Vol. 34 (4), June 1987, 241–316 ISSN 0931–1793 A 20821 E

Journal of Veterinary Medicine Series B
Zentralblatt für Veterinärmedizin Reihe B

Infectious Diseases, Immunology, Food Hygiene, Veterinary Public Health

Contents

Original Papers

Short Communications

PAUL PAREY Scientific Publishers Berlin and Hamburg

J. Vet. Med. B 34, 310—313 (1987)

ISSN 0931—1793

Short Communications

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Changes in Restriction Enzyme Pattern of the Equine Herpes Virus Type 1 (EHV-1) Strain Rac H DNA During Attenuation

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With one figure

(Received for publication February 2, 1987)

Summary

Equine herpesvirus type 1 (EHV-1) strain Rac H is used after attenuation (256 passages in embryonic porcine kidney cells) as a live vaccine against rhinopneumonitis and abortion. The genome of this strain shows a different DNA pattern after digestion with the restriction endonuclease Bam HI from that of the abortigenic virus isolates. A virus subpopulation revealing this pattern existed already at passage 182. This pattern is likely to serve as an *in vitro* marker for differentiation of field virus from the vaccine strain.

Key words: EHV-1, restriction enzyme pattern, attenuation, herpesvirus

Introduction

Infection of horses with Equine herpesvirus type 1 (EHV-1) can cause respiratory distress, neurological disorders and, if pregnant mares become infected, abortion, which is the main economic impact of the infection. For the prevention of these diseases the high-passage strain Rac H is widely used as an attenuated live vaccine. An important advantage of this vaccine is that while virulence is reduced, the immunogenicity remains stable. *In vitro* markers for the differentiation of the vaccine strain from field strains were established specially for the licensing of this vaccine in Sweden. Borgen (3) described the specific ability of the vaccine strain Rac H to produce plaques in a clone of mouse c-fibroblasts (L-marker). Klingenborn and Dinter (6) tested the relative sensitivity of various EHV-1 strains to dithiothreitol treatment (DTT marker), and found the vaccine strain Rac H most resistant to inactivation.

Recent characterization of EHV-1 strains by restriction endonuclease analysis of their DNA revealed some differences among EHV-1 isolates (1, 2, 10). In the present study we investigated changes in the DNA-fingerprinting-pattern of the strain Rac H during its attenuation to the vaccine strain.

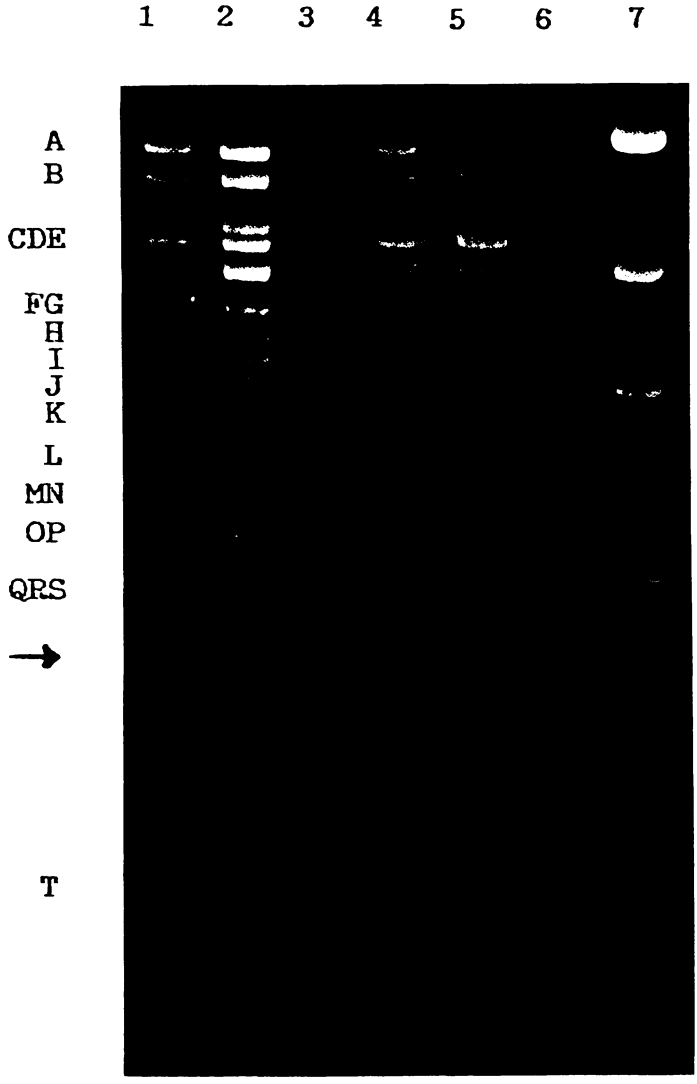

Fig. 1. Bam HI restriction profiles of the DNAs of EHV-1 isolates. The labelling of the fragments A—T is true for lane 1, which is similar to an isolate described by WHALLEY et al. (12). 1. Kent.-D strain; 2. Rac H at passage 12; 3. Rac H at passage 182; 4. a subpopulation of Rac H (182. P) after purification by limiting dilution; 5. Rac H at passage 256; 6. Field isolate PV 52; 7. MW-standard lambda Hind III. Arrow indicates new fragment of Rac H at passage 182 (MG approx. 2.5 Megadalton)

Material and Methods

Viruses

EHV-1 strain Kentucky D was obtained from ATCC, strain PV 52 was isolated from an aborted foal (by courtesy of Prof. K. PETZOLDT, Hannover), and vaccine strain Rac H was obtained at the 12th, 182nd, 256th, and 421th passage in primary porcine kidney cells (9). All viruses were propagated in embryonic equine lung cells.

DNA purification

The virus was harvested when cells showed 80—90 % cytopathic effect by freeze-thawing twice; cell debris was removed by low-speed centrifugation. Virus particles were purified by centrifugation at 65,000 g for 90 min and the pellet was then layered onto a preformed 20—60 % (w/w) sucrose gradient. The virus band was harvested and DNA was extracted by lysis of virions with SDS-sarcosyl and proteinase K, followed by extraction with phenol and chloroform (8). DNA was precipitated with ethanol and resuspended in 10 mM Tris-HCl and 1 mM EDTA, pH 7.4. Digestion with restriction endonuclease Bam HI was performed according to the manufacturer's instructions (Boehringer, Mannheim; BRL, Heidelberg). Cleavage fragments and a DNA standard marker were separated on a 0.6 % agarose gel at 2 V/cm for 18 hours. DNA fragments were photographed under UV-light (302 nm).

Results

Comparing the restriction profiles of a European field isolate (Fig. 1, lane 6) and the reference strain Kentucky D (lane 1) some minor variations in the mobility of fragments E, M/N, O/P, Q/R/S are obvious. These fragments are located within the inverted repeat region of the genome and their highly varied mobility has been reported in isolates from epidemiologically unrelated abortions (2).

The low passaged (12) Rac H strain (lane 2) shows a similar variation of these fragments and additionally the loss of BamH1 fragment T.

At passage 182 (lane 3) the loss of BamH1 fragment L and a shortened fragment with about 2.5 Megadaltons was observed. A single passage of this virus in a rabbit kidney cell line (RK_{13}) led to a variation in the restriction profile (data not shown) indicating a heterogeneity of the viral stock at passage 182. After cloning the original passage 182 and passaging the virus once in RK_{13} by limiting dilution, we obtained two different subpopulations. One restriction profile is shown in lane 4; the other turned out to be identical to passage 256, which is actually used in vaccine production (lane 5). This pattern, missing fragment M/N, proved to be stable at least up to passage 421 (data not shown).

Discussion

Analysis of viral DNA with restriction endonucleases provides a sensitive method for measuring the heterogeneity of the EHV-1-strains. Variations in restriction sites can be grouped into two types. The first group involves loss or gain of cleavage sites due to mutation or deletion resulting in the generation of new fragments; the second type leads to different mobilities of fragments (E, M/N, O/P, Q/R/S), located in the inverted repeat regions (11). ALLEN et al. (1) suggest that different molecular sizes depend on a variation in the number of copies of repetitive sequences contained in those fragments. Addition or deletion can occur during the replication of the genome.

Both types of variation are probably selected during laboratory manipulations, such as plaque purification or adaptation by serial passage to semipermissive, non-equine cell lines or animal hosts. The unique DNA restriction patterns of various attenuated, live vaccine strains of EHV-1 are undoubtedly the result of a large number of laboratory passages of these strains in non-equine cell lines (1). As the fetal isolate Rac H was propagated in Syrian hamsters and porcine cells, we do not know the DNA cleavage pattern of the original isolate, but during attenuation loss and gain as well as different mobilities of fragments occurred. Loss of Bam HI fragment L and M coincides with the appearance of a new fragment, which is probably co-migrating with D and has the size of L plus M (7.0 Md). The variation in length of the inverted repeats shows a characteristic shortening for Rac H (HÜBERT, in prep.). This cleavage pattern could be detected by purification of the viral stock at passage level 182. It remained stable at least up to passage 421. We consider this pattern to be an *in vitro* marker for differentiation of field virus and vaccine.

The fact that experimental infections of horses and hamsters with the vaccine strain did not cause disease may be attributed to reduced virulence. This might also be true for

two isolates with a DNA-cleavage pattern very similar to Rac H (4), causing only mild respiratory disease. We assume that reduced virulence is due to deletions located within the inverted repeat region which is significantly shorter in Rac H than in abortigenic isolates. Deletions in the inverted repeat regions of non-virulent strains have been reported for other herpesviruses e. g. pseudorabies virus (PRV) and Herpes simplex virus (HSV) (7, 5), Further investigations require exact physical mapping of Rac H virus DNA and location of genes on the repetitive sequences.

Acknowledgements

We gratefully acknowledge the technical assistance of S. SCHLABITZ and D. KRONTHALER, and the secretarial assistance of S. SCOTT.

Zusammenfassung

Änderungen im Restriktionsenzymschnittmuster der DNS des equinen Herpesvirus Typ 1 (EHV-1) Stammes Rac H während der Attenuierung

Der durch 256 Passagen auf embryonalen Schweinenierenzellen attenuierte Stamm Rac H wird als Lebendvakzine gegen Rhinopneumonitis/Stutenabort (EHV-1) eingesetzt. Das Genom dieses Stammes wies nach Verdau mit der Restriktions-Endonuclease Bam HI ein von Abortisolaten deutlich unterscheidbares DNS-Schnittmuster auf. Es wird dargestellt, daß bereits zum Zeitpunkt der 182. Passage eine Subpopulation mit diesem Schnittmuster existierte.

Dieses Muster ist im Sinne eines „*in vitro* markers" zur Unterscheidung von Impfstamm und Feldvirusisolaten geeignet.

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