



NOTE

Wildlife Science

Monitoring relative abundance index and age ratios of wild boar (*Sus scrofa*) in small scale population in Gifu prefecture, Japan during classical swine fever outbreak

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ABSTRACT. Although the first cases of classical swine fever were reported in 2018, no studies have explored this impact on wild boar populations in Japan. Comparing the relative abundance indices and age ratios in the wild boar population before and after the outbreak, we investigated the impact of classical swine fever virus on wild boar population dynamics in August 2017–December 2019. Relative abundance indices declined from 2017 to 2019 drastically, while there were no significant differences in age ratios throughout the study period. Consequently, wildlife managers should consider that wild boars continue to contract classical swine fever virus, and they should intensively implement countermeasures in agricultural lands and in pig farms, in addition to wild boar population management.

KEY WORDS: camera trap survey, classical swine fever, piglet, wild boar

Classical swine fever (CSF) reemerged in Japan in 2018. The first CSF cases were reported in Gifu City, Gifu Prefecture, central Japan, in pigs and wild boars on September 9 and 13, 2018, respectively. Thereafter, CSF virus (CSFV) spread rapidly to neighboring prefectures and municipalities. CSFV outbreaks have been reported on 58 pig farms in 10 prefectures (March 12, 2020) and in 2,046 wild boars in 12 prefectures (March 13, 2020) [18]. Particularly, 1,175 cases of CSF in wild boars have been reported in Gifu Prefecture, and there is a high possibility that a large proportion of CSFV spread is attributable to wild boars [11].

The whole-genome sequence of a CSFV (JPN/1/2018) isolate collected in September 2018 from an infected pig in Gifu Prefecture showed that JPN/1/2018 is closely related to isolates in eastern and southeast Asia, and this was the first detection of a CSFV sub-genotype 2.1 in Japan [21, 23]. The analysis of CSFV genes isolated from pig farms during the outbreak from the 1st to 28th and from wild boars revealed that the strains were very similar, with a maximum difference of one base [17]. In addition, the experimental infection of pigs with CSFV/JPN/1/2018 revealed that pathogenicity of the JPN/1/2018 strain was milder than that of the highly virulent ALD strain [13]. The same reports suggested that CSFV infections that have persisted since 2018 in Japan have minor impacts on wild boar populations. However, no studies have explored the impact of CSFV outbreaks on wild boar populations in Japan.

We evaluated the relative population abundance and diel activity patterns of wild boars in Gifu City before the CSFV outbreak [9] and thus could compare the population status before and after the outbreak. To establish appropriate wild boar population management strategies and CSFV countermeasures, wildlife managers need to determine how CSFV influences wild boar populations. In the present study, we focused on monitoring the relative abundance indices and age ratios in the wild boar population of Gifu Prefecture to investigate the impact of CSFV on the dynamics of this population on a relatively small scale.

Our study was conducted on Mt. Kinka (35°26'00", 136°46'53") in Gifu Prefecture. The mountain covers an area of approximately 597 ha and has a highest elevation of 328.8 m. Wild boars were first reported on the mountain in 1997, and they were associated with damage to home gardens and hiking courses, in addition to traffic accidents. We suggest that this population is isolated from neighboring populations by a large river and steep topography in the northern area, as well as residential land in southern, western, and south-eastern areas. Although there is a possibility that wild boar in neighboring populations immigrated from the north-eastern area to this mountain, we defined this population as an almost closed population. Thus, there was almost no influence of hunting and nuisance controls in the neighboring area on this population. Gifu City has conducted nuisance controls

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Table 1. The number of wild boars captured, classical swine fever virus (CSFV) positive wild boars by reverse transcriptase polymerase chain reaction (RT-PCR; n=21) and by enzyme-linked immunosorbent assay (ELISA; n=17) in August 2017–December 2019. We classified the study period into a period before the CSFV outbreak (from August 2017 to August 2018) and the period after the CSFV outbreak (from September 2018 to December 2019)

Year	2017					2018					2019																		
Period	Before the CSFV outbreak										After the CSFV outbreak																		
Month	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D
The number of wild boars captured	15	10	5	5	0	5	5	5	5	7	5	6	5	4	0	0	0	2	5	0	0	6	2	0	2	0	0	1	8
The number of positive wild boars by RT-PCR test (n=21)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0/1	-	-	4/4	5/5	-	-	3/6	1/2	0/1	0/2	-	-	-	-
The number of positive wild boars by ELISA test (n=17)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0/1	-	-	1/2	1/3	-	-	6/6	2/2	1/1	1/2	-	-	-	-

The interruptions by culling programs took place ranged from September 15, 2018, to October 21, 2018, and from October 1, 2019, to November 27, 2019.

and captured 510 wild boars on this mountain during 2009–2019 to reduce such problems and prevent human injuries, and this capture effort has ensnared 6.00 wild boars/month during August 2017 and August 2018 (Table 1; before the CSFV outbreak).

The study area was within 10 km of the sites where CSFV was reported in pigs and wild boars on September 9 and 13, 2018, respectively. Following previous diagnosis manual for CSF [16], Gifu prefecture tested wild boars using reverse transcriptase polymerase chain reaction (RT-PCR) with tonsil samples and enzyme-linked immunosorbent assay (ELISA) with serum samples. Therefore, 19 positive cases of CSFV infection in wild boars have been reported based on RT-PCR and/or ELISA on 21 carcasses (4 wild boars; natural mortality) or live trapped wild boars (17 wild boars; nuisance controls) on the mountain. Gifu prefecture have partially published diagnosis results of RT-PCR [5], but not those of ELISA. Thereafter, the capture effort decreased drastically during September 2018 and December 2019 (Table 1; after the CSFV outbreak: 1.88 boars per month). The decrease in capture efforts could have been caused not only by the CSFV outbreak but also by several factors including the interruption of culling programs (from September 15, 2018, to October 21, 2018, and from October 1, 2019, to November 27, 2019), a decrease in capture efforts, and a decline in wild boar population abundance.

We used 20 infrared-triggered cameras (Ltl Acorn 6210; Ltl Acorn Outdoors, Green Bay, WI, USA) in August 2017–December 2019 and recorded the number of wild boars captured in all photos, in addition to the dates and times and other species. To obtain information on the wild boar population evenly throughout the study area, we first divided the study area into 28 quadrats (42.5 ha; 650 m × 650 m) and placed cameras within 100 m of the center of each quadrat. Because a 100 m buffer in eight quadrats did not include this mountain, we placed 20 cameras near signs of wild boars (e.g. trails and digging) throughout the study area. Details of our study area and the design of the camera trap survey have been reported in a previous study [9].

According to the method of a previous study [10], we classified wild boars as adults and piglets based on age in the month of June. To avoid overestimation owing to double counts, only one photo of each wild boar captured within 1 hr was used in the analysis. Thereafter, we calculated the monthly relative abundance index (RAI) [22] for each camera, year, and month according to the number of wild boars photographed and trap days, in addition to age ratios for each day from the sum of wild boars photographed and the number of piglets photographed. We defined RAI as the number of wild boars photographed per 100 trap days. A previous study [7] highlighted the potential misidentification of individuals between the sub-adult and the piglet classes when piglets are 8–12-months-old. Therefore, age ratios in the present study were calculated based on the proportion of striped piglets for 7 months (June 1 and December 31) in all study years.

All statistical analyses were performed in R v3.4.0 [24]. First, we used a generalized linear mixed model (GLMM) with a Poisson distribution in the lme4 package [2] and multcomp package [8] of R to investigate differences in RAIs among the years and the months. We set the total monthly number of wild boars photographed for each year and camera as the response variable, the year and the month as the explanatory variables, each camera as a random factor, and the trap days for each camera and season as offset terms. Because we focused on the influence of CSFV on wild boar populations, we set 2018 as the counterpart of other years. Second, we compared the age ratios across years using GLMM with a Poisson distribution in the lme4 and multcomp packages [2, 8]. We set the daily number of piglets photographed for each year and camera as the response variable, the year as the explanatory variables, each camera and each month as a random factor, and the daily number of adults and piglets photographed for each year and camera as offset terms. Thereafter, we tested the differences in RAIs among the months and age ratios among the years using the multiple comparison test.

We photographed a total of 4,732 wild boars from 16,876 camera trap days in August 2017–December 2019 and calculated monthly RAIs and daily age ratios using 3,566 wild boars. RAIs in 2017 were significantly higher than those in 2018 ($P < 0.01$), whereas RAIs in 2019 were significantly lower than those in 2018 ($P < 0.01$). Additionally, the monthly RAIs exhibited seasonal variations; RAI in September was significantly higher than that in other months (Table 2, Fig. 1). Overall, RAIs tended to become high during August and November (Table 2, Fig. 1). Conversely, the yearly age ratios (\pm standard error) were 26.32 (\pm 2.11), 26.67 (\pm 1.80), and 28.52 (\pm 2.10) in 2017, 2018, and 2019, respectively. We did not observe any significant differences among the ratios

Table 2. Results (Estimates ± SE) of the multiple comparison test to investigate differences in relative abundance indices among the months

Month	January	February	March	April	May	June	July	August	September	October	November
February	-0.21 ± 0.11	-	-	-	-	-	-	-	-	-	-
March	-0.15 ± 0.11	0.06 ± 0.12	-	-	-	-	-	-	-	-	-
April	-0.12 ± 0.11	0.09 ± 0.12	0.03 ± 0.11	-	-	-	-	-	-	-	-
May	0.05 ± 0.10	0.26 ± 0.11	0.20 ± 0.11	0.17 ± 0.11	-	-	-	-	-	-	-
June	0.16 ± 0.10	0.37 ± 0.11 ^{c)}	0.31 ± 0.11	0.28 ± 0.11	0.11 ± 0.10	-	-	-	-	-	-
July	0.18 ± 0.10	0.39 ± 0.11 ^{c)}	0.33 ± 0.11	0.30 ± 0.11	0.13 ± 0.10	0.02 ± 0.10	-	-	-	-	-
August	0.37 ± 0.09 ^{b)}	0.58 ± 0.10 ^{a)}	0.52 ± 0.10 ^{a)}	0.49 ± 0.10 ^{a)}	0.32 ± 0.09 ^{c)}	0.21 ± 0.09	0.09 ± 0.09	-	-	-	-
September	0.86 ± 0.09 ^{a)}	1.07 ± 0.10 ^{a)}	1.01 ± 0.09 ^{a)}	0.98 ± 0.09 ^{a)}	0.81 ± 0.08 ^{a)}	0.71 ± 0.08 ^{a)}	0.68 ± 0.08 ^{a)}	0.50 ± 0.07 ^{a)}	-	-	-
October	0.63 ± 0.09 ^{a)}	0.84 ± 0.10 ^{a)}	0.78 ± 0.09 ^{a)}	0.75 ± 0.09 ^{a)}	0.58 ± 0.09 ^{a)}	0.47 ± 0.08 ^{a)}	0.45 ± 0.08 ^{a)}	0.26 ± 0.07 ^{b)}	-0.24 ± 0.06 ^{b)}	-	-
November	0.45 ± 0.09 ^{a)}	0.66 ± 0.10 ^{a)}	0.60 ± 0.09 ^{a)}	0.57 ± 0.09 ^{a)}	0.40 ± 0.09 ^{a)}	0.29 ± 0.09 ^{c)}	0.27 ± 0.08	0.08 ± 0.07	-0.41 ± 0.06 ^{a)}	-0.18 ± 0.06	-
December	0.06 ± 0.09	0.27 ± 0.10	0.21 ± 0.10	0.18 ± 0.10	0.01 ± 0.09	-0.09 ± 0.09	-0.12 ± 0.09	-0.30 ± 0.08 ^{b)}	-0.80 ± 0.07 ^{a)}	-0.56 ± 0.07 ^{a)}	-0.39 ± 0.07 ^{a)}

We set the total monthly number of wild boars photographed for each year and camera as the response variable, the year and the month as the explanatory variables, each camera as a random factor, and the trap days for each camera and season as offset terms, using a generalized linear mixed model with a Poisson distribution in the lme4 package and multcomp package. a), b), and c) indicates $P < 0.001$, $P < 0.01$, and $P < 0.05$, respectively.

(2017–2018: $P = 0.58$, 2017–2019: $P = 0.81$, 2018–2019: $P = 0.94$).

Our seasonal RAI trends were similar to those of a previous study, which found peaks in September and October [7]. This previous study suggested that monthly relative abundances were linked to the availability of fruit and mast seeding and piglet movement ability. Thus, wild boars frequently moved near the camera sites, resulting in an increase in RAI in this and the previous study. Considering similar seasonal RAI trends before and after the CSFV outbreak, the potential influence of CSFV on seasonal RAI trends was very low or non-existent.

Further, RAIs in 2019 were significantly lower than those in 2017 and 2018. First, the number of wild boars captured in 2017 from August to December was 35, and the number decreased drastically during similar periods in 2018 (nine boars) and 2019 (11 boars). Although culling programs interrupted the trends in both years, the periods of interruption were only 1 month and 2 months in 2018 and 2019, respectively. Therefore, the decrease in the number of wild boars captured was due to population decline rather than a decrease in capture efforts. Second, we observed no significant decrease in age ratios throughout the study period, indicating that there were no changes in natural mortality in the piglet class before and after the CSFV outbreak. A previous study reported that the most important period for piglets is during the first 3 months after birth, followed by during the autumn months (October and November), as 82.1% of piglets died before December [12]. Thus, our results reflected the initial natural mortality of piglets. However, population abundance would be influenced not only by natural mortality but also by birth rate. If the birth rate was relatively stable, it is possible there would be no negative impact of CSFV on population decline in the following year. Because we did not investigate the birth rate, however, population decline would not always be influenced only by CSFV.

Therefore, in the semi-isolated wild boar population on Mt. Kinka, CSFV had a negative impact on the population abundance of the wild boars temporarily. However, we did not investigate the relationship between RAI and food availability in this study area. A previous study in Shimane prefecture, western Japan, reported the positive impact of acorn food availability on the nutritional condition of wild boars [14], supporting the importance of rich or poor mast availability for wild boar population management [6]. However, wild boar inhabiting forested areas mainly feed not only bamboo and acorn but also on the underground parts of plants throughout the year [15]. Therefore, there is a possibility that yearly RAIs were influenced by food availability and rich or poor mast availability. A further study should carefully monitor the relationship between wild boar abundance and food availability in surrounding areas, in addition to the CSFV outbreak.

Following the outbreak of CSFV on Mt. Kinka in 2018, some boars infected with CSFV died and the relative population

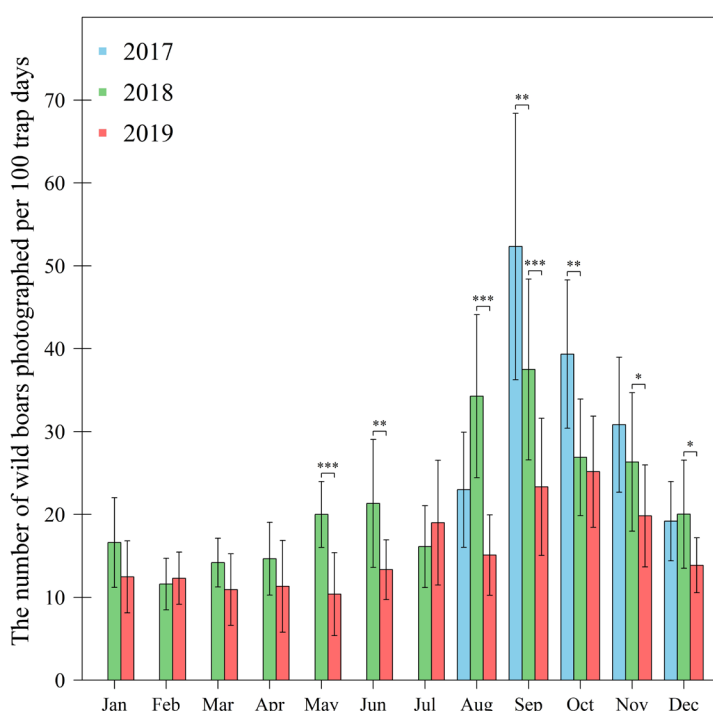


Fig. 1. Monthly number of wild boars photographed per 100 trap days and standard errors throughout the study period on Mt. Kinka, Gifu Prefecture, Japan.

abundance decreased temporarily. However, most adult pigs recovered and convalescent animals exhibited stable lifelong immunity against CSFV [19]. After the CSFV outbreak on Mt. Kinka, 10 live-captured adults were tested using RT-PCR and ELISA for the diagnosis of CSF. Three samples from the adults showed negative RT-PCR and positive ELISA results. Because a CSFV oral vaccine was first administered in December 2019 in the surrounding area located approximately 3 km from the study area, CSFV-positive boars on Mt. Kinka were considered infected naturally. Furthermore, considering the milder pathogenicity of the JPN/1/2018 strain [13], many adult animals with stable, lifelong immunity against CSFV potentially remain on Mt. Kinka. The sustained supply of piglets and/or the low mortality rates of susceptible piglets after the disappearance of the maternal antibodies were thought to be some of the factors leading to the lack of differences in age ratios annually before and after the CSFV outbreak on Mt. Kinka.

The main goal of CSF management in wild boars is to stop virus transmission by reducing the population abundance of susceptible individuals in the infected area up to the threshold level [1, 3]. The threshold for low-virulence CSFV variants was estimated to be one susceptible boar/km² [4]. However, although it was difficult to estimate the wild boar population abundance, several estimation methods without the need for individual recognition using camera traps have been developed recently [20, 25]. To facilitate the implementation of effective CSFV countermeasures, further studies that estimate wild boar population abundance using such approaches are required.

In the present study, a relatively small wild boar population showed no drastic decreases following a CSFV outbreak. Therefore, wildlife managers should consider that wild boars continue to contract CSFV, and they should intensively implement countermeasures in agricultural lands and in pig farms, in addition to wild boar population management. For example, it could be necessary to place wire fences to prevent invasion into agricultural lands and pig farms and to monitor the presence of wild boars in surrounding areas. Conversely, although CSFV outbreaks have been reported in 12 prefectures, no study has reported the impacts of CSFV on wild boar population dynamics. Considering that national authorities in Europe have adopted countermeasures that were ineffective against African swine fever when it broke out in 2007, it would be critical for wildlife managers to adopt countermeasures that are backed by the results of research to effectively manage wildlife disease [26]. Consequently, wildlife managers and researchers need to obtain ecological data on wild boar populations from various sources, which would facilitate the implementation of appropriate CSFV and wild boar population management strategies informed by research findings. Our results obtained from field data could facilitate the formulation of appropriate CSFV countermeasures.

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