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Development of a novel lateral flow assay for detection of African swine fever in blood

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Abstract

Background: African swine fever (ASF) is a viral infectious disease of domestic and wild suids of all breeds and ages, causing a wide range of hemorrhagic syndromes and frequently characterized by high mortality. The disease is endemic in Sub-Saharan Africa and Sardinia. Since 2007, it has also been present in different countries of Eastern Europe, where control measures have not been effective so far. The continued spread poses a serious threat to the swine industry worldwide. In the absence of vaccine, early detection of infected animals is of paramount importance for control of the outbreak, to prevent the transmission of the virus to healthy animals and subsequent spreading of the disease. Current laboratory diagnosis is mainly based on virological methods (antigen and genome detection) and serodiagnosis.

Results: In the present work, a Lateral Flow Assay (LFA) for antigen detection has been developed and evaluated. The test is based on the use of a MAb against VP72 protein of ASFV, the major viral capsid protein and highly immunogenic. First experiments using VP72 viral and recombinant protein or inactivated culture virus showed promising results with a sensitivity similar to that of a commercially available Antigen-ELISA. Moreover, these strips were tested with blood from experimentally infected pigs and field animals and the results compared with those of PCR and Antigen-ELISA. For the experimentally infected samples, there was an excellent correlation between the LFA and the ELISA, while the PCR always showed to be more sensitive (38 % positive samples by PCR versus 27 % by LFA). The LFA was demonstrated to be positive for animals with circulating virus levels exceeding 10^4 HAU. With the field samples, once again, the PCR detected more positives than either the Antigen-ELISA or LFA, although here the number of positive samples scored by the LFA exceeded the values obtained with the Antigen-ELISA, showing 60 % positivity vs 48 % for the ELISA. For the two groups of sera, the specificity was close to 100 % indicating that hardly any false positive samples were found.

Conclusions: The newly developed LFA allows rapid and reliable detection of ASFV, at field and laboratory level, providing a new useful tool for control programs and in situations where laboratory support and skilled personnel are limited.

Keywords: African swine fever virus, Lateral flow assay, Diagnosis

Background

African swine fever virus (ASFV) is a large, enveloped, icosahedral double-stranded DNA virus that belongs to the *Asfviridae* family, genus *Asfivirus* [1]. ASFV was first identified in 1921 in Kenya as the cause of lethal hemorrhagic disease in domestic pigs [2]. In Europe, ASF was introduced to Portugal in 1957, and from 1960, in other countries such as Spain or Italy and the Caribbean islands, but finally eradicated. Currently, the disease is

endemic in the majority of Sub-Saharan countries and Sardinia (Italy) [3, 4]. Since the introduction of ASFV into Georgia in 2007 from East Africa, several cases have been declared in Armenia, Azerbaijan and in the Russian Federation, where continued uncontrolled spreading poses a serious threat to the swine industry worldwide [5–8]. The disease again manifested itself in early 2014, when the first cases of ASF in wild boar in Lithuania and Poland were reported in areas bordering on Belarus. Since then, the ASFV has spread in Estonia, Latvia, Lithuania and Poland, mostly affecting wild boar and to a lesser extent domestic pigs [9, 10]. Presently, the disease is threatening other

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