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PSEUDORABIES IN THE EUROPEAN WILD BOAR FROM EASTERN GERMANY

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ABSTRACT: Sera collected from European wild boar (*Sus scrofa*) shot in Eastern Germany between January 1991 and December 1994 were tested for antibodies to pseudorabies virus (PRV). Of 3,143 sera tested, 281 (8.9%) and 13 (0.4%) were positive and suspect in an enzyme-linked immunosorbent assay (ELISA), respectively. The specificity of the reactions was confirmed by detection of neutralizing antibodies in 220 sera (74.8%) and by immunoblotting. Analysis of host age and sex of the animals, temporal and spatial factors showed significantly higher seroprevalences in older animals than in younger individuals, but no differences between males and females. Pseudorabies virus infections have been endemic in this wild boar population for several years and the extreme eastern part of the study area had significantly higher seroprevalences (≤22%) than other areas. In the area covered by this study, pseudorabies virus was eradicated in the domestic animal populations in 1985. Thus, the infections in the wild boar population appear to be endemic and persist completely separately and without affecting the domestic pig population.

Key words: Aujeszky's disease, epidemiology, pseudorabies, seroprevalence, *Sus scrofa*, wild boar.

INTRODUCTION

Although most mammals are susceptible to infections with pseudorabies virus (PRV) (Fenner et al., 1987), pigs seem to represent the main host reservoir (Pensaert and Kluge, 1989). From an epidemiological point of view, it is important to characterize the susceptibility and reservoir function of wildlife for economically important diseases. Due to its high impact on the economy of pork production, PRV requires particular consideration (Blancou and Barrat, 1984, Corn et al., 1986). However, only limited data exist about natural infections of wildlife animals with this virus.

Due to its evolutionary relatedness with the domestic pig, the European wild boar (*Sus scrofa*) may serve as a reservoir for PRV (Corn et al., 1986, van der Leek et al., 1993; Kirkpatrick et al., 1980). Natural infections of feral swine with PRV have been reported from the USA, where the populations in 11 of 17 states with feral swine or wild boar are seropositive (Nettles and Erickson, 1984, Nettles, 1991). The overall seroprevalence among these feral swine populations amounts to 19%, especially in the southeastern states (Nettles, 1989), while other regions appear to be free of PRV infections (New et al., 1994). Although the extent of clinical disease and the prevalence of latent infections in wild boar is unknown (van der Leek et al., 1993), PRV-infected feral swine represent a potential source of infection for domestic pigs in North America (Creswell, 1989, Ormiston, 1989). By contrast, reports on PRV-infections of wild boar in Europe are scarce (Dahle et al., 1993). Wild boar populations in different regions of France appeared to be free of PRV (Baradel et al., 1988). Circumstantial evidence exists for sporadic PRV-infection of wild boar in Germany (Dedek et al., 1989, Dahle et al., 1993, Oslage et al., 1994) and the Netherlands (Cromwijk, 1995). Eastern Germany is known for high population densities in wildlife, especially wild boar (Briedermann, 1982).

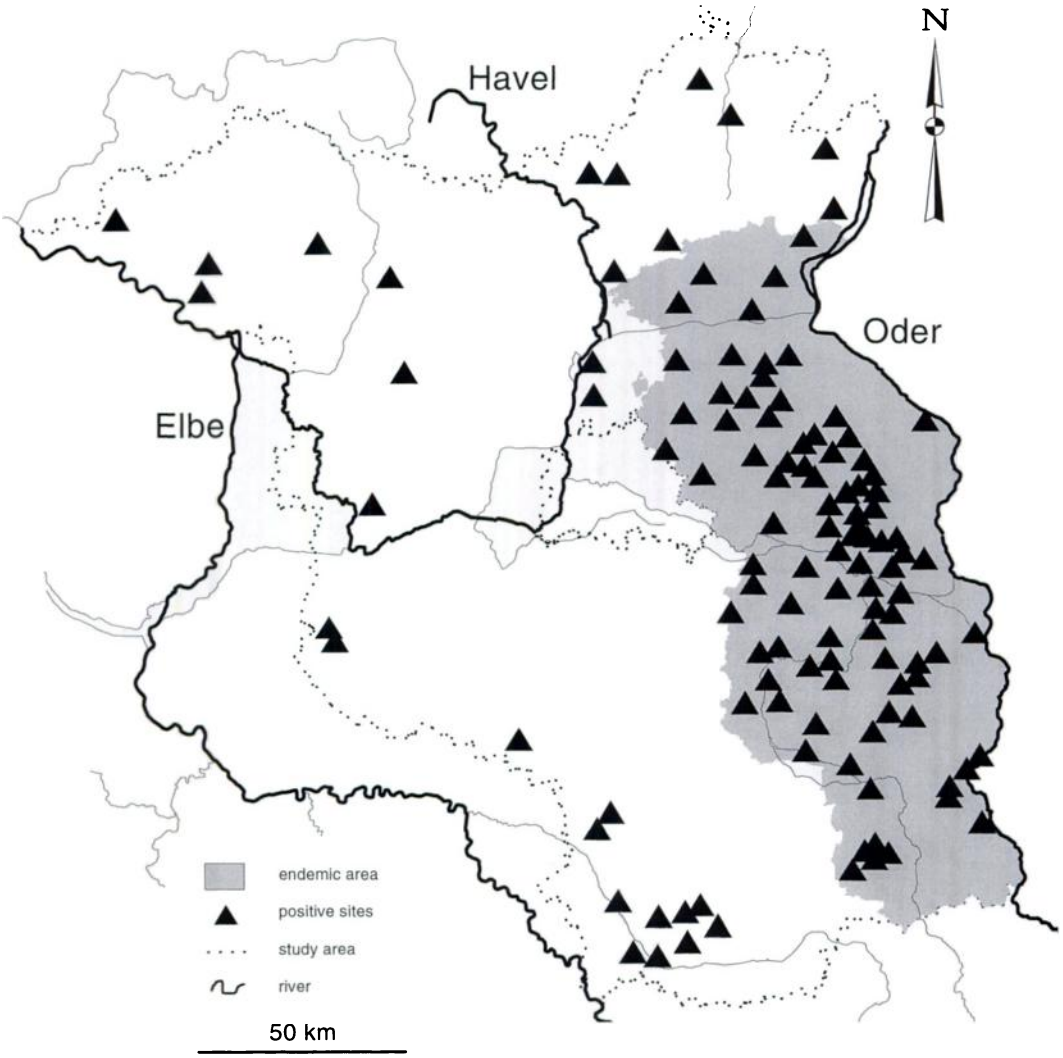

FIGURE 1. Origin of pseudorabies-seropositive samples (triangles) from individual wild boar based on municipality level plotted in a map of the study area in eastern Germany. Municipalities with significantly higher seroprevalences ($P < 0.05$) during the observation period as compared with other regions of the study area were defined as the endemic area which is marked by a gray background. The Elbe, Havel and Oder rivers are indicated.

From 1991 to 1994 a wildlife disease monitoring project on the occurrence of selected viral diseases was conducted in parts of eastern Germany. The objective of the study presented here was to assess the occurrence of PRV-infection in the indigenous wild boar population.

MATERIALS AND METHODS

The study area comprised a region in the eastern part of Germany (53°23′ to 51°22′N; 11°45′ to 14°42′E) covering 29,480 km^2 (Fig. 1). Sera were obtained from wild boar shot between January 1991 and December 1994. Samples were collected throughout the year with special emphasis on the main hunting season in fall and winter (end of October until end of January) either by members of local hunting associations, state forest officers or veterinarians, and were subsequently sent to the laboratory (Institute for Epidemiological Diagnostics, Federal Research Centre for Virus Diseases of Animals, D-16868 Wusterhausen, Germany; State Veterinary and Food Investigation

Centre Frankfurt, D-15234 Frankfurt/Oder, Germany) by courier. Delivery of the sera took 1 to 3 days. Prior to testing, the sera were centrifuged and stored in 1 ml aliquots at −30 C. Sex, age of the animals, origin and sampling time were recorded for descriptive epidemiological analysis. Determination of the age structure of shot wild boar was established using tooth eruption patterns (Matschke, 1967). Animals were divided into three categories consisting of animals <12-mo-old and 12- to <24-mo-old (juveniles), and >24-mo-old (adults).

A commercially available enzyme-linked immunosorbent assay (ELISA; Chekit® Aujeszky-ELISA, Dr. Bommeli AG, CH-3097 Liebefeld/Bern, Switzerland) licensed for routine diagnosis in domestic pigs was used for the detection of PRV-specific antibodies according to the instructions of the manufacturer. The test represents an indirect assay employing PRV antigen-coated microtiter plates. In brief, sera were reacted with the antigen and bound antibodies detected with an anti-pig-IgG-peroxidase-conjugate. The optical density (OD) of a field serum was compared with the OD of positive control (PC) and negative control sera (NC) using the following equation: $\%\text{-}OD_{492}$ (sample) $= [(OD_{sample} - OD_{NC})/(OD_{PC} - OD_{NC})] \times 100\%$. Values of ≤30% were regarded as negative, values of ≥50% were considered positive. Wild boar sera with values between 30% and 50% were regarded as suspect.

All ELISA-positive and -suspect wild boar sera were also investigated in a virus neutralization test (VNT) against plaque-purified field PRV (strain Stendal/64) essentially as described by Dahle et al. (1993), but without guinea pig complement. Following inactivation at 56 C for 30 min and centrifugation for 10 min at 2,000 × g, sera were titrated in $\log_2$ steps. Serum dilutions were incubated with 100 $TCID_{50}$ PRV for 24 h at 37 C in a total volume of 200 µl (Bitsch and Eskildsen, 1982). Sera with titers ≥1:4 were considered positive. In selected sera (n = 10), the specificity of anti-PRV antibodies was also confirmed by immunoblotting as described by Sherba et al. (1991).

For geographical analysis, results were plotted on maps using the desktop mapping software "RegioGraph" (Version 1.2a, Macon Markt und Konzept, Waghäusel, Germany). Depending on the sample size, chi-square or Fisher's exact test (Freeman and Halton, 1951) served for the detection of statistical differences in seroprevalences between subpopulations (sex, age groups). The calculations were conducted using the software package EPI-INFO, version 5.0 (April 1990, Public Domain Software for Epidemiology and Disease Surveillance, Centers for Disease Control and Prevention, Epidemiology Program Office, Atlanta, Georgia, USA; Statcalc Epi Calculator). The significance level was set to $P = 0.05$. The 95% ($P = 0.05$) confidence interval (CI) limits for the estimation of the true seroprevalence within the wild boar population were determined according to the method of Willer (1982). A time series analysis (bivariat fourier cross spectrum analysis) was applied to proof correlations between PRV-positive findings and sampling densities over time using the software package Statistica 5.0 (StatSoft. Ltd., Tulsa, Oklahoma, USA).

RESULTS

Of 3,143 wild boar sera assayed by ELISA, 281 (8.9%) yielded positive readings. In addition, 13 sera (0.5%) were classified as suspect. When positive and suspect sera (294) were tested by VNT, 220 sera were confirmed as positive and had neutralizing antibody titers ranging between 1:4 and 1:2048, with an average of 1:16 to 1:128 (Fig. 2). The overall seroprevalence for the whole observation period and the entire study area was 8.9% with 95% CI limits of 8.0% and 10.2% (Table 1). Statistically significant differences of the seroprevalences in different years of the observation period could not be detected. Selected PRV-positive and suspect sera (n = 10) strongly reacted with several viral antigens on westernblots, whereas no reactivity was observed when negative sera were assayed (data not shown).

While seroprevalences did not differ between male and female wild boar, there was a significant positive correlation ($P < 0.05$) with the age of the animals (Fig. 3). The seroprevalence of animals <12-mo-old and 12- to <24-mo-old was determined as 4.7% and 10.1%, respectively. Adults showed the highest seroprevalence at 16.7%. The temporal differences between the seroprevalence and the monthly sampling density during the observation period could not be confirmed by a bivariat fourier (cross spectrum) analysis (Fig. 4).

Pseudorabies virus-positive sera were found in 105 of 1,696 municipalities within the study area. Ninety four percent of the

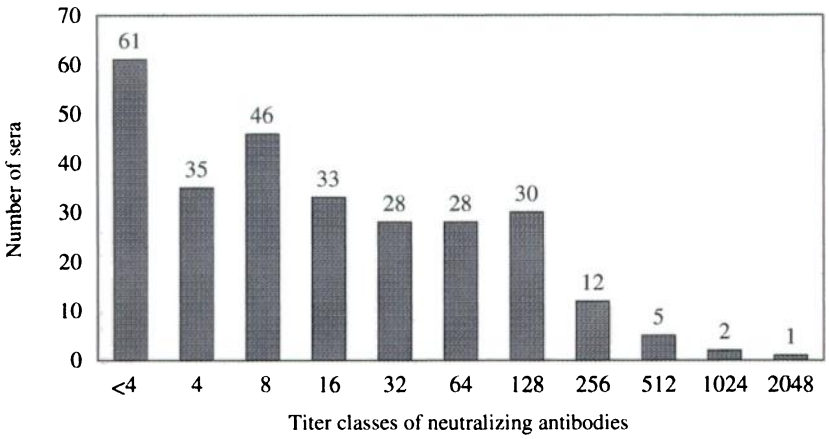

FIGURE 2. Number of ELISA-positive and suspect wild boar sera tested in the virus neutralization test plotted against the titer classes in the neutralization test for pseudorabies in the wild boar population from Germany.

PRV-positive sera originated from the most eastern parts of the study area adjacent to the Oder River, whereas in the western parts of the study area positive serum samples were found only sporadically (Fig. 1). The seroprevalences in the eastern region were significantly higher ($P < 0.05$) than in the remaining area and reached levels of 22% and above in certain municipalities (Fig. 1).

DISCUSSION

The collection of sera with a higher sampling frequency during the main hunting season was the only possible way to obtain large, representative sample sizes from free-ranging wild boar. However, hemolysis and dilution of samples are potential disadvantages of this sampling method (van der Leek et al., 1993).

TABLE 1. Estimated percentages of ELISA-positive wild boar sera 1991–94 and calculated 95% confidence intervals of the true pseudorabies virus seroprevalence in the wild boar population from eastern Germany.

Year	Number sampled	ELISA-positive	Seroprevalence in %	95%-confidence interval
1991	169	9	5.3	2.5–11.7
1992	377	34	9.0	6.5–12.9
1993	1,411	125	8.9	7.6–10.8
1994	1,186	113	9.5	8.2–11.4
Total	3,143	281	8.9	8.0–10.1

Of 294 sera tested positive or suspect by ELISA, 220 (74.8%) contained neutralizing antibodies and frequently had high titers (Fig. 2). This finding is in accord with the results of Martin et al. (1983), who showed that the ELISA is more sensitive and thus detects PRV-infections earlier than the VNT. Comparable high neutralizing antibody titers against PRV were reported from North American feral swine populations (Nettles and Erickson, 1984). Immunoblotting of selected sera confirmed the specificity of the antibodies for PRV (Ben-Porat et al., 1986; Eloit et al., 1988).

The percentage of PRV-positive sera (8.9%) detected in this study is distinctly higher than seroprevalences previously reported from most other regions in Germany. Only Lutz and Wurm (1996) detected a comparable seroprevalence (7%) in North Rhine-Westphalia, whereas Dedek et al. (1989) and Oslage et al. (1994) found seroprevalences of 0.3% and 0.9% in regions north and west of our study area. From Lower Saxony (Germany) (Dahle et al., 1993) and the Netherlands (Cromwijk, 1995) seroprevalences of 1.7% and 2.6% were reported. Clark et al. (1983) found 3.0% in certain regions of

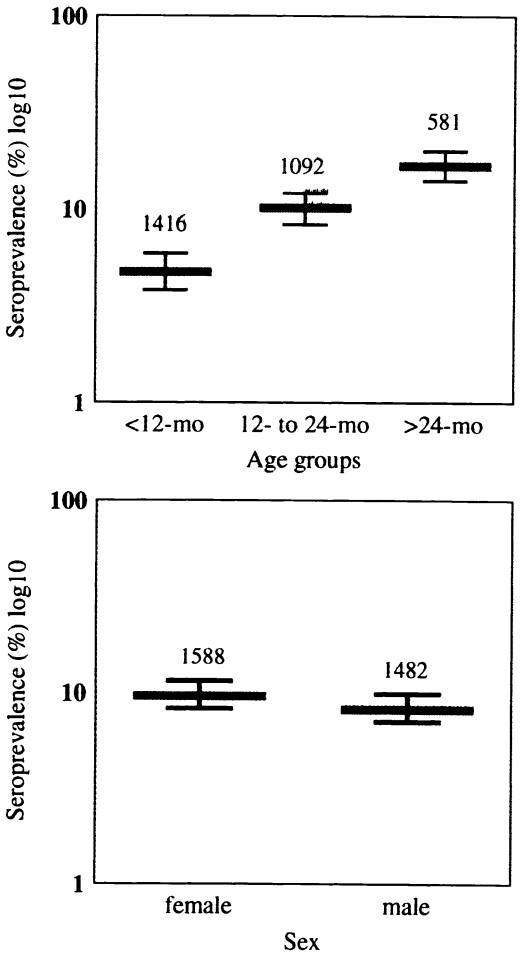

FIGURE 3. Distribution of pseudorabies antibodies by host age and sex as determined by an enzyme-linked immunosorbent assay in the wild boar population from eastern Germany. Estimated seroprevalences (%) and 95%-confidence intervals are shown.

California (USA). With respect to the upper limits of the confidence intervals of the true seroprevalences (Table 1), the situation in the study area seems to resemble PRV infections in feral swine populations in the USA, where the overall seroprevalence is estimated as 19% nationwide (Nettles, 1991). However, from an epidemiological point of view it has to be recognized that wild boar represent genuine wildlife in Germany. Feral swine in the USA originated from two ancestral pools including (1) hogs descendent from domestic stock that had run wild during the last four centuries and (2) Russian wild boar (European wild boar) introduced for hunting purposes. Today's feral swine are all hybrids to some extent (Nettles and Erickson, 1984).

Since the seroprevalences between males and females did not differ significantly, there is no indication that the sexes are exposed to an infection with PRV in different ways. However, our data indicate that the risk of infection increases with the age of the animals. The significant differences between the age groups (Fig. 3) indicates a higher risk of older individuals (>24-mo-old) in contracting a PRV infection. These results agree with findings of van der Leek et al. (1993) and Pirtle et al. (1989) who found up to 70.0% or 28.5%, respectively, of adult animals seropositive.

Piglets can possess maternal antibodies for approximately 15 wk as demonstrated for domestic pigs (Pensaert and Kluge, 1989). Since hunting wild boar piglets of <3-mo-old is prohibited in Germany, nothing is known about the persistence of maternal antibodies in wild boar. As judged by the age-stratified seroprevalences, young wild boar (3- to 12-mo) appear to be at a lower risk of PRV infection and may contract the infection by direct contact. Alternatively, PRV infections may be highly lethal for young animals thus leading to an underrepresentation of seropositive wild boar in the respective age group. Experimental infections show, however, that PRV isolates obtained from wild boar do not lead to clinical disease in wild boar (T. Müller et al., unpubl. data). Recently it was shown that venereal transmission of PRV is the main route of infection in a feral swine population in Florida (Romero et al., 1997). The significantly higher PRV seroprevalence in older, sexually mature animals may indicate that this route of infection may also play a role in European wild boar. In any case, the differences of the prevalences in different age classes show that the spread of the infection in the population is relatively slow.

Although the project was launched in

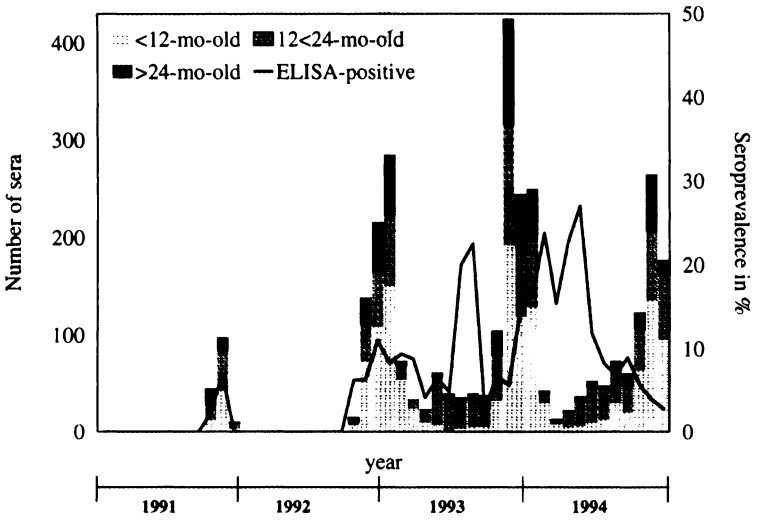

FIGURE 4. Monthly sample sizes (number of sera) for pseudorabies samples from wild boar in eastern Germany and seroprevalences plotted against time for the years 1991 until 1994.

1991, most of the sera were collected during the hunting seasons between 1992 and 1994. The seroprevalence seemed to undergo oscillations with peaks in summer 1993 and winter 1994 with maxima in August and February, respectively. The bivariat fourier (time series) analysis showed statistically significant differences ($P < 0.05$) indicating that this seasonality does not reflect a biological aspect of the infection itself because the oscillation of the seroprevalence heavily depended on the monthly sampling density. Furthermore, the variation of the seroprevalence may also be influenced by the age distribution of the sample (Fig. 4).

The implementation of the monitoring project by local veterinary and forest authorities warranted a balanced spatial distribution of the samples. Although the origin of serum samples is certainly influenced by hunting activities, the relatively homogeneous spatial distribution of the samples mirrors the fact that the study area represents an optimal habitat for wild boar (Briedermann, 1982). Seropositive animals were repeatedly found in distinct locations near the Oder River east of the study area resulting in significantly higher seroprevalences ($P < 0.05$) in this region as compared with other regions of the study area. The epidemiological situation in certain districts within this endemic region parallels that described for Florida (USA) (van der Leek et al., 1993), Texas (USA) (Corn et al., 1986) and Hawaii (USA) (Nettles and Erickson, 1984) with reported seroprevalences of 34.8%, 36.0% and 46.0%, respectively, and underlines the endemic character of the infection in this region (Fig. 1).

The fact that we found no significant differences in the seroprevalences over time indicates a constant infection pressure in the wild boar population. Yet, during the past 2 yr we also observed small numbers of seropositive animals in regions which were previously considered non endemic (Fig. 1). To some extent, an increase in hunting activities may have lead to more disturbances of the animals resulting in migration, higher contact rates and possible spread of PRV infections.

While in the western parts of Germany efforts have been undertaken to control PRV by national eradication programs using gI-deleted PRV-vaccines (Pittler and Rohjahn, 1990), PRV had already been eradicated in most parts of Eastern Germany since 1985. It has been suspected

that PRV can be transmitted by aerosol over several miles (Christensen et al., 1990), thus PRV-infected wild boar may endanger domestic animals and the success of eradication programs. However, no evidence of disease transmission to domestic pigs has been found in the study area, despite the presence of an endemic focus.

The importance of clinical disease and the prevalence of latent PRV infections in free-ranging wild boar is unknown (van der Leek et al. 1993). Experimental infections of wild boar with PRV, however, caused no clinical signs (Tozzini et al., 1982). The high proportion of sera with low neutralizing antibody titers as detected in our study may indicate latency or a subclinical character of PRV infections in wild boar (Dahle et al., 1993).

Predators consuming wild boar carcasses can act as bioindicators for the presence of PRV infection in the natural environment. The only relevant predator species reaching high population densities in central Europe is the red fox (*Vulpes vulpes*) (Lloyd, 1980). Interestingly, 5.5% out of 309 red fox sera (CI = 3.6%–9.5%, P = 0.05) from regions with PRV infection in wild boar contained PRV-specific neutralizing antibodies (T. Müller et al., unpubl. data). PRV infections of secondary hosts are thought to be invariably lethal (Fenner et al., 1987), but clinical or histopathological signs in red foxes have never been observed in the endemic region. However, additional efforts have to be undertaken to isolate the causative agent and to evaluate its pathogenicity for wild boar and domestic pigs.

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