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Oral immunization of wild boar and domestic pigs with attenuated live vaccine protects against Pseudorabies virus infection

Christina Maresch, Elke Lange, Jens P. Teifke, Walter Fuchs, Barbara Klupp, Thomas Müller, Thomas C. Mettenleiter, Thomas W. Vahlenkamp *

Friedrich-Loeffler-Institut, Federal Research Institute for Animal Health, 17493 Greifswald-Insel Riems, Germany

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ABSTRACT

In domestic pigs strict control measures and the use of gene-deleted marker vaccines resulted in the elimination of pseudorabies virus (PrV) infections in many parts of Europe and North America. In free-roaming feral pigs and wild boar populations, however, serological surveys and monitoring in The Americas, Europe and North Africa provided serological and virological evidence that PrV is more widely distributed than previously assumed. Thus, there is a constant risk of spillover of PrV infection from wild pig populations to domestic animals which could require intervention to limit the infection in wild pigs. To investigate whether oral immunization of wild boar by live-attenuated PrV could be an option, wild boar and domestic pigs were orally immunized with 2×10^6 $TCID_{50}$ of the attenuated live PrV vaccine strain Bartha supplied either with a syringe or within a blister, and subsequently intranasally challenged with 10^6 $TCID_{50}$ of the highly virulent PrV strain NIA-3. Oral immunization with live-attenuated PrV was able to confer protection against clinical signs in wild boar and against transmission of challenge virus to naïve contact animals. Only two vaccinated domestic pigs developed neurological signs after challenge infection. Our results demonstrate that oral immunization against PrV infection in wild boar is possible. In case increasing PrV infection rates in wild boar may enhance the risk for spillover into domestic pig populations, oral immunization of wild boar against PrV in endemic areas might be a feasible control strategy.

1. Introduction

Aujeszky's disease (AD, pseudorabies) is a notifiable disease caused by Suid herpesvirus 1 (SuHV1), often designated as Pseudorabies Virus (PrV) or Aujeszky's disease virus (ADV) (Mettenleiter, 2000). The disease has a worldwide distribution particularly in regions with dense populations of domestic pigs. In recent decades increased control efforts and the strict implementation of national eradication programs based on large scale vaccination with inactivated and/or live-attenuated vaccines, including genetically modified live-attenuated vaccine viruses and gene-deleted (so-called 'marker') vaccines, resulted in virtual disappearance of AD from domestic pigs in several parts of the world. In Europe, PrV has been eliminated from domestic pig populations in many countries including Austria, Cyprus, Czech Republic, Denmark, Finland, France, Germany, Hungary, Luxembourg, the Netherlands, Sweden, Switzerland, Slovakia, England, Scotland and Wales (Müller et al., 2003, 2011). In AD-free countries vaccination of domestic pigs against PrV is prohibited. Despite the tremendous progress made to control and eliminate the disease in domestic pigs, PrV infections seem to be widespread in populations of non-domestic swine, including feral pigs, wild boar and hybrids, across the world (Müller et al., 2011).

* Corresponding author at: Institute of Virology, Faculty of Veterinary Medicine, University Leipzig, Germany.
Tel.: +49 341 97 38 200; fax: +49 341 97 38 219.
E-mail address: thomas.vahlenkamp@uni-leipzig.de (T.W. Vahlenkamp).

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PrV can be transmitted via different routes. The virus is spread primarily by direct contact between swine or by contact with fomites, e.g. contaminated bedding and water, meat products, carcasses of rats, raccoons, swine, and other infected animals. The mucosae of the nose and oral cavity are the main entry points. Transmission among pigs can also occur during breeding from exposure to contaminated vaginal mucosa or semen. Virological and serological evidence for PrV infections in wild boar populations was documented during the last 20 years in the USA and in several European countries including, Czech Republic, Croatia, France, Germany, Italy, Netherlands, Slovenia, Poland, Russia, Switzerland and Spain (Oslage et al., 1994; Szweda et al., 1998; Albina et al., 2000; Müller et al., 1998, 2000; Zupancic et al., 2002; Gortazar et al., 2002; Vengust et al., 2006; Shcherbakov et al., 2007; Leuenberger et al., 2007; Sedlak et al., 2008; Pannwitz et al., 2012). Transmission of PrV to hunting dogs and other wild carnivores by direct contact with infected animals was reported during recent years in Austria, Belgium, France, Germany, and the USA (Glass et al., 1994; Thaller et al., 2006; Cay and Letellier, 2009; Müller et al., 2010; Cramer et al., 2011) which supports the notion that PrV is more widespread among wild boar populations than previously assumed.

Despite increasing PrV antibody prevalence in wild boar no spillover infections from wild boar to domestic pigs have been reported in Germany during an observation period of more than two decades. Therefore, under the prevailing epidemiological conditions PrV-infected non-domestic swine appear to pose only a limited risk to domestic animals (Pannwitz et al., 2012).

In PrV infected non-domestic swine, clinical signs are rare indicating that prevailing PrV variants are highly adapted to the host population (Müller et al., 2001). Only from Spain and Germany, rare cases of spontaneous clinical AD in juvenile wild boar have been reported indicating that these field viruses can induce disease in wild pigs that is clinically and pathologically identical to AD in domestic pigs (Gortazar et al., 2002; Schulze et al., 2010).

Although reports of PrV transmission from feral pigs and wild boar to domestic pigs are surprisingly rare across the world, outdoor pig farms are at higher risk and, therefore, deserve special risk mitigating measures and close attention in serological monitoring for the maintenance of an AD-free status (Müller et al., 2011). As a possible spillover cannot completely be ruled out potential strategies to prevent transmission of PrV from endemically infected wild boar to domestic pigs have to be investigated. Oral vaccination has been shown to be a powerful intervention strategy to combat infectious diseases in wildlife. It was successful in foxes, raccoon dogs and wild boar against rabies and classical swine fever (CSF), respectively (Rupprecht et al., 2006; Kaden et al., 2000). So far, experiments to test for efficacy of oral vaccination of wild boar against PrV, however, have not yet been performed. Therefore, the objective of the current study was to investigate (i) whether experimental oral application of a modified-live PrV vaccine causes clinical signs and/or the production of virus neutralizing antibodies, (ii) whether orally immunized pigs are protected from virulent PrV challenge infection, and (iii) whether oral immunization using a modified-live PrV strain results in virus excretion or transmission of the vaccine virus to naïve contact animals.

2. Materials and methods

2.1. Animals

Wild boar were bred at the animal facility of the Friedrich-Loeffler-Institut (FLI) and used in the studies at the age of five months. Ten-week-old domestic pigs were derived from a local PrV-free breeder. For the vaccination and challenge experiments all pigs were housed at the high containment animal facility (BSL3+) at the FLI. Animals were seronegative against PrV at the beginning of the experiments as determined by seroneutralization assay. Animals were housed in pens, and feed and water were available ad libitum and were replenished once a day. Animal experiments were approved by the ethics committee and performed in accordance with the German Animal Welfare Act.

2.2. Experimental design

2.2.1. Experiment 1

To investigate whether oral immunization by live-attenuated PrV can elicit neutralizing antibodies and confer protection against a lethal challenge infection, four domestic pigs and four wild boars were immunized orally with 2×10^6 $TCID_{50}$ of the attenuated PrV modified live-vaccine strain Bartha. The virus was orally inoculated without anesthesia into the oral cavity with a syringe in 2 ml volume. The animals were physically examined daily and nasal swabs were taken at regular intervals to monitor virus excretion after vaccination and after challenge infection. EDTA-blood and serum samples for serological analysis were taken from domestic pigs and wild boar at day 21 and 28 post vaccination (p.v.), when animals were challenge infected intranasally with 10^6 $TCID_{50}$ in 1 ml of the highly virulent PrV strain NIA-3 (Pol et al., 1989). Four naïve animals (one wild boar and three domestic pigs) were similarly infected and served as infection controls. The animals were physically examined daily and nasal swabs were taken at regular intervals after vaccination and post challenge infection (p.i.) to monitor virus excretion. EDTA-blood and serum samples were taken on days 0, 3, 5, 7, 10, 14, and 21 p.i. Animals were euthanized and investigated by necropsy on day 21 p.i. unless they developed clinical symptoms and had to be euthanized before.

2.2.2. Experiment 2

Four pigs and three wild boar were immunized orally with 2×10^6 $TCID_{50}$ of the attenuated live PrV vaccine strain Bartha. In contrast to the first experiment the virus was given in a 2 ml volume within a blister. For this purpose the pigs and wild boar were anesthesized and manually forced to bite the blister. The animals were physically examined daily. EDTA-blood and serum samples for serological analysis were taken from the wild boar and pigs on days 14 and 21 p.v., respectively. The animals were intranasally challenged with 10^6 $TCID_{50}$ of PrV strain NIA-3 on day 28 p.v. Four PrV naïve