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Molecular characterization of Belgian pseudorabies virus isolates from domestic swine and wild boar

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ABSTRACT

Aujeszky's disease is an economically important disease in domestic swine caused by suid herpesvirus 1, also called pseudorabies virus (PRV). In several European countries, including Belgium, the virus has successfully been eradicated from the domestic swine population. The presence of PRV in the wild boar population however poses a risk for possible reintroduction of the virus into the domestic pig population. It is therefore important to assess the genetic relatedness between circulating strains and possible epidemiological links. In this study, nine historical Belgian domestic swine isolates that circulated before 1990 and five recent wild boar isolates obtained since 2006 from Belgium and the Grand Duchy of Luxembourg were genetically characterized by restriction fragment length polymorphism (RFLP) analysis and phylogenetic analysis. While all wild boar isolates were characterized as type I RFLP genotypes, the RFLP patterns of the domestic swine isolates suggest that a shift from genotype I to genotype II might have occurred in the 1980s in the domestic population. By phylogenetic analysis, Belgian wild boar isolates belonging to both clade A and B were observed, while all domestic swine isolates clustered within clade A. The joint phylogenetic analysis of both wild boar and domestic swine strains showed that some isolates with identical sequences were present within both populations, raising the question whether these strains represent an increased risk for reintroduction of the virus into the domestic population.

1. Introduction

Aujeszky's disease is an economically important disease in domestic swine caused by suid herpesvirus 1, also called Aujeszky's disease virus (ADV) or pseudorabies virus (PRV). The virus belongs to the family *Herpesviridae*, subfamily *Alphaherpesvirinae*, genus *Varicellovirus*. PRV infection is characterized by respiratory, reproductive, and neurological symptoms which depend on the age of the pigs and the virulence of the strain (Pomeranz et al., 2005). In several European countries, including Belgium, the virus was successfully eradicated from the domestic swine population due to large scale vaccination programs (Decision 2011/648/EU). The virus remains however present in the wild boar population (Czaplicki et al., 2006; Müller et al., 2011) and thereby poses a possible risk for reintroduction of the virus into the currently unprotected domestic pig population.

Conventional restriction fragment length polymorphism (RFLP) analysis of genomic DNA has allowed differentiation of PRV isolates in four major types and several subtypes (Herrmann et al., 1984). In Europe, wild boar PRV strains are mainly genotype I (Müller et al., 2010), while in domestic swine both type I and II are found (Herrmann et al., 1984). Type III and type IV PRV strains are restricted to Northern Europe and Asia, respectively,

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(Herrmann et al., 1984). More recently, phylogenetic analysis of a partial sequence of the glycoprotein C (gC) gene has been used for molecular typing (Goldberg et al., 2001) and showed that European wild boar isolates differentiate in two clades (Müller et al., 2010). One clade consists of strains originating from eastern Europe, while the other clade is made up of strains originating from southwestern Europe, but both clades seem to overlap geographically in Central Europe (Müller et al., 2010). Only limited information is available for European domestic swine strains (Sozzi et al., 2013).

In this study, we genetically characterized Belgian PRV strains from wild boar and domestic swine origin by RFLP analysis and phylogenetic analysis to get a better insight into the genetic relatedness between strains in both populations and to compare Belgian strains with strains circulating in neighboring countries.

2. Materials and methods

2.1. Virus isolation and virus identification

Brain or lung tissue from animals diagnosed with Aujeszky's disease was used for virus isolation. A 10% weight/volume suspension of tissue in phosphate buffered saline (PBS) was prepared by mechanical disruption using an ultraturrax and centrifuged at 1750 × *g* for 20 min. The supernatant was collected, filtered through a 0.45 μm filter and inoculated onto a confluent monolayer of PK15 cells, grown on six-well plates. The inoculum was removed after 1 h at 37 °C. Cells were washed with PBS and MEM, supplemented with fetal calf serum (10%), penicillin (1000 U/mL), gentamicin (50 μg/mL) and fungizone (250 ng/mL), was added to the cells and incubated at 37 °C. The cells were daily assessed for cytopathic effect typical for herpesvirus infection. The supernatant of cell cultures with a cytopathic effect was collected and cells were fixed with methanol. Immunofluorescence staining using a PRV-specific fluorescein-conjugated antiserum was performed to confirm the presence of PRV.

In total nine domestic swine isolates from the period between 1973 and 1989 were obtained from eight domestic swine and one bovine that got infected after being in close contact with pigs (Table 1). Five PRV strains from wild boar origin were obtained between 2006 and 2011: one directly from a wild boar, and three from hunting dogs and one from a wolf that had been fed with wild boar offal (Cay and Letellier, 2009; Verpoest et al., 2014). One of these wild boar isolates originated from a hunting dog from the Grand Duchy of Luxembourg. Brain tissue from the animal was sent to the Belgian reference laboratory for Aujeszky's disease at CODA–CERVA for virus isolation and was therefore included in this study. Virus could be isolated from all animals, except from the wild boar. The latter could therefore only be examined by phylogenetic analysis and not by RFLP analysis.

2.2. RFLP

To determine the restriction fragment pattern, the PRV isolates were passaged once on ST cells followed by one freeze–thaw cycle. The cellular debris was removed by low speed centrifugation at 1750 × *g* for 20 min. The supernatant was ultra-centrifuged at 100,000 × *g* and 4 °C for 90 min. The virus pellet was resuspended in PBS, followed by DNA extraction using the QiAmp DNA extraction kit (Qiagen, Germany). One μg of DNA was digested with 10 U *Bam*HI (Roche, Switzerland) for 3 h at 37 °C. The same procedure was applied to PRV reference strains Bartha and Kaplan. The digests were loaded on a 0.6% agarose gel and run for 6 h at 50 V. Bands were visualized using a 0.5 μg/mL ethidium bromide solution (Sigma-Aldrich, Belgium).

2.3. Sequencing and phylogenetic analysis

For sequencing purposes, a part of the gC sequence of the virus isolates was amplified with the DyNazyme Ext DNA polymerase (Thermo Fisher Scientific, USA) using previous published primers (Hahn et al., 2010; Müller et al., 2010). The amplicon of the expected size was excised from an agarose gel and DNA was purified using the Qiaquick Gel Extraction Protocol (Qiagen). Approximately 5 ng of PCR product was used as template for sequencing using the PCR primers described above and the BigDye Terminator Sequencing Kit (Applied Biosystems, USA).

Table 1
Overview of Belgian PRV isolates from wild boar and domestic swine.

Isolate ID	Host species	Year	Country	Provence	*Bam*HI RFLP pattern	Phylogeny	Accession number
BEL10053	Wild boar	2006	Belgium	Antwerp	n.a.	A	KF779456
BEL20070	Hunting dog	2007	Belgium	Namur	Ip	B	KF779457
BEL20075	Hunting dog	2007	Belgium	Luxembourg	Ip	B	KF779458
LUX20484	Hunting dog	2010	Luxembourg	n.a.	Ip	B	KF779459
BEL24043	Wolf	2011	Belgium	Namur	Ip	B	KF415193
BEL50	Domestic swine	1973	Belgium	n.a.	Ip	A	KF779461
BEL55	Domestic swine	1976	Belgium	n.a.	Ii	A	KF779462
BEL2	Domestic swine	1988	Belgium	Antwerp	Ip	A	KF779460
BEL60	Domestic swine	1988	Belgium	West Flanders	IIi	A	KF779463
BEL62	Domestic swine	1988	Belgium	West Flanders	IIp	A	KF779464
BEL63	Domestic swine	1988	Belgium	Antwerp	IIp	A	KF779465
BEL68	Bovine	1988	Belgium	West Flanders	IIi	A	KF779466
BEL69	Domestic swine	1988	Belgium	Liège	IIp	A	KF779467
BEL71	Domestic swine	1989	Belgium	n.a.	IIp	A	KF779468

n.a.: Not available.