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A survey of biosecurity measures and serological status for bovine viral diarrhoea virus and bovine herpesvirus 1 on dairy cattle farms in north-west and north-east Spain

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ABSTRACT

Background Biosecurity is a key measure to reduce and prevent the introduction of diseases to farms and minimise spread of diseases within a herd. The aim of the study was to characterise the current application of biosecurity measures on dairy cattle farms in Spain along with their bovine viral diarrhoea and infectious bovine rhinotracheitis status.

Methods Data on biosecurity measures for 124 dairy herds were collected using a questionnaire. The sanitary status of these farms for bovine viral diarrhoea and infectious bovine rhinotracheitis was also assessed using antibody ELISA. Data were analysed using multiple correspondence analysis and a two-step cluster analysis.

Results Three main clusters of farms were identified: clusters 1 and 2 included herds of small and intermediate sizes. These, particularly cluster 1, showed the most deficiencies in the control of vehicles and visitors. However, laboratory tests were always performed on purchased animals. 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 more often.

Conclusion The study indicated that, in the study population, there are serious shortcomings in the application of biosecurity measures on dairy farms, exposing them to disease transmission. This survey also highlights regional and herd size-related differences in the implementation of biosecurity. Collecting data is an important first step to identification of specific weaknesses in different farm typologies, and an adequate follow-up is needed to ensure that measures are implemented correctly on farms.

INTRODUCTION

Infectious agents that affect livestock may be transmitted by various routes such as live infected animals, trucks and other vehicles, people, aerosols, fomites, wildlife and other animal vectors. Biosecurity has been estimated to be the most cost-effective protection against animal diseases.[1–3]

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.[4] 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.[5 6]

The importance of biosecurity is highlighted in the EU health strategy. From 2007 onwards, the EU embraced a new motto as part of its Animal Health Strategy, 'prevention is better than cure', implemented by the EC.[7] In 2019, the Council of the European Union still emphasised the key role of biosecurity, which should be seen not only as the concrete management and physical measures designed to reduce the risk of transmission of animal diseases at the farm level, but also as a wider concept, as a strategic and holistic approach to managing relevant risks.[8]

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.[9] In addition, in pig herds, a link between biosecurity and antimicrobial treatment-related criteria has been demonstrated and quantified.[10]

Despite these benefits, implementation of biosecurity on dairy cattle farms is often insufficient. Poor or inappropriate knowledge transfer is often cited as a potential cause of disease spread.[11] The main limitations and strengths of the biosecurity measures applied in dairy cattle farms have been studied recently in several countries.[11–16] In Spain, despite the economic importance of milk production in some regions, current biosecurity practices on dairy farms have been scarcely studied, except for a recent survey assessing perceptions and practices applied by rural veterinarians.[17]

Moreover, bovine viral diarrhoea (BDV) and infectious bovine rhinotracheitis (IBR) have been often considered in previous biosecurity studies, as the implementation of their related biosecurity measures should be prioritised in biosecurity programmes.[14 17] The sanitary status of dairy cattle farms with respect to these two endemic diseases is highly variable among Spanish regions. In some, the situation is unknown and the approach to managing outbreaks depends mainly on the actions of individual veterinarians and farmers. In others, mainly in the north-west, voluntary programmes were established some time ago and now involve a large proportion of the cattle population.[18]

BDV and IBR, caused by the bovine viral diarrhoea virus (BVDV) and the bovine herpesvirus 1 (BoHV-1), respectively, are highly contagious diseases of economic and trade importance for the livestock industry worldwide. Several European countries have implemented official voluntary or compulsory programmes to eradicate both diseases.[19 20]

The aim of the present study was to assess and characterise the current application of biosecurity measures on dairy cattle farms in two autonomous communities (ACs) of Spain (first-level administrative division): Galicia and Catalonia. Considering that BVD and IBR are endemic in most countries with cattle production in the absence of control programmes and given that many key biosecurity measures for cattle herds relate to these diseases,[14 17] the present study analysed the sanitary status of farms in relation to BVD and IBR along with biosecurity data.

MATERIALS AND METHODS

Area description and herds surveyed

The study was conducted in two ACs of Spain: Galicia (north-west) and Catalonia (north-east). Galicia is the main dairy cattle area of the country, 55 per cent of farms and 38 per cent of milk production. The mean herd size per farm is 43 cows, lower than the national mean of 59.3, and farms are still predominantly family owned and managed. In Galicia, an official voluntary BoHV-1 and BVDV control programme has been in place since 2004. This region is representative of the production type prevailing in the north-west and Cantabric area of the country. By contrast, farms in Catalonia have a mean herd size of 144 cows, with 4 per cent of the farms nationally yielding 11 per cent of milk production.[21] In this region, no official control programme is established. The farm typology in this region could be considered representative of the rest of Spain.

One hundred and twenty-four dairy farms were selected, 93 from Galicia and 31 from Catalonia, as part of a larger national project on risk analysis for the introduction of BVDV and BoHV-1 to dairy cattle herds. They represented 1.3 per cent and 5.7 per cent of the dairy farms from Galicia and Catalonia, respectively. For farm selection, the project was presented to the veterinarians responsible for health management in the different dairy cattle areas from both regions. During their routine farm visits, veterinarians willing to take part explained the project to the farmers. Those interested in participating volunteered to enrol in this study, which was not incentivised. Thereby, the selection of farms was not random, and the sample size corresponds to those farmers who voluntarily joined the project (conditional on their veterinarians' willingness to participate).

Biosecurity questionnaire

Biosecurity data were obtained using a questionnaire. The questionnaire was designed based on a literature review,[9–13] and discussions with researchers and veterinarians at the Santiago de Compostela University and Autonomous University of Barcelona, regional government veterinarians and cattle veterinarians of diverse subspecialties in both ACs (online supplementary file 1).

The questionnaire (available in Spanish and English on request) consisted of closed questions and included four sections: (1) general farm information (eg, location, herd size, vaccination programmes); (2) animal movements (eg, origin of the animals, frequency of introductions, test, quarantine facilities, external rearing farms, cattle fairs or competitions, pasture) and neighbouring herds (ie, other ruminant farms in a 1-km radius); (3) movements and types of vehicles and equipment (for live and dead animal transport, manure, slurry and feeding vehicles, machinery) and biosecurity-related measures (eg, vehicles may enter inside the farm perimeter, vehicles may enter inside the farm perimeter with animals from other farms); and (4) visitors and staff (eg, external workers; frequency of visits by professionals such as veterinarians and technicians, or non-professionals such as neighbouring farmers, who come in contact with the animals; use of protective clothing).

The questionnaire was first administered to five test farms (three in Galicia and two in Catalonia, all of which are not included in the main sample) to assess for clarity of the question items and whether respondents had suggestions for improvement. As a result of this survey, minor modifications were made to the questions.

Finally, in the study farms, the questionnaire was completed by a researcher from Santiago de Compostela University (Galicia) or from Autonomous University of Barcelona (Catalonia) (authors of the present study) during farm visits, with the farmer and the veterinarian responsible for managing the health of each farm

being present. The interview appointments were scheduled according to participants' convenience. Ensuring the presence of the veterinarian (who knows the usual management and biosecurity practices of each farm) and scheduling the interview during the visit to the farm itself sought to improve the accuracy of the data collected. In addition, farmers were informed that questionnaires were processed anonymously and that no individual farm data would be disclosed.

Each farm was visited once between July 2017 and April 2018. The approximate time needed to complete the questionnaire and the farm visit was 2.5 hours.

Sanitary status

The BVDV and BoHV-1 infection status of the farms was determined using antibody ELISA following the guidelines from the Animal Health and Production Laboratory of Galicia[22] and taking into consideration the fact that farms that applied vaccines used inactivated vaccines in case of BVDV and marker vaccines (live or inactivated) in case of BoHV-1.

Samples for antibody ELISA were collected by the veterinarians responsible for each farm involved in the project within three months since the completion of the questionnaire. Briefly, blood was collected from all cattle from nine months to 24 months. At the same time, a bulk tank milk (BTM) sample was collected from each farm. In case these samples tested ELISA-negative, blood was also collected and analysed for antibodies from all cattle older than two years to determine free status. Samples were stored at −70°C until analysis.

For BVDV, antibodies to the p80 antigen were determined using a commercial blocking ELISA (BVD p80 Ab, IDEXX Laboratories, The Netherlands). In animals vaccinated with inactivated vaccines, the antibodies mainly react with structural proteins rather than the p80 antigen (non-structural).[23] This allowed for differentiation between animals exposed to wild-type BVDV and the vaccine virus since live vaccines were not used in the herds examined here. For BoHV-1, two different tests were used depending on whether vaccines were used on the herd (IDEXX IBR gE antibody, IDEXX Laboratories) or not (IDEXX IBR gB antibody, IDEXX Laboratories). All tests were run in accordance with the manufacturer's instructions.

Using the results of ELISA tests, three different farm profiles were established for each of the two viruses: (1) farms with recent or active infection (seropositivity in heifers from nine months to 24 months born on the farm); (2) farms with seropositive adult animals (positive BTM or negative BTM with at least one seropositive cow >24 months) but all rearing heifers (9–24 months) seronegative; or (3) free farms (BTM and all individual animals tested seronegative).[22 24 25]

Statistical analysis

All statistical tests were performed with SPSS V.15.0. Study data is provided as online supplemental file 2. Initially, the frequencies of the different BoHV-1 and BVDV profiles and biosecurity measures were analysed.

A multiple correspondence analysis (MCA) was performed[26] to characterise the current application of biosecurity measures in dairy cattle farms from two ACs in Spain. MCA aims to reduce a set of possibly correlated variables (including all the biosecurity variables and the sanitary status of the farms) to a smaller group of linearly uncorrelated dimensions. The number of dimensions was set to two, to facilitate two-dimensional graphical representation. The position of the full set of categories for each investigated variable (category points) on the MCA graph is the basis for revealing relationships among variables: variable categories with a similar profile tend to group together, whereas negatively correlated categories are located on opposite sides of the graph. The origin of the graph reflects the weighted mean of the categories for each variable considered in the study (centroid). As a result, the distance from the origin to a category point is the reflection of the variation from the mean pattern (the most frequent category for each variable). In addition, a two-step cluster analysis (TSCA)[27] was performed. TSCA aims to classify a sample of subjects (farms) on the basis of a set of measured variables into a number of different groups such that similar subjects are placed in the same group. Thereby, clusters of farmers with similar implemented biosecurity measures and BVDV and BoHV-1 profiles were identified. The frequencies of entry of animals, vehicles and visitors were categorised in quartiles for MCA and TSCA but were included in the final results' tables as mean and median frequencies.

RESULTS

BVDV and BoHV-1 profiles of the 124 farms are summarised in table 1. The proportion of farms categorised as recent or active infection for both BVDV and BoHV-1 was 5.6 per cent (7 of 124). Thirty per cent of the farms used inactivated vaccines against BVDV, whereas the remainder did not vaccinate; 27 per cent used marker vaccines for BoHV-1, whereas the remainder did not vaccinate.

Table 2 describes the biosecurity measures related to animal movements and possible contact with other

Table 1 BVDV and BoHV-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)
	Free farm	56 (44.3)

BoHV-1, bovine herpesvirus 1; BVDV, bovine viral diarrhoea virus.

Table 2 Biosecurity measures related to purchase of cattle, or possible contact 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)
Mean (median; Q1–Q3*) frequency of heifers or cows purchased per year, when applicable (46 farms)	5.25 (3; 2–6)
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
Embryo transfer	
No	118 (95.2)
Yes (sanitary status of donor cows known)	6 (4.8)
Yes (sanitary status of donor cows unknown)	0 (0)
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 on 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)

*Q1: 25th percentile; Q3: 75th percentile.
†Separate buildings, outside farm perimeter and animals quarantined for an adequate length of time to confirm their sanitary status.
‡Raising in specialised farms with animals of other farms.
§Rearing farm is under the official BVD/IBR control programme (ie, Livestock Health Defense Association) or equivalent to the official one.
BDV, bovine viral diarrhoea; IBR, infectious bovine rhinotracheitis.

domestic ruminants. The mean of purchases was 5.2 animals per year in farms that purchased cattle (46 of 124, 37.1 per cent). It is noteworthy that 11 of 46 (23.9 per cent) farms purchased animals without any testing, and 120 of 124 farms (98.6 per cent) lacked adequate quarantine facilities. Animal movement to cattle fairs or competitions took place only in seven of 124 farms (5.7 per cent). Embryo transfer was only performed in six of 124 (4.8 per cent) farms. Access to pasture was reported by 54 of 124 farms (43.6 per cent), and 51.8 per cent (28 of 54) reported the possibility of contact with other domestic ruminants at the pasture.

Vehicles visiting the farms represented an infection risk for most of the surveyed farms because nearly all vehicles entered inside the perimeter of all farms (table 3). The most frequently entering vehicles were feeder wagons (mean 7.1 times/week), followed by those collecting live animals (for slaughter or calves for feedlot) (mean 2.6 times/month). Moreover, in 114 of 124 farms (92.6 per cent), vehicles in the latter two categories were permitted to enter the farms carrying domestic ruminants from other farms.

Control of visits and staff also showed room for improvement (table 4). Most farms (111 of 124) had no perimeter fences (89.3 per cent), and visitors' parking was usually (120 of 124) inside the farm perimeter (96.7 per cent). Farms also commonly (115 of 124) could receive visitors who, without protective clothing, had contact with the animals (92.6 per cent).

The MCA with standardised data explained 38.9 per cent of the variance in biosecurity and sanitary status among the 124 farms. The percentage of variance explained by the first dimension was 24.7 per cent and for the second dimension was 14.2 per cent.

The main results of the MCA (joint plot of category points) are presented graphically in figure 1. Figure 2 represents the clustered farms and their location on the MCA chart. Three main clusters were formed that included 122 out of the 124 herds. Online supplementary table S1 (within-cluster percentages) shows how each sanitary category or biosecurity variable is split within each cluster.

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). They were most frequently BVDV-free and BoHV-1-free. These farms 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. Access to pasture 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 was very rare. Farms mostly lacked

Table 3 Biosecurity measures related to vehicles and equipment in 124 farms in Spain

	n (%)
Shared feeder wagon	
No	48 (38.7)
Yes	76 (61.3)
Mean (median; Q1–Q3*) number of entries of the feeder wagon per week, when shared	7.1 (7; 7–7)
Shared machinery (tractors, wagons, cistern, slurry whisk, baler or others)	
No	60 (47.5)
Yes	64 (52.5)
Shared manure vehicle	
No	103 (80.1)
Yes	21 (18.9)
Mean (median; Q1–Q3) number of entries of the manure vehicle per month, when shared	0.39 (0.17; 0.12–0.54)
Shared materials (ear tag applicators, calving materials, cleaning materials, harvesting materials or others)	
No	100 (80.3)
Yes	24 (19.7)
Shared slurry vehicle	
No	82 (66.2)
Yes	42 (33.8)
Mean (median; Q1–Q3) number of entries of the slurry vehicle per month, when shared	0.33 (0.08; 0.08–0.60)
Carcase deposit area	
Outside farm perimeter	27 (20.5)
Inside farm perimeter	97 (79.5)
Mean (median; Q1–Q3) number of entries of the carcase vehicle per month inside the farm perimeter (when applicable)	0.34 (0.17; 0.12–0.51)
Vehicle (slaughter/feedlot) may arrive carrying animals from outside the farm	
No	5 (3.3)
Not known	5 (4.1)
Yes	114 (92.6)
Vehicles (slaughter/feedlot) may enter inside farm perimeter	
No	6 (4.9)
Yes	118 (95.1)
Mean (median; Q1–Q3) number of entries of the slaughter/feedlot vehicle per month inside farm perimeter	2.6 (2; 1.5–5.3)

*Q1: 25th percentile; Q3: 75th percentile.

structures such as perimeter fencing or outdoor parking. Interestingly, they received numerous visitors despite their small size. Although the use of suitable protective clothing emerged as deficient in the entire sample of farms, it was especially inadequate in this cluster.

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

	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 closed	5 (4.1)
Yes, not always closed	8 (6.6)
No	111 (89.3)
Vehicle parking for visitors	
Outside farm perimeter	4 (3.3)
Inside farm perimeter	120 (96.7)
Mean (median; Q1–Q3*) number of visitors per month that can contact animals	7.2 (5; 3–9)
Visitors always use protective clothing†	
Yes	9 (7.4)
No	115 (92.6)

*Q1: 25th percentile; Q3: 75th percentile.
†Coveralls or overcoats and boots that are provided by the farmer before contacting cattle area.

Cluster 2 (n=31) included herds also from Galicia, often of intermediate size (mean 63 cows). From the sanitary point of view, seropositivity against BoHV-1 or BVDV without evidence of recent or active infection was most frequently observed. These farms purchased animals less often than those in other clusters. When they did so, purchases often entailed few animals that were tested against BVDV and BoHV-1. This was also the cluster with more control over the transport of purchased animals, avoiding contact with external cattle during transport. As in the previous cluster, these farms were located in high-density cattle areas, and most also had other small ruminant farms within 1 km. The feeder wagon was very frequently shared, being a vehicle that entered the farms daily (this particularity also occurred in cluster 1, although at a lower proportion). Structures such as perimeter fencing or outdoor parking for visitors were also scarce. Numbers of external workers were intermediate between those for clusters 1 and 3. Although cluster 2 farms received, on average, less visitors than clusters 1 and 3, the use of adequate protective clothing was still scarce.

Cluster 3 (n=29) comprised all herds from Catalonia, with the largest herds (mean 122 cows). This cluster had the highest proportions of active or recent infections with BVDV and BoHV-1. Purchase of replacement animals was most common, and the number of purchased animals was usually high. Notably, the 11 farms that purchased animals without any testing were included in this cluster. Although infrequent, it was also the cluster with the highest frequency of participation in cattle fairs

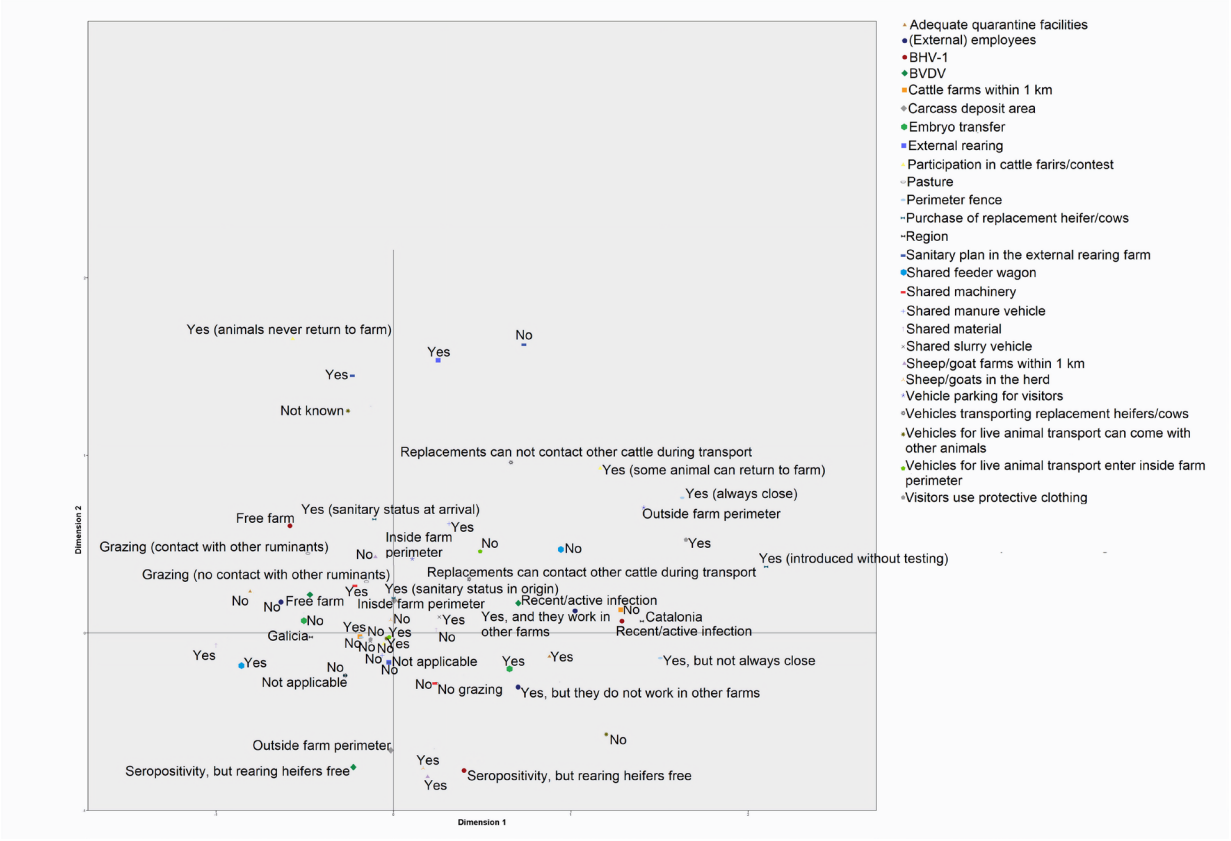

Figure 1 Joint MCA plot of category points for the different biosecurity measures and the BVDV/BoHV-1 profiles that resulted from MCA. Frequencies of cattle purchases and entries of vehicles and visitors, each with four categories based on the quartiles of these frequencies, are not shown. BoHV-1, bovine herpesvirus 1; BVDV, bovine viral diarrhoea virus; MCA, multiple correspondence analysis.

or contest. Farms in this cluster shared machinery or materials less frequently than did the smaller farms in clusters 1 and 2, although manure vehicles were shared much more often than in the other two. Structures such as perimeter fencing or outdoor parking for visitors were scarce, but nonetheless more frequent than in the other clusters. All cluster 3 farms employed external workers who often worked also on other farms. The use of protective clothing by visitors was also somewhat more common in this cluster.

DISCUSSION

The present study shows important shortcomings in the application of biosecurity measures, particularly highlighting room for improvement of such measures controlling various potential routes of disease introduction.

Fortunately, many farms do not purchase animals, reflecting a higher percentage of 'closed' herds than those described in other biosecurity studies in Europe,[9 11–13] but still lower than that reported in Denmark, where 76.3 per cent of surveyed farms had not purchased animals in the previous year.[16] Among those who do, there are still farms that do not regularly assess the sanitary status of these animals. The scarcity of quarantine facilities on farms is remarkable and has previously been observed in other populations.[13 28] Appropriate quarantine facilities were considered separate buildings and outside farm perimeter and with animals quarantined for an adequate length of time to confirm their sanitary status (ie, to test the animals—if they have not been tested in origin or the transport conditions cannot be controlled—and at least until the laboratory results are received).[22] Other movements that may pose a risk (ie, rental/shared bulls) do not occur in the study population. In pasture, the concept of contact with other ruminants comprised any contact, either direct or indirect, with organic matter of ruminants from other farms or with animals themselves (in pasture or as the ruminants were herded into the pasture). In the study population, the cows were consistently herded into meadows in close vicinity of the farm but, given the high density of farms in many of the areas under observation, contact was likely to take place.

Several types of vehicles, especially feeding vehicles, frequently enter farms. Biosecurity measures related to visitors should also be improved: for example, nearly 93 per cent of the farms could have visitors that have contact with the animals and did not use protective clothing. Adequate protective clothing was defined as coveralls or overcoats and boots that are provided by the farmer before contacting the cattle area. A similar lack has been observed on dairy farms in Europe,[9 13 17] although

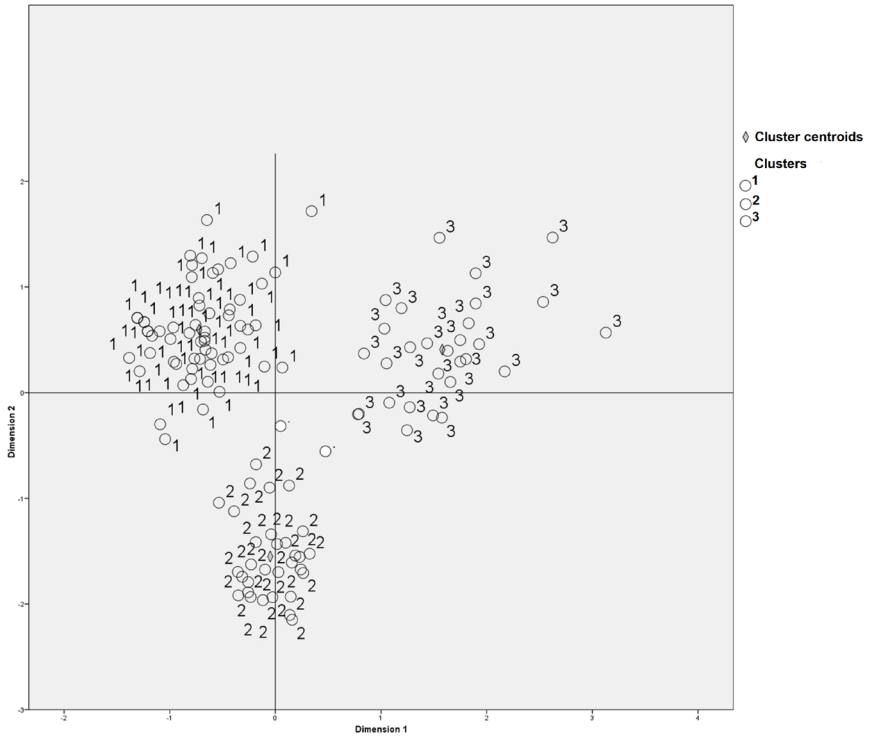

Figure 2 Clustered farms and their location on the multiple correspondence analysis chart. Two-step cluster solution identifying three main clusters including 122 of the 124 study herds; the remaining two are shown in white in the centre of the chart.

countries in Northern Europe reported better implementation of biosecurity measures in this regard.[12 29]

The implementation of biosecurity plans on dairy farms is voluntary in almost all countries. One exception within the UE is Denmark.[30] The biosecurity legislation in this country may have contributed to adoption of good biosecurity practices[31] and has been recently extended from only large (>380 animals) to all farms.[30] Regulation (EU) No 429/2016, which shall apply from 2021 and will affect EU animal health legislation, recognises and addresses the importance of biosecurity.[32] Therefore, farmers need to be motivated both to change existing behaviours and to implement effective biosecurity practices to reduce the risk of disease introduction.[33–35]

MCA and TSCA show that there are three main farm typologies in relation to the implementation of biosecurity measures differentiated mainly by region (which implies different implementation of BVDV and BoHV-1 control programmes) and herd size. Although some biosecurity limitations were common, others were more specific to each cluster, similar to the observation made by Sarrazin and others.[13]

Therefore, cluster 1 included the smallest herds in which low turnovers would pose a challenge to investment, which may limit biosecurity (ie, these farms may frequently be forced to share some vehicles, machinery or materials, and infrastructures such as perimeter fencing or parking are scarce). Additionally, the high number of visitors they receive may be related to the fact that small farms more frequently require timely collaboration from neighbouring farmers (eg, in the case of a calving). They also often receive courtesy visits from them, since such farms function with close links between the farm and the farmer's own house, as has been described elsewhere.[36]

Despite the lack of several biosecurity measures, the proportion of such farms with recent or active BoHV-1 or BVDV infection was low, possibly due to several factors. The most prominent refers to the nature of the aforementioned BVD and IBR control programmes in Spain: these are voluntary and executed only in some ACs. In Galicia and other regions in the north-west and the Cantabric area, such programmes are conducted mainly through Livestock Health Defense Associations (known as ADSG in Spanish) established by the regional governments in 2004. In Catalonia, these programmes are absent.

Farms included in cluster 1 (and cluster 2) were all from the Galicia region, where ADSGs included 55.2 per cent of the herds and 65.0 per cent of Galician bovine census and are therefore under BVD and IBR control programmes.[18] These programmes are based on serological surveillance of herds and progressive reduction of seroprevalence (mainly by controlling replacements; seropositive animals are not culled). Only in the case of BVDV, when tested samples indicated possible persistent infection (PI) in an animal (ie, when a positive result was obtained for a young heifer in serological test), the PI cattle was identified and eliminated, where appropriate. To prevent new infections, it is mandatory to test all purchased animals against BVDV and BoHV-1 using established protocols.[22] This measure is controlled through the official register on livestock movements (REMO, by its Spanish acronym), and the non-performance of these

tests implies economic penalties.[22] However, for herds in ADSG, there is no obligation over other biosecurity actions, although recommendations are provided to each farm.

Thus, the existence of control programmes has an impact on the sanitary status of these farms; however, apart from the control of purchased animals (mandatory), the ADSG programmes do not seem to have significant influence on the application of many other measures concerning biosecurity (recommended but not mandatory), judging by the critical deficiencies that have been observed. Although purchased cattle are considered to be the main risk factor for disease entry to dairy farms,[37–41] the importance of other measures should not be underestimated and may result in less use of monetary and labour resources intended for control programmes.

In cluster 2, the most frequent sanitary status was the presence of seropositive animals without evidence of recent or active infections. In the case of BVDV, the percentage of farms with active infection is higher than in cluster 1, although still far from that observed in cluster 3. The virus is more likely to persist over time in larger herds. Viral persistence may increase with herd size because self-clearance may be more frequent in small herds due to stochastic events.[42]

Cluster 3 included the largest herds in the study, located in Catalonia. Herd size has been previously described as a cluster variable for several biosecurity risks such as increased purchase of animals, increased visitors (veterinary practitioners, technicians) and the presence of external workers, all of which will increase the likelihood of disease introduction and maintenance.[24] Cluster 3 farms in the present study met all these characteristics and often introduced animals without testing. Relevantly, as mentioned, no official control programme has been established in this region. Thus, although some biosecurity facilities or biosecurity measures were more frequent in these farms than in the other two clusters, cluster 3 showed the poorest sanitary level, especially in relation to BVDV.

Additionally, it should be noted that when comparing clusters 1 and 2, it was observed that farms that purchase animals were somewhat more numerous in cluster 1, so herd size and number of animal purchases were not directly related in these two clusters. The smallest farms sometimes combine dairy production with other professional activities, using dairy farming to supplement family income.[43] Thus, the lack of labour and even facilities for rearing heifers could explain the higher frequency of purchases than larger farms in cluster 2.

The influence of the study's methodology on these results must be considered. Questionnaires were completed during face-to-face interviews on farms in the presence of the veterinarian responsible for the sanitary programme of the farm. This enabled clear explanation of questions and control of bias related to social desirability response, although the authors may have been able to obtain a larger sample size using mailed questionnaires. However, it is important to keep in mind that farmers enrolled voluntarily in the study, and therefore the results cannot be extrapolated directly to all dairy farms in Spain due to possible selection bias. Farmers more concerned with biosecurity might have decided to participate in the project, resulting in over-representation of farms with relatively good implementation of biosecurity measures in the sample. Additionally, the existence of voluntary ADSG BoHV-1 and BVDV control programmes in Galicia but not in other regions may have reduced the representative value of the sample. In addition, MCA is an exploratory method and interpretations of the results may not extend to the magnitude of the effects of biosecurity measures on BVD and IBR status.[44]

The study confirms previous perceptions that there is a substantial lack in the application of biosecurity measures,[17] even on farms under sanitary programmes. The fact that there are still herds that introduce untested animals and that there is lack of quarantine facilities in most farms indicates that there is a need for improvement in this regard. Additionally, in view of the frequency of non-farm and shared vehicles entering inside farm perimeter, measures should be taken to reduce these entries to essential ones and when necessary cleaning and disinfection programmes should be followed for these vehicles. Visitor control, and the associated biosecurity measures (ie, use of protective clothing), also appears to be a priority, given the observed deficiencies and the small distances to neighbouring farms (most farms had other cattle farms within 1 km). This survey also highlights regional and herd size-related differences in the implementation of biosecurity measures.

In Spain, regional and national governments are increasingly involved in generating debate, awareness campaigns and training in promoting the implementation of biosecurity as a previous step to develop specific legislation to force farmers to implement some recommendations.[36] Despite the inherent limitations of this study the authors believe it to have provided a comprehensive overview of the main biosecurity shortcomings in the dairy sector of Spain and to identify clusters in terms of biosecurity measures and sanitary status. Such data should be useful to focus training and awareness programmes and to improve risk reduction strategies in this economically important industry.

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