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Short Communication

Detection and molecular analysis of Pseudorabies virus strains isolated from dogs and a wild boar in Italy

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ABSTRACT

Aujeszky's disease (AD) is one of the most economically important diseases of farmed pigs. Wild boars can act as reservoirs and might represent a potential threat for domestic animals, including dogs. The aim of this study was to report the results of an AD survey based on the Pseudorabies virus (PRV) genome detection in samples of dogs clinically suspected of AD and of wild boars collected during four consecutive hunting seasons in the period 2010–2014. Genomic characterization was based on the partial gC sequence of the Italian strains and the comparison with those from domestic pigs and European PRV strains circulating in wild boars. The Italian PRV strains were mainly distributed into three different clusters and revealed two interesting findings. First, there was a clear distinction between the viral strains that were isolated from dogs used for hunting and subsequently traced back to wild boars and the strains that were isolated from working dogs and subsequently found to be closely related to domestic pigs. Second, the Italian epidemiological situation was found to be different from those of European countries in that the Italian situation was characterized by the presence of both the typical Italian clades 1 and 2 and supported by new patterns of aa deletions/insertions. Italian clade 1 included strains from hunting dogs and two Italian wild boars, and Italian clade 2 grouped with recent strains from dogs that were unable to hunt and domestic pigs that were related to one old reference strain (S66) and not included elsewhere. Molecular and phylogenetic analyses of PRV strains are therefore necessary to improve the understanding of the distribution of the PRV clusters and their evolution.

1. Introduction

Pseudorabies virus (PRV) is the causal agent of Aujeszky's disease (AD), an animal disease primarily affecting pigs but also known to occur occasionally in cattle, sheep, goats, horses, dogs and cats. Suids are the natural reservoir of the virus, and the disease is self-limiting in the other species. AD is a contagious infection and is mainly transmitted by direct and indirect contact between pigs.

The causative agent is an enveloped DNA virus, which belongs to the Alphaherpesvirinae subfamily of the

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Herpesviridae family. AD is a notifiable disease that causes substantial economic losses to the swine industry and has major economic impact due to trade implications and income losses for farmers.

Italian pig production is concentrated (over 80%) in the four Northern Italian regions of Lombardia, Emilia-Romagna, Piemonte and Veneto. The Lombardia region is the major producer, with 50% of the national pig production (Maiorano, 2009). Compared with the pig production of other countries, Italian pig production differs in the high live weight of pigs at slaughter; they are slaughtered at 10–12 months of age when they reach a live weight of 150–170 kg and provide carcasses of 125–140 kg maintaining proper adiposity.

The European general policy is to eradicate AD in order to support free intra-EU trade. However, eradication may take several years depending on the epidemiological situation in countries where the disease is endemic. In Italy, an AD national monitoring program was implemented in 1997. However, since 2011, with the issuing of Decree 30/12/2010 and the latest update in 2014, the National Authority has put in place more strict measures to ensure a substantial reduction in virus circulation on pig farms. Thereafter, extraordinary regional control plans were implemented, especially in the Northern Italian regions with the highest concentrations of pig industry (Lombardia Region, D.d.s. 9/05/2014 - n. 3822; Emilia Romagna Region, Delibera Giunta Regionale n. 1588–13/10/2014; Veneto Region, Delibera Giunta Regionale n. 2061–11/10/2012), to achieve AD eradication status and to be included in annex two of the EU Decision 2008/185/EC. Important tools in AD eradication plans included the use of DIVA gE-deleted vaccines, movement restrictions, more intensive serological testing and the application of stamping out or slaughter policies to remove infected animals. Only the Bolzano province was classified in this annex that includes member states or regions that applied disease controls programs and have already eradicated AD or are in an advanced stage of eradication.

Although PRV has been eliminated in domestic pigs in many European countries, AD is being continuously reported in wild boar populations and in related hunting dogs (Albina et al., 2000; Gortazar et al., 2002; Lari et al., 2006; Leuenberger et al., 2007; Lutz et al., 2003; Muller et al., 2010; Pannwitz et al., 2012; Roic et al., 2012; Steinrigl et al., 2012; Verin et al., 2014; Vengust et al., 2005; Verpoest et al., 2014). Consequently, the possible impact of wild boars on the application and success of AD eradication programs and the risk they pose to the PRV-free status should be taken into account (Boadella et al., 2012). The role of wild boars as potential reservoirs of PRV has become increasingly important; thus, a deeper investigation on the distribution of PRV strains in wild boars and their genomic characterization at a regional level became necessary. AD surveillance plans were carried out (a) by testing for the presence of anti-PRV antibodies in sera samples collected within the wildlife national monitoring program in different regions of Italy (Lari et al., 2006, Montagnaro et al., 2010, Verin et al., 2014) and (b) by attempting PRV genome detection in wild boar samples voluntary submitted by hunters of some provinces of North Italy. In a previous study conducted on swine and dog strains isolated before 2010, we showed a clear distinction between the strains isolated from hunting dogs exposed to wild boars and those originated from domestic pigs (Sozzi et al., 2014). In the following years, we continued to monitor the epidemiology of AD in Italy to better understand the distribution of the virus clusters and their evolution. In this study, we report the results of an AD survey based on PRV genome detection in samples from dogs clinically suspected of AD and from wild boars collected during four consecutive hunting seasons, 2010–2014. Moreover, the genome characterization of two strains isolated from dogs in 1993 and 1994 was included. Dogs in which AD was clinically suspected were examined by using histopathological and virological methods, resulting in the isolation of 13 PRV strains from dead dogs. One strain isolated from a wild boar during the same period and region was also genetically characterized. The phylogenetic analysis was based on a partial sequence of the gC gene, and the results were compared with the sequences available in GenBank.

2. Materials and methods

2.1. Animals

A total of 11 dogs of different breeds, which were conferred to IZSLER laboratories in 2010–2014, and two dogs collected in 1993 and 1994 were included in the study. Of the dogs, eight were hunting dogs, four were dogs living in or close to pig farms (farm dogs), and although the last dog's exposure was unknown, the dog was unable to hunt. The origins and years of the PRV strain identifications are reported in Table 1. All of the hunting dogs had been used for hunting in the days immediately before the onset of clinical signs. Epidemiological investigations were conducted by the field veterinary services, and in all cases, the dogs' owners declared that animals had direct contact with wild boars or had been fed PRV-infected meat and/or offal. The clinical signs in the dogs included neurological signs, such as tremor, trismus, spasms of the muscles of the larynx and pharynx, dyspnea, vomiting and pruritus. Death occurred within 24–48 h.

Wild boar tissue samples of lungs and tonsils were voluntarily collected by hunters in several provinces of North Italy during the period 2011–2014. The sampling sites were divided according two different ecologic areas, the Alps (AP) and the Apennines (AN). A total of 176 samples originated from AP and 155 from AN with the following per annum distribution: 2011- 86 AP, 3 AN; 2012- 89 AP, 3 AN; 2013- 1 AP, 99 AN; 2014- 48 AN.

2.2. Laboratory investigations

Complete necropsies followed by virological examinations of selected organs were performed.

The presence of PRV DNA in the field samples was systematically determined by real-time PCR tests based on the specific detection of the gE gene as described by Yoon et al., 2005.