The Genome Type of Aujeszky’s Disease Virus Isolated from a Cat in Japan

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In Japan, Aujeszky’s disease (AD) has spread mainly in Tohoku and Kanto areas since its first outbreak in Yamagata Prefecture in 1981 [5]. First AD in swine was discovered in May, 1984 in Kanagawa Prefecture. Since then, it has caused considerable economic losses in pig husbandry (personal communication). Aujeszky’s disease virus (ADV) was isolated from an infected companion cat in Chigasaki City, Kanagawa Prefecture in January, 1990. At almost the same time (in December, 1989), ADV infection in a dog was discovered in Kawasaki City, Kanagawa Prefecture. By etiological investigation, pork was suspected to be a mediator of infection because the owner stated that the dog was fed on raw minced pork. The source of infection in the former was unknown. These cases of infection in companion animals indicated the possibility of widespread prevalence of AD in Kanagawa district as discussed previously [1]. Recently, Nishimori et al. reported that the field isolates in Japan were classified into two groups by restriction endonuclease analysis (REA) [4]. To differentiate the two viruses isolated from

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**Fig. 1.** DNA cleavage patterns of Aujeszky’s disease viruses digested with BamHI. Lane M, size marker; lane 1, Yamagata S-81 from a pig; lane 2, Saitama S-85 from a pig; lane 3, Saitama B-85 from cattle; lane 4, Saitama C-86 from a dog; lane 5, Azabu C-85 from a dog; lane 6, Azabu C-89 from a dog; lane 7, Azabu F-90 from a cat. Designation of Yamagata S-81 BamHI fragments is indicated at the right, Arrows; fragments 3 and 4.
the companion animals in the same prefecture, viral DNAs were extracted from the ADV-infected cells and their genotypes were determined by REA.

The viral strains used consisted of Yamagata S-81 isolated from a pig in Yamagata Prefecture in 1981, Saitama S-85 and Saitama B-85 isolated from a pig and cattle respectively in Saitama Prefecture in 1985 [3], and Saitama C-86 isolated from a dog in the same prefecture in 1986. Viral DNAs extracted from these strains were kindly supplied by Dr. Matsuoka (Ohmiya Livestock Hygiene Service Center of Saitama Prefecture). In addition, three strains, Azabu C-85 isolated from a dog in Ibaraki Prefecture in 1985 [1], and Azabu C-89 and Azabu F-90 both isolated newly from the above-mentioned dog and cat respectively in Kanagawa Prefecture were used. The CRFK cells of feline kidney cell line were grown in a 500-ml culture bottle as described previously [1] and were infected with 1 ml of viral fluid (10^3 TCID_{50}/ml). The infected cells were harvested in order to observe an extensive cytopathic effect, washed three times in TBS buffer (10 mM Tris-HCl, 0.15 M NaCl, pH 7.4) and then suspended in the same buffer. To the cell suspension, SDS and proteinase K were added to make final concentrations of 0.6% and 400 μg/ml respectively. After incubation at 37°C for 3 hr, the lysate was extracted three times with a mixture (2:1:1.05) of phenol, chloroform and isoamylalcohol. DNAs were precipitated with ethanol and dissolved in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0). The DNA solution was incubated with 100 μg/ml of RNase at 37°C for 3 hr and subsequently with 200 μg/ml of proteinase K at 37°C for 3 hr. DNAs were extracted three times with a mixture of phenol, chloroform and isoamylalcohol, precipitated with ethanol and then dissolved in TE buffer. Purified DNAs were digested with BamH I at 37°C for 3 hr and with BstE II at 60°C for 3 hr in the same buffer system as recommended by an enzyme manufacturer. After digestion of DNAs electrophoresis was performed for 24 hr at 40 V using 0.8% agar. DNAs were then stained with ethidium bromide for examination.

When cleaved by BamH I, Azabu F-90 isolated from the cat in Kanagawa Prefecture demonstrated the same cleavage pattern as Yamagata S-81 isolated from a pig.

![Fig. 2. DNA cleavage patterns of Aujeszky's disease viruses digested with BstE II. Lane M, size maker; lane 1, Yamagata S-81 from a pig; lane 2, Saitama S-85 from a pig; lane 3, Saitama B-85 from cattle; lane 4, Saitama S-86 from a dog; lane 5, Azabu C-85 from a dog; lane 6, Azabu C-89 from a dog; lane 7, Azabu F-90 from a cat. Designation of Yamagata S-81 BstE II fragments is indicated at the right, Arrow; fragment 6.](image-url)
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(Fig. 1). These two strains formed two bands (arrows) near 9.4 Kb, in contrast to the other strains that formed three bands. These were considered to fit in the first group of type II according to the classification of Nishimori et al. [4]. The other strains were considered to correspond to the second group of type II according to their REA classification [4]. As shown in Fig. 2, the BstE II cleavage patterns of all the strains corresponded to group v1 of type I reported by Herrmann et al. [2], although the electrophoretic mobility of fragment 6 below 9.4 Kb on lanes 4 and 6 was slightly different (arrow). The two lanes represent the viruses isolated from the dogs in Saitama and Kanagawa Prefectures, respectively.

Herrmann et al. examined 150 ADV strains by REA and classified them into six (I, Ii, II, II i, III and IV) different groups and five (I, Iv1, Iv2, II and III) different groups on the basis of the cleavage patterns produced by digestion with BamH I and BstE II respectively. Type II in BamH I cleavage lacks one fragment (fragment 2) of type I but possesses two new fragments, one each on the upper and lower sides of fragment 4 [2]. Nishimori et al. classified type II produced by digestion with BamH I into two more groups. The first group possessed fragment 8, and the second group did not possess fragments 5 and 8 but acquired a fragment migrating between fragments 3 and 4 [4].

The isolate from the cat was presumed to belong to the first group because of the possession of fragment 8 in digestion with BamH I, and the isolate from the dog to the second group because of the loss of fragments 5 and 8 in digestion with BamH I. Furthermore, the strains isolated from the dog and cat in Kanagawa Prefecture and from a dog in Saitama Prefecture in 1986 demonstrated slightly different cleavage patterns by digestion with BstE II. The above finding seemed to indicate that the sources of ADV infection in the two cases found in Kanagawa Prefecture differed from each other, and this may suggest the prevalence of two different genotypes in Kanagawa Prefecture.

REFERENCES