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African swine fever virus excretion patterns in persistently infected animals: A quantitative approach

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ABSTRACT

The continuing circulation of African swine fever (ASF) in Russia and in the Trans-Caucasian countries has led to increased efforts in characterizing the epidemiology of ASF. For a better insight in epidemiology, quantitative data on virus excretion is required. Until now, excretion data has mainly focused on the initial stages of the disease. In our study we have studied ASF virus (ASFV) excretion dynamics in persistently infected animals. For this purpose, virus excretion through different routes was quantified over 70 days after infection. Three virus isolates of moderate virulence were used: the Brazil'78, the Malta'78 (a low and a high inoculation dose) and the Netherlands'86 isolate. For each isolate or dose, 10 animals were used. All (Brazil'78 group), or three animals per group were inoculated and the other animals served as contact animals. It was shown that dose (Malta'78 low or high) or infection route (inoculated or naturally infected) did not influence the ASFV excretion ($p > 0.05$). Nasal, ocular and vaginal excretions showed the lowest ASFV titres. Virus was consistently present in the oropharyngeal swabs, showing two peaks, for up to 70 days. Virus was occasionally present in the faeces, occasionally with very high titres. Viral DNA persisted in blood for up to 70 days. The results presented in this study show that a high proportion of persistently infected animals shed virus into the environment for at least 70 days, representing a possible risk for transmission and that should be considered in future epidemiological analysis of ASF.

1. Introduction

African swine fever (ASF) is a highly contagious haemorrhagic disease of pigs, caused by the African swine fever virus (ASFV), an enveloped double-stranded DNA virus from the Asfarviridae, genus Asfivirus. In Africa, an intricate cycle between warthogs and soft ticks perpetuates the endemic state of ASF and constitutes a risk for repeated introductions in the local domestic pig population (Ekue et al., 1989). In Europe, every ASF introduction had been followed by successful eradication, with the exception of Sardinia. But in 2007, ASF was introduced into Georgia, spreading rapidly within the Caucasus and to Russia, where it currently circulates in domestic pigs and wild boars. In these countries, there has been no evidence of transmission by an arthropod vector up to this date (EFSA Panel on Animal and Welfare, 2010). Since there is no vaccine available against ASF, control strategies involve movement restrictions, biosecurity measures and stamping out (OIE, 2011).

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ASFV can give rise to per acute, acute, or sub-acute disease with a high case-fatality rate (Mebus, 1988). Animals that survive an ASF infection often become persistently infected. In this case, they may either show signs of chronic disease or look apparently healthy. In the latter, ASFV can latently persist in some tissues (Wilkinson, 1984). However, such a latent infection may be reactivated by stress, resulting in viraemia and shedding of the virus (Hamdy and Dardiri, 1984; Sanchez Botija, 1982; Wilkinson, 1984).

ASFV shedding starts from 1 to 7 days post inoculation (pi) (Ekue et al., 1989; Greig and Plowright, 1970; McVicar, 1984), depending on the isolate and on the route of infection. Highest virus titres have been reported in the oronasal fluid, while lower titres were seen in conjunctival fluid and in the genital fluid (Ekue et al., 1989; Greig and Plowright, 1970). Reports on virus shedding through the faecal route vary considerably, ranging from intermittent shedding periods with low virus titres (Ekue et al., 1989) to continuous shedding with high virus titres in the faeces during the acute and sub-acute phase (McVicar, 1984). ASFV shedding in persistently infected animals is less clear, although studies have shown the presence of infectious virus in tissues up to 180 days pi (Hamdy and Dardiri, 1984; Mebus and Dardiri, 1980; Wilkinson, 1984; Wilkinson et al., 1981), in blood up to 456 days pi (Detray, 1957) and viral DNA in leukocytes up to 500 days pi (Carrillo et al., 1994). The successful eradication of ASF in Spain has been associated with the screening and removal of persistently infected pigs (Arias and Sanchez-Vizcaino, 2002).

So far, studies on ASFV excretion have focused mostly on the initial stages of disease (up to 30 days pi) and quantitative data on virus excretion and virus transmission after this period is still lacking. As a result, the role of persistently infected animals in the overall epidemiology of ASF is still unclear. Quantitative data on ASFV excretion can improve existing knowledge on ASF epidemiology and further help in predicting the effect of intervention measures.

In this study, we examined excretion dynamics of ASFV after inoculation or natural infection, using three different isolates of varying virulence and using different doses. Excretion of virus was measured frequently in various sample types for up to 70 days after infection.

2. Materials and methods

2.1. Study design

The study was carried out with 4 groups of 10 pigs each. In each group, all or a fraction of the pigs were inoculated with ASFV. Remaining pigs served as contacts, mimicking a natural infection. Details regarding the age of the pigs, the virus isolates used, dose and type of sample can be found in Table 1. The animal experiment was approved by the Ethical Committee for Animal Experiments of the Animal Sciences Group of Wageningen UR.

2.2. Virus and inoculation

The Brazil'78 ASFV isolate, considered to be a moderately virulent isolate (Mebus and Dardiri, 1979, 1980), was used in the "Brazil" group. The Malta'78 ASFV isolate, also considered moderately virulent (Wilkinson et al., 1981), was used in two doses (Table 1, groups designated "Malta low" and "Malta high"). The Netherlands'86 ASFV isolate, also considered moderately virulent (Terpstra and Wensvoort, 1986), was used to inoculate the animals in the "Netherlands" group.

In each group, the pigs were inoculated intranasally with 1 ml of virus suspension (0.5 ml per nostril). When contact pigs were used, they were added to the group 24 h after inoculation, to avoid infection due to residual virus from the inoculum. The inocula were back titrated to confirm the administered dose.

2.3. Clinical examination

Pigs were examined daily for clinical signs (Table 2), including body temperature, until either a pig was euthanized or day 70 post inoculation (pi), when the experiment was ended. Pigs were examined twice per day when the body temperature was over 41 °C or when body temperature was over 40 °C for two consecutive days, and were placed under analgesic treatment (Buprenorphine) if signs of moderate to severe discomfort were seen. If no improvement was seen within a few days, or pigs deteriorated further, they were euthanized intravenously with T61. Fever was defined as body temperature higher

Table 1
Study design used in each experimental group. Numbers between brackets show the number of successful inoculations and true number of naturally infected animals after failure of initial inoculation.

Groups	Brazil	Malta low	Malta high	Netherlands
Age of pigs (weeks)	8	12	12	12
Number of inoculated pigs	10	5 (3)	5	5 (3)
Number of naturally infected pigs	0	5 (7)	5	5 (7)
Inoculation dose	4.5 $\log_{10}$ $TCID_{50}$	3 $\log_{10}$ $TCID_{50}$	4 $\log_{10}$ $TCID_{50}$	3.5 $\log_{10}$ $TCID_{50}$
Samples	Blood	Blood	Blood	Blood
	OPF[a]	OPF	OPF	OPF
	Faeces	Faeces	Faeces	Faeces
	Vaginal swabs			
	Ocular swabs			
	Nasal swabs			

[a]Oropharyngeal fluid.