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NOTE

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Influenza A virus infection in Japanese wild boars (*Sus scrofa leucomystax*)

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ABSTRACT. Serum samples were collected from 385 wild boars between 2010 and 2013 to examine the seroprevalence of influenza A virus (IAV) in Japan. Antibodies against IAV were identified using a commercial kit in 13 wild boars (3.4%). To identify the serotypes, positive sera were examined by virus-neutralization test using representative serotypes and strains. Three wild boars in Yamaguchi and four in Tochigi showed the highest antibody titers against the pandemic H1N1 2009 virus and classical swine H1N1 virus strains, respectively. These data indicate that wild boars may have close contact with humans and domestic pigs and therefore that there is potential for IAVs to reassort in wild boars as they have been shown to do in pigs.

KEY WORDS: influenza A virus, seroepidemiology, wild boar

Influenza A virus (IAV) is endemic in pig populations worldwide with genetically distinct lineages of H1N1, H1N2 and H3N2 [7]. Pigs are susceptible to infection with both avian and human influenza viruses, and are believed to play an important role in human influenza ecology. In 2009, a new virus of swine origin, named pandemic H1N1 2009 virus (H1N1pdm09), emerged in humans and caused a worldwide pandemic [2]. Immediately after the emergence of H1N1pdm09, human-to-swine virus transmission was observed in many countries, including Japan [6, 11].

Japanese wild boars (*Sus scrofa leucomystax*), which are ancestors of domestic pigs, have drastically increased throughout Japan in recent decades, resulting in significant agricultural damage. Wild boars play an important role in the spread of viruses, including African swine fever virus [13], hepatitis E virus [16, 20], pseudorabies virus [9, 14] and Japanese encephalitis virus [12], to domestic animals and humans. However, there is currently no information about the IAV infection status in wild boars in Japan. In this study, we investigated the seroprevalence of IAV in Japanese wild boars in three different prefectures, Tochigi, Yamaguchi and Oita. Furthermore, we serologically assessed the IAV subtypes circulating in the wild boar population.

We collected 385 serum samples from wild boars captured under the official population control program between 2010 and 2013 in the prefectures of Tochigi (153 samples), Yamaguchi (192 samples) and Oita (40 samples), which are located in eastern and western parts of Honshu island and Kyushu island, respectively. We performed an ELISA using an Influenza A virus antibody test kit purchased from IDEXX laboratories (Westbrook, ME, U.S.A.) to screen for IAV-specific antibodies in the sera. The results showed that 3.4% (13 of 385) of wild boars had antibodies against IAV. Although 5.9% (9 of 153) and 2.1% (4 of 192) of wild boars in Tochigi and Yamaguchi prefectures, respectively, were seropositive for IAV, none of 40 wild boars in Oita prefecture were seropositive (Table 1).

To determine which subtype of IAV had infected the seropositive wild boars, virus-neutralization (VN) tests were performed using H1N1pdm2009 (A/Osaka/364/2009), seasonal H1N1 (A/Kawasaki/UTK4/2009), swine H1N1 (A/swine/Hokkaido/2/1981), swine H1N2 (A/swine/Miyagi/5/2003), swine H3N2 (A/swine/Obihiro/10/85) viruses and highly pathogenic H5N1 (clade 2.5) avian influenza virus (A/chicken/Yamaguchi/8/2004). Serum samples were used after receptor-destroying enzyme treatment and

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Table 1. Summary of the results of serological tests using a commercial kit

Region/period	No. positive/no. samples (%)	ID	Date of collection	Body weight (kg)	Sex	IDEXX Ab test [a]
Oita Prefecture						
2012	0/40 (0)	-	-	-	-	-
Yamaguchi Prefecture						
2010	0/57 (0)	-	-	-	-	-
2011	1/27 (4)	11-94	2011 Dec 25	35	Female	0.21
2012	1/51 (2)	12-25	2012 Oct 7	38	Female	0.35
2013	2/57 (4)	12-99	2013 Feb 3	15	n.r.[b]	0.58
		13-62	2013 Sep 14	52	Female	0.21
Tochigi Prefecture						
2011–2012	9/153 (5.9)	11013	2011 May 13	24	Female	0.38
		11028	2011 Jun 11	44	Male	0.10
		11032	2011 Jun 21	31	Female	0.44
		11056	2011 Aug 17	35	Male	0.59
		11072	2011 Sep 14	43	Male	0.30
		11076	2011 Sep 22	44	Female	0.20
		11080	2011 Sep 30	58	Male	0.52
		11087	2011 Oct 10	46	Female	0.17
		11123	2011 Nov 18	43	Female	0.20
Total	13/385 (3.4)					

a) S/P value of less than 0.6 was considered as an antibody positive sample. b) Not recorded.

Table 2. Virus-neutralization test with sera from wild boars in Japan

ID	Virus-neutralization test [a]					
	2009pdm H1N1	Seasonal H1N1	Swine H1N1	Swine H1N2	Swine H3N2	H5N1
11-94	**1,024**[b]	<8	16	256	<8	<8
12-25	**2,048**	NT	256	256	<8	<8
12-99	<16	<16	<16	<16	<16	<16
13-62	**12,800**	16	512	3,200	<16	<16
11032	128	NT	**256**	64	<8	<8
11080	512	<8	**1,024**	128	<8	<8
11087	128	<8	**256**	64	<8	<8
11123	1,024	<8	**4,096**	512	<8	<8

a) A/Osaka/364/2009 (2009pdm H1N1), A/Kawasaki/UTK4/2009 (Seasonal H1N1), A/swine/Hokkaido/2/1981 (Swine H1N1), A/swine/Miyagi/5/2003 (Swine H1N2), A/swine/Obihiro/10/85 (Swine H3N2) and A/chicken/Yamaguchi/8/2004 (H5N1; clade 2.5) were used for the virus-neutralization test. Representative data from 3 independent experiments are shown. b) The highest titer in sera is shown in bold.

heat-inactivation of the sera to remove non-specific inhibitors. The five positive sera, 11013, 11028, 11056, 11072 and 11076, were not examined by VN test, because of the lack of volume. The results of the VN test using eight selected positive sera are shown in Table 2. Seven seropositive sera had VN antibodies against the H1 subtype of IAV. One serum sample did not show any VN activity against any of the viruses including H1, H3 and H5 subtypes of IAV. Interestingly, wild boars from Yamaguchi showed the highest VN activity against H1N1pdm09 virus, while wild boars from Tochigi showed the highest VN activity against classical swine H1N1 virus.

We next purchased ImunoAce Flu from Tauns Laboratories, Inc. (Shizuoka, Japan) to use for the antigen screening of influenza virus among wild boars in Yamaguchi prefecture from 2012 to 2013. However, none of 106 nasal swabs from wild boars were positive for influenza A or B virus antigens (data not shown).

Our data indicated that wild boars in Yamaguchi had been infected with human H1N1pdm09 virus. Since there were no pig farms in the area where wild boars were captured, it is assumed that they might have been infected with H1N1pdm09 from humans or other wild animals. However, only 1.5% (3/192) of wild boars in Yamaguchi prefecture were seropositive for H1N1pdm09 virus, indicating that it might not be spreading among wild boars. One wild boar which was seropositive for IAV using the commercial kit was negative for VN antibody against all of the examined IAV subtypes, suggesting that it might have been infected with another IAV subtype.

The seroprevalence of IAV among wild boars varies from 0% in Finland, France and Slovenia, up to 74% in China [1, 3, 8, 19, 22, 23]. In this study, the seroprevalence of IAV in wild boars was lower than in domestic pigs (8.4–22.7%) in Japan [18]. This

difference may be due to the population density because domestic pigs are raised in densely populated pigpens, while wild boars live in small groups in the field.

In our study, 5.9% of wild boars in Tochigi were seropositive for swine H1N1 virus. In 2008, swine influenza virus was reported in pig farms in Tochigi [21]. Although there was no direct evidence of transmission of influenza viruses between domestic pigs and wild boars, transmission of other viruses, such as African swine fever virus [13], hepatitis E virus [16] and pseudorabies virus [14], has been reported between wild boars and domestic pigs. Therefore, this result might indicate the transmission of influenza virus between wild boars and domestic pigs.

In Japan, several outbreaks of avian influenza virus (AIV) H5N1 in poultry farms have been reported since 2004 [10, 15]. In Yamaguchi, two outbreaks occurred in 2004 and 2014 near the area where wild boars were captured [5, 10]. AIV H5N1 is thought to be transferred by birds migrating from the Asian continent to Japan and has been introduced into poultry farms, causing lethal infections [17]. Therefore, we suspected that AIV H5N1 might be more prevalent in Japan, including at Yamaguchi. We have previously reported that there are many AIV H5N1-positive wild raccoons in Japan, even in places where there have been no outbreaks of AIV H5N1 [4]. However, there was no evidence of transmission of AIV H5N1 from birds to wild boars.

Recently, wild boars have appeared in residential areas, resulting in an increased probability of contact directly or indirectly with humans and domestic animals. Our data indicate that transmission of IAV between wild boars and domestic pigs or humans is possible and that special attention is therefore required to monitor IAV infection in wild boars.

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