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Evaluation of a Commercial Field Test to Detect African Swine Fever

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ABSTRACT: African swine fever (ASF) is one of the most important and complex infectious diseases affecting pigs (*Sus scrofa*). The disease has been present in Sardinia, Italy, since 1978. Factors influencing the presence of the disease on the island are the presence of illegally bred pigs, uncontrolled movements of animals, and local traditions. Implementation of public health programs is essential for controlling ASF. The use of new diagnostic techniques on both wild boar (WB) and illegally bred pigs would provide tools for faster and more inexpensive control of the disease. We evaluated a commercial serological test kit (Pen-side [PS]) for use in the field. We sampled 113 hunter-harvested WB during the 2014–15 season, collecting blood and lung samples to conduct serological analyses and to screen for the ASF virus. Although the sensitivity (81.8%) and specificity (95.9%) of tests performed in the field were reduced compared to the same test in laboratory, they nevertheless allowed for rapid diagnosis and reduced unnecessary carcass destruction. The test, conducted in the field, was less expensive than in the laboratory and required less manpower. Therefore, we conclude that the combined use of antibody PS test and antigen PS test may be a valuable emergency management method during an outbreak as well as a useful tool for conducting regular monitoring activities as a preventive policy.

Key words: African Swine Fever, antibody, control strategy, Pen-side, risk, wild boar.

African swine fever (ASF) is a complex infectious disease affecting swine species. This disease is caused by a large double-stranded DNA virus, the African swine fever virus (ASFV), which is the only member of the *Asfarviridae* family (Dixon 2005). The ASFV also replicates in *Ornithodoros* soft ticks, which then act as a virus reservoir. Vaccine development is hampered by the absence of neutralizing antibodies, the genetic variability of ASFV, and the lack of knowledge of the pathogenesis of ASF. There is currently no vaccine available to prevent the disease and control its spread (Sánchez-Vizcaino et al. 2014). The disease has been present in Sardinia, Italy, since 1978 (Fig. 1). The presence of asymptomatic carrier pigs in the wild, contact between livestock and wild boar (WB), lack of biosecurity, and poor sanitary measures of pig breeding are major risk factors that leads to the persistence of ASF in many areas of the island. The high number of illegally bred pigs increases the risk of ASF. Free-range grazing on public portions of territories rich in acorns and chestnuts is used to reduce the costs of feeding pigs. This activity is a pivotal factor for the persistence of ASF in Sardinia (Mur et al. 2014). From 2011 to 2014, there was an increase of ASF incidence, which spread swiftly into territories outside the endemic area. In February 2014, in accordance with the European Commission, the Sardinian Region Authority developed a new Plan of Eradication of ASF 2015-18 (PE-ASF15-18; Regional Decree Number 50/17, 16 December 2014). In order to speed up the diagnosis of ASF in hunted WB, we evaluated the use of the one-step immunochromatographic Pen-side (PS) rapid test (INgezim PPA CROM, Ingenasa, Madrid, Spain), capable of quickly detecting anti-ASFV antibodies in WB blood samples during field operations. Our aim was to assess the practicality and cost efficiency of ASF antibody screening, particularly in the high ASF risk zones, where WB live closely with illegal pigs. In Sardinia, pigs are defined as "illegal" when they are not identified and registered, or if they are bred free-ranging, according to the PE-ASF15-18 and National Legislative De-

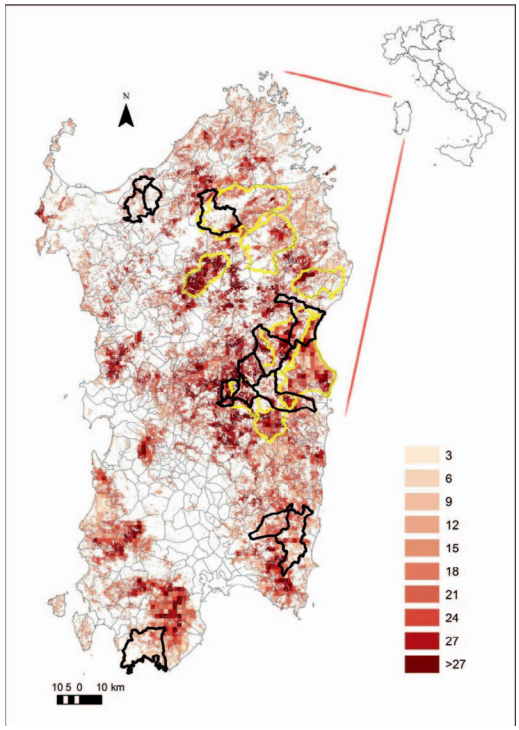

FIGURE 1. Wild boar (WB) density on the island of Sardinia, Italy. The numbers of animals are shown in different shades of red. The historical area of infection based on WB disease prevalence is shown in yellow outline, and black outlines the area from which WB were hunted and samples collected for detection of African swine fever.

cree Number 200 (2008/71/CE) on pig registration and identification. As demonstrated by Perez et al. (2011) for the experimental detection of ASFV-specific antibody, the PS test met the sensitivity and specificity parameters (sensitivity: 99%; specificity: 100%) set for serological assays by the World Organization for Animal Health (OIE 2016).

The hunting season span was set between 1 November and 31 January in accordance with the plan for eradication (PE-ASF15-18). Serology and viral testing was conducted for detecting ASFV in WB populations all over the island. For the last 6 yr, an annual average number of 8,225 (range: 3,137–11,386) animals were examined. Five veterinarians were trained for collecting samples and conducting the PS test. Four age categories of WB were considered in the study: 6 mo, 7–18 mo, 19–30 mo, and >30 mo (Table 1). The distribution of WB in Sardinia is vastly widespread, with a density of 3–27 animals/km^2 (Fig. 1). Data about the hunt area, sex, age, and health parameters were collected for each animal. This allowed us to connect each WB to its hunting area therefore reducing, in the data analysis, the probability of attributing wrong geographical data resulting from WB captured in different locations other than their usual territory. For this study, we used samples of spleen, blood, and lungs (3 cm^3) collected from all WB killed during the 2014–15 hunting season. Serum samples were frozen at –20° C and organs at –80° C until laboratory tests were conducted. The blood samples were tested for ASFV antibodies using INgezim, in accordance with the manufacturer's instructions. For better comparison with other virus detection procedures, sera samples were collected by the same WB. Since not all sera were sufficient in volume and some were in deteriorated condition, some of them were examined for the presence of antibodies using the enzyme-linked immunosorbent assay (ELISA) test INgezim PPA Compac (Ingenasa), while an Immunoperoxidase Monolayer Assay (IPMA) on fluid expressed from lung samples was done on the remaining samples. The IPMA has been optimized and is an alternative to the most common test used to detect antibodies against ASFV (Arias et al. 1993). The IPMA proved to be more sensitive than the ELISA test for detecting antibodies in earlier stages of infection (Pastor et al. 1990). For all these reasons we considered it equally reliable to compare the ELISA or IPMA to the PS test. Virus detection was carried out on lung and spleen samples of WB using the real-time PCR method (King et al. 2003) described in the OIE manual (OIE 2016).

This study had a planned sample size of 110 animals in order to detect, with a power of 80% and α error=0.05, a prevalence of 11% in ASF virus presence, starting from a baseline of 2.7%, based on historical data observed in our region over the previous years (2013–14). Considering a 10% drop-out, a total of 122 animals was sampled at a postmortem exam-

TABLE 1. Sex and age of hunter-killed wild boar (*Sus scrofa*) in Sardinia, Italy in 2014–15 from which blood samples were collected for field testing for African swine fever virus antibodies in a comparison of a commercial test (Pen-side [PS]) suitable for use in the field and the gold standard tests (enzyme-linked immunosorbent assay [ELISA] or immunoperoxidase monolayer assay [IPMA]) used for detection of antibodies. A total of 122 animals were sampled, but nine and 12 animals were not tested by the PS test and ELISA or IPMA, respectively. The total numbers of tested samples were 113 and 110, for the PS test and the ELISA or IPMA, respectively.

	PS, no. (%)	ELISA or IPMA, no. (%)
Sex		
Male	54 (48)	53 (48)
Female	59 (52)	57 (52)
Age (months)		
≥6	9 (8)	9 (8)
7–18	29 (26)	27 (25)
19–30	35 (31)	34 (31)
>30	40 (35)	40 (36)
Results		
Positive	11 (10)	12 (11)
Negative	102 (90)	98 (89)

TABLE 2. The confounding variable table based on the Pen-side (PS) test and compared to the enzyme-linked immunosorbent assay (ELISA) or immunoperoxidase monolayer assay (IPMA) tests for African swine fever to obtain an indication of the occurrence of false positives and false negatives, used to fit the receiver operator characteristics curve. The total numbers of tested samples were 110; 99 gave a negative result with the PS test. In comparison, the ELISA or IPMA test showed 96% (95/99) true negative and 4% (4/99) false negative. Of the 11 samples that tested positive with the PS test, 9 (82%) of them were true positives and two (18%) were false positives.

	Infected wild boar (ELISA or IPMA test), no. (%)	Noninfected wild boar (ELISA or IPMA test), no. (%)	Total
PS negative	4 (4)	95 (96)	99
PS positive	9 (82)	2 (18)	11
Total	13	97	110

ination, with an average time delay of 3 h (range: 1–5 h). Nine animals were excluded from sample collection because the average postmortem time exceeded the criterium (>5 h). On the basis of geolocations obtained by hunters, we established that 52 animals came from low-risk areas and 70 from high-risk areas (Fig. 1). A PS test was conducted on 113 WB blood samples; 38 of the 113 sera collected were examined using IPMA test, and the other 75 were examined for the presence of antibodies using the ELISA test. To compare the PS test with the serological test currently used (ELISA or IPMA), we analyzed the empirical (nonparametric) receiver operating characteristic (ROC) curve, to evaluate the specificity and sensitivity of the PS (Grzybowski et al. 1997). The area under the curve is widely recognized as the measure of a diagnostic test's discriminatory power and is computed using the trapezoidal rule, and a Wald test was used to compare the curves (Fan et al. 2006). In order to assess possible confounding variables, such as locations where animals were hunted and possible human error committed by technicians while carrying out evaluation tasks, a univariate logistic regression model was performed. All tests were two-sided, with $P<0.05$ considered significant. The analyses were performed using the STATA 13.1 software (Stata Statistical Software: Release 13, StataCorp. 2013, College Station, Texas, USA).

The WB we tested with the PS test and the ELISA were evenly distributed in terms of sex and age classes (Table 1), with a similar number of males and females and a prevalence of older animals (36%). Age was determined via tooth analysis of each animal and is an essential factor for determination of the original infection time. We carried out PS tests on WB captured in the infected areas as well as in other territories. The confounding matrix (Table 2) based on the PS test and the ELISA or IPMA give a preliminary indication on false positives and false negatives, used to fit the ROC curve. Based on ROC analysis (area under the curve=0.83, SE=0.067, 95% confidence interval=0.70–0.96; P=0.014; Fig. 2), the PS test was moderately accurate

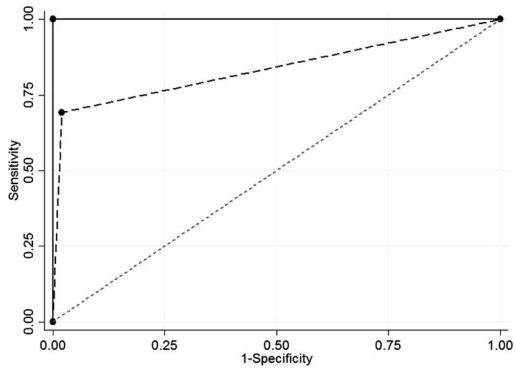

FIGURE 2. Empirical (nonparametric) receiver operating characteristic curve to evaluate the specificity and sensitivity of the Pen-side test compared with enzyme-linked immunosorbent assay (ELISA) or immunoperoxidase monolayer assay (IPMA) for detection of African swine fever in wild boar in Sardinia, Italy. The dashed black line represents the Pen-side test curve; the black solid line the ELISA-IPMA test curve; the gray dashed line the reference of no power.

(sensitivity=81.8%; specificity=95.9%; positive predictive value=69.3%; negative predictive value=97.9%; Swets 1988). The hypothesis of a possible confounding role of the operator was rejected since there were no significant differences in the probability of obtaining a positive or negative PS test based on the technician's performance (relative risk=1.33; P=0.99); likewise, there was no significant difference between capture locations (relative risk=1.27; P=0.599).

The results between PS test and the ELISA or IPMA were interesting, considering the different conditions in which they were performed. The PS test demonstrated relatively high efficiency in terms of sensitivity and specificity, compared to the ELISA or IPMA. The working relationships with hunting parties and their degrees of collaboration were not consistent across sample collection areas and throughout the hunting season. Several factors, including the hunters' habit of eating the hunted animal's entrails, the time and place of the hunt, and adverse weather conditions, did not allow for complete sample collection. When a collection was not possible at the hunt site, the carcass was transported to nearby authorized locations. Who collected samples and performed the test, or where the boar was hunted, did not affect our results. A great advantage to the PS test is that it can be performed directly in the field. This was further supported because our PS test results compared favorably with two different serological tests (ELISA or IPMA). Recent developments in ASF diagnostic tests have improved and facilitated the likelihood of ASF early detection, which is an essential factor that enables a better control of the disease. The rapidity in obtaining results by utilizing the PS test reduces the costs and time required for standard testing, as was previously demonstrated (Cappai et al. 2016). The PS test can also be used for the detection of ASF in new areas such as hunting reserves that have not been previously tested. Indeed, ASFV antibodies can be detected in oral fluids postinfection, meaning that oral fluids can be used when target organs are not collected (Mur et al. 2013). In the future, the combination use of the antibody PS test and the antigen PS tests could be a useful tool during outbreaks as well as during normal surveillance.

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