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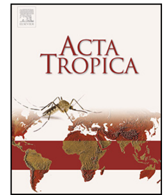

African and classical swine fever situation in Ivory-Coast and neighboring countries, 2008–2013

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ABSTRACT

This study was conducted from 2008 to 2013 to determine the animal health status of Ivory Coast and neighboring countries (Burkina Faso, Ghana, Togo and Benin) for African swine fever (ASF) and classical swine fever (CSF), and to assess the risk factors for ASF introduction in Ivory Coast. Ivory Coast had probably been free from ASF from 1998 to 2014 when it was re-introduced in this country. However, the ASF virus was found in all neighboring countries. In contrast, no evidence of CSF infection was found so far in Ivory Coast and neighboring countries. To assess the risk of ASF reintroduction in Ivory Coast, we surveyed 59 modern pig farms, and 169 pig owners in 19 villages and in two towns. For the village livestock, the major risk factor was the high frequency of pig exchanges with Burkinabe villages. In the commercial sector, many inadequate management practices were observed with respect to ASF. Their identification should enable farmers and other stakeholders to implement a training and prevention program to reduce the introduction risk of ASF in their farms.

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1. Introduction

In Ivory-Coast, the development of pig production is encouraged by the government. Ivorian pig stock is estimated at >360,000 heads (FAOSTAT, 2014), reared in smallholder and commercial farms. Smallholder farming system is found in most rural regions and in some urban areas. Free-ranging pigs represent 78% of the national stock. In contrast, farmers in the commercial system rear crossbreed or exotic pigs in pigsties where they are provided with specific feeding and medical cares (MIRAH, 2014).

West Africa is an enzootic area for African swine fever (ASF), a virus disease with serious socio-economic impacts on regional and international trade (Costard et al., 2009a,b). ASF virus (ASFV) is the unique member of the *Asfivirus* genus within the family *Asfarviridae*. It is highly contagious and lethal for domestic pigs. It induces acute, subacute or chronic clinical forms with high mortality rates. During chronic infections, most of the pigs die after one to three months, still being contagious during this period. ASFV transmission occurs through direct contact with an infected animal, ingestion of contaminated feed stuffs, and more generally contact with infected fomites, or bites of infected soft ticks of *Ornithodoros* genus (Penrith et al., 2004).

ASF must be differentiated from other diseases in particular classical swine fever (CSF) which shows similar clinical signs and lesions and can also generate huge socio-economic losses (Vandeputte and Chappuis, 1999; Edwards et al., 2000). CSF is caused by a virus (CSFV) belonging to the *Pestivirus* genus within the *Flaviviridae* family (Heinz et al., 2000 Wengler, 1991). It has never been reported in West Africa. In contrast, Ivory- Coast became

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infected by ASF during the large 1996 epizootic. It was followed by Benin, Cape Verde, Togo, and Nigeria in 1997, Senegal and Ghana in 1999 (El-Hicheri et al., 1998; Odemuyiwa et al., 2000) and Burkina Faso in 2003 (OIE, 2004). In Ivory-Coast, ASFV killed 135,000 pigs (29% of the pig population), mostly from the commercial farming system. The global cost of the epizootic was estimated at US$ 18 million in Ivory Coast (El-Hicheri et al., 1998) and US$ 6 million in Benin (FAO, 1997). In Togo, 62 ASF outbreaks were reported between 1997 and 1999 leading to the destruction of 17,000 pigs (direct losses and stamping out) (FAO, 1999; Edoukou, 2000; Kagnaya, 1999). In Ghana, 200,000 pigs were slaughtered in 1999.

Though ASF has not been reported in Ivory-Coast since 1998 and up to 2014 when it reemerged, the infection is still widespread in West Africa. Therefore, because of socio-economic exchanges with neighboring countries, Ivory-Coast is continuously exposed to the risk of ASFV reintroduction. Consequently, the purposes of this study were (i) to assess the ASFV and CSFV status of neighbor countries, (ii) to assess whether undetected circulation of either ASFV or CSFV occurred in Ivory-Coast, and (ii) to identify risk factors for ASF reintroduction in this country.

2. Material and methods

2.1. Sampling

Preliminary studies and national reports showed that Ivory-Coast is surrounded by ASF-infected countries at its eastern and north-eastern borders. The virus is present in Burkina Faso, Ghana. Togo and Benin, and human and commercial exchanges are intense with these countries. No ASF report was available from the other neighboring countries (Liberia, Guinea and Mali). Therefore, epidemiological surveys were designed to assess the risk of ASF introduction in Ivory-Coast, targeting the borders with Ghana (regions of Bouna, Bondoukou, Agnibilekrou, Abengourou, Aboisso) and Burkina Faso (Bouna), as well as the District of Abidjan (Lagune region) harboring the main seaport and airport and Agneby region in the south (close to Lagune region) near Abidjan which the breeder depend to this city. Biological samples were taken and questionnaire surveys were implemented to assess the risk of ASF introduction (see below). In each region, at least five villages were randomly selected from a known list of villages. Within a selected village, at least five pig owners were selected and at least 30 free-ranging pigs were bled from the selected pig owners. This pig sample size was chosen to ensure a 95% probability to detect at least one positive pig if the seroprevalence rate was at least 10%. This threshold was chosen according to seroprevalence rates reported in southern Senegal, with similar pig farming systems and trans-boundary exchanges with infected countries (Etter et al., 2011). In addition, samples were collected in the main pig slaughterhouse to assess the situation in commercial farms. At each visit, five owners were selected representing the average of the number of farmers received per day for the slaughter of their animals and minimum five of their pigs were randomly sampled for blood and organs (lung, kidney and spleen). The owner sample size was adjusted to represent 10% of the commercial production system.

Collaboration was established with national veterinary services and diagnostic laboratories of neighboring countries reporting ASF outbreaks. Samples taken in suspected ASF outbreaks from Burkina Faso, Ghana, Togo and Benin were sent to the Lanada/Virology Laboratory. When blood was collected for serum preparation, two drops were used to soak a Whatman 3MM filter paper (VWR, Fontenay-Sous-Bois, France). Filter papers (FPs) were then dried and stored at −80 °C until use.

2.2. Laboratory analysis

Antibody detection in sera was performed using a commercial competition ELISA: Ingezim PPA COMPAC 11.PPA.K.3, (Ingenasa, Spain) and IDEXX CHEKITR CSF-SERO CST1131T (IDEXX, France) for ASF and CSF according to manufacturer' instructions.

Organ samples and dried FPs were screened for ASF and CSF virus genomes. Twenty mg of tissue sample were distributed in each well of a 96 well deep square plate and crushed in 600 μL of PBS 1X by two 3 mm diameter steel beads in a Tissue Lyser II (Quiagen) set at 30 Hz during 3 min. Homogenates were clarified 10 min at 5600 g and nucleic acids extraction was then performed on 100 μL of the supernatant using an automated extraction platform (BIOMEK FXP, Beckman Coulters) and the NucleoSpinR 96 RNA Tissue Core Kit (MACHEREY-NAGEL GmbH & Co. KG, Germany) according to the manufacturer's instructions.

Molecular detection of ASFV DNA was performed by a real-time PCR targeting the B646L gene encoding for the capsid protein VP72 (King et al., 2003). Briefly, 2 μL of extracted nucleic acids were added to a reaction mix containing 0.4 μM of each primer [King-s (5′-C T G C T C A T G G T A T C A A T C T T A T C G A-3′) and King-r (5′-G A T A C C A C A A G A T C R G C C G T-3′)], 0.25 μM of a TaqMan probe (5′-[6-carboxy-fluorescein (FAM)]-C C A C G G G A G G A A T A C C A A C C C A G T G-3′-[6-carboxy-tetramethyl-rhodamine (TAMRA)]) using QuantiFast Probe polymerase chain reaction (PCR) kit (Qiagen, Netherland). Following a denaturation of 5 min at 95 °C, DNA amplification consisted in 45 cycles of 10 s at 95 °C and 30 s at 58 °C.

Positive samples were then submitted to a conventional PCR for subsequent sequencing. Briefly, a 478-bp fragment of B646L gene was amplified by using the primers p72-U (5′-G G C A C A A G T T C G G A C A T G T-3′) and p72-D (5′-G T A C T G T A A C G C A G C A C A G-3′) (Bastos et al., 2003) and 2.5 units of Pfu polymerase (Promega, Madison, USA).

Dried FPs were only tested for ASFV DNA according to Randriamparany et al. (2016) using Universal Probe Library (UPL) real-time PCR developed by Fernandez-Pinero et al. (2013). Briefly, a piece of 1.25 mm^2 of dried FPs was placed directly into 18 μL of ultra-pure water and heat up at 95 °C for 10 min. Then 22 μL of a reaction mix containing 0.4 μM of each primer (ASF-VP72-R 5′-C C C A G G R G A T A A A A T G A C T G-3′ and ASF-VP72-F 5′-C A C T R G T T C C C T C C A C C G A-3′), 0.1 μM of UPL # 162 probe (5′-6FAM-GGCCAGGA-dark quencher-3′) and 20 μL of Light Cycler 480 Probes Master mix 2X (Applied Biosystem) were added. DNA amplification and detection were then performed after 5 min at 95 °C and 45 cycles of 10 s at 95 °C and 30 s at 60 °C.

Molecular detection of CSF viral RNA was performed by real-time RT-PCR using ADIAVET CSF REALTIME one-step Kit as prescribed by the manufacturer (AES Luminex, France, Le Dimna et al., 2008).

PCR products from five, two and four samples from Burkina Faso, Togo and Ghana, were sequenced for virus genotyping. The sequences were deposited in Genbank under the accession numbers KT368176 for Togo6, KT368177 for Ghana4 and KT368178 for Burkina-Faso5. Phylogenetic analyses were conducted on a set of 70 isolates including the 22 ASFV genotypes, except the 23rd genotype recently described (Achenbach et al., 2016). Multiple sequence alignments were achieved using Mega version 6 (Tamura et al., 2013). Best fit evolutionary model was determined using TREEFINDER version March 2011 (Jobb et al., 2004) according to the corrected Akaike information criterion (AICc) and Bayesian information criterion (BIC). The tree was constructed under HKY + $\Gamma 5$ by Bayesian inference using Monte Carlo Markov Chain (MCMC) implemented in MrBayes software v. 3.2 (Ronquist et al., 2012). MCMC was run for 10 million trees with 1/1000 tree sampled. The consensus tree was generated after having discarded the first 25%

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