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Potential use of oral fluid samples for serological diagnosis of African swine fever

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ABSTRACT

African swine fever (ASF) is a complex, highly lethal, notifiable disease of swine. ASF is wide-spread in sub-Saharan Africa and East European countries and there is presently a great risk of spread to neighboring countries. Since there is no vaccine for ASF virus (ASFV), control is based on rapid and early detection of the disease via surveillance. This approach requires collecting blood samples from large number of animals. Laborious and expensive of itself, this process also presents an additional risk because ASFV is present at high concentrations in the blood. The objective of this study was to initiate studies into the potential use of oral fluid as an alternative to serum for ASF diagnosis, for latter studying its possible use in surveillance and control programs. To this end, oral fluid samples collected at different times post infection from eight pigs experimentally inoculated with an attenuated ASFV were assayed using modified protocols of the two validated serological techniques, the enzyme-immune-liked assay (ELISA) and immunoperoxidase technique (IPT). Antibodies against ASFV were detected in oral fluid samples of all animals from early post infection through the end of the experiment by ELISA and IPT. These results confirmed the presence of ASFV antibodies in swine oral fluids samples, the possibility of an oral fluid-based approach in ASF diagnosis and, potentially in ASF surveillance.

1. Introduction

African swine fever (ASF) is one of the most complex infectious diseases of swine species. It is caused by a large doubled-stranded DNA virus, ASF virus (ASFV) belonging to the *Asfaviridae* family (Dixon et al., 2005). The epidemiology of ASF is complex, involving domestic and wild suids of different ages and breeds, as well as ticks from *Ornithodoros* genus. In affected countries, e.g., most of the sub-Saharan African countries, ASFV imposes a severe economic and social burden due to high mortality rates and international trade restrictions. In addition to Africa, ASFV is endemic in the island of Sardinia (Italy) and, since 2007, in a number of Eastern European countries, including the Russian Federation and the Trans Caucasian countries. In the recently infected areas of East Europe, ASFV's rapid expansion into north-western Russia demonstrated its capacity for rapid and far-flung spread, with numerous outbreaks occurring in such geographically distant regions as St Petersburg and the Baltic Sea, i.e., very close to European Union (EU) countries. The current situation in this region, in combination with its increased presence in African continent, highlights ASFV's potential for devastation and the risk it poses to the global pig industry (Sanchez-Vizcaino et al., 2012).

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There are no effective vaccines against ASFV. Therefore control is based on the implementation of strict prevention measures and the early detection of the disease, primarily through rapid laboratory diagnosis. At present, good antibody assays are available for the diagnosis of ASFV infection. These assays are suitable for use both in well-equipped international and national reference laboratories, as well as in basic regional and local laboratories. More recently, rapid assays have been developed for on-site ("point-of-care") use. Antibodies appear early in ASFV infection and persist for a long time, thereby serving as good markers of infection and providing for the detection of carrier animals. Antibody assays are economical, compatible with automation, and suitable for high-throughput screening. These factors make antibody testing the best option for ASFV surveillance (large scale screening) and eradication programmes (Arias and Sanchez-Vizcaino, 2002; 2012).

The limitation of this approach is the expense of collecting and testing blood samples. In particular, the need to handle and bleed animals presents a significant risk for further spread of ASFV as a consequence of the high levels of ASFV present in blood (McVicar, 1984). Oral fluid is an alternative diagnostic specimen that could potentially address these problems. Oral fluid has been proven to be a good diagnostic specimen for the detection of a number of pathogens of swine, including porcine reproductive and respiratory syndrome virus (PRRSV) and porcine circovirus type 2 (PCV2), by both nucleic acid, and antibody detection (Prickett and Zimmerman, 2010; Prickett et al., 2011; Ramirez et al., 2012).

Given the current risk for the spread of ASFV, more cost-effective and rapid methods for ASFV surveillance are needed. Therefore, the objective of this study was to determine if antibodies against ASFV could be detected in oral fluid samples from experimentally infected animals as the first step in evaluating its potential use in ASF surveillance and eradication programmes.

2. Material and methods

2.1. Cells and viruses

A Spanish strain of ASFV isolated in 1970 (E70) and adapted to grow in a monkey stable (MS) kidney cell line (ECACC, 91070510) was used for OIE-antigen production following the method described in the Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (OIE, 2012). For the experimental in vivo studies, the attenuated and non haemadsorbing Portugal ASFV isolate NH/P68 (NHV) belonging to p72 genotype I and the virulent Armenia ASFV Arm07 isolate (genotype II) were used. Finally, isolate BA71V, recovered in Spain in 1971 and adapted to Vero cells (ATCC CCL 81) was employed for the production of the fixed indirect immunoperoxidase plates. The ASFV isolates were propagated and titrated according with Carrascosa et al. (2011). All ASFV isolates were obtained from the collections of the European Union Reference laboratory for ASF (CISA-INIA).

2.2. Experimental design and sampling collection

The ability to detect antibodies against ASF in oral fluid was evaluated using samples collected over time in two independent studies involving experimentally-infected pigs: (i) four Landrace × Large White pigs intramuscularly inoculated with 10^5 TCID50/ml of the ASFV isolate NH/P68 (NHV) and challenged at day 30 post infection (dpi) with 10 HAD50/ml of the heterologous virulent Armenia ASFV Arm07 isolate; and (ii) four Landrace × Large White pigs intramuscularly inoculated with 10^3 TCID50/ml of the attenuated and non haemadsorbing Portugal ASFV isolate NH/P68 (NHV). Paired oral fluid and serum samples were obtained at 0, 11, 14, 18, 21, 30, 37, 44, 52, 58 and 65 dpi(s). Both animal experiments were conducted at the BSL3 animal facilities at CISA-INIA and performed in accordance with the EC Directive 86/609/EEC, following the recommendation 2007/526/EC for the accommodation and care of animals used for experimental and other scientific purposes.

Oral fluid samples were collected from individual pigs using cotton rope 12 mm diameter and 25 cm length. Pigs were allowed to chew the rope for 10 min, i.e., until the rope was sufficiently wet. The wet end of the rope was cut, placed in a syringe (50 ml), and compressed to recover the oral fluid. A volume of ~5 ml was obtained from each animal on each collection day. Serum samples were collected using conventional methods.

2.3. ASF antibody detection in serum and oral fluid samples

ASFV antibody in serum samples was measured using the OIE indirect ELISA (OIE, 2012) and the Indirect Immunoperoxidase test (IPT). The IPT was performed using fixed VERO cells infected with isolate Ba71V ASFV following the same procedure described in COS1-cells by Gallardo et al., 2012.

The ASF ELISA and IPT serological tests were adapted to anti-ASFV antibody detection in oral fluid samples by adjusting incubation time, incubation temperatures, blocking buffers, concentrations of the antigen and the oral fluid samples, as well as the type and concentration of the conjugate (secondary anti-pig and/or protein A horseradish peroxidase [HRP] conjugated).

3. Results

3.1. Modification of OIE ASFV indirect ELISA and IPT protocols for oral fluid specimens

Optimization of the OIE ELISA and IPT tests for the detection of ASFV antibody in oral fluid specimens was carried out by comparing the responses of varying concentrations of antigen and conjugates using a checkerboard titration procedure. The optimum response for the indirect ELISA was achieved when microtitration plates were coated with 1 μg of ASFV cytoplasmic antigen per well, which correlated to a 1:800 working dilution. In addition, the OIE ASFV ELISA protocol was modified to enhance the detection of antibody in oral fluid, e.g., oral fluid samples were assayed without