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Research article

Seroprevalence and risk factors of swine influenza in Spain

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ABSTRACT

Swine influenza is caused by type A influenza virus. Pigs can be infected by both avian and human influenza viruses; therefore, the influenza virus infection in pigs is considered an important public health concern. The aims of present study were to asses the seroprevalence of swine influenza subtypes in Spain and explore the risk factors associated with the spread of those infections. Serum samples from 2151 pigs of 98 randomly selected farms were analyzed by an indirect ELISA for detection of antibodies against nucleoprotein A of influenza viruses and by the hemagglutination inhibition (HI) using H1N1, H1N2 and H3N2 swine influenza viruses (SIV) as antigens. Data gathered in questionnaires filled for each farm were used to explore risk factors associated with swine influenza. For that purpose, data were analyzed using the generalized estimating equations method and, in parallel by means of a logistic regression. By ELISA, 92 farms (93.9%; $CI_{95\%}$: 89.1–98.7%) had at least one positive animal and, in total, 1340/2151 animals (62.3%; $CI_{95\%}$: 60.2–64.3%) were seropositive. A total of 1622 animals (75.4%; $CI_{95\%}$: 73.6–77.2%) were positive in at least one of the HI tests. Of the 98 farms, 91 (92.9%; $CI_{95\%}$: 87.7–98.1%) had H1N1 seropositive animals; 63 (64.3%; $CI_{95\%}$: 54.6–73.9%) had H1N2 seropositive pigs and 91 (92.9%; $CI_{95\%}$: 87.7–98.1%) were positive to H3N2. Mixed infections were detected in 88 farms (89.8; $CI_{95\%}$: 83.7–95.9%). Three risk factors were associated with seroprevalences of SIV: increased replacement rates in pregnancy units and, for fatteners, existence of open partitions between pens and uncontrolled entrance to the farm.

1. Introduction

Type A Influenza virus infections in swine are usually described as explosive outbreaks of acute respiratory disease similar in clinical course to human influenza (Olsen et al., 2006). A common assumption is that under certain circumstances related mostly to population dynamics, an epidemic outbreak of influenza in a pig farm may lead to the establishment of an endemic infection where SIV can circulate within the population without producing clearly noticeable outbreaks (Elbers et al., 1992)

The three commonest subtypes of SIV are H1N1, H1N2 and H3N2. In Europe, since 1979 the dominant H1N1 viruses have been 'avian-like' H1N1 viruses (Brown et al., 2000), while the most common H3N2 strains have been human-avian reassortants between hemagglutinin (HA) and neuraminidase (NA) genes from human-like swine H3N2 virus and the internal proteins from avian-like swine H1N1 virus (Castrucci et al., 1993; Campitelli et al., 1997). Finally, "triple reassortant" H1N2 contains HA from H1N1 human influenza virus, NA from swine H3N2 and internal proteins from avian-like swine H1N1 virus (Brown et al., 1998). However, the origin and nature of swine influenza

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Abbreviation: ELISA, enzyme linked immunoassay.

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strains are different depending on their geographical location (Olsen et al., 2006). Thus, European H1N1, H3N2 and H1N2 SIV subtypes are genetically and antigenically different from those of North America (Kothalawala et al., 2006).

The HI, which is the classical serological test for detecting antibodies against SIV, is subtype-specific and is thought to be precise enough to discriminate between infections with different SIV subtypes provided that the viruses used as antigens in the test and the strains circulating in a region are antigenically close (Brown et al., 1998; Van Reeth et al., 2000, 2006).

The recent emergence of the new human pandemic A/H1N1 influenza virus, a triple human-swine-avian reassortant, was an example of the importance of pigs in the epidemiology of influenza. In Spain, as well as in other countries of Europe, non-random serological surveys revealed a high seroprevalence of H1N1, H1N2 and H3N2 in sows (Maldonado et al., 2006) and fattening pigs (Fraile et al., 2009) but knowledge on the risk factors for the introduction and spread of the infection in farms is scarce. The aims of the present study were: (i) to estimate the seroprevalence of H1N1, H1N2 and H3N2 subtypes in sows and fattening pigs and (ii) to evaluate the potential risk factors associated with seropositivity to the different SIV subtypes in pig farms from Spain.

2. Materials and methods

2.1. Study design

A cross-sectional survey (2008–2009) was designed to estimate the seroprevalence of different SIV subtypes. Sampling was restricted to Spanish pig census, which includes approximately 25,000,000 pigs located in about 94,000 herds. Approximately, 52% of the Spanish farms have an average stocking rate of more than 120 large animal units (Anonymous, 2010). Sampling was planned to be initially conducted in farms having more than 80 sows but finishing herds were excluded. With this restriction, 10 regions which accounted for 96.2% of the Spanish farms were included. Considering the previous data (Maldonado et al., 2006), an expected prevalence of 50% was assumed. Then, given the number of farms in Spain ($n > 10{,}000$), the precision (which was set at ±10%) and the confidence level (which was set at 95%), the sample size (97 farms) was obtained.

For practical reasons, one hundred farms were considered, and the sampling was stratified by regions according to the proportion of farms in each one. Within each farm, 14 sows were randomly sampled – which was enough to detect antibodies against a given subtype if it was present in more than 20% of the sows – and, when available, samples from 10 finishing pigs were also obtained; enough to detect antibodies against a certain subtype if it affected more than 25% of fatteners. Ages of fattening pigs sampled ranged from 11 to 20 weeks, ensuring the absence of maternal antibodies and time enough for the exposure to influenza viruses. In total, 1400 sows and 849 fattening pigs were sampled. Unfortunately, sows from seven farms had been vaccinated against influenza in the previous 2 years, and as a consequence 98 sows samples were excluded from the analysis. In regions where the animal health authorities participated in the sampling (Andalusia, Castilla la Mancha, Catalonia, Extremadura, Galicia, and Navarra; representing 55% of the total Spanish census), farms were fully selected at random (random numbers applied to the registry reference number of the farm). In the other four regions, full random sampling was not always possible and, when needed, a convenience sampling, based on the availability of swine practitioners, was used to complete the selection of farms. At the end of the study a total of 85 farrow-to-finish and 13 farrow-to-weaning farms were surveyed. In total 1302 samples from sows collected in 93 pregnancy units (considering a unit as the whole group of sows of each farm), and 849 samples from fattening pigs collected in 85 fattening units (pigs between 11 and 20 weeks of age of each farm) were analysed. The geographical location of the farms is represented in Fig. 1.

2.2. Data collection: the questionnaire

Epidemiological data were gathered through an on-farm interview with the farmer. The questionnaire was designed using only "close-ended" questions to avoid ambiguities. Variables were grouped by topic: (a) general data: identification, location, herd size, presence of other domestic animal species (cats, dogs, birds or cattle), all-in/all-out (AIAO) management system and distance to the nearest farm; (b) production and health parameters: number of pigs per production phase (sows, weaners, fatteners and finishing pigs, boars), percentage of mortality in suckling pigs, weaners and fatteners, vaccination program and records of enteric and respiratory disease outbreaks during the last year; (c) facilities: floor type, floor material, presence of outdoor pens, type of waterers and feeders; (d) biosecurity: origin of replacement gilts

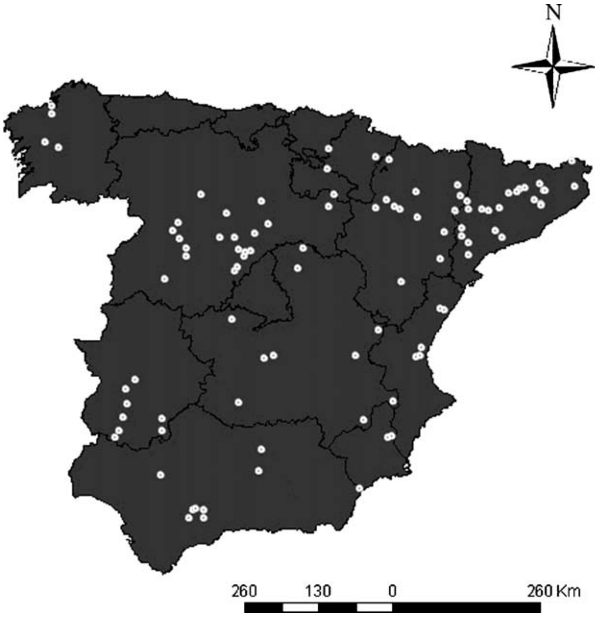

Fig. 1. Distribution of analysed farms (white dots) in Spain.