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Comparative evaluation of novel African swine fever virus (ASF) antibody detection techniques derived from specific ASF viral genotypes with the OIE internationally prescribed serological tests

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ABSTRACT

The presence of antibodies against African swine fever (ASF), a complex fatal notifiable OIE disease of swine, is always indicative of previous infection, since there is no vaccine that is currently used in the field. The early appearance and subsequent long-term persistence of antibodies combined with cost-effectiveness make antibody detection techniques essential in control programmes. Recent reports appear to indicate that the serological tests recommended by the OIE for ASF monitoring are much less effective in East and Southern Africa where viral genetic and antigenic diversity is the greatest. We report herein an extensive analysis including more than 1000 field and experimental infection sera, in which the OIE recommended tests are compared with antigen-specific ELISAs and immuno-peroxidase staining of cells (IPT). The antibody detection results generated using new antigen-specific tests, developed in this study, which are based on production of antigen fractions generated by infection and virus purification from COS-1 cells, showed strong concordance with the OIE tests. We therefore conclude that the lack of success is not attributable to antigenic polymorphism and may be related to the specific characteristics of the local breeds African pigs.

1. Introduction

African swine fever (ASF) is a complex and lethal viral disease of swine with significant socio-economic impact in the developed and developing world. The disease has a major negative effect on national, regional and international trade and constrains pig production by livestock farmers in affected areas in Sub-Saharan Africa (Penrith et al., 2004) and in the Caucasus region, where the disease was first identified in 2007 (Rowlands et al., 2008). The devastating acute form of the disease is characterized among others by functional and congestive-haemorrhagic disorders of the digestive and

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respiratory systems, causing to 100% mortality of infected pigs.

African swine fever virus (ASFV), the causative agent of ASF, is a large double-stranded DNA virus and the only member of *Asfaviridae* family, genus *Asfivirus* (Dixon et al., 2000, 2005). Transmission of ASFV can occur in a sylvatic or in a domestic pig cycle, with or without tick involvement. Depending on the presence or absence of wild suids and arthropod vectors and the type of pig production system, the epidemiology varies substantially between countries, regions and continents. In East and South Africa the disease has been maintained, since the first description in 1920s (Montgomery, 1921), in a sylvatic cycle involving asymptomatic wild African pigs (*Phacochoerus* and *Potamochoerus* spp.) and soft ticks of the genus *Ornithodoros*, mainly *O. porcinus* (Plowright et al., 1969; Wilkinson et al., 1988; Kleiboeker and Scoles, 2001; Penrith et al., 2004; Penrith, 2009; Jori and Bastos, 2009; Costard et al., 2009). In contrast, in West Africa the virus appears to be maintained by transmission between domestic pigs and the existence of a sylvatic cycle has never been demonstrated except for a single record in Sierra Leone (Penrith et al., 2004; Vial et al., 2007). Outside Africa, wild boar (*Sus scrofa*) and feral pigs are susceptible to ASFV and show similar clinical signs and mortality to domestic pigs. Long-term persistence of ASFV caused by the presence of the soft tick vector *Ornithodoros erraticus* was also reported in the Iberian Peninsula (Oleaga et al., 1990; Oleaga-Pérez et al., 1990; Boinas et al., 2011).

ASF is endemic in most sub-Sahara Africa with an increase of intermittently reported ASF outbreaks from the late-2000s onwards that continue to this day. In Europe, until 2007 and since the eradication of the disease in the Iberian Peninsula, ASF has been confined to Sardinia (Italy). In April 2007 its remarkable potential for trans-boundary spread was demonstrated by the appearance of the virus in the Republic of Georgia with subsequent outbreaks in Armenia, Azerbaijan and Southern Russia. Currently ASF is considered to be established in the southern part of the Russian Federation with sporadic reported outbreaks in Georgia and Armenia (Penrith, 2009; FAO, 2009a, 2010; World Organization for Animal Health, OIE, 2007–2012). The endemicity of ASF in the Caucasus threatens Europe, central Asia and even China, which has the largest pig population in the world.

Since there is no vaccine available, rapid and specific diagnostic procedures are an essential component of a control strategy in affected countries. Available conventional and Real Time PCR assays have been shown to be highly sensitive as diagnostic tools in acute and subacute forms of the disease caused by current circulating ASFV isolates in Europe and Africa (Aguero et al., 2003; King et al., 2003; Zsack et al., 2005; McKillen et al., 2010; James et al., 2010; Ronish et al., 2011; Tignon et al., 2011; Fernández-Pinero et al., 2012). Based on long term observations of p72 genotype I ASFV infections in European and West African pigs, it is apparent that antibodies to ASFV persist for long periods after the infection. Due to the absence of vaccine currently deployed for control of ASFV these antibodies are a good indicator of infection. Therefore the detection of seropositive animals is the most important and cost effective method for the control of the disease even when chronic or apparently asymptomatic pigs are present in the field, as it was demonstrated during the Spanish and Portuguese eradication programs (Bech-Nielsen et al., 1993a,b; Arias and Sanchez-Vizcaino, 2002). However recent investigations have indicated a low incidence of sero-positive ASFV infected virus-positive animals in East African countries, such as Kenya and Uganda, using recombinant antigens and OIE-approved tests based on crude antigen extracts (Perez-Filgueira et al., 2006; Gallardo et al., 2009b, 2011a). The comparison of the gene sequences of ASFV antigenic proteins p30 and p54 revealed higher levels of genetic variation located in areas of the proteins containing predicted antigenic determinants in the eastern African ASFV isolates comparing with sequences obtained from viruses circulating in West Africa and Europe (Perez-Filgueira et al., 2006; Sun et al., 1995). A similar pattern was observed by the genotyping of ASFV viruses using the C-terminal end of the ASFV p72 structural protein. This enabled discrimination of 22 different ASFV p72 genotypes in eastern and southern African regions. By contrast the West African, American and European ASFV isolates has been traditionally classified in a single p72 genotype I (Lubisi et al., 2005; Boshoff et al., 2007; Gallardo et al., 2009a, 2011a,b; Giammarioli et al., 2011). This historically broad distribution of genotype I viruses in Europe was altered in 2007 in Georgia when the first non-genotpye I

Table 1
ASFV isolates employed in this study.

ASFV isolate	Origin		Host species	Year collection	P72 genotype	Virulence	Haemadsorbing	Reference
	Country	Town/province						
E70[a]	Spain	Pontevedra	Pig	1970	I	vir	+	Zsak et al. (2005)
E75	Spain	Lerida	Pig	1975	I	vir	+	de Villiers et al. (2010)
Moz64[a]	Mozambique	NK	Pig	1964	V	vir	+	Gallardo et al. (2009a,b)
MwLil 20/1[a]	Malawi	Chalaswa	Tick	1983	VIII	vir	+	Haresnape et al. (1988)
Ken06.Bus[a]	Kenya	Busia	Pig	2006	IX	vir	+	Gallardo et al. (2009b)
Ken05/Tk1	Kenya	Machakos	Tick	2005	X	mod vir	+	Gallardo et al. (2011a)
Ken08/Tk2.1[a]	Kenya	Machakos	Tick	2008	X	mod vir	+	Gallardo et al. (2011a)
Arm07[a]	Armenia	Dilijan	Pig	2007	II	vir	+	Unpublished CISA-INIA
SS08/47223	Italy	Sardinia	Pig	2008	I	vir	+	Giammarioli et al. (2011)
NH/P68	Portugal		Pig	1967	I	att	–	Gil et al. (2003)

att: attenuated; vir: virulent; mod vir: moderately virulent.
[a] ASF viruses selected for ASFV genotypes-specific-antigens production.