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# Effect of husbandry methods on seropositivity to African swine fever virus in Sardinian swine herds

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## Abstract

Multiple logistic regression was used on serological data collected in the context of the Sardinian African swine fever (ASF) eradication program from pig farms in the province of Nuoro, Sardinia. The monthly percentage of ASFV-positive herds decreased significantly from October 1994 through March 1996 ( $P < 0.001$ ). The farm-level risk of seropositivity to African swine fever virus (ASFV) was higher in free-range farms than in partial-confinement farms (odds ratios (OR) varied between 4.9 in October 1994, and 5.7 in March 1996,  $P < 0.001$ ). The risk of infection for total-confinement farms was one-fifth of the risk for partial-confinement farms in October 1994 (OR = 0.2,  $P < 0.001$ ), whereas in March 1996, the estimated OR was 0.57 and not significant (upper confidence limit = 1.1). The maintenance of ASFV in Sardinia was primarily associated with free-range pig farms. The natural logarithm of the number of pigs tested per visit in a farm was positively associated with the risk of herd seropositivity (OR = 2.6,  $P < 0.001$ ).  
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*Keywords:* Pig-microbiological disease; African swine fever; Logistic regression; Italy

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## 1. Introduction

African swine fever (ASF) is one of the most severe diseases of pigs. Acute ASF is characterized by hemorrhage and high mortality (Sanchez-Vizcaino, 1992). Subclinical infections may also occur, depending upon host factors and the virulence of the etiological agent (Mebus, 1988). The unique and complex DNA virus that causes ASF (ASFV) is maintained, in sub-Saharan Africa, in a transmission cycle between argasid

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ticks (*Ornithodoros* sp.), and warthogs (*Phacochoerus aethiopicus*) and bushpigs (*Potamochoerus porcus*), with no disease consequences for the vertebrate hosts (Wilkinson, 1989).

The first report of ASF outside the African continent came from Portugal in 1957. In the following decades, ASF epidemics occurred in several European and American countries (Sanchez-Vizcaino, 1992). In the absence of a vaccine, and because of the catastrophic impact of ASF on the pig industry, control of ASF epidemics has been achieved by slaughtering susceptible pigs; the entire swine population of Malta (80 000 pigs) was sacrificed because of an outbreak of ASF in 1978 (Wilkinson et al., 1981).

ASF was eradicated from the Iberian Peninsula in 1995, and Sardinia is the only non-African region where the infection is still present (Laddomada, 1996). The disease appeared in southern Sardinia in 1978, allegedly introduced from the Iberian Peninsula via garbage containing raw pork, which was subsequently fed to pigs (Contini et al., 1982). The infection was successfully eradicated from the southern part of the island, but an endemic area persists in the province of Nuoro, in eastern-central Sardinia (Laddomada et al., 1991). In this mountainous area ( $\approx 1/4$  of the island), traditional pig husbandry practices (such as grazing free-range herds on publicly-owned land) is considered a major obstacle to disease control.

*Ornithodoros* sp. ticks were an important factor in the persistence of ASF in Spain (Perez-Sanchez et al., 1994), but have not been found in Sardinia (Ruiu et al., 1989). The wild boar (*Sus scrofa ferus*) is susceptible to ASF, and its role as a reservoir of ASFV needs further study. In Sardinia, cases of ASF in this species were consistently attributed to primary infections in domestic pigs (Firinù and Scarano, 1988). Therefore, the maintenance of ASF in Sardinia is probably based on the transmission of ASFV among domestic pigs.

In addition to acutely-ill animals, asymptomatic or recovered pigs are potential sources of ASFV (Wilkinson, 1984). The serological identification of these 'permanently infected virus carriers' is critical to the eradication of the disease (Sanchez-Vizcaino, 1992; Bech-Nielsen et al., 1995). To evaluate the time trend of ASFV in the endemic area of Nuoro, and the role of three traditional pig husbandry systems in the persistence of the infection, we carried out epidemiological analyses of serological data collected from October 1994 through March 1996.

## 2. Materials and methods

### 2.1. The Sardinian ASF eradication program

In accordance with the guidelines of the Sardinian ASF eradication program, funded by the European Union (EU Official Bulletin No. L 116, 1990), serological exams were carried out on pigs older than 3 months from registered farms<sup>1</sup> in Health Districts No. 7, 8, 9, 10, and 11, of Nuoro. Serum samples obtained by veterinarians were analyzed for ASFV antibodies at the Istituto Zooprofilattico Sperimentale of Sassari, using a

<sup>1</sup> All pig farms were registered in a list at the Health District's Veterinary Office.

previously described enzyme-linked immunosorbent assay (ELISA) (Pastor et al., 1990). Confirmatory western blotting (WB) was carried out on samples that tested positive by ELISA (Escribano et al., 1990). Seropositive pigs were to be destroyed and, on farms where seropositive pigs were found, serological exams on all remaining animals were to be repeated after one month.

The ASF eradication program was monitored through a computerized database, consisting of farm type, number of pigs present (NPRES), number of pigs serologically tested (NTEST), and number of pigs that tested positive (NPOS).

## 2.2. Epidemiological analyses

Pig farms were divided into three types: (a) free-range farms, with pigs grazing on vast publicly owned areas during the entire year; (b) partial confinement farms, where animals are grazing on public areas during the fall (to utilize acorns produced by evergreen oaks) and kept indoors for the rest of the year; (c) total confinement farms, with a small number of animals reared for family consumption and kept indoors permanently (Contini et al., 1982).

For each farm visit, within-farm seroprevalence (NPOS/NTEST) was calculated, and the herd was classified as ASFV-positive if seroprevalence  $> 0$ . The percentage of ASFV + farms [(No. of ASFV + farms/visited farms)  $\times 100$ ] was calculated for each farm type. The median and the first and third quartiles ( $Q_1$ ,  $Q_3$ ) of within-farm ASFV seroprevalence were calculated for ASFV + farms, and differences in seroprevalence among farm types were tested by Kruskal-Wallis nonparametric ANOVA (Zar, 1984).

The secular trend of the percentage of ASFV + farms was analyzed by 12-month moving average (Smith, 1995), and by simple linear regression of monthly (actual) data using MONTH as the independent variable (two-tailed significance level  $\alpha = 0.05$ ) (SAS, 1990).

Multiple logistic regression (unconditional maximum likelihood method; SAS, 1990) was used to evaluate the adjusted effects of farm type,  $\ln(\text{NTEST})$  (the natural logarithm of NTEST; Willeberg et al., 1994), and MONTH on farm seropositivity. Two design ('dummy') variables were created to compare farm types, using partial-confinement farms as the reference group. The likelihood ratio test was used to assess the overall significance of the model (two-tailed significance level  $\alpha = 0.05$ ) and of interactions between the variables coding for the comparisons among farm types and the continuous independent variables ( $\ln(\text{NTEST})$ , and MONTH) (two-tailed significance level  $\alpha = 0.1$ ) (Hosmer and Lemeshow, 1989). The significance of each term retained in the final model was tested by Wald's  $\chi^2$ . Estimated odds ratios (OR) for the comparison between farm types at different levels of the interacting continuous variable, were obtained (Hosmer and Lemeshow, 1989).

## 3. Results

Serological tests were carried out on 97 283 pigs, from 5744 traditional farms (out of 6409 registered) in the ASFV endemic area in Nuoro, from October 1994 through March

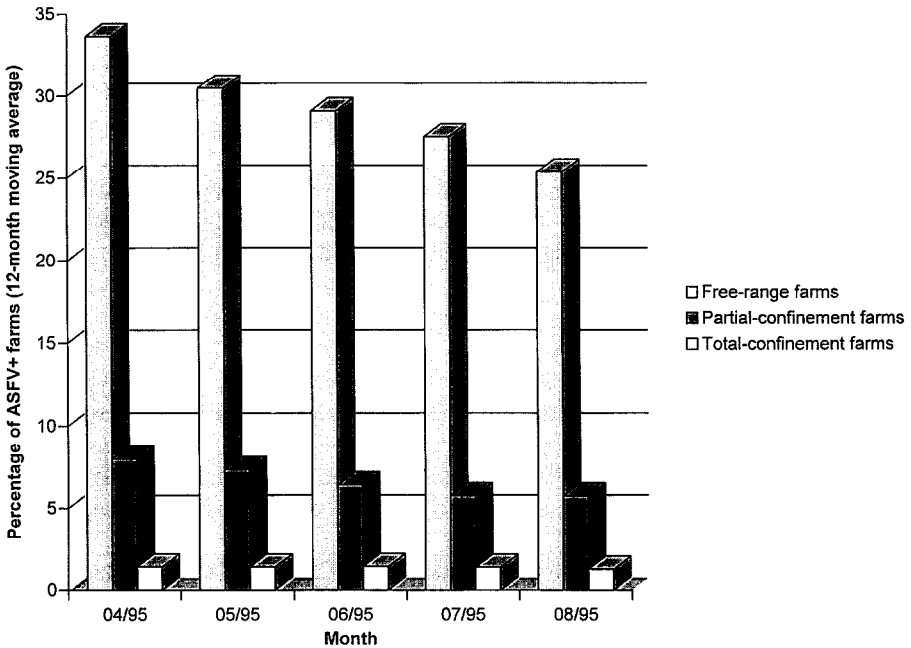


Fig. 1. Time trend of the percentage of seropositivity to ASFV among three types of traditional swine farms, in the endemic area of the province of Nuoro, from October 1994 through March 1996.

1996. In traditional farms, 9.84% of pigs that were examined tested positive for ASFV<sup>2</sup>.

The 12-month moving average of the percentage of ASFV + farms is shown in Fig. 1. There was a significant negative time trend in the percentage of ASFV + farms ( $\hat{y} = 5.7 - 0.22 \cdot \text{MONTH}$ ,  $R^2 = 0.43$ ,  $P < 0.01$ ). The within-farm seroprevalence differed significantly among farm types (Kruskal-Wallis  $H = 12.2$ , 2 d.f.,  $P < 0.005$ , Table 1).

The likelihood ratio test for the logistic regression model was significant (likelihood ratio  $\chi^2 = 1015.3$ , d.f. = 6,  $P < 0.001$ ; Table 2). The interaction between farm type and MONTH (likelihood ratio  $\chi^2 = 4.79$ , d.f. = 2,  $P < 0.10$ ) was included in the final model. In fact, the decrease in the risk of infection over time was greater for partial-confinement than for total-confinement farms (Wald's  $\chi^2 = 3.81$ ,  $P < 0.06$ ), while the risk for free-range and partial-confinement farms had similar time trends (Wald's  $\chi^2 = 0.091$ ,  $P > 0.1$ ). Estimated OR's for the comparisons among farm types (controlling for MONTH) are shown in Table 3.

<sup>2</sup> No seropositive pig was found, out of the 7497 that were tested, in 181 visits in intensive nontraditional farms. Data relative to this husbandry system were not included in our analyses.

Table 1

Results of serological analyses for African swine fever virus in the endemic area of the province of Nuoro, Sardinia, from October 1994 through March 1996

| Farm type           | No. of farm visits | Median NTEST (Q <sub>1</sub> –Q <sub>3</sub> ) | % ASFV + farms | Median % seroprevalence in ASFV + farms (Q <sub>1</sub> –Q <sub>3</sub> ) |
|---------------------|--------------------|--|----------------|---|
| Free-range          | 703                | 9 (4–17)                                       | 29.45          | 38.10 (14.29–80.00)   |
| Partial-confinement | 1318               | 8 (4–12)                                       | 7.13           | 16.67 (9.09–50.00)  |
| Total-confinement   | 8339               | 3 (2–6)  | 1.21           | 33.33 (16.13–66.67)   |
| Overall             | 10360              | 4 (2–7)  | 3.88           | 31.00 (12.50–66.67)   |

NTEST = number of pigs that were tested in a visit in a farm.

Q<sub>1</sub> = first quartile.

Q<sub>3</sub> = third quartile.

Table 2

Logistic regression of the effects of farm type, MONTH of sampling, and natural logarithm of the number of tested pigs per visit, ln(NTEST), on the seropositivity to ASFV

| Variable test                                 | $\beta$ | OR   | Wald's $\chi^2$ | P       |
|---|---------|------|-----------------|---------|
| Free-range vs. partial-confinement (1)        | 1.58    | –    | 35.60           | < 0.001 |
| Total-confinement vs. partial-confinement (2) | –1.61   | –    | 33.72           | < 0.001 |
| MONTH   | –0.101  | –    | 18.13           | < 0.001 |
| ln(NTEST)                                     | 0.973   | 2.65 | 177.49          | < 0.001 |
| Interaction 1 $\times$ MONTH                  | –0.009  | –    | 0.09            | > 0.1   |
| Interaction 2 $\times$ MONTH                  | 0.061   | –    | 3.81            | < 0.06  |

Swine herds in the endemic area of the province of Nuoro, from October 1994 through March 1996.

Table 3

Estimated odds ratios (OR) and 95% confidence interval for the risk of ASFV in free-range farms and in total-confinement farms (compared to partial-confinement farms), controlling for MONTH

| MONTH   |             | 10/94            | 03/95            | 09/95            | 03/96           |
|---|-------------|------------------|------------------|------------------|-----------------|
| Free-range farms compared to partial-confinement farms        | OR (95% CI) | 4.9 (2.9–8.2)    | 5.1 (3.7–7.0)    | 5.4 (3.8–7.7)    | 5.7 (3.0–11.0)  |
| Total-confinement farms compared to partial-confinement farms | OR (95% CI) | 0.20 (0.12–0.35) | 0.27 (0.19–0.38) | 0.39 (0.27–0.58) | 0.57 (0.29–1.1) |

Endemic area of the province of Nuoro, Sardinia, October 1994–March 1996.

The interaction between farm type and ln(NTEST) did not significantly improve the model (likelihood ratio  $\chi^2 = 2.17$ , d.f. = 2,  $P > 0.10$ ), and was not included in the final regression.

#### 4. Discussion

The results of our analyses supported the hypothesis that the maintenance of ASFV in the endemic area of Nuoro was primarily associated with free-range pig farms (Tables 1

and 3). The percentage of ASFV + farms and the seroprevalence of ASFV that we observed in such herds, suggested an intense viral circulation among pigs. In free-range farms, the application of disease-control measures is particularly difficult, and animals belonging to different herds can share the same grazing areas (facilitating viral transmission) (Wilkinson, 1984; Bech-Nielsen et al., 1995).

Other epidemiological studies on viral diseases of pigs have shown that risk of infection in commercial, 'industrial' farms is associated with high confinement level and animal density, which create physiological stress and facilitate viral transmission among pigs through secretions and aerosol (Weigel et al., 1992; Austin et al., 1993). Conversely, in our study area, total-confinement farms only have small numbers of animals reared for family consumption. Raw pork in garbage fed to pigs is the most likely source of infection for totally confined pigs in Nuoro.

The percentage of ASFV + farms decreased significantly since 1994 (Fig. 1). However, the interaction between farm type and MONTH showed that disease control was less efficacious in total-confinement than in the other farm types. In fact, the estimated risk of infection for total-confinement farms was only one-fifth of the risk for partial confinement farms in October 1994 (OR = 0.20, Table 3), whereas in March 1996, the estimated OR for the comparison between these two husbandry systems was 0.57, and not statistically significant (upper confidence limit = 1.1).

The natural logarithm of the number of tested pigs per visit in a farm was associated with an increased ASFV seropositivity (Table 2). However, a logistic regression coefficient for  $\ln(\text{NTEST})$   $\beta \cong 1$  suggested that the risk of infection for a single animal was not affected by herd size (Willeberg et al., 1994; Flori et al., 1995).

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