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Aujeszky's Disease in Red Fox (*Vulpes vulpes*): Phylogenetic Analysis Unravels an Unexpected Epidemiologic Link

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ABSTRACT: We describe Aujeszky's disease in a female of red fox (*Vulpes vulpes*). Although wild boar (*Sus scrofa*) would be the expected source of infection, phylogenetic analysis suggested a domestic rather than a wild source of virus, underscoring the importance of biosecurity measures in pig farms to prevent contact with wild animals.

Aujeszky's disease (AD) or pseudorabies is an infectious disease caused by *Suid herpesvirus* 1 (SuHV-1 or pseudorabies virus, PRV), a DNA virus of the family *Herpesviridae*. The natural hosts for PRV are members of the family Suidae, in which infection results in clinical or subclinical disease as well as latent infection with the possibility of viral reactivation. Other wild and domestic animals (e.g., cattle [*Bos taurus*]; dogs [*Canis lupus familiaris*]; cats [*Felis catus*]; rats [*Rattus norvegicus*]; raccoons [*Procyon lotor*]; opossums [*Didelphis marsupialis*]; etc.) and several fur-bearing animals are susceptible to infection. In these species, infection leads to neurologic disease resulting in death within a few days of the onset of clinical signs. Restriction fragment length polymorphism (RFLP) analysis has been used for genomic characterization of PRV, revealing three major genetically distinct PRV groups, or genomic types. More recently, investigators have used *UL44* gene (*gC* gene, encoding glycoprotein C) sequencing for molecular characterization of PRV, introducing the concept of phylogenetic clusters to classify viral strains (Fonseca et al. 2010, 2012; Serena et al. 2011; Sozzi et al. 2013).

We describe a case of AD in a female red fox (*Vulpes vulpes*) in the Piedmont region of northwestern Italy. The fox was found alive in February 2012 near the village of Centallo (Province of Cuneo) by the local veterinary service. It displayed atypical behavior with motor incoordination, head-scratching, rolling in the snow, lunging, and biting branches and shrubs. Clinical signs persisted and death followed 2 days after observation of signs. At necropsy, the fox appeared in good general condition as determined by fat deposits and body weight. Gross lesions were limited to the head and consisted of subcutaneous edema and multiple skin abrasions from scratching. Rabies, canine distemper, and AD were considered in the differential diagnosis based on clinical manifestations. Rabies and canine distemper were ruled out by direct immunofluorescence assay on central nervous system sections using specific monoclonal antibodies (Mab) against rhabdovirus (Bio-Rad, Richmond, Virginia, USA) and canine distemper virus (VMRD).

Viral isolation was carried out on fresh brain tissue homogenate in rabbit kidney cells (RK13, American Type Culture Collection, Manassas, Virginia, USA). Three days after infection, the cell monolayer showed focal cytopathic effects with refractive cells and large syncytia. Staining of infected cells with Mab against SuHV-1 (EuroClone, Pero Milan, Italy) was positive. Nested PCR (Yoon et al. 2006) on brain tissue and RK13 supernatant confirmed the diagnosis.

We sequenced the *gC* gene according to Goldberg et al. (2001) and performed phylogenetic analysis on 37 *gC* gene sequences using MrBayes 3 (Ronquist and Huelsenbeck 2003). The red fox PRV strain was classified into cluster B

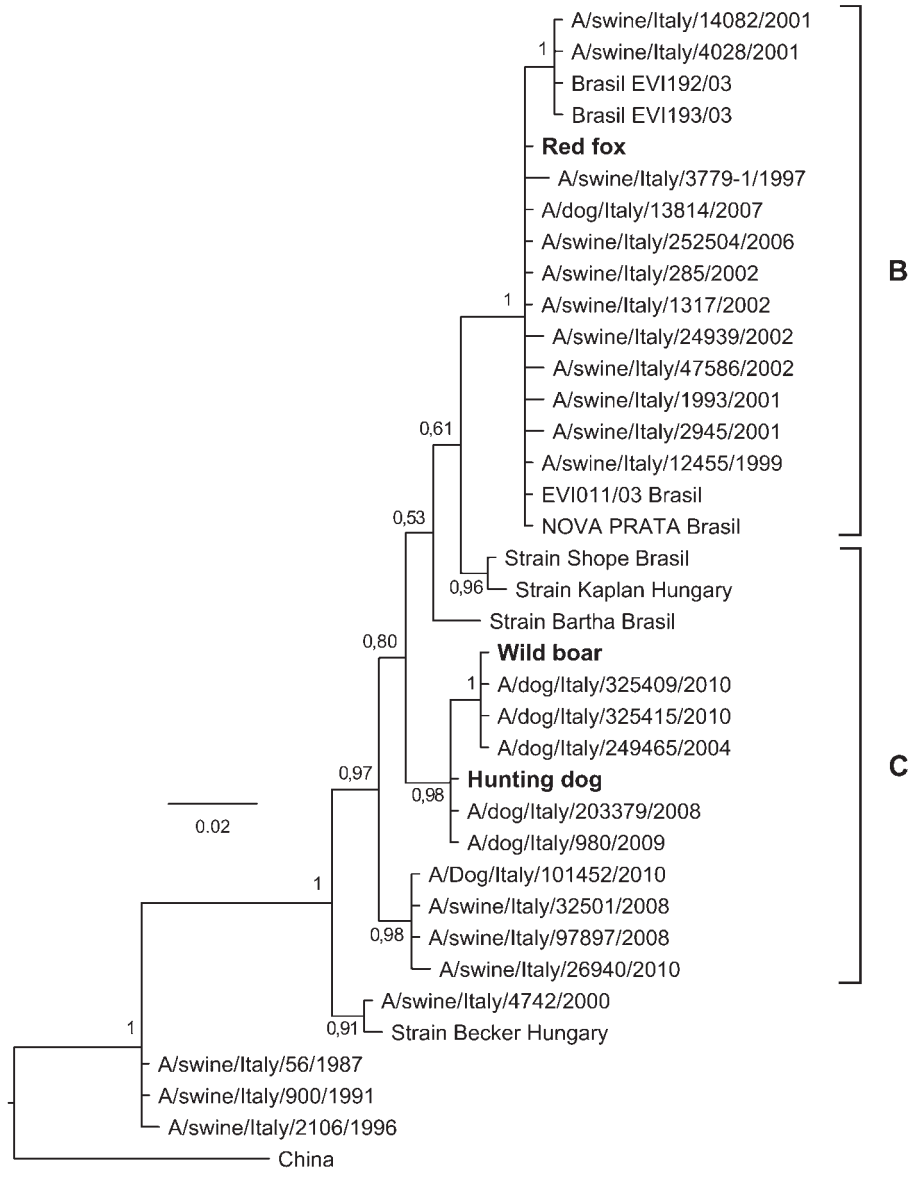

FIGURE 1. Phylogenetic tree of 37 partial *gC* gene sequences. The phylogenetic tree was obtained by an alignment of 692 base pairs including three new sequences (*Vulpes vulpes*, red fox; *Sus scropha* wild boar; *Canis lupus familiaris*, hunting dog, in bold) from the study area and 34 sequences from GenBank (see Supplementary Material). The prior settings for the Bayesian inference within MrBayes 3 from the output of jModelTest2 (3 models +G +I +F) were as follows: nucleotide substitution model: HKY+G; base frequencies: A = 0.12, C = 0.41, G = 0.36, T = 0.11; gamma shape = 0.021; transition frequency=1.84; transversion frequency=1.00. Node support is reported as posterior probability. Clusters B and C are marked and strongly supported by the posterior probability. The red fox pseudorabies virus strain was classified into cluster B and occupied the same phylogenetic branch as eight sequences from farm pigs and one sequence from a domestic dog detected in northern Italy.

(Fig. 1), strongly supported by the posterior probability. Two PRV strains previously isolated from a wild boar (*Sus scrofa*) and a hunting dog from the same area were also analyzed. Both samples were classified into cluster C (Fig. 1) with a statistically significant posterior probability. In Italy, the isolation of PRV from wild animals has been limited to wild boar (Capua et al. 1997) and captive brown bears (*Ursus arctos*) (Zanin et al. 1997); these were linked to the consumption of raw pork. In the US, infections in captive coyotes (*Canis latrans*), Florida panther (*Felis concolor coryi*), and hunting dogs have been reported (Glass et al. 1994; Raymond et al. 1997; Cramer et al. 2011); in Morocco, PRV was isolated from African wild dogs (*Lycaon pictus*) held in captivity after being fed wild boar meat (Haddane and Essalhi unpubl. data). Between 1964 and 1969, outbreaks of AD were reported in red foxes in Denmark, but no molecular characterization was done to investigate epidemiologic links (Bitsch et al. 1969).

Wild carnivores may be infected with PRV in several ways. Consumption of a viral source from the wild, such as wild boar meat, may be the most likely means of transmission. Serosurveys revealed PRV infection in wild boar in at least 14 European countries (Muller et al. 2011). In the epidemiology of AD in Italy, the wild boar is an important reservoir of PRV, and circulation of the virus has been demonstrated by serologic surveys (overall antibody prevalence between 2011 and 2013 was 37–65% based on 968 tested serum samples; C.C. unpubl. data) of wild boar living in the same areas as the red fox. Nevertheless, molecular epidemiology did not support this hypothesis. Phylogeny reconstruction showed that the PRV strain identified in the red fox belongs to cluster B. This phylogenetic cluster seems to include exclusively PRV *gC* sequences recovered in swine and in working dogs from pig farms, while the PRV strains circulating in wild boar and, consequently, in hunting dogs are distinct and grouped into cluster C (Steinrigl et al. 2012; Sozzi et al. 2013). Phylogenetic analyses were also carried out on two archived PRV strains isolated from a wild boar and a hunting dog from the same area in 2011 and 2012, respectively. As expected, they were classified into cluster C, suggesting that locally circulating PRV strains are not an exception to known epidemiologic correlations between phylogenetic clusters and domestic or feral swine and related dog categories.

Our results implicate domestic swine as the primary source of infection for the red fox. Potential transmission routes include direct contact with infected swine, drinking contaminated water (especially in cool, damp weather, which aids virus survival), and feeding on rodents. Consuming virus-contaminated organic waste is another possible source of infection. This is unlikely, however, due to the territorial structure and the inaccessibility of areas relegated to waste disposal. Airborne transmission of AD has also been documented. Our findings underscore the importance of biosecurity measures on pig farms and of proper disposal of dead pigs to prevent pathogen transmission to wild animals.

SUPPLEMENTARY MATERIAL

Supplementary material for this article is online at http://dx.doi.org/10.7589/2013-11-312.

LITERATURE CITED

Bitsch V, Knox B, Munch B. 1969. Cases of pseudorabies in free-living red foxes (*Vulpes vulpes*) and in captive blue foxes (*Alopex lagopus*) in Denmark. *Acta Vet Scand* 10:195–196.

Capua I, Fico R, Banks M, Tamba M, Calzetta G. 1997. Isolation and characterisation of an Aujeszky's disease virus naturally infecting a wild boar. *Vet Microbiol* 55:141–146.

Cramer SD, Campbell G, Njaa BL, Morgan S, Smith S, McLin W, Brodersen B, Wise A, Scherba G, Langohr I, et al. 2011. Pseudorabies virus in Oklahoma hunting dogs. *J Vet Diag Invest* 23:915–923.

Fonseca AA Jr, Camargos MF, de Oliveira AM, Ciacci-Zanella JR, Patrício MA, Braga AC, Cunha ES, D'Ambros R, Heinemann MB, Leite RC, et al. 2010. Molecular epidemiology of Brazilian pseudorabies viral isolates. *Vet Microbiol* 141:238–245.

Fonseca AA Jr, Camargos MF, Sales ML, Heinemann MB, Leite RC, Reis JK. 2012. Pseudorabies virus can be classified into five genotypes using partial sequences of UL44. *Braz J Microbiol* 43:1632–1640.

Goldberg TL, Weigel RM, Hahn EC, Scherba G. 2001. Comparative utility of restriction fragment length polymorphism analysis and gene sequencing to the molecular epidemiological investigation of a viral outbreak. *Epidemiol Infect* 126:415–424.

Steinrigl A, Revilla-Fernandez S, Koloodziejek J, Wodak E, Bago Z, Nowotny N, Schmoll F, Köfer J. 2012. Detection and molecular characterization of Suid herpesvirus type 1 in Austrian wild boar and hunting dogs. *Vet Microbiol* 157:276–284.

Glass CM, Mclean RG, Katz JB, Maehr DS, Cropp CB, Kirk LJ, McKeirnan AJ, Evermann F. 1994. Isolation of pseudorabies virus from Florida panther. *J Wildl Dis* 30:180–184.

Muller T, Hahn EC, Tottewitz F, Kramer M, Klupp BG, Mettenleiter TC, Freuling C. 2011. Pseudorabies virus in wild swine: A global perspective. *Arch Virol* 156:1691–1705.

Raymond TJ, Gillespie R, Woodruff M, Janovitz B. 1997. Pseudorabies in captive coyotes. *J Wildl Dis* 33:916–918.

Ronquist F, Huelsenbeck JP. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19:1572–1574.

Serena MS, Metz GE, Mórtola EC, Echeverría MG. 2011. Phylogenetic analysis of Suid herpesvirus 1 isolates from Argentina. *Vet Microbiol* 154:78–85.

Sozzi E, Moreno A, Lelli D, Cinotti S, Alborali GL, Nigrelli A, Luppi A, Bresaola M, Catella A, Cordioli P. 2013. Genomic characterization of pseudorabies virus strains isolated in Italy. *Transbound Emerg Dis*. doi: 10.1111/tbed.12038

Yoon HA, Eo SK, Aleyas AG, Cha SY, Lee JH, Chae JS, Jang HK, Ch JG, Song HJ. 2006. Investigation of pseudorabies virus latency in nervous tissues of seropositive pigs exposed to field strain. *J Vet Med Sci* 68:143–148.

Zanin E, Capua I, Casaccia C, Zuin A, Moresco A. 1997. Isolation and characterization of Aujeszky's disease virus in captive brown bears from Italy. *J Wildl Dis* 33:632–634.

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