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RESTRICTION ENDONUCLEASE PATTERNS OF THE GENOME OF PSEUDORABIES VIRUSES ISOLATED FROM PIGS

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Several field isolates of the pseudorabies vins (PrV) obtained from outbreaks on pig farms were examined by restriction fragment pattern (RFP) analysis. DNA was digested using Bam HI and Kpn I enzymes. All isolates provided similar Bam HI and Kpn I restriction patterns, with a slight heterogeneity between them. These differences are a useful tool in epizootological studies.

Key words: Herpes virus, Pseudorabies virus, Restriction endonucleases, Epizootological studes.

INTRODUCTION

According to their clinical specificity and genome structure, viruses of the family Herpesviridae have been divided into three subfamilies: Alphaherpesvirinae, Betaherpes virinal and Gamaherpesvirinae. Two viruses of the family Herpesviridae have been isolated from swine: pseudorabies virus (PrV) suid herpesvirus 1, classified as Alphaherpesvirinae and a porcine cytomegalovirus suid herpesvirus 2, belonging to the subfamily of Betaherpesvirinae (Fenner, 1987).

The PrV genome consists of linear double DNA about 150 kpb in size, containing approximately 100 genes. Such a large genome is the result of the complex life cycle of herpesviruses, including infection, multiplication in host cells, as well as the establishment of an inapparent latent infective state.

Restriction fragment pattern (RFP) analysis of the PrV genome by restriction endonucleases can detect differences in genome patterns between the isolates, i. e. various viral strains. The number and size of fragments obtained using different restriction enzymes provide information abount the genetic patterns of virus populations. Some differences in the number and size of restriction fragments may be conditioned by events. Thus the loss of a cleavage site between fragments appears as fusion (Ben-Porat et al., 1984). The addition or loss of a sequence of nucleotides changes the molecular weight, i. e. fragment size, and their migration characteristics. These changes may also be the result of translocation of sequences of nucleotides from one into another region. These dif-

ferences are very useful in epizootological studies (Ben-Porat et al., 1984; Gielkens A. L. J. 1984; Harrmann S. et al. 1984).

The aim of this investigation was to analyse several field isolates obtained from outbreaks on pig farms in Serbia over a period of three years. The results of serological studies (Pančić et al.,1987) reveal a high percentage of infected herds in Serbia. This survey indicated a seroprevalence of 13,9% - 26,7%, when clinical signs of PrV infection are not present. The high percentage of reactors shows that this infection is constantly present on farms and that it may have a tendency to spread. Up to now, no special attention has been paid to this problem, although it has been obligatory to report it to the authorities. The farmers themselves are allowed to decide about the choice of vaccination.

MATERIALS AND METHODS

Isolation and identification of viruses - Isolation of viruses from suspected materials (tonsil and medulla oblongata) of pigs was performed on a PK-15 cell line. The isolates were identified by the method of fluorescent antibodies.

Purification of viruses - Purification was carried out on a TBDA-30% sucrose cushion at 20.000 rpm for 1 hour.

DNA extraction - The purified virus was treated with 2% Na-sacrosyl at 60^0C for 15 min. and then digested by the enzyme nuclease-free-pronase, 5 mg/ml, at 37^0C for 2 hours. DNA was extracted four times using phenol-chloroform-isoamylalcohol (50:48:20 vol/vol/vol).

DNA digestion - were used. Two restriction enzymes, Bam HI and Kpn I, for DNA digestion at 37^0C during four hours.

DNA electrophoresis - Electrophoresis of restriction DNA fragments was performed in 1% agarose gel stained in ethidium bromide, and photographed under UV illumination.

RESULTS

In the present study ten field isolates obtained from outbreaks on pig farms over a period of three years were analysed by restriction enzymes. RFP analysis was used to detect differences between virus isolates. using Bam HI and Kpn I enzymes. Bam HI is the enzyme of choice if only 15 major fragments are produced. All of the strains used in this study resembled genome type I. All isolates produced similar Bam HI patterns, but minor variations in the mobility of fragments 5, 5' and 6 were evident. There were variations in the position of J, J' and K fragments if the Kpn I enzyme was used (figure 1. and 2.). Strains number 6, 8 and 10 were isolated from outbreaks on the same farm, during two years. On the basis of the mobility of Bam HI fragments 5, 5' and 6, the results show that this pattern of restriction fragments remained stable for three years.

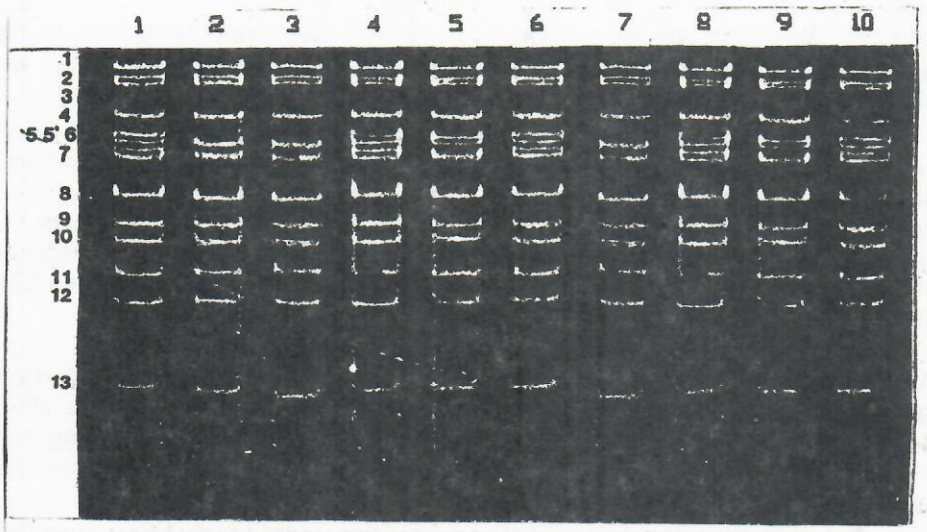

Figure 1. DNA cleavage patterns of ten PrV isolates digested with the restriction endonuclease Bam HI.

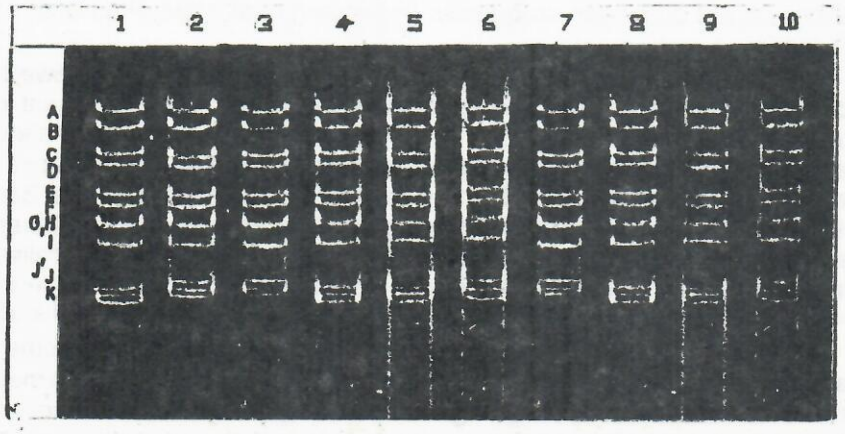

Figure 2. DNA cleavage patterns of ten PrV isolates digested with the restriction endonuclease Kpn I.

DISCUSSION

The purpose of this report was to analyse the genome of PrV isolates by restriction endonucleases. Our isolates originated from a relatively small locality of approximately 400 km in diameter, and were collected during three years from pigs having clinical features of the disease. Purified DNA were digested with restriction endonucleases Bam HI and Kpn I. This restriction pattern analysis was

used for detecting differences between virus isolates and variations in the genome. All analysed isolates provided similar Bam HI fragments which are similar to the genome type of group I (figure 1.).

In the study of Harrmann et al. (1984), a series of PrV isolates from the whole world collected over 20 years were analysed by restriction endonucleases and significant heterogeneity was found. When the Bam HI enzyme was used, considerable differences were found, and on the basis of these results the genomes of the isolates were grouped into four genome types, with corresponding subgroups. The genome of type I is similar or identical to the genome of the laboratory PrV (Ka) strain (Harrmann et al., 1984; Ben-Porat et al. 1984).

The genome of group I type mainly persists in the regions of Central Europe, America and Northern Ireland. All vaccine strains (Dassau, Bartha, Norden) generally contain genome type I, but significantly differ from it by the loss of the sequence of nucleotides of fragment 7 using Bam HI digestion and of fragment I when DNA is digested by the Kpn I enzyme (Harrmann et al. 1984); Lomniczi et al. 1984).

Belonging to a certain group of genomes has undoubtedly been correlated with the time of virus isolation as well as with the geographie site, as a result of viral evolution under different regional conditions. These genome alterations are infrequent and only a limited number of variants exist, indicating that natural mutations do not occur very frequently. (Harrmann et al. 1984; Paul et al. 1982; Oliver 1989).

The analysis of several field isolates (Ben-Porat et al., 1984), showed that genome variations are most frequently localised in the inverted repeat or in sequences localised at the left end of the genome. Our results have shown a slight heterogeneity among the examined isolates. The study demonstrated differences in the position of 5, 5' and 6 fragments under conditions of Bam HI digestion, i. e. of J. J' and K fragments using the Kpn I enzyme. These differences were evident from the position of fragments 5 and 5' in which they localised in the inverted repeat. The results for isolates number 6, 8 and 10, punified from outbreaks on the same farm during two years show that RFP remained stable.

Although all the strains showed a significant heterology of genome patterns, all of them were serologically uniform (Ben-Porat et al., 1984; Harrmann et al., 1984). Correlation between the genome structure and viral virulence could not be established either. Variations in virulence of the strain isolates were unrelated to the RFP subtypes (Jestin et al., 1990). When the genome patterns and the clinical features of the disease were considered, correlation could not be established, contrary to herpesvirus infection in horses and cattle, where subtypes producing particular clinical features of the disease can be differentiated by RFP analysis (Harrmann et al., 1984).

RFP analysis is a modern technique which can demonstrate the apizootological background. Actual differences in fragment position are relevant since they enable the follow-up of viral spread within the porcine population. These differences are useful tools for epizootological studies.

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ANALIZA GENOMA PSEUDORABIES VIRUSA TERENSKIH IZOLATA PRASADI RESTRIKCIONIM ENDONUKLEAZAMA

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SADRŽAJ

Deset terenskih izolata pseudorabies virusa (PrV), dobijenih za vreme naleta bolesti, od prasadi sa farmi svinja Srbije, u vremenskom periodu od tri godine, analizirani su restrikcionim endonukleazama. Digestija DNK je rađena sa enzimima Bam HI i Kpn I. Položaj restrikcionih fragmenata izolata bio je skoro identičan sa malim varijacijama. Te razlike su veoma korisne za praćenje širenja virusa u populaciji svinja, odnosno epizootološke situacije.