind III and the DNA fragments so generated were analysed by agarose gel electrophoresis. The restriction patterns and the fragment sizes are shown in Figs. 1 and 2. Fragments are lettered in order of decreasing size. It can be seen that there is a close degree of similarity between the restriction patterns. Twelve of the fragments were present in all the capripoxvirus DNAs studied. A more detailed analysis of the Hind III restriction endonuclease pattern is given in Black et al. (1).

In individual restriction digests, co-migrating fragments of the same molecular weight were identified by the enhanced intensity appearing on the exposed X-ray film. In some examples, the terminal fragments were detectable by their reduced intensity (e.g. fragment F, Nigeria sheep pox).

COMPARISON OF RESTRICTION PATTERNS BETWEEN CAPRIPOXVIRUSES

The maximum number (31 out of 33) of fragments common to digests of two different viruses was observed between lumpy skin disease (LSD) virus and the Kenya sheep and goat pox isolate. Similarly, the minimum number (16 out of 33) was observed between the DNAs of India goat pox and Iraq sheep pox viruses. Within this overall relatedness, several distinct characteristics could be observed. The restriction patterns of the sheep pox virus DNAs contained 23 fragments with the same mobilities. Those fragments which have the same mobilities as fragments A, B, O, V and W of Nigeria sheep pox DNA were readily identifiable as being characteristic of sheep pox virus DNAs. An apparent exception was the pattern obtained from the DNA of Oman sheep pox virus.

* Department of Biochemistry, Animal Virus Research Institute, Pirbright, Surrey GU24 0NF, United Kingdom.
FIG. 1

Hind III restriction fragments of DNA from capripoxvirus isolates separated by agarose gel electrophoresis

Nigeria sheep pox (NS), India goat pox (InG), India sheep pox (InS), Oman sheep pox (OS), Iraq goat pox (IrG), Iraq sheep pox (IrS), lumpy skin disease (LSD), Kenya sheep and goat pox (K).

(Reproduced from Black et al., 1986, Virus Research, 5).
FIG. 2

Hind III restriction fragments of DNA from vaccinia virus WR strain (V) and capripoxvirus isolates separated by agarose gel electrophoresis

Kenya sheep and goat pox (K), Oman sheep pox (OS), Nigeria sheep pox (NS), Stavropol sheep pox (RS), "Lyovariovis" (FS), Saudi sheep pox vaccine (SS), India goat pox (InG), Iraq goat pox (IrG), Oman goat pox (OG).

(Reproduced from Black et al., 1986, Virus Research, 5.)
The restriction endonuclease patterns of all the goat pox virus DNAs have 21 fragments in common. The fragments with the same mobilities as fragments A, B, C, M and T of India goat pox could be identified as characteristic of goat pox virus DNAs.

The molecular weight of the DNA of sheep pox and goat pox isolates was estimated by summation of the molecular weight of individual restriction digest fragments. The estimated molecular weights ranged from 73 to 91 megadaltons (1). The use of end-labelled fragments allowed easy visualisation of very small fragments and the detection, by their double intensity, of co-migrating fragments. The values obtained are all considerably lower than those reported for orthopox virus DNA (10), but similar to the reported values for parapox virus DNA (11).

**COMPARISON OF CAPRIPOXVIRUSES WITH OTHER POXVIRUSES**

The DNA from ortho- and parapox viruses has been shown to contain covalent terminal cross-links (4, 7, 9). Following digestion of the virus DNA with a restriction endonuclease, it is possible to identify the terminal fragments by their ability to re-anneal rapidly following denaturation. A Hind III digest of Nigeria sheep pox DNA was denatured by boiling and then rapidly cooled to 0°C in ice-water. Agarose gel electrophoresis showed the presence of only two fragments (co-migrating with Nigeria fragments E and F). These fragments were thus identified as the terminal fragments. Using a Hind III digest of Yemen goat pox DNA, the terminal fragments were identified as F and G.

Comparisons between the Hind III restriction digest patterns of capripoxvirus DNAs and of vaccinia and orf virus DNA (representative ortho- and parapox viruses, respectively) showed no similarities between the three genera (1). Attempts to hybridise the Hind III P fragment of Nigeria sheep pox virus to any of the orf or vaccinia Hind III digest fragments were unsuccessful, even under low stringency conditions (1).

**GENOME HOMOLOGY BETWEEN CAPRIPOXVIRUSES**

Previous investigations (2, 3, 5) have shown that strains of capripoxvirus cannot be distinguished except by their host preference. However, strains such as Kenya sheep and goat pox, Oman sheep pox and Yemen goat pox cannot be readily identified even by this criterion. Schumperli et al. (12) have calculated that, with restriction endonuclease digests using a six nucleotide cutter (such as Hind III), a genomic homology of greater than 80% can be assumed between two DNAs if their restriction patterns contain co-migrating fragments. Figs. 1 and 2 show that selected pairs of capripoxvirus DNA digests contained between 61% and 93% fragments with the same electrophoretic mobility. In addition, 12 restriction fragments (i.e. 36% and 43%, dependent on the virus isolate considered) were present in the Hind III digest patterns of all the capripoxvirus DNAs used in the present study. On the basis of the calculations of Schumperli et al. (12), the genome homology of the capripoxvirus must be more than 80% and, thus, the capripox viruses used in this study can be considered as very closely related, regardless of the animal of origin. This conclusion was strongly supported by Southern Cross blotting data, which showed a high degree of genome homology between three capripox isolates (1). While suggesting that the different viruses are separate isolates of a single virus, the results show that isolates from different animal hosts have specific differences in their restriction pattern.

Oman sheep pox does not readily fit into either group. Its DNA contains Hind III A and B fragments, which appear to be smaller than the typical sheep pox virus.
DNA Hind III A and B fragments, and a fragment C with the same mobility as the C fragment characteristic of a typical goat pox virus DNA Hind III digest. In addition, the Oman sheep pox DNA Hind III digest contained the doublet fragments T and U characteristic of goat pox virus DNAs and a fragment (P) which appeared to be similar to the Hind III O fragment of Nigeria sheep pox virus DNA (characteristic of Hind III digests of DNA from sheep isolates of capripoxviruses).

These results indicate either that the virus had undergone modification during its passage through the specific animal hosts or, more interestingly, that genomic recombination had occurred between sheep and goat viruses within a single host. More detailed work on specific fragment hybridisation is necessary to elucidate this relationship.

The Hind III restriction patterns of the capripoxvirus DNAs were widely different from those obtained with both the ortho- and parapox virus DNAs. Thus, it would be relatively easy to identify a capripoxvirus on the basis of its Hind III DNA restriction pattern. Despite the smaller DNA of the capripoxviruses, their Hind III digest patterns contained many more fragments than either vaccinia or orf virus DNA Hind III digests. This suggested that the genome sequences of the capripoxviruses are highly divergent from those of the ortho- and parapox viruses. On the other hand, the presence of restriction fragments which rapidly re-annealed following denaturation showed that, as with all other poxvirus DNAs so far studied, the DNA of capripoxvirus contained terminal covalent cross-links (1).

**CONCLUSION**

The results reported here show that sheep pox, goat pox, sheep and goat pox and LSD are caused by very closely related isolates of capripoxvirus. This not only supports the proposal that the malignant pox diseases of sheep and goats should be referred to by the single term “capripox” (5) but also indicates that LSD should be included. It is therefore proposed that all the malignant pox diseases of ruminants be referred to as “capripox”.

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**LE GÉNOME DES CAPRIPOXVIRUS. — D.N. Black.**

*Résumé :* La classification des capripoxvirus selon la maladie qu'ils déterminent chez les ovins, les caprins ou les bovins ne peut plus être considérée comme adéquate. Les recherches de Capstick et, plus récemment, de Kitching...
et Taylor, ont montré que les souches de capripoxvirus ne sont pas spécifiques d'un hôte, bien que beaucoup d'entre elles montrent une nette affinité pour un hôte. L'emploi des épreuves de séroneutralisation ou d'immunodiffusion en gélose n'a pas permis de différencier les souches de capripoxvirus d'origine ovine, caprine ou bovine. En revanche, les endonucléases de restriction se sont révélées très utiles dans l'identification des différences entre ADN de génomes appartenant à des orthopoxvirus et des parapoxvirus étroitement apparentés entre eux. Cet article présente les résultats de l'utilisation de ces enzymes pour mettre en évidence la parenté des génomes dans des souches de capripoxvirus.


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EL GENOMA DE LOS CAPRIPOXVIRUS. — D.N. Black.

Resumen : Ya no se puede considerar adecuada la clasificación de los capripoxvirus según la enfermedad que determinan en los ovinos, caprinos o bovinos. Las investigaciones de Capstick y, más recientemente, de Kitching y Taylor, han demostrado que las cepas de capripoxvirus no son específicas de un huésped, aunque muchas de las mismas manifiestan una afinidad neta por un huésped. El uso de las pruebas de seroneutralización o de inmunodifusión en agar no permitió diferenciar las cepas de los capripoxvirus de origen ovino, caprino o bovino. En cambio, resultaron de suma utilidad las endonucleasas de restricción en la identificación de las diferencias entre ADN de genomas que pertenecen a orthopoxvirus y parapoxvirus estrechamente emparentados entre sí. Se presentan en el artículo los resultados del uso de estas enzimas para evidenciar el parentesco de los genomas en cepas de capripoxvirus.


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REFERENCES


