The Journal of Veterinary Medical Science

NOTE

Virology

A serological survey of porcine reproductive and respiratory syndrome virus in wild boar in Gifu Prefecture, Japan

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J. Vet. Med. Sci.
84(10): 1406–1409, 2022
doi: 10.1292/jvms.21-0554

Received: 15 October 2021
Accepted: 29 July 2022
Advanced Epub:
17 August 2022

ABSTRACT. Porcine reproductive and respiratory syndrome (PRRS) is an infectious swine disease caused by the PRRS virus (PRRSV) that results in economic loss to the pig-rearing industry. To study PRRSV infection in wild boars and pigs, we conducted a serological survey in Gifu Prefecture, Japan, from 2020 to 2021. Three out of 453 (0.7%) wild boar sera were positive for PRRSV antibodies in a commercial ELISA. However, given that PRRSV RNA was not detected in these three wild boars and the specificity and sensitivity of the test kit, these are considered as false positives. Although seropositive pigs were found in multiple pig farms in the study area, the role of wild boars as a source of PRRS to pig farms appeared to be minimal.

KEYWORDS: antibody, Gifu, porcine reproductive and respiratory syndrome virus (PRRSV), wild boar

Porcine reproductive and respiratory syndrome (PRRS) is an infectious disease of pigs that is characterized by reproductive failure in pregnant sows and respiratory tract disorder in piglets [9]. The etiological agent of PRRS is the PRRS virus (PRRSV), an arterivirus that belongs to the order Nidovirales, family Arteriviridae [1]. As PRRSV is present in the secretions and excretions of infected animals, it is mainly transmitted through contact between infected animals [1]. PRRS has been previously reported in wild boars (*Sus scrofa*) in several countries, Korea, Germany, Spain, and Lithuania [2, 6–8]. In Japan, a serological survey of wild boars has been conducted in 41 out of 47 prefectures by the Ministry of Agriculture, Forestry and Fisheries (MAFF) between 2014 and 2017. In this study, more than a thousand individuals were examined and a low seroprevalence of PRRS (10/1,382, 0.7%) was reported with suspicion of all these samples being false-positive reactions [5]. On the other hand, as the sample size for each prefecture was small, ranging from 6–75, the possibility of the presence of PRRS in very low prevalence could not be ruled out. In Gifu Prefecture, Japan, classical swine fever (CSF) reemerged in domestic pigs in 2018 and subsequently spread to wild boars and CSF virus (CSFV) is suspected to be transmitted between wild boars and domestic pigs [3]. In this study, in order to investigate the role of wild boars as a source of PRRSV to pig farms, serological tests were conducted for wild boars in Gifu Prefecture.

As a part of CSF control strategies, the Gifu Prefectural Government has been conducting wild boar capture surveys since 2018. In this survey, information on the captured wild boars was recorded, including capture point, sex, and body length. Serum samples of wild boar collected in this survey were used in this study. According to the Regulations on Animal Care and Use in Research of Gifu University, this study was not considered as involving animal experiments requiring committee approval because no wild boars were killed specifically for this study.

Serum samples were collected from wild boars captured at 353 points in Gifu Prefecture between September 4, 2020 and March 4, 2021, by the Gifu Prefectural Government (see Supplementary Table 1 for sample detail). During this period, more than 99.2% of these boars were negative for CSF by PCR. Due to the restrictions imposed by the administrative regulations, only sera from animals with negative results of CSF PCR were used in this study. PCR negative samples were from 32 out of the 42 municipalities in Gifu prefecture. A total of 453 serum samples were selected to include at least one sample in each municipality (Supplementary Fig. 1). Detailed information relating to the capture point, sex, and body length was recorded for each sample. The differentiation between adult and young was based on the body length: adult >90 cm and young ≤90 cm. Serum samples were collected from 453 wild boars consisting of young (205 samples, 45.3%) and adults (248 samples, 54.7%); males (235 samples, 51.9%), females (217 samples,

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(Supplementary material: refer to PMC https://www.ncbi.nlm.nih.gov/pmc/journals/2350/)

47.9%), and sex unknown (1 sample, 0.2%). In addition, pig serum samples in the study area were also investigated. In the study area, Gifu Prefecture has collected 11 serum samples per farm from all farms in operation for the PRRSV Antibody Survey Project. In this study, the pig serum samples collected in 2019 and 2020 were tested. In these sampling, 11 specimens per farm were taken with the priority of selecting animals kept on each farm for at least one year.

The presence of PRRSV antibodies in the serum samples of wild boars and pigs was tested using a commercial ELISA kit, IDEXX PRRS X3 Ab Test (IDEXX, Westbrook, ME, USA), in accordance with the manufacturer's instructions. The wild boar capture points were mapped using QGIS (version 3.10.14) with the administrative boundary data. The administrative boundary data were downloaded from the Administrative Zones Data Version 2.4, National Land Information Boundary, National Spatial Planning and Regional Policy Bureau, MLIT of Japan (https://nlftp.mlit.go.jp/ksj/gml/datalist/KsjTmplt-N03-v2_4.html).

Of the 453 wild boar sera, only three samples were judged to be positive for PRRSV antibodies (0.7%, 95% CI: 0.1–1.9%) (Supplementary Table 1 and Fig. 1A). Whereas, considering the specificity and sensitivity indicated in the instruction of the commercial kit (99.9% and 98.8%, respectively), the possibility of false positives cannot be excluded for these three positive samples. Despite these samples were taken from wild boars captured in distant locations (Figs. 1A–2), all three samples were included in the same trial conducted on the same day using the same ELISA plate (Supplementary Table 1 and Supplementary Fig. 2). To examine the reliability of the test result, these three serum samples were re-examined using another commercial ELISA kit, an AniGen PRRS ELISA 4.0 (Bayer, Leverkusen, Germany). As a result, two of three samples (No.115 and No.145) showed positive whereas another one (No.159) showed negative. Additionally, these three samples were tested by the reverse transcription-polymerase chain reaction

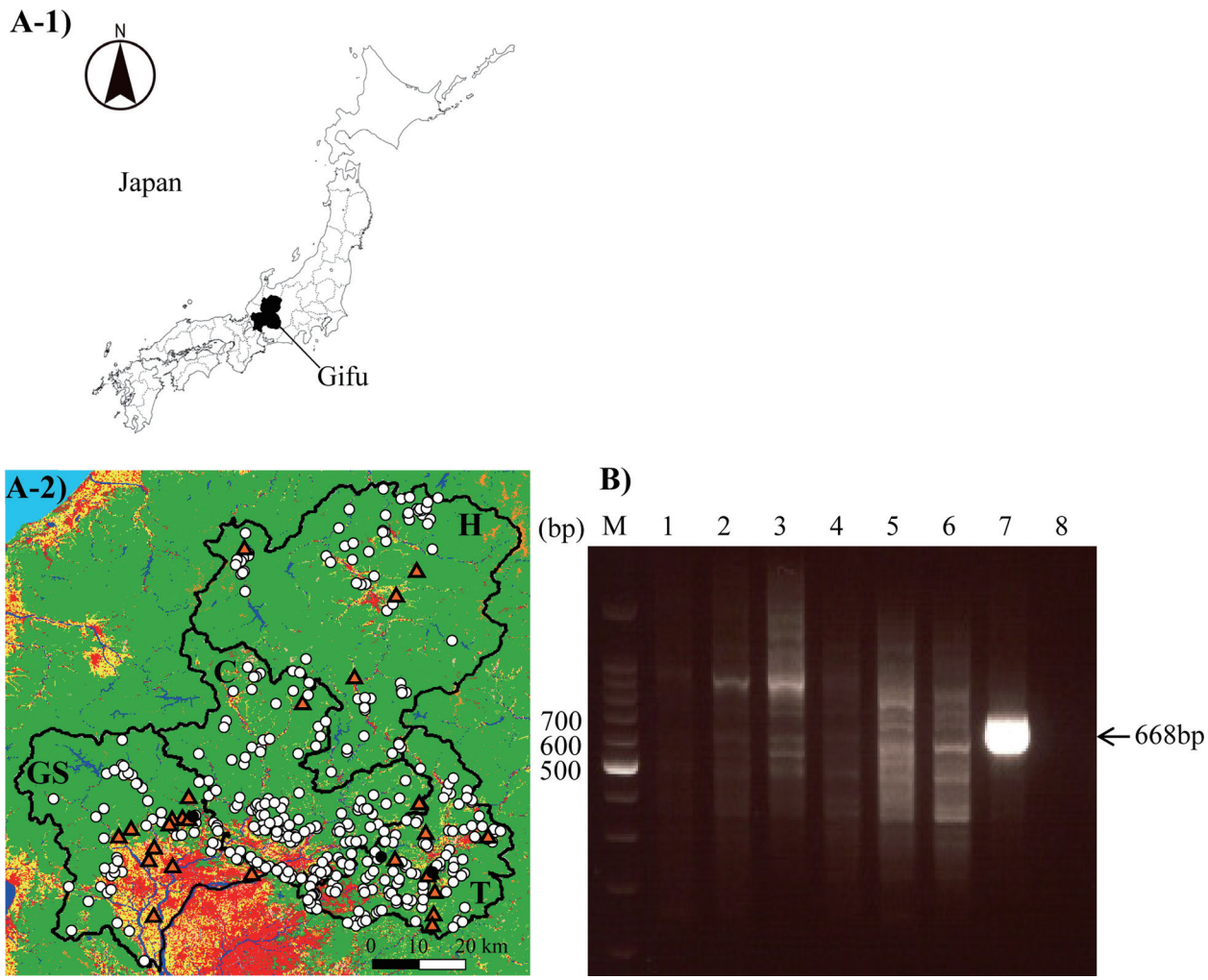

Fig. 1. Distribution of porcine reproductive and respiratory syndrome virus (PRRSV) seropositive wild boars and detection of PRRSV in seropositive wild boars. (**A**) Distribution of wild boars positive for PRRSV antibody in Gifu Prefecture, Japan. Solid and open circles indicate each capture point of seropositive and seronegative wild boar, respectively. Triangles indicate location of pig farms. Bold letters indicate each area: GS, Gifu-Seino; C, Chuno; H, Hida; T, Tono. The colors on the maps represent the land use type: forest (green), residential area (red), rice fields (yellow), other agricultural land (pale orange), wastelands (orange), river and lakes (blue), and golf courses (light green). (**B**) RT-PCR to detect PRRSV. Lane 1–3 show antibody-positive wild boars and lanes 4–6 show antibody-negative wild boars. The summary of each lane sample and wild boar individual number are as follows: 1: No.115, 2: No.145, 3: No.159, 4: No.118, 5: No.127, 6: No.142, 7: pig positive for PRRSV, 8: Negative control using distilled water instead of template. M: marker.

Table 1. Seroprevalence of porcine reproductive and respiratory syndrome virus (PRRSV) among pigs

		Seroprevalence of PRRSV (%)	
		Year 2019	Year 2020
Vaccinated pig farm	Farm A	6/11 (54.5%)	4/11 (36.4%)
	Farm B	6/11 (54.5%)	7/11 (63.6%)
	Farm C	2/11 (18.2%)	6/11 (54.5%)
	Farm D	10/11 (90.9%)	11/11 (100.0%)
	Farm E	non operated	9/11 (81.8%)
Unvaccinated pig farm	Farm F	4/11 (36.4%)	9/11 (81.8%)
	Farm G	8/11 (72.7%)	2/11 (18.2%)
	Farm H	0/11 (0%)	0/11 (0%)
	Farm I	0/11 (0%)	0/11 (0%)
	Farm J	Non operated	0/11 (0%)
	Farm K	Non operated	0/11 (0%)
	Farm L	Non operated	0/11 (0%)
	Farm M	Non operated	0/11 (0%)
	Farm N	Non operated	0/11 (0%)
	Farm O	Non operated	0/11 (0%)
	Farm P	Non operated	0/11 (0%)
	Farm Q	Non operated	0/11 (0%)
	Farm R	Non operated	0/11 (0%)
	Farm S	Non operated	0/11 (0%)
	Farm T	Non operated	0/11 (0%)
	Farm U	Non operated	0/11 (0%)
	Farm V	0/11 (0%)	Non operated
	Farm W	0/11 (0%)	0/11 (0%)
	Farm X	0/11 (0%)	0/11 (0%)
	Farm Y	0/11 (0%)	0/11 (0%)
	Farm Z	0/11 (0%)	0/11 (0%)
	Farm AA	0/11 (0%)	0/11 (0%)

(RT-PCR). For RT-PCR, total RNA was extracted from these sera using QIAamp Viral RNA Mini Kit (QIAGEN, Venlo, Netherlands) according to the manufacturer's protocol. Two µL of sample RNA was subjected to RT-PCR with PrimeScript OneStep RT-PCR Kit Ver.2 (TaKaRa Bio, Kusatsu, Japan), and the PRRSV-specific primer set [4], according to the manufacturer's instructions. As a result, no PRRSV was detected in these three samples (Fig. 1B). Taking all these results into consideration, it is highly likely that these three samples were false positives for PRRS antibody. Therefore, PRRSV infection among wild boars seemed to be absent or in very low level if present in the study area.

In contrast, seropositive pigs were found both in 2019 and 2020. In Gifu prefecture, 14 and 26 pig farms were operated in 2019 and 2020, respectively. Among them, PRRSV vaccine was used in 4 and 5 farms in 2019 and 2020. From the same two unvaccinated farms, several seropositive pigs were found in both 2019 and 2020 (Table 1). These results indicated that PRRSV continues to exist in pig farms in the study area. A previous study in Spain showed that PRRSV-positive wild boars tended to be found in the areas with higher densities of domestic pigs, although the finding was not statistically significant [7]. In addition, PRRSV nucleotide sequences in wild boars in South Korea were related to those found in domestic pigs [2]. These findings suggested the transmission of PRRSV between pigs and wild boars could occur. In contrast, our study indicated the absence or very low prevalence of PRRSV among wild boars despite the continuous PRRSV infection was observed in unvaccinated pig farms in the study area. Nonetheless, in order to prevent PRRSV intrusion to PRRSV-negative pig farms in this area, it will be better to keep a higher biosecurity level as well as prevent wildlife from entering the farm.

CONFLICT OF INTEREST. The authors declare no conflicts of interest associated with this manuscript.

ACKNOWLEDGMENTS. This work was partially supported by the Research Program on Emerging and Re-emerging Infectious Diseases of the Japan Agency for Medical Research and Development (21fk0108137s0102) and by commissioned projects of the Gifu Prefectural Government.

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