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## Temporal and spatial patterns of African swine fever in Sardinia

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### Abstract

Temporal patterns and spatial distribution of African swine fever (ASF) were studied through the analysis of routinely collected data in the ASF-endemic area of the Province of Nuoro, Sardinia. During 1993–1996, ASF outbreaks were reported from 45 out of the 82 municipalities of the study area. Overall farm-level incidence rate (IR) was 1.3 outbreaks per 100 farms-year. ASF peaked in 1995 (IR=1.8) and declined in 1996 (IR=0.82). Significant ( $P<0.05$ ) spring peaks of ASF outbreaks and affected municipalities were detected using statistical methods for circular distributions. Spatial clustering of ASF-affected municipalities, as evaluated by join-count statistics, was significant in 1993 ( $Z_{jc}=-3.0$ ,  $P<0.01$ ) and 1994 ( $Z_{jc}=-3.2$ ,  $P<0.01$ ) but not in 1995 ( $Z_{jc}=-0.6$ ,  $P=0.55$ ) and 1996 ( $Z_{jc}=-1.2$ ,  $P=0.23$ ). Extensive pig farming and ASF were spatially co-distributed ( $\kappa=0.51$ , 95% CI=0.33–0.70). © 1998 Elsevier Science B.V.

*Keywords:* Pig-microbiological diseases; African swine fever virus; Spatial analysis; Circular data analysis

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### 1. Introduction

African swine fever (ASF) – a disease of domestic pigs and wild boars (*Sus scrofa ferus*) caused by a unique and complex DNA virus (ASFV) – has a catastrophic impact on pig industry due to drastic disease control measures and limitations to the commerce of pigs and pig products (Sanchez-Vizcaino, 1992).

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Outside the African continent— where ASFV is perpetuated in a clinically silent cycle involving *Ornithodoros* ticks and wild pigs (warthogs and bushpigs) (Plowright et al., 1969; Wilkinson, 1989) – an ASF-endemic area persists in the Province of Nuoro, Sardinia (Contini et al., 1982; Laddomada et al., 1991; Laddomada, 1996).

In Nuoro, pigs are farmed according to traditional practices, such as grazing free-range herds on vast communal lands to utilize acorns produced by evergreen oaks. In the absence of *Ornithodoros* ticks, free-ranging pigs are considered the primary reservoirs of ASFV in Sardinia – whereas wild boars probably play a secondary role (Firinu and Scarano, 1988; Ruiu et al., 1989; Laddomada et al., 1994a). The rough topography of grazing areas – where pigs may join different herds, the uncontrolled introduction of pigs from unknown sources, and the feeding to pigs of waste food containing pork were considered major factors in the persistence of ASF in Nuoro (Contini et al., 1982; Wilkinson, 1984).

In a study on sociological factors that may present obstacles to disease control in the traditional Sardinian husbandry system, Firinu et al., 1988 found the pig farming was mainly a secondary activity practiced by dairy-sheep farmers, and reliable information on the actual numbers of pigs and pig herds was lacking. It was also concluded that extensive pig husbandry was deeply rooted in the region's culture, and changes towards intensive nontraditional farming practices were unlikely.

In 1993, a European Union-funded ASF eradication program (EU Official Bulletin no. L 116, 1990) was adopted. According to the guidelines of the program, all pigs in ASF-affected farms and pigs that tested positive to ASFV by serological tests were slaughtered and prompt refunds made to farmers (Patta et al., 1994). Starting in 1996, pigs from unaffected farms that were suspected of infection (i.e. free-ranging pigs sharing pastures with ASF-affected herds) were also sacrificed.

In this study, we applied analytical techniques to temporal and spatial patterns of ASF occurrence in the endemic area of Nuoro. Through the analysis of ASF time trend and seasonality, we evaluated the impact of the EU-funded eradication plan and generated hypotheses on risk factors. Using spatial statistics, we tested clustering of ASF and codistribution of extensive pig farming and disease to target control efforts and further research on spatial determinants of ASFV transmission.

## 2. Materials and methods

Clinical ASF in pigs and wild boars was confirmed by direct immunofluorescence (DIF) and polymerase chain reaction (PCR) (Sanchez-Vizcaino, 1992; Caccia et al., 1992; Laddomada et al., 1994b) carried out on kidney, spleen, and lymph nodes, at the Istituto Zooprofilattico Sperimentale (IZS) of Sassari and Nuoro.

For epidemiological analyses, we used records (collected at the IZS) on monthly occurrence of outbreaks from 1993 through 1996, in 82 municipalities of Health District nos. 7–11 of the province of Nuoro. Outbreaks were defined as pig farms where ASF was confirmed. No information on affected herd size and management type, and on numbers of affected pigs during each outbreak was available for this study.

Pigs farms that were registered at the Health Districts' Veterinary Offices (during a census carried out in 1996) were used as the population at risk. 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 small numbers of animals reared for family consumption and kept permanently indoors.

Reliable information on herd and pig population size was not available for the years 1993–1995. Nevertheless, wide year-to-year fluctuations in the traditional pig husbandry system were unlikely (A. Firinu, IZS Nuoro, personal communication), and the 1996 data were used as the population at risk for all years.

Approximations of ASF incidence rates (IR=outbreaks per 100 farm-year) in 1993–1994 (pooled data) vs. 1995, and in 1995 vs. 1996 were compared by  $\chi^2$  (significance level  $\alpha=0.05$ ) and relative risk (RR) estimates (Kelsey et al., 1996).

The seasonal pattern of the disease was evaluated by inspection of the 3-month moving average of the reported numbers of outbreaks and ASF-affected municipalities (Smith, 1995). Subsequently, seasonal fluctuations were tested, for each year, using statistical methods for circular distributions. The months of the year were considered to have been placed around a circumference of a circle, and the monthly frequency of outbreaks was assigned to the mid point of the angular interval corresponding to each month (i.e.  $15^\circ$  for January;  $45^\circ$  for February). The mean time of ASF occurrence was then determined from the mean angle. Dispersion was evaluated by the angular deviation  $s$ , which ranges from a minimum of zero to a maximum of  $81.03^\circ$  and is inversely related to  $r$ , the length of the mean vector from the geometric center of the circle to the center of gravity (average location) of outbreaks. Rayleigh's  $z$  was used to test the significance of the mean time of outbreak occurrence ( $\alpha=0.05$ ) (Zar, 1984). Calculations were carried out using a Microsoft Excel 97 spreadsheet (Microsoft Corporation, Redmond, WA). No adjustment was made for length of month or leap year, and for seasonal variations of the population at risk. Between-year differences in ASF seasonal patterns were tested by  $\chi^2$  for contingency tables ( $\alpha=0.05$ ) using bimonthly data to attain sufficiently large frequencies for a  $\chi^2$  analysis (Zar, 1984).

The spatial clustering of ASF in the municipalities of the province of Nuoro was evaluated by binary join-count statistics (Odland, 1987; Hungerford and Smith, 1996), using Microsoft Excel 97. The numbers of joins (shared borders) that were observed among municipalities where ASF was reported and unaffected municipalities (discordant joins) were compared – for each year from 1993 through 1996 – to the numbers of discordant joins to be expected according to a random distribution of the disease. Autocorrelation was tested by calculating the standard normal deviate  $Z$  ( $\alpha=0.05$ ).

ASF IR during 1993–1996 was calculated for each municipality of the ASF-endemic area of Nuoro. Municipalities were then cross-classified into a  $2 \times 2$  table based upon IR and the proportion of extensive farms (free-range and partial confinement) out of the registered farms, dichotomized at the medians. Agreement between the two classification criteria – as a measure of the spatial co-distribution of extensive pig farming and ASF – was tested by Kappa statistic ( $\kappa$ ) using the FREQ procedure in the SAS system, version 6.12 (AGREE option) (Fleiss, 1981; Hungerford, 1991; Hungerford and Smith, 1996;

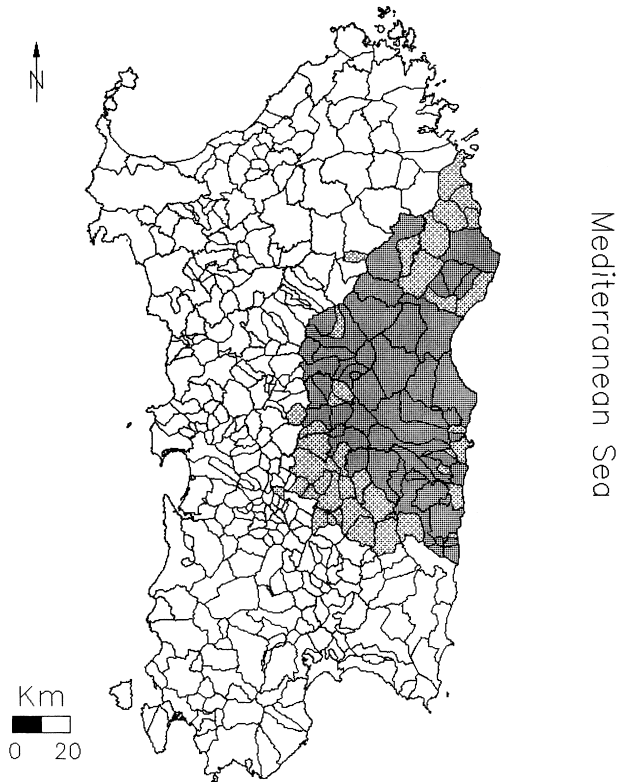


Fig. 1. Distribution of ASF-affected municipalities (dark shading) in the ASF-endemic area of the province of Nuoro (light shading), Sardinia, during the period 1993–1996.

SAS, 1996). The medians of the numbers of pigs and of registered pig farms in ASF+ and ASF– municipalities were compared by Wilcoxon Two-Sample test (SAS, 1990).

### 3. Results

During the period 1993–1996, 400 ASF outbreaks were reported in 7670 registered pig farms, from 45 out of 82 municipalities (54.9%) of the endemic area of the province of Nuoro (Fig. 1). Overall IR was 1.3 outbreaks per 100 farm-years. The median ( $Q_1$ – $Q_3$ )<sup>1</sup> IR in all municipalities was 0.27 (0–1.8), whereas, in ASF-affected municipalities the median ( $Q_1$ – $Q_3$ ) IR was 1.3 (0.50–4.4). Five municipalities (6.1%) (Orgosolo, Talana, Urzulei, Villagrande, Orune) were affected by ASF during each year and accounted for 4.6 outbreaks per 100 farm-years. Twelve municipalities (14.6%) were affected by one outbreak only. ASF in wild boars was reported from six municipalities (7.3%).

<sup>1</sup>  $Q_1$ =first quartile;  $Q_3$ =third quartile.

Table 1

Reported ASF occurrence in pig farms and wild boars in the endemic area of the province of Nuoro, Sardinia, from 1993 through 1996

Variable	1993	1994	1995	1996
Outbreaks in pig farms	100	89	140	63
IR <sup>a</sup>	1.3	1.2	1.8	0.82
Outbreaks in wild boars	1	0	5	2
Municipalities affected by ASF outbreaks (%)	23 (28.0)	21 (25.6)	25 (30.5)	20 (24.4)

<sup>a</sup> Outbreaks per 100 farm-years.

Table 2

Analysis of circular data for monthly occurrence of ASF outbreaks and affected municipalities in the endemic area of the province of Nuoro, Sardinia, from 1993 through 1996

Year		Mean angle	Month	<i>s</i> <sup>a</sup>	<i>z</i> <sup>b</sup>	<i>P</i>
1993	Outbreaks	118.6°	April	65.7	11.6	<0.001
	Municipalities	90.2°	April	65.7	6.0	<0.01
1994	Outbreaks	82.3°	March	53.9	27.0	<0.001
	Municipalities	89.8°	March	61.6	7.2	<0.001
1995	Outbreaks	111.8°	April	72.0	6.2	<0.001
	Municipalities	113.7°	April	73.4	2.3	NS <sup>c</sup>
1996	Outbreaks	129.6°	May	56.4	16.9	<0.001
	Municipalities	118.2°	April	57.8	10.9	<0.001

<sup>a</sup> Angular deviation.

<sup>b</sup> Rayleigh's *z*.

<sup>c</sup> Not significant.

ASF peaked in 1995 and declined in 1996 (Table 1). In fact, IR was significantly higher in 1995 than in 1993–1994 (RR=1.5,  $\chi^2=12.5$ , d.f.=1,  $P<0.001$ ), and 1996 (RR=2.2,  $\chi^2=29.2$ , d.f.=1,  $P<0.001$ ). The seasonal pattern of disease occurrence was characterized by significant spring peaks (Fig. 2; Table 2). But bimonthly frequency of outbreaks differed significantly among years ( $\chi^2=68.9$ , d.f.=15,  $P<0.001$ ).

Spatial clustering of ASF-affected municipalities, as evaluated by discordant join counts, was statistically significant in 1993 ( $Z_{jc}=-3.0$ ,  $P<0.01$ ) and 1994 ( $Z_{jc}=-3.2$ ,  $P<0.01$ ) but not in 1995 ( $Z_{jc}=-0.6$ ,  $P=0.55$ ) and 1996 ( $Z_{jc}=-1.2$ ,  $P=0.23$ ).

There was good agreement between classification of municipalities based upon extensive pig farming and ASF IR ( $\kappa=0.51$ , 95% CI=0.33–0.70). The numbers of pigs and pig farms did not differ significantly between ASF-affected and unaffected municipalities: medians ( $Q_1$ – $Q_3$ ) were 605 (374–1269) vs. 488 (308–731) for pigs ( $P=0.12$ ), and 72 (52–141) vs. 75 (55–92) for farms ( $P=0.72$ ).

#### 4. Discussion

The persistence of ASF in Sardinia poses a continuous threat to the European pig industry. In fact, the illegal introduction of pork from Sardinia caused an ASF outbreak in northern Italy in 1983. Moreover, countries of Southern Europe, such as Portugal and

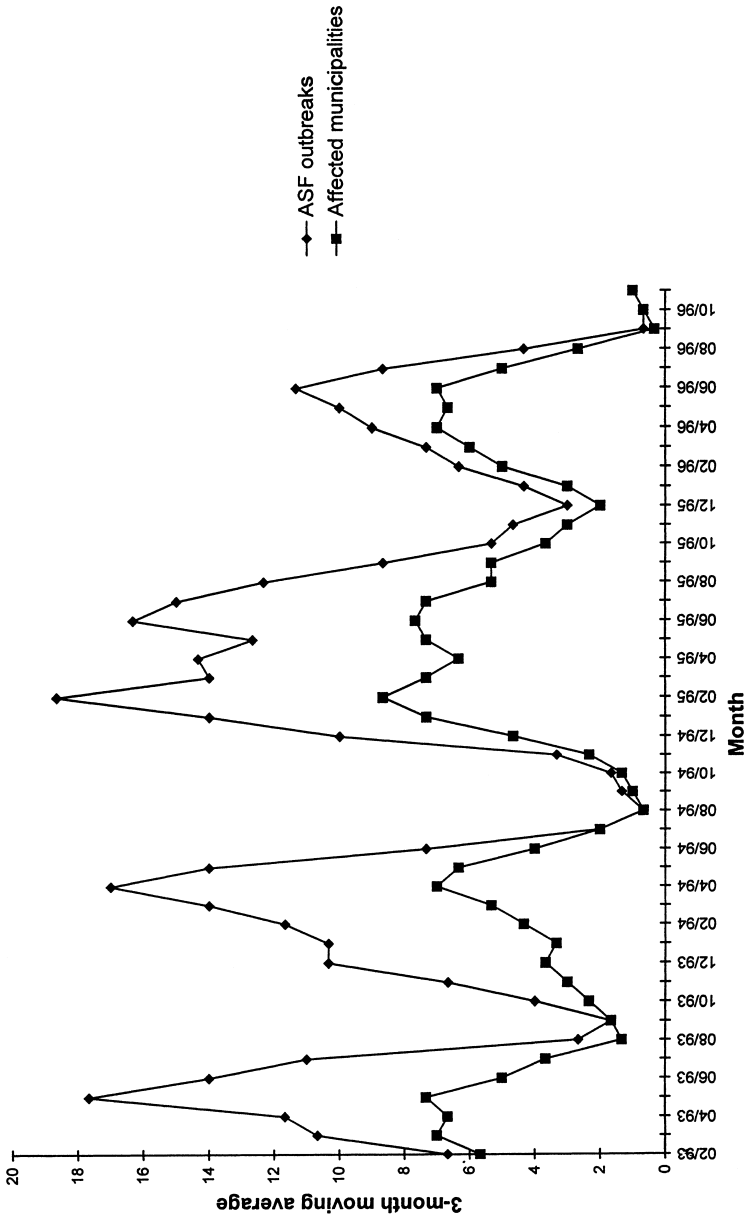


Fig. 2. Seasonality of ASF outbreaks and ASF-affected municipalities in the endemic area of the province of Nuoro, Sardinia, from 1993 through 1996.

Spain, that were recently freed from the infection and where habitat and pig husbandry conditions are similar to those found in Nuoro, are at risk for the re-establishment of endemic ASFV transmission cycles (Wilkinson, 1984).

Analytical techniques – applied to data that are routinely collected in the context of disease control activities – provide valuable information on the impact of intervention and guide further research and control measures. Through the analysis of temporal and spatial patterns of ASF reports in Nuoro, we evaluated the effect of a EU funded eradication plan.

The annual frequency of ASF outbreaks that was reported in Sardinia during 1993–1996 (average $\pm$ SD=100 $\pm$ 33.5 outbreaks per year, Table 1) was higher than disease frequency reported from 1978 through 1992 (38 $\pm$ 20.8 outbreaks per year; IZS, unpublished). Prompt reimbursement for pigs that were slaughtered following ASF outbreaks was established in 1993 (as part of the EU-funded eradication plan) and might have improved farmers' compliance in reporting the disease resulting in an increased number of diagnosed outbreaks. In an attempt to eliminate a major factor in ASF persistence, free-range pig farming was declared illegal from 1981 through 1993 but was never completely abandoned (Firinu et al., 1988). Therefore, ASF outbreaks that occurred in free-range herds in the period 1981–1993 were allegedly not reported – resulting in a relatively low disease frequency. The ban on free-range pig farming was lifted in 1993, when the ASF eradication plan started.

The significant twofold decline of ASF IR between 1995 and 1996 (Table 1) might have been caused by the implementation of strict disease control measures following ASF outbreaks and the elimination of pigs that were positive to ASFV by serological tests (Patta et al., 1994). In agreement with a decline in ASF outbreaks, the percentage of ASFV-seropositive pig farms in Nuoro declined significantly from 1994 through 1996 (Mannelli et al., 1997a, b).

Using statistical techniques for circular data, we obtained measures of central tendency and dispersion, and tested statistical significance of seasonal patterns of ASF in Sardinia. The mean time of ASF occurrence varied between March (1994), April (1993 and 1995) and May (1996) (Fig. 2; Table 2).

Management practices that are typical of the Sardinian pig husbandry system might affect the seasonality of ASF. In the fall – and through winter and spring – free-ranging pigs are concentrated in the publicly-owned areas where acorns are most abundant. Under these circumstances, the transmission of ASFV from “permanently infected virus carriers” and susceptible animals may occur leading to clinical outbreaks in the following months (Wilkinson, 1984; Bech-Nielsen et al., 1995). The spring peaks of outbreaks that we observed were probably due to the gradual diffusion of the infection across neighboring areas through the movements of infected free-range pigs and wild boars. Accordingly, the numbers of municipalities that were affected by ASF also peaked in spring (Fig. 2; Table 2) and were spatially clustered.

Other factors that may affect ASF seasonality include the slaughtering of pigs for family consumption and wild boar hunting, that are carried out in fall and winter and may favor ASFV transmission through infected pork fed to pigs. Conversely, solar irradiation reduces ASFV viability on pig pasture (Perestrelo Vieira, 1993) and may account for the low disease frequency that we observed in summer.



Seasonal fluctuations of Sardinian pig population (population at risk) were not taken into account in our analysis. We, therefore, studied seasonal patterns of ASF incidence rather than IR. In the Sardinian traditional husbandry system, pig reproduction occurs throughout the entire year and it is not a cause of variations in the population at risk.

Good agreement between the classifications of municipalities based upon the proportion pig farms that were of the free-range and partial confinement type, and ASF IR ( $\kappa=0.51$ ) demonstrated a spatial co-distribution of extensive pig husbandry and ASF. The farm-level association between such a husbandry system and seropositivity to ASFV was previously shown (Mannelli et al., 1997a).

Spatial clustering of ASF+ areas suggested that ASF in a municipality was associated with the occurrence of the disease in bordering areas. Similarities among neighboring areas with regard to habitat, animal husbandry, and sociological factors underlies clustering of diseases. In Nuoro, movements of ASFV-infected free-ranging pig or wild boars across bordering municipalities, and the role of mechanical vectors (such as flies) in ASFV transmission should be investigated (Mellor and Wilkinson, 1985).

In agreement with the hypothesis that the risk of the disease was primarily associated with extensive pig husbandry and spatial factors, ASF in municipalities was not associated with the numbers of farms and pigs.

Binary join count analysis was significant in 1993 and 1994. In the following years, ASF was reported from municipalities that were not previously affected and not sharing borders with affected areas. Consequently, spatial autocorrelation in 1995 and 1996 was low. Infected pork or pigs illegally moved from the ASF-endemic area are possible routes of ASFV transmission among distant municipalities.

## 5. Conclusions

In Nuoro, ASF control measures should be focused on municipalities at highest endemicity, that are the most likely sources of infection for other areas. The unique combination of environmental and social factors underlying the maintenance of ASFV in those municipalities must be taken into account. Particularly, the rationalization of extensive pig farming – a traditional method to utilize unproductive lands in the Mediterranean area – is a critical step for disease eradication and for the prevention of further infections. Delimiting pastures with fences, as an example, could limit movements of free range pigs and prevent contacts among herds of different areas – reducing the risk of ASFV transmission. Moreover, keeping all pigs in enclosed areas during months when pastures are poor is proposed to allow an effective control of animals (Laddomada et al., 1991).

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