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NOTE

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Serological survey of antibodies to four pathogens in wild boars in Nagano Prefecture, Japan

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ABSTRACT. Wild boars (*Sus scrofa*) captured or found dead in Nagano Prefecture were surveyed for antibodies to Aujeszky's disease virus (ADV), *Erysipelothrix rhusiopathiae*, porcine reproductive and respiratory syndrome virus (PRRSV), and *Toxoplasma gondii*. While all 168 samples tested were negative for anti-ADV antibodies and all 140 samples tested were negative for anti-PRRSV antibodies, all 190 samples tested were positive for anti-*E. rhusiopathiae* antibodies and 12 of 180 samples were positive for anti-toxoplasma antibodies. These results suggest that since *E. rhusiopathiae* and *T. gondii* cause zoonotic diseases, in addition to wild boars being a potential source of infection for domestic pigs, caution should be taken when handling wild boars or eating wild boar meat because of the possibility of human infection.

KEY WORDS: Aujeszky's disease, *Erysipelothrix rhusiopathiae*, porcine reproductive and respiratory syndrome, *Toxoplasma gondii*, wild boar

The distribution of wild boars (*Sus scrofa*) has been expanding in Japan [13], including in Nagano Prefecture [15], as well as in Europe and other countries [6, 14], and opportunities for direct and indirect contact between wild boars and livestock and humans have been increasing. Wild boars and domestic pigs are the same species (*Sus scrofa*) and viruses, bacteria, and parasites that cause swine diseases can also infect wild boars, increasing the risk that wild boars will become reservoirs of pathogens and a source of swine disease transmission to domestic pigs [18].

However, there is little information on the status of livestock infectious diseases and zoonotic diseases in wild boars in Japan. Therefore, to evaluate the risk to the livestock industry and humans, we investigated the prevalence of antibodies for four diseases that are important for livestock and human epidemics in wild boars.

Nagano Prefecture is in the center of Honshu, Japan (Fig. 1). While some areas in the south of the prefecture have a warm, humid climate, most has a humid continental climate, the high altitude areas are cool, and the alpine zones of the Hida, Kiso, and Akaishi Mountains have a subarctic climate. Forest covers about 80% of the prefecture, with about 60% of the forest being coniferous and the remainder broad-leaved trees such as sawtooth oak (*Quercus acutissima*), Japanese beech (*Fagus crenata*), Japanese oak (*Quercus crispula*), and Konara oak (*Quercus serrata*). The most common coniferous tree is Japanese larch (*Larix kaempferi*), which accounts for about a quarter of the total, followed by Japanese cypress (*Chamaecyparis obtusa*), Japanese false cypress (*Chamaecyparis pisifera*), Japanese red pine (*Pinus densiflora*), and Japanese cedar (*Cryptomeria japonica*).

Serum samples were collected from wild boars that were captured or found dead in Nagano Prefecture from April, 2020, to January, 2021; in addition to the tonsil samples used for the porcine circovirus study (Porcine circoviruses in wild boars in Nagano Prefecture, Japan), the serum samples were originally collected for surveillance of classical and African swine fevers. When wild boars were hunted or carcasses were reported to local municipalities, veterinarians and other officials were deployed to collect blood samples. These samples were kept in cold storage and delivered to Matsumoto Livestock Hygiene Service Center, where they were centrifuged and the resulting serum was stored at −80°C until examination. The latitude and longitude of the sample collection sites were obtained using Google Earth (https://www.google.com/earth/).

The seroprevalence tests for each disease were performed as follows. Enzyme-linked immunosorbent assays (ELISAs) were performed to test for the presence of antibodies to Aujeszky's disease virus (ADV), porcine reproductive and respiratory syndrome virus (PRRSV), and *Toxoplasma gondii* using commercial kits according to the manufacturers' instructions [ADV (S) ELISA kit (IDEXX Laboratories, Tokyo, Japan) for ADV; PRRS X3 ELISA kit (IDEXX Laboratories) for PRRSV, and Porcine

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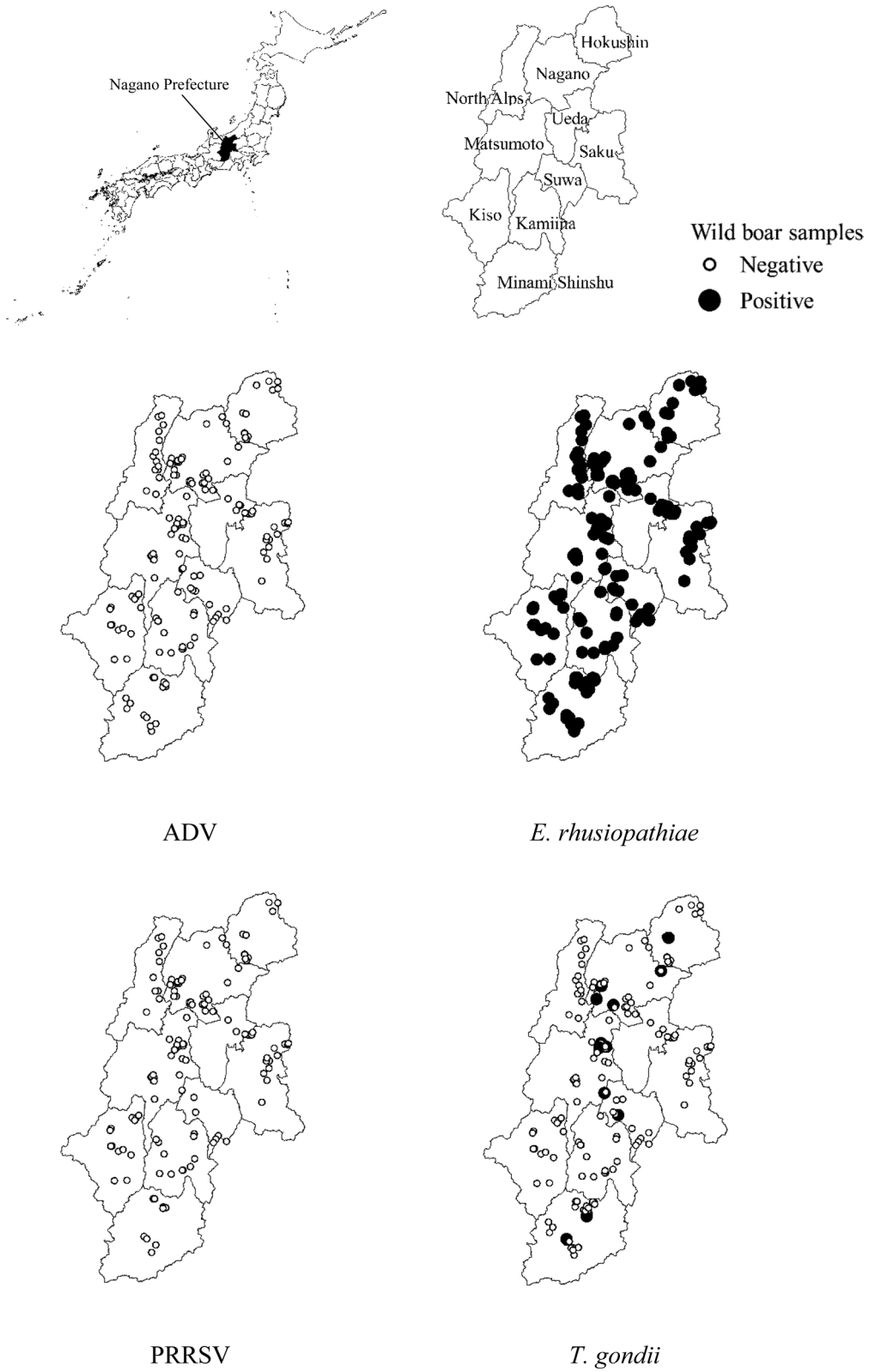

Fig. 1. Locations in Nagano Prefecture where wild boars were captured or carcasses discovered. White circle, negative test result; black circle, positive test result. Top left, the location of Nagano Prefecture in Japan; top right, the 10 wide areas in Nagano Prefecture. ADV, Aujeszky's disease virus; *E. rhusiopathiae*, *Erysipelothrix rhusiopathiae*; PRRSV, porcine reproductive and respiratory syndrome virus; *T. gondii*, *Toxoplasma gondii*.

Toxoplasma Ab Kit (Frontier Institute, Ishikari, Japan) for *T. gondii*]. For ADV, ELISA-positive samples were then tested using a latex agglutination (LA) assay using the AD-antigen latex "Kashiken" (Scientific Feed Laboratory, Tokyo, Japan) [16]. For *Erysipelothrix rhusiopathiae*, a growth agglutination (GA) test was performed to detect anti-*E. rhusiopathiae* antibodies in wild boar serum. First, 50 µl of each serum sample was incubated with 50 µl of 0.2 M 2-mercaptoethanol (Wako, Osaka, Japan) for 1 hr at 37°C. Then, each serum sample was diluted two-fold from 1/2 to 1/2,048 with brain heart infusion (BHI) broth (Becton, Dickinson and Co., Japan, Tokyo, Japan) containing 0.1% Tween 80 (Nacalai Tesque, Kyoto, Japan), 0.3% Tris (Sigma-Aldrich Japan, Tokyo, Japan), kanamycin (100 µg/ml), and gentamicin (50 µg/ml) in 96-well plates. Subsequently, 50 µl of BHI broth containing 10% of BHI culture of *E. rhusiopathiae* strain Marienfelde (serovar 1a) was added to each well. The agglutinating antibody titer was determined after incubation for 24 hr at 37°C; the titer values were expressed as the reciprocal of the highest serum dilution that showed agglutination. Samples with an agglutination titer of 4 or greater were assessed as positive.

The wild boar capture locations and those where carcasses were found were mapped using QGIS ver. 3.4 (http://www.qgis.org). The boundaries for 10 wide areas in Nagano Prefecture were produced by manipulating GIS data downloaded from the National Land Information Division, National Spatial Planning and Regional Policy Bureau, MLIT of Japan (https://nlftp.mlit.go.jp/ksj/gml/datalist/KsjTmplt-N03-v2_4.html).

ADV: Of 168 samples tested, none had anti-ADV antibodies. Although 22 samples had positive ELISAs, none of these tested positive in the LA assay. Although we found no ADV-positive samples, several wild boars captured in the Kinki region and Kyushu possessed anti-ADV antibodies [11, 12, 16]. Furthermore, live ADV was isolated from two hunting dogs that developed neurological symptoms after eating wild boar meat in Oita Prefecture, Kyushu, in 2018 [4]. Therefore, ADV seems to be maintained in wild boar populations in the limited area in Japan, although domestic pigs in those regions are categorized as ADV-free, as in Nagano Prefecture. As such, there is a risk that ADV could be introduced from those regions via contaminated vehicles and clothing. In addition to taking measures to prevent the spread of ADV, it is essential to perform regular wild boar monitoring surveys so that ADV invasion can be detected promptly.

E. rhusiopathiae: All 190 specimens tested had GA antibody titers greater than 4-fold, which implies that they possess anti-*E. rhusiopathiae* antibodies. Positive wild boars were found in all 10 wide areas of Nagano Prefecture (Fig. 1 and Table 1). In a recent nationwide survey of the seroprevalence of anti-*E. rhusiopathiae* antibodies in wild boars, 1,312 of 1,372 wild boars (95.6%) possessed antibodies, including all 16 wild boars (100%) captured in Nagano Prefecture [10, 23]. This concurs with our results that wild boars inhabiting Nagano Prefecture are very likely to be exposed to *E. rhusiopathiae*. Shimoji *et al*. isolated *E. rhusiopathiae* from the tonsils of 18 of 20 (90%) wild boars [23], also suggesting that wild boars carry *E. rhusiopathiae* and are a likely source of infection for domestic pigs.

In addition to wild boars and domestic pigs, *E. rhusiopathiae* can infect chickens and humans, causing economic damage and health hazards [1]. Therefore, in areas inhabited by wild boars, it is necessary to take wildlife measures to prevent the introduction of *E. rhusiopathiae* into livestock barns and to ensure hygiene management. The number of wild boars captured is increasing and the use of gibier meat has been promoted. Therefore, care must be taken to avoid infection with *E. rhusiopathiae*.

PRRSV: All 140 samples tested were negative and none had anti-PRRSV antibodies. The seroprevalence of antibodies and viral nucleic acids for PRRSV in wild boars have also been examined in Spain [19], Germany [2], and Canada [8], but few antibodies and genes were detected, and when detected, they were less common than other swine diseases [9]. There is no solid evidence that wild boars are reservoirs of PRRSV in Japan or elsewhere [9–10, 16]. Since no history of PRRSV infection has been confirmed in wild boars in Nagano Prefecture, it is important to focus on measures to prevent the spread of PRRSV among domestic pigs.

T. gondii: Twelve of 180 (6.7%) samples possessed antibodies against *T. gondii*. These 12 positive samples included four from Nagano, three from Matsumoto, two from Suwa, two from Minami Shinshu, and one from Hokushin area (Fig. 1 and Table 1). All the positives were adult wild boars (four adult males and eight adult females), and all 36 juveniles were negative. Kobayashi *et al.* and other studies have reported that the prevalence of anti-toxoplasma antibodies is low in young animals [3, 5, 17, 24], and this

Table 1. Positivity rate of each disease in 10 wide areas in Nagano Prefecture

Ten Wide Area	ADV	*E. rhusiopathiae*	PRRSV	*T. gondii*
Hokushin	0.0% (0/15)	100.0% (15/15)	0.0% (0/11)	6.7% (1/15)
North Alps	0.0% (0/15)	100.0% (16/16)	0.0% (0/11)	0.0% (0/15)
Nagano	0.0% (0/29)	100.0% (29/29)	0.0% (0/29)	13.8% (4/29)
Ueda	0.0% (0/5)	100.0% (9/9)	0.0% (0/2)	0.0% (0/3)
Matsumoto	0.0% (0/28)	100.0% (28/28)	0.0% (0/28)	10.7% (3/28)
Saku	0.0% (0/16)	100.0% (16/16)	0.0% (0/16)	0.0% (0/16)
Suwa	0.0% (0/15)	100.0% (16/16)	0.0% (0/9)	13.3% (2/15)
Kiso	0.0% (0/15)	100.0% (15/15)	0.0% (0/14)	0.0% (0/15)
Kamiina	0.0% (0/15)	100.0% (16/16)	0.0% (0/11)	0.0% (0/15)
Minami Shinshu	0.0% (0/15)	100.0% (30/30)	0.0% (0/9)	6.9% (2/29)
Total	0.0% (0/168)	100.0% (190/190)	0.0% (0/140)	6.7% (12/180)

Values are percentages (positive samples/total samples). ADV, Aujeszky's disease virus; *E. rhusiopathiae, Erysipelothrix rhusiopathiae*; PRRSV, porcine reproductive and respiratory syndrome virus; *T. gondii, Toxoplasma gondii*.

tendency was also observed in this study. A survey of anti-toxoplasma antibodies in wild boars in neighboring prefectures, found that 6.3% were positive in Gunma Prefecture (east of Nagano Prefecture) [7] and 22.7% were positive in Gifu Prefecture (west of Nagano Prefecture) [20]. Furthermore, in the Hida region, a sparsely populated, mountainous area in northern Gifu Prefecture close to the center of Nagano Prefecture, the positive rate was 16.9%. Therefore, our finding that 6.7% of wild boars inhabiting Nagano Prefecture were positive for anti-toxoplasma antibodies is reasonable. In a recent nationwide survey of the seroprevalence of anti-toxoplasma antibodies in wild boars, 670 of 1,881 wild boars (35.6%) possessed antibodies, and in Nagano prefecture, 3 of 16 wild boars (18.8%) possessed antibodies [11]. Considering the small sample size in Nagano Prefecture, it cannot be simply compared with the results obtained in this study, however there are few positive wild boars in Nagano Prefecture when viewed from a national perspective.

Toxoplasmosis, a zoonosis caused by *T. gondii* infection, can have serious effects on the fetus when it infects pregnant women or cause severe illness in patients suffering from immunodeficiency diseases such as acquired immunodeficiency syndrome [22]. One route of toxoplasma infection is the ingestion of raw or undercooked meat containing *T. gondii* cysts, so wild boar meat must be cooked properly before consumption [21].

CONFLICT OF INTEREST. The authors declare no conflicts of interest regarding the contents of this manuscript.

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