Pseudorabies Virus Infection in Wild Boars in Japan

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ABSTRACT. In Japan, most pig populations are now free from pseudorabies virus (PRV) due to the recent success of an extensive eradication program. However, PRV infection persists in Japanese wild boars (Sus scrofa leucomystax), representing another potential reservoir for the virus in Japan. In this study, the seroprevalence of PRV in wild boars captured in three different prefectures was ascertained. A virus neutralization (VN) test showed that 6 of 173 serum samples (3%) were positive for VN antibody; glycoprotein E-ELISA revealed infection with the wild-type, but not the available vaccine strain, PRV. These results indicate that PRV has continued to spread among wild boars in Japan.

KEY WORDS: Aujeszky’s disease, pseudorabies virus, wild boar.

NOTE Virology

Pseudorabies virus (PRV), a member of the genus Vari-cellivirus in the subfamily Alphaherpesvirinae of the family Herpesviridae [9], is the causative agent of a contagious and epidemic disease, Aujeszky’s disease, which affects swine at various production phases, causing high mortality in naive and newborn piglets and abortion in pregnant sows. This disease results in significant economic losses for the swine industry [3].

In Japan, a PRV eradication program in domestic pigs has been successfully implemented; consequently, most domestic pig populations are now free from wild-type PRV and therefore are not vaccinated with a live attenuated vaccine. As this vaccine virus has a deletion in the glycoprotein E (gE) gene, vaccinated pigs can be serologically differentiated from those infected with wild-type PRV by the gE-ELISA. In 1997, a PRV epidemic in Nara Prefecture, Japan, resulted in the deaths of 24 hunting dogs due to PRV infection acquired after eating raw wild boar (Sus scrofa leucomystax) meat (Kouda, T. et al., In the 129th Annual Meeting of the Japanese Society of Veterinary Science, Tsukuba, 2000). This event suggested that wild boars may represent a potential source of PRV infection for other animals. However, there is currently little information about the present situation of PRV prevalence among wild boars in Japan. Here, we present the results of a seroepidemiological study of PRV in wild boars in Japan in order to clarify the seroprevalence of PRV in this population.

A total of 173 serum samples were collected from wild boars in three different prefectures A, B and C, of Japan (Table 1). These three prefectures were located in the western part of Japan, were not next to each other and were free from PRV in their pig populations. Most animals were hunted with government permission during the winter season. Sera were inactivated by incubation at 56°C for 30 min and then kept at –20°C until use.

Cloned porcine kidney (CPK) cells were kindly provided by the National Institute of Animal Health in Japan; these were cultured in Dulbecco’s Modified Eagle’s Medium (DMEM; GIBCO, NY, U.S.A.) supplemented with 10% fetal calf serum (FCS), 100 U/ml penicillin and 100 μg/ml streptomycin. Subsequently, the PRV Indiana strain was propagated in the cultured CPK cells. To assess the presence of virus neutralization (VN) antibody to PRV, sera were serially twofold diluted in DMEM containing 2% FCS. Diluted sera and medium (control) samples were mixed with equal volumes of a solution containing 50 plaque-forming units (PFU)/100 μl of PRV, and then incubated at 37°C for 1 hr. CPK cells were then inoculated with these samples. After adsorption for 60 min at 37°C in 5% CO2, treated cells were washed twice with DMEM and then overlaid with 0.8% agarose (SeaPlaque GTG Agarose, Lonza, ME, U.S.A.) in DMEM containing 10% FCS. The plates were then incubated at 37°C in 5% CO2 for 3 days. The cells were fixed with 5% buffered formaldehyde for 1 hr, and the agarose layers were removed. After staining with crystal violet, plaques were counted. Sera that reduced the number of plaques by more than 80%, as compared with the mean number of plaques in control wells, were considered positive.

The results of VN assays revealed that at least 6 sera (3%) had VN antibodies against PRV (Table 1). PRV seropositivity was found in 2 of 50 wild boars (4%) in Prefecture A, 4 of 71 (6%) in Prefecture B, and none of the 52 in Prefecture C. The VN titers in the 6 positive sera ranged from 1:40 to 1:160 (Table 2). PRV-positive boars were over three years old and relatively heavy (49–81 kg), indicating that infection may have occurred a few years before sampling took place and may not have occurred recently.
To confirm PRV seropositivity, PRV gE-ELISA was carried out using HerdChek PRV g1 (gE) Antibody ELISA (IDEXX Laboratories, Westbrook, ME, U.S.A.). This assay detects the antibody to wild-type PRV gE by competition with a monoclonal antibody, thereby distinguishing the antibody induced by wild-type PRV from that induced by the vaccine strain [11]. The results showed that all 6 VN-positive sera contained antibodies to wild-type, virulent PRV gE, and not the vaccine strain.

The present results were consistent with the PRV epidemic in hunting dogs in 1997, because prefecture B is next to Nara Prefecture, suggesting that virulent PRV continues to spread among wild boars in Japan. However, the reported seroprevalences of PRV in wild boars in western (10%) and eastern (9%) Germany [10, 12], France (6%) [1], south-central Spain (56%) [5], Croatia (55%) [16] and the southern U.S.A. (34–61%) [2, 6, 7, 14] seem to be higher than that in Japan (3%). In addition, only one previous report indicated that wild boars in Shikoku, in southern Japan, were free from PRV [8]. These results suggest that the low seroprevalence of PRV in wild boars in Japan might be indicative of the success of the PRV eradication program in domestic pigs.

PRV-seropositive wild boars can act as a source of infection for other wildlife species, including wild canids and hunting dogs [13]. Mortality associated with PRV infection has also been documented in endangered species such as the Florida panther (Felis concolor) in the U.S.A. [4], and PRV has been suggested as a possible factor contributing to the declining numbers of the Eurasian lynx (Lynx lynx) in Slovenia [15]. Taken together with our results, these trends indicate that PRV infection in wild boars should become a target for eradication programs like the one implemented in the domestic pig population in Japan.

In conclusion, since PRV continues to infect wild boars in Japan, even in the prefectures without PRV in pigs, it is necessary to take precautions concerning possible transmission of PRV from wild boars to pigs.

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REFERENCES


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**Table 1. Seroprevalence of PRV in wild boars**

<table>
<thead>
<tr>
<th>Prefecture</th>
<th>Period</th>
<th>Number of examined sera</th>
<th>Number of PRV-positive sera (% of PRV-positive sera)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Sep. 2009 – Nov. 2010</td>
<td>50</td>
<td>2 (4%)</td>
</tr>
<tr>
<td>B</td>
<td>Nov. 2007 – Mar. 2010</td>
<td>71</td>
<td>4 (6%)</td>
</tr>
<tr>
<td>C</td>
<td>Jan. 2010 – Dec. 2010</td>
<td>52</td>
<td>0 (0%)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td>173</td>
<td>6 (3%)</td>
</tr>
</tbody>
</table>

**Table 2. VN titers and antibodies to PRV gE in PRV-positive sera**

<table>
<thead>
<tr>
<th>Prefecture</th>
<th>Date</th>
<th>Sex</th>
<th>Age(a) (Body weight)</th>
<th>gE ELISA S/N ratio(b)</th>
<th>VN titer</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Nov. 26, 2010</td>
<td>F</td>
<td>Over 3 (65 kg)</td>
<td>0.04</td>
<td>1:160</td>
</tr>
<tr>
<td>A</td>
<td>Oct. 4, 2010</td>
<td>F</td>
<td>Over 3 (73 kg)</td>
<td>0.19</td>
<td>1:80</td>
</tr>
<tr>
<td>B</td>
<td>Feb. 15, 2008</td>
<td>M</td>
<td>N.A. (52 kg)</td>
<td>0.05</td>
<td>1:160</td>
</tr>
<tr>
<td>B</td>
<td>Feb. 19, 2008</td>
<td>M</td>
<td>N.A. (53 kg)</td>
<td>0.19</td>
<td>1:80</td>
</tr>
<tr>
<td>B</td>
<td>Mar. 3, 2010</td>
<td>M</td>
<td>N.A. (81 kg)</td>
<td>0.10</td>
<td>1:40</td>
</tr>
<tr>
<td>B</td>
<td>Feb. 16, 2010</td>
<td>M</td>
<td>N.A. (49 kg)</td>
<td>0.15</td>
<td>1:80</td>
</tr>
</tbody>
</table>

a) Age was presumed from their teeth.

b) S/N ratio was calculated as a ratio of the absorbance of a well with serum to the absorbance of a control well without serum. Serum with an S/N ratio of ≤0.60 was judged as positive.

N.A.) Data is not available.


