

Figure 1. Uterine cervical tissue showing presence of cytoskeleton at 60 kV ($\times 9700$).

clumped together without any identity. The addition of lysine to triton extracted cytoskeleton of the cells which has been shown to be useful in demonstrating the nature of the cellular actin filaments. It has been shown by Swift *et al.*⁴, that addition of lysine to the fixative

allows the lipoprotein particles to be better visualized.

It is suggested that glutaraldehyde reacts with the lysine, forming complex pyridines and pyridiniums. These products are generally weak bases, capable of interacting with osmium tetroxide. During aldehyde fixation and staining weak bases might react with the osmium tetroxide. In aldehyde fixation amines of the tissue are affected. The effect of added amines may act to prevent some of the changes developed as a result of blockade of free amino groups of the tissue. The addition of lysine in glutaraldehyde fixation and its role in preserving internal cell structures were also reported by Boyles⁵, Courtoy and Boyles⁶.

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Received 18 January 1993; accepted 8 February 1993.

Restriction enzyme digestion patterns of DNA from an Indian isolate of equine herpes virus-1

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The DNA fingerprints of EHV-1 isolate 'UPPAL', encountered in a respiratory disease outbreak in horses at Turf Club in India in 1989, were compared with two known abortigenic and one respiratory EHV-1 isolates using *Bam*HI and *Pst*I restriction endonucleases. All the four viruses comprising two from respiratory tract ('UPPAL' and 'Sheva') and two from abortion cases (4056A and 14208A) had the same pattern as abortigenic strains. The two isolates, 14208A and Sheva were comparable whereas, 'UPPAL' and 4056A were close but not identical. However, minor differences observed in the *Bam*HI as well as *Pst*I fingerprint patterns can be expected as these viruses were of different epizootiological origin.

FOUR distinct herpes viruses, viz. equine herpes virus-1, 2, 3 and 4 (EHV-1, EHV-2, EHV-3, and EHV-4) have been isolated from horses^{1,2}. EHV-1 is responsible for causing abortions in mares, still birth, neonatal foal mortality, paralysis and respiratory infections^{3,4}. Two

antigenic subtypes of EHV-1, viz. subtype-1 and 2, which were previously recognized on the basis of serological tests, major differences in structural proteins and restriction endonuclease cleavage patterns are now designated as EHV-1 and EHV-4 respectively^{2,5,6}. EHV-1, though encountered both from respiratory as well as abortion and paralytic (myeloencephalitic) syndromes, is mostly associated with abortions. On the other hand, EHV-4 mainly causes respiratory disease but at times has been found to cause abortions as well⁷.

Since both EHV-1 and EHV-4 can be isolated from the respiratory or abortion syndromes, laboratory tests are necessary to identify the type involved in a particular syndrome. Restriction endonuclease fingerprints of viral DNA not only provide a powerful tool to differentiate between these two EHV types but also an important basis for the study of molecular epizootiology of the disease as the two types have been shown to possess distinct fingerprints⁵. The present study was undertaken to compare the DNA fingerprints of a recent EHV-1 isolate, viz. 'UPPAL', encountered in a respiratory disease outbreak in race horses⁸ during 1989 with two known abortigenic EHV-1 strains and one EHV-1 strain isolated from respiratory tract of a horse.

The isolates of EHV-1 analysed are listed in Table 1. These were grown in CCL-64 cell monolayers at a low multiplicity. The cells were maintained in RPMI 1640

Table 1. Summary of EHV-1 isolates studied for restriction endonuclease cleavage patterns

Isolate	Source	No. of cleavage sites	
		<i>Bam</i> H1	<i>Pst</i> I
UPPAL	Respiratory	13	16
14208A	Abortion	13	15
Sheva	Respiratory	13	15
4056A	Abortion	15	16

medium (Flow) containing 1.2% glucose, 1% each of glutamine and sodium pyruvate, 100 IU/ml penicillin, 50 µg/ml streptomycin and 5% inactivated foetal calf serum. After 48 h post infection when about 80 per cent cells showed cytopathic effect (CPE), these were processed for virus concentration, purification and digestion of DNA as below:

The cells were scraped off and separated from the supernatant by low speed centrifugation (5000 rpm for 20 min). Pooled supernatants and infected cell pellets were both used for DNA extraction. The cell-free supernatant was centrifuged at 10,000 rpm for 20 min.

The supernatant was pelleted at 30,000 *g* for 3 h. The cell pellet was suspended in 300 µl of 10 mM Tris HCl, 1 mM EDTA, pH 7.4. This was sonicated. The supernatant obtained after centrifugation at 5000 rpm for 20 min was processed to obtain a virus pellet as described above for the cell-free supernatant from infected cultures. The virus pellets suspended in TNE buffer were loaded on to a sucrose cushion (35% w/v) and centrifuged at 40,000 rpm overnight in a Beckman centrifuge. The light blue band was harvested with a syringe. The virus pellets were resuspended in 250 µl of TNE buffer to which 200 µl of 10 per cent SDS and 50 µg/ml proteinase K were added. After incubation at 37° C, DNA was extracted with phenol, chloroform and isoamyl alcohol and precipitated with ethanol (-20° C). The DNA was redissolved 0.1 X SSC (0.15 M NaCl and 0.015 M Na citrate) and stored at 4° C.

One µg viral DNA was digested with 25 units of *Bam*H1 or *Pst*I as recommended by the supplier. The resulting cleavage fragments along with molecular weight markers were separated by electrophoresis on 0.6% agarose gels containing ethidium bromide (0.5

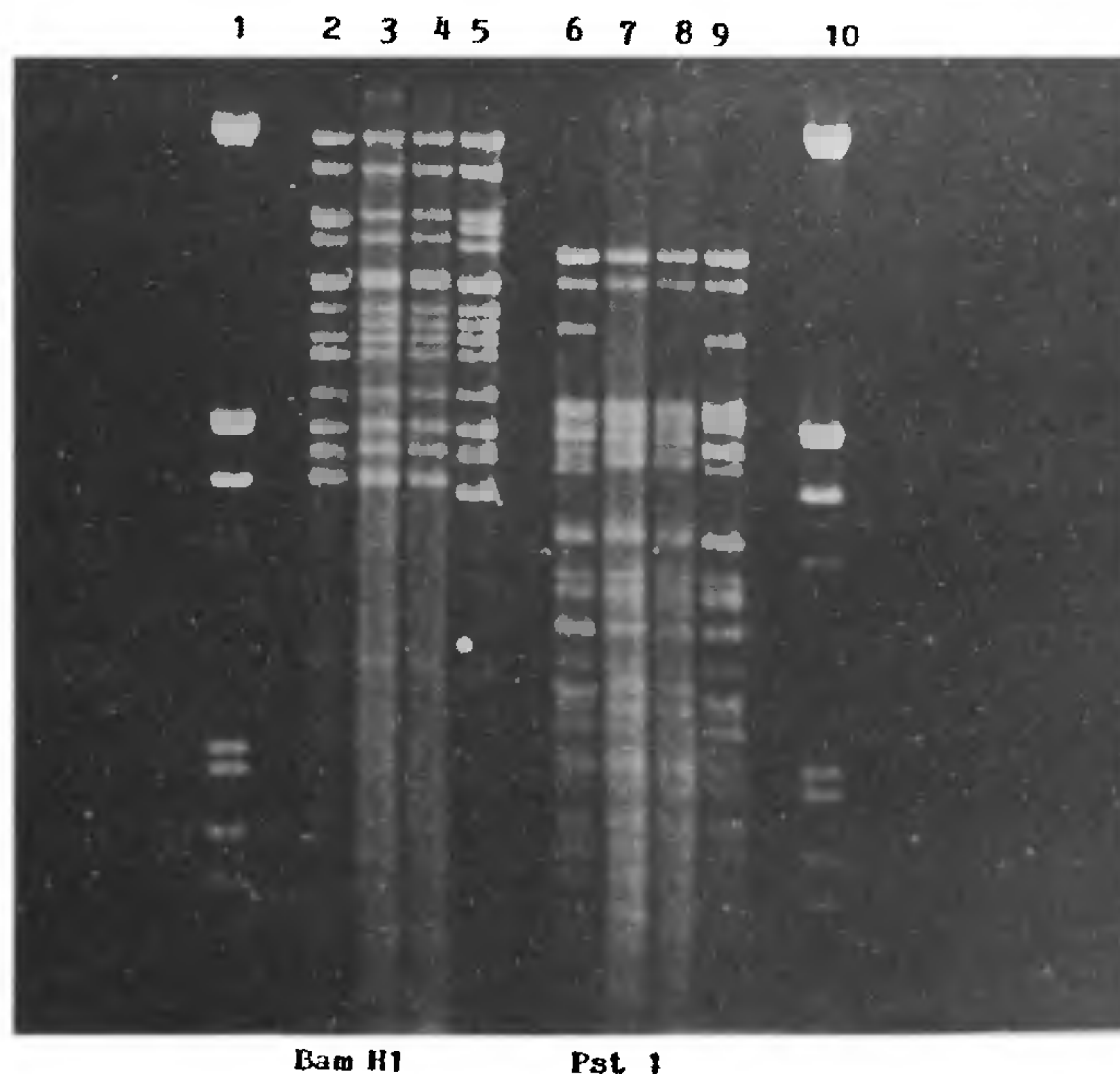


Figure 1. DNA fingerprints of four EHV-1 isolates of different epizootiological origin. Lane 1 and 10 represent marker DNA while lanes 2, 3, 4 and 5 show *Bam*HI patterns of isolates 'UPPAL', Sheva, 14208A and 4056A respectively. Similarly, the *Pst*I patterns of these isolates are shown in lanes 6, 7, 8 and 9 respectively.

$\mu\text{g/ml}$). The gels were run at 20 V for 18 h in TLE. The DNA fragment bands were photographed under a UV transillumination lamp (302 nm) through a yellow filter using polaroid film.

The *Bam*H1 and *Pst*I fingerprints of the four EHV-1 isolates are shown in Figure 1. It can be seen from the restriction cleavage patterns that the 'UPPAL', 14208A, Sheva and 4056A isolates exhibited 13, 13, 13 and 15 cleavage sites, respectively with *Bam*H1. However, differences were noticed at three cleavage sites. While all cleavage sites were possessed by 4056A, the isolates 14208A and Sheva lacked bands 3 bis and 12 bis. The isolate 'UPPAL' lacked band 7 and 12 bis but possessed band 3 bis.

With *Pst*I, the only difference observed among the four viruses was for band 3 which is present in 'UPPAL' and 4056A and not in 14208A and Sheva.

The genome of isolate 'UPPAL' possesses about 121306 base pairs in the 14 different fragments of various sizes obtained with *Bam*H1. Amongst the fragments the largest and smallest have about 20893 and 2570 base pairs respectively.

A comparison of *Bam*H1 and *Pst*I restriction endonuclease DNA fingerprints of the 'UPPAL' isolate of EHV-1 with two known abortigenic strains, i.e. 4056A and 14208A indicated that it has the same pattern as abortigenic strains. Among the four viruses studied, the two isolates 14208A and Sheva are comparable whereas 'UPPAL' and 4056A were close but not identical. It is interesting to note that the 'UPPAL' virus was isolated from respiratory tract of horses suffering with an acute respiratory disease at a race tract in concurrence with equine infectious anaemia (EIA). Since there were no breeding mares, behaviour of the virus in pregnant mares could not be known. The DNA fingerprints, however, establishes the virus to be abortigenic (EHV-1). EHV-1, though mainly responsible for abortion, has also been occasionally found to be involved in respiratory illness^{1,2,7}. The *Bam*H1 fingerprints obtained in the present study for 'UPPAL' and other abortigenic viruses are in close agreement with those reported for foetal strains⁵. Minor differences observed in the *Bam*H1 and *Pst*I fingerprints of the four viruses studied can be expected being of different epizootiological origin⁹. The Sheva and 4056A isolates appear to closely resemble with the IP prototype⁷ of EHV-1 subtype-1 (now designated as EHV-1).

The EHV-1 viruses so far reported from India have been from abortion, still births, neonatal deaths and paralytic syndromes. Some of these have been identified serologically as subtype-1. The viruses isolate 'UPPAL' recovered from respiratory disease also fell in this category. The severe respiratory manifestation observed in the outbreak could be due to the concurrent EIA outbreak in these horses. Although in recent years some

evidence has been gathered in serological studies for the possible presence of EHV-1 subtype-2 (EHV-4) in Indian horses (authors' unpublished data), this virus has not been isolated so far.

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Received 22 August 1992; revised accepted 6 January 1993

On the possibility of allochthonous peat on the inner shelf off Karwar

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Peat deposits occupying an area of more than 139 km², intercalated in sediments from the inner shelf of Karwar-Kumta, have been identified. Such organic-rich nearshore facies are rare and hence their presence is intriguing. Considering the fact that peat directly reflects massive plant production, and since it is well known that mangroves are prolific producers of peat, it would imply that vegetation once flourished as far as 11 to 18 km from the present shoreline. Since mangrove populations are capable of advancing seaward or retreating landward, they play a dual role in changing the tropical coastlines. But considering the geomorphic features of the coastal and offshore zones of Karwar-Kumta (open ocean, wave-dominated high energy environment, lack of favourable substrates for mangrove colonization), it is obvious that mangroves could not have prograded 18 km seaward as they essentially require a low energy and sheltered habitat. We therefore propose that these peat deposits were not laid down *in situ*, but judging from their physical, mineralogical and chemical characteristics, these intercalated peat layers on the inner shelf appear to have undergone transport and hence are of allochthonous origin.

THE accumulation and preservation of recent coastal deposits are governed by the rate of sea level rise, sediment influx, incident wave energy and antecedent topography¹. Coastal Holocene sequences, from tropical regions in particular, are marked by the presence of