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Serological and molecular prevalence of severe fever with thrombocytopenia syndrome virus in wild boar and poultry

멧돼지와 가금류에서의 중증열성혈소판감소증후군 바이러스에 대한 분자학적 및 혈청학적 유병률

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Abstract

Severe fever with thrombocytopenia syndrome (SFTS) is an emerging infectious, zoonotic disease and caused by *Dabie bandavirus* that belongs to the genus *Bandavirus* in the family *Phenuiviridae* and order Bunyavirales. It is transmitted by hard ticks and has been found in Asian countries. SFTS virus

(SFTSV) has been detected in several tick species, various animals, and humans. Many previous studies of SFTSV have been conducted in animals, but there is no or lack of information on wild boar and poultry such as chickens, ducks, and wild geese. Thus, the purpose of this study was to detect antigens and antibodies of SFTSV in wild boar and poultry including chickens, ducks, and wild geese (farm-raised) in the Republic of Korea (ROK).

A total of 768 wild boar and 606 poultry sera were collected all over the country in the ROK. 768 wild boar sera were collected between January and December, and poultry sera were collected with 312 from chickens, 249 from ducks, and 45 from wild geese.

To detect antigen of SFTSV, one-step reverse transcriptase polymerase chain reaction (RT-PCR) and nested PCR was performed to amplify the S segment of SFTSV. The phylogenetic analysis and alignment of the gene were carried out after the sequencing the gene. IgG antibodies against SFTSV was measured with indirect enzyme-linked immunosorbent assay (ELISA). In addition, immuno-fluorescence assay (IFA) with wild boar sera and neutralization test (NT) with poultry sera were also conducted as complementary tests to the ELISA. Correlation analysis of ELISA with IFA and NT were performed with wild boar and poultry sera, respectively with calculation of Cohen's kappa coefficient.

Of wild boar sera, 40 (5.2%) were antigen positive. Two hundred twenty-one (28.8%) and 159 (20.7%) were seropositive by ELISA and IFA, respectively. 110 (14.3%) wild boar sera were seropositive by both tests. Comparing to the ELISA, the sensitivity of IFA was 69.2% and the specificity was 81.8%. ELISA and IFA were statistically significant difference (p <0.05). Of 40 detected SFTSV by RT-PCR, 33 (82.5%) and 7 (17.5%) of them were classified as the genotype B-3 and D, respectively.

A total of 606 poultry sera, 15 (2.5%) were antigen positive; 7 (2.2%) were from chickens, 3 (1.2%) from ducks, and 5 (11.1%) from wild geese. From the 15 positive sera for SFTSV, 3 were from chickens, 3 from ducks, and one from wild geese were classified as the genotype B-2, one serum from chickens was classified as the genotype B-3, 3 from chickens and 4 from wild geese were classified as the genotype D. Out of the 568 sera tested by ELISA, 83 (28.0%) from chickens, 81 (32.9%) from ducks, and 8 (30.8%) from wild geese were seropositive.

Of the 539 poultry sera for which an NT was performed, 113 (38.6%) were from chickens and 75 (30.5%) from ducks were positive. Comparing to the ELISA in chicken sera, the sensitivity of NT was 51.3% and the specificity was 87.8%. In the duck sera, the sensitivity of NT was 76.0% and the specificity was 86.0%. ELISA and NT were statistically significant in both chicken and duck sera through statistical analysis (p <0.05).

The results of this study suggested the possibility on transmission of SFTSV through the various animal species including wild boar, chickens, ducks, and wild geese in the ROK. In addition, the presence of several genotypes of SFTSV was demonstrated in this study. These results might be useful to develop the strategies to prevent and control SFTSV.

Keywords: Severe fever with thrombocytopenia syndrome virus, tick-borne diseases, wild boar, poultry, chickens, ducks, wild geese

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List of Abbreviations

CB	Chungcheongbuk-do
CN	Chungcheongnam-do
DMEM	Dulbecco's modified Eagle medium
DR	Detection rate
ELISA	Enzyme-linked immunosorbent assay
FITC	Fluorescein isothiocyanate
GB	Gyeongsangbuk-do
GG	Gyeonggi-do
GN	Gyeongsangnam-do
GW	Gangwon-do
HRP	Horseradish peroxidase
IFA	Immunofluorescence assay
IPTG	Isopropyl β–D-thiogalactoside
IQR	Interquartile range
JB	Jeollabuk-do
JJ	Jeju-do
JN	Jeollanam-do
mAb	Monoclonal antibody
NP	Nucleoprotein
NS	Nonstructural

NT	Neutralization test
OD	Optical density
PCC	Pearson correlation coefficient
PR	Positive rate
SFTS	Severe fever with thrombocytopenia syndrome
SFTSV	Severe fever with thrombocytopenia syndrome virus
qRT-PCR	Quantitative real-time polymerase chain reaction
RdRp	RNA-dependent RNA polymerase
ROK	Republic of Korea
RT	Room temperature
RT-PCR	Reverse transcriptase polymerase chain reaction
SU	Seoul

1. Introduction

Dabie bandavirus, generally called severe fever with thrombocytopenia syndrome virus (SFTSV), is the causative agent of severe fever with thrombocytopenia syndrome (SFTS) and belongs to the genus *Bandavirus*, family *Phenuiviridae*, and order Bunyavirales (Loeffelholz and Fenwick, 2021). SFTS is an emerging infectious disease and the major clinical manifestations are fever, thrombocytopenia, leukopenia, and gastrointestinal symptoms (Xu et al., 2011; Yu et al., 2011; Yokomizo et al., 2022). SFTSV is spherical and 80-100 nm in diameter (Tiwari and Talreja, 2020). It has negative sense, single stranded RNA segments including three of S, M, and L segments (Yun et al., 2020). S segment contains nonstructural protein (NS) and nucleoprotein (NP) and encoded by these proteins. M segment encodes the Gn and Gc of glycoproteins and L segment encodes the RNA-dependent RNA polymerase (RdRp).

SFTSV has been detected in hard tick species such as *Haemaphysalis longicornis, Haemaphysalis flava, Amblyomma testudinarium, Ixodes nipponensis*, and *Rhipicephalus microplus* (Yun et al., 2014; Jung et al., 2019). The main vector of SFTSV transmission is known as *Haemaphysalis longicornis* (Zhuang et al., 2018). SFTSV has been found in Asian countries including China, Japan, Republic of Korea (ROK), Vietnam, Taiwan, and has recently also been reported in Thailand, Pakistan, and Myanmar (Kim et al., 2013; Takahashi et al., 2014; Zhan et al., 2017; Tran et al., 2019; Lin et al., 2020; Ongkittikul et al., 2020; Win et al., 2020; Zohaib et al., 2020).

In China, SFTS was identified in 2009 and 571 confirmed cases from 13 provinces were reported according to the national surveillance in 2011 (Yu et al., 2011; Liu et al., 2014a). Since 2010, SFTS cases increased gradually and more than 7,000 cases were reported in at least 23 provinces of China with average mortality rate of 5.3% from 2013 to 2016 (Zhan et al., 2017; Huang et al., 2020). Confirmed cases of SFTS and fatal cases of SFTSV infection have been reported in Japan and

the ROK (Kim et al., 2013; Takahashi et al., 2014; Li et al., 2015; Wei et al., 2015; Liang et al., 2021). Since the first SFTS case was confirmed in 2013 in Japan, 40 to 90 cases have been reported between 2013 and 2017 from 23 of the 47 prefectures with total reported number of 303 SFTS cases (Kobayashi et al., 2020; Yokomizo et al., 2022). In the ROK, the number of patients infected with SFTSV have increased since the first SFTS patients were reported in 2013, and more than 200 patients with SFTS have been reported since 2017 (Choi et al., 2021). During 7 years of 2013 to 2019, there was a total of 1,089 patients with SFTS, and the number of deaths were 214 with a fatality rate of 19.7%.

Studies on human-to-human transmission cases caused by SFTSV (Gai et al., 2011; Liu et al., 2012; Chen et al., 2013; Tang et al., 2013; Jiang et al., 2015) and studies that suggesting the possibility of aerosol transmission on SFTSV (Gong et al., 2015; Moon et al., 2018) have been reported. Recently, a secondary transmission case from cat to human SFTS was reported in Japan (Kida et al., 2019; Yamanaka et al., 2020). Thus, it is important to investigate infection and transmission routes of SFTSV in various animal species as well as in humans.

Many epidemiological studies have been performed to survey the prevalence or seroprevalence of SFTSV in domesticated and wild animals including dogs, cats, goats, chickens, cattle, pigs, rodents, geese, hedgehogs, wild boar, minks, sheep, yellow weasels, hares, Elk, Deer, Shrews, *Anser cygnoides*, and *Streptopelia chinensis* (Ding et al., 2014a; Li et al., 2014; Hayasaka et al., 2016; Wang et al., 2017; Chen et al., 2019; Fujii et al., 2019; Huang et al., 2019). Various studies of SFTSV detection have been conducted in the ROK from Korean water deer, wild boar, reptiles, cats, dogs, domestic pigs, and black goats (Oh et al., 2016; Suh et al., 2016; Hwang et al., 2017; Lee et al., 2017b; Kang et al., 2018a, 2018b; Han et al., 2020; Lee et al., 2020).

Although previous studies of SFTSV detection in wild boar have been reported, they did not provide enough information about the antigens and

antibodies of SFTSV in wild boar throughout the ROK. In addition, there is no or a lack of information on chickens, ducks, and wild geese despite many of the previous studies of SFTSV in animals. Thus, the aim of this study is to investigate the serological and molecular prevalence of SFTSV in wild boar and poultry including chickens, ducks, and wild geese (farm-raised) in the ROK.

2. Materials and methods

2.1. Ethics statement

This study was approved by the Seoul National University Institutional Animal Care and Use Committee (IACUC No. SNU-190524-2-1) for test from wild boar and poultry, Jeonbuk National University Institutional Biosafety Committee (IBC No. JBNU2022-03-002) for test from poultry, and performed with strict accordance to the recommendations of the National Guidelines.

2.2. Sample collection and storage

Blood samples were collected from wild boar, chickens, ducks, and wild geese (farm-raised) using a SST blood tube (BD Vacutainer®). Wild boar samples were collected from January to December 2019 in all ten provinces of the ROK. Poultry samples such as chickens, ducks, and wild geese were collected in Gyeonggi-do (GG), Chungcheongnam-do (CN), Jeollabuk-do (JB), Jeollanam-do (JN), and Gyeongsangnam-do (GN). All sera samples were stored at -80℃ until analysis.

2.3. RNA preparation

The RNA was extracted from 200 μL serum using the Viral Gene-spin Viral DNA/RNA Extraction Kit (iNtRON Biotechnology) according to the manufacturer's instructions and the extracted viral RNA was stored at -80℃ until use.

2.4. Reverse transcriptase polymerase chain reaction (RT-PCR)

To detect the small (S) segment of SFTSV, each sample was tested using one-step reverse transcriptase polymerase chain reaction (RT-PCR) and nested PCR assays. Primary PCR was performed using DiaStar One-Step RT-PCR premix (Solgent) with previously designed primers (Table 1). The primary reaction was performed with an initial step of 30 min at 50℃ and 15 min at 95℃ for denaturation, followed by 40 cycles of 20 s at 95℃, 40 s at 52℃, 30 s at 72℃, with a final extension step of 5 min at 72℃. Nested PCR was conducted using 1 μL of the primary PCR product as a template with HiPi PCR premix (Elpis-Biotech) and previously described primers (Table 1). The reaction for the nested PCR consisted of 25 cycles of 20 s at 94℃, 40 s at 55℃, and 30 s at 72℃. The PCR products were analyzed using gel electrophoresis in 1.2% agarose gel and a 100 bp ladder molecular weight DNA size marker (iNtRON Biotechnology).

2.5. Nucleotide sequencing

To analyze PCR products of SFTSV, positive PCR amplicons were directly sequenced by sequencing service (Bionics, Seoul, ROK) using Applied Biosystems 3730XL DNA Analyzer (Thermo Scientific, Waltham, MA). The nucleotide sequences in this study were obtained by several times of RT-PCR for the accurate sequencing analysis. These sequences were aligned using ClustalW of MEGA7 and compared with the sequences of reference strain.

2.6. Phylogenetic analysis

A phylogenetic tree was constructed based on nucleotide alignments using the maximum-likelihood method implemented in the MEGA 7 software (Kumar et al., 2016) and bootstrap analysis was performed to evaluate the robustness with 1,000 replicates. The nucleotide sequences obtained in this study were submitted to

the GenBank database under the accession numbers MT502531-MT502570 and MZ593709-MZ593723.

2.7. Indirect enzyme-linked immunosorbent assay (ELISA)

To detect antibodies against SFTSV in wild boar and poultry sera, 96-well plates (Thermo Scientific) were coated with 100 ng/well of purified recombinant nucleoprotein (NP) at 4°C overnight. For preparation of recombinant proteins, gene encoding the NP was cloned into pET28a (Novagen) and the proteins were purified from *Escherichia coli* BL21 strain. After induction with 0.1 mM isopropyl β–D-thiogalactoside (IPTG), the proteins were purified using HisTrap HP histidine-tagged protein columns (GE Healthcare) according to the manufacturer's instructions. After coating, the 96-well plates were blocked with 5% skim milk in PBST (containing 0.05% Tween 20) for 2 hrs at room temperature (RT) and subsequently incubated for 1 hr at RT with 1 μL of wild boar serum per well in 5% skim milk. Detection in the wild boar serum samples was performed using horseradish peroxidase (HRP)-conjugated goat anti-pig IgG (Abcam) and HRP-conjugated goat anti-chicken IgG (Novus biologicals) was used for detection in the chicken serum samples. For detection in the duck and wild goose serum samples, HRP-conjugated goat anti-duck IgG (SeraCare) was used. 3,3',5,5'-tetramethylbenzidine substrate solution was added for development of color and the reaction was performed for 10 min. Subsequently, stop solution (2N hydrosulfuric acid) was added to each well. The optical density (OD) was measured at 450 nm of wavelength by a microplate reader (BioTek Instruments, Winooski, VT).

2.8. Indirect immunofluorescence assay (IFA) from wild boar

IFA slides were prepared using SFTSV-infected Vero E6 cells. The Vero cells were resuspended at a density of 5×10^3 cells/well in medium (2% fetal bovine serum in Dulbecco's modified Eagle medium (DMEM)), added to 24-well slides, and incubated for 16 hrs in a 5% CO_2 incubator. The slides were fixed with 100 % acetone at −20 °C for 10 min. After blocking with 5% rabbit serum for 2 hrs, the diluted serum was applied to IFA antigen slides and incubated in the 5% CO_2 incubator for 90 min. The serum samples were diluted to 1:50, 1:100, 1:200, 1:400 with PBS for attaching the primary antibody. Anti-pig IgG fluorescein isothiocyanate (FITC)-antibody (Sigma-Aldrich) was added to each well of the antigen slides after washing and incubated in the 5% CO_2 incubator for 1 hr. Visualization of the IFA slides was performed using EVOS™ M7000 Imaging System (Invitrogen, Frederick, MD).

2.9. Neutralization test (NT) from poultry

To confirm the presence of the antibody against SFTSV in poultry sera, equal volumes (100 μL) of chicken and duck sera, and SFTSV (100 $TCID_{50}$) were incubated for 1 hr in 96-well plate. To determinate the cut-off dilution ratio, two ELISA positive and two ELISA negative sera from both chicken and duck samples were prepared with dilution ratios from 1:2 to 1:64. Cut-off dilution ratio of chicken and duck sera was determined to 1:4. Cell control and virus back titration was used to compare the antibody positive and negative sera. After incubation at 37℃ in a 5% CO_2 incubator for 1 hr, Vero E6 cells were added and cultured for 3~4 days. Subsequently, 80% acetone was added for 30 min at -20℃ to fix the samples and SFTSV NP specific monoclonal antibody (mAb) provided by VetAll (VetAll Laboratories®) with dilution ratio of 1:1,000 was added to the fixed samples for 1 hr at 37℃ incubator. Anti-mouse IgG FITC antibody (Sigma-

Aldrich) with a dilution ratio of 1:100 was added after the reaction with mAb and a EVOS^{TM} M7000 microscope (Thermo Scientific, Waltham, MA) was used to confirm if chicken and duck sera samples were antibody positive or negative for SFTSV. When confirming results using an EVOS^{TM} M7000 microscope, it was determined as positive for SFTSV antibodies if fluorescence did not appear, and it was determined as negative for SFTSV antibodies if fluorescence appeared.

2.10. Statistical analysis

The Chi-square test and Pearson correlation analysis were performed to determine if there was a statistically significant difference between ELISA and the IFA from wild boar and ELISA and the NT from poultry. Cohen's kappa coefficient was also used to measure the agreement of the ELISA and IFA, and the ELISA and NT. The SPSS Statistics software version 26.0 (IBM, Armonk, NY) was used to conduct the Chi-square test and Pearson correlation analysis and to calculate the Cohen's kappa coefficient. If the *p* value of the Chi-square test was <0.05, the results were considered to be statistically significant and these experiments were considered to have a quantitative linear relationship when the Pearson correlation coefficient (PCC) was closer to 1. Strength of agreement on Cohen's kappa coefficient was determined according to the interpretation by Landis and Koch (Landis and Koch., 1977).

3. Results

3.1. Detection of antigen and antibody on SFTSV in wild boar

3.1.1. Antigen test by RT-PCR

A total of 768 wild boar sera were collected from all ten provinces of ROK. Forty (5.2%) of 768 sera were positive for SFTSV by RT-PCR. Specifically, SFTSV genomes were found in seven out of the ten provinces and detection rate (DR) for SFTSV was the highest in Seoul (SU) (16.7%), followed by GN (8.6%), Chungcheongbuk-do (CB) (8.1%), and Gyeongsangbuk-do (GB) (7.3%) provinces (Fig. 1). The SFTSV antigen was most frequently detected in July, but was not detected in January, April, May, August, September, and October (Fig. 2). The detection of antigen by each month is described in Figure 2.

3.1.2. Nucleotide sequencing and phylogenetic analysis

According to a novel classification method of SFTSV based on phylogenetic analysis, 33 out of 40 positive wild boar serum samples were classified as genotype B-3 with 29 samples (wild boar 81) containing same sequences and the other four (wild boar 215, wild boar 306, wild boar 346, and wild boar 385) containing different sequences (Fig. 3). Compared to the wild boar 81, all four samples (wild boar 215, wild boar 306, wild boar 346, and wild boar 385) were different in one nucleotide position (Table 2). The rest of the seven sera samples were classified as genotype D, one nucleotide position was different between four samples (wild boar 3974) and the other three samples (wild boar 4030).

3.1.3. Antibody test by ELISA and IFA

Of the 768 sera, 221 (28.8%) sera were seropositive for SFTSV based on ELISA with exceeding OD value of 1 at 450 nm. Eight (50.0 %) wild boar sera from Jeju-do (JJ) and 24 (63.2%) sera collected in February had the highest positive rate (PR) by ELISA (Fig. 1 and 2). No seropositive sera were detected in JB using ELISA. SFTSV antibodies showed the lowest detection in April based on ELISA, 2 (6.9%) sera were seropositive. There was no cross-reactivity between SFTSV and *Anaplasma* based on the ELISA of SFTSV from dog samples experimentally infected with *Anaplasma* (data not shown).

Using IFA, 159 (20.7%), 82 (10.7%), 32 (4.2%), and 8 (1.0%) wild boar sera samples were found to be seropositive at more than 1:50 (≥50), 1:100 (≥100), 1:200 (≥200), and 1:400 (≥400) dilution ratios, respectively. Based on the EVOS™ M7000 Imaging System, wild boar sera that were positive for SFTSV antibodies were confirmed at ≥50, ≥100, ≥200, and ≥400 (Fig. 4). Of the seropositive wild boar samples, 1 (25.0%) of ≥50 from JB, 37 (13.2%) of ≥100 from Gangwon-do (GW), 5 (6.8%) of ≥200 from CB, and 5 (1.8%) of ≥400 from GW provinces based on IFA had the highest PR (Fig. 1). The SFTSV antibodies were the most frequently detected in August based on IFA in 8 (47.1%) of ≥50, 5 (29.4%) of ≥100, and 2 (11.8%) samples of ≥200 (Fig. 2). In case of ≥400 based on IFA, 3 (6.3%) sera in January had the highest PR for SFTSV antibodies. None of wild boar sera were seropositive for SFTSV using IFA in SU and except ≥50 based on IFA, none of seropositive sera were detected in JB province (Fig. 1). In case of ≥200 and ≥400 by IFA, SFTSV antibodies were not detected in wild boar sera in JJ, CN, and JN provinces. At ≥400 based on IFA, antibodies for SFTSV were not detected in the GG province. The lowest detection of SFTSV antibodies was in June at ≥50, ≥100 and ≥400 based on IFA; 2 (3.2%) and 1 (1.6%) sera were seropositive at ≥50

and ≥100, respectively, and seropositive sera were not detected at ≥400 (Fig. 2). At ≥200 and ≥400 based on IFA, none of serum samples were seropositive for SFTSV in April and May. In case of ≥400 based on IFA, antibodies for SFTSV were not detected in July, August, September, and November.

Out of the seropositive wild boar sera that were detected simultaneously by both ELISA and IFA, 110 (14.3%) of 768 sera were positive for SFTSV antibodies. Unlike the results of RT-PCR, SFTSV antibodies were mostly found in the CB province (18.9%) by both ELISA and IFA (Fig. 5). Of the 74 sera in CB province using both ELISA and IFA, 14 samples were found to be seropositive for SFTSV. The lowest detection of SFTSV antibodies by both ELISA and IFA was in June (Fig. 6). As shown in Figure 6, the PR was the highest in August (41.2%), as detected by both ELISA and IFA. Comparing the result concordance of ELISA and IFA, the sensitivity of IFA compared to the ELISA was 69.2% and the specificity was 81.8% (Table 3). Considering the above results, the PCC was 0.458 and p value was <0.05 in the Pearson correlation analysis and the Chi-square test, respectively, and kappa value was measured at 0.448. Compared to the antigen test by RT-PCR, total prevalence (%) based on the antibody test for both ELISA and IFA (14.3%) was more than double that of the antigen test (5.2%). PR of antibody test was high from late summer to early winter season, while DR of antigen test was low in the same period (Fig. 6). As spring and winter progresses, results of antigen and antibody test showed decreasing and increasing tendency, respectively. In addition, the PR found using the antibody test increased after DR found using the antigen test increased.

3.2. Detection of antigen and antibody on SFTSV in poultry

3.2.1. Antigen test by RT-PCR

Of the 606 poultry sera, 312 were from chicken sera, 249 from duck sera, and 45 from wild goose sera were collected (Table 4). Of these sera, total of 15 (2.5%) poultry sera including 7 from chickens, 3 from ducks, and 5 from wild geese were positive for SFTSV by RT-PCR. The highest DR was in wild goose sera (11.1%), followed by chicken (2.2%) and duck (1.2%) sera. Poultry sera were collected from 5 provinces, GG (*n*=110), CN (*n*=50), JB (*n*=50), JN (*n*=345), and GN (*n*=51). DR of SFTSV antigen from poultry sera by provinces are described in Figure 7. The chicken sera were collected from GG (*n*=110), CN (*n*=50), JN (*n*=146), and GN (*n*=6) (Fig. 7). Chicken sera of GN indicate the highest DR (33.3%) against SFTSV antigen. For the duck sera, 50 and 199 sera were collected from JB and JN, respectively. Three (1.5%) duck sera in JN were positive for SFTSV antigen, and SFTSV was not detected in the duck sera from JB. Wild goose sera were only collected from GN and five (11.1%) of the 45 wild goose sera were positive against SFTSV antigen.

3.2.2. Nucleotide sequencing and phylogenetic analysis

Based on phylogenetic analysis, three of seven positive chicken sera, all of three positive duck, and one of the five positive wild goose sera were classified as the genotype B-2 according to a novel classification method of SFTSV (Fig. 8). Of a total of 15 positive poultry sera, only one positive chicken serum was belonged to the genotype B-3. The remaining four positive chicken and four

positive wild goose sera were classified as the genotype D. Through sequence analysis according to nucleotide position, three positive chicken (KGNCh41, KGNCh44, KJNCh51) and three positive wild goose sera (KGNWG27, KGNWG33, KGNWG44) contained the same sequence (Table 5). In the genotype B-2, two pairs of positive chicken and duck sera contained same sequences each other; KICCh26 of chicken and KJNDu15 of duck sera, and KICCh34 of chicken and KJNDu74 of duck sera.

3.2.3. Antibody test by ELISA and NT

Of a total of 606 collected poultry sera, 568 sera that included 296 from chickens, 246 from ducks, and 26 from wild geese were tested to detect SFTSV antibodies using ELISA. Cut-off OD values were determined to be 0.93, 1.82, and 0.92 in chickens, ducks, and wild geese, respectively. Seropositive sera were determined to excess cut-off OD value of each species. Antibody positive and negative sera of SFTSV by NT were described in Figure 9. One hundred and seventy-two (30.3%) out of 568 sera were seropositive for SFTSV antibodies (Table 4). In detail, 83 chicken sera, 81 duck sera, and 8 wild goose sera were seropositive. Duck sera showed the highest PR (32.9%), followed by wild goose (30.8%) and chicken (28.0%) sera. In chicken sera, samples from GG (n=94), CN (n=50), JN (n=146), and GN (n=6), were tested by ELISA for the detection of SFTSV antibodies (Fig. 7). Of a total of 296 chicken sera performed using ELISA, 41 from GG, one from CN, and 41 from JN were seropositive and SFTSV antibodies were not detected in sera from GN. Sera of GG showed the highest PR (43.6%) in chicken sera. Of a total of 246 duck sera, 28 from JB and 53 from JN were seropositive and sera in JB demonstrating the highest PR (59.6%) out of all poultry sera. In case of wild goose sera, eight from GN were seropositive.

Of the total 606 poultry sera collected, 539 sera containing 293 chicken

and 246 duck sera were tested by NT to confirm the antibody positive to SFTSV and to compare with the results of ELISA (Table 4). Sera of antibody positive to SFTSV were decided to those that did not display fluorescence at cut-off dilution ratio. Of a total of 539 poultry sera tested by NT, 188 sera were antibody positive to SFTSV. In detail, 113 chicken and 75 duck sera were antibody positive to SFTSV with PR of 38.6% and 30.5%, respectively. Comparing the result concordance of ELISA and NT, there were 58 antibody positive chicken sera for both ELISA and NT and 158 antibody negative chicken sera for both ELISA and NT. The sensitivity of NT compared to the ELISA was 51.3% and the specificity was 87.8% (Table 6). Considering the above results, the PCC was 0.427 and *p* value was <0.05 in the Pearson correlation analysis and the Chi-square test, respectively, and kappa value was measured at 0.414. For the duck sera, 57 were antibody positive and 147 were antibody negative for ELISA and NT, with a sensitivity of NT compared to the ELISA was 76.0% and specificity was 86.0%. Based on the Pearson correlation analysis and the Chi-square test between ELISA and NT, the PCC was 0.607 and *p* value was <0.05 from duck, and a 0.606 kappa value was calculated.

4. Discussion

In previous studies of animals that have shown the prevalence and seroprevalence of SFTSV, 1.6% (3/184) of rodents, 5.1% (8/159) of hedgehogs, 3.8% (18/472) of sheep, 4.2% (35/842) of cattle, 5.3% (19/359) of dogs, 2.3% (3/129) of water deer, and 1.9% (4/207) of goats were positive for SFTSV by antigen tests (Niu et al., 2013; Li et al., 2016; Yu et al., 2018; Lee et al., 2020). In addition, 8.3% (7/184) of rodents, 12.6% (20/159) of hedgehogs, 2.4% (2/85) of birds, 69.5% (328/472) of sheep, 60.5% (509/842) of cattle, 37.9% (136/359) of dogs, 23.8% (5/21) of water deer, and 83.0% (111/134) of goats were seropositive for SFTSV by antibody tests (Zhao et al., 2012; Niu et al., 2013; Ding et al., 2014a; Li et al., 2016; Oh et al., 2016).

According to previous studies on pigs and wild boar, 2.6% (22/839) of pigs, and 3.7% (2/54) of wild boar were positive for SFTSV by RT-PCR and quantitative real-time PCR (qRT-PCR), and 3.1% (26/839) of pigs, 18.9% (36/190) (Hayasaka et al., 2016) and 1.9% (1/54) of wild boar were seropositive for SFTSV based on ELISA and IFA (Niu et al., 2013; Oh et al., 2016). In previous studies of detection on SFTSV antigen in poultry, nine out of 527 chickens were positive for SFTSV antigen with infection rate of 1.7% using the qRT-PCR (Niu et al., 2013). Moreover, 250 out of 527 chickens were positive for SFTSV antibody with positivity of 47.4% and two out of 120 geese were seropositive for SFTSV with positivity of 1.7% by ELISA (Niu et al., 2013; Li et al., 2014). There were no enough studies on the prevalence or seroprevalence on SFTSV in poultry such as ducks and wild geese.

To the best of our knowledge, this is the first study to investigate the wild boar sera by month throughout the ROK and to determine the serological and

molecular prevalence of SFTSV in wild boar and poultry including wild geese as well as chickens and ducks in the ROK using RT-PCR, ELISA, IFA and NT.

In this study, two wild boar sera from SU and one from CN were detected for SFTSV antigen by RT-PCR, and SFTSV were not detected in JB, JN, and JJ, where only a small number of sera were collected (Fig. 1). Thus, it is necessary to test a larger number of sera samples from SU, CN, JB, JN, and JJ provinces. According to the DR results of SFTSV in wild boar based on RT-PCR, DR was high in March and July (Fig. 2). To our knowledge, this result suggests that the transmission cycles of SFTSV in tick populations are consistent with the increase in DR. Wild boar from SU, GG, and GW were positive for SFTSV in December. It was found that ticks attach to wild boar and suck their blood even in the winter season (unpublished data). This result reveals the possibility of transmission from ticks to wild boar in the early winter season. It is prudent for hunters and authorized people to be careful to prevent secondary infections when dealing with carcasses of wild boar infected with SFTSV in the winter season for public health.

The phylogenetic analysis of the SFTSV viral genome revealed that SFTSV was classified into two clades, Japanese and Chinese (Yoshikawa et al., 2015). However, according to the new classification method of SFTSV genome, its sequences were classified into at least six genotypes (A-F) and reassorted groups of at least nine genotypes (R-1 to R-9) (Yun et al., 2020). In this study, 33 wild boar seropositive sera were found to belong to genotype B-3 and 7 wild boar seropositive sera were found to belong to genotype D. In a previous study, genotype B-3 was the second most detected group, while genotype D showed a low number of detected cases (Yun et al., 2017).

In the results of antibody test using ELISA and IFA, SFTSV antibodies

were detected in wild boar samples obtained in February (winter) and August (summer) (≥400 in IFA from sample taken in August was not detected). These results show that the PR of SFTSV antibody was high in the periods of summer and winter. According to periods of activity of nymphs in March and adults in July (Zheng et al., 2011), the DR of SFTSV antigen by RT-PCR also increased in March and July and this may be related to the life cycle of tick populations. Further studies are required to investigate the correlation of patterns of antigen and antibodies of SFTSV and activities of ticks. The results of ELISA and IFA showed high PR (14.3%, 110 in 768 sera based on both ELISA and IFA). High values of PR of SFTSV antibodies indicate that infection has been occurred not only in the past but also in the present throughout the ROK. Although there are few studies showing data of both ELISA and IFA, some previous studies have shown high sensitivity of ELISA by nonspecific reactions and specificity of IFA to confirm false-positive reactions (Gokuden et al., 2018; Kimura et al., 2018). However, there are limitations of existing cross-reactivity in ELISA (Wu et al., 2014). Thus, we adjusted this using specificity of IFA. In this study, the wild boar sera that were seropositive by both ELISA and IFA may provide useful information for determining the seroprevalence of SFTSV. To confirm the cross-reactivity between SFTSV and other organisms, we performed the ELISA of SFTSV from dogs experimentally infected with *Anaplasma*. Anaplasmosis is one of the most frequent tick-borne diseases and the vector of *Anaplasma* is same as that of SFTS (Rymaszewska and Grenda, 2008; Seong et al., 2015). Through this experiment, we confirmed that there was no cross-reactivity between SFTSV and *Anaplasma*. In this study, the sensitivity and specificity of IFA compared to the ELISA were 69.2% and 81.8%, indicating that the specificity of the IFA is higher than the sensitivity. As a result of Chi-square test, the p value was <0.05 in wild boar, therefore, the results of ELISA and IFA were considered to be statistically significant. In the Pearson correlation analysis, PCC showed that there is a

quantitative linear relationship between ELISA and IFA. In the result of calculation on Cohen's kappa coefficient, kappa value indicated that strength of agreement was moderate in wild boar. Comparing the antigen test to the antibody test for SFTSV in wild boar, the DR of antigen was lower than that of antibody, which suggests that the infected animals experience low level or a short period of viremia, and rapid production of antibodies against SFTSV (Oh et al., 2016; Kang et al., 2018a). Thus, further studies, such as investigation of SFTSV distribution, survey of circulation routes in other animals, and analysis of SFTSV antigen and antibodies are necessary to understand the prevalence of SFTSV in animals and its trends (Kang et al., 2018a).

Among poultry sera tested by RT-PCR, chicken sera in GN province had the highest DR (Fig. 7). Moreover, the SFTSV was detected in all sampling areas except for JB province and it showed that the SFTSV was detected in various provinces of the ROK. This study provided meaningful information for investigating the prevalence of SFTSV among poultry in the ROK. The detection of SFTSV antigen in five provinces indicated that the poultry of those provinces were exposed to SFTSV. In this regard, poultry farmers and poultry-related workers should be vigilant with their poultry management to prevent exposure of ticks. It is necessary to collect poultry samples and test them with RT-PCR monthly throughout the ROK to monitor the DR and observe any changes.

Based on sequencing and the phylogenetic tree, poultry sequences of positive for SFTSV were classified into three genotypes, genotype B-2, B-3, and D (Table 5, Fig. 8). This is following the new classification method on SFTSV genome as genotype A to F of seven genotypes and B-1 to B-3 as sub-genotypes of genotype B, do not follow the classification method of the two clades, Chinese and Japanese clades (Yoshikawa et al., 2015; Yun et al., 2020). The genotypes mainly

found in poultry sequences of this study were genotype B-2 and D (Table 5, Fig. 8). The genotype B-2 has been known to be one of the most prevalent genotypes in the ROK (Seo et al., 2021). With a rate of 86.0%, the genotype B-2 is also the most prevalent genotype in Japan as well (Yun et al., 2020; Casel et al., 2021). In China, the most prevalent genotype is the genotype F at a rate of 43.6% followed by the genotype A (Fu et al., 2016). The genotype D has not been frequently detected in the ROK (Yun et al., 2017), but in this study, it was detected in more than half of the poultry sequences, and this indicates that the genotype D is also a prevalent genotype in wild geese as well as chickens.

Due to the insufficient volume of samples, sera from 16 chickens, 3 ducks, and 19 wild geese were not evaluated by ELISA. ELISA was performed on the remaining 568 sera of the total 606 poultry sera used in RT-PCR (Table 4). The cut-off value of ELISA for each poultry species, except for wild geese, was calculated by multiplying 30 samples of negative for SFTSV antibody by a constant according to the 99% of reliability and adding the average of the 30 samples (Frey et al., 1998). In the case of wild geese, it was determined by calculating the cut-off value from 6 samples of negative for SFTSV antibody. Comparing the antigen DR by RT-PCR and the PR by ELISA on SFTSV, the DRs were 2.2% in chickens, 1.2% in ducks, and 11.1% in wild geese with a total DR of 2.5% in poultry. The PR was 28.0% in chickens, 32.9% in ducks, and 30.8% in wild geese with a total of 30.3% PR in poultry (Table 4). Overall, PR is higher than the DR of SFTSV, and this high seroprevalence may put people who come into close contact with poultry in the environment susceptible to transmission of SFTSV. In addition, as animal to human of SFTSV infection has been reported and the possibility that animals could be a source of SFTSV transmission to humans has been emerged (Kida et al., 2019;

Kobayashi et al., 2020; Yamanaka et al., 2020), people who have direct contact with animals should make an effort to prevent the risk of secondary transmissions between animals and humans. Further studies on the establishment of surveillance systems to control the SFTSV transmission between animals and humans are necessary.

To understand the relationship between the two experiments (ELISA and NT), NT was performed to complement the limitations of ELISA. The sample volumes of 19 chickens, three ducks, and all the wild goose sera were insufficient, thus the NT was conducted on 539 chicken and duck sera out the total 606 poultry sera collected (Table 4). ELISA has a relatively higher sensitivity than specificity, and NT has higher specificity than sensitivity (Li, 2013; Liu et al., 2014b; Gokuden et al., 2018; Noh et al., 2021). In this study, the sensitivity and specificity of NT compared to the ELISA were 51.3% and 87.8% in chickens, and 76.0% and 86.0% in ducks, respectively (Table 6), indicating that the specificity of the NT is higher than the sensitivity. Chi-square test, Pearson correlation analysis, and Cohen's kappa coefficient have been used to perform statistical analysis in various studies (Ding et al., 2014b; Lee et al., 2017a; Matsuu et al., 2021). In this study, statistical analysis was performed using Chi-square test, Pearson correlation analysis, and Cohen's kappa coefficient. In the results of Chi-square test, the p value was <0.05 in both chickens and ducks, therefore, the results of ELISA and NT were considered to be statistically significant. In the Pearson correlation analysis, PCC showed that there is a quantitative linear relationship between ELISA and NT. As a result of calculation on Cohen's kappa coefficient, kappa value indicated that strength of agreement was moderate in chickens and substantial in ducks.

5. Conclusions

This study showed the serological and molecular prevalence of SFTSV from wild boar and poultry including chickens, ducks, and wild geese using RT-PCR, ELISA, IFA, and NT in the ROK and indicated the possibility of circulation of SFTSV in these animal species. In case of wild boar, we tested sera during an entire year throughout the ROK. This will provide useful information to other studies for understanding the SFTSV infection and transmission between wild boar and poultry as well as wild animals. Wild boar and poultry samples in this study were classified as genotype B-2, B-3, and D (Fig. 10). Further studies on the detection of SFTSV in other wild animals are needed, as this study reported SFTSV infections in wild boar and poultry.

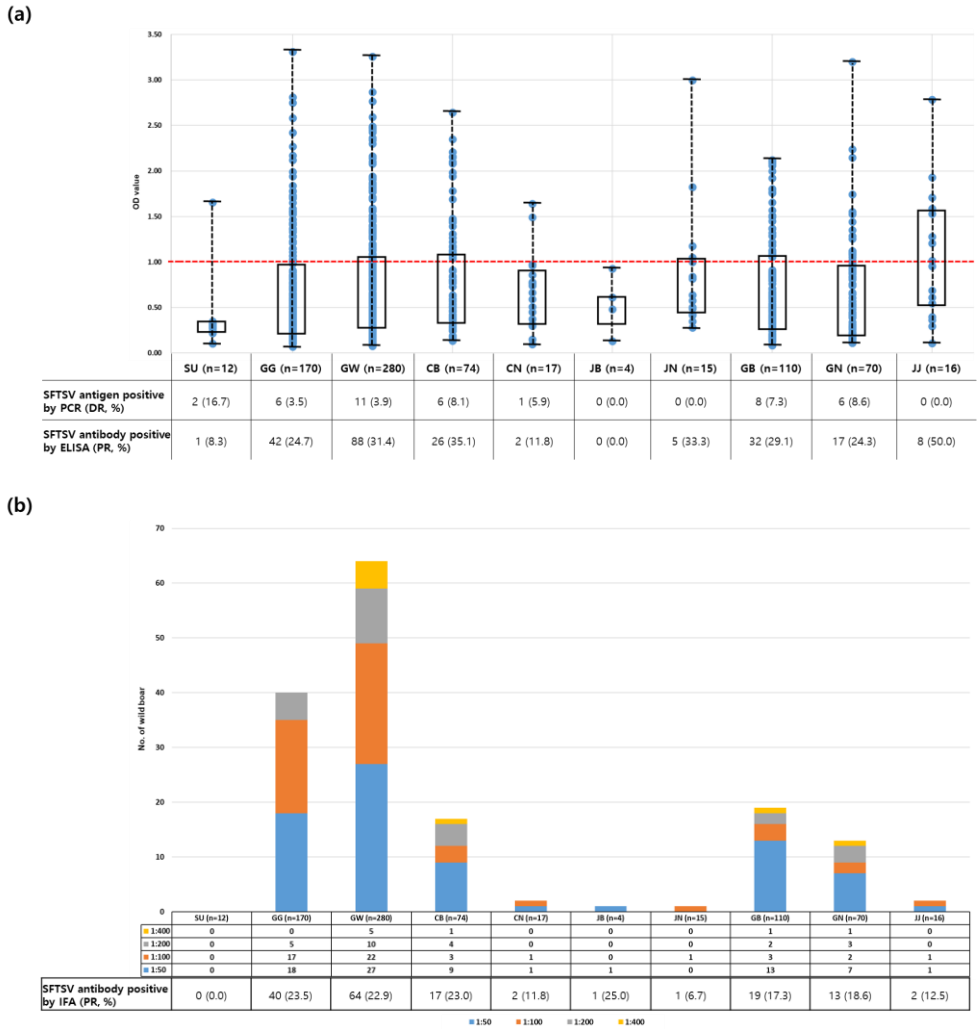
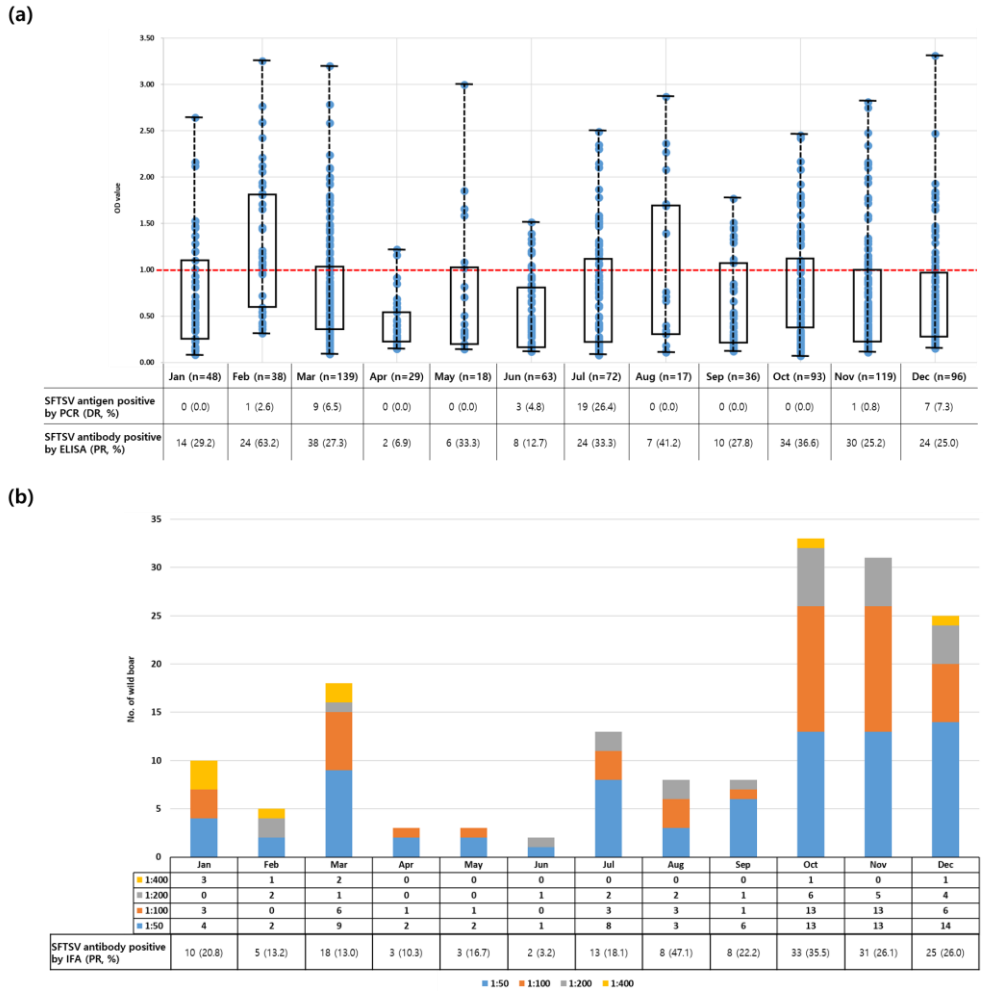


Figure 1. Regional distribution of SFTSV antigen and antibody in the ROK. (a) Regional distribution of SFTSV antigen and antibody in wild boar using RT-PCR and ELISA, respectively. Cut-off OD value was indicated with red dotted line. IQR of the box plot was indicated to show the middle 50% of values. (b) Regional distribution of SFTSV antibody in wild boar by IFA. OD, optical density; SU, Seoul; GG, Gyeonggi-do; GW, Gangwon-do; CB, Chungcheongbuk-do; CN, Chungcheongnam-do; JB, Jeollabuk-do; JN, Jeollanam-do; GB, Gyeongsangbuk-do; GN, Gyeongsangnam-do; JJ, Jeju-do; IQR, Interquartile range; RT-PCR, reverse transcriptase polymerase chain reaction; DR, detection rate; PR, positive rate; IFA, immunofluorescence assay; SFTSV, severe fever with thrombocytopenia syndrome virus; ROK, Republic of Korea; ELISA, enzyme-linked immunosorbent assay.



	Jan (n=48)	Feb (n=38)	Mar (n=139)	Apr (n=29)	May (n=18)	Jun (n=63)	Jul (n=72)	Aug (n=17)	Sep (n=36)	Oct (n=93)	Nov (n=119)	Dec (n=96)
SFTSV antigen positive by PCR (DR, %)	0 (0.0)	1 (2.6)	9 (6.5)	0 (0.0)	0 (0.0)	3 (4.8)	19 (26.4)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.8)	7 (7.3)
SFTSV antibody positive by ELISA (PR, %)	14 (29.2)	24 (63.2)	38 (27.3)	2 (6.9)	6 (33.3)	8 (12.7)	24 (33.3)	7 (41.2)	10 (27.8)	34 (36.6)	30 (25.2)	24 (25.0)

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
1:400	3	1	2	0	0	0	0	0	0	1	0	1
1:200	0	2	1	0	0	1	2	2	1	6	5	4
1:100	3	0	6	1	1	0	3	3	1	13	13	6
1:50	4	2	9	2	2	1	8	3	6	13	13	14
SFTSV antibody positive by IFA (PR, %)	10 (20.8)	5 (13.2)	18 (13.0)	3 (10.3)	3 (16.7)	2 (3.2)	13 (18.1)	8 (47.1)	8 (22.2)	33 (35.5)	31 (26.1)	25 (26.0)

Figure 2. Monthly distribution of SFTSV antigen and antibody in the ROK. (a) Monthly distribution of SFTSV antigen and antibody in wild boar using RT-PCR and ELISA, respectively. Cut-off OD value was indicated with red dotted line. IQR of the box plot was indicated to show the middle 50% of values. (b) Regional distribution of SFTSV antibody in wild boar by IFA. OD, optical density; SU, Seoul; GG, Gyeonggi-do; GW, Gangwon-do; CB, Chungcheongbuk-do; CN, Chungcheongnam-do; JB, Jeollabuk-do; JN, Jeollanam-do; GB, Gyeongsangbuk-do; GN, Gyeongsangnam-do; JJ, Jeju-do; IQR, Interquartile range; RT-PCR, reverse transcriptase polymerase chain reaction; DR, detection rate; PR, positive rate; IFA, immunofluorescence assay; SFTSV, severe fever with thrombocytopenia syndrome virus; ROK, Republic of Korea; ELISA, enzyme-linked immunosorbent assay.

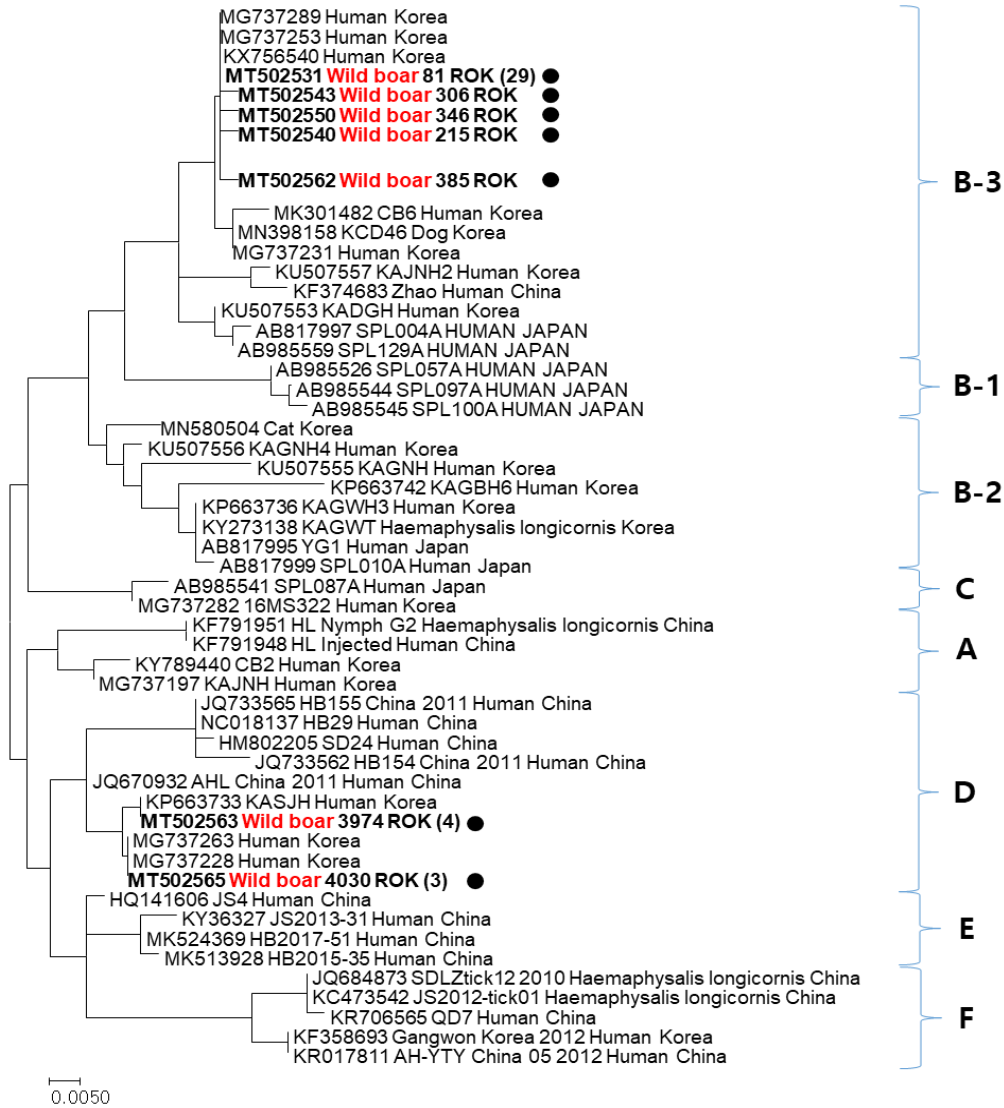


Figure 3. Phylogenetic tree and genotypes of wild boar sequences on severe fever with thrombocytopenia syndrome virus (SFTSV) based on the partial small (S) segment sequences (346 bp). The sequences identified in this study are indicated by bold letters. The maximum-likelihood method was used to construct the phylogenetic tree based on the Kimura two-parameter model (1,000 bootstrap replicates). The percentage of trees in which associated taxa are clustered together is shown next to the branches. Black dots indicate the sequences of wild boar sera positive for SFTSV in this study. The number inside the bracket indicates the number of detection samples of SFTSV.

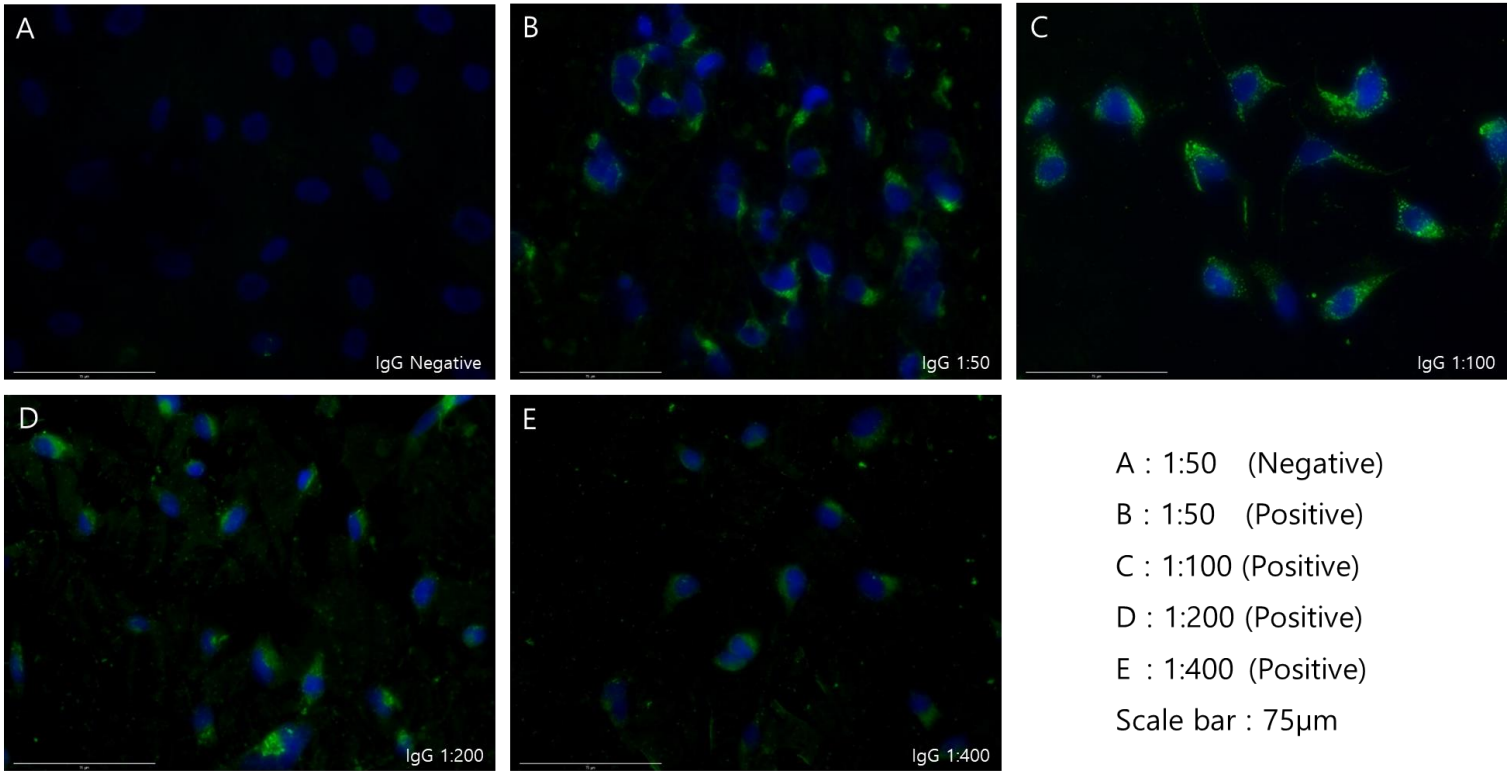


Figure 4. Results of immunofluorescence assay (IFA) for diagnosis of severe fever with thrombocytopenia syndrome (SFTS) from wild boar. A, negative control; B, 1:50 of dilution ratio in positive serum; C, 1:100 of dilution ratio in positive serum; D, 1:200 of dilution ratio in positive serum; E, 1:400 of dilution ratio in positive serum.

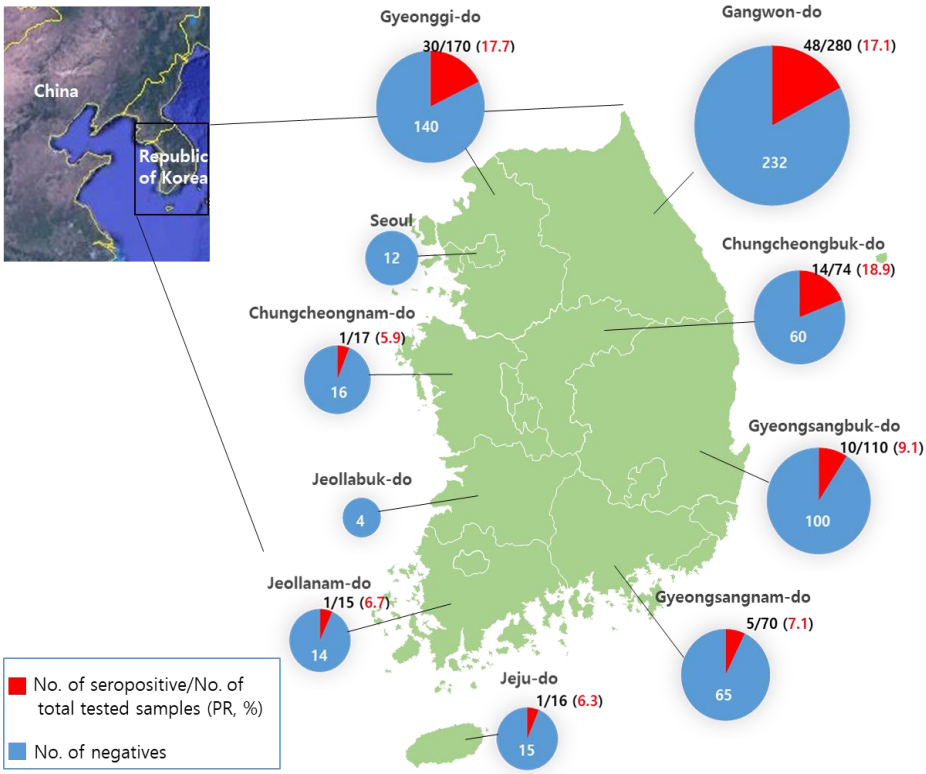


Figure 5. Map of seroprevalence in wild boar sera for severe fever with thrombocytopenia syndrome virus (SFTSV) and positive rate (PR) of each province from the Republic of Korea (ROK). The number inside the bracket indicates the PR (%) in each province. Seropositive samples are indicated with results of both immunofluorescence assay (IFA) and enzyme-linked immunosorbent assay (ELISA): antibodies were detected at more than 1:50 of dilution ration in IFA and optical density (OD) value at 450 nm exceeded 1 in ELISA.

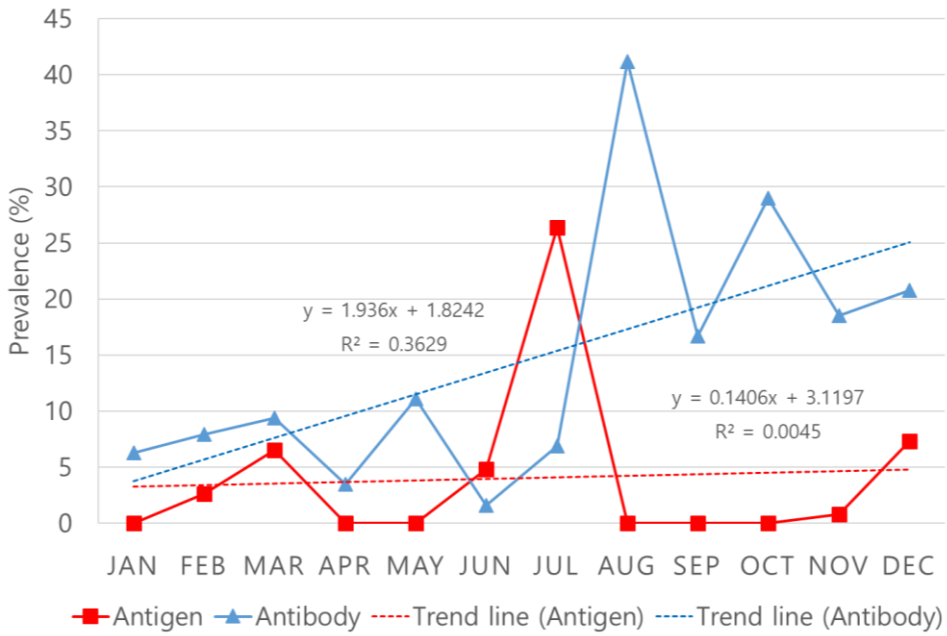


Figure 6. Comparison of prevalence (%) of severe fever with thrombocytopenia syndrome virus (SFTSV) between antigen and antibody test by reverse transcriptase polymerase chain reaction (RT-PCR) and both immunofluorescence assay (IFA) and enzyme-linked immunosorbent assay (ELISA) from wild boar (n=768). Positive rate (PR) for antibody test is indicated by using both IFA and ELISA. R^2 indicates a coefficient of determination for each trend line of antigen and antibody test.

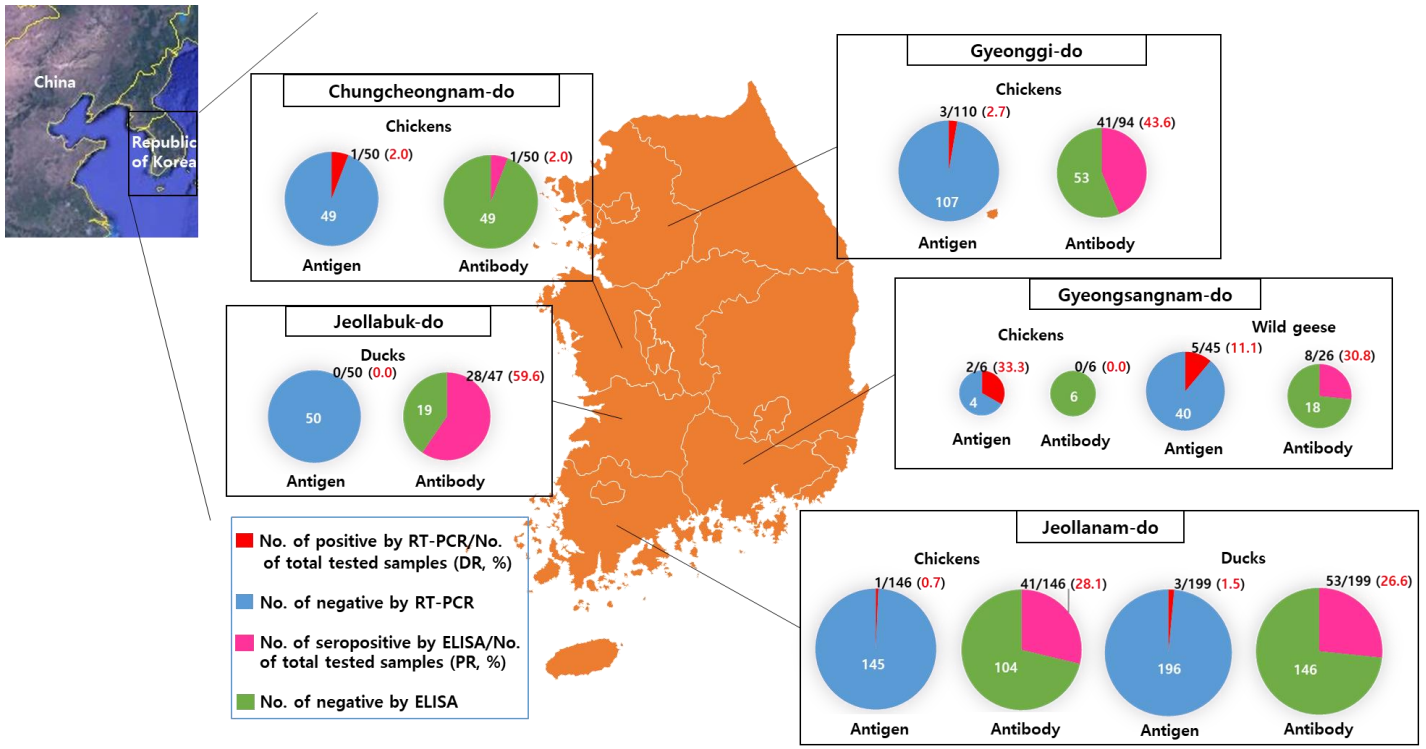


Figure 7. Map of antigen detection and seroprevalence from poultry on severe fever with thrombocytopenia syndrome virus (SFTSV) of each province from the Republic of Korea (ROK). Detection of antigen and antibody for SFTSV was performed by reverse transcriptase polymerase chain reaction (RT-PCR) and indirect enzyme-linked immunosorbent assay (ELISA), respectively. The numbers inside the brackets indicate the detection rate (DR, %) and positive rate (PR, %) in each province. White numbers inside the circular graphs indicate the number of negative poultry sera for SFTSV antigen and antibody.

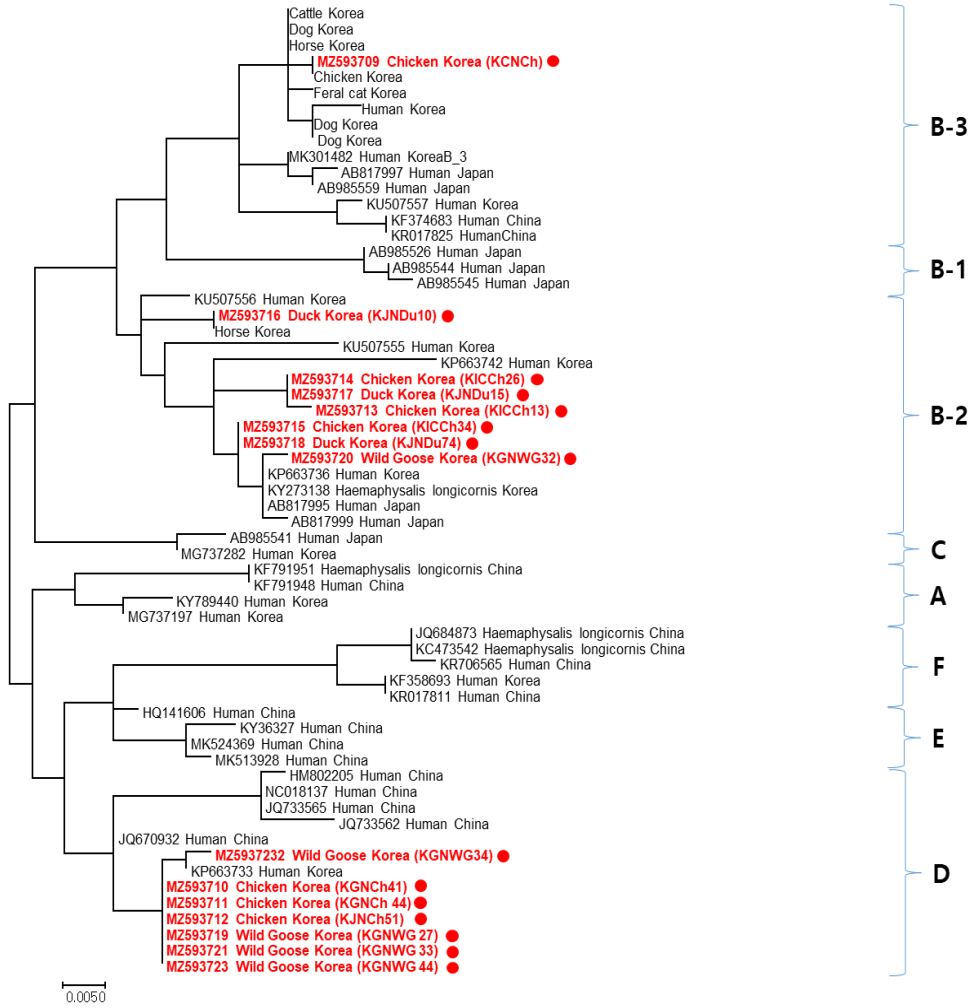


Figure 8. Phylogenetic tree and genotypes of poultry sequences on severe fever with thrombocytopenia syndrome virus (SFTSV) by the partial small (S) segment sequences (346 bp). The sequences identified in this study are indicated by bold letters. The maximum-likelihood method was used to construct the phylogenetic tree based on the Kimura two-parameter model (1,000 bootstrap replicates). Red dots indicate the sequences of poultry sera including chickens, ducks, and wild geese (farm-raised) positive for SFTSV antigen in this study. The letters inside the bracket indicate the name of detection poultry samples on SFTSV.

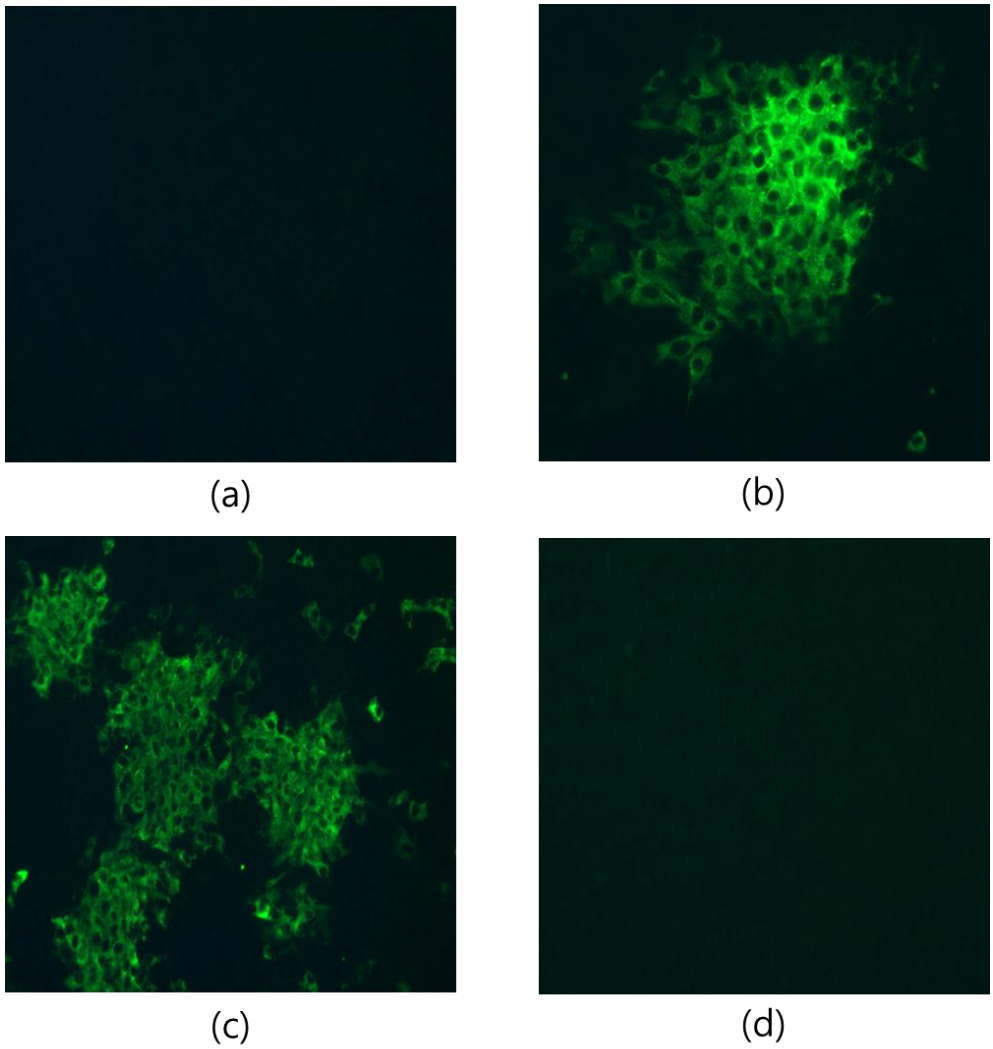


Figure 9. Results of neutralization test (NT) in poultry for detection of antibodies on severe fever with thrombocytopenia syndrome virus (SFTSV). (a) Antibody positive serum of SFTSV. (b) Antibody negative serum of SFTSV. (c) SFTSV control. (d) Cell control.

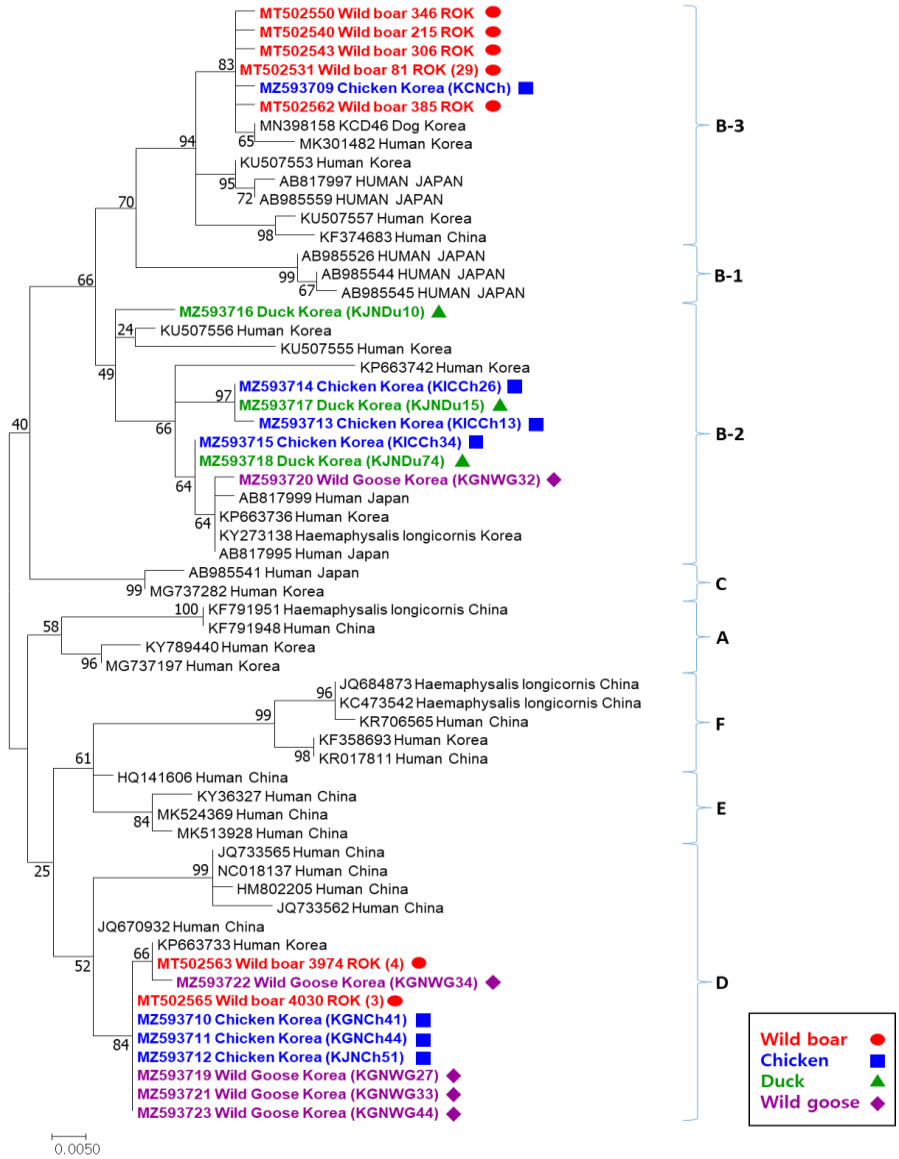


Figure 10. Phylogenetic tree and genotypes of wild boar and poultry sequences on severe fever with thrombocytopenia syndrome virus (SFTSV) by the partial small (S) segment sequences (346 bp). The sequences identified in this study are indicated by bold letters. The maximum-likelihood method was used to construct the phylogenetic tree based on the Kimura two-parameter model (1,000 bootstrap replicates). The letters and numbers inside the bracket indicate the name of detection poultry samples on SFTSV and number of same sequences in wild boar, respectively.

Table 1. Primer sets used for RT-PCR and nested PCR

Primer names	Nucleotide sequences	References
NP-2F	5'-CAT CAT TGT CTT TGC CCT GA-3'	Yoshikawa et al., 2014
NP-2R	5'-AGA AGA CAG AGT TCA CAG CA-3'	
N2-F	5'-AAY AAG ATC GTC AAG GCA TCA-3'	Oh et al., 2016
N2-R	5'-TAG TCT TGG TGA AGG CAT CTT-3'	

RT-PCR, reverse transcriptase polymerase chain reaction.

Table 2. Nucleotide differences by position in genotype B-3 and D of positive wild boar sera for severe fever with thrombocytopenia syndrome virus (SFTSV) based on sequence analysis

Sample names	Genotypes (n)	Nucleotide positions (bp)																					
		3	33	45	55	63	96	105	126	129	153	171	186	192	198	225	231	255	258	273	282	294	315
Wild boar 81	B-3 (29)	T	A	T	A	T	A	C	A	A	C	A	A	C	T	C	A	G	T	A	A	G	C
Wild boar 215	B-3 (1)	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	T
Wild boar 306	B-3 (1)	·	·	·	G	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
Wild boar 346	B-3 (1)	·	·	·	·	·	·	T	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
Wild boar 385	B-3 (1)	·	G	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
Wild boar 3974	D (4)	C	·	C	·	C	G	·	T	G	T	G	T	G	C	T	T	A	C	G	T	C	·
Wild boar 4030	D (3)	·	·	C	·	C	G	·	T	G	T	G	T	G	C	T	T	A	C	G	T	C	·

Black dots (·) indicate same nucleotides compared with Wild boar 81 by position.

Table 3. Sensitivity and specificity of the immunofluorescence assay (IFA) to indirect enzyme-linked immunosorbent assay (ELISA) from wild boar

		IFA		PCC	*p* value (Chi-square test)	*k* value
		Positive (≥50)	Negative (<50)			
ELISA	Positive (OD>1)	110	111	0.458	<0.05	0.448
	Negative (OD≤1)	49	498			
Sensitivity (%)		69.2				
Specificity (%)		81.8				

IFA, immunofluorescence assay; ELISA, enzyme-linked immunosorbent assay; PCC, Pearson correlation coefficient; OD, optical density.

Table 4. Antigen and antibody tests for severe fever with thrombocytopenia syndrome virus (SFTSV) using reverse transcriptase polymerase chain reaction (RT-PCR), indirect enzyme-linked immunosorbent assay (ELISA), and neutralization test (NT) from poultry sera, including chickens, ducks, and wild geese (farm-raised) in the Republic of Korea (ROK)

Animals	No. of positive by RT-PCR/No. of sample	Detection rates (DR, %)	No. of seropositive by ELISA/No. of sample (OD value)	Positive rates by ELISA (PR, %)	No. of seropositive by NT/No. of sample (titers)	Positive rates by NT (PR, %)
Chickens	7/312	2.2	83/296 (OD>0.93)	28.0	113/293 (<4)	38.6
Ducks	3/249	1.2	81/246 (OD>1.82)	32.9	75/246 (<4)	30.5
Wild geese (farm-raised)	5/45	11.1	8/26 (OD>0.92)	30.8	NA	NA
Total	15/606	2.5	172/568	30.3	188/539	34.9

RT-PCR, reverse transcriptase-polymerase chain reaction; DR, detection rate; ELISA, enzyme-linked immunosorbent assay; OD, optical density; PR, positive rate; NT, neutralization test; NA, not available.

Table 5. Nucleotide sequence differences by position of poultry sera positive for severe fever with thrombocytopenia syndrome virus (SFTSV) in genotypes B-2, B-3, and D based on sequence analysis

Genotypes	N	Animals	Sample names	Nucleotide positions (bp)															
				3	39	45	52	63	78	96	126	129	135	153	169	171	174	186	192
D	3	Chicken	KGNCh41, KGNCh44, KJNCh51	T	A	C	T	C	A	G	T	G	C	T	T	G	G	T	G
D	3	Wild goose	KGNWG27, KGNWG33, KGNWG44	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
D	1	Wild goose	KGNWG34	C	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
B-2	1	Chicken	KICCh13	·	T	T	·	·	G	A	·	A	·	·	C	·	·	A	·
B-2	1	Chicken	KICCh26	·	T	T	·	·	G	A	·	A	·	·	C	·	·	A	·
B-2	1	Chicken	KICCh34	·	T	T	·	·	G	A	·	A	·	·	C	A	·	A	·
B-2	1	Duck	KJNDu15	·	T	T	·	·	G	A	·	A	·	·	C	·	·	A	·
B-2	1	Duck	KJNDu74	·	T	T	·	·	G	A	·	A	·	·	C	A	·	A	·
B-2	1	Duck	KJNDu10	·	·	T	C	·	·	A	·	A	·	·	·	A	·	A	·
B-2	1	Wild goose	KGNWG32	C	T	T	·	·	G	A	·	A	·	·	·	A	T	A	·
B-3	1	Chicken	KCNCh	·	·	T	·	T	·	A	A	A	A	C	·	A	·	A	C

Genotypes	N	Animals	Sample names	Nucleotide positions (bp)															
				198	201	208	219	225	231	255	258	273	279	282	288	294	319	322	
D	3	Chicken	KGNCh41, KGNCh44, KJNCh51	C	C	C	G	T	T	A	C	G	A	T	C	C	C	T	
D	3	Wild goose	KGNWG27, KGNWG33, KGNWG44	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	
D	1	Wild goose	KGNWG34	·	·	·	·	·	·	·	·	·	·	·	·	·	·	C	
B-2	1	Chicken	KICCh13	T	T	·	·	·	·	G	·	A	G	·	T	G	·	·	
B-2	1	Chicken	KICCh26	T	T	·	·	·	·	G	·	A	·	·	T	G	·	·	
B-2	1	Chicken	KICCh34	T	·	·	A	·	·	G	·	A	·	·	·	G	·	·	
B-2	1	Duck	KJNDu15	T	T	·	·	·	·	G	·	A	·	·	T	G	·	·	
B-2	1	Duck	KJNDu74	T	·	·	A	·	·	G	·	A	·	·	·	G	·	·	
B-2	1	Duck	KJNDu10	T	·	T	·	·	·	G	·	A	·	·	·	G	T	·	
B-2	1	Wild goose	KGNWG32	T	·	·	A	·	·	G	·	A	·	·	·	G	·	·	
B-3	1	Chicken	KCNCh	T	·	·	·	C	A	G	T	A	·	A	·	G	·	·	

Black dots (·) indicate the same nucleotides in the nucleotide sequence of KGNCh41 by position.

Table 6. Sensitivity and specificity of the neutralization test (NT) to indirect enzyme-linked immunosorbent assay (ELISA) from chicken and duck sera

Chickens		NT		PCC	*p* value (Chi-square test)	*k* value	Ducks		NT		PCC	*p* value (Chi-square test)	*k* value
		Positive (≥4)	Negative (<4)						Positive (≥4)	Negative (<4)			
ELISA	Positive (OD>0.93)	58	22	0.427	<0.05	0.414	ELISA	Positive (OD>1.82)	57	24	0.607	<0.05	0.606
	Negative (OD≤0.93)	55	158					Negative (OD≤1.82)	18	147			
Sensitivity (%)		51.3					Sensitivity (%)		76.0				
Specificity (%)		87.8					Specificity (%)		86.0				

NT, neutralization test; ELISA, enzyme-linked immunosorbent assay; PCC, Pearson correlation coefficient; OD, optical density.

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국 문 초 록

멧돼지와 가금류에서의 중증열성혈소판감소증후군 바이러스에 대한 분자학적 및 혈청학적 유병률

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중증열성혈소판감소증후군(severe fever with thrombocytopenia syndrome, SFTS)은 신종 인수공통감염병으로 Bunyavirales 목, *Phenuiviridae* 과, *Bandavirus* 속에 속하는 *Dabie bandavirus*에 의해 발병한다. 이것은 참진드기에 의해 전파되고 여러 아시아 국가에서 발견되고 있다. SFTS virus (SFTSV)는 여러 참진드기 종과 다양한 동물 종 및 사람에서 검출되어

왔다. SFTSV에 대한 이전의 많은 연구가 동물에서 수행되어 왔음에도 불구하고 멧돼지와 가금류에 속하는 닭, 오리, 기러기에 대한 정보가 부족하거나 연구된 바 없었다. 따라서, 본 연구는 국내의 멧돼지와 닭, 오리, 기러기(농장 사육)를 포함한 가금류에서 SFTSV의 항원 및 항체 검출을 목적으로 하였다.

국내에서 총 768마리의 멧돼지와 606마리의 가금류 혈청 샘플을 수집하였다. 768마리의 멧돼지 혈청은 1월부터 12월 사이의 샘플로 확보하였고, 가금류 혈청은 닭 312마리, 오리 249마리, 기러기 45마리에서 샘플을 수집하였다.

SFTSV의 항원을 검출하기 위하여 1단계 역전사중합효소연쇄반응(reverse transcriptase-polymerase chain reaction, RT-PCR)과 SFTSV의 S절편을 증폭시키기 위한 중첩 PCR을 수행하였다. 유전자 염기서열을 분석한 후 계통학적 분석과 정렬을 시행하였다. 간접효소면역측정법(enzyme-linked immunosorbent assay, ELISA)을 수행하여 SFTSV의 IgG 항체를 검사하였다. 덧붙여, ELISA를 보완하기 위해 멧돼지에서 면역형광측정법(immunofluorescence assay, IFA)이, 가금류에서 중화항체측정(neutralization test, NT)이 수행되었다. 멧돼지와 가금류 혈청에서 각각 ELISA에 대한 IFA와 NT의 Pearson correlation analysis를 수행하였고 Cohen's kappa coefficient를 계산하였다.

멧돼지 혈청 중에서 40마리(5.2%)가 항원 양성이었다. ELISA와 IFA에서 각각 221마리(28.8%)와 159마리(20.7%)가 항체 양성으로 나타났다. 두 실험에서 모두 항체 양성인 멧돼지 혈청은 110마리(14.3%)였다. ELISA와 비교했을 때, IFA의 민감도는 69.2%, 특이도는 81.8%로 나타났다. ELISA와 IFA는 통계적으로 유의하게 다른 것으로 나타났다(p <0.05). RT-PCR에서 SFTSV가 검출되었던 40마리 중에서 33마리(82.5%)와 7마리(17.5%)의 혈청이 각각 B-3와 D 유전자형으로 분류되었다.

총 606마리의 가금류 혈청 중에서 15마리(2.5%)가 항원 양성이었는데 닭이 7마리(2.2%), 오리가 3마리(1.2%), 그리고 기러기가 5마리(11.1%)였다. 15마리의 항원 양성 혈청 중에서 닭 3마리, 오리 3마리, 그리고 기러기 1마리가 B-2 유전자형으로 분류되었고, 닭 1마리가 B-3 유전자형에

속했으며 닭 3마리와 기러기 4마리가 D 유전자형으로 나타났다. ELISA를 수행한 568마리의 혈청에 대하여 닭 83마리(28.0%), 오리 81마리(32.9%), 그리고 기러기 8마리(30.8%)가 항체 양성으로 나타났다. 539마리의 가금류 혈청에서 NT를 수행한 결과, 닭 113마리(38.6%)와 오리 75마리(30.5%)가 SFTSV 항체 양성이었다. 닭 혈청 샘플에서 ELISA와 비교한 NT의 민감도는 51.3%, 특이도는 87.8%였다. 오리 혈청에서의 NT 민감도는 76.0%, 특이도는 86.0%였다. 통계적 분석에 따라 ELISA와 NT는 닭과 오리 혈청에서 모두 통계적으로 유의한 것으로 나타났다(p <0.05).

본 연구 결과는 국내의 멧돼지, 닭, 오리, 기러기를 포함한 다양한 동물 종에서의 SFTSV 전파 가능성을 시사하였다. 또한, 동물 SFTSV의 B-2, B-3, D 유전자형이 본 연구에서 확인되었다. 이러한 결과들은 향후 SFTSV에 대한 예방 및 관리 전략을 수립하는 데 유용하게 활용될 것이다.

키워드: 중증열성혈소판감소증후군 바이러스, 진드기매개질병, 멧돼지, 가금류, 닭, 오리, 기러기

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