

Biosecurity assessment of Argentinian pig farms

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ABSTRACT

The pig industry is growing very fast in Argentina with an increasing need for replacement animals, feedstuff and transportation of animals. One of the main competitive advantages of the Argentinian pig industry is its being free of most major pig diseases. Within this context, applying measures aimed to reduce the risk of introduction and spread of pathogens is critical. The aim of the present study was to assess the biosecurity of Argentinian pig farms. Two types of farms were assessed: firstly, all official suppliers of high-genetic-value ($n = 110$) and secondly, a sample from commercial farms ($n = 192$). Data on the external and internal biosecurity practices applied on the farms was collected with a questionnaire. Data was analysed using a correspondence analysis and a hierarchical clustering analysis, which allowed identification of types of farms with regard to the biosecurity measures applied. Key variables characterizing the clusters were identified through an indicator value analysis. In addition, the external biosecurity of the farms was evaluated by using risk assessment tools with respect to the potential introduction of porcine epidemic diarrhoea virus. Results made evident three clusters: the first one which, amongst other measures, applied several barriers to prevent the entry of people, trucks and other vehicles, and could be considered as a group of high biosecurity, and the two other groups which applied a lower number of external and internal biosecurity measures. The results of the risk assessment showed that the routes with the highest risk of disease introduction were: replacement animals, vehicles transporting feed or animals, and visitors. The assessment of the external biosecurity showed that most Argentinian farms were not prepared for the contingency of a pathogen such as porcine epidemic diarrhoea virus. Special efforts should be made in official suppliers of high-genetic-value farms with poor biosecurity scores since they are at the top of the pig production chain and can be key for the spread of diseases.

1. Introduction

Biosecurity is defined as the "... implementation of measures that reduce the risk of the introduction and spread of disease agents; it requires the adoption of a set of attitudes and behaviours by people to reduce risk in all activities involving domestic, captive/exotic and wild animals and their products" (FAO, 2008). At the farm level, biosecurity measures may focus either on reducing the risk of entry of new pathogens (external biosecurity) or on reducing the internal dissemination of pathogens (internal biosecurity) (FAO, 2010).

Biosecurity is founded on knowledge of the epidemiology of transmissible diseases, including the duration of the contagiousness period in infected animals, the main routes of pathogen shedding, the survival of

the pathogen in the environment, and the routes of infection. This knowledge allows technically appropriate measures to be designed. However, it is also important to consider the socioeconomic aspects of proposed measures, as these will have an impact on their compliance (FAO, 2010).

In pig farms, a lack of biosecurity measures or the application of poorly chosen ones may lead to several disease outbreaks, including foot and mouth disease (FMD), classical swine fever (CSF), Aujeszky's disease, and porcine epidemic diarrhoea (PED) (Elbers et al., 2001; Amass et al., 2004; Olugasa and Ijagbone, 2007; Ellis-Iversen et al., 2011; Dekker, 2014; Lowe et al., 2014; Kim et al., 2017).

Argentina has a very strong tradition of livestock production, mainly for beef. However, in recent years, pig production has been

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growing at a rate of > 5% per year, reaching about 1 million in commercial and genetic farms in 2017, of which around 255,000 were in medium and large farms, according to official statistics (<http://www.senasa.gob.ar/cadena-animal/porcinos/informacion/informesyestadisticas>).

One of the competitive advantages of Argentinian pig production is that it is free of some of the most important pig diseases, such as the porcine reproductive and respiratory syndrome (PRRS) (Monterubbianesi et al., 2016; Carpinetti et al., 2017), CSF, FMD, and PED, which are still present in many countries of South America (http://www.oie.int/wahis_2/public/wahid.php/Countryinformation/Animalsituation).

However, the sustained growth and the high intensification of the new farms create a need for more replacement animals of higher genetic value and more movement to and from farms to ship the animals and the feedstuff. Within this scenario the need to apply more biosecurity measures is evident. Based on the above, the aim of the present study was to assess the biosecurity of Argentinian pig farms by: i) describing the biosecurity measures applied in pig farms supplying replacement animals; ii) identifying typologies of farms based on the level of application of biosecurity measures; and iii) evaluating their external biosecurity with risk assessment tools.

2. Material and methods

2.1. Farms, data collection and validation of the questionnaires

The survey was conducted in Argentinian pig farms in 2015 and 2016 included two studies. The first one, carried out during 2015, comprised all the farms officially registered by the Argentinian authorities as companies supplying breeders of high genetic value ($n = 110$). These farms were geographically distributed as follows: 38 (34.5%) in Buenos Aires province, 30 (27.2%) in Córdoba province, 18 (16.3%) in Santa Fe province, 5 (4.5%) in Entre Ríos province - these being the main pig-producing provinces in Argentina - and the remaining 19 farms (17.5%) in Chaco, Chubut, San Juan, Neuquén, La Pampa, La Rioja, Río Negro, and San Luis provinces. The second study, performed during 2016, focused on the evaluation of 355 commercial farms, 319 of which had 100–500 sows (125 in Buenos Aires province, 93 in Córdoba province, 21 in Entre Ríos province, and 80 in Santa Fe province) and 36 had ≥ 500 sows (12 in Buenos Aires province, 14 in Córdoba province, 4 in Entre Ríos province, and 6 in Santa Fe province). Of both kinds of (genetic and commercial) farms, 98–98.5 % operated as farrow-to-finish farms while the remaining 1.5–2% were exclusively breeding and nursery farms. In this second study, the sample size ($n = 355$) was calculated considering a variation in the frequency of application of biosecurity measures of 50%, a confidence level of 95%, and a 5% accuracy. The study population (i.e. farms with more than 100 sows/farm) was classified by province and number of sows on the farm. The farms included in the study were randomly selected within each class based on the official registry number of each farm.

The questionnaire used for collecting data on the biosecurity measures applied was the same for both the genetic and commercial farms. This questionnaire included a total of 126 questions and was divided into sections, including: a) general data of the farm such as location, number of sows, distance to neighbouring farms, etc., b) external biosecurity measures related to replacement animals, vehicles, visitors and geographic risk (e.g. perimeter fence), and c) internal biosecurity measures as regards to management, installations, cleaning and personnel routines. Supplementary Table S1 shows the number of questions for the different categories included in the questionnaire.

A draft questionnaire was first tested for clarity and adequacy in four farms (two with > 100 sows and two with ≥ 500 sows) by means of a personal interview with the farmer. After making some amendments, the questionnaire (available in Spanish on request) and the

guidelines for completing it, were distributed through the Argentinian National Service for Health and AgriFood Quality (SENASA) to veterinary officers, who visited the farms and supervised the collection of the data. Before the on-farm data collection, the veterinarians in charge of that task attended a workshop where they were instructed on the correct way to complete the questionnaire.

Since it was the first time that this type of survey was carried out at a national level, in the first study, two thirds of the questionnaires were verified by means of a telephone call to the farmer, the veterinarians, and the laboratories. This was done to assess the quality of the information collected. Data was stored in a database created with the Epi Info software (Dean et al., 2011). In the second study, using the same questionnaire, and given the results of the assessment from the first survey which showed no major discrepancies between submitted and checked data, the collected data was not verified.

2.2. Assessment of farm type based on the biosecurity measures applied

About 40% (50/125) of the questions were excluded from this analysis because they were determined to be redundant to the main question or had a relatively low rate of response. Variables which were included in this analysis can be found in the Supplementary Tables S2a and S2b. Since most of the questions were categorical, continuous variables (4/70) were categorized to allow a similar analysis for the entire questionnaire. The frequency of application of the different biosecurity measures were calculated and the confidence interval for the resulting proportions were estimated using the VassarStats website (<http://vassarstats.net/>), whose calculations are based on methods described by Newcombe Robert (1998). To explore the existence of farms with different models of external and internal biosecurity measures, a correspondence analysis and a hierarchical grouping analysis were performed. To avoid the bias derived from the fact that some farms used only external replacement stock while others used only internal replacements, data were analysed in two ways: 1) including all farms disregarding variables related to replacement animals and 2) those farms with external replacements exclusively.

The Multi-Response Permutation Procedure Test (MRPP), a non-parametric method to test multivariate differences among pre-defined groups, was used to test the statistical significance of the clusters. After determining the existence of different significant clusters, an indicator value analysis was performed (Dufrene and Legendre, 1997) to determine which variables could significantly characterize each group. The observed Indicator Value (OIV) of variable I in group p is the product of two quantities: $A \cdot B$, where $A = np/n$ and $B = np/Np$ (Np : total number of farms belonging to group p (target farms group), n : number of occurrences of the variable I among all farms, np : number of occurrences of the variable I among the target farms group p). Then A is the proportion of farms with security variable I that belong to the target group p and B is the relative frequency of the variable i among the farms belonging to the target group p (Caceres and Legendre, 2009). All the analyses were done using the PC ORD v6 software (McCune and Mefford, 2011).

2.3. Evaluation of the external biosecurity by using risk assessment tools

To evaluate the external biosecurity of the herds, we used the risk assessment tool developed by Allepuz et al. (2018) in a hypothetical scenario of an epidemic episode of porcine epidemic diarrhoea virus (PEDV) in Argentina. PEDV is a highly contagious enteric virus of pigs transmitted by the fecal-oral route. In farms with no previous immunity, suckling piglets suffer severe watery diarrhoea with fatality rates reaching 50–100% (Straw et al., 2006). The above-mentioned approach allowed both the estimation of a score for the annual probability of disease introduction and to decide where to concentrate the effort to reduce this risk. Briefly, the approach comprises five steps: i) identifying the possible routes of disease introduction and key

parameters for each route (e.g. herd prevalence and within-herd prevalence in affected farms); ii) calculating a score for the probability of each route harbouring the disease agent upon arrival at the farm; iii) conducting an expert opinion workshop to obtain a score for the different input parameters; iv) calculating the risk mitigation (reduction of the probability of introduction by a given route after applying a biosecurity measure); and v) calculating a final score of the probability of disease introduction for each route.

Based on the epidemiology of PEDV and on the Argentinian context, six routes of introduction of the disease were considered: i) replacement animals, ii) vehicles transporting replacement animals, iii) vehicles transporting animals to the slaughterhouse, iv) vehicles transporting feed, v) people visiting the farm, and vi) geographical risk (i.e. from a neighbouring farm, a slaughterhouse or a road). The risk associated with trucks transporting cadavers or manure was not considered since in Argentina each farm eliminates these materials by itself (e.g. through pits, composting, etc.). Parameters considered for the arrival of PEDV at the farm through the different routes are described in the Supplementary Table S3. An expert opinion workshop aiming to obtain the scores for the different input parameters was carried out following the OIE recommendations (OIE, 2004). The workshop was a one-day meeting with 18 veterinarians and researchers actively involved in swine practice and animal health in Argentina. The Supplementary Table S4 provides details of the selected experts, including their background, years of expertise, and main area of work. Specifically, the meeting began by presenting the concept of what an expert opinion workshop is, followed by instructions on how to assign values. Experts were asked to provide ordinal values in a 0–9 scale, as proposed by Dufour et al. (2011) for expert opinion panels. This was done individually without discussion allowed at this stage. Subsequently, answers were compiled and histograms showing the distribution of the values assigned by the members were shown for group discussion. During this discussion, the members had the chance to change their values.

For the input parameters representing proportions (such as the PEDV herd prevalence), a uniform probability distribution was used. This type of distribution is defined with a minimum and a maximum value, and a continuous spectrum of values occurs with the same probability within those values. Pert probability distributions were used for the input parameters representing the importance of biosecurity measures to reduce the probability of virus introduction obtained from the workshop. These distributions are defined by the minimum, the most likely, and the maximum values, which are useful to model expert opinion (OIE, 2004).

The models were run using the mc2d package (Pouillot and Delignette-Muller, 2010), implemented in R (R Development Core Team, 2008). Monte Carlo simulations (10,000 iterations) were performed and all non-fixed input parameters were included as uncertain parameters. The values for the prevalence of PEDV-infected herds and infected animals within infected herds were obtained from Beam et al. (2015), who studied 222 sites in the United States during the 2013 PEDV epidemic.

3. Results

3.1. Official suppliers of high-genetic-value farms

3.1.1. Response rates and application of different biosecurity measures

All the genetic suppliers answered the questionnaire. The frequencies of application of the different external and internal biosecurity measures in those farms are shown in the Supplementary Tables S2a and S2b. The question response rate was: 100% for 68/126 questions (54.0%; lower and upper limits of the 95% confidence interval = $CI_{95\%}$: 45.7–62.4); between 95% and 99.9% for 31/126 questions (24.6%; $CI_{95\%}$: 17.9–32.8); between 90% and 95% for 8/126 questions (6.4%; $CI_{95\%}$: 3.3–12.0); and between 80 and 90% for 10/126 questions (7.9%;

$CI_{95\%}$: 4.4–14.0) of which six were related to the trucks transporting animals from quarantines. Only two questions (1.6%; $CI_{95\%}$: 0.4–5.6) had a response rate < 80%.

It is worth mentioning that most of the genetic farms introduced gilts from external sources (77/110; $CI_{95\%}$: 63.6%; 54.5–72.0) and that nearly half of them (35/77, 45.5%; $CI_{95\%}$: 34.8–56.5) introduced them at least four times a year. Several farms introducing external gilts (36/77, 47.0% $CI_{95\%}$: 35.4–58.4) transported the animals by using trucks that had been in contact on the same day with other farms or pigs of other origins. In addition to this, most of the farms that reported introducing external gilts (38/77, 49.4%; $CI_{95\%}$: 38.5–60.3) did not have quarantine facilities.

With regard to vehicles arriving at the farm, trucks transporting feedstuff, trucks that collected pigs to be sent to the slaughterhouse, and private vehicles were allowed to enter the farm premises in 73/110 (66.4%, $CI_{95\%}$: 57.1–74.5), 77/110 (70.0%, $CI_{95\%}$: 60.9–77.8), and 39/110 (35.5%; $CI_{95\%}$: 27.1–44.7) of the farms, respectively. In addition, 63/77 (81.8%; $CI_{95\%}$: 71.8–88.9) farms introducing external gilts lacked specific loading/unloading docks for them.

With respect to visitors, 49/110 (44.6%; $CI_{95\%}$: 35.6–53.9) of the farms received more than one visit per week. Also, 33/110 (30.0%; $CI_{95\%}$: 22.2–39.1) of the farms had a compulsory shower on entry, 69/110 (62.7%; $CI_{95\%}$: 53.4–71.2) required the use of clean clothes exclusively provided by the farm, and only 19/110 (17.3%; $CI_{95\%}$: 11.3–24.4) had a written biosecurity protocol for visitors.

Regarding internal biosecurity, between one third and one half of the farms did not apply basic internal biosecurity measures, such as an ‘all-in/all-out’ policy (namely the moving of entire batches of animals in or out of the facilities to avoid mixing).

3.1.2. Correspondence and cluster analysis

In the correspondence analysis done with the 110 genetic farms (Fig. 1a and b), axes 1 and 2 explained 17.9% and 6.9% of the variance, respectively. When the analysis was performed with the farms that only used external sources of gilts ($n = 77$), axes 1 and 2 explained 19.4% and 7.7% of the variance, respectively. The hierarchical cluster analysis resulted in the identification of three significant groups (MRPP, $p < 0.0001$) when considering all 110 farms and equally when analysing the farms that only purchased external replacements (Fig. 1). The indicator values are calculated to measure the strength of association of each variable with the different farms groups. For predictive purposes, the list of variables strongly associated to the farm groups has a great interest as diagnostic variables. The observed Indicator Value (OIV) associated with each cluster within the commercial and genetic farms are shown in Tables 1 and 2. Cluster 1 was associated with 26 external and 20 internal biosecurity measures. With respect to external biosecurity, the measures related to the entry of personnel on the farm were important; for example: a compulsory shower (OIV: 86.4%), a compulsory hand wash (OIV: 83.5%), and compulsory use of clean boots and clothes (OIV: 56.4%), followed by measures related to the entry of animals such as the use of a loading dock with clean and dirty areas (OIV: 43.1%) and the restriction of entry for trucks into the farm perimeter (OIV: 30.5%). The other clusters were associated only with two or three measures with lower OIV (25–40%).

3.2. Commercial farms

3.2.1. Response rates and application of different biosecurity measures

In the case of the commercial farms, the response rate was 55.8% (198/355; $CI_{95\%}$: 50.6–60.9). In the subsequent analysis, six questionnaires were discarded due to a low response rate of the questions, and thus only 192 farms were analysed. Of these, 185/198 were farms with 100 to 500 sows and 13/198 with > 500 sows. By provinces, 90/198 (45.5%; $CI_{95\%}$: 38.7–52.4) farms were from Buenos Aires, 52/198 (26.3%; $CI_{95\%}$: 20.6–32.8) from Santa Fe, 34/198 (17.2%; $CI_{95\%}$: 12.6–23.0) from Córdoba, and 22/198 (11.1%; $CI_{95\%}$: 7.5–16.3) from

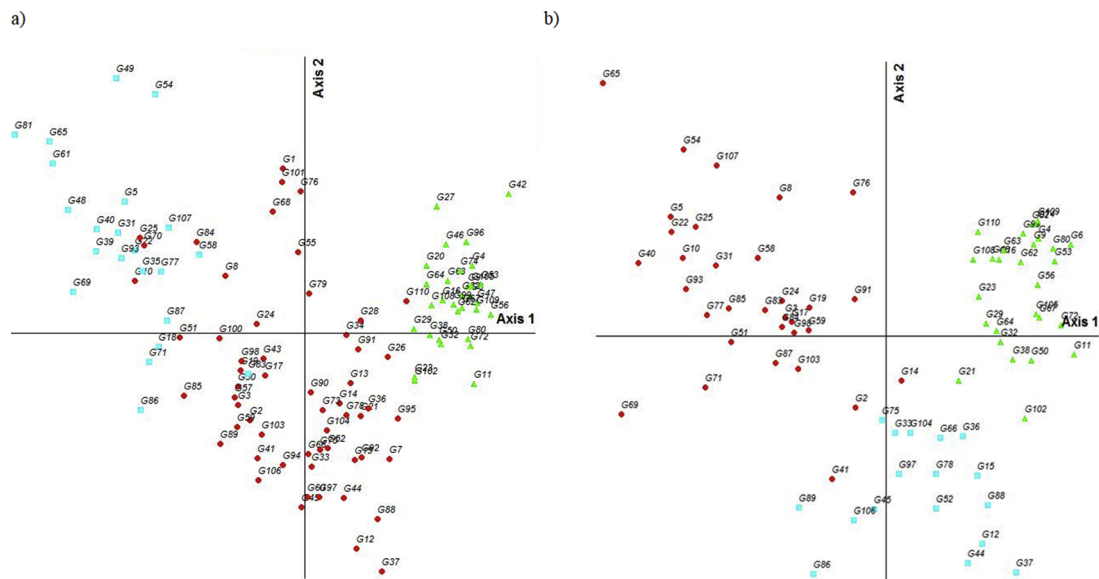


Fig. 1. Results of the cluster analysis for farms producing gilts of high genetic value. References: a) ▲ number of farms = 31, distance = 0.3, average of sows in production = 1275, ● number of farms = 52, distance = 0.57, average of sows in production = 171, ■ number of farms = 27, distance = 0.62, average of sows in production = 56. b) ▲ number of farms = 27, distance = 0.34, average of sows in production = 1226, ■ number of farms = 19, distance = 0.54, average of sows in production = 215, ● number of farms = 31, distance = 0.59, average of sows in production = 45.

Entre Ríos, thus resulting in a representative sample of the country.

The frequencies of application of different external and internal biosecurity measures in these farms are shown in the Supplementary Tables S2a and S2b. As can be observed, 156/192 (81.3%; CI_{95%}: 75.1–86.1) of the farms in this group purchased replacement gilts from external facilities and 49/156 (31.4%; CI_{95%}: 24.7–39.1) of these used two or more sources. In 79/156 (50.6%; CI_{95%}: 42.9–58.4) of the farms that purchased external gilts, replacement animals were transported in vehicles that could have visited other farms on the same day, whereas in 49/156 (31.4%; CI_{95%}: 24.7–39.1) of the cases, gilts from different origins could have been transported on the same truck. In 93/156 (59.6%; CI_{95%}: 51.8–67.0) of these farms, gilts arrived at the farm every 90 days or less. Only one farm had the quarantine unit outside the premises, at more than 1,000 m distance.

In 141/192 commercial farms analysed (73.4%; CI_{95%}: 66.8–79.2), trucks that transported animals to the slaughterhouse belonged to external companies, and in 42/192 (21.9%; CI_{95%}: 16.6–28.3) they could have loaded or unloaded pigs in other farms on the same day. In addition, 111/192 (57.8%; CI_{95%}: 50.7–64.6) of the farms did not have loading docks with delimited clean and dirty areas.

Concerning visitors, 82/192 (42.7%; CI_{95%}: 35.9–49.8) of the farms had less than one visitor per week and 64/192 (33.3%; CI_{95%}: 27.1–40.3) had a policy to restrict visitors. Clothes and boots were provided to visitors in 116/192 (60.4%; CI_{95%}: 53.4–67.1) and 143/192 (74.5%; CI_{95%}: 67.9–80.1) of the farms, respectively. Internal biosecurity measures within this group of farms (Fig. 2a and b) were also rarely applied and, in fact, no cleaning or disinfection procedures were carried out between different animal batches in 51/192 (26.6%; CI_{95%}: 20.8–33.2) of the farrowing rooms, in 41/192 (21.4%; CI_{95%}: 16.2–27.7) of the nursery units, and in 77/192 (40.1%; CI_{95%}: 33.3–47.2) of the fattening facilities.

3.2.2. Correspondence and cluster analysis

In the correspondence analysis in relation to the whole population of commercial farms analysed ($n = 192$), axes 1 and 2 explained 13.5% and 6.32% of the variance. In the analysis of the farms using external sources of gilts ($n = 153$), axes 1 and 2 explained 11.3% and 5.9% of the variance. The hierarchical cluster analysis for the whole population and equally that for farms with external gilts showed three significant

groups (MRPP, $p < 0.0001$) (Fig. 2). The indicator values that distinguished to a greater degree between groups of farms are shown in Tables 1 and 2. As shown in Table 1, clustering was defined by 41 variables, 38 of which were strongly associated with cluster 1. Furthermore, the dressing room with separate dirty and clean areas, the compulsory shower for visits, and the compulsory hand wash between stages of production were highly associated with that cluster (percentage of perfect indication = 64%, 52.6%, and 58.4%, respectively). Clusters 2 and 3 were characterized by three and five variables, respectively.

3.3. Risk scoring: evaluation of the external biosecurity

3.3.1. Expert panel meeting

The Supplementary Table S5 shows the scores provided by the experts for the different parameters. All members agreed on the importance that vehicles intended to transport animals to the slaughterhouse must not arrive loaded with animals from other farms (scores 8–9 on the 0–9 scale). However, with respect to the importance of disinfection of the truck after visiting the slaughterhouse, disagreement was higher (range of 0–9). There was also a high variability in the perception between experts about: the importance of measures related to quarantine (such as the location of quarantine facilities, use of exclusive personnel, or the importance of examining incoming gilts); the importance of barrier measures (such as sanitary fords or loading docks); the importance of workers not having contact with other pigs; and the measures related to visitors (e.g., the requirement of using boots and clothes provided by the farm).

3.3.2. Risk assessment

Figs. 3 and 4 show the mean values of the initial score for the probability of introduction, the risk mitigation, and the final score for the probability of PEDV introduction by each route for both the genetic and commercial farms. The results for both groups of farms were quite similar, showing that the routes with higher initial and final scores were: i) the introduction of replacement animals, ii) the vehicles transporting feedstuff, iii) the vehicles transporting animals, and iv) the visitors. The results also revealed that the application of biosecurity measures was quite variable in both groups. The risk mitigation for the

Table 1

Indicator Variables (% of perfect indication) for each biosecurity cluster not related to the replacement of animals in genetic and commercial farms.

Biosecurity Measures	Genetic Farms			Commercial farms		
	ID cluster	OIV (%)	<i>p</i> (value) *	ID cluster	OIV (%)	<i>p</i> (value) *
Semen produced in the farm	3	35.1	0.0326			
Presence of sanitary ford	1	46.7	0.0002	1	37.9	0.0002
Presence of disinfection arch	1	43.2	0.0002	1	17.5	0.0002
Presence of loading dock for each production phase	1	28.8	0.0234			
Truck for market animals:						
It belongs to the farm/company	3	33.7	0.0228	1	20.2	0.0076
It does not go to other farms on the same day				1	45.4	0.0002
It does not arrive with animals				1	40.3	0.0002
It is disinfected between every loading/unloading of animals	1	38.5	0.0034	1	46.2	0.0002
It is disinfected after taking the animals to the slaughterhouse				1	42.4	0.0002
It does not enter the perimeter of the farm	1	36.9	0.0006	1	32.6	0.0002
The dock has an enclosed clean / dirty area	1	45.3	0.0002	1	44.0	0.0002
Restrictions to the truck driver regarding the access to the farm	1	46.0	0.0002			
Treatment of carcasses by well	3	39.5	0.0020			
Treatment of carcasses by incineration	2	25.6	0.0206	3	17.2	0.0074
Treatment of carcasses by composting	1	18.2	0.0348			
Number of visitors (less than 1 per week)				1	34.5	0.0008
There is a policy restricting entry of persons	1	54.5	0.0002	1	43.4	0.0002
There is a record of visits	1	54.7	0.0002	1	46.2	0.0002
There is an office	1	52.5	0.0002	1	36.4	0.0006
There is a sign with instructions at the entry	1	36.7	0.0002			
Visitors must use boots provided by the farm (required)	1	53.8	0.0002	1	42.3	0.0002
Visitors must use clothes provided by the farm (required)	1	67.4	0.0002	1	44.0	0.0002
There is a dressing room	1	70.3	0.0002	1	48.3	0.0002
Showers are present	1	79.4	0.0002	1	48.0	0.0002
Visitors should take a shower upon arrival at the farm	1	86.4	0.0002	1	52.6	0.0002
The dressing room have dirty and clean areas are separate	1	89.6	0.0002	1	64.0	0.0002
Visitors must wash their hands before entering	1	83.5	0.0002	1	51.2	0.0002
The material used belongs to the farm	1	36.0	0.0226			
It is verified that the tools have been disinfected and not used on another farm	1	48.5	0.0002	1	41.8	0.0002
Tools and supplies of off-farm workers are washed and disinfected before being introduced in the farm	1	40.4	0.0006	1	53.9	0.0002
Farm workers must take a shower on entering the farm	1	88.0	0.0002	1	46.4	0.0002
Farm workers must change their clothes and boots upon arrival at the farm	1	56.6	0.0002	1	40.3	0.0002
Farm workers must wash their hands before moving between stages of production	1	54.8	0.0002	1	58.4	0.0002
The farm workers must change their boots in and out of each stage of production	1	54.6	0.0002	1	46.1	0.0002
There is a routine in the internal circulation of the farm workers	1	45.5	0.0002	1	41.5	0.0002
There is a perimeter fence	1	46.5	0.0002	1	34.0	0.0120
A systematic rodent control program is implemented	1	37.3	0.0034	1	35.1	0.0370
There are nets or meshes in the windows to prevent the entry of birds	1	65.3	0.0002	1	30.4	0.0258
A systematic disinfestation program is followed	1	39.2	0.0006	1	33.4	0.0022
The farm operates organized into groups to inseminate sows	1	53.8	0.0002			
There is a policy of adoption or movement of piglets	1	53.9	0.0002	2	36.3	0.0072
All in-All out in maternity	1	56.5	0.0002	2	38.2	0.0004
Animals from different weaning batches are not mixed	1	36.6	0.0060	3	31.3	0.0002
Animals from different weaning batches of weaning are mixed				2	37.2	0.0008
All in-All out in weaning	1	47.8	0.0002	2	35.5	0.0098
Uses Circovirus vaccine	1	40.9	0.0004	2	36.2	0.0040
Uses Mycoplasma vaccine	2	34.8	0.0486			
Animals from different fattening batches are not mixed	1	36.6	0.0022			
Animals from different fattening batches are mixed				2	31.2	0.0120
All in-All out in fattening units	1	39.1	0.0006			
The farm treat effluents	1	51.8	0.0002			
The effluent tank is located outside the perimeter of the farm	1	46.5	0.0002			
Drinking water for animals is potabilized	1	36.1	0.0016			
The farm uses hot pressurized water for cleaning	1	12.9	0.0084	1	9.8	0.0080
The farm uses cold pressurized water for cleaning	1	41.3	0.0002			
Brushed for cleaning	1	25.4	0.0314	1	22.8	0.0002
Allow to dry before disinfecting	1	39.5	0.0018			

References: ID Cluster = identification of the group to which each biosecurity measure constitutes an indicator value; OIV (%) = observed indicator values for each biosecurity measure; *p* (value).

* = Monte Carlo test of significance of the observed maximum indicator value based on 1000 randomizations for the hypothesis of no differences between groups.

different routes ranged from 0 to 0.95, indicating that some farms did not implement any measures while others had a high level of biosecurity. In addition, the median for the proportion of risk reduction was below 40% in all routes from both groups.

The introduction of replacement animals was one of the routes with the lowest application of biosecurity measures. The median for the proportion of risk reduction for this route was 7.3% in the genetic farms

and 12.8% in the commercial farms and for about 50% of the farms biosecurity measures to block this route were extremely low. On the other hand, the geographic risk had a low initial and final score for the probability of disease introduction, which correlates to the low pig density in the country.

Table 2
Indicator Variables (% of perfect indication) for each biosecurity clusters related to the replacement of animals, in both genetic and commercial farms.

Biosecurity measures	Indicator Values (%) Genetic Farms with external replacement - 2015		
	ID Cluster	OIV (%)	p (value)*
Location of replacement animals (Outside. > 1000 meters)	1	17.2	0.0354
Duration of the quarantine period (> 6 weeks)	1	24.3	0.0034
Replacement animals are analysed	1	25.8	0.0022
The truck transporting replacement animals does not enter the perimeter of the farm	1	30.5	0.015
The loading dock has clearly indicated clean/dirty areas	1	43.1	0.0002
Restrictions to the truck driver regarding the access to the farm	1	49	0.0002
Frequency of introduction of genetic animals (≥ 13 weeks)	3	39.9	0.001
The truck for transport of animals from official suppliers of high-genetic value belongs to the farm or company	3	54.4	0.0002
The truck transporting replacement animals does not go to other farms on the same day	3	48	0.0006
The truck transporting replacement animals does not arrive with animals	3	33.4	0.0448

Biosecurity measures	Indicator Values (%) Commercial farms with external replacement -2016		
	ID Cluster	OIV (%)	p (value)*
Duration of the quarantine period (> 6 weeks)	1	30.6	0.005
The truck transporting replacement animals is disinfected after each loading / unloading of animals	1	28.3	0.016

Reference: ID Cluster = identification of the group to which each biosecurity measure constitutes an indicator value; OIV (%) = observed indicator values for each biosecurity measure; p (value).

* = Monte Carlo test of significance of the observed maximum indicator value based on 1000 randomizations for the hypothesis of no differences between groups.

4. Discussion

The present study intended to assess the biosecurity of pig farms of Argentina, a country experiencing a very rapid growth in the pig population. The study focused firstly on farms producing replacement animals of high genetic value. Those farms are essential to sustain the continuous increase in pig production but the introduction of a major pathogen in one of them could have a catastrophic national impact.

The survey of genetic suppliers was exhaustive because it was compulsory as a part of the national pig health program. Since this was the first time that this type of survey was conducted in Argentina, the data was additionally verified by means of a telephonic interview with the veterinarians in charge of the farm or directly by visit. This additional verification of the data assured a very accurate picture of this

type of farm, reducing potential measurement biases. This verification was not performed in the group of commercial farms. For this second group, some measurement bias might exist as some farmers might have not answered what they really do on their farm. On the other hand, for some questions (mostly those related to quarantines) the response rate was low. In our opinion, the lack of an answer was related to the fact that some farms actually lacked quarantine facilities and also to the lack of knowledge of the importance of some biosecurity measures. These could have introduced some classification bias in the analysis. It would be desirable to do a future follow-up in order to update results, as the implementation of measures might change over time. For commercial farms the enrolment was voluntary, which resulted in a lower participation rate of about half of the farms within the categories examined. This voluntary participation could have introduced some selection bias.

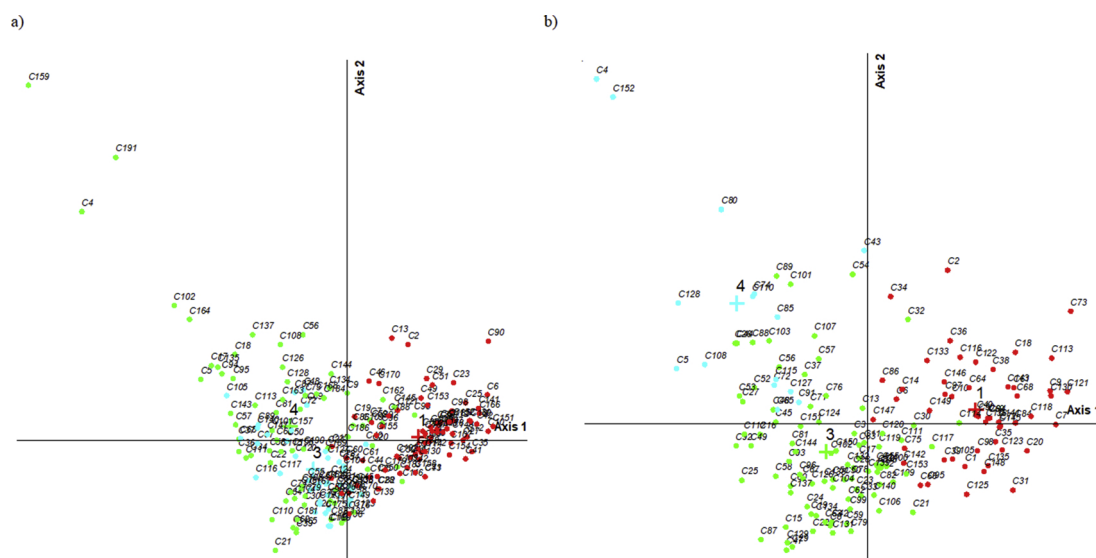


Fig. 2. Results of the cluster analysis for commercial farms: a) all farms disregarding variables related to replacement animals, and b) farms with external replacements and all variables. References: a) ●: number of farms = 44, distance = 0.35, average of sows in production = 329, ▲: number of farms = 72, distance = 0.46, average of sows in production = 214, ■: number of farms = 78, distance = 0.6, average of sows in production = 151. b) ●: number of farms = 51, distance = 0.43, average of sows in production = 303, ■: number of farms = 87, distance = 0.54, average of sows in production = 178, ▲: number of farms = 15, distance = 0.94, average of sows in production = 158.

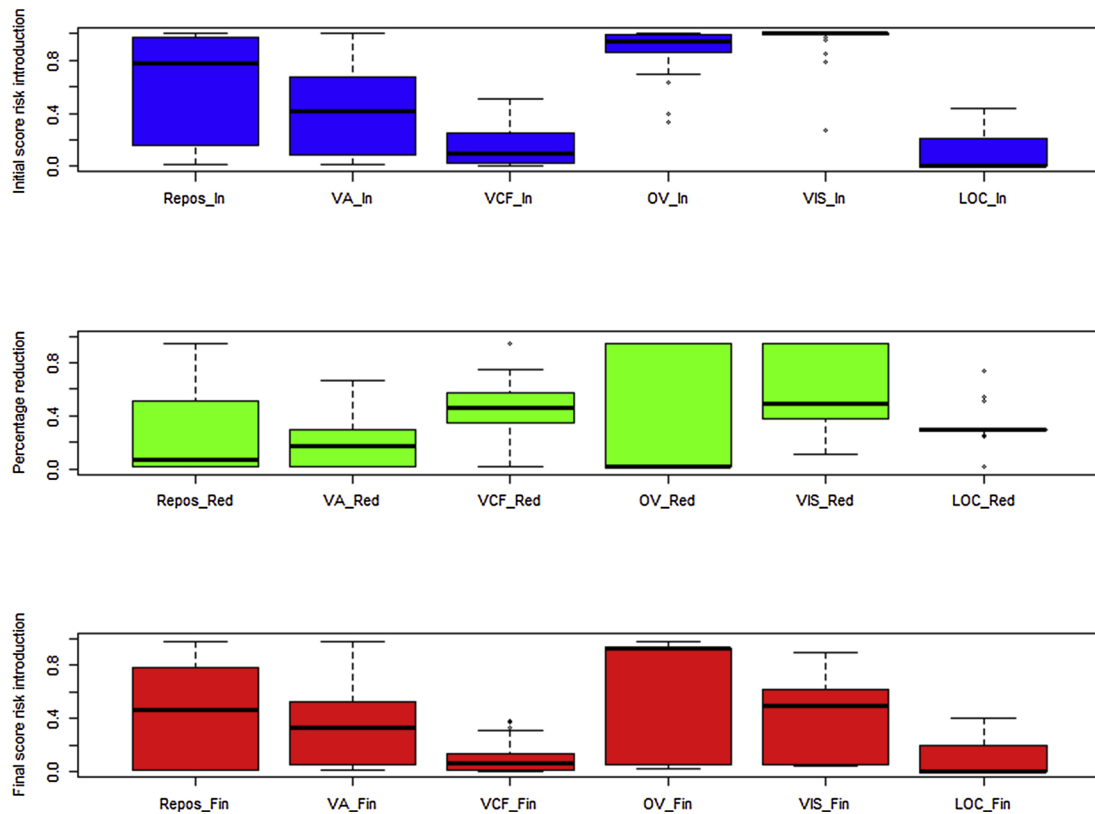


Fig. 3. The boxplots show the mean values for the initial risk of introduction, the percentage of reduction (i.e. risk mitigation) and the final score for the risk of PEDV introduction, for the 110 genetic farms assessed considering the different routes (Repos = replacement animals; VA = vehicles transporting replacement animals; VCF = vehicles transporting animals to the slaughterhouse; OV = vehicles transporting feed; VIS = visits, including farm workers; LOC = geographic risk).

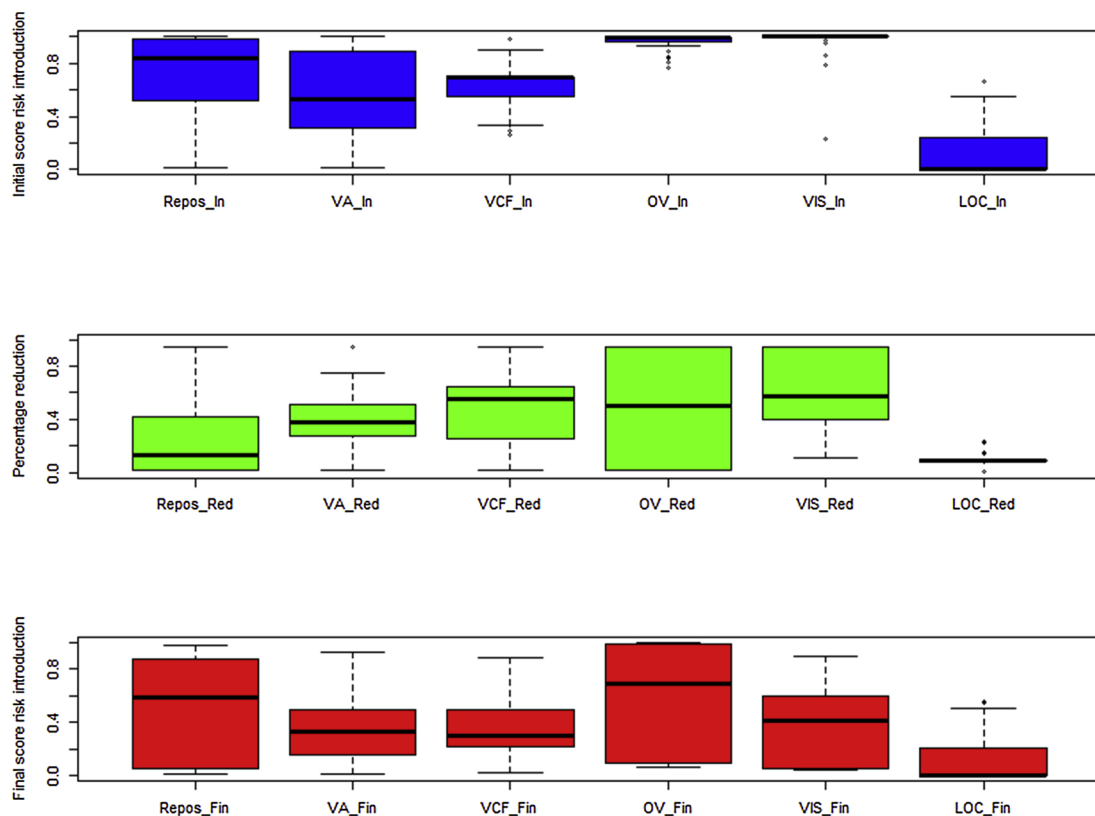


Fig. 4. The boxplots show the mean values for the initial risk of introduction, the percentage of reduction (i.e. risk mitigation), and the final score for the risk of PEDV introduction for the 192 commercial farms examined considering the different routes. (Repos = replacement animals; VA = replacement animals transport vehicles; VCF = slaughterhouse transport vehicle; OV = feed transport vehicle; VIS = visits, including farm workers; LOC = geographic risk).

Hierarchical cluster analysis revealed three types of farms in terms of the biosecurity practices, across both providers of genetic and commercial farms. These three clusters were also significant within all farms and only those purchasing external gilts. This reinforces the notion that the clusters found truly represented the types of farms present in Argentina.

For both types of farms, the first cluster had in common several measures such as the existence of strict barriers preventing the entry of people, trucks and other vehicles to the farm, with clear indication of clean and dirty areas, representing in all likelihood high biosecurity operations. Most of the farms in this group were new or belonged to large companies. The second and third clusters represented farms with an intermediate and a low level of biosecurity, respectively. These results agree with those found by [Bottoms et al. \(2013\)](#) and [Laanen et al. \(2013\)](#), who observed that the larger and more modern farms implement more biosecurity measures. In our case, most of the larger farms are new and belong to large companies with a high technical standard.

Although three types of farms were identified, the percentage of variance explained by the analysis was relatively low (25–27% in breeders and 17–20% in commercial farms). This suggests that the combination of biosecurity measures adopted by a given farm has a certain degree of randomness and, consequently, the clusters contain some internal heterogeneity. In our opinion, this is an indication of the complexity surrounding decision making and the implementation of biosecurity measures. Beyond the technical level or the size of the farm, the diversity probably arises from the diverse level of expertise and experience of veterinarians and producers, their personalities, and their connection with sources of technical information ([Racicot et al., 2012](#); [Alarcon et al., 2013](#); [Simon-Grifé et al., 2013](#); [Nantima et al., 2016](#)). Besides this, the fact that Argentina is free of most of the main pig diseases may also influence the perception of any need to implement biosecurity programs. Indeed, previous research noticed an increase in the biosecurity standards after the introduction of a new disease in neighbouring countries such as Uruguay and Chile ([Nöremark et al., 2009](#)).

In the present study, the evaluation of external biosecurity with regard to the introduction of PEDV showed that most Argentinian farms are not prepared for such eventuality. PEDV is an extremely transmissible agent with a very low minimum infective dose ([Thomas et al., 2015](#)). If introduced in Argentina it would be very difficult to prevent its entry in the farms as has happened recently in different countries of America.

There are some tools that may be used to compare the biosecurity status between pig farms or for farm-specific counseling ([Pinto and Urcelay, 2003](#); [Laanen et al., 2013](#); [Postma et al., 2016](#); [Holtkamp et al., 2013b](#)). All these tools are based on values obtained through expert opinion panels. In the present study, we used a methodology based on the risk assessment tool recently developed by our group ([Allepuz et al., 2018](#)), also using expert opinion panels. Since the opinion of experts may vary depending on the features of a given disease, the epidemiological circumstances of a country, or the prevailing ideas at a given moment, scoring systems based on perceptions must be adapted to each situation. Here, we conducted an expert opinion workshop with Argentinian veterinarians to adapt the values to the context and situation of the country. The 18-person panel was composed of veterinarians working in the pig sector whose expertise included the most common profiles (health, husbandry, etc.). Because of this diversity, some persons could be more sensitive to risks than others.

In our analysis, the routes identified with the greatest risk for the entry of PEDV into farms were the transport vehicles of replacement animals and feed, the visitors, and the replacement of animals. Our results are consistent with those of other studies where the vehicles and their drivers, the clothing and boots, the workers and materials for the farm were identified as ways of transmitting PEDV ([Kim et al., 2017](#); [Lowe et al., 2014](#); [Dee et al., 2015](#); [Scott et al., 2016](#)). It is worth mentioning that Argentina is a very large country and movements of

animals between farms or slaughterhouses can involve distances of up to one thousand kilometres. For this reason, the costs of transportation are high and the distribution of young sows to medium and small farms or the transportation of animals to the slaughterhouse is usually carried out by a truck serves several farms on the same day, with the consequent risks.

The lack of significant differences in the external biosecurity scores when comparing farms that sell high-genetic-value animals and commercial farms is in some way surprising. However, this could be due to the heterogeneous composition of the group of genetic farms. In Argentina, high genetic value are sold by large modern farms with high biosecurity standards, but also by some small farms (on average about 50 sows) with a low level of compliance with biosecurity measures. Two facts stand out in relation to the application of biosecurity measures: the diversity of measures applied and the lack of basic measures, such as an isolated quarantine in many of them. The first fact suggests a lack of consensus on the minimum biosecurity standard of this type of Argentinian farm. We believe that this consensus is necessary to establish appropriate biosafety guidelines. In this regard, international actions leading to the development of such consensus guidelines would be of great help for the pig industry in Argentina and elsewhere. The second fact, or the lack of some basic measures, is more local and implies a serious risk because of the central role of genetic farms as providers of replacement animals and therefore a potential disseminator of diseases in this country.

In summary, the present study shows a nationwide application of a biosecurity assessment methodology that allowed the characterization of pig farms and their typological classification. This methodology allowed detecting biosecurity gaps and identifying farms with poor biosecurity that could be critical to the whole pig production system. The results of the present study may help veterinarians, producers, and health authorities to establish plans to improve biosecurity against enteric pathogens such as PEDV. The results may also be useful for the design of education programs on biosecurity. The combination of this methodology with others, such as the analysis of movement networks, can greatly improve the biosecurity of pig farms at a regional scale. In the present case, the introduction of PEDV was used as a scenario, but the results could be easily extrapolated to other pathogens and countries.

5. Conclusion

The application of biosecurity measures in Argentinian pig farms was diverse and some of the biosecurity gaps identified in this study represent a high risk for the pig sector. Special efforts to improve should be made by the suppliers of breeder animals with poor biosecurity standards, since they are at the top of the production chain. Based on this study and the identification of the routes with higher risk of introduction of enteric pathogens such as PEDV to Argentinian farms, veterinarians and farmers should pay special attention to the biosecurity measures related to the movement of replacement animals, the transport of feedstuff, and visits. The results of this study could be useful to improve the application of biosecurity measures, and thus reduce the risk of disease dissemination. Moreover, it provides information on the points that should be addressed in the training of professionals and farmers in the country.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the

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References

- Alarcon, P., Wieland, B., Mateus, A.L.P., Dewberry, C., 2013. Pig farmers' perceptions, attitudes, influences and management of information in the decision-making process for disease control. *Prev. Vet. Med.* 116, 223–242.
- Allepuz, A., Martín-Valls, G.E., Casal, J., Mateu, E., 2018. Development of a risk assessment tool for improving biosecurity on pig farms. *Prev. Vet. Med.* 153, 56–63.
- Amass, S., Mason, P., Pacheco, J., Miller, C., Ramirez, A., Clark, L., Ragland, D., Schneider, J., Kenyon, S., 2004. Procedures for preventing transmission of foot-and-mouth disease virus (O/TAW/97) by people. *Vet. Microbiol.* 103, 143–149.
- Beam, A., Goede, D., Fox, A., Mccool, M.J., Wall, G., Haley, C., Morrison, R., 2015. A porcine epidemic diarrhea virus outbreak in one geographic region of the United States: descriptive epidemiology and investigation of the possibility of airborne virus spread. *PLoS One* 10 (12), e0144818. <https://doi.org/10.1371/journal.pone.0144818>.
- Bottoms, K., Poljak, Z., Friendship, R., Deardon, R., Alsop, J., Dewey, C., 2013. An assessment of external biosecurity on southern Ontario swine farms and its application to surveillance on a geographic level. *Can. J. Vet. Res.* 77, 241–253.
- Caceres, M., Legendre, P., 2009. Associations between species and groups of site: indices and statistical inference. *Ecology* 90, 3566–3574.
- Carpinetti, B., Castresana, G., Rojas, P., Grant, J., Marcos, A., Monterubbiansi, M., Sanguinetti, H.R., Serena, M.S., Echeverría, M.G., Garciaarena, M., Aleksa, A., 2017. Determinación de anticuerpos contra patógenos virales y bacterianos seleccionados en la población de cerdos silvestres (*Sus scrofa*) de la Reserva Natural Bahía Samborombón, Argentina. *Analecta Vet* 37, 21–27.
- Dean, A.G., Arner, T.G., Sunki, G.G., Friedman, R., Lantinga, M., Sangam, S., Zubieta, J.C., Sullivan, K.M., Brendel, K.A., Gao, Z., Fontaine, N., Shu, M., Fuller, G., Smith, D.C., Nitschke, D.A., Fagan, R.F., 2011. Epi Info™, A Database and Statistics Program for Public Health Professionals. CDC, Atlanta, GA, USA.
- Dee, S., Neill, C., Clement, T., Singrey, A., Christopher-Hennings, J., Nelson, E., 2015. An evaluation of porcine epidemic diarrhea virus survival in individual feed ingredients in the presence or absence of a liquid antimicrobial. *Porcine Health Manag.* 9 (1), 9.
- Dekker, A., 2014. Biosecurity and FMD Transmission. *Veterinary Record*, pp. 126–127.
- Dufour, B., Plée, L., Moutou, F., Boisseleau, D., Chartier, C., Durand, B., Ganière, J.P., Guillotin, J., Lancelot, R., Saegerman, C., Thébault, A., Hattenberger, A.M., Toma, B., 2011. A qualitative risk assessment methodology for scientific expert panels. *Rev. Sci. Tech.* 30, 673–681.
- Dufrene, M., Legendre, P., 1997. Species assemblages and indicator species: the need for a flexible asymmetrical approach. *Ecol. Monogr.* 67, 345–366.
- Elbers, A., Sregeman, J., Jong, M., 2001. Factors associated with the introduction of classical swine fever virus into pig herds in the central area of the 1997/1998 epidemic in The Netherlands. *Vet. Rec.* 149, 377–382.
- Ellis-Iversen, J., Smith, R., Gibbens, J., Sharpe, C., Dominguez, M., Cook, A., 2011. Risk factors for transmission of foot and mouth disease during an outbreak in southern England in 2007. *Vet. Rec.* <https://doi.org/10.1136/vr.c6364>.
- Food and Agriculture Organization of United States, 2008. Biosecurity for Highly Pathogenic Avian Influenza. Issues and options, Rome 73 pp.
- Food and Agriculture Organization of United States, 2010. Good Practices for Biosecurity in the Pig Sector. the food and agriculture organization of the United Nations the World organization for animal health the world bank, Rome 2012.
- Holtkamp, D., Lin, H., Wang, C., Polson, D., 2013b. Development and validation of an objective risk scoring system for assessing the likelihood of virus introduction in porcine reproductive and respiratory syndrome virus-free sow farms in the US. *Open J. Vet. Med.* 3, 168–175.
- Kim, Y., Yang, M., Goyal, S.M., Cheeran, M.C.-J., Torremorell, M., 2017. Evaluation of biosecurity measures to prevent indirect transmission of porcine epidemic diarrhea virus. *BMC Vet. Res.* 13, 89.
- Laanen, M., Persoons, D., Ribbens, S., De Jong, E., Callens, B., Strubbe, M., Maes, D., Dewulf, J., 2013. Relationship between biosecurity and production/antimicrobial treatment in pig herds. *Vet. J.* 198, 508–512.
- Lowe, J., Gauger, P., Harmon, K., Zhang, J., Connor, J., Yeske, P., Loula, T., Levis, I., Dufresne, L., Main, R., 2014. Role of transportation in spread of porcine epidemic diarrhea virus infection, United States. *Emerg. Infect. Dis.* 20 (5).
- McCune, B., Mefford, M.J., 2011. PC-ORD. Multivariate Analysis of Ecological Data. Version 6. MjM Software, Gleneden Beach, Oregon, U.S.A.
- Monterubbiansi, M., Vidal, M., Debenedetti, R., Suárez, M., Barral, L., Duffy, S., 2016. Serological Surveillance of the Porcine Reproductive Respiratory Syndrome in the Argentine (2010-2015). XIII Congreso Nacional De Producción Porcina. Memorias XIX Jornadas De Actualización Porcina. VIII Congreso De Producción Porcina Del Mercosur. Page 149. .
- Nantima, N., Davies, J., Dione, M., Ocaido, M., Okoth, E., Mugisha, A., Bishop, R., 2016. Enhancing knowledge and awareness of biosecurity practices for control of African swine fever among smallholder pig farmers in four districts along the Kenya-Uganda border. *Trop. Anim. Health Prod.* 48, 727–734 RESEA.
- Newcombe Robert, G., 1998. Two-sides confidence intervals for the single proportion: comparison of seven methods. *Statist. Med.* 17, 857–872.
- Nöremark, M., Lindberg, A., Vagsholm, I., Sternberg Lewerin, S., 2009. Disease awareness, information retrieval and change in biosecurity routines among pig farmers in association with the first PRRS outbreak in Sweden. *Prev. Vet. Med.* 90, 1–9.
- OIE, 2004. Handbook on Import Risk Analysis for Animals and Animal Products, vol. 2. World Organization for Animal Health (Office International des Epizooties), Paris, France.
- Olugasa, B., Ijagbone, I., 2007. Pattern of spread of African swine fever in south-western Nigeria, 1997-2005. *Vet. Ital.* 43, 621–628.
- Pinto, C.J., Urcelay, S., 2003. Biosecurity practices on intensive pig production systems in Chile. *Prev. Vet. Med.* 59, 139–145.
- Postma, M., Backhans, A., Collineau, L., Loesken, S., Sjölund, M., Belloc, C., Emanuelson, U., Grosse Beilage, E., Stärk, K.D., Dewulf, J., 2016. The biosecurity status and its associations with production and management characteristics in farrow-to-finish pig herds. *Animal* 10, 478–489.
- Pouillot, R., Delignette-Muller, M.L., 2010. Evaluating variability and uncertainty in microbial quantitative risk assessment using two R packages. *Int. J. Food Microbiol.* 142, 330–340.
- R Development Core Team, 2008. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing. Vienna, Austria. <http://www.Rproject.org>.
- Racicot, M., Venne, D., Durivage, A., Vaillancourt, J.P., 2012. Evaluation of the relationship between personality traits, experience, education and biosecurity compliance on poultry farms in Québec, Canada. *Prev. Vet. Med.* 103, 201–207.
- Scott, A., McCluskey, B., Brown-Reid, M., Grear, D., Pitcher, P., Ramos, G., Spencer, D., Singrey, A., 2016. Porcine epidemic diarrhea virus introduction into the United States: root cause investigation. *Prev. Vet. Med.* 123, 192–201.
- Simon-Grifé, M., Martín-Valls, G.E., Vilar-Ares, M.J., Garcia-Bocanegra, I., Martín, M., Mateu, E., Casal, J., 2013. Biosecurity practices in Spanish pig herds: perceptions of farmers and veterinarians of the most important biosecurity measures. *Prev. Vet. Med.* 110, 223–231.
- Straw, B., Zimmerman, J., D'Allaire, S., Taylor, D., 2006. Diseases of Swine, 9th edition. Ames, Iowa, U.S.A.
- Thomas, J.T., Chen, Q., Gauger, P.C., Giménez-Lirola, L.G., Sinha, A., Harmon, K.M., Madson, D.M., Burrough, E.R., Magstadt, D.R., Salzbrenner, H.M., Welch, M.W., Yoon, K.J., Zimmerman, J.J., Zhang, J., 2015. Effect of porcine epidemic diarrhea virus infectious doses on infection outcomes in naïve conventional neonatal and weaned pigs. *PLoS One* 10 (October (10)), e0139266. <https://doi.org/10.1371/journal.pone.0139266>. eCollection.

Web References

- Food and Agriculture Organization of United States, 2014: <http://www.fao.org/ag/AGInfo/themes/es/pigs/production.html>.
- National Service for Health and AgriFood Quality, 2016 National Service for Health and AgriFood Quality, 2016. Statistics Report: <http://www.senasa.gov.ar/cadena-animal/porcinos/informacion/informesyestadisticas>.
- Word Organization for Animal Health, 2018: http://www.oie.int/wahis_2/public/wahid.php/Countryinformation/Animalsituation.