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Short communication

Molecular tracing of classical swine fever viruses isolated from wild boars and pigs in France from 2002 to 2011

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ABSTRACT

There were three outbreaks of classical swine fever (CSF) in north-eastern France between 2002 and 2011. The first two occurred in April 2002 in the Moselle department, in a wild boar and pig herd, respectively, while the third occurred in April 2003, in the Bas-Rhin department, in a wild boar. A survey was subsequently implemented in wild boar and domestic pig populations, during which 43 CSF viruses (CSFVs) were genetically characterized to provide information on virus sources, trace virus evolution and help in the monitoring of effective control measures. Phylogenetic analyses, based on fragments of the 5′NTR, E2 and NS5B genes, showed that all French CSFVs could be assigned to genotype 2, subgenotype 2.3. CSFVs isolated in Moselle were classified in the "Rostock" lineage, a strain first described in 2001 in wild boar populations in the Eifel region of north-western Rhineland-Palatinate in Germany, and in Luxemburg. In contrast, the CSFVs isolated in Bas-Rhin were homologous to strains from the "Uelzen" lineage, a strain previously isolated from wild boars in south-eastern Rhineland-Palatinate, Germany, as well as in Vosges du Nord, France, during a previous outbreak that had occurred in wild boars between 1992 and 2001. The outbreak in Moselle domestic pigs was quickly resolved as it concerned only one herd. The infection in wild boars from Moselle was extinguished after a few months whereas wild boars from Bas-Rhin remained infected until 2007. Molecular tracing showed that the Bas-Rhin index virus strain evolved slightly during the period but that no strain from a novel lineage was introduced until this outbreak ended after application of a vaccination scheme for six years.

Classical swine fever (CSF) is an immunosuppressive viral disease that affects pigs and can be responsible for severe economic losses to the pig industry. Wild boars are also susceptible to its causative agent, the CSF virus (CSFV) and constitute the natural reservoir and a source of transmission to domestic animal populations. CSFV is a single-stranded RNA virus belonging to the genus pestivirus in the *Flaviviridae* family. CSFV can be divided into three genotypes (1, 2 and 3), each comprising three to four subgenotypes (1.1–1.4; 2.1–2.3; 3.1–3.4) (Lowings et al., 1996; Paton et al., 2000; Postel et al., 2013). In the case of CSF outbreak, phylogenetic analyses can be helpful to trace the virus source and its evolution, and provide a useful tool in its control (Depner et al., 2006).

During the 1990s, an endemic outbreak occurred for ten years in wild boars in the north-eastern part of France (Vosges du Nord) and was considered eradicated in

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January 2002 (Rossi et al., 2005). However, in April 2002, one wild boar and a pig herd in the administrative department of Moselle (number 57) were successively found to be CSFV positive. A third outbreak occurred in April 2003 in one wild boar in the Bas-Rhin department (number 67). This paper reports molecular tracing of the causative viruses, and provides information on their origin and evolution over time, from the beginning to extinction of the three corresponding outbreaks that occurred during the last decade in this north-eastern area of France, which is close to borders with Luxemburg and Germany.

The first outbreak that occurred in Moselle in April 2002 (Moselle-1) was traced back to a female wild boar (index case) found dead by a hunter at Basse-Rentgen (Fig. 1). An epidemiological survey was carried out to trace and control the causative CSFV, as described previously (Pol et al., 2008). Briefly, all hunted or dead wild boars, located in a zone 10 to 15 km wide in the five French departments bordering Luxembourg and Germany, were spleen sampled for virological analyses (Fig. 1). Spleens were first analyzed by Reverse Transcription (RT)-Polymerase Chain Reaction (PCR) assays specific for all CSFV genotypes (Le Dimna et al., 2008; Mc Goldrick et al., 1999). PCR positive samples were then subjected to virus isolation (VI) on porcine kidney (PK15) endothelial cell cultures, as described in the European Community Manual for CSF diagnosis (Anonymous, 2002). Genetic typing of CSFV isolates included the sequencing of 150 nt located at the 5′-non-translated region (5′NTR), 190 nt located within the E2 glycoprotein gene and 409 nt located at the 3′ end of the NS5B polymerase gene (Paton et al., 2000). Briefly, viral RNA was extracted from cell culture supernatant using RNeasy Mini or QiaAmp Viral RNA kit (Qiagen). Synthesis of cDNA was carried out using the M-MLV Reverse Transcriptase enzyme (Promega) and either random hexamers (Promega) or the reverse primers used for subsequent PCR and listed thereafter. All primers, except 5NTR-Fo2, were described by Paton et al. (2000). The 5NTR-Fo2 sequence (5′ GTATACGAGGTTAGTTCATTC 3′) was kindly provided by the European Union Reference Laboratory for CSF, Hannover, Germany. Parts of 5′NTR (421 nt and 514 nt), the E2 gene (671 nt) and the NS5B gene (449 nt) were amplified by four polymerase chain reactions (PCRs) using primer pairs 5NTR-Fo1/5NTR-Rev1 (PCR1), 5NTR-Fo2/5NTR-Rev1 (PCR2), E2-Fo1/E2-Rev1 (PCR3) and NS5B-Fo1/NS5B-Rev1 (PCR4), respectively. PCRs were carried out with Taq DNA

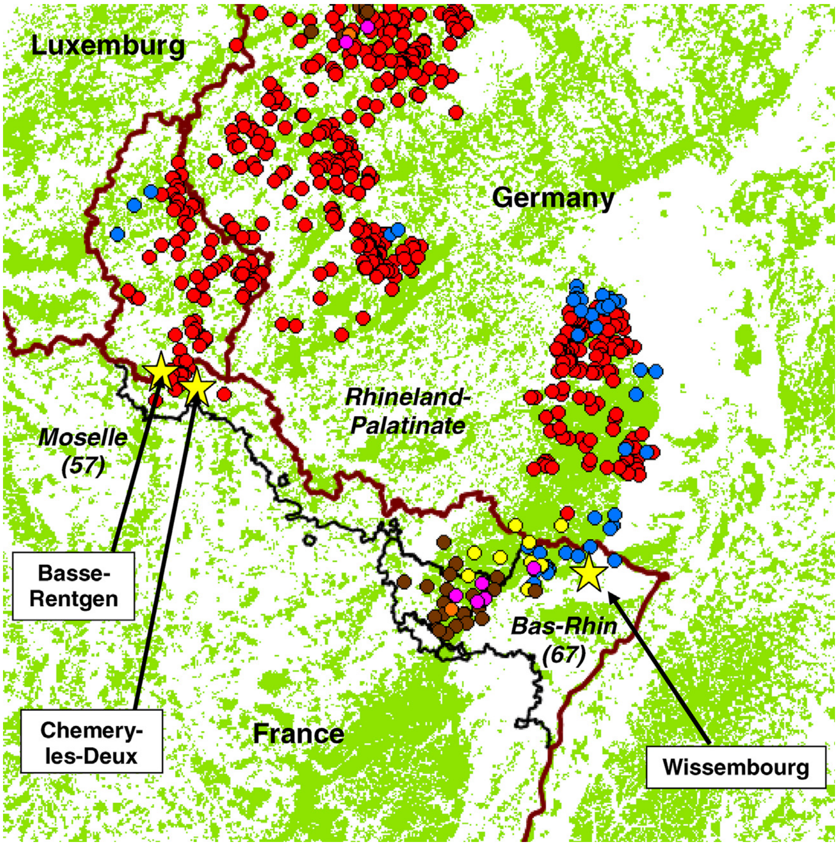

Fig. 1. Geographical localization of CSFV isolates obtained between 2002 and 2007 in France and bordering areas in Germany and Luxemburg. Borders between countries are indicated with a brown line. The sampling areas that were implemented in Moselle (department 57) and Bas-Rhin (department 67) are delimited with a black line. Dots represent virus isolates as follows: Red dots indicate isolates from 2002; blue dots, isolates from 2003; yellow dots, isolates from 2004; brown dots, isolates from 2005; pink dots, isolates from 2006; orange dots, isolates from 2007. The yellow stars locate the three outbreak index cases: a wild boar at Basse-Rentgen (index of the Moselle 1 outbreak), a pig herd at Chemery-les-Deux (index of the Moselle 2 outbreak), and a wild boar at Wissembourg (index of the Bas-Rhin outbreak).

Adapted from a map kindly provided by the EURL for CSF, Hannover, Germany.