Arthropods as potential vectors of African swine fever virus outbreaks on pig farms in the Republic of Korea

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Abstract

The seasonality of African swine fever (ASF), with cases concentrated over the summer in Europe, in addition to outbreaks on farms with high levels of biosecurity, suggest that ASF virus (ASFV) may be transmitted by arthropod vectors. In this study, arthropods were collected from Korean pig farms with ASF outbreaks to determine the role of arthropods as a potential vector of ASFV. Arthropods were collected from 14 farms with ASF outbreaks, from September 27 to October 31, 2019. A total of 28,729 arthropods, including 28,508 (99.2%) Diptera, were collected using blacklight traps, insect nets, and yellow sticky strips. All arthropods samples were negative for ASFV genomic DNA according to laboratory tests using real-time polymerase chain reaction. Nevertheless, it is premature to conclude that arthropods do not play any role in ASFV transmission.

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The seasonality of African swine fever (ASF), with cases concentrated over the summer in Europe, in addition to outbreaks on farms with high levels of biosecurity, suggest that ASF virus (ASFV) may be transmitted by arthropod vectors. In this study, arthropods were collected from Korean pig farms with ASF outbreaks to determine the role of arthropods as a potential vector of ASFV. Arthropods were collected from 14 farms with ASF outbreaks, from September 27 to October 31, 2019. A total of 28,729 arthropods, including 28,508 (99.2%) Diptera, were collected using blacklight traps, insect nets, and yellow sticky strips. All arthropods samples were negative for ASFV genomic DNA according to laboratory tests using real-time polymerase chain reaction. Nevertheless, it is premature to conclude that arthropods do not play any role in ASFV transmission.

Keywords: African swine fever, arthropod, Diptera, transmission, Republic of Korea

Introduction

African swine fever (ASF) is a hemorrhagic fatal disease for both domestic pigs and wild boars. Recently, there has been a drastic increase in the areas affected by ASF in Asia, Europe, and Africa. Since the first suspected case on a pig farm in Paju, Gyeonggi-do Province on September 16, 2019, ASF outbreaks were confirmed on a total of 14 pig farms in the Republic of Korea (hereafter Korea) by October 9, 2019. Farms with ASF outbreaks were confirmed in four counties in Gyeonggi-do (Paju, Yeoncheon, and Gimpo) and Incheon Metropolitan City (Ganghwa) (Yoon et al., 2020). On October 3, 2019, the ASF virus (ASFV) was first detected in a wild boar carcass found in the demilitarized zone on the border of the Democratic People's Republic of Korea (North Korea) (Jo & Gortazar, 2020); reports of ASF cases in wild boars have continued until October 2020 (Kim et al., 2020; Yoo, Kim, Lee, & Yoo, 2020).

The seasonality of ASF, with cases concentrated over the summer in Europe, combined with outbreaks occurring on farms with high levels of biosecurity, suggests that ASFV may be transmitted by arthropod vectors (Petrasiunas, Bernotiene, & Turcinaviciene, 2018). Soft ticks of the genus *Ornithodoros* are known to be biological vectors (Sur, 2019), but there has only been one report of this in Korea, on an uninhabited island (Han et al., 2019). Experimental studies have reported the possibility of the mechanical transmission of ASFV by stable flies (*Stomoxys calcitrans*) and blowflies (Calliphoridae) (Forth, Amendt, Blome, Depner, & Kampen, 2018; Mellor, Kitching, & Wilkinson, 1987). More recently, stable flies that fed on ASFV-spiked blood had detectable levels of ASFV 3 and 12 hours after feeding; moreover, the pigs upon which these flies fed became infected with ASFV (Olesen, Hansen, et al., 2018; Olesen, Lohse, et al., 2018). Arthropods, especially flies and mosquitoes, exist all over the world. Therefore, in this study, arthropods were collected from pig farms with ASF outbreaks to determine their role as a potential vector of ASFV.

Materials and Methods

Arthropods were collected from 14 farms with ASF outbreaks, located in 4 regions (counties): 5 farms in Paju, 2 in Yeoncheon, 2 in Gimpo, and 5 in Ganghwa. Arthropod specimens were collected using blacklight traps (UV-LED Blackholeplus®, Biotrap Ltd.), insect nets, and yellow sticky traps. These traps were installed inside and around the pig houses, and were not targeted to catch specific arthropods. These tools were left in place for at least 24 hours before the trapped arthropods were retrieved. Arthropod collection was performed in three ways: via collaboration between the Animal and Plant Quarantine Agency (APQA) and the Korea Centers for Disease Control and Prevention (KCDC), via APQA alone, or via a private company (Biogenoci Ltd.) under contract by APQA.

Arthropods collected using blacklight traps and insect nets were classified in the field by researchers from the KCDC or Biogenoci Ltd., then confirmed in the APQA laboratory using Optinity KS-200® with 0.8 to 5× magnification. Yellow sticky strips were sent directly to the laboratory without on-site classification. Specimens were pooled according to location and insect type to enable the detection of ASFV genomic DNA using real-time polymerase chain reaction (PCR). Laboratory tests were conducted by APQA and results were released through the government official document system of Korea.

Results

A total of 28,729 arthropod specimens were collected through 111 collecting operations carried out between September 27 and October 31, 2019. The median number of insects collected per farm was 1,047 (minimum: 341, maximum: 11,359). The time interval between the ASF outbreak and arthropod collection varied from 1 to 44 days. On one farm in Paju and another farm in Gangwha, collection was performed during the on-farm outbreak investigation.

The most common (99.2%) arthropod collected belonged to the order Diptera: 28,508 individuals from 10 families were collected (Table 1). In the order Diptera, the majority belonged to Muscidae (N = 21,559, 75.6%) and Calliphoridae (n = 5,285, 18.5%; Table 2). Most Muscidae flies were *Musca domestica* , (houseflies, n = 21,553), but*Stomoxy calcitrans* (stable flies, n = 6) were also collected from 3 farms (Supplementary Table 1). In addition, small numbers of Blattaria (n = 24), Ixodidae (n = 11), Lepidoptera (n = 5), and Coleoptera

(n = 1) were collected, but only in some regions. One hundred-eighty (0.6%) specimens could not be classified (Table 1). All 28,729 collected arthropods were negative for ASFV genomic DNA.

Discussion

Flies, mosquitoes (Diptera), and cockroaches (Blattaria) are hygiene pests that are capable of harming animals, both directly and indirectly. They can be hematophagous or necrophagous. Hygiene pests may bite animals to injure the skin, spread pathogens through the wound, and take blood and nutrients. Even when they do not bite, these pests may transmit pathogens mechanically or biologically. ASFV is known to persist for extended periods in wild boar carcasses with the colonization of necrophagous pests, mainly belonging to the orders Diptera and Coleoptera (Bonnet et al., 2020); however, to date, there have been no reports of the spread of ASFV via these pests, and there is only anecdotal support for this hypothesis.

Similar research studies have been conducted in other countries. In August 2016, during an epidemiological investigation of an ASF-positive pig farm in Estonia, 13 flies (*Musca domestica* , n = 9, and*Drosophila* spp. , n = 4) and 2 mosquitoes found in close contact with pigs in the affected unit were randomly caught using an aerial net. ASFV DNA was detected in two of the flies (one *Musca domestica* and the other *Drosophila*) and the pooled mosquitoes; however, high Ct (threshold cycles) values indicated that only very small quantities of the virus were present (Herm, Tummeleht, Jurison, Vilem, & Viltrop, 2020). In Poland, ASFV DNA was detected in stable flies collected on a pig farm with an ASF outbreak; however, the exact sampling period was not reported (Mazur-Panasiuk, Zmudzki, & Wozniakowski, 2019). In Lithuania, a study collected blood-feeding insects, including Tabanidae and Muscidae *Stomoxy* spp. Most insects were collected during the summer (July and August), but the*Stomoxys* specimens, the most well-known blood-feeding fly species, were mostly collected in the autumn (October) (Petrasiunas et al., 2018). The aforementioned Eastern European countries are located at higher latitudes (46°–54° North) than Korea; however, their average temperatures in July and August, when insect collection was performed, were higher than 20°C (AccuWeather, 2020; KoreaMeteorologicalAdministration, 2020). Korean farms with ASF outbreaks were located at latitudes 37°–38° North, with average daily temperatures of 7.4°C–16.1°C (in Paju and Yeoncheon) and 8.6°C–17.7°C (in Gimpo and Ganghwa) over the main collection period (KoreaMeteorologicalAdministration, 2020). Due to the low fall temperatures in Korea, the environment in which the insects were collected may be critically different from that of the Eastern European countries at the time of insect collection. Nevertheless, the species of collected flies in Korea were similar to those collected from farms in Germany over a study period from June to September, with *Musca domestica* being the most commonly collected insects in both studies (Forster et al., 2007).

The number of insects collected in this study was especially high on a farm in Yeoncheon (Supplementary Table 1), likely because collection was conducted while pigs were still present in the barns. Calliphoridae, a necrophagous insect, was the second most common family of Diptera (Table 2). In an experimental study of Calliphoridae larvae (*Lucilia sericata* and *Calliphora vicina*), ASFV genomic DNA was detected inside and on the body surface of larvae that fed on ASFV-infected tissues; however, when the larvae became pupae 10 days later, very small quantities of virus DNA were detected in only a few individuals, suggesting that the virus failed to replicate within the bodies of the larvae (Forth et al., 2018). Although ASFV can survive for 2 days in*Stomoxy calcitrans* without a reduction in titer or ability to spread (Mellor et al., 1987), the number of specimens collected in this study was too small (2 of each, for a total of 6 over 3 farms) to assess its role in the transmission of ASFV in Korea. Small species, such as stable flies and blowflies, do not travel long distances and may thus be involved in the spread of the virus within a single farm. Although not collected in this study, Tabanidae (horsefly) travel farther distances and could therefore be involved in the spread of ASFV between farms or in the wild boar/domestic pig transmission interface (Fila & Wozniakowski, 2020).

Conclusions

In this study, a total of 28,729 arthropods were collected from farms with ASF outbreaks. No trace of ASFV genomic DNA was detected by laboratory analysis. This result may reflect the effects of cleaning and disinfection in the early phase of infection following the detection of an ASF outbreak (Yoon et al., 2020).

The results of the present study, combined with previously conducted experimental research and fieldwork in other countries, suggest that it is premature to conclude that arthropods play a role in the transmission of ASFV; however, the possibility of the virus spreading *via* arthropods 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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Table 1.

Number of arthropods collected on pig farms with ASF outbreak, by order and region

Region Order	Paju (5 farms)	Paju (5 farms)	Yeoncheon (2 farms)	Yeoncheon (2 farms)	Gimpo (2 farms)	Gimpo
	Number	(%)	Number	(%)	Number	(%)
Diptera	3,300	(98.6)	13,642	(99.8)	1,707	(98.2)
Blattaria	0	-	20	(0.1)	3	(0.2)
Ixodidae	0	-	0	-	0	-
Lepidoptera	0	-	0	-	0	-
Coleoptera	1	(<0.1)	0	-	0	-
Unclassified	45	(1.3)	10	(0.1)	28	(1.6)
Total	3,346	(100)	13,672	(100)	1,738	(100)

Table 2.

Number of insects belonging to the order Diptera, by family and region, collected on pig farms with ASF outbreak

Region Family	Paju (5 farms)	Paju (5 farms)	Yeoncheon (2 farms)	Yeoncheon (2 farms)	Gimpo (2 farms)	Gi
	Number	(%)	Number	(%)	Number	(%
Muscidae	1,770	(53.6)	11,464	(84.0)	1,181	(69
Calliphoridae	1,059	(32.1)	2,030	(14.9)	347	(20
Culicidae	297	(9.0)	51	(0.4)	114	(6.7
Ceratopogonidae	132	(4.0)	46	(0.3)	1	(0.1
Tipulidae	0	-	0	-	43	(2.5
Scathophagidae	16	(0.5)	15	(0.1)	1	(0.1
Sarcophagidae	25	(0.8)	33	(0.2)	10	(0.6
Chironomidae	0	-	0	-	10	(0.6
Psychodidae	0	-	2	(<0.1)	0	-

Region Family	Paju (5 farms)	Paju (5 farms)	Yeoncheon (2 farms)	Yeoncheon (2 farms)	Gimpo (2 farms)	Gi
Stratiomyidae	1	(<0.1)	1	(<0.1)	0	-
Total	3,300	(100)	13,642	(100)	1,707	(10