

# Insects as potential vectors of African swine fever virus in the Republic of Korea

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## Abstract

African swine fever (ASF) is a fatal disease in swine. Concentration of cases over summer and outbreaks in farms with high levels of biosecurity might support the hypothesis of ASF virus transmission via insects. A total of 28,718 insects were collected from 14 pig farms affected by ASF in the Republic of Korea. The insects collected were flies (N = 27,310, 95.1%), mosquitoes (N = 1,198, 4.2%), and cockroaches (N = 24, 0.1%). The remaining 0.6% (N = 186) could not be taxonomically classified. All the insects collected showed no trace of ASF Virus-DNA was detected by laboratory analysis. It is premature to conclude on the role of insects in the transmission of ASFV. However, the possibility of the virus spreading via insects should not be overlooked.

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## Summary

African swine fever (ASF) is a fatal disease in swine. Concentration of cases over summer and outbreaks in farms with high levels of biosecurity might support the hypothesis of ASF virus transmission via insects. A total of 28,718 insects were collected from 14 pig farms affected by ASF in the Republic of Korea. The insects collected were flies (N = 27,310, 95.1%), mosquitoes (N = 1,198, 4.2%), and cockroaches (N = 24, 0.1%). The remaining 0.6% (N = 186) could not be taxonomically classified. All the insects collected showed no trace of ASF Virus-DNA was detected by laboratory analysis. It is premature to conclude on the role of insects in the transmission of ASFV. However, the possibility of the virus spreading *via* insects should not be overlooked.

**Keywords:** African swine fever, Insect, Fly, Mosquito, Republic of Korea

## Introduction

African swine fever (ASF) is a hemorrhagic fatal disease in swine. Recently, there has been a drastic increase the affected areas, worldwide especially in Europe and Asia. Since the first notification of suspect cases at a pig farm in Paju, Gyeonggi-do Province, on September 16, 2019, the ASF outbreak had been confirmed

in a total of 14 pig farms by October 9, 2019, in Gyeonggi-do (Paju, Yeoncheon, and Gimpo) and Incheon Metropolitan City (Ganghwa) in the Republic of Korea (South Korea, hereafter Korea) [Kim et al., 2020]. On October 3, 2019, the ASF virus (ASFV) was first detected in a wild boar found dead in the demilitarized zone facing the border of the Democratic People's Republic of Korea (North Korea) [Jo & Gotyazar, 2020], and the documentation of ASF cases in wild boars continued until April 2020 [Yoo et al., 2020].

In Europe, the seasonality of ASF with a concentration of cases over summer and outbreaks occurring even in farms with high levels of biosecurity might support the hypothesis that ASFV is a vector-borne disease [Petrasianus et al., 2018]. The possibility of mechanical transmission of ASFV by the stable fly (*Stomoxys calcitrans*) and the blowfly (Calliphoridae) has been reported in experimental studies [Mellor et al., 1987; Forth et al., 2018]. More recently, it has been found that ASFV could be detected in stable flies that fed on ASFV-spiked blood 3 h and 12 h post-feeding. Moreover, pigs on which these flies fed were infected with ASFV [Olesen et al., 2018a; Olesen et al., 2018b]. Flies and mosquitoes exist all over the world, and the possibility of ASFV transmission by insects cannot be completely ruled out in Korea. Therefore, in this study, insects were collected and identified in the ASF outbreak pig farms to determine the role of insects as a potential vector of ASFV.

## Materials and Methods

Insects were collected in the 14 ASF outbreak farms, inside and around pig houses, both with and without virus-positive pigs, by the reverse transcription polymerase chain reaction (RT-PCR) test. Sampling was performed by installing collection tools, such as blacklight traps, aerial nets, and sticky strips. These tools had been in place for at least 24 h before being retrieved. The collection was performed jointly by the Animal and Plant Quarantine Agency (APQA) and the Korea Centers for Disease Control and Prevention (KCDC), APQA alone, or a private company (Biogenoci Ltd.) under consignment by APQA for the collection service. Laboratory tests for the detection of ASFV-DNA were conducted by APQA using RT-PCR according to the method described in previous studies by APQA [Kim et al., 2019; Kim et al., 2020]. Raw data on the insect collection and laboratory tests were derived from the government official document system of Korea. The data that support the findings of this study are available in the Veterinary Epidemiology Division of APQA.

## Results

A total of 28,718 insects were collected from 14 pig farms affected by the ASF outbreak in 4 regions: Paju (5 farms), Yeoncheon (2 farms), Gimpo (2 farms), and Ganghwa (5 farms). The first two collections were performed on September 27, 2019 in Ganghwa and October 2, 2019 in Paju during a field epidemiological investigation. Only one fly was caught on each farm. All other samplings were conducted between October 10, 2019 and October 31, 2019. In the three outbreak farms, insect collection was performed in pig houses the day after the virus has been detected in one or more of the pigs. The interval between the outbreak and the insect sampling varied from 1 to 44 days.

The most common type of insect was flies (nine species,  $N = 27,310$ , 95.1%), followed by mosquitoes (four or more species,  $N = 1,198$ , 4.2%). Cockroaches ( $N = 24$ , 0.1%) were also collected in all regions, except for Paju. The remaining 0.6% ( $N = 186$ ) of all collected insects could not be taxonomically classified. The largest number of insects was collected in Yeoncheon ( $N = 13,672$ ), most ( $N = 13,545$ , 99.1%) of which were flies. The proportion of mosquitoes was higher in Paju (12.8%) than in other three regions (0.7% in Yeoncheon, 5.6% in Ganghwa, and 6.6% in Gimpo, respectively) (Supplementary Table 1).

The flies collected were mostly houseflies (*Musca domestica*,  $N = 21,553$ ) and blowflies (or blue bottle flies, *Calliphora vomitoria*,  $N = 5,285$ ), which together accounted for 98.3% of all collected flies. The number of yellow dung flies (*Scatophaga stercoraria*,  $N = 144$ ) and flesh flies (*Sarcophaga* spp.,  $N = 108$ ) was small; however, the two species were found in all four regions. In Gimpo and its neighboring region, Ganghwa, crane flies (Tipulidae,  $N = 200$ ) were collected. Blood-feeding flies, including non-biting midges (Chironomidae,  $N = 10$  in Gimpo), stable flies (*S. calcitrans*,  $N = 6$  in Paju and Yeoncheon), and drain flies (*Psychodidae* spp.,  $N = 2$  in Yeoncheon), were found only in some regions. One each black soldier fly (*Hermetia illucens*) was found in Paju and Yeoncheon, respectively (Supplementary Table 2).

The most common type of classified mosquitoes was the biting midge (*Culicoides* spp., N = 324, 27.0%). The second most common type was yellow fever mosquitoes (*Aedes aegypti* spp., N = 77), the number of which was not even one fourth that of biting midges (Supplementary Table 3). *Aedes vexans* was found in Paju (N = 8) and Yeoncheon (N = 3), and the Korean yellow fever mosquito (*Aedes koreicus*, N = 1) was found only in Paju. Anopheles mosquitoes (*Anopheles* spp., N = 7) were found in regions other than Paju, whereas *Culex pipiens* (*Culex* spp., N = 4) was found in regions other than Yeoncheon. The *Culex orientalis* (N = 1) was found only in Paju. More than half (65.6%) of the collected mosquitoes could not be classified.

All 28,718 insects (flies, mosquitoes, and cockroaches) collected from ASF outbreak farms in four regions were proved to be ASFV-DNA-negative by RT-PCR.

## Discussion

Flies, mosquitoes, and cockroaches are hygiene pests that may harm animals, both directly and indirectly. The hygiene pests may bite animals to injure the skin, spread pathogens through the wound, and take blood and nutrients from the animals. Even when they do not bite, they may transmit pathogens mechanically or biologically. Several cases have been documented where hygiene pests have spread pathogens in pig farms [Baldacchino et al., 2013; Fila et al., 2020]; however, no clear evidence of the spread of ASFV has been reported, and there is only anecdotal support for the hypothesis. In a pig farm in Estonia in 2016, clinical symptoms suggestive of ASF were firstly recognized in mid-August, followed by the confirmation of the outbreak in 8 days. At the time of the epidemiological investigation conducted on the following day, 13 flies (*M. domestica*, N = 9, and *Drosophila* spp., N = 4) and 2 mosquitoes in close contact with pigs in the affected unit were randomly caught using an aerial net. ASFV-DNA was detected in two flies (one was *M. domestica* and the other was *Drosophila*) and two pooled mosquitoes; however, high Ct (threshold cycles) values indicated only very small quantities of the virus [Herm et al., 2019]. In Poland, ASFV-DNA was detected in stable flies collected during the ASF outbreak in pigs; however, the exact sampling period was not reported [Mazur-Panasiuk et al., 2019]. In Estonia, ASFV-DNA was detected in farm insects in August during summer [Herm et al., 2019]. In Lithuania, a study was conducted to collect blood-feeding insects (Diptera: Tabanidae and Muscidae [*Stomoxys*]) using the NZI traps; these insects are likely to play a role in the ASFV transmission. Most insects were collected in July and August (summer). Contrarily, the specimens of *Stomoxys*, the most well-known species of blood-feeding flies, were mostly collected in October (fall) [Petrasiunas et al., 2018]. The aforementioned East European countries, including Poland, Lithuania, and Estonia, are located at higher latitudes (46°–54° North) than Korea; however, the average temperature in July–August when the insect collection was performed in these countries was higher than 20 °C [AccuWeather, 2020; Korea Meteorological Administration, 2020]. The farms affected by the ASF outbreak in Korea were located at latitudes of 37°–38° North and the average daily temperatures in the affected regions from October 10 to 31, which is the collection period, were 7.4°C–16.1°C (in Paju and Yeoncheon) and 8.6°C–17.7°C (in Gimpo and Ganghwa) [Korea Meteorological Administration, 2020]. Due to the low temperatures in Korea in the fall, the environment where the insects were collected may be critically different from that of the Eastern European countries at the time of the sampling. Nevertheless, the species of collected flies in Korea were similar to those collected from the farms in Germany in June–September [Forster et al., 2007]. The housefly (*M. domestica*) was the most common species among the collected insects in the present study and in the study conducted in Germany. However, there is little evidence to support the hypothesis that these insects are spreaders of ASFV, although they have the potential owing to their ecological characteristics, in addition to the reported evidence of transmitting other diseases [Fila et al., 2020]. The blowfly, which is a type of necrophagous, was the second most common species of the collected flies in the present study. In an experimental study of the larvae of *Lucilia sericata* and *Calliphora vicina*, which are species of blowflies, ASFV genomic DNA was detected inside and on the body surface of the larvae that fed on ASFV-infected tissues. However, the study reported that when the larvae became pupae 10 days later, very small quantities of virus DNA were detected in only a few individuals. This observation suggested that the virus failed to replicate in the bodies of the larvae [Forth et al., 2018]. Although an experimental study has shown that ASFV can survive for 2 days in *S. calcitrans* (stable flies) without losing titers or the ability to spread

[Mellor et al., 1987], the number of collected specimens in this study was too small to assess its role in the transmission of ASFV in Korea. Small species, such as stable flies and blowflies, do not move around for long distances and may thus be involved in the spread of the virus within a farm. Although missing in this study, the Tabanidae, a large species called “horsefly,” travels farther and could therefore be involved in the spread of the virus between farms or in the wild boar/domestic pig transmission interface [Fila et al., 2020].

## Conclusions

In this study, a total of 28,718 insects were collected in ASF outbreak farms, and no trace of ASFV-DNA was detected by laboratory analysis. This may reflect the effects of cleaning and disinfection, following the detection of ASF outbreak in the early phase of infection [Yoon et al., 2020]. The results of the present study and the experimental research and fieldwork previously conducted in other countries suggest that it is premature to conclude on the role of insects in the transmission of ASFV. However, the possibility of the virus spreading *via* insects should not be overlooked.

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## Ethics Statement

The authors confirm that the ethical policies of the journal, as noted on the journal’s author guidelines page, have been adhered to. No ethical approval was required as this article is based on field work not involving any experiment.

## Conflict of Interest Statement

None

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