Food and Agriculture Organization of the United Nations

Case studies of the use of agricultural biotechnologies to meet the needs of smallholders in developing countries

Case studies of the use of agricultural biotechnologies to meet the needs of smallholders in developing countries

Edited by
John Ruane, Chikelu Mba, Paul Boettcher, Jarkko Koskela,
Graham Mair and Selvaraju Ramasamy

Food and Agriculture Organization of the United Nations
Rome, 2023

Required citation:
Ruane, J., Mba, C., Boettcher, P., Koskela, J., Mair, G. & Ramasamy, S., eds. 2023. *Case studies of the use of agricultural biotechnologies to meet the needs of smallholders in developing countries*. Rome, FAO.
https://doi.org/10.4060/cc8940en

ISBN 978-92-5-138424-4

Contents

Abbreviations

AATF	African Agricultural Technology Foundation
AGI	Agricultural Genetics Institute (Viet Nam)
AI	artificial insemination
ASF	African swine fever
BMGF	Bill and Melinda Gates Foundation
Bt	bacillus thuringiensis
CIMMYT	International Maize and Wheat Improvement Center
CS	case study
DNA	deoxyribonucleic acid
EFSB	eggplant fruit and shoot borer
ELISA	enzyme-linked immunosorbent assay
FAO	Food and Agriculture Organization of the United Nations
FAW	fall armyworm
GAB	genomics-assisted breeding
GEF	Global Environment Facility
GM(O)	genetically modified (organism)
IAEA	International Atomic Energy Agency
ICAR	Indian Council of Agricultural Research
ICRISAT	International Crops Research Institute for the Semi-Arid Tropics
IFGTB	Institute of Forest Genetics and Tree Breeding (India)
IFPRI	International Food Policy Research Institute
IPM	integrated pest management
MABC	marker-assisted backcrossing
MARS	marker-assisted recurrent selection
MAS	marker-assisted selection
NARS	national agricultural research systems
NGO	non-governmental organization
PCC	Philippine Carabao Center
PCR	polymerase chain reaction
PPP	public–private partnership
QTL	quantitative trait locus
R&D	research and development
RPS	relative percent survival
SIT	sterile insect technique

SNP	single nucleotide polymorphism
SSA	Sub-Saharan Africa
SSR	simple sequence repeat
SSS	sex-sorted semen
UN	United Nations
USAID	United States Agency for International Development
USD	US dollars

2.4 Use of filter paper to rapidly diagnose African swine fever in Madagascar

Tantely Randriamparany,[1] Fleurette Ravaomanana,[1] Nirharosoa de Borgia Randriamora,[1] Miatrana Voahangielisoa Rasamoelina,[2] Rianja Tsanta Ny Aina Rakotoarivony,[2] Daouda Kassie,[3] Diana Edith Andria-Mananjara,[2] Mihajamanana Rakotoarinoro,[2] Herilantonirina Ramaroson,[2] Modestine Raliniaina,[2] Ferran Jori Massana[4] and Eric Etter[5]

[1] Laboratoire National de Diagnostic Vétérinaire, Ministry of Agriculture and Livestock, Antananarivo, Madagascar
[2] Centre National de la Recherche Appliquée au Développement de Recherches Zootechniques Vétérinaires et Piscicoles, FOFIFA/DRZVP, Antananarivo, Madagascar
[3] French Agricultural Research Centre for International Development (CIRAD), Antananarivo, Madagascar
[4] ASTRE, Université Montpellier, CIRAD, INRAE, Montpellier, France
[5] UMR 117 ASTRE (Animals, health, Territories, Risks, Ecosystems), CIRAD, Guadeloupe, France

Email: t.randriamparany@gmail.com

Background

The world's population is currently growing, but stocks of vital natural resources such as animal genetic resources, agricultural land and water are gradually diminishing. To feed the world in this situation, the development of short-cycle livestock, especially pork, would be a plausible approach. It has a positive impact on a large part of the population through its value chain which involves several types of actors including producers, live pig traders, pork processors, consumers and many other actors who use pork and its by-products for traditional, cultural, religious and medicinal purposes.

African swine fever (ASF) is a highly contagious viral disease, caused by a virus belonging to the family Asfarviridae and genus Asfivirus (Dixon *et al.*, eds., 2005). It is transmissible, affects wild and domestic swine and has very severe epidemic characteristics with very high mortality rates (Atuhairwe *et al.*, 2013). Worldwide, ASF remains a major health threat and limits the development of pig farming, compromising the food security of many countries (Rasamoelina-Andriamanivo, Porphyre and Jambou, 2013).

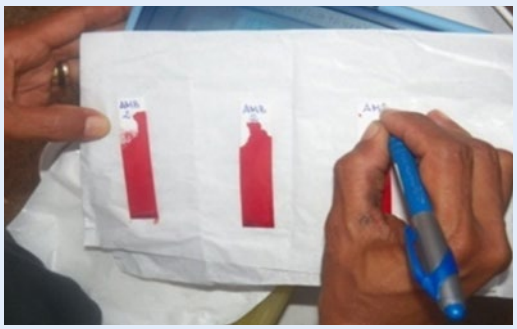
In Madagascar, this disease was unknown until it had the first diagnosis in December 1998. Thereafter, it spread over almost all the country (Humbert, 2006). ASF caused numerous economic losses and major disruptions in the pig industry, as well as the disappearance of many improved livestock farms (Franco, 2007; Grangé, 2016).

There is still no effective treatment or vaccine against ASF, so the disease can only be controlled by preventive sanitary measures. The lack of medical prophylaxis and treatment of ASF requires rapid diagnosis in order to control and eradicate the disease (Randriamparany *et al.*, 2016). In tropical countries, however, the diagnosis of human or animal viral infections is often hampered by the need to maintain a cold chain for the preservation of samples to the laboratory. The cold chain is necessary to transport infectious material for initial or confirmatory diagnosis of ASF infection

to local, regional or international laboratories. In remote areas, especially in developing countries without adequate infrastructure, it is often impossible to maintain a cold chain for the storage of biological samples. There is therefore an urgent need for a method of sampling and transport from farm to laboratory that does not rely on the cold chain.

In recent years, studies have demonstrated that samples could be poured and stored on filter papers at room temperature for relatively long periods (Uttenthal *et al.*, 2013). The use of blood samples dried on filter papers was described as a possible alternative to preserving human and animal samples for testing (Abdelwhab *et al.*, 2011; Joseph and Melrose, 2010).

Figure 1. Room temperature storage of filter papers



©LNDV/Tantely Randriamparany

The diagnosis of ASF is based on virus or antibody detection. Many techniques can be used but the choice of a test depends on financial means, materials and techniques, the level of urgency and the virus strain in question (Franco, 2007). Whatman 3MM filter paper was used to collect pig blood (Figure 1) in this study, because it is difficult to send conventional samples from the field to the laboratory due to the cold chain requirement. The same sample was used to detect both the ASF virus, using the polymerase chain reaction (PCR) technique, and the presence of circulating antibodies during viral infection by the enzyme-linked immunosorbent assay (ELISA) technique.

PCR is a laboratory technique for rapidly producing and amplifying millions to billions of copies of a specific segment of DNA, which can be studied in greater detail. ELISA is used for detecting the presence of an antigen in biological samples. An ELISA, like other types of immunoassays, relies on antibodies to detect a target antigen using highly specific antibody-antigen interactions.

Case study

This case study involves suspected outbreaks of ASF in different localities of Madagascar from late 2019 to early 2022. Investigations were carried out in seven different communes during outbreaks (Figure 2). During the outbreaks, samples were collected from the sick pigs still on the infected farms and also from the apparently non-infected surrounding farms. A total of 198 filter paper samples impregnated with pig blood were collected, and analysis of the samples was carried out at the Laboratoire National de Diagnostic Vétérinaire (LNDV, the National Laboratory of Veterinary Diagnosis), located at Itaosy, Antananarivo.

The types of farms and husbandry practices involved a very low level of biosecurity and farms are not fenced. As previously indicated, Whatman 3MM filter papers, which are often used for storage and detection of genetic or protein material, were selected for this study (Randriamparany *et al.*, 2016). The filter papers were cut into 5 x 0.5 cm strips and then impregnated with blood from slaughtered pigs and farms after making a small incision in the throat, then dried and stored in envelopes at room temperature until use. Once dried, samples can be sent, such as to an analysis

laboratory, quickly and easily. This technique can be used in surveillance or vigilance studies and allows early detection of virus circulation, and thus allows farmers to take control and protection measures for their animals in a timely manner.

Diagnosis procedures

Conventional direct PCR

The highly conserved region of the genome encoding the p72 protein of the ASF virus was amplified by PCR using the five prime Mastermix (Eppendorf, Montesson, France). Pieces of 3MM filter papers with dried blood from infected pigs were placed directly into PCR tubes without prior nucleic acid extraction. A 2 mm^2 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 for proper immersion of the filter papers. The reaction mixture contained 0.4 µM of direct primer: 5'-T CGGAGATGTTCCAGGTAGG-3' and reverse primer: 5'-GCAAAAGGATTTGGTGAAT-3'. The PCR was run 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 visualized on an agarose gel. A negative control from an uninfected pig was included. Fragment size was defined by comparison with DNA ladders.

Antibody detection by ELISA

A 40 mm^2 piece of 3MM filter paper containing dried blood was collected and added to a 100µl volume of ELISA buffer (Ingezim PPA Compac, Ingenasa, Spain). After incubation for two hours, the eluate was collected and tested with a designed ELISA kit.

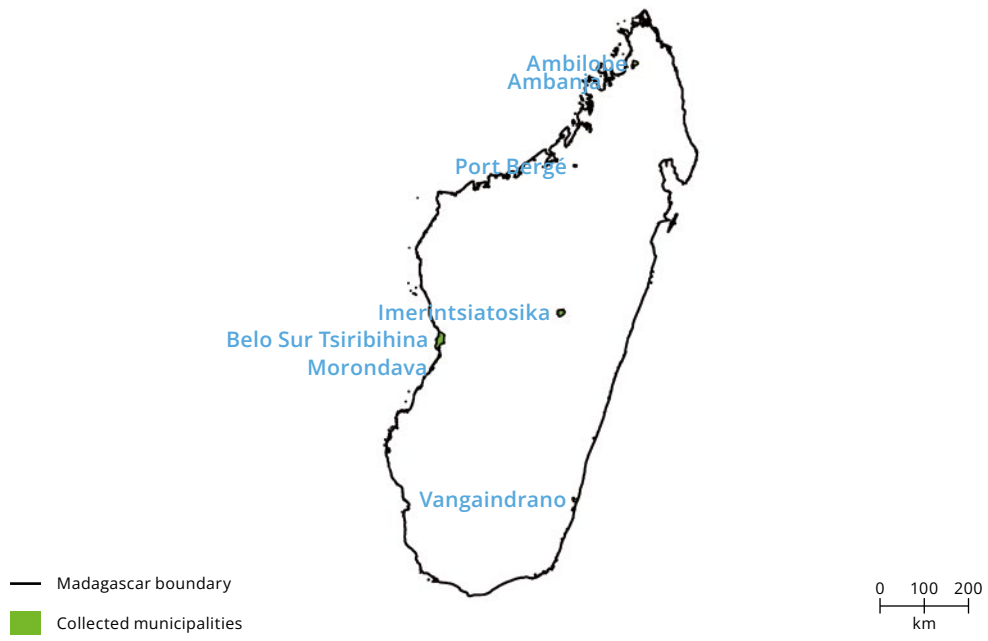
Results

Using 3MM blotting paper for the samples, the detection of the ASF virus by direct PCR gave positive results in 39 of the 198 samples (an overall 19.7 percent prevalence rate). No positive results were observed for the detection of ASF antibodies. The results are summarized in Table 1.

Table 1. Detection of the ASF virus by the PCR technique using 3MM filter paper samples impregnated with pig blood, according to year and location

Year	Region	District	Commune	3MM filter papers		Pig numbers in the municipality
				Collected	Positive	
2019	Diana	Ambanja	Ambanja	1	1	9 500
		Ambilobe	Ambilobe	68	7	24 000
2020	Menabe	Belo	Belo	7	3	4 000
		Morondava	Morondava	20	1	6 000
2021	Itasy	Arivonimamo	Imeritsiatosika	14	9	22 000
	Sofia	Port Bergé	Port Bergé	26	14	600
	Fitovinany	Vangaindrano	Vangaindrano	25	3	400
2022	Menabe	Morondava	Morondava	37	1	6 000
Total				**198**	**39**	**72 500**

Figure 2. Map of Madagascar with the locations of African swine fever virus outbreaks



Discussion

This technique was successfully validated with experimentally infected pig blood (Michaud *et al.*, 2007). Another validation of this technique was performed by Randriamparany *et al.* (2016) by comparing filter papers with standard field samples.

In this case study, we did not find any positive samples for antibodies when testing 3MM blotting papers using the ELISA kit. This result is consistent with the circumstances by which antibodies were not yet circulating in the animals' blood at the time of sampling. Antibodies are usually detected around the 14th day of virus incubation (Gallardo, Fernández-Pinero and Arias, 2019). Another possible explanation is that low concentrations of antibodies may be less detectable in 3MM filter papers compared to sera. On the other hand, a prevalence of 19.7 percent (95 percent CI: 14.4–25.6 percent) was found using molecular techniques (PCR) which are the most popular diagnostic procedures used for rapid identification of animal and human diseases.

Based on these results, this study suggests that the Whatman 3MM filter papers can be used for ASF diagnosis using a series of currently available tests that were originally designed for the detection of ASF virus and antibodies in conventional biological samples. No direct comparison with conventional samples was done, however, in this study.

Whatman 3MM filter papers do not contain additives. Therefore, they can preserve infectivity and can theoretically be used for further amplification of pathogens. Another advantage is that they do not contain PCR inhibitors and can be used directly in conventional PCR without prior nucleic acid extraction, as previously demonstrated in the detection of ASF virus (Michaud *et al.*, 2007; Randriamparany *et al.*, 2016). This technique is quick, easy to perform and does not use cold chains which usually cause problems in most developing countries.

In this study, the performance of Whatman 3MM filter papers for blood collection on blotting paper and storage at room temperature with current diagnostic procedures yielded convincing results for the detection of ASF. The observed specificity for this test on filter papers containing dried blood was excellent (100 percent) in a previous study (Randriamparany, *et al.*, 2016). It was also shown that 3MM filter papers have a remarkable advantage over conventional biological materials because nucleic acid extraction is not required. Thus, to be able to perform conventional direct PCR, there is a considerable reduction in the time needed for molecular diagnosis and cost. In addition, another potential benefit is the reduction of potential contamination during sample processing because samples are dried and prepared on small pieces of 3MM filter paper.

It was possible to use the Whatman 3MM filter paper technique for very early detection of infection by conventional direct PCR. Early detection is important because it has been reported that, in cases of ASF outbreaks, farmers in Madagascar rush to sell or slaughter their pigs to avoid economic losses (Randriamparany *et al.*, 2005). The same is true in other African countries where ASF is prevalent (Dione *et al*, 2014). In Madagascar, the government orders the slaughter of pigs on ASF-infected farms. Pigs on the farms where the 39 positive animals were detected were therefore slaughtered.

Conclusion

Whatman 3MM filter papers are an inexpensive, simple and fast way to collect blood, store samples and diagnose ASF disease by ELISA and conventional direct PCR. Advantages of 3MM filter 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 multi-purpose medium for versatile diagnosis under tropical conditions.

African swine fever is a major health problem for the pig industry. One of the challenges of diagnosis, especially for African countries, is to be able to rapidly diagnose the presence of the virus on samples that are easy to collect and that do not require special equipment for storage. The test we used meets these requirements. It is a direct PCR test (conventional) on blood samples taken on blotting paper. After impregnation, the blotting paper is dried and a fragment of about 2 mm^2 is placed directly into a PCR tube, into which the reaction mixture is added. The use of this technique has shown promising results and has saved several farms in Madagascar since 2019.

In this case study, Whatman 3MM blotting paper proved to be a good support for the collection and storage of pig blood coming from a distant locality without using a cold chain to detect the presence of ASF in Madagascar. The method is currently being used by the LNDV to detect and analyze ASF outbreaks.

Acknowledgment

This study was partially funded by the project "Unraveling the Effect of Contact Networks & Socio-Economic Factors in the Emergence of Infectious Diseases at the Wild-Domestic Interface", USDA Award Number: 2019-67015-28981 (Madagascar-Mozambique-South Africa) (www.asf-nifnaf.org/)

References

Abdelwhab, E.M., Lüschow, D., Harder, T C. & Hafez, H.M. 2011. The use of FTA filter papers for diagnosis of avian influenza virus. *Journal of Virological Methods*, 174, 120–122. **https://doi.org/10.1016/j.jviromet.2011.03.017**

Atuhairwe, K.D., Ochwo, S., Afayoa, M., Mwine, F., M, Kokas, I., Arinaitwe, E., Ademun-Okurut, R.A. *et al.*, 2013. Epidemiological overview of African swine fever in Uganda (2001–2012). *Journal of Veterinary Medicine*, 9, 263. **http://dx.doi.org/10.1155/2013/949638**

Dione. M.M., Ouma, E.A., Roesel K., Kungu, J., Lule, P & Pezo, D. 2014. Participatory assessment of animal health and husbandry practices in smallholder pig production systems in three high poverty districts in Uganda. *Preventive Veterinary Medicine,* 117, 566–576. **https://doi.org/10.1016/j.prevetmed.2014.10.012**

Dixon, L.K., Escribano, J.M., Martins, C., Rock, D.L., Salas, M.L. & Wilkinson, P.J. 2005 Asfarviridae. In: C.M. Fauquet, M.A. Mayo, J. Maniloff, U. Desselberger & L.A. Ball, eds. *Virus taxonomy: Eighth report of the International Committee on Taxonomy of Viruses*, pp. 135–143. London, Elsevier Academic Press.

Franco, S. 2007. Epidémiologie de la peste porcine africaine dans la région du lac Alaotra (Madagascar): Etude des facteurs de risque et estimation de la prévalence. Toulouse, France, Ecole Nationale Vétérinaire de Toulouse. Thesis.

Gallardo, C., Fernández-Pinero, J. & Arias, M. 2019. African swine fever (ASF) diagnosis, an essential tool in the epidemiological investigation. *Virus Research*, *271*. **https://doi.org/10.1016/j.virusres.2019.197676**

Grangé, T. 2016. 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). Toulouse, France, Ecole Nationale Vétérinaire de Toulouse. Thesis.

Humbert, C. 2006. Etude épidémiologique de la peste porcine africaine dans la région de Marovoay (Madagascar): Etude de la filière porcine selon une approche participative et étude de prévalence. Toulouse, France, Ecole Nationale Vétérinaire de Toulouse. Thesis.

Joseph, H.M. & Melrose, W. 2010. Applicability of the filter paper technique for detection of antifilarial IgG_4 Antibodies using the Bm14 Filariasis CELISA. *Journal of Parasitology Research*, 2010. **https://doi.org/10.1155/2010/594687**

Michaud, V., Gil, P., Kwiatek, O., Prome, S., Dixon, L., Romero, L., Le Potier, M.-F. *et al.*, 2007. Long-term storage at tropical temperature of dried-blood filter papers for detection and genotyping of RNA and DNA viruses by direct PCR. *Journal of Virological Methods*, 146, 257–265. **https://doi.org/10.1016/j.jviromet.2007.07.006**

Randriamparany, T., Grenier, A., Tourette, I., Maharavo Rahantamalala, C.Y., Rousset, D. & Lancelot, R. 2005. 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 Médecine Vétérinaire des Pays Tropicaux*, 58, 15–20.

Randriamparany, T., Kouakou, K. V., Michaud, V., Fernández-Pinero, J., Gallardo, C., Le Potier, M.-F., Rabenarivahiny, R., Couacy-Hymann, E., Raherimandimby, M. & Albina, E. 2016. African swine fever diagnosis adapted to tropical conditions by the use of dried-blood filter papers. *Transboundary and Emerging Diseases,* 63, 379–388. **https://doi.org/10.1111/tbed.12295**

Rasamoelina-Andriamanivo, H., Porphyre, V. & Jambou, R. 2013. Control of cysticercosis in Madagascar: beware of the pitfalls. *Trends in Parasitology*, 29, 538–547. **https://doi.org/10.1016/j.pt.2013.09.002**

Uttenthal, Å., Braae, U.C., Ngowi, H.A., Rasmussen, T.B., Nielsen, J., & Johansen, M.V. 2013. ASFV in Tanzania: Asymptomatic pigs harbor virus of molecular similarity to Georgia 2007. *Veterinary microbiology*, 165, 173–176. **https://doi.org/10.1016/j.vetmic.2013.01.003**