A survey of biosecurity measures applied on dairy cattle farms in Spain

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Abstract

Attention to biosecurity has been highlighted as the most important measure to reduce and prevent the introduction of diseases to farms. There is little published information about the biosecurity of dairy cattle in Spain. We therefore aimed to assess and characterize the current application of biosecurity measures on dairy cattle farms in Spain, and relate these to bovine viral diarrhea and infectious bovine rhinotracheitis. From July 2017 to April 2018, data on biosecurity measures for 124 dairy herds were collected using a questionnaire. We also assessed the sanitary status of these farms (efficacy of measures implemented against both diseases using antibody ELISA. Data were analyzed descriptively, and using multiple correspondence analysis and a two-step cluster analysis. Measures to prevent disease introduction were often poorly implemented. Three main clusters of farms were identified: Clusters 1 and 2 included herds of small and intermediate sizes, respectively. These, particularly cluster 1, showed the most deficiencies in the control of vehicles and visitors. However, individual purchases usually involved low numbers of animals, especially in cluster 2, and animals were tested for bovine viral diarrhea and infectious bovine rhinotracheitis at their places of origin or on arrival at farms. Farms in clusters 1 and 2 were frequently under voluntary control programs. Cluster 3 had the largest herd sizes, with somewhat better biosecurity control of vehicles and visitors. However, farms in this cluster also purchased the most animals, sometimes without testing, and hired external workers most often. Farms in cluster 1 showed the best sanitary level, followed by clusters 2 and 3. Collecting data such as these is an important first step to identification of biosecurity shortcomings, and to structuring of adequate follow-up to ensure that measures are implemented correctly on farms in Spain.

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Introduction

Infectious agents that affect livestock may be transmitted by various routes such as live infected animals, trucks and other vehicles, people, aerosols, fomites, or wildlife or insect vectors. Thereby, prevention through biosecurity is the most cost-effective protection (1).

Within the context of animal production, biosecurity is defined as management activities that reduce the opportunities for infectious agents to gain access to, or spread within, a production unit (2).Thus, it has two main components: external and internal biosecurity. External biosecurity entails preventive measures and risk reduction strategies designed to avoid the introduction of pathogenic infections (hazards), whereas internal biosecurity entails measures to limit within-farm transmission of infectious hazards between animals (3).

The importance of biosecurity is highlighted in the European Union health strategy. From 2007 onwards, the EU embraced a new motto as part of its Animal Health Strategy: "prevention is better than cure", now implemented by the European Commission. The aim was to focus on preventive measures, disease surveillance, controls, and research to reduce the incidence of animal diseases and minimize the impact of outbreaks (4).

The putative benefits of undertaking biosecurity for disease prevention and/or control include improvements in production efficiency (hence greater profits), animal welfare, immune responses to vaccines, and job satisfaction for producers, herd health professionals, and other agricultural workers (5). In addition, in pig herds, a link between biosecurity and antimicrobial treatment-related criteria has been demonstrated and quantified (6).

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79   Despite these benefits, implementation of biosecurity on dairy cattle farms is

80   often sub-optimal, and poor or inappropriate knowledge transfer is often cited as a

81   potential cause of disease spread (7).The main limitations and strengths of the

82   biosecurity measures applied in dairy cattle farms have been studied recently in several

83   countries (7-10), as a preparatory step to develop greater awareness of the importance of

84   each measure and the factors that might restrict its application. In Spain, despite the

85   economic importance of milk production in some regions, current biosecurity practices

86   on dairy farms have not been studied empirically although a recent study assessed

87   perceptions and practices applied by rural veterinarians (11).

88   The sanitary status of dairy cattle farms with respect to endemic diseases such as

89   bovine viral diarrhea (BVD) and infectious bovine rhinotracheitis (IBR) is highly

90   variable among Spanish regions. In some, the situation is unknown and the approach to

91   outbreaks depends mainly on the actions of individual veterinarians and farmers. In

92   others, mainly in the northwest, voluntary programs were established some time ago,

93   and now involve a large proportion of the cattle population (12).

94   BVD and IBR, both caused by the BVD virus (BVDV), and bovine herpesvirus

95   1 (BoHV-1) are highly contagious diseases of economic and trade importance for the

96   livestock industry worldwide. They can cause serious economic losses, with pathogenic

97   effects including reduced milk yield, infertility, abortion, respiratory disease, and an

98   increase in opportunistic infections such as calf pneumonia and scours (13,14). For all

99   these reasons, several European countries have implemented official voluntary or

100   compulsory programs to eradicate both diseases (15,16).

101   The aim of the present paper was to assess and characterize the current

102   application of biosecurity measures on dairy cattle farms in Spain, and the relationship

103   of these to BVD and IBR.

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Materials and methods

Area description and herds surveyed

We conducted our study in two Autonomous Communities (AC) of Spain: Galicia (NW), and Catalonia (NE). Galicia is the main dairy cattle area of the country, with 55% of the farms and 38% of the milk production. The mean herd size per farm is 43 cows, lower than the national average of 59.3, and farms are still predominantly family owned and managed. This region is representative of the production type prevailing in the NW and Cantabric area of the country. In contrasts, farms in Catalonia have a mean herd size of 144 cows, with 4% of the farms nationally yielding 11% of the milk production (17). The farm typology in this region could be considered representative of the rest of Spain.

We selected124 dairy farms, 93 from Galicia and 31 from Catalonia, as part of a larger national project on risk analysis for the introduction of BVDV and BoHV-1todairy cattle herds. For farm selection, the project was presented to the veterinarians responsible for health management in the main dairy cattle areas from both regions. Subsequently, together with the veterinarians, the project was communicated to farmers and those interested in participation were enrolled in the study.

Biosecurity questionnaire

Data were obtained using a questionnaire which was completed during personal interviews with farmers and the veterinarian responsible for health management of each farm. Farms were visited between July 2017 and April 2018.

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126   The questionnaire, which is available in Spanish on request from the authors,

127   included four sections: I) general farm information (e.g. location, herd size, vaccination

128   programs); II) animal movements (e.g. origin of the animals, frequency of introductions,

129   test, quarantine facilities, external rearing farms, cattle fairs or competitions, pasture)

130   and neighborhood (i.e. other ruminant farms in a 1 km radius); III) movements and

131   types of vehicles and equipment (for live and dead animal transport, manure, slurry and

132   feeding vehicles, machinery or materials) and biosecurity-related measures (e.g.

133   vehicles may enter inside the farm perimeter, vehicles may enter with other animals.)

134   and IV) visitors and staff (e.g. external workers, frequency of professional or non-

135   professional visitors that may contact animals, use of protective clothing).

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Health status

138 The BVDV and BoHV-1 sanitary status of the farms was determined using antibody

139 ELISA, taking into consideration the fact that farms that applied vaccines used

140 inactivated vaccines in case of BVDV and marker vaccines (live or inactivated) in case

141 of BoHV-1.

142   For BVDV, antibodies against the p80 antigen were determined using a

143 commercial blocking ELISA(BVD p80 Ab, IDEXX laboratories, the Netherlands),

144 since the antibodies of animals vaccinated with inactivated vaccines react mainly with

145 structural proteins rather than the p125 or p80 antigens (18). Additionally, in Galicia,

146 when tested samples indicated possible persistent infection (PI) in an animal (i.e., when

147 a positive result was obtained fora young heifer), this was confirmed by antigen capture

148 ELISA (IDEXX BVDV Ag Serum Plus, IDEXX Laboratories; the Netherlands) based

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149   on detection of the E^{rns} viral protein. Two samples positive for BVDV from the same

150   animal taken 3–4 weeks apart were considered to confirm persistent infection.

151   For the case of BoHV-1, two different tests were used depending on whether

152   vaccines were used on the herd (IDEXX IBR gE antibody, IDEXX Laboratories; the

153   Netherlands) or not (IDEXX IBR gB antibody, IDEXX Laboratories; the Netherlands).

154   All analyses were performed following the recommendations of the manufacturer.

155   Using the results of these tests, three different BoHV-1 and BVDV farm profiles

156   were established: (1) farms with recent or active infection (BVDV seropositivity in

157   heifers from 9 to 24 months born on the farm and/or PI confirmed), (2) farms with

158   seropositive adult animals but all rearing heifers (9-24 month) seronegative, or(3) free

159   farms (all animals tested seronegative).

160

Statistical analysis

162   All statistical tests were performed with SPSS 15.0. Initially, the frequencies of the

163   different BoHV-1 and BVDV profiles and biosecurity measures were analyzed.

164   After that, to characterize the current application of biosecurity measures in

165   dairy cattle farms from Spain, a multiple correspondence analysis (MCA) was

166   performed. MCA aims to reduce a set of possibly correlated variables (including all the

167   biosecurity variables and the sanitary status of the farms) to a smaller group of linearly

168   uncorrelated dimensions. We set the number of dimensions to two, to facilitate two-

169   dimensional graphical representation. The position of the full set of categories for each

170   investigated variable (category-points) on the MCA graph is the basis for revealing

171   relationships among variables: variable categories with a similar profile tend to group

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172   together, whereas negatively correlated categories are located on opposite sides of the

173   graph. In addition, a two-step cluster analysis (TSCA) was performed to identify

174   clusters of farmers with similar biosecurity levels and BVDV and BoHV-1 profiles. For

175   this analysis, the frequencies of entry of animals, vehicles, and visits (collected as

176   numerical values) were processed as categorical (4 categories based on the quartiles of

177   the variables), but finally were transferred to the results as mean and median frequencies

178   of entry.

179

Results

181   BVDV and BoHV-1 profiles of the124 farms are summarized in Table 1. The

182   serologically free farm category represented similar proportion of the total for both

183   viruses (50% and 44.3% for BoHV-1 and BVDV, respectively). However, the

184   proportion of farms categorized as recent/active infection was much higher for BVDV

185   (36.1% *versus* 10.7%). Thirty-seven of 124 farms used inactivated vaccines against

186   BVDV, whereas the remainder did not vaccinate; 33 used marker vaccines for BoHV-1

187   whereas the remainder did not vaccinate.

188   **Table 1. BVDV and BHV-1 sanitary status of 124 farms in Spain**

	Sanitary status	**N (%)**
BoHV-1	Recent/active infection	13 (10.7%)
	Seropositive animals (but rearing heifers free)	48 (39.3%)
	Free farm	63 (50.0%)
BVDV	Recent/active infection	44 (36.1%)
	Seropositive animals (but rearing heifers free)	24 (19.7%)

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	Free farm	56 (44.3%)

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190   Table 2 describes biosecurity measures related to animal movements and
191   possible contact with other domestic ruminants. For most farms, the risk of introduction
192   of diseases through the purchase of animals (heifers or cows) was presumably
193   negligible, as most reared their own replacements. Farms that purchased cattle (46/124,
194   37.1%) had a low mean frequency of introductions (5.2 purchased animals/year).
195   However, it is noteworthy that 11/46 (23.9%) farms purchased animals without any
196   testing, 39/46 farms (84.8%) purchased cattle that could contact other cattle during
197   transport, and most farms lacked adequate quarantine facilities. Animal movement to
198   cattle fairs or competitions was a possible pathway of disease transmission for only
199   7/124 farms (5.7%), but it is noteworthy that nearly all farms reported that animals
200   returned to the farm without any quarantine. Movements to pasture were much more
201   frequent: 54/124 farms (43.6%) reported this type of movement, and half of these
202   reported the possibility of contact with other domestic ruminants at the pasture.
203   Moreover, most farms had other cattle or sheep/goat farms within a 1 km radius.

204   **Table 2. Biosecurity measures related to purchase of cattle, or possible contact**
205   **with other ruminants, for 124 farms in Spain**

		N (%)
Purchase of animals (heifers/cows)	No	78 (62.9%)
	Yes (sanitary status assessed at origin)	18 (14.5%)
	Yes (sanitary status assessed on arrival)	17 (13.7%)
	Yes, without testing	11 (8.9%)
Average (median) frequency of heifers or cows purchased/year, when	5.25 (3)	

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applicable		
Transport of purchased heifers/cows	Cannot contact other ruminants during transport	39 (84.8%)
	Can contact other ruminants	7 (15.2%)
	Not applicable	78
Adequate quarantine facilities	Yes	4 (3.2%)
	No	120 (96.8%)
External rearing	No	112 (90.2%)
	Yes	12 (9.8%)
Sanitary plan in the external rearing farm	Yes	6 (50.0%)
	No	6 (50.0%)
	Not applicable	112
Cattle farms within 1 km	No	16 (12.9%)
	Yes	108 (87.1%)
Sheep/goat farms within 1 km	No	81 (65.3%)
	Yes	43 (34.7%)
Sheep/goats in the farm	No	113 (92.6%)
	Yes	11 (7.4%)
Participation in cattle fairs/competitions	No	117 (94.3%)
	Yes (no return)	1 (0.8%)
	Yes (possible return)	6 (4.9%)
Pasture	No	70 (56.4%)
	Yes, no contact with other ruminants	28 (22.6%)

	Yes, possible contact with other ruminants	26 (21.0%)

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207   Vehicles visiting the farms represented an infection risk for most of the surveyed

208   farms, because nearly all vehicles entered inside the perimeter of all farms (Table 3).

209   The most frequently entering vehicles were feeder wagons, followed by those collecting

210   animals for slaughter, or calves for feedlot. Moreover, in most farms (92.6%), vehicles

211   in the latter two categories were permitted to arrive carrying domestic ruminants from

212   other farms. Several farms shared machinery or other vehicles, such as manure or slurry

213   vehicles, with other cattle farms.

214   **Table 3. Biosecurity measures related to vehicles and equipment in 124 farms in**

215   **Spain**

		N (%)
Shared feeder wagon	No	48 (38.7%)
	Yes	76 (61.3%)
Average (median) number of entries of the feeder wagon per week, when shared	7.1 (7)	
Shared machinery	No	60 (47.5%)
	Yes	64 (52.5%)
Shared manure vehicle	No	103 (80.1%)
	Yes	21 (18.9%)
Average (median) number of entries of the manure vehicle per month, when shared	0.39 (0.17)	

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Shared materials (ear tag applicators, calving materials, cleaning materials or others)	No	100 (80.3%)
	Yes	24 (19.7%)
Shared slurry vehicle	No	82 (66.2%)
	Yes	42 (33.8%)
Average (median) number of entries of the slurry vehicle per month (when shared)	0.33 (0.08)	
Carcass deposit area	Outside farm perimeter	27 (20.5%)
	Inside farm perimeter	97 (79.5%)
Average (median) number of entries of the carcass vehicle per month inside the farm perimeter (when applicable)	0.34 (0.17)	
Vehicle (slaughter/feedlot) may arrive carrying animals from outside the farm	No	5 (3.3%)
	Not know	5 (4.1%)
	Yes	114 (92.6%)
Vehicles (slaughter/feedlot) may enter inside farm perimeter	No	6 (4.9%)
	Yes	118 (95.1%)
Average (median) number of entries of the slaughter/feedlot vehicle per month inside farm perimeter	2.6 (2)	

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217   Control of visits and staff also showed room for improvement (Table 4). Most

218   farms had no perimeter fences (89.3%), and visitors' parking was usually inside the

219   farm perimeter (96.7%). Farms also commonly received visitors who, without

220   protective clothing, had contact with the animals (92.6%).

221   **Table 4. Biosecurity measures related to visitors and staff in 124 farms in Spain**

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		N (%)
(External) employees	No	68 (55.7%)
	Yes, but do not work in other farms	44 (34.4%)
	Yes, and they work in other farms	12 (9.8%)
Perimeter fence	Yes, always close	5 (4.1%)
	Yes, not always close	8 (6.6%)
	No	111 (89.3%)
Vehicle parking for visitors	Outside farm perimeter	4 (3.3%)
	Inside farm perimeter	120 (96.7%)
Average (median) number of visitors per month that can contact animals	7.2 (5)	
Visitors use protective clothing	Yes	9 (7.4%)
	No	115 (92.6%)

222

223   The MCA with standardized data explained 38.9% of the variance in biosecurity

224   and health status among the 124farms. The percentage of variance explained by the first

225   dimension was 24.7%, and for the second dimension was 14.2%.

226   The main results of the MCA are presented graphically in Fig 1. The object

227   scores obtained from the MCA, together with the solutions of the TSCA, are presented

228   in Fig 2. Three main clusters were formed that included 122 out of the 124 herds. S1

229   Table (within-cluster percentages) shows how each sanitary category or biosecurity

230   variable is split within each cluster.

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Fig 1. Joint MCA plot of category points for the different biosecurity measures, and BVDV/BoHV-1 profiles resulted from multiple correspondence analysis. Frequencies of cattle purchases, and entries of vehicles and visitors, each with 4 categories based on the quartiles of these frequencies, are not shown.

Fig 2. Object scores of the multiple correspondent analysis, and two-step cluster solution identifying 3 main clusters including 122 of the 124 study herds; the remaining 2 are shown in white in the center of the chart.

239

Cluster 1 (n=62) comprised herds located mainly in the upper left quadrant of the MCA chart. All these herds were from Galicia, with low mean herd size (51 cows). These farms were most frequently BVDV and BoHV-1-free.

These farms always checked the sanitary status of purchased animals, either at the farm of origin or on arrival. However, they did not have adequate quarantine facilities. This cluster was the one that most often used external rearing farms. Grazing was observed more frequently than in the other clusters, including possible contact between the farm's cattle and ruminants from other farms. In addition, these herds were located in high-density cattle areas, with most located within 1 km of other cattle farms.

This cluster was also the one that most frequently shared machinery and materials with other farms.

The presence of external workers on Cluster 1 farms was very rare. Farms mostly lacked structures such as perimeter fencing or outdoor parking. Interestingly, these farms received numerous visitors despite their small size. Although the use of

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254 suitable protective clothing emerged as deficient in the entire sample of farms, it was

255 especially inadequate in this cluster.

256 Cluster 2 (n=31) included herds also from Galicia, often of intermediate size

257 (mean 63 cows). From the sanitary point of view, these farms often had seropositive

258 animals but without evidence of recent or active infection.

259 These farms purchased animals less frequently than did those in other clusters.

260 When they did so, purchases often entailed few animals that were tested, usually at their

261 origin, against BVDV and BoHV-1. This was also the cluster with more control over

262 the transport of purchased animals, avoiding contact with external cattle during

263 transport. As in the previous cluster, these farms were located in high-density cattle

264 areas, and most also had other small ruminant farms within 1 km.

265 Structures such as perimeter fencing or outdoor parking for visitors were also

266 scarce in cluster 2 farms. Numbers of external workers were intermediate between those

267 for clusters 1 and 3. Although cluster 2 farms received less visitors, the use of adequate

268 protective clothing was still scarce.

269 Cluster 3 (n=29) comprised all herds from Catalonia, with the largest herds

270 (mean 122 cows). This cluster had the highest proportions of active/recent infections

271 with BVDV and BoHV-1.

272 Purchase of replacement animals was most common in cluster 3, and the number

273 of purchased animals was usually high. Notably, the 11 farms that purchased animals

274 without any testing were included in this cluster. Although infrequent, it was also the

275 cluster with the highest frequency of participation in cattle fairs or contest.

276 Farms in this cluster shared machinery or materials less frequently than did the

277 smaller farms in clusters 1 and 2,but manure vehicles were shared much more often than

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278   in the other two clusters. The frequency of entry of manure and slurry vehicles was also

279   highest in cluster 3 farms, but shared use of feeder wagons was less frequently observed

280   than in the other two clusters.

281   Structures such as perimeter fencing or outdoor parking for visitors were scarce,

282   but nonetheless more frequent than in the other clusters. All cluster 3 farms employed

283   external workers who often worked also on other farms. The use of protective clothing

284   by visitors was somewhat more common in this cluster.

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Discussion

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287   Spain is the eighth-largest dairy producer in the E.U., being responsible for 4.5% of the

288   milk produced (19). Despite this, published information about biosecurity on Spanish

289   dairy cattle farms is very scarce, and official or private initiatives for the

290   implementation of biosecurity programs in this livestock sector are few.

291   Our study showed important short comings in the application of biosecurity

292   measures in this sector, particularly highlighting room for improvement of such

293   measures controlling various potential routes of disease introduction. Fortunately, most

294   farms do not purchase animals, but among those who do there are still farms that do not

295   test these animals. In addition, purchased cattle are habitually permitted to contact other

296   cattle during transport process. Several types of vehicles, especially feeding vehicles,

297   frequently enter farms. Policies related to visitors should also be improved: for example,

298   nearly 93% of visitors that have contact with the animals do not use protective clothing.

299   A similarly lack of implementation of biosecurity measures has been observed

300   on dairy farms elsewhere in Europe (7,11,20). The implementation of biosecurity plans

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301 on dairy farms is voluntary in almost all countries, with the exception of larger dairy

302 farms in Denmark (21). Reg. (EU) No. 429/2016, which shall apply from 2021 and will

303 affect EU animal health legislation, recognizes and addresses the importance of

304 biosecurity (22). Therefore, farmers need to be motivated both to change existing

305 behaviors, and to implement effective biosecurity practices to reduce the risk of disease

306 introduction (23).

307 Our MCA and TSCA analyses show that there are different farm-typologies in

308 relation to the implementation of biosecurity measures and their health status.

309 Cluster 1 includes farms with small herds and low turnovers that would pose a

310 challenge to investment in infrastructure such as perimeter fencing or parking. For the

311 same reason, such farms may frequently be forced to share machinery or materials.

312 Another important point is that these small farms nonetheless receive a high number of

313 visitors who come into contact with animals, both professional (veterinarians,

314 technicians, etc.) and non-professional (neighboring farmers, etc.). Small farms more

315 frequently require timely collaboration from neighboring farmers, for example, in the

316 case of a calving, or receive courtesy visits from them, since such farms function with

317 close links between the farm and the farmer's own house, as has been described

318 elsewhere (24). Despite the abovementioned lack of biosecurity measures, the

319 proportion of such farms with recent/active BoHV-1 or BVDV infection was very low,

320 possibly because of several factors.

321 One important factor is the nature of BVD and IBR control programs in Spain:

322 these are voluntary, and executed only in some AC. In Galicia and other regions in the

323 north-west and the Cantabric area, such programs are conducted mainly through

324 Livestock Health Defense Associations (known as ADSG in Spanish) established by the

325 regional governments in 2004.In Catalonia, these programs are absent. Farms included

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326   in cluster1 (and in cluster 2) were all from the Galicia region. Currently, ADSG groups

327   55.2% of the herds and 65.0% of Galician bovine census (12) and therefore are under

328   control programs. For farms participating in ADSG, it is compulsory to test all

329   purchased animals against BVDV and BoHV-1using established protocols (25). In the

330   dairy cattle sector, the presence of the ADSG has increased awareness of the importance

331   of incorporation of biosecurity controls into farming practices. Many farms that do not

332   belong to ADSG now also practice such controls. Purchased cattle are considered to be

333   the main risk factor for disease entry to dairy farms (3,26-29).

334   In contrast, cluster 3 included the largest herds in our study, located in Catalonia.

335   Herd size has been previously described as a cluster variable for several biosecurity

336   risks such as increased purchase of animals, increased visitors (veterinary practitioners,

337   technicians), and the presence of external workers, all of which will increase the

338   likelihood of disease introduction and maintenance (30). Cluster 3 farms in our study

339   met all these characteristics, and, in addition, sometimes introduced animals without

340   testing. Thus, although some facilities (i.e. perimeter fencing, outdoor parking) or

341   biosecurity measures (use of protective clothing by visitors or scarce use of shared

342   material or equipment) are more frequent in these farms, the cluster 3 showed the

343   poorest sanitary level.

344   On the other hand, when comparing clusters 2 and 1, we observed that farms that

345   purchase animals are somewhat more numerous in cluster 1, so herd size and number of

346   animal purchases are not directly related in these two clusters. The smallest farms

347   sometimes combine dairy production with other professional activities, using dairy

348   farming to supplement family income (31). Thus, the lack of labor and even facilities

349   for rearing heifers could explain the higher frequency of purchases than larger farms in

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350   cluster 2. Cluster 1 farms also use external rearing farms more frequently than do farms

351   in cluster 2.

352   The most frequent sanitary status for cluster 2 was the presence of seropositive

353   animals without evidence of recent or active infection.

354   The farms of cluster 2 are located in areas of high cattle density and very often

355   close to small ruminant farms. In Spain, the particular case of BVDV circulation in

356   sheep herds has been described (32,33);owners of these herds almost never implement

357   BVDV and border disease virus control programs, and act only in cases of clinical

358   outbreaks. Thereby, the risk they could pose is unknown.

359   The influence of our methodology on these results must be considered.

360   Questionnaires were completed during face-to-face interviews on farms. This enabled

361   us to explain the questions clearly, and to control for the bias related to social

362   desirability response; in addition, the veterinarian responsible for the sanitary program

363   of the farm was present. However, it is important to keep in mind that farmers enrolled

364   voluntarily in the study, and therefore our results cannot be extrapolated directly to all

365   dairy farms in Spain due to possible selection bias. Farmers more concerned with

366   biosecurity might have decided to participate in the project, resulting in over-

367   representation of farms with relatively good implementation of biosecurity measures in

368   our sample. Additionally, the existence of voluntary ADSG BoHV-1 and BVDV control

369   programs in Galicia but not in other regions may have reduced the representative value

370   of our sample.

371   Nevertheless, despite the inherent limitations of this study we believe it to have

372   provided a comprehensive overview of the main biosecurity shortcomings in the dairy

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373   sector of Spain. Such data should be useful to focus future training and to improve risk

374   reduction strategies in this economically important industry.

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Supporting information

S1 Table. Composition of sanitary and biosecurity profiles within 3 clusters

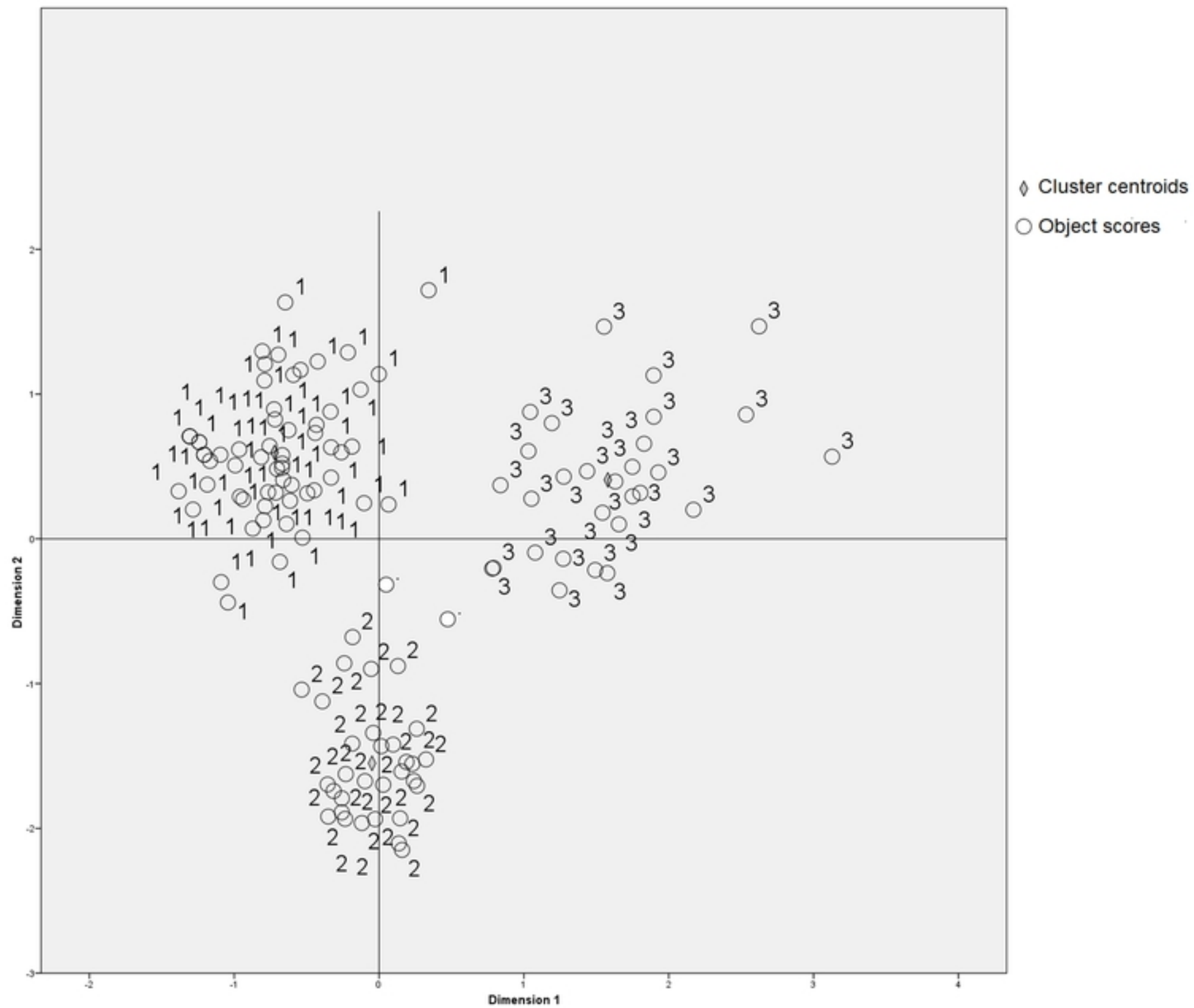**obtained through a two-step cluster analysis**

27

Legend:
◇ Adequate quarantine facilities
○ (External) employees
◉ BoHV-1
◇ BVDV
⊡ Cattle farms within 1 km
□ External rearing
△ Participation in cattle fairs/contest
⌣ Pasture
⊝ Perimeter fence
⋈ Purchase of replacement heifers/cows
⋈ Region
▭ Sanitary plan in the external rearing farm
▥ Shared machinery
+ Shared manure vehicle
Ⱶ Shared material
× Shared slurry vehicle
△ Sheep/goat farms within 1 km
⊥ Sheep/goats in the herd
✳ Vehicle parking for visitors
⊙ Vehicles transporting replacement heifers/cows
⊙ Vehicles transporting animals for slaughter or for fattering can enter inside farm perimeter
○ Vehicles transporting animals for slaughter or for fattering can come with other animals
⊙ Visitors use protective clothing

Yes, but animals never return to farm

No

Yes

Yes

Not known

Yes, some animal can return to farm

Replacements can contact with other cattle during transport

Yes, always close

Yes (sanitary status at arrival)

Free farm

Outside farm perimeter

+Yes

Access to pasture (possible contact with other ruminants)

No Inside farm perimeter No

Yes

Access to pasture (no contact with other ruminants)

Yes, introduced without testing

Replacements can not contact with other cattle during transport

No Free farm Yes Yes (sanitary status in origin) Recent/active infection

No

No

No

Catalonia

Yes, and they work in other farms

Recent/active infection

Galicia

Yes No Yes Yes
No No No Yes No

Yes

No Not applicable

Yes, but not always close

Not applicable No
No

No access to pasture

Yes, but do not work in other fams

Not applicable

No

No

Does not have

Yes
Yes Seropositivity (rearing heifers free)

Seropositivity (rearing heifers free)

Dimension 2

Dimension 1

Fig1

Fig2