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Isolation and Characterization of Aujeszky's Disease Virus in Captive Brown Bears from Italy

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ABSTRACT: An epizootic of Aujeszky's disease (pseudorabies) in four captive European brown bears (*Ursus arctos*) in November 1994, in the Val di Non, Trentino Region, Italy, was linked to consumption of raw pork. Affected animals had severe pruritus resulting in self-mutilation, and all four died within 24 hr after onset of clinical signs. Aujeszky's disease virus was isolated on first passage from the brain and was characterized by means of restriction endonuclease analysis. Based on these data, we believe that bears are extremely susceptible to the disease, and that wildlife managers should consider pseudorabies as a potential risk for the captive and wild bear populations.

Key words: Aujeszky's disease, pseudorabies, European brown bear, *Ursus arctos*, restriction fragment length polymorphism (RFLP), case report, Italy, wildlife park.

Aujeszky's disease (pseudorabies) has been reported in a variety of wild animals (Mohanty, 1990); however among bears, we believe that Aujeszky's disease virus (ADV) has been isolated only once from an American black bear (*Ursus americanus*) (Schultze et al., 1986). The only other evidence of ADV infection in a bear was the presence of antibodies in a black bear cub (Pirtle et al., 1986) in Florida (USA). Herein, we report an epizootic of Aujeszky's disease in four of five European brown bears (*Ursus arctos*) and describe the characterization of the virus isolate by means of restriction endonuclease analysis.

Bears were housed together in a large outdoor cage in a wildlife park in the Val di Non, in the Trentino region of northern Italy (46°59′N, 10°44′E). The group consisted of four males and one female ranging from 18- to 27-yr-old. Bears were fed vegetables, fruit, bread, milk and freeze-dried dog food daily; once a week they were fed alternately with fresh pork offal or fish. Each bear was fed approximately 5 kg of pork, originating from a local abattoir every 2 wk. The one 27-yr-old female member of the group did not feed on pork.

On 7 November 1994, one female died; the three males died on the 9, 11, and 12 November. The only bear which survived was the 27-yr-old female which did not feed on pork. Clinical signs in the male bears were very similar in all the affected animals. Depression, anorexia, and hypersalivation occurred first, followed by a pruritus characterized by violent scratching particularly around the right ear. These symptoms were followed by serious respiratory distress and paralysis which eventually led to death within 24 hr from the onset of the clinical signs. Scratching resulted from pruritus led to mutilation of the ear of one bear. No medical treatment was administered to any of the animals. Upon post-mortem examination we did not observe any significant lesions, apart from the severe self-mutilation.

A 10% weight/volume suspension of the brain was prepared in phosphate buffer solution (PBS) containing 10,000 units/ml of penicillin (Sigma Chemical Co., Milan, Italy), 10 mg/ml streptomycin (Sigma) 5,000 units/ml of mycostatin (Sigma) and 250 μg/ml of gentamycin (Sigma). Each suspension was filtered through a 450 nm filter, and inoculated onto a confluent monolayer of feline embryonic fibroblasts (FEA cells, Istituto Zooprofilattico, Padova, Italy). The first foci of cytopathic effect appeared approximately 48 hr after infection: the culture was subpassaged onto rabbit kidney RK13 continuous cell line (ATCC CCL37, American Type Culture

Collection, Rockville, Maryland, USA), and the virus was identified by means of serum neutralization test with a reference serum against ADV (Toma and Eloit, 1991). Briefly, ten-fold serial dilutions of the virus were neutralized by a 1:5 dilution of the hyperimmune serum on RK13 cells.

The virus was further characterized by means of restriction fragment length polymorphism (Banks, 1993). The virus isolate, together with reference ADV types I, II and II intermediate (Herrmann et al., 1984) were grown on RK13 cells at a multiplicity of infection of 1 and harvested after 24 hr. The virus suspensions were centrifuged at 1400 × *G* for 10 min., and the resulting pellets were treated with lysis buffer composed of 0.01M pH 7.5 Tris HCl, 0.01M KCl, 0.0015M $MgCl_2$, and 0.5% Nonidet P 40 (Sigma) at 0 C for 10 min. The suspensions were recentrifuged as above and the supernatants ultracentrifuged at 130,000 × *G* for 1 hr. Supernatants were then discarded and the pellets were resuspended in 2.5 ml of TE buffer (Tris HCl 100 mM (Sigma), EDTA 10 mM (Sigma), pH 8). The virus suspensions were treated with sodium-dodecyl-sulphate (SDS, Sigma) (final concentration 0.5%) for 20 min. at 37 C in order to dissolve the pellets. The viral DNA was extracted twice collecting the aqueous phase after gently mixing the suspension with equal volumes of phenol and chloroform (1:1), and once with chloroform-isoamyl alcohol (24:1). The DNA was precipitated in three volumes of absolute ethanol overnight at −20 C and pelleted by centrifugation for 1 hr at 15000 × *G*. The amount of DNA was estimated by electrophoresis on a 0.75% agarose gel (Sigma) and stained with ethidium bromide (Sigma).

According to the amount of DNA present, 1 to 2 µl were digested overnight with one unit of *Bam HI* (Sigma). The digests were run at 50 V for 4 to 6 hr on a 14 × 20 cm horizontal 0.75% agarose electrophoresis gel. The restriction fragment pattern of the bear isolate was similar to that of a type II Aujeszky's disease virus, according to the classification of Herrmann et al. (1984) (Fig. 1). Nevertheless, although the DNA migration pattern of the bear isolate resembled that of a type II strain, it appeared to have a larger fragment 5 than is characteristic for a type II virus, and in this respect it was more like a type I strain. Further investigations are necessary to clarify these findings. Without further molecular characterization of the isolate, it is not possible to draw any conclusions on the nature and implications of this diversity. Type II isolates are the predominant contemporary strains in the pig population of central Europe (Jestin et al., 1989) and in Italy (Luini et al., 1989); therefore, the results obtained from the restriction fragment length polymorphism analysis support the likelihood that the virus originated from pig meat.

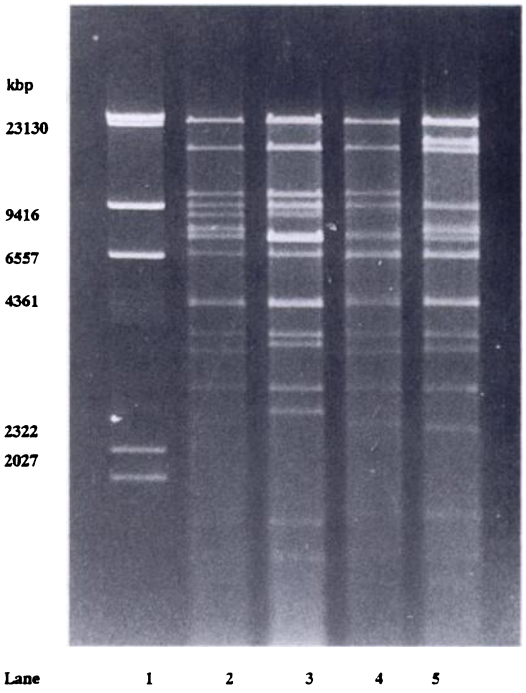

FIGURE 1. Characterization of Aujeszky's disease virus by means of restriction fragment length polymorphism with *Bam HI*. Lane 1: Molecular weight marker Lambda *Hind* III digest. Lane 2: Bear ADV isolate. Lane 3: Reference type II ADV isolate. Lane 4: Reference type IIi isolate. Lane 5: Reference type I ADV isolate; kbp is kilobase pairs.

Based on our evidence, captive and wild

bears as well as other susceptible animals should be protected from Aujeszky's disease, especially considering that some wild bear populations, such as the one present in the Trentino region are extremely endangered. Feeding them raw pork originating from countries in which Aujeszky's disease is present should be avoided; in fact, this strategy has been adopted by the rangers in the park in Trentino. The second management possibility for these bears is vaccinating susceptible animals with an inactivated vaccine. This has been suggested for the Florida panther (*Felis concolor coryi*) by Glass et al. (1994), although vaccination has not been proven efficacious.

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