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SHORT COMMUNICATION

Prevalence of *Haemophilus parasuis* infection in hunted wild boars (*Sus scrofa*) in Germany

Gerald Reiner · Christina Fresen · Sebastian Bronnert · Ingo Haack · Hermann Willems

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Abstract *Haemophilus parasuis* is the etiological agent of Glässer's disease, often involved in pneumonia, and also an early colonizer of the upper respiratory tract of healthy domestic pigs. Little information is available on *H. parasuis* in wild boars. The aim of the present study was to evaluate *H. parasuis* infection in wild boars in Germany. Tissue samples from the lungs and tonsils of 531 wild boars from 52 hunts during the hunting seasons 2004/2005 to 2006/2007 were examined independently for *H. parasuis* by PCR because *H. parasuis* is a fastidious organism, which hampers its isolation from clinical samples. The overall prevalence of *H. parasuis* in wild boars in Germany was 74.2%. *H. parasuis* was detected in 69.1% of tonsils and 40.4% of lungs. In conclusion, the present study demonstrates a wide distribution of *H. parasuis* in German wild boar populations and further research is required to understand the virulence of *H. parasuis* strains in wild boars, as well as the distribution and potential exchange of different strains between wild boars and domestic pigs.

Keywords *Haemophilus parasuis* · Epidemiology · Wild boar

Introduction

Haemophilus parasuis infection is considered one of the economically most important diseases in swine because of the high costs of antibiotic treatments and loss of animals (Oliveira et al. 2001). The gram-negative, non-haemolytic, nicotinamide adenine dinucleotide-dependent bacterium is the etiological agent of Glässer's disease, a syndrome characterized by fibrinous polyserositis, arthritis, and meningitis. The bacterium is an early colonizer of pigs and is commonly isolated from the upper respiratory tract of healthy individuals (Oliveira et al. 2001; Oliveira and Pijoan 2004; Rapp-Gabrielson et al. 2006). Regarding the lower respiratory tract, *H. parasuis* has been isolated from domestic pigs with pneumonia, but the organism is not commonly isolated from normal lungs (Little 1970; Moller et al. 1993). Isolation of *H. parasuis* from pigs with pneumonia has increased substantially during the past few years (Rapp-Gabrielson et al. 2006).

There is little information available regarding *H. parasuis* infection in wild boars (*Sus scrofa*). Glässer's disease has never been reported (Olvera et al. 2007), and in one of two studies that have attempted to detect *H. parasuis* in wild boar by serology, no antibodies were detected in 78 animals from southcentral Spain (Vicente et al. 2002). In the second study, 18% of wild boars in Slovenia were seropositive (Vengust et al. 2006). In an attempt to isolate *H. parasuis* from the respiratory tract of wild boars in Spain, Olvera et al. (2007) cultured two strains of *H. parasuis* from nasal swaps of 4.8% of boars sampled.

Diagnosis of *H. parasuis* infections is mostly based on clinical signs, presence of lesions, and isolation of the organism. *H. parasuis*, however, is a fastidious organism, which hampers its isolation from clinical samples (Rapp-Gabrielson et al. 2006).

In the present study, we used a PCR assay to evaluate the prevalence and distribution of *H. parasuis* infection in wild boars in Germany. To determine if there is a difference between the prevalence of infection between the upper and

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G. Reiner (✉) · C. Fresen · S. Bronnert · I. Haack · H. Willems
Department of Veterinary Clinical Sciences, Swine Diseases,
University of Giessen,
Frankfurter Strasse 112,
35392 Giessen, Germany
e-mail: gerald.reiner@vetmed.uni-giessen.de

lower respiratory tract of wild boars, we analysed the tonsils (Oliveira et al. 2001; Rapp-Gabrielson et al. 2006) and lung tissue (Little 1970; Moller et al. 1993) from each boar separately.

Materials and methods

The total area of Germany covers 357,000 km^2. Actual numbers of wild boars are unknown, however, during each hunting season from 2004 to 2007, an average of 400,000 wild boars were shot (DJV 2009). Samples for the present study were collected between latitudes 48.1° and 54.5° N and between longitudes 6.1° and 14.0° E.

Tonsils and lung samples were collected from 531 boars during 52 hunts. The hunts took place in 14 of the 16 federal states of Germany. Samples were collected from boars hunted from November 2004 to March 2007. Samples were taken randomly. Age was determined based on eruption of teeth (Stubbe 2001). The sex ratio of the sampled wild boars was 44%:56% (males/females). The average age was 11 months (range, 1 to 84 months) and the average carcass weight was 35 kg (range, 5 to 120 kg).

Samples of approximately 5 cm^3 were taken 0.5 to 2 h after death using new sterile disposable gloves and scalpel blades to collect each tonsil and lung sample. Tonsils were excised from the dorsal part of the pharynx. Lung samples were removed from the right dorsal lobe. They were stored in individual freezer bags at−20°C until further usage.

DNA was extracted separately from lung and tonsil samples with the Qiagen Viral RNA Mini kit (Qiagen, Hilden, Germany) as described elsewhere (Reiner et al. 2010).

H. parasuis-specific DNA was amplified as described by Reiner et al. (2010), but with hairpin-shaped primers HPS-forward (5′-TGA TGA GGA AGG GTG GTG T-3′) and HPS-reverse (5′-GGC TTC GTC ACC CTC TGT-3′) (Oliveira et al. 2001) and an annealing temperature of 63°C. The sensitivity of this PCR is 10^2CFU/mL (Olvera et al. 2007). The PCR covers all *H. parasuis* serotypes (Oliveira et al. 2001) and excludes other *Pasteurellaceae* or less-related bacteria, except *Actinobacillus indolicus*. In order to exclude amplification of *A. indolicus*, we used a Taq DNA polymerase, which makes it possible to increase the annealing temperature from 59°C (Oliveira et al. 2001) to 63°C and to decrease the extension time from 2 to 1 min. Success of this approach was tested with up to 10^8 DNA copies of *A. indolicus* isolate 46KC2 (German Collection of Microorganisms and Cell Cultures, DSMZ) per PCR reaction, which did not provide any signal.

Statistics were calculated with the Statistical Package for Social Sciences, version 15.0 (SPSS, Inc., Chicago, IL, USA). Differences in tissue prevalence and effects of hunting ground, season, and sex were calculated with Chi^2-test. Effects of age and body weight were calculated by the Spearman correlation analysis. The threshold for statistical significance was set to <0.05.

Results

The overall prevalence of *H. parasuis* in wild boars (tissue independent) in Germany was 74.2%. *H. parasuis* was detected in 69.1% of the tonsils and 40.4% of the lungs. *H. parasuis* was detected in the tonsils but not in the lungs in 33.8%, in the lungs but not in the tonsils in 5.1%, and in both tissues in 35.5% of the sampled wild boars. Figure 1 shows the spatiotemporal distribution of hunting grounds and the respective prevalence of *H. parasuis*. Over the complete time period, there were significant differences in prevalence between states, with overall lowest values in Berlin, Saarland, Hesse, and Brandenburg and highest values in Schleswig–Holstein, Bavaria, Baden-Wurtemburg, and Mecklenburg–West Pomerania (Fig. 1).

Whether wild boars were infected with *H. parasuis* or not was not significantly influenced by sex, age, or body weight.

Discussion

The present study provides prevalence of *H. parasuis* in wild boars in Germany. *H. parasuis* DNA was detected in 74.2% of the sampled individuals by PCR. Seroprevalences reported in recent studies from Spain (Vicente et al. 2002) and Slovenia (Vengust et al. 2006) were low (0 to 18%) and Olvera et al. (2007) detected *H. parasuis* in 4.8% of wild boars from Spain by culture and PCR. Although these studies represent different methods, prevalences are consistently lower than those identified in the present study. The difference may thus be based more on the hunting location than on the methodology.

H. parasuis is regularly isolated from the upper respiratory tract of healthy pigs (Oliveira et al. 2001; Oliveira and Pijoan 2004; Rapp-Gabrielson et al. 2006), indicating that pigs can be a reservoir for this pathogen without the necessity to develop disease. Thus, the usefulness of the PCR with the nasal or tonsillar swabs in clinical diagnostics has been questioned (Olvera et al. 2007). With regard to the lower respiratory tract, *H. parasuis* is more frequently isolated from cases with pneumonia than from normal lungs (Little 1970; Moller et al. 1993). Significant differences in virulence between *H. parasuis* are well documented and susceptibility of pigs to *H. parasuis*-associated disease depends on the individual resistance and immunity as well as on distinct risk factors. Thus, whether an *H. parasuis* infection stays in the upper or

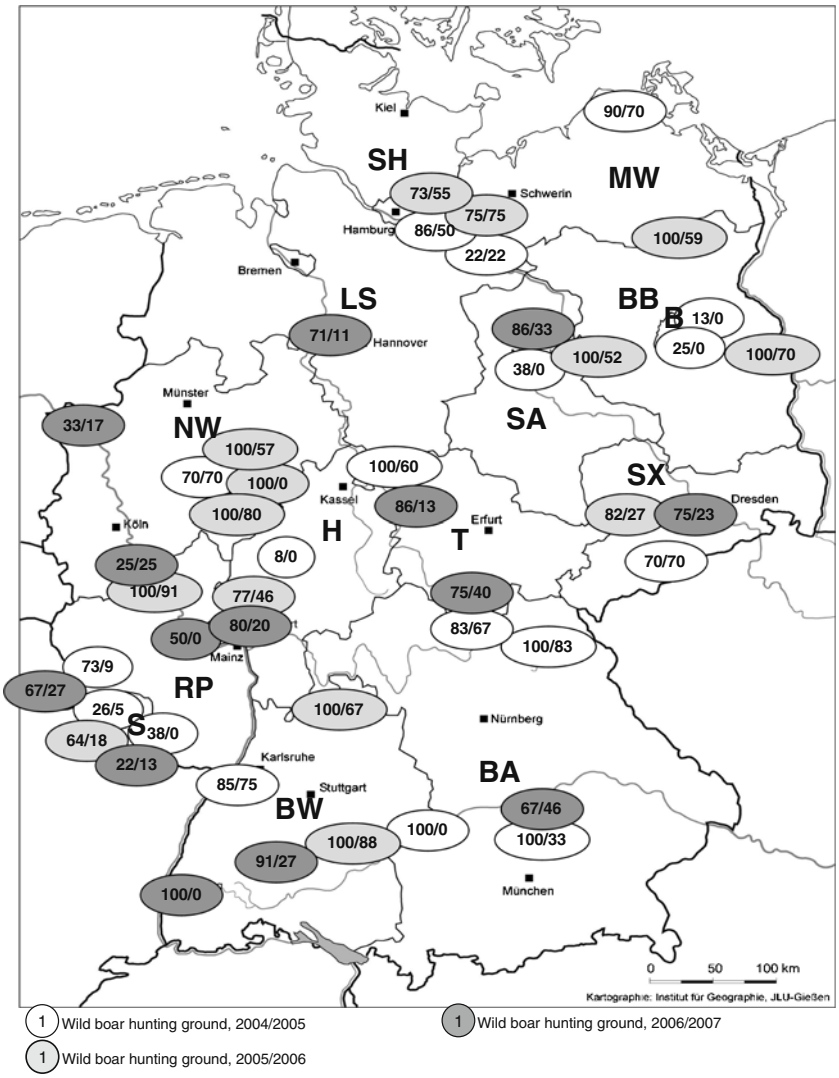

Fig. 1 Spatiotemporal distribution of wild boars infected with *H. parasuis* in Germany. *Numbers* show prevalence by hunting ground (*H. parasuis* DNA in the tonsils *left* and lung tissue *right*, respectively). *White circles* hunting season 2004/2005, *light grey circles* hunting season 2005/2006, *dark grey circles* hunting season 2006/2007. *Bold letters B* Berlin, *BA* Bavaria, *BB* Brandenburg, *BW* Baden-Wurtemburg, *H* Hesse, *MW* Mecklenburg–West Pomerania, *NW* North Rhine–Westphalia, *LS* Lower Saxony, *RP* Rhineland-Palatinate, *SX* Saxony, *SA* Saxony-Anhalt, *SH* Schleswig–Holstein, *S* Saarland, *T* Thuringia

reaches the lower respiratory tract, depends on both, factors of the agent and of the host that have not been addressed by the present study. Considerable differences in the prevalence of *Actinobacillus pleuropneumoniae* between the lungs (6.4%) and the tonsils (34.8%) argue for the carrier status of wild boars regarding a further member of the *Pasteurellaceae* (Reiner et al. 2010). We have, therefore, isolated DNA from the tonsils and lung tissue separately. With the improved PCR assay, prevalences of the *H. parasuis* DNA turned out to be high in both tissues. However, in spite of the high prevalence of *H. parasuis* in the lung tissue, the sampled wild boars did not show any gross pathological signs of disease.

In conclusion, wild boars have to be interpreted as a reservoir for *H. parasuis*. While cross contamination between samples can be excluded because of the sampling technique, these high lung prevalences without signs of disease might be explained by either low virulence of the strains or high resistance of the wild boar to *H. parasuis* or both. The high prevalence of *H. parasuis* in wild boars in Germany might be interpreted as a high risk for domestic pig infection as the spread of pathogens from wild boars to domestic pigs is not uncommon. On the other hand, wild boar populations might be under risk to be infected with *H. parasuis* from domestic pig herd emissions. Further research should aim at the identification of *H. parasuis* strains involved and their respective virulence and at comparing them with strains isolated from domestic pigs. This will provide further insight into the degree and impact of exchange of *H. parasuis* between both species.

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