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1 **Biosecurity assessment of Argentinian pig farms**

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28 **ABSTRACT**

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

51 **ARTICLE INFO**

52 Keywords: Pigs Biosecurity, Correspondence analysis Cluster analysis, Risk assessment, Porcine
53 epidemic diarrhoea virus

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57 **1. Introduction**

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

64 Biosecurity is founded on knowledge of the epidemiology of transmissible diseases, including
65 the duration of the contagiousness period in infected animals, the main routes of pathogen
66 shedding, the survival of the pathogen in the environment, and the routes of infection. This
67 knowledge allows technically appropriate measures to be designed. However, it is also
68 important to consider the socioeconomic aspects of proposed measures, as these will have an
69 impact on their compliance (FAO, 2010).

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

75 Argentina has a very strong tradition of livestock production, mainly for beef. However, in recent
76 years, pig production has been growing at a rate of > 5% per year, reaching about 1 million in
77 commercial and genetic farms in 2017, of which around 255,000 were in medium and large
78 farms, according to official statistics ([http://www.senasa.gob.ar/cadena-](http://www.senasa.gob.ar/cadena-animal/porcinos/informacion/informesyestadisticas)
79 [animal/porcinos/informacion/informesyestadisticas](http://www.senasa.gob.ar/cadena-animal/porcinos/informacion/informesyestadisticas)).

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

85 However, the sustained growth and the high intensification of the new farms create a need for
86 more replacement animals of higher genetic value and more movement to and from farms to

87 ship the animals and the feedstuff. Within this scenario the need to apply more biosecurity
88 measures is evident. Based on the above, the aim of the present study was to assess the
89 biosecurity of Argentinian pig farms by: i) de- scribing the biosecurity measures applied in pig
90 farms supplying re- placement animals; ii) identifying typologies of farms based on the level of
91 application of biosecurity measures; and iii) evaluating their external biosecurity with risk
92 assessment tools.

93 **2. Material and methods**

94 2.1. Farms, data collection and validation of the questionnaires

95 The survey was conducted in Argentinian pig farms in 2015 and 2016 included two studies. The
96 first one, carried out during 2015, comprised all the farms officially registered by the Argentinian
97 authorities as companies supplying breeders of high genetic value (n = 110). These farms were
98 geographically distributed as follows: 38 (34.5%) in Buenos Aires province, 30 (27.2%) in Córdoba
99 province, 18 (16.3%) in Santa Fe province, 5 (4.5%) in Entre Ríos province - these being the main
100 pig-producing provinces in Argentina - and the remaining 19 farms (17.5%) in Chaco, Chubut,
101 San Juan, Neuquén, La Pampa, La Rioja, Río Negro, and San Luis provinces. The second study,
102 performed during 2016, focused on the evaluation of 355 commercial farms, 319 of which had
103 100–500 sows (125 in Buenos Aires province, 93 in Córdoba province, 21 in Entre Ríos province,
104 and 80 in Santa Fe province) and 36 had ≥ 500 sows (12 in Buenos Aires province, 14 in Córdoba
105 province, 4 in Entre Ríos province, and 6 in Santa Fe province). Of both kinds of (genetic and
106 commercial) farms, 98–98.5% operated as farrow-to-finish farms while the remaining 1.5–2%
107 were exclusively breeding and nursery farms. In this second study, the sample size (n = 355) was
108 calculated considering a variation in the frequency of application of biosecurity measures of
109 50%, a confidence level of 95%, and a 5% accuracy. The study population (i.e. farms with more
110 than 100 sows/farm) was classified by province and number of sows on the farm. The farms
111 included in the study were randomly selected within each class based on the official registry
112 number of each farm.

113 The questionnaire used for collecting data on the biosecurity measures applied was the same
114 for both the genetic and commercial farms. This questionnaire included a total of 126 questions
115 and was divided into sections, including: a) general data of the farm such as location, number of
116 sows, distance to neighbouring farms, etc., b) external bio- security measures related to
117 replacement animals, vehicles, visitors and geographic risk (e.g. perimeter fence), and c) internal

118 biosecurity measures as regards to management, installations, cleaning and personnel routines.
119 Supplementary Table S1 shows the number of questions for the different categories included in
120 the questionnaire.

121 A draft questionnaire was first tested for clarity and adequacy in four farms (two with > 100
122 sows and two with ≥ 500 sows) by means of a personal interview with the farmer. After making
123 some amendments, the questionnaire (available in Spanish on request) and the guidelines for
124 completing it, were distributed through the Argentinian National Service for Health and
125 AgriFood Quality (SENASA) to veterinary officers, who visited the farms and supervised the
126 collection of the data. Before the on-farm data collection, the veterinarians in charge of that
127 task attended a workshop where they were instructed on the correct way to complete the
128 questionnaire.

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

136 2.2. Assessment of farm type based on the biosecurity measures applied

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

149 variables related to replacement animals and 2) those farms with external replacements
150 exclusively.

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

163 2.3. Evaluation of the external biosecurity by using risk assessment tools

164 To evaluate the external biosecurity of the herds, we used the risk assessment tool developed
165 by Allepuz et al. (2018) in a hypothetical scenario of an epidemic episode of porcine epidemic
166 diarrhoea virus (PEDV) in Argentina. PEDV is a highly contagious enteric virus of pigs transmitted
167 by the fecal-oral route. In farms with no previous immunity, suckling piglets suffer severe watery
168 diarrhoea with fatality rates reaching 50–100% (Straw et al., 2006). The above-mentioned
169 approach allowed both the estimation of a score for the annual probability of disease
170 introduction and to decide where to concentrate the effort to reduce this risk. Briefly, the
171 approach comprises five steps: i) identifying the possible routes of disease introduction and key
172 parameters for each route (e.g. herd prevalence and within-herd pre- valence in affected farms);
173 ii) calculating a score for the probability of each route harbouring the disease agent upon arrival
174 at the farm; iii) conducting an expert opinion workshop to obtain a score for the different input
175 parameters; iv) calculating the risk mitigation (reduction of the probability of introduction by a
176 given route after applying a bio- security measure); and v) calculating a final score of the
177 probability of disease introduction for each route.

178 Based on the epidemiology of PEDV and on the Argentinian context, six routes of introduction
179 of the disease were considered: i) replacement animals, ii) vehicles transporting replacement

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

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

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

210 **3. Results**

211 3.1. Official suppliers of high-genetic-value farms

212 3.1.1. Response rates and application of different biosecurity measures

213 All the genetic suppliers answered the questionnaire. The frequencies of application of the
214 different external and internal biosecurity measures in those farms are shown in the
215 Supplementary Tables S2a and S2b. The question response rate was: 100% for 68/126 questions
216 (54.0%; lower and upper limits of the 95% confidence interval = $CI_{95\%}$: 45.7–62.4); between 95%
217 and 99.9% for 31/126 questions (24.6%; $CI_{95\%}$: 17.9–32.8); between 90% and 95% for 8/126
218 questions (6.4%; $CI_{95\%}$: 3.3–12.0); and between 80 and 90% for 10/126 questions (7.9%; $CI_{95\%}$:
219 4.4–14.0) of which six were related to the trucks transporting animals from quarantines. Only
220 two questions (1.6%; $CI_{95\%}$: 0.4–5.6) had a response rate < 80%.

221 It is worth mentioning that most of the genetic farms introduced gilts from external sources
222 (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)
223 introduced them at least four times a year. Several farms introducing external gilts (36/ 77,
224 47.0% $CI_{95\%}$: 35.4–58.4) transported the animals by using trucks that had been in contact on the
225 same day with other farms or pigs of other origins. In addition to this, most of the farms that
226 reported introducing external gilts (38/77, 49.4%; $CI_{95\%}$: 38.5–60.3) did not have quarantine
227 facilities.

228 With regard to vehicles arriving at the farm, trucks transporting feedstuff, trucks that collected
229 pigs to be sent to the slaughterhouse, and private vehicles were allowed to enter the farm
230 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
231 (35.5%; $CI_{95\%}$: 27.1–44.7) of the farms, respectively. In addition, 63/77 (81.8%; $CI_{95\%}$: 71.8–88.9)
232 farms introducing external gilts lacked specific loading/unloading docks for them.

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

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

241 3.1.2. Correspondence and cluster analysis

242 In the correspondence analysis done with the 110 genetic farms (Fig. 1a and b), axes 1 and 2
243 explained 17.9% and 6.9% of the variance, respectively. When the analysis was performed with
244 the farms that only used external sources of gilts ($n = 77$), axes 1 and 2 explained 19.4% and
245 7.7% of the variance, respectively. The hierarchical cluster analysis resulted in the identification
246 of three significant groups (MRPP, $p < 0.0001$) when considering all 110 farms and equally when
247 analysing the farms that only purchased external replacements (Fig. 1). The indicator values are
248 calculated to measure the strength of association of each variable with the different farms
249 groups. For predictive purposes, the list of variables strongly associated to the farm groups has
250 a great interest as diagnostic variables. The observed Indicator Value (OIV) associated with each
251 cluster within the commercial and genetic farms are shown in Tables 1 and 2. Cluster 1 was
252 associated with 26 external and 20 internal biosecurity measures. With respect to external
253 biosecurity, the measures related to the entry of personnel on the farm were important; for
254 example: a compulsory shower (OIV: 86.4%), a compulsory hand wash (OIV: 83.5%), and
255 compulsory use of clean boots and clothes (OIV: 56.4%), followed by measures related to the
256 entry of animals such as the use of a loading dock with clean and dirty areas (OIV: 43.1%) and
257 the restriction of entry for trucks into the farm perimeter (OIV: 30.5%). The other clusters were
258 associated only with two or three measures with lower OIV (25–40%).

259 3.2. Commercial farms

260 3.2.1. Response rates and application of different biosecurity measures

261 In the case of the commercial farms, the response rate was 55.8% (198/355; $CI_{95\%}$: 50.6–60.9).
262 In the subsequent analysis, six questionnaires were discarded due to a low response rate of the
263 questions, and thus only 192 farms were analysed. Of these, 185/198 were farms with 100 to
264 500 sows and 13/198 with > 500 sows. By provinces, 90/ 198 (45.5%; $CI_{95\%}$: 38.7–52.4) farms
265 were from Buenos Aires, 52/198 (26.3%; $CI_{95\%}$: 20.6–32.8) from Santa Fe, 34/198 (17.2%;
266 $CI_{95\%}$:12.6–23.0) from Córdoba, and 22/198 (11.1%; $CI_{95\%}$: 7.5–16.3) from Entre Ríos, thus
267 resulting in a representative sample of the country.

268 The frequencies of application of different external and internal biosecurity measures in these
269 farms are shown in the Supplementary Tables S2a and S2b. As can be observed, 156/192 (81.3%;
270 $CI_{95\%}$: 75.1–86.1) of the farms in this group purchased replacement gilts from external facilities

271 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%}:
272 42.9–58.4) of the farms that purchased external gilts, replacement animals were transported in
273 vehicles that could have visited other farms on the same day, whereas in 49/156 (31.4%; CI_{95%}:
274 24.7–39.1) of the cases, gilts from different origins could have been transported on the same
275 truck. In 93/156 (59.6%; CI_{95%}: 51.8–67.0) of these farms, gilts arrived at the farm every 90 days
276 or less. Only one farm had the quarantine unit outside the premises, at more than 1,000 m
277 distance.

278 In 141/192 commercial farms analysed (73.4%; CI_{95%}: 66.8–79.2), trucks that transported animals
279 to the slaughterhouse belonged to external companies, and in 42/192 (21.9%; CI_{95%}: 16.6–28.3)
280 they could have loaded or unloaded pigs in other farms on the same day. In addition, 111/192
281 (57.8%; CI_{95%}: 50.7–64.6) of the farms did not have loading docks with delimited clean and dirty
282 areas.

283 Concerning visitors, 82/192 (42.7%; CI_{95%}: 35.9–49.8) of the farms had less than one visitor per
284 week and 64/192 (33.3%; CI_{95%}: 27.1–40.3) had a policy to restrict visitors. Clothes and boots
285 were provided to visitors in 116/192 (60.4%; CI_{95%}: 53.4–67.1) and 143/192 (74.5%; CI_{95%}: 67.9–
286 80.1) of the farms, respectively. Internal biosecurity measures within this group of farms (Fig. 2a
287 and b) were also rarely applied and, in fact, no cleaning or disinfection procedures were carried
288 out between different animal batches in 51/192 (26.6%; CI_{95%}: 20.8–33.2) of the farrowing
289 rooms, in 41/192 (21.4%; CI_{95%}: 16.2–27.7) of the nursery units, and in 77/192 (40.1%; CI_{95%}:
290 33.3–47.2) of the fattening facilities.

291 3.2.2. Correspondence and cluster analysis

292 In the correspondence analysis in relation to the whole population of commercial farms analysed
293 (n = 192), axes 1 and 2 explained 13.5% and 6.32% of the variance. In the analysis of the farms
294 using external sources of gilts (n = 153), axes 1 and 2 explained 11.3% and 5.9% of the variance.
295 The hierarchical cluster analysis for the whole population and equally that for farms with
296 external gilts showed three significant groups (MRPP, p < 0.0001) (Fig. 2). The indicator values
297 that distinguished to a greater degree between groups of farms are shown in Tables 1 and 2. As
298 shown in Table 1, clustering was defined by 41 variables, 38 of which were strongly associated
299 with cluster 1. Furthermore, the dressing room with separate dirty and clean areas, the
300 compulsory shower for visits, and the compulsory hand wash between stages of production
301 were highly associated with that cluster (percentage of perfect indication = 64%, 52.6%, and

302 584%, respectively). Clusters 2 and 3 were characterized by three and five variables,
303 respectively.

304 3.3. Risk scoring: EVALUATION of the EXTERNAL biosecurity

305 3.3.1. Expert PANEL meeting

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

316 3.3.2. Risk assessment

317 Figs. 3 and 4 show the mean values of the initial score for the probability of introduction, the
318 risk mitigation, and the final score for the probability of PEDV introduction by each route for
319 both the genetic and commercial farms. The results for both groups of farms were quite similar,
320 showing that the routes with higher initial and final scores were: i) the introduction of
321 replacement animals, ii) the vehicles transporting feedstuff, iii) the vehicles transporting
322 animals, and iv) the visitors. The results also revealed that the application of biosecurity
323 measures was quite variable in both groups. The risk mitigation for the different routes ranged
324 from 0 to 0.95, indicating that some farms did not implement any measures while others had a
325 high level of biosecurity. In addition, the median for the proportion of risk reduction was below
326 40% in all routes from both groups.

327 The introduction of replacement animals was one of the routes with the lowest application of
328 biosecurity measures. The median for the proportion of risk reduction for this route was 7.3% in
329 the genetic farms and 12.8% in the commercial farms and for about 50% of the farms biosecurity
330 measures to block this route were extremely low. On the other hand, the geographic risk had a

331 low initial and final score for the probability of disease introduction, which correlates to the low
332 pig density in the country.

333 **4. Discussion**

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

339 The survey of genetic suppliers was exhaustive because it was compulsory as a part of the
340 national pig health program. Since this was the first time that this type of survey was conducted
341 in Argentina, the data was additionally verified by means of a telephonic interview with the
342 veterinarians in charge of the farm or directly by visit. This additional verification of the data
343 assured a very accurate picture of this type of farm, reducing potential measurement biases.
344 This verification was not performed in the group of commercial farms. For this second group,
345 some measurement bias might exist as some farmers might have not answered what they really
346 do on their farm. On the other hand, for some questions (mostly those related to quarantines)
347 the response rate was low. In our opinion, the lack of an answer was related to the fact that
348 some farms actually lacked quarantine facilities and also to the lack of knowledge of the
349 importance of some biosecurity measures. These could have introduced some classification
350 bias in the analysis. It would be desirable to do a future follow-up in order to update results,
351 as the implementation of measures might change over time. For commercial farms the
352 enrolment was voluntary, which resulted in a lower participation rate of about half of the
353 farms within the categories examined. This voluntary participation could have introduced
354 some selection bias.

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

359 For both types of farms, the first cluster had in common several measures such as the existence
360 of strict barriers preventing the entry of people, trucks and other vehicles to the farm, with clear

361 indication of clean and dirty areas, representing in all likelihood high biosecurity operations.
362 Most of the farms in this group were new or belonged to large companies. The second and third
363 clusters represented farms with an intermediate and a low level of biosecurity, respectively.
364 These results agree with those found by Bottoms et al. (2013) and Laanen et al. (2013), who
365 observed that the larger and more modern farms implement more biosecurity measures. In our
366 case, most of the larger farms are new and belong to large companies with a high technical
367 standard.

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

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

386 There are some tools that may be used to compare the biosecurity status between pig farms or
387 for farm-specific counseling (Pinto and Urcelay, 2003; Laanen et al., 2013; Postma et al., 2016;
388 Holtkamp et al., 2013b). All these tools are based on values obtained through expert opinion
389 panels. In the present study, we used a methodology based on the risk assessment tool recently
390 developed by our group (Allepuz et al., 2018), also using expert opinion panels. Since the opinion
391 of experts may vary depending on the features of a given disease, the epidemiological

392 circumstances of a country, or the prevailing ideas at a given moment, scoring systems based on
393 perceptions must be adapted to each situation. Here, we conducted an expert opinion workshop
394 with Argentinian veterinarians to adapt the values to the context and situation of the country.
395 The 18-person panel was composed of veterinarians working in the pig sector whose expertise
396 included the most common profiles (health, husbandry, etc.). Because of this diversity, some
397 persons could be more sensitive to risks than others.

398 In our analysis, the routes identified with the greatest risk for the entry of PEDV into farms were
399 the transport vehicles of replacement animals and feed, the visitors, and the replacement of
400 animals. Our results are consistent with those of other studies where the vehicles and their
401 drivers, the clothing and boots, the workers and materials for the farm were identified as ways
402 of transmitting PEDV (Kim et al., 2017; Lowe et al., 2014; Dee et al., 2015; Scott et al., 2016). It
403 is worth mentioning that Argentina is a very large country and movements of animals between
404 farms or slaughterhouses can involve distances of up to one thousand kilometres. For this
405 reason, the costs of transportation are high and the distribution of young sows to medium and
406 small farms or the transportation of animals to the slaughterhouse is usually carried out by a
407 truck serves several farms on the same day, with the consequent risks.

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

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

432 **5. Conclusion**

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

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446 **Appendix A. Supplementary data**

447 Supplementary material related to this article can be found, in the online version, at doi:
448 <https://doi.org/10.1016/j.prevetmed.2019.02.012>.

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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				
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				
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				
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				
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				
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				
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				
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				
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				
Brushed for cleaning	1	25.4	0.0314	1	22.8	0.0002
Allow to dry before disinfecting	1	39.5				

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.

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 (%)	<i>p</i> (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 (%)	<i>p</i> (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

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.

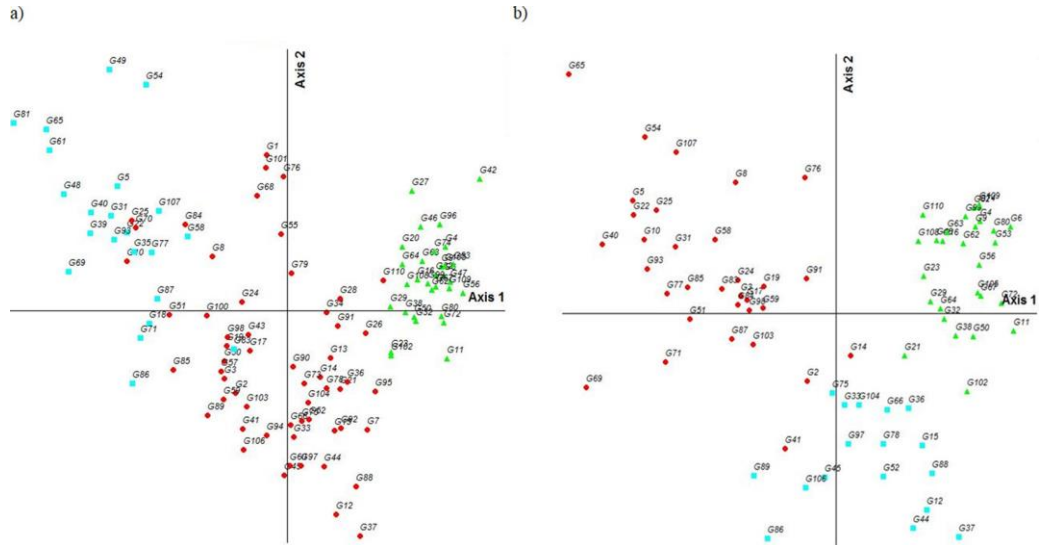


Fig. 1. Results of the cluster analysis for farms producing gilts of high genetic value. References: a) ▲ number of farms = 31, distance = 0.3, average of sows in production = 1275, ● number of farms = 52, distance = 0.57, average of sows in production = 171, ■ number of farms = 27, distance = 0.62,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

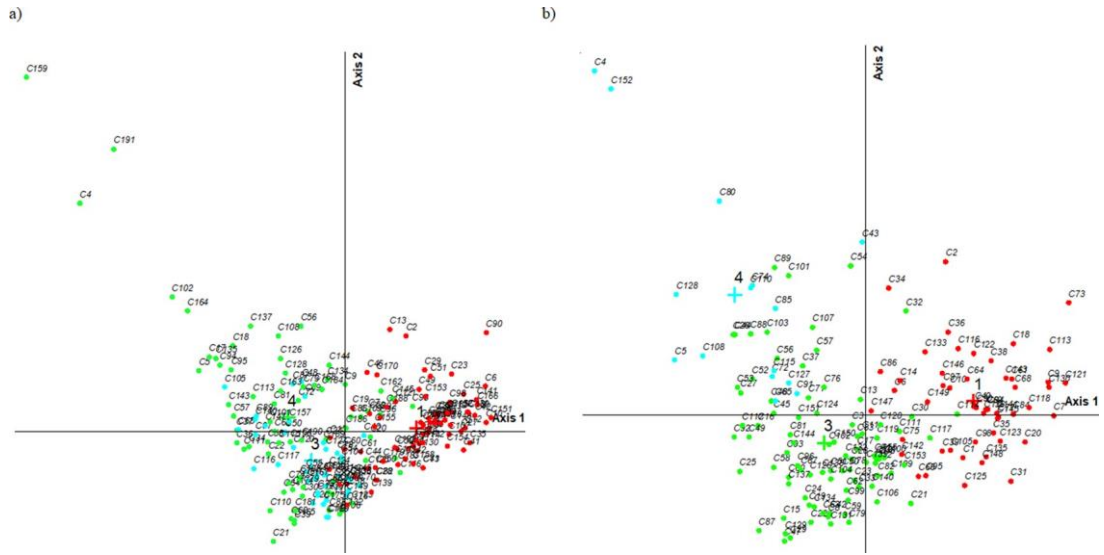


Fig. 2. Results of the cluster analysis for commercial farms: a) all farms disregarding variables related to replacement animals, and b) farms with external replacements and all variables. References: a) ● number of farms = 44, distance = 0.35, average of sows in production = 329, ● number of farms = 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.

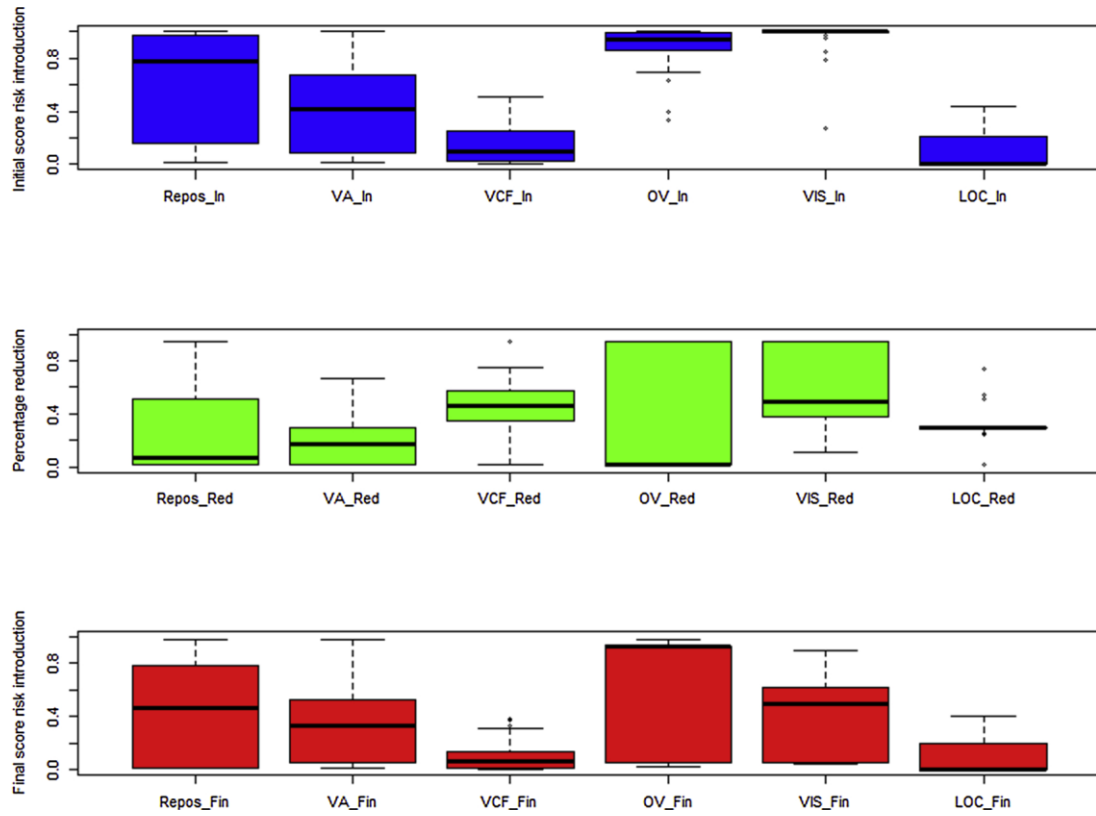


Fig. 3. The boxplots show the mean values for the initial risk of introduction, the percentage of reduction (i.e. risk mitigation) and the final score for the risk of PEDV introduction, for the 110 genetic farms assessed considering the different routes (Repos = replacement animals; VA = vehicles transporting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

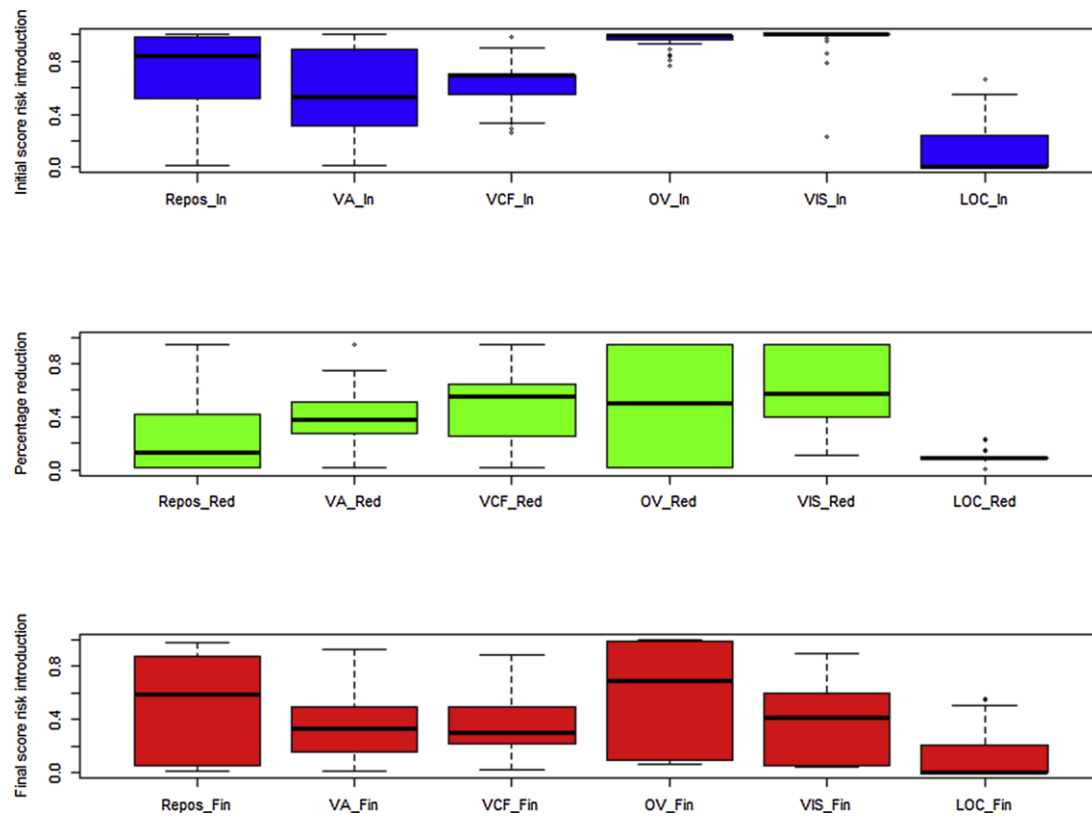


Fig. 4. The boxplots show the mean values for the initial risk of introduction, the percentage of reduction (i.e. risk mitigation), and the final score for the risk of PEDV introduction for the 192 commercial farms examined considering the different routes. (Repos = replacement animals; VA = replacement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