



Descriptive epidemiology of endemic Classical Swine Fever in Cuba

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Abstract

In Cuba, Classical Swine Fever (CSF) has become an endemic disease since 1993 with several outbreaks each year despite the compulsory vaccination program implemented. To deepen the disease characterization is essential for improving the CSF control measures and to achieve its eradication. The aim of this study was to describe the epidemiological characteristics of CSF occurrences in Cuba during a seven-year period within the endemic situation. Data on CSF occurrence from January 2010 to December 2016 were analyzed. The seven-year period shows a tendency of the number of affected premises to increase ($r=0.31$, $p=0.005$) over time (month). Directional distribution (1SD ellipse) indicated a great dispersion of affected premises by year across the country with a trend to a higher occurrence to the west. It was demonstrated by the negative correlation ($r=-0.893$, $p=0.007$) between the longitude of the mean center of the ellipses over the years. The Kernel density indicated that the disease was spatially distributed across the whole country, but four hot spots were found in the western (Pinar del Río and Artemisa) and eastern (Guantánamo and Holguín) regions. The clinical sign most frequently reported in affected premises was fever, followed by loss of appetite, conjunctivitis, and diarrhea. The most frequent observed clinical signs were non-specific, which complicates the disease recognition in the field. The obtained results have a practical importance for improving the efficiency of the CSF control program implemented in the country and contribute to enhance epidemiological surveillance taking into account the risk based principles.

Additional keywords: swine health; clinical signs; spatial distribution; temporal trend.

Abbreviations used: CSF (Classical Swine Fever); CSFV (Classical Swine Fever virus); DIP (direct immunoperoxidase); LNDV (National Laboratory for Veterinary Diagnostic).

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Introduction

Classical Swine Fever (CSF) is one of the major economic threats to pig production worldwide affecting farms with different production system (Monger *et al.*, 2015; Pannhorst *et al.*, 2015). Its etiological agent, Classical Swine Fever virus (CSFV), is a member of the *Pestivirus* genus within the *Flaviviridae* family. Although a number of efficacious and safe vaccines is available to control CSF, the disease remains endemic

in certain areas of Asia, Europe, Central and South America, the Caribbean, and parts of Africa (Coronado *et al.*, 2017). Due to a wide range of clinical signs and similarity to other diseases, it has become a challenging task to diagnose the CSF accurately (Blome *et al.*, 2017).

Depending on the virulence of the strain, varying degrees of disease severity have been observed, ranging from acute or chronic to subclinical forms (Moennig, 2015). In general, while infections with virulent strains

result in acute hemorrhagic disease, the infection caused by less virulent isolates can become chronic or subclinical. Pigs infected with low virulent strains can shed the virus continuously or intermittently for months, representing a constant source of reinfection in endemic areas and a threat to virus-free countries (Muñoz-González *et al.*, 2015).

In Cuba, since 1993 CSF has become an endemic disease with several outbreaks each year (Díaz de Arce *et al.*, 1999, 2005) despite the regular and compulsory programmed vaccination with a lapinized live attenuated vaccine (C strain, Labiofam) (IMV, 2005). Interestingly, a trend towards milder, chronic presentations of the disease has been observed among the animals in the last years, and a high rate of non-synonymous mutations in E2 of CSFV among the isolates is associated with this trend (Pérez *et al.*, 2012). The molecular studies within this epidemiological situation showed that the most likely origin of the Cuban outbreaks in 1993 was due to the reintroduction of the Margarita strain to the field (Díaz de Arce *et al.*, 1999, 2005).

Against this background, Díaz de Arce *et al.* (2005) suggested that evolution of the virus in the country could be supported by a positive selection pressure linked to inefficient vaccination programs, which could lead to a lower severity in the clinical signs developed by most of the affected animals. According to Pérez *et al.* (2012), since 2009, the CSF virus genetic variability has decreased even more, most likely due to bottleneck caused by the vaccine in use. Thus, the strains currently circulating might have been positive selected by the vaccine pressure. Recently, phylogenetic analysis of CSFV strains isolated in Cuba demonstrated the existence of a new sub-genotype 1.4 (Postel *et al.*, 2013; Silva *et al.*, 2016).

Food and Agriculture Organization of the United Nations (FAO) have developed a CSF eradication plan for the Americas. The goal of this plan is to achieve the CSF eradication for 2020, and also it is an important compromise to Cuba. The eradication of CSF in Cuba could contribute to increase the swine production, reduce the economic losses, and rise the efficiency of the productive process. Regional control programs in South and Central America demonstrated to be successful in the progressive control and elimination of CSF in the past decade, with the industrial and public sectors sharing common objectives for the disease control and eradication (Pinto *et al.*, 2011).

A description of time, place and population for the epidemiological characterization of the disease is useful initially (Thrusfield, 2013). The integration of the spatial dimension into epidemiological investigations provides an opportunity for conducting more inform-

ative descriptive analyses and gaining additional insights into the causal processes under investigation (Pfeiffer *et al.*, 2008).

Since CSF reemergence in Cuba, the epidemiological analysis of the disease evolution in whole country has been carried out systematically by the official veterinary service. To deepen the disease epidemiological characterization is necessary for the improving CSF control measures and achieve its eradication.

Previous studies on CSF affected premises distribution and disease spreading in Pinar del Río province, Cuba, from 2009–2015 had been carried out (Fonseca *et al.*, 2014, 2015a,b,c, 2016; Fonseca, 2016). However, similar studies are necessary to be developed at national level to create a baseline for a more holistic analysis to support the decision-making process for implementing a most efficient control measures and achieving disease eradication.

The aim of this study was to describe and update the epidemiological characteristics of CSF occurrence in Cuba within its known endemic situation.

Material and methods

Data sources

Data on CSF occurrence from January 2010 to December 2016 were obtained from the National Laboratory for Veterinary Diagnostic (LNDV), which is responsible for CSF diagnosis from samples received from all suspected premises throughout the whole country. In addition, LNDV provided the epidemiological information included in a questionnaire applied to investigated premises. The direct immunoperoxidase (DIP) technique was used for case confirmation (De Smit *et al.*, 2000; Blome *et al.*, 2017). Case definition in place considers a premise to be affected when at least one tested animal has a positive result to DIP (IMV, 2005).

The veterinary staff of the official veterinary service at the local level (*i.e.*, municipality) and also the veterinarians of different commercial farms send samples for disease diagnosis with the official annexed questionnaire. Data obtained through the questionnaire from each affected premise were registered in electronic spreadsheets (Microsoft Excel® 2010) by the personnel of the LNDV as part of the established laboratory routine. They included the premise or owner's name, province, municipality, date of confirmation of the CSF cases, clinical signs and geographic location using the epidemiological quadrant of 1-km², according to the National Information and Surveillance Epizootiological System.

Statistical analysis

A monthly time trend graph was created in Excel 2010 with the number of CSF affected premises. Thus, a linear regression was used to model the tendency of the number of affected premises per month during the studied period. The linear regression was performed using the software package Jamovi 0.8.1.10 (Jamovi Project, 2017).

Directional distribution (1 standard deviation ellipse) tool in ArcGIS 9.3.1 (ESRI, Redlands, USA) was used to describe the spatio-temporal pattern of CSF occurrences by year throughout the whole country. Correlation analyses were carried out using Spearman Rho to test whether the mean center longitude of the ellipses had a higher degree of correlation with years to identify the movement tendency with an east–west direction. Correlation analysis was performed using Software package Jamovi 0.8.1.10 (Jamovi_Project, 2017).

Kernel density smoothing technique in ArcGIS 9.3.1 (ESRI, Redlands, USA) was applied to describe the spatial distribution of affected swine premises during the study period taking into account the latitude and longitude (decimal degrees) of the epidemiological quadrant (polygon) centroid. The

centroid was calculated using the Calculate Geometry command. All affected premises within 1-km² were aggregated in a single point through spatial join tool. The band width was calculated using Moran's Index and was determined in 19.82 km and an output cell size of 1-km² were used to create the kernel-smoothed maps.

A frequency distribution of clinical signs reported by affected premises was created. Also, the proportion of the reported clinical signs were compared using Jamovi 0.8.1.10 (Jamovi Project, 2017).

Results

During the study period (2010–2016), LNDV confirmed the presence of 846 swine premises affected by CSF distributed in all provinces. The annual average of CSF affected swine premises was of 120.86 (CI95%: 119.06–122.66).

The time trend graph (Fig. 1) shows the monthly occurrence of a great number of cases with an apparent upward trend. It was posteriorly confirmed through a linear regression ($r=0.31$, $p=0.005$) which showed a tendency of the number of affected premises to increase through the years studied (Table 1).

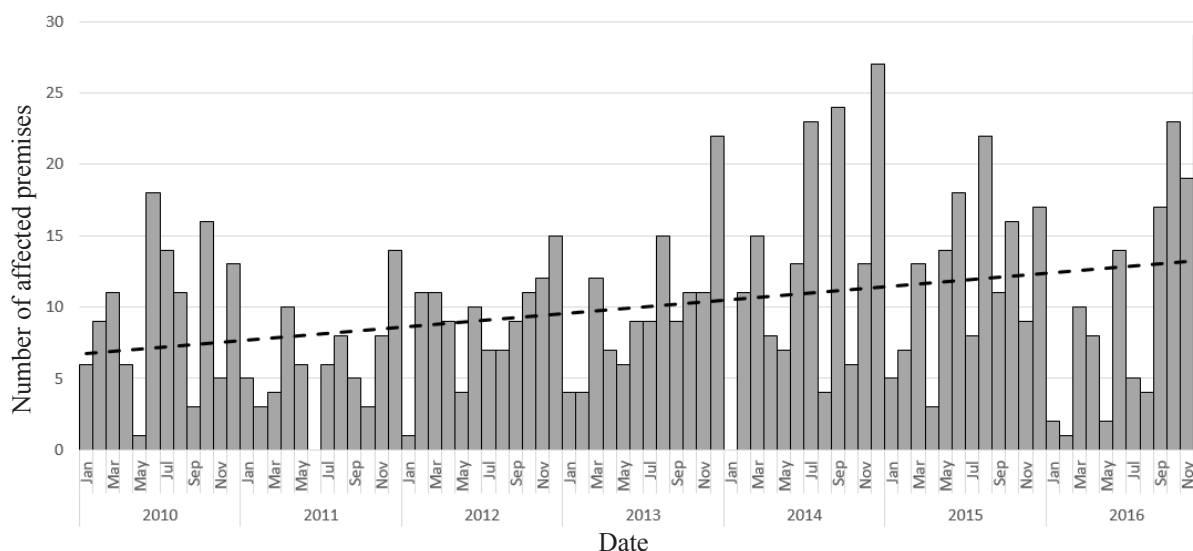


Figure 1. Time trend graph of Classical Swine Fever affected premises by month in Cuba (2010–2016). Dashed line shows the linear tendency during the studied period.

Table 1. Trend analysis of premises monthly affected by CSF (2010–2016).

	β	SE	t	p value	95% CI	
					Lower	Upper
Intercept	6.633	1.334	4.971	0.000	3.979	9.287
Month (January/2010 - December/2016)	0.079	0.027	2.895	0.005	0.025	0.133

β : coefficient (slope). SE: standard error. p value: significance ($p < 0.05$). CI: confidence interval.

A great dispersion of swine premise affected by year was revealed across the country; also, a discrete trend to occur more to the west during the studied period (Fig. 2). Ellipses showed a little spatial moving from east to west during the studied period. Likewise, there was a negative correlation ($r=-0.893$, $p=0.007$) between the centroid mean center longitude of the ellipses and years, confirming the tendency to occur a higher number of CSF occurrence to the west.

The disease was spatially distributed across the whole country (Fig. 3), but a higher density of affected premises was observed in the western (Pinar del Río and Artemisa) and eastern (Guantánamo and Holguín) regions of the island. Nevertheless, areas with medium densities of CSF affected premises occurred in all the provinces with variable extension.

The clinical sign most frequently reported in affected swine premises was fever, followed by loss of appetite, conjunctivitis and diarrhea. On the contrary, reproductive disorders like mummifications and malformations, abortions, and stillbirths were the clinical signs less frequently reported in the affected premises (Fig. 4).

Discussion

CSF is a serious problem for pig industry, despite the enormous efforts carried out during the last decades to eradicate the disease. Many factors, such as globalization and increased swine trade and movement, the progressive growth of pig populations with increased high-density areas, as well as the circulation of low and moderate virulence strains make difficult the diagnosis and control of the disease (Ganges *et al.*, 2007; Muñoz-González *et al.*, 2015).

In Cuba, the lapinized live attenuated vaccine is used as part of the measures established by the National Control Program against CSF (Frías Lepoureau, 2003; Ganges *et al.*, 2007). Nevertheless, the disease remains endemic in the country despite the intensive vaccination program implemented. This failure in the responses to vaccination is caused by a combination of several problems such as vaccine quality and availability and gaps in the cold chain between vaccine production and application, among others (Díaz de Arce *et al.*, 1999, 2005; Frías Lepoureau, 2003; Pérez *et al.*, 2012; Coronado *et al.*, 2017).

Characterizing the presentation of the diseases through an epidemiological analysis has a paramount importance to develop more efficient strategies for effective control and eradication measures (Mur Gil, 2015). A common method of displaying the spatial distribution of disease and related factors is by drawing

maps. This is of value not only in registering the areas where diseases exist but also in investigating the mode and direction of transmission of infectious diseases (Thrusfield, 2013).

The period studied showed a slight tendency of the disease occurrence to increase over years (Fig. 1 and Table 1). Moreover, the affected premises were distributed along the country, although there were areas where the disease was concentrated. The disease occurrence had a behavior according to an endemic condition and an unsuccessful disease control, which propitiated the viral transmission (Blome *et al.*, 2017).

Directional distribution (standard deviational ellipse) helps to understand the spatiotemporal patterns of disease occurrences by visualization of the central tendency, dispersion and directional trends (Ward *et al.*, 2008; Oganessian *et al.*, 2012). A trend of movement of the ellipses to a western direction (Fig. 2) is really alarming, because there is a higher concentration of swine populations and premises, areas with significant swine production. This is also showed in the spatial analysis (Kernel density) of affected premises (Fig. 3), which identified important hot spots in those regions.

The Kernel density estimation is an interpolation technique that creates a continuous surface derived from a point pattern and allows an easier identification of densely distributed features (Smith & Bruce, 2008). Thus, it has been used to detect and visualize the spatial distribution of affected premises by a specific condition of disease (Kauhl *et al.*, 2015). Two important hot spots located in the eastern region of the country (Guantánamo and Holguín provinces) and two hot spots in the western provinces (Pinar del Río and Artemisa) were identified (Fig. 3). It is interesting to mention that the hot spots occurring in the western region could have had epidemiological relationships with a hot spot detected to the north of Isla de la Juventud, because this territory has a high number of pig producers closely linked to the provinces of Pinar del Río and Artemisa through the frequent and significant animal movements (DSA, 2016).

Despite the relevance of CSF in Cuba due to its negative impact on the economy and animal welfare, few epidemiological analyses applying spatial and spatiotemporal analysis have been carried out. A lack of digitalized information is the most significant obstacle for developing a more detailed epidemiological analysis. Previous spatiotemporal analysis (Fonseca, 2016) were made using the aggregated information at municipality level. The available information at that moment allowed carrying out a space-time cluster analysis of affected and non-affected municipalities in Cuba from 2007 to 2015 by using the Bernoulli probabilistic model in SaTScan™ 9.3 (Kulldorff, 2014).

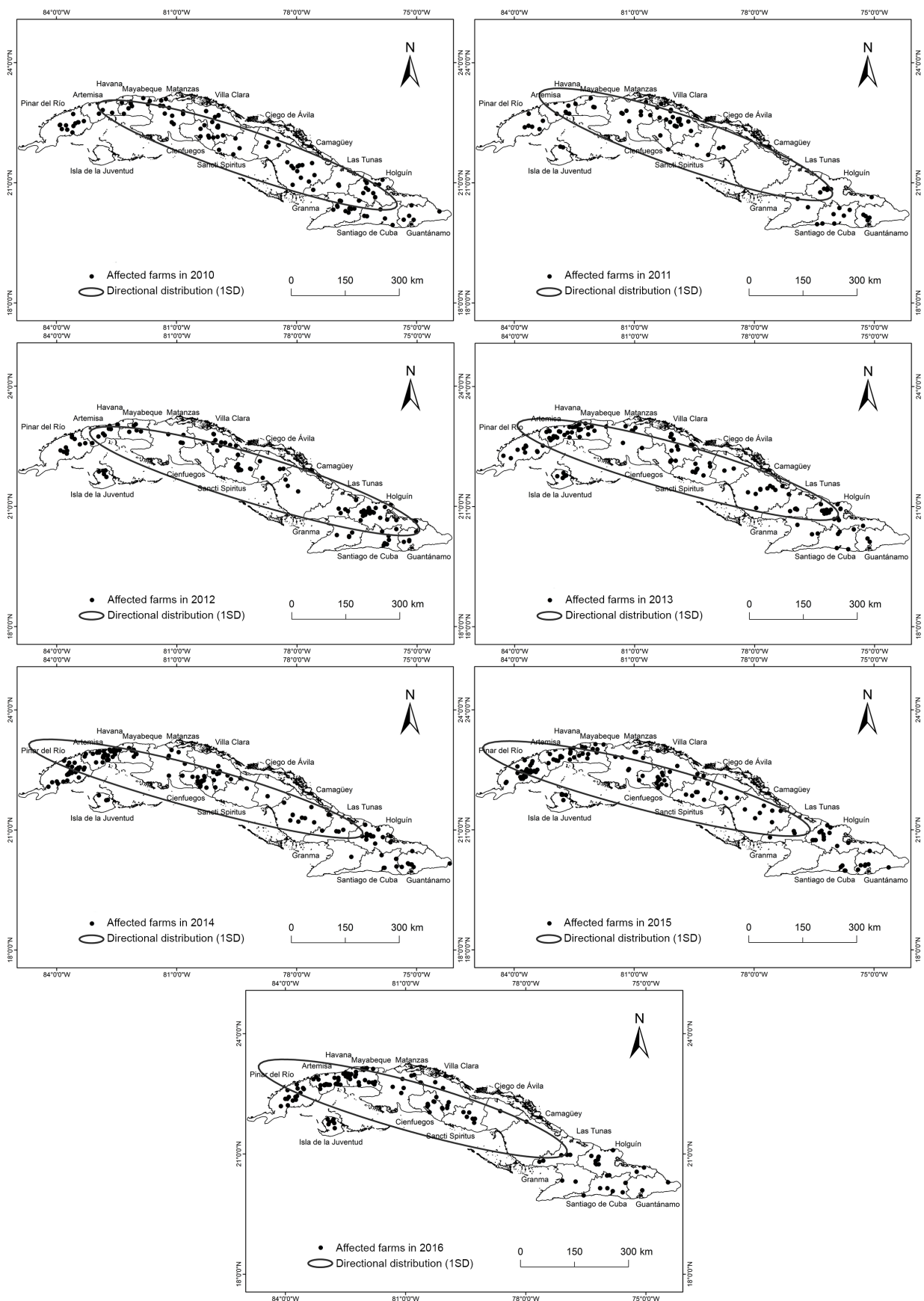


Figure 2. Directional distribution of Classical Swine Fever affected premises by year in Cuba (2010–2016) (one standard deviation ellipses).

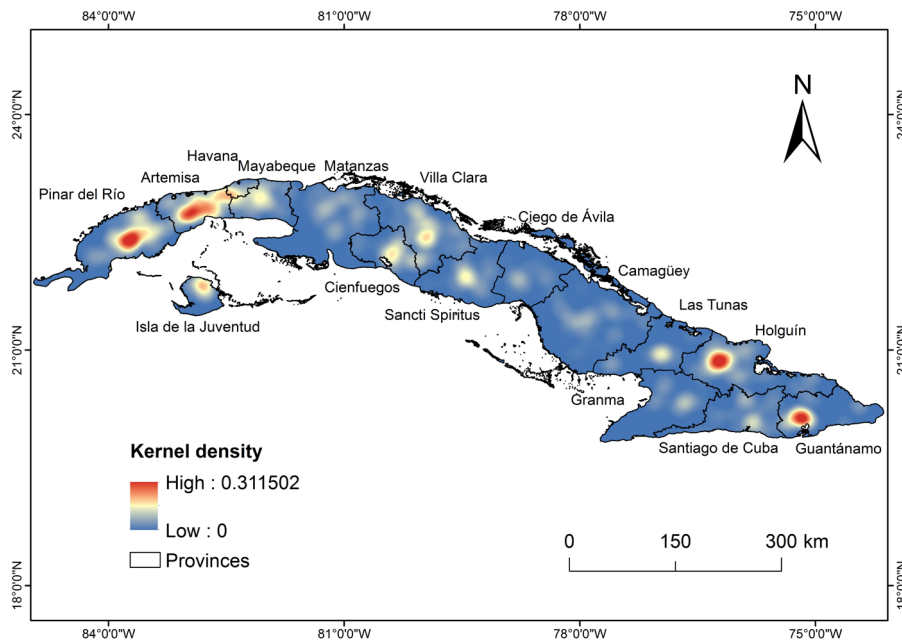


Figure 3. Kernel density of premises affected by Classical Swine Fever during 2010–2016 in Cuba (1 km² grid cells, 19.82 km bandwidth).

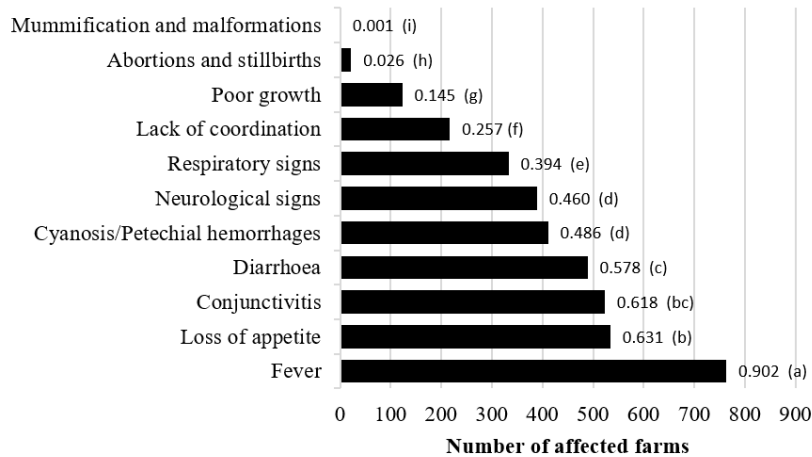


Figure 4. Frequency of clinical signs observed in premises affected by Classical Swine Fever in Cuba (2010–2016). Horizontal bars show the proportion, and different letters show significant differences ($p < 0.05$).

This previous study identified three spatiotemporal high-rate clusters of affected municipalities in the three regions, East (2007–2010), Central (2007–2009) and West (2011–2014) (Fonseca, 2016). In the western region, the high-rate clusters were spatially overlaid with the hot spots showed in this study by Kernel density on the provinces of Pinar del Río and Artemisa. Otherwise, the high-rate cluster identified previously in the eastern region overlaps also the hot spot detected by Kernel density in Holguín. Both coincidences confirm the relevance of these territories identified as hot spots for the control disease strategy and the risk based surveillance.

The identification of four hot spots of the CSF occurrence despite the endemic behavior of the disease

in whole country could be indicative of the paramount epicenters for the disease dissemination from those territories. In this case, the strengthening of CSF surveillance and control in those areas could have also a great impact on neighboring areas by reducing the disease spread.

Kernel density estimation has been used as a complementary tool to visualize the spatial distribution of events within the spatial clusters (Kauhl *et al.*, 2015). However, an important hot spot revealed by Kernel density in Guantánamo is not inside the clustered area detected by previous space-time analysis using Scan Statistic. It occurs due to Kernel density does not take the time into account and a few regular number

of affected premises occurred each year near to the Guantánamo Bay establishing a hot spot (Fig. 3).

The trend of ellipses to move to the western direction (Fig. 2) as indicative of current higher concentration of CSF affected premises in the western region over time. It agrees with the spatiotemporal analysis of the disease developed by Fonseca (2016), showing that the most recent high-rate clusters were located in the western region (2011–2014). Whereas the most recent low-rate cluster (2014–2015) was found in the eastern region.

In this background, when a shorter period (2007–2013) was analyzed using the same method (Bernoulli probabilistic model in SaTScan™ 9.3), a similar pattern occurred and some clusters of high-rates were spatially overlapped by the later occurrence of low-rate clusters (Fonseca *et al.*, 2016). However, the aggregation of cases to administrative divisions may negatively affect analysis accuracy (Jones & Kulldorff, 2012). Thus, more precise methods of cluster detection at country level must be performed with a lower level of aggregation to obtain more accurate results of the distribution of CSF occurrences.

Likewise, the previously mentioned studies (Fonseca, 2016; Fonseca *et al.*, 2016) did not analyze the spatial or spatiotemporal distribution of affected premises within Isla de la Juventud, meanwhile the Kernel density analysis detected a hot spot in the north of this island. Therefore, a more detailed analysis must be carried out in this territory to strengthen the sanitary measures toward the disease control in the framework of CSF eradication strategy by zones.

On the other hand, eleven clinical signs were reported during epidemiological investigations in farms affected by CSF (Fig. 4). The clinical sign most frequently reported in affected swine premises was fever, followed by loss of appetite, conjunctivitis and diarrhea. Similar results were described by Allepuz *et al.* (2007) during the outbreaks occurred in 2001–2002 in Catalonia, Spain, where fever was also the most frequent clinical sign followed by loss of appetite. Similarly, in Belgium in 1993–1994, Koenen *et al.* (1996) described these clinical signs as the most often reported at the first visit made by the veterinary services. Furthermore, in the Netherlands, the symptoms reported in outbreaks in 1997–1998 were also mainly non-specific: fever, apathy, and ataxia were the symptoms most prominently observed in infected herds (Elbers *et al.*, 1999).

It is noteworthy that when the most frequent clinical signs are milder and non-specific, it is very difficult to recognize the disease clinically. Additionally, it should be noted that easier recognized clinical signs, such as cyanosis and petechial hemorrhages, were the 5th more reported signs. The tendency to increase circulation of middle and low virulence strains (Díaz de Arce *et al.*,

2005; Pérez *et al.*, 2012) could contribute to affect the detection of the disease in the premises (Postel *et al.*, 2015). The experience obtained after many years of CSF control in Cuba demonstrates the importance of warning the suspicion of CSFV infection when high temperatures in pigs are detected and to issue guidelines for the practical outbreak control by slaughtering of infected pigs detected based on their daily thermometry.

During the first epizootic period in Cuba, from 1993 to 1997, most of the affected animals showed acute clinical signs of the disease. Nevertheless, a trend towards milder, chronic and subclinical presentations of the disease has been observed among the animals during recent years (Díaz de Arce *et al.*, 2005; Pérez *et al.*, 2012).

In this line, other studies are required to compare high and low-rate clusters of CSF occurrences to analyze the frequency of clinical signs in affected premises and to characterize the clinical signs caused by the circulating CSFV strain identified in areas where these clusters were detected. Low-rate clusters or cold spots could be associated with middle and low pathogenicity CSFV strains that cause under-detection of cases through the passive surveillance based on clinical methods. A significant problem in transmissible disease control is the under-detection of cases (Bailey *et al.*, 2005). The proposed analysis could help to modify some methods that could improve the sensitivity of the Cuban surveillance system by the inclusion of the risk based surveillance principles.

In summary, the occurrence of CSF affected premises showed a temporal increasing tendency during the studied period. The disease was spatially more concentrated in certain areas (hot spots) and the occurrence of cases tended to move mainly in an east-west direction. Additionally, the most frequent observed clinical signs were non-specific, which complicates the disease recognition in the field. Therefore, these results have a practical importance to improve the CSF control in the disease endemic situation in the whole country, because it highlights the hot spots as an epicenter for the disease dissemination, and enhances the surveillance taking into account the risk based principles.

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References

- Allepuz A, Casal J, Pujols J, Jové R, Selga I, Porcar J, Domingo M, 2007. Descriptive epidemiology of the

- outbreak of classical swine fever in Catalonia (Spain), 2001/02. *Vet Rec* 160 (12): 398-403. <https://doi.org/10.1136/vr.160.12.398>
- Bailey TC, Carvalho MS, Lapa TM, Souza WV, Brewer MJ, 2005. Modeling of under-detection of cases in disease surveillance. *Ann Epidemiol* 15 (5): 335-343. <https://doi.org/10.1016/j.annepidem.2004.09.013>
- Blome S, Staubach C, Henke J, Carlson J, Beer M, 2017. Classical Swine Fever—An updated review. *Viruses* 9 (4): E86. <https://doi.org/10.3390/v9040086>
- Coronado L, Liniger M, Muñoz-González S, Postel A, Pérez LJ, Pérez-Simó M, Perera CL, Frías-Lepoureau MT, Rosell R, Grundhoff A, *et al.*, 2017. Novel poly-uridine insertion in the 3' UTR and E2 amino acid substitutions in a low virulent classical swine fever virus. *Vet Microbiol* 201: 103-112. <https://doi.org/10.1016/j.vetmic.2017.01.013>
- De Smit A, Eble P, de Kluijver E, Bloemraad M, Bouma A, 2000. Laboratory experience during the classical swine fever virus epizootic in the Netherlands in 1997-1998. *Vet Microbiol* 73 (2-3): 197-208. [https://doi.org/10.1016/S0378-1135\(00\)00145-0](https://doi.org/10.1016/S0378-1135(00)00145-0)
- Díaz de Arce H, Núñez JI, Ganges L, Barreras M, Teresa Frías M, Sobrino F, 1999. Molecular epidemiology of classical swine fever in Cuba. *Virus Res* 64 (1): 61-67. [https://doi.org/10.1016/S0168-1702\(99\)00077-5](https://doi.org/10.1016/S0168-1702(99)00077-5)
- Díaz de Arce H, Ganges L, Barrera M, Naranjo D, Sobrino F, Frías M, Nunez J, 2005. Origin and evolution of viruses causing classical swine fever in Cuba. *Virus Res* 112 (1-2): 123-131. <https://doi.org/10.1016/j.virusres.2005.03.018>
- DSA, 2016. Libro de traslados del Sistema de Vigilancia Epizootiológica de la Dirección de Salud Animal de la República de Cuba. Cuba, Work Document.
- Elbers ARW, Stegeman A, Moser H, Ekker HM, Smak JA, Pluimers FH, 1999. The classical swine fever epidemic 1997-1998 in the Netherlands: descriptive epidemiology. *Prev Vet Med* 42 (3-4): 157-184. [https://doi.org/10.1016/S0167-5877\(99\)00074-4](https://doi.org/10.1016/S0167-5877(99)00074-4)
- Fonseca O, 2016. Caracterización espaciotemporal y factores de riesgo del comportamiento endémico de la peste porcina clásica en Cuba. Doctoral Thesis, Agricultural University of Havana, Cuba.
- Fonseca O, Percedo MI, Rutili D, Alfonso P, Conte A, Ferrer E, Abeledo MA, Fernandez O, Calistri P, 2014. Simulation model for assessing the risk of classical swine fever spreading in Pinar del Río province, Cuba. 2nd Int Conf on Animal Health Surveillance (ICAHS2), Havana (Cuba), 7-9 May.
- Fonseca O, Grisi-Filho JHH, Santoro KR, Alfonso P, Abeledo MA, Fernández O, Blanco M, Rabelo Y, Percedo MI, 2015a. Network analysis of pig industry in a proposed zone for Classical Swine Fever eradication in Cuba. OIE Global Conf on Biol Threat Reduction. Paris, 30 June-2 July.
- Fonseca O, Percedo MI, Grisi-Filho JH, Alfonso P, Abeledo MA, Fernández O, Blanco M, Gutiérrez Y, Santoro KR, 2015b. Network analysis applied to classical swine fever epidemiology in Cuba. *Int Cong Eur Soc of Vet Virol (ESVV)*. Montpellier (France), 31 Aug-03 Sept.
- Fonseca O, Santoro K, Alfonso P, Abeledo M, Fernández O, Blanco M, Rabelo Y, Percedo M, 2015c. Spatial analysis of Classical Swine Fever outbreaks in Pinar del Río province, Cuba. 14th Int Symp on Vet Epidemiol Econ (ISVEE14), Merida, Yucatan (Mexico) 3-7 Nov.
- Fonseca O, Santoro KR, Abeledo MA, Capdevila Y, Fernández O, Alfonso P, Ayala J, Percedo MI, 2016. Spatiotemporal distribution of classical swine fever in Cuba, 2007-2013. *Rev Salud Anim* 38 (1): 30-38.
- Frías Lepoureau M, 2003. Reemergence of Classical Swine Fever in Cuba 1993-1997. *Rev Salud Anim* 25 (1): 1-4.
- Ganges L, Barrera M, Díaz de Arce H, Vega A, Núñez J, Sobrino F, Frías M, 2007. Antigenic, biological and molecular characterization of the Cuban CSFV isolate "Margarita". *Rev Salud Anim* 29 (3): 182-192.
- IMV, 2005. Programa de Prevención y Control de la peste porcina clásica en la República de Cuba. Ministerio de la Agricultura Press, Cuba.
- Jamovi_Project, 2017. Jamovi vers 0.8 [Computer Software]. <https://www.jamovi.org>
- Jones SG, Kulldorff M, 2012. Influence of spatial resolution on space-time disease cluster detection. *PLoS ONE* 7 (10): e48036. <https://doi.org/10.1371/journal.pone.0048036>
- Kauhl B, Pilot E, Rao R, Gruebner O, Schweikart J, Krafft T, 2015. Estimating the spatial distribution of acute undifferentiated fever (AUF) and associated risk factors using emergency call data in India. A symptom-based approach for public health surveillance. *Health & Place* 31: 111-119. <https://doi.org/10.1016/j.healthplace.2014.11.002>
- Koenen F, Van Caenegem G, Vermeersch J, Vandenheede J, Deluyker H, 1996. Epidemiological characteristics of an outbreak of classical swine fever in an area of high pig density. *Vet Rec* 139 (15): 367-371. <https://doi.org/10.1136/vr.139.15.367>
- Kulldorff M, 2014. SatScan v9.3: Software for the spatial and space-time scan statistics. Information Management Services Inc, Silver Spring, MD, USA.
- Moennig V, 2015. The control of classical swine fever in wild boar. *Front Microbiol* 6: 1211. <https://doi.org/10.3389/fmicb.2015.01211>
- Monger VR, Stegeman JA, Dukpa K, Gurung RB, Loeffen WL, 2015. Evaluation of oral bait vaccine efficacy against classical swine fever in village backyard pig farms in Bhutan. *Transb Emerg Dis* 63 (6): e211-e218. <https://doi.org/10.1111/tbed.12333>
- Muñoz-González S, Ruggli N, Rosell R, Pérez LJ, Frías-Lepoureau MT, Fraile L, Montoya M, Cordoba L, Domingo M, Ehrensperger F, Summerfield A, Ganges L,

2015. Postnatal persistent infection with classical Swine Fever virus and its immunological implications. *PLoS ONE* 10 (5): e0125692. <https://doi.org/10.1371/journal.pone.0125692>
- Mur Gil L, 2015. Nuevas estrategias para la prevención y control de la peste porcina africana. Doctoral Thesis. Veterinary School. Universidad Complutense de Madrid, Spain.
- Oganesyan AS, Petrova ON, Korennoy FI, Bardina NS, Gogin AE, Dudnikov SA, 2012. African swine fever in the Russian Federation: Spatio-temporal analysis and epidemiological overview. *Virus Res* 173 (1): 204-211. <https://doi.org/10.1016/j.virusres.2012.12.009>
- Pannhorst K, Frohlich A, Staubach C, Meyer D, Blome S, Becher P, 2015. Evaluation of an Erns-based enzyme-linked immunosorbent assay to distinguish Classical swine fever virus-infected pigs from pigs vaccinated with CP7_E2alf. *J Vet Diagn Invest* 27 (4): 449-460. <https://doi.org/10.1177/1040638715592446>
- Pérez LJ, Diaz de Arce H, Perera CL, Rosell R, Frias MT, Percedo MI, Tarradas J, Dominguez P, Nunez JI, Ganges L, 2012. Positive selection pressure on the B/C domains of the E2-gene of classical swine fever virus in endemic areas under C-strain vaccination. *Infect Genet Evol* 12 (7): 1405-1412. <https://doi.org/10.1016/j.meegid.2012.04.030>
- Pfeiffer D, Robinson T, Stevenson M, Stevens K, Rogers D, Clements A, 2008. Spatial analysis in epidemiology. Oxford University Press, NY. FAO, Rome. <https://doi.org/10.1093/acprof:oso/9780198509882.001.0001>
- Pinto J, Depner KR, Vargas-Terán M, 2011. Overview of classical swine fever: learning from regional disease control strategies. *EMPRES Transboundary Animal Diseases Bulletin* 39: 46-51. FAO Anim Prod & Health Div.
- Postel A, Pérez LJ, Perera CL, Schmeiser S, Meyer D, Meindl-Boehmer A, Rios L, Austermann-Busch S, Frias-Lepoureau MT, Becher P, 2015. Development of a new LAMP assay for the detection of CSFV strains from Cuba: A proof-of-concept study. *Arch Virol* 160 (6): 1435-1448. <https://doi.org/10.1007/s00705-015-2407-1>
- Postel A, Schmeiser S, Perera CL, Rodríguez LJP, Frias-Lepoureau MT, Becher P, 2013. Classical swine fever virus isolates from Cuba form a new subgenotype 1.4. *Vet Microbiol* 161 (3-4): 334-338. <https://doi.org/10.1016/j.vetmic.2012.07.045>
- Silva MNF, Silva DMF, Leite AS, Gomes ALV, Freitas AC, Pinheiro-Junior JW, Castro RS, Jesus ALS, 2016. Identification and genetic characterization of classical swine fever virus isolates in Brazil: A new subgenotype. *Arch Virol* 162 (3): 817-822. <https://doi.org/10.1007/s00705-016-3145-8>
- Smith SC, Bruce CW, 2008. CrimeStat III user workbook. The National Institute of Justice, Washington DC.
- Thrusfield M, 2013. *Veterinary epidemiology*. Elsevier.
- Ward MP, Maftai D, Apostu C, Suru A, 2008. Geostatistical visualisation and spatial statistics for evaluation of the dispersion of epidemic highly pathogenic avian influenza subtype H5N1. *Vet Res* 39 (3): 22. <https://doi.org/10.1051/vetres:2007063>