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Detection of ASF using Whatman 3MM filter paper in 2021 in Madagascar

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Abstract: *The requirement for cold chains sometimes prevents samples from being sent from the field to the laboratory, which is necessary for diagnosing this disease.*
The method using Whatman 3MM blotting paper to transport and analyse African Swine Fever during sporadic cases has been put forward for the diagnosis of ASF in three localities in Madagascar. It involves a direct PCR test (conventional) and an ELISA test on blood samples taken on blotting paper. After impregnation, the blotting paper was dried and a fragment of approximately 2mm2 or 40mm2 was placed directly into a PCR tube or ELISA plate respectively, into which the reaction mixture was added.
A total of 65 pigs were analysed in this study. The overall prevalence of infection was 40% (CI95%: 27.85 - 52.15) for viral detection by direct PCR using Whatman 3MM blotting paper, and 0% for antibody detection by ELISA serology.
The use of this technique has produced promising results and has saved several farms in Madagascar since 2019. In this study, the Whatman 3MM blotting paper proved to be a medium for collecting and storing pig blood from a distant locality without using the cold chain to detect the presence of African swine fever in 2021.

I. Introduction

African swine fever (ASF) is a highly contagious viral disease caused by a virus belonging to the Asfaviridae family and the Asfivirus genus [1](Dixon, 2005). It is transmissible and affects wild and domestic swine, and has very severe epidemic characteristics with very high mortality rates [2, 3] (Grangé, 2016; Atuhairwe, 2013). In Madagascar, this disease was ignored until it was first diagnosed in December 1998.

The lack of medical prophylaxis and treatment for ASF means that the disease must be diagnosed quickly if it is to be controlled and eradicated [4] (Randriamparany, et al., 2016). Diagnosis of ASF is based on detection of the virus or detection of antibodies. Numerous techniques can be used, but the choice of test depends on financial resources, equipment and techniques, the level of urgency and the virus strain in question [5] (Franco, 2006). However, low-income countries are vulnerable to this disease. Whatman 3MM filter papers contain no additives, so they can preserve infectivity and can theoretically be used for further amplification of pathogens. Another advantage is that they contain no PCR inhibitors and can be used directly in conventional PCR without prior nucleic acid extraction, as has already been demonstrated in the detection of ASFV [6] (Michaud et al.,

2007). On the basis of these results, this study was designed to assess whether Whatman 3-MM filter papers can be used to diagnose ASF using a series of currently available tests that were originally designed for the detection of ASF virus and antibodies in conventional biological samples.

The aim of this study is to use Whatman 3MM blotting paper to collect pig blood. This same sample is used to detect both the ASF virus using the PCR technique, and the presence of circulating antibodies to viral infection using Enzyme-Linked Immunosorbent Assay (ELISA). This technique is fast, easy to perform and does not use cold chains, which usually cause problems in most developing countries.

II. Materials and methods

This study is being carried out in three different localities in Madagascar to diagnose suspected cases of ASF in Arivonimamo, Port Bergé and Vangaindrano in 2021. The samples used are blotting papers that have been impregnated with the blood of slaughtered pigs, then dried and stored in an envelope at room temperature until use. The samples were then sent to the National Veterinary Diagnostic Laboratory in Itaosy, Antananarivo, where they were tested. The samples were analysed at the Laboratoire National de Diagnostic Vétérinaire (LNDV), located in Itaosy, Antananarivo 102, Madagascar.

II.1 Methods

II.1.1. Preparation of samples

a) Preparation of filter paper

Whatman 3MM blotting papers, which are often used for the storage and detection of genetic or protein material, were selected for this study. The 3MM blotting papers were cut into 5 x 0.5 cm strips. The strips were soaked (Figure 1) in whole blood taken from slaughtered pigs and left to dry.

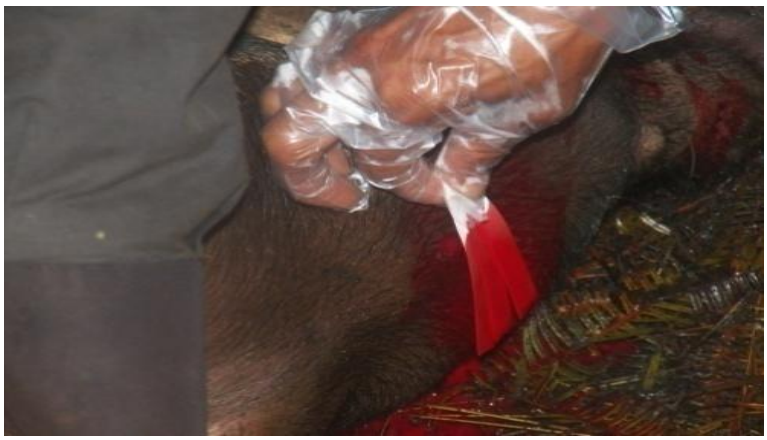

Figure 1: Collection of samples on Whatman 3MM blotting paper during the bleeding of a pig

Once dried, the filter papers were stored at room temperature (22-25°C) or at 37°C until use.

b) Conventional samples

For tissue and blood samples, viral nucleic acids were prepared using the QIAamp viral DNA mini kit (QIAGEN, Venlo, The Netherlands) as described by King and colleagues in 2003 and stored at -80°C until subsequent PCR analysis.

II.2.1. Diagnostic procedures

a) Conventional direct PCR

The highly conserved region of the genome coding for the p72 protein of the ASF virus was amplified by PCR using 5 prime Mastermix (Eppendorf, Montesson, France). Dried blood from infected pigs was placed directly into PCR tubes without prior nucleic acid extraction. A 2 mm2 piece of filter paper was placed in each 0.2 ml PCR tube. The reaction mixture was added to a final volume of 50 µl to allow correct immersion of the filter papers. The reaction mixture contained 0.4 µM of forward primer: 5'-T C G G A G A TG T C A G G T A G G-3' and reverse primer: 5'-G C A AAA G G A T TT G G T G A A T-3'. PCR was performed as follows: (i) 5 min at 95°C; (ii) 35 cycles for 30 s at 95°C, 30 s at 55°C, and 30 s at 72°C; (iii) 7 min at 72°C.

A PCR fragment of 346 base pairs was visualised on an agarose gel. A negative control from an uninfected pig was included. Fragment size was defined by comparison with DNA ladders.

Using the same master mix and primers, 15 µl of DNA extracted from blood and tissue was analysed in final volumes of 50 µl. The reaction mixture was processed as follows: (i) 5 min at 95°C; (ii) 35 cycles, 30 s at 95°C, 30 s at 60°C and 30 s at 72°C; (iii) 10 min at 72°C.

b) Elution of antibodies from 3MM filter papers for antibody detection by ELISA and evaluation of assay performance.

Paired samples (3-MM filter papers containing blood and dried sera) were taken from experimentally infected pigs.

A 40 mm2 piece of 3-MM filter paper containing dried blood was removed and added to a 100 µl volume of ELISA buffer (Ingezim PPA Compac, Ingenasa, Spain). After incubation for 2 hours, the eluate was collected and tested in the company's specially designed ELISA kit. Sera were tested in the commercial version of this kit according to the manufacturer's instructions.

III. Results

During the year 2021, sixty-five (65) samples of blotting paper soaked in pig blood were collected and received at the laboratory. In the Commune of Imeritsiatosika, 14 samples were collected at the request of a pig farmer in March 2021. 25 pig samples were received from the Vangaindrano slaughterhouse in June 2021. 26 samples were then recovered following a mission to farms in the Commune of Antafiakatsaka, Port-Bergé district, in December 2021.

The samples received were analysed using two ASF diagnostic techniques. These were the detection of antibodies by ELISA and the search for viral circulation by PCR.

Using 3MM blotting paper as the sample, detection of African swine fever virus by PCR gave us 26/65 positive samples, giving an overall prevalence of 40% (IC95%: 27.85 - 52.15). No positive results were found for the detection of antibodies to ASF in 65 samples. The results are summarised in Table 1.

Table 1: Detection of PPA virus by PCR technique using samples of 3MM filter paper soaked in pig blood

Year	Region	District	Commune	Filtre Papiers 3MM		
				Collected	Pos PCR (%)	Pos Anticorps
2021	Itasy	Arivonimamo	Imeritsiatosika	14	9 (64,29)	0
	Sofia	Port Bergé	Antafiakatsaka	26	14 (53,84)	0
	Fitovinany	Vangaindrano	Vangaindrano	25	3 (12)	0
			Total	**65**	**26 (40)**	**0**

Pos : positive

IV. Discussion

In this study, the performance of Whatman 3MM filter papers for blood collection on blotting paper and their storage at room temperature (>22°C) with current diagnostic procedures gave convincing results for the detection of African swine fever.

We did not find any positive samples when analysing the 3MM blotting papers using the ELISA Kit. This result suggests that there are no antibodies circulating in the animal's blood that are detected around the 14th day of incubation of the virus. Another idea is that low concentrations of antibodies may not be detected in 3MM filter papers compared with sera.

In contrast, a prevalence of 40% was found using molecular techniques, which are the most popular diagnostic procedures used for the rapid identification of animal and human diseases.

In this study, it was demonstrated that 3MM filter papers offer a remarkable advantage over conventional biological materials, as nucleic acid extraction is not required. Thus, to be able to carry out direct, conventional PCR implies a considerable reduction in the time required for molecular diagnosis and in the cost. Another potential advantage is the reduction in potential contamination during sample processing. It was possible to use Whatman 3MM filter papers collected as early as 2-3 days after infection in conventional direct PCR or real-time PCR for early detection or real-time PCR for early detection of the ASF genome in the three different experiments. According to Randriamparany et al 2016, ASF virus was detected for at least 9 months at room temperature (22-25°C) and it is likely that even higher temperatures do not interfere with material preservation and virus detection, as shown by other authors [6, 7] (Michaud et al., 2007; Uttenthal et al., 2013).

V. Conclusion

Whatman 3MM blotting papers are an inexpensive, simple and rapid means of blood collection, reservation and diagnosis of African swine fever disease by ELISA and conventional direct PCR. Advantages of 3MM blotting paper strips include the smaller volume of blood required and the ability to collect a large number of samples in the field. Whatman 3MM blotting papers can be used as a multivalent medium for versatile diagnosis in tropical conditions. In the Materials and methods section, 3MM filter papers stored at room temperature must be eluted for several minutes or hours by shaking in the appropriate buffer to allow antibody recovery. However, this disadvantage does not exist for molecular techniques which, after a serological test, are the most popular diagnostic procedures used for the rapid identification of animal and human diseases. The disadvantages found could be improved by optimising this technique.

References

[1] Dixon LK, Escribano JM, Martins C, Rock DL, Salas ML et Wilkinson PJ. Asfarviridae. In: Fauquet CM, Mayo MA, Maniloff J, Desselberger U et Ball(editors). Virus Taxonomy. VIII. Rep ort of the ICTV, Elsevier, Academic Press, London; 2005. pp. 135–143.

[2] Grangé, Tania. Risques épidémiologiques associés à l'élevage porcin à Madagascar : cas particulier de la peste porcine africaine dans les zones d'interface avec le potamochère (potamochoerus larvatus). Thèse d'exercice, Médecine vétérinaire, Ecole Nationale Vétérinaire de Toulouse - ENVT, 2016, 156 p.

[3] Atuhairwe KD, Ochwo S, Afayoa M, Mwine FM, Kokas I, Arinaitwe E et al. Epidemiological Overview of African Swine Fever in Uganda (2001–2012). Journal of Veterinary Medicine. 2013; 9: 263(Article ID 949638).

[4] Franco, 2007. Epidémiologie de la peste porcine africaine dans la région du lac Alaotra (Madagascar) ; Etude des facteurs derisque et estimation de la prévalence. Thèse Médecine vétérinaire, Ecole Nationale Vétérinaire de Toulouse - ENVT, 150p

[5] Randriamparany T, Grenier A, Tourette I, Maharavo Rahantamalala CY, Rousset D, Lancelot R. Epidemiological situation of African swine fever in Lake Alaotra Region (Madagascar) and possible consequences on the organization of disease control and surveillance. Revue d'Elevage et de Me´decine Ve´ te´ rinaire des Pays Tropicaux. 2005; 58: 15–20.

[6] Randriamparany T, Kouakou KV, Michaud V, Ferna´ndez-Pinero J, Gallardo C, Le Potier MF, et al. African Swine Fever Diagnosis Adapted to Tropical Conditions by the Use of Dried-blood Filter Papers. Transbound Emerg Dis. 2016 Aug; 63(4):379–88. https://doi.org/10.1111/tbed.12295 Epub 2014 Nov 27. PMID: 25430732

[7] Michaud, V., P. Gil, O. Kwiatek, S. Prome, L. Dixon, L. Romero, M.-F. Le Potier, M. Arias, E. Couacy-Hymann, F. Roger, G. Libeau, and E. Albina, 2007: Long-term storage at tropical temperature of dried-blood filter papers for detection and genotyping of RNA and DNA viruses by direct PCR. J. Virol. Methods 146, 257–265.

[8] Uttenthal, A., U. C. Braae, H. A. Ngowi, T. B. Rasmussen, J. Nielsen, and M. V. Johansen, 2013: ASFV in Tanzania: asymptomatic pigs harbor virus of molecular similarity to Georgia 2007. Vet. Microbiol. 165, 173–176.