Veterinary Microbiology 211 (2017) 92–102

Contents lists available at ScienceDirect

Veterinary Microbiology

journal homepage: www.elsevier.com/locate/vetmic

Transmission of African swine fever virus from infected pigs by direct contact and aerosol routes

Ann Sofie Olesen[a], Louise Lohse[a], Anette Boklund[b], Tariq Halasa[b], Carmina Gallardo[c], Zygmunt Pejsak[d], Graham J. Belsham[a], Thomas Bruun Rasmussen[a], Anette Bøtner[a,*]

[a] DTU National Veterinary Institute, Technical University of Denmark, Lindholm, DK-4771 Kalvehave, Denmark
[b] DTU National Veterinary Institute, Technical University of Denmark, Kemitorv, Building 204, DK-2800, Kgs. Lyngby, Denmark
[c] European Union Reference Laboratory (EURL) for African swine fever, INIA-CISA, 28130 Valdeolmos, Madrid, Spain
[d] National Veterinary Research Institute, Department of Swine Diseases, Partyzanrow 57, 24-100 Pulawy, Poland

ARTICLE INFO

Keywords:
ASF
Poland
Virus transmission
Air sampling
Haemorrhagic disease

ABSTRACT

In 2014, African swine fever virus (ASFV) was introduced into the Baltic states and Poland. Since then, the disease has continued to spread within these regions, and recently, cases were reported in the Czech Republic and Romania. Currently, there is an increasing risk of ASFV introduction into Western Europe. Hence, there is an urgent need to assess current contingency plans. For this purpose, knowledge of modes-of-transmission and clinical outcome in pigs infected with new European ASFV strains is needed.

In the present study, two experiments were conducted in pigs using an isolate of ASFV from Poland (designated here POL/2015/Podlaskie/Lindholm). In both studies, pigs were inoculated intranasally with the virus and contact pigs were exposed to the experimentally infected pigs, either directly (contact within and between pens) or by air.

Pigs exposed to the virus by intranasal inoculation, by direct contact to infected animals and by aerosol developed acute disease characterized by viremia, fever and depression. Infectious virus was first detected in blood obtained from the inoculated pigs and then sequentially among the within-pen, between-pen and air-contact pigs. ASFV DNA and occasionally infectious virus was found in nasal-, oral-, and rectal swabs obtained from the pigs, and ASFV DNA was detected in air samples. No anti-ASFV antibodies were detected in sera.

In conclusion, the study shows that the currently circulating strain of ASFV can be efficiently transmitted via direct contact and by aerosols. Also, the results provide quantitative transmission parameters and knowledge of infection stages in pigs infected with this ASFV.

1. Introduction

African Swine Fever (ASF) is a severe viral haemorrhagic disease affecting swine (Mebus, 1988). The disease is caused by African swine fever virus (ASFV) which is a large, enveloped, DNA virus and the sole member of the genus *Asfivirus* within the family *Asfarviridae* (Dixon et al., 2005).

In 2007, ASFV was introduced into Georgia and subsequently into other Transcaucasian countries, the Russian Federation, Ukraine and Belarus (EFSA Panel on Animal Health and Welfare, 2014). In the beginning of 2014, outbreaks of the disease occurred in the Baltic states and Poland, within wild boar and domestic pigs (EFSA Panel on Animal Health and Welfare, 2015). Outbreaks have continued to occur in the Baltic states and Poland, and more recently, in 2017, ASFV has been reported in wild boar in the Czech Republic and in domestic pigs in Romania

(http://www.oie.int/wahis_2/public/wahid.php/Diseaseinformation/WI).

The continued circulation of the virus in Eastern Europe means that there is a risk of further spread of ASFV into Western Europe. In European countries, with a large swine production and substantial exports of swine products, it is predicted that ASF outbreaks will have huge economic consequences, especially due to export restrictions (Halasa et al., 2016a,b). Hence, enforcement of current ASF contingency plans to achieve early detection and eradication of the disease in these countries is of major importance in order to limit these costs (Halasa et al., 2016c). Currently, since no vaccine or treatment options are available to prevent the infection (Zakaryan and Revilla, 2016), the

* Corresponding author at: Division for Diagnostics and Scientific Advice, DTU National Veterinary Institute, Technical University of Denmark, Lindholm, DK-4771, Kalvehave, Denmark.
E-mail address: aneb@vet.dtu.dk (A. Bøtner).

http://dx.doi.org/10.1016/j.vetmic.2017.10.004
Received 24 July 2017; Received in revised form 3 October 2017; Accepted 3 October 2017

disease can only be controlled by administrative and regulatory measures. The procedures applied in the event of an outbreak are based on classical disease control strategies (EFSA Panel on Animal Health and Welfare, 2014; CEC, 2002; Gallardo et al., 2015). These include epidemiological investigations, tracing of pigs, stamping out on infected holdings, surveillance and strict movement control (The European Commision, 2013). In order to choose the most effective control strategies, modelling of the epidemiological and economic consequences of an outbreak can be a valuable tool, and recently such analyses of within-herd and between-herd ASFV transmission have been reported (Halasa et al., 2016a,b). However, in order for these models to reflect the actual conditions that may occur in an outbreak situation, accurate quantitative parameters concerning the nature of ASFV transmission from domestic pigs infected with the currently circulating European ASFV strains are necessary. Also, knowledge of the clinical and pathological aspects of the infection by these strains is important to facilitate early detection of ASFV by farmers, veterinary practitioners and the authorities.

In the present study, we have conducted two experiments in pigs with an ASFV isolate obtained from an infected wild boar in Poland in February 2015, this virus is designated here as POL/2015/Podlaskie/Lindholm. Transmission of the virus via direct contact between experimentally infected pigs and susceptible, within-pen- and between-pen contact pigs was investigated. In addition, potential aerosol transmission over a short distance, between pigs within separated pens was analyzed. Previously, transmission studies in pigs using ASFVs from the Georgian incursion of the disease have investigated direct-contact transmission (Guinat et al., 2014; Gallardo et al., 2017), but airborne transmission have only been clearly demonstrated once − using an African isolate of the virus (Wilkinson and Donaldson, 1977). To our knowledge, this is the first study to investigate transmission between pigs via air using a recent European ASFV.

2. Materials and methods

2.1. Animals

Twenty two pigs, eight to nine weeks of age, 18–25 kg, were included in each of the two studies. The 44 pigs were obtained from a conventional Danish swine herd (Landrace × Yorkshire × Duroc hybrids) with specific pathogen free (SPF) status. This means freedom from atrophic rhinitis, enzootic pneumoniae, porcine reproductive and respiratory syndrome, swine dysentery and most serotypes of *Actinobacillus pleuropneumoniae*. On arrival at the research facility, one week before the start of the experiment, all pigs were found to be healthy by veterinary inspection. The pigs were fed a commercial diet for weaned pigs once a day, and water was provided *ad libitum*. Straw was used for bedding.

Animal care and maintenance, experimental procedures and euthanasia were conducted in accordance with Danish and EU legislation on animal experimentation (Consolidation Act 474 15/05/2014 and EU Directive 2010/63/EU) and with the approval from the Danish Animal Experimentation Inspectorate (license number 2015-15-0201-00606).

2.2. Challenge virus

Spleen material from a dead wild boar in Podlaskie voivodeship (province), Poland, collected in February 2015, was obtained via the EU Reference Laboratory (EURL) for ASF (Valdeolmos, Spain). At the EURL, the sample (designated as Pol14/WB-17397#13) was found positive for ASFV by the UPL real-time polymerase chain reaction (PCR) (Fernández-Pinero et al., 2013) and after one passage in porcine blood monocytes (PBM) (Carrascosa et al., 2011) showing the characteristic haemadsorbing pattern (data not shown).

For the experimental infections, virus was isolated from the spleen material (as described below) in porcine pulmonary alveolar macrophages (PPAM). These cells were obtained as described by Bøtner et al. (1994), and resuspended in Eagle's Minimum Essential Medium (EMEM) supplemented with streptomycin (Sigma-Aldrich), neomycin (Sigma-Aldrich) and 5% fetal calf serum (suppl. EMEM) to a final concentration of 2×10^6 cells/mL.

For isolation of the virus, Nunc™ cell culture flasks (Thermo Fisher Scientific), containing 10 mL of the cell suspension, were inoculated with 200 µL of a clarified 10% spleen suspension. After three days incubation at 37 °C, in an atmosphere with 5% CO_2, the first passage virus was harvested by freezing and thawing. For the second passage, 200 µL of this virus harvest was added to 10 mL fresh PPAM, then incubated and harvested as described for the first passage.

The titre of the second passage was determined by end-point titration in PPAM. Following three days incubation (as above), virus infected cells were identified following fixation and staining of the cells using an immunoperioxidase monolayer assay (IPMA) as described for the detection of PRRSV (Bøtner et al., 1994). The cells were stained using ASFV antibody positive swine serum, protein A-conjugated horseradish peroxidase (Sigma-Aldrich) and hydrogen peroxide and the infected (red-stained) cells were counted using a light microscope. The virus titre (as $TCID_{50}$/mL) was calculated using the method described by Reed and Muench (1938).

For inoculation of pigs, the second passage virus was diluted in suppl. EMEM to 4 $\log_{10}$ $TCID_{50}$ per 2 mL. At the time of inoculation, back titration of the inoculum was carried out in PPAM to confirm the administered dose.

2.3. Study design

Upon arrival at the research facility, pigs were randomly allocated into two separate high containment stable units (BSL3 animal isolation facilities), termed unit *I* and unit *II* (Fig. 1). Inside unit *I*, the pigs were divided into the following groups; 1) inoculated pigs, 2) within-pen contact pigs, 3) between-pen contact pigs, and 4) air-contact pigs. Inside unit *II*, pigs were kept in one group as mock-inoculated control pigs. In study *a*, 16 pigs (pigs 1–16 in groups 1*a*-4*a*) were allocated to unit *I* and six pigs (pigs 17–22 in group 5*a*) to unit *II*. In study *b*, 18 pigs (pigs 23–40 in groups 1*b*-4*b*) were allocated to unit *I* and four pigs (pigs 41–44 in group 5*b*) to unit *II* (Fig. 1). Unit *I* had a room volume of 155 m^3 and the air within the room was changed 5–10 times/h. Air was recirculated, following HEPA filtration, into the room above pens 1 and 2. The air exit was located adjacent to pen 3 (Fig. 1). Unit *I* had an average temperature of 20.6 °C (± 0.4 °C) and a relative humidity of 44% (± 4%).

Following an acclimatization period of one week, the four pigs in groups 1*a* (pigs 1–4) and 1*b* (pigs 23–26) were inoculated intranasally

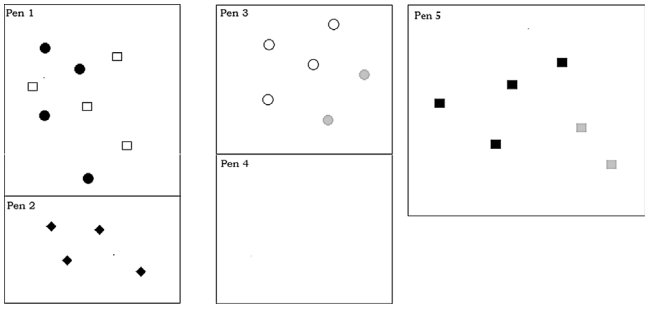

Fig. 1. Illustration of the pens and groups in the two studies, *a* and *b*. Black dots = inoculated pigs in group 1*a* (pigs 1–4) and 1*b* (pigs 23–26) in pen 1; white squares = within-pen contact pigs in group 2*a* (pigs 5–8) and 2*b* (pigs 27–30) in pen 1; black rhombus = between-pen contact pigs in group 3*a* (pigs 9–12) and 3*b* (pigs 31–34) in pen 2; white dots = air-contact pigs in group 4*a* (pigs 13–16) in pen 3 and white + grey dots = air-contact pigs in group 4*b* (pigs 35–40) in pen 3; black + grey squares = control pigs in group 5*a* (pigs 17–22) in pen 5 and black squares = control pigs in group 5*b* (pigs 41–44) in pen 5.