The Genotype of Aujeszky's Disease Viruses Isolated in Argentina

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ABSTRACT. Genomes of four Argentine isolates of Aujeszky's disease virus (ADV) (Rio Cuarto/79, Mercedes, Chanar Ladeado-7 and Chanar Ladeado-15) from pigs were characterized and compared with four ADV strains obtained from U.S.A. (Indiana-S), Sweden (Sweden 66), France (Alfort) and Japan (Yamagata-S81) by restriction endonuclease (RE) analysis. Although three Argentine isolates were classified into type I of *Bam*HI cleavage pattern, one isolate, Mercedes, belonged to type II, according to the classification by Herrmann *et al.* [6]. Since this type II virus was first isolated in 1981, no outbreak of ADV infection by this type has so far been reported in Argentina. This may imply that the immediate measures by total slaughter of pigs in the farm led successful eradication of the type II ADV infection in Argentina. This report is the first epidemiological study using RE analysis on ADV strains in this country.—KEY WORDS: Aujeszky's disease virus, restriction endonuclease analysis.

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Aujeszky's disease (AD) is a viral infection of swine manifested by various degrees of respiratory distress, nervous and genital disorders, and mortality, according to the age of the host and the virulence of the virus strain involved. Also, after recovery, latent infection is established. Aujeszky's disease virus (ADV) is classified as Suid Herpesvirus-1, a member of subfamily Alphaherpesvirinae, and has a linear double stranded DNA genome of approximately 90 megadaltons, composed of the long unique and the short unique sequences. The smaller is bracketed by the internal and terminal repeat sequences (IR and TR respectively) [3].

Restriction endonuclease (RE) analysis of ADV genome has been used to differentiate ADV isolates which could not been differentiated by other techniques, such as plaque morphology, heat or trypsin sensitivity and virulence for laboratory animals [6, 7]. Herrmann *et al.* [6] reported that there are four major genome types among world-wide isolates of ADV in *Bam*HI cleavage pattern, clustering in distinct geographic areas. In general, type I belongs to U.S.A. and Middle Europe (Germany and Belgium). Type II belongs to Middle Europe. Type III is limited to isolates originating from Northern Europe as Denmark and Sweden. Type IV has been isolated only in Thailand [6].

As reported by many others, this classification has much advantage to epidemiological survey of ADV infection. The purpose of the present study is to characterize the genomes of Argentine strains of ADV and compare with other country's strains using the RE analysis with *Bam*HI.

Argentine strains used are Rio Cuarto/79 (RC/79) [1], Mercedes (Mer) [9], Chanar Ladeado 7 (CL-7) and Chanar Ladeado 15 (CL-15) [4] strains. Reference strains used are Yamagata-S81 (YS-81) [5], Indiana (Ind-S) [7], Alfort (Alf) [6] and Sweden 66 (S-66) [8] strains. All strains were cloned twice using CPK cells. CPK cells were grown in Dulbecco's modified Eagle's medium (Nissui, Tokyo, Japan) supplemented with 10% heat-inactivated fetal calf serum and antibiotics. For viral DNA extraction, confluent monolayers of CPK cells were grown in petri dishes of 9.5 cm in diameter and infected with each of the ADV strains at a multiplicity of infection between 1 and 5. The infected cells were washed once with phosphate-buffered saline without Mg and harvested when extensive cytopathic effects were observed. Then, the cells were suspended in TEN buffer consisting of 100 mM Tris-HCl (pH 7.5), 12.5 mM EDTA (pH 8.0), 150 mM NaCl and 1% SDS. Proteinase K at a final concentration of 0.2 mg/m*l* were then added and incubated at 50°C for 4 hr. The lysate was extracted once with TE (10 mM Tris-HCl pH 8.0 and 1 mM EDTA pH 8.0)-saturated phenol and once with a mixture of phenol-chloroform-isoamylalcohol (25:24:1). Finally, the total DNA were precipitated with two volumes of 99% ethanol, rinsed twice with 70% ethanol, dried and dissolved in 0.04 m*l* of sterilized distilled water. The DNA solution was digested overnight with *Bam*HI at 37°C. The digested DNA were subjected to an electrophoresis at 30 V for 14 hr on 0.6% or 0.8% agarose gel (140 × 150 × 5 mm) in buffer consisting of 40 mM Tris-acetate pH 7.8, 5 mM sodium acetate, and 1 mM EDTA. Bacteriophage lamda DNA cleaved with *Hind*III was used as a size marker. After electrophoresis, the gels were stained with ethidium bromide, observed and photographed under short-wave ultraviolet light.

The *Bam*HI restriction patterns of ADV strains obtained are shown in Fig. 1. The numbering of each fragment was based on the reports of Ind-S and YS-81 strains [2, 10]. As previously reported, Alf, Ind-S, and YS-81 strains presented the *Bam*HI cleavage patterns of type I, type I intermediary (type Ii) and type II, respectively [6, 10]. S-66 strain was considered to be variation of type I, though isolates in Northern Europe reportedly belonged to type III [6]. Among Argentine strains, RC/79, CL-7 and CL-15 strains belonged to type I because of the presence of fragment 2 and quite similarity to Alf strain's cleavage pattern. On the other hand, Mer strain belonged to type II, based on the absence of fragment 2 and the presence of fragments 2a and 2b. Mer

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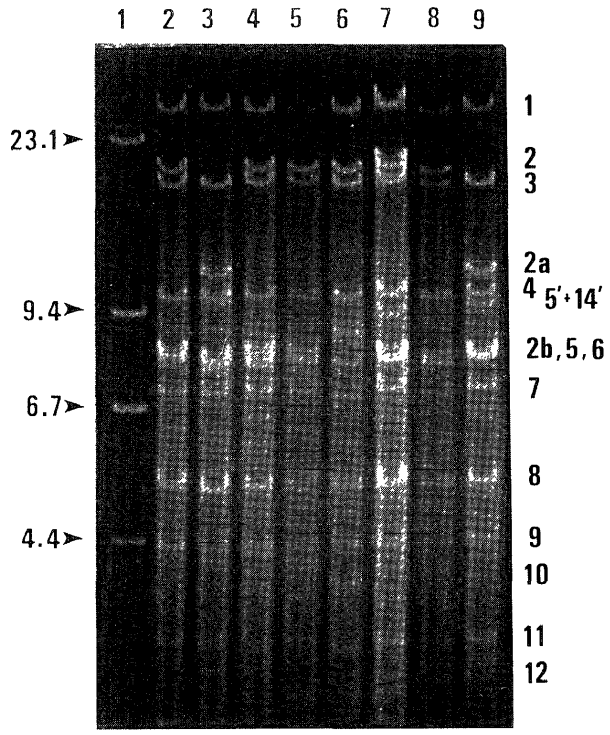

Fig. 1. Restriction fragments obtained with *Bam*HI of 8 strains. Lane 1, size marker; lane 2, Rio Cuarto (RC/79); lane 3, Mercedes (Mer); lane 4, Chanar Ladeado 7 (CL-7); lane 5, Chanar Ladeado 15 (CL-15); lane 6, Sweden (S-66); lane 7, Indiana (Ind-S); lane 8, Alfort (Alf); lane 9, Yamagata (YS-81). Fragments are numbered according to the reports of Ind-S and YS-81 strains [2, 10].

strain was similar to YS-81 strain but had slight differences in mobility of fragment 10. Although RC/79 strain had almost the same pattern with CL-7 and CL-15 strains, the fragment 10 of RC/79 seemed to be shifted to the position of fragment 9. As the variation of fragments 10 and 12 was known even in viruses isolated in the same area, the differences found between CL-7 and CL-15 might be due to those mobilities.

In addition to 3 Argentine isolates being classified as type I, our latest isolates in 1992 also belonged to type I (data not shown). Therefore, since the first outbreak of AD in Argentina occurred in 1979, major outbreaks have been caused by type I. However, Mer strain belonging to type II was isolated from the outbreak occurred in 1980 in a city located in San Luis Province, where the swine production is on a small scale and the farm is isolated from other swine production area in Argentina. The outbreak started among pigs imported from Holland shortly after they arrived in Argentina. The reason why this viral strain seemed to have not spread throughout the country may be that 100% of the piglets died after showing symptoms and the survived adults were totally sacrificed (Fondevila, personal communication).

Since specific sequences within ADV genome are expected to be related with biological functions of the virus, we examined the 8 ADV strains if they possess different biological properties (data not shown). However, it was difficult to differentiate those differences clearly. Although these strains showed some degree of antigenic variation when they were analyzed by virus nerutralization test using monoclonal antibodies directed against gII (data not shown), it was not sufficient to identify one by one. On the other hand, the several variations were clearly found in their restriction patterns as described above. This indicates the advantage of RE assay in the classification of ADV, because the assay can allow the further differences into groups and is much easier to carry out.

In conclusion, we have had at least 2 genome types of ADV strain in Argentina, types I and II, according to the classification by *Bam*HI cleavage pattern. However, major outbreaks have been caused by type I and the outbreak of type II has occurred so far only once. This may imply that the immediate measures by total slaughter of pigs in the affected farm was successful to prevent the spread of type II ADV. As the present data are not sufficient to know the prevalence of AD in this country, the same effort of these analyses should be continued for survailance of ADV infection in Argentina.

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