Evidence of aerosol transmission of African swine fever virus in piggeries under field conditions: a case report

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Case Report

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Abstract

Background

African swine fever (ASF) is a devastating and economically significant infectious disease and has caused huge losses in the commercial pig sector in China since 2018. The major transmission route of African swine fever virus (ASFV), the causative agent of ASF, is direct pig-to-pig contact or indirect contact with virus-contaminated objects. Although aerosol transmission route of ASFV has been reported under experimental conditions previously, whereas no reports described under field conditions.

Case presentation

In the three ASFV-positive piggeries, environmental samples, including floors, aerosols, dusts and air outlets, tested positive in the monitoring period. Aerosol and floor samples always tested positive firstly at the same time. ASFV-positive aerosols were associated with live ASFV-positive pigs in piggeries. Dust and air outlet samples remained positive for several days after all positive pigs were eliminated, and the times of positive and persistent positive of dust and air outlet samples were always close.

Conclusions

This is the first report to provide some evidence of airborne transmission route of ASFV in field piggeries, and more research is needed to study aerosol-spread laws of ASFV under field conditions, assess the risk of ASFV aerosol transmission, and develop effective strategies for air disinfection to create low-risk fresh air for pig herds.

Background

ASF is an acute, febrile, highly contagious infectious disease listed by the World Organization for Animal Health (OIE) as a disease of obligatory declaration [1], with a morbidity and mortality rate as high as 100% in domestic pigs [2, 3]. ASFV, the only member of *Asfivirus* genus within the *Asfarviridae* family, is the causative agent of ASF and first reported in 1921 in East Africa, and spreads to other Africa countries rapidly [4]. ASFV outbreak is first described in China in 2018 [5, 6], and has caused 1.193 million deaths of pigs by November 2021 [7]. The domestic pig population in China accounts for more than 50% of the whole pig population worldwide [8]. Such huge losses have forced people to find new strategies to prevent ASFV infection. The major transmission route of ASFV is direct pig-to-pig contact or indirect contact with virus-contaminated objects, including excretory materials [9, 10], feed [11], water [11, 12], needles [2] and so on. Based on this, the partitioning approach is developed and proved to be an effective method to prevent ASFV diffusion and preserve other healthy pigs to the greatest extent possible. Briefly, pig farms improves the biosecurity level to reduce the risk of ASF introduction, strengthens monitoring process to enhance early detection, removes and culls positive groups to eliminate pollution sources, and uses strict disinfection measures to further eliminate pollution sources and cut off transmission routes

[13]. However, with the emergence of some mutant ASFV strains, the details of this strategy also need to be improved in the future.

Aerosol transmission is another important route for ASFV spreads. The definition of aerosol transmission is that susceptible animals inhale pathogen-carrying particles with the diameters 5μm or less [14]. Aerosol usually contains solid or liquid particles suspended in air [14]. One study in 1977 shows that the longest distance of ASFV transmission is 2.3 meters in a confined space, but no detection of ASFV in the air. Until 2012, an air sampling method is proved to be effective and ASFV particles are detected in the air. And so far, only a few studies have reported the airborne transmission of ASFV in experimental conditions [15, 16], whereas no reports in field conditions. Here, we presented some evidence of ASFV-carrying aerosols in piggeries under field conditions.

Case Presentation

In winter, the ventilation mode of pig farms in northern China is usually switched to winter mode, whose ventilation is smaller than that in the summer mode. Therefore, the monitoring and control of environmental risks is more important in ASF-positive piggeries in winter. In this study, we tracked the detection of ASFV in environmental samples from three different ASFV-positive piggeries for 24 to 27 days since the first day (defined as Day 0) with the first confirmed case of ASFV-positive pigs. Piggery 1 was confirmed to be infected with a highly virulent strain [17]; Piggery 2 and 3 were confirmed to be infected with two different naturally lower virulent strains respectively [18, 19]. The definite diagnoses of ASFV-positive pigs were by testing serum samples and nasal swabs. The environmental samples included floor samples of columns with ASFV-positive pigs, aerosol samples in piggeries, dust samples on the surface of feeding device, and air outlet samples on the surface of air filters. Floor, dust and air outlet samples were collected by wiping with a gauze (10cm×10cm), and then eluting with 10 mL normal saline. Aerosol samples were collected by the MD8 air scan sampling device (Sartorius, Nieuwegein, The Netherlands) at the air speed of 50 m^3/min for 20 min and the sterile gelatine filters of 3 mm pore size and 80 mm diameter (type 17528-80-ACD, Sartorius) were then dissolved in 5 mL normal saline. All the samples above were tested by RT-PCR targeting the viral p72 gene in a specialized testing laboratory of our company, and a CT value of < 40 was considered to be positive.

In Piggery 1, as shown in Fig. 1A, pigs tested positive from Day 0 to Day 15, and the whole pig group were culled after Day 15. Floor samples and aerosol samples first tested positive at Day 0; then dust samples and air outlet samples both tested positive at Day 9. Floor samples were positive throughout the testing period, even after the pig depopulation, indicating that ASFV in pig residues lasted on the floor for a long time. The positive duration for aerosol samples was 9 days, from Day 0 to Day 9. Aerosol samples and pigs tested both negative at Day 12, and aerosol samples came to be negative before the pig depopulation. Positive durations for dust samples and air outlet samples were 15 days and 18 days respectively, and these samples remained positive for several days after the pig depopulation. The CT values of these samples were shown in **Additional file 1**.

In Piggery 2, as shown in Fig. 1B, positive pigs have been detected throughout the testing period, from Day 0 to Day 24. Floor samples and aerosol samples first tested positive at Day 6; then air outlet samples tested positive at Day 9; finally, dust samples tested positive at Day 12. Floor samples were positive throughout the monitoring period for 21 days since they first tested positive. The positive duration for aerosol samples was 18 days, from Day 6 to Day 24, whereas the test results were negative on Day 12 and Day 15, which may be related to the amount of virus released from the positive pigs. Positive durations for dust samples and air outlet samples were 12 days and 15 days respectively, though these results showed discontinuities. Moreover, these samples remained positive until the last day of testing, as those in pigs. The CT values of these samples were shown in **Additional file 1**.

In Piggery 3, as shown in Fig. 1C, positive pigs have been detected for 18 days, from Day 0 to Day 18, and no positive-pigs were detected after Day18, indicating all positive pigs have been eliminated. Floor samples and aerosol samples first tested positive at Day 6; then dust samples and air outlet samples both tested positive at Day 9, which were the same as those in Piggery 1. Floor samples were positive for 12 days, and came to be negative at the same time with pigs at Day 18. The positive duration for aerosol samples was 9 days, from Day 6 to Day 12, and aerosol samples came to be negative before all positive pigs were eliminated, which was the same with that in Piggery 1. Positive durations for dust samples and air outlet samples were 18 days and 15 days respectively, and air outlet samples were discontinuously positive in this period. Moreover, these samples remained positive for several days after all positive pigs were eliminated. The CT values of these samples were shown in **Additional file 1**.

Based the results from the above three cases, we found some common rules between environmental samples and ASFV-positive pigs in piggeries. First, floor and aerosol samples were always positive at the same time. Second, aerosol samples came to be negative before all positive pigs were eliminated. Third, dust and air outlet samples remained positive for some time after all positive pigs were eliminated. Last, the times of positive and persistent positive of dust and air outlet samples were always close.

Discussion And Conclusions

Aerosol transmission of infectious agents are the most difficult to control [20], especially in swine virus. Some swine virus has been reported to spread by aerosols, such as foot-and-mouth disease virus (FMDV) [21], porcine reproductive and respiratory syndrome virus (PRRSV) [22], porcine epidemic diarrhea virus (PEDV) [23], and influenza A virus (IAV) [24]. However, ASFV has long been considered as a highly contagious disease, though a few studies provide some evidence on the aerosol transmission in experimental conditions [15, 16]. And also, the present prevention strategies are lacking in targeting the risk of airborne transmission. In this report, we found some evidence that aerosol transmission of ASFV occurred in piggeries in field conditions, including a highly virulent strain and two naturally lower virulent strains. In the three piggeries, floor and aerosol samples were earliest and simultaneously positive. It mostly like due to that the excrements of live ASFV-positive pigs, including urine, sneezes and feces, were released on the floor and into the air, then formed ASFV-positive aerosols. A previous study has proven that the positive aerosols were associated with viruses in faeces [15], which could support our conjecture.

Interestingly, in Piggery 1 and 3, when the floor samples remained positive at the later stage, no viruses were detected in aerosols, indicating that the main source of ASFV in aerosols was not from the excrements on the floor, but from the fresh excrements from live ASFV-positive pigs. And the fact that no positives were detected in aerosols after eliminating positive pigs further supported our opinion. ASFV-positive floor and aerosol samples started earlier than those of moderately virulent strains in Piggery 2 and 3, indicating virus excretion earlier in highly virulent infection, which was consistent with the results under experimental conditions [15]. Dusts are often overlooked by farmers because they are often in hard-to-reach locations. If these ASFV-positive dusts cause vigorous activity for some reason, like sudden changes of wind directions or feeding activities in piggeries, this can be dangerous for the entire herd. Though ASFV dusts stated later than floor or aerosol samples, they remained positive for some time after all positive pigs were eliminated. ASFV dusts may be from feed contaminated with positive saliva or sneeze, because feed has been reported to be a main risk of ASFV transmission [25, 26]. Therefore, it is important to clean up the dust in piggeries in time for the removal of ASFV. Air outlet samples were positive in the three piggeries, indicating a big risk of ASFV traveling outside and even to adjacent piggeries. Air filtration systems has been proved to be effective in preventing of airborne transmission of PRRSV [27]. Therefore, air filtration systems should be considered as an important part of the farm biosecurity against ASFV and other pathogens, especially in most small farms with poor biosecurity in China [7].

In conclusion, this case report showed some evidence of airborne transmission of ASFV. As shown in Fig. 2, ASFV-positive aerosols are from live ASFV-positive pigs, excrements and contaminated feed, and then spread to air in piggeries. We call for that farmers should take into account the filtration of the air inlets and outlets of piggeries, strength the air disinfection and dust reduction in piggeries, so as to create low-risk fresh air for pig herds.

Abbreviations

ASF: African swine fever; ASFV: African swine fever virus; OIE: the World Organization for Animal Health; FMDV: foot-and-mouth disease virus; PRRSV: porcine reproductive and respiratory syndrome virus; PEDV: porcine epidemic diarrhea virus; IAV: influenza A virus; RT-PCR: Reverse Transcription-Polymerase Chain Reaction.

Declarations

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Author contributions

ZY and XL conceived and designed the analyzation method. XL, ZH and MF collected and analyzed the data, and also wrote the original draft. ZY and XL reviewed and edited the manuscript. WW, WG, LB, WL,

XT and XJ contributed to supply environmental samples from field farms and the data of RT-PCR test. All authors read and approved the final manuscript.

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Availability of data and materials

The data generated for the current case report are kept and stored by the corresponding authors. The data are available from the corresponding authors.

Ethics approval and consent to participate

Ethical review and approval was not required for the animal study because the manuscript is a case report of spontaneous disease. Written informed consent was obtained from the owners for the participation of their animals in this study.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures

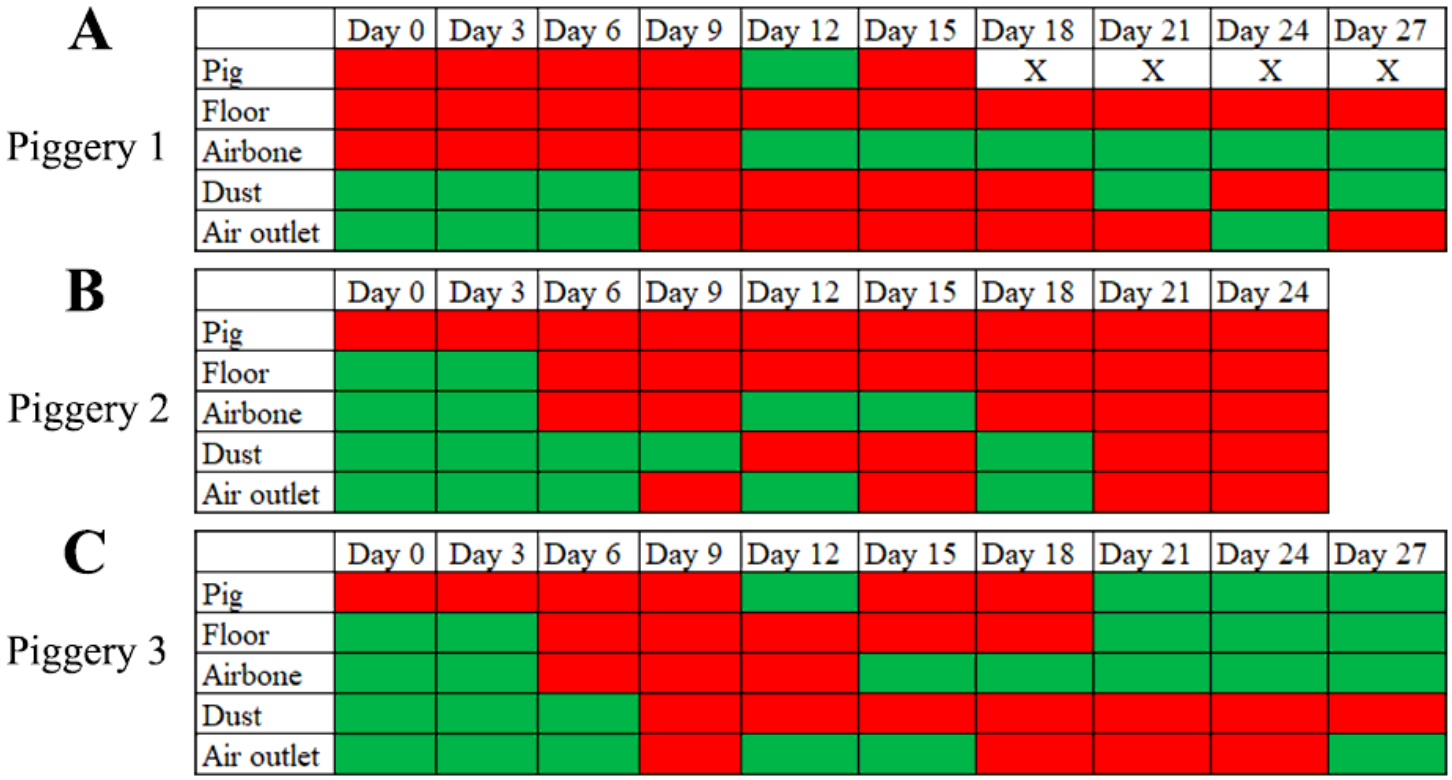

Figure 1

The status and time of ASFV detection in various samples in piggeries. **(A)** Piggery 1 with a highly virulent ASFV strain infection; **(B and C)** Piggery 2 and Piggery 3 with naturally lower virulent strain infections. “X” means no pigs in the piggery.

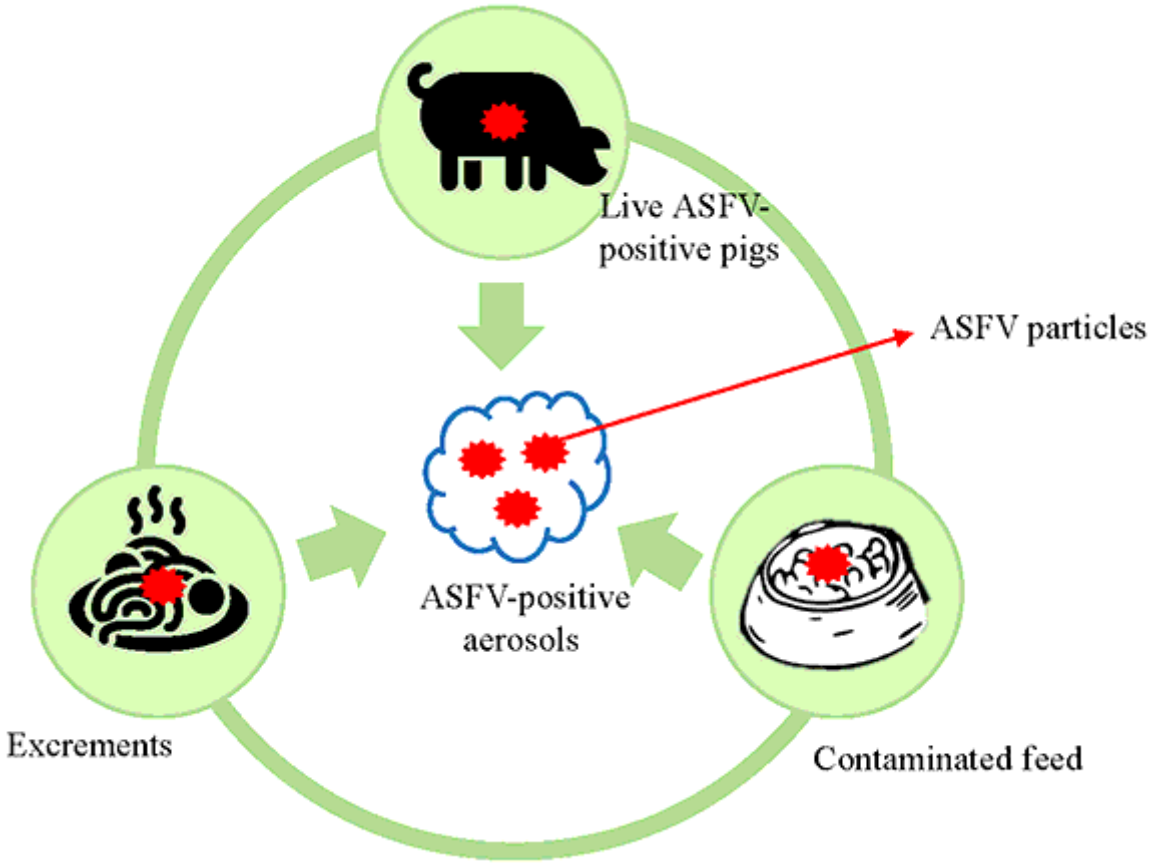

Figure 2

Proposed model of ASFV-positive aerosol production in a piggery.

Supplementary Files

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