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# **Biosecurity in cattle production: animal transport, assessment methods, and cost-effectiveness of measures**

**Fernando Javier Duarte Godoy**

**PhD thesis**

**2025**

**UAB**  
Universitat Autònoma  
de Barcelona



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**Departament de Sanitat i d'Anatomia Animals**

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Tesi doctoral presentada per **Fernando Javier Duarte Godoy** per accedir al grau de Doctor en Veterinària dins del programa de Doctorat en Medicina i Sanitat Animals de la Facultat de Veterinària de la Universitat Autònoma de Barcelona, sota la direcció del Dr. Alberto Allepuz Palau i la Dra. Giovanna Ciaravino.

Bellaterra (Cerdanyola del Vallès), 2025



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Declaren:

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I per a que consti als efectes oportuns, signen la present declaració a Bellaterra, 1 de juliol de 2025:

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**Fernando Javier Duarte Godoy**

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**Doctorand**



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*Prevention is better than cure*

*Prevenir es mejor que curar*

*Prevenir és millor que curar*

Latin proverb



# TABLE OF CONTENTS

List of abbreviations and acronyms .....	vii
Summary .....	ix
Resumen .....	xi
Resum .....	xv
Publications.....	xvii
<b>Chapter I: Introduction .....</b>	<b>1</b>
1    Introduction .....	3
1.1    Definition and importance of biosecurity on animal farms .....	3
1.1.1    Assessing on-farm biosecurity.....	6
1.2    Biosecurity in animal farms .....	8
1.2.1    External biosecurity .....	8
1.2.1.1    Animal introduction.....	9
1.2.1.2    Cattle grazing outdoors .....	11
1.2.1.3    Visits.....	12
1.2.1.4    Vehicles entering the farm .....	12
1.2.1.5    Feed and water.....	13
1.2.2    Internal biosecurity .....	14
1.2.2.1    Farm management.....	15
1.2.2.2    Disease management.....	16
1.2.2.3    Flies, rodents, dogs and cats .....	16

1.3	Live cattle transport .....	17
1.3.1	Biosecurity measures applied during the transport of animals .....	19
1.3.1.1	Loading and unloading procedures .....	20
1.3.1.2	Cleaning and disinfection of transport vehicles .....	21
1.3.1.3	Vehicle assignment and transport policies .....	23
1.3.2	Legislation on biosecurity in the transport of live animals .....	23
1.4	Economic costs and benefits of biosecurity on farms .....	25
1.5	References .....	31
<b>Chapter II: Objectives</b>	.....	<b>49</b>
2	Objectives .....	51
<b>Chapter III: Research articles</b>	.....	<b>53</b>
3	Study I: Characterization of biosecurity practices among cattle transport drivers in Spain	55
3.1	Abstract .....	57
3.2	Introduction.....	58
3.3	Material and methods .....	59
3.3.1	Sampling design and sample selection .....	59
3.3.2	Survey .....	60
3.3.3	Definitions used in this study.....	61
3.3.3.1	Journey .....	61
3.3.3.2	Categories of transported animals .....	62
3.3.3.3	Shared journey .....	62
3.3.4	Data analysis .....	63

3.4	Results .....	64
3.4.1	Descriptive analysis .....	64
3.4.2	Multiple correspondence analysis .....	71
3.4.3	Hierarchical clustering on principal components .....	73
3.5	Discussion .....	75
3.6	Conclusions .....	79
3.7	Acknowledgements .....	80
3.8	Financial disclosure statement .....	80
3.9	Ethics statement.....	80
3.10	References .....	81
3.11	Supplementary material.....	86
3.11.1	Supplementary material 1: Survey .....	86
3.11.2	Supplementary material 2 .....	89
3.11.3	Supplementary material 3 .....	90
4	Study II: Methods to assess on-farm biosecurity in Europe and beyond .....	97
4.1	Abstract .....	99
4.2	Introduction.....	99
4.3	Material and methods .....	100
4.3.1	Survey design and data collection.....	100
4.3.2	Data analysis .....	102
4.4	Results .....	103
4.4.1	General characteristics of the BAMs .....	103

4.4.2	How the assessment was done .....	106
4.4.3	Output of the biosecurity assessment.....	108
4.4.4	Exploratory clustering .....	109
4.5	Discussion .....	111
4.6	Conclusions .....	113
4.7	Acknowledgments .....	113
4.8	Financial disclosure statement .....	114
4.9	Ethics statement.....	114
4.10	References .....	115
4.11	Supplementary material.....	119
4.11.1	Supplementary material 1: Survey .....	119
4.11.2	Supplementary material 2 .....	134
5	Study III: Estimate the most cost-effective option for improving biosecurity on dairy cattle farms to support informed decision making.....	141
5.1	Abstract .....	143
5.2	Introduction.....	144
5.3	Material and methods .....	145
5.3.1	Estimation of biosecurity cost .....	146
5.3.2	Estimating the cost of a disease outbreak .....	149
5.3.3	Estimating the probability of BVD introduction .....	150
5.3.4	Estimating the cost-effectiveness of farm biosecurity.....	150
5.3.4.1	Sensitivity analysis .....	152

5.3.4.2	Data collection.....	152
5.4	Results .....	153
5.4.1	Farm biosecurity cost.....	153
5.4.2	Risk of BVD introduction and cost of an outbreak .....	154
5.4.3	Decision analysis on biosecurity improvements .....	155
5.4.3.1	Sensitivity analysis .....	156
5.5	Discussion .....	157
5.6	Conclusions .....	161
5.7	Acknowledgements .....	161
5.8	Financial disclosure statement .....	161
5.9	Ethics statement.....	161
5.10	References .....	162
5.11	Supplementary material.....	168
5.11.1	Supplementary material 1: Cost estimation of biosecurity measures. ....	168
5.11.2	Supplementary material 2: Outputs obtained from the SimHerd simulation ...	183
5.11.3	Supplementary material 3 : Decision analysis on the most cost-effective biosecurity improvements .....	185
<b>Chapter IV: General discussion</b>	.....	<b>193</b>
6	General discussion .....	195
6.1	References .....	199
<b>Chapter V: Conclusions</b>	.....	<b>203</b>
7	Conclusions.....	205



## List of abbreviations and acronyms

<b>AN</b>	Andalusia
<b>AR</b>	Aragon
<b>BAMs</b>	Biosecurity assessment methods
<b>BS</b>	Biosecurity
<b>BVD</b>	Bovine viral diarrhoea
<b>CA/BETTER</b>	Cost Action CA20103 Biosecurity Enhanced Through Training, Evaluation and Raising Awareness
<b>CB</b>	Cantabria
<b>CFPs</b>	Country focal points
<b>CI</b>	Confidence interval
<b>CL</b>	Castile and León
<b>CM</b>	Castilla-La Mancha
<b>CT</b>	Catalonia
<b>EMV</b>	Expected monetary value
<b>EC</b>	European Commission
<b>EU</b>	European Union
<b>EX</b>	Extremadura
<b>FAO</b>	Food and Agriculture Organization of the United Nations
<b>FMD</b>	Foot and mouth disease
<b>GA</b>	Galicia
<b>GM</b>	Gross margin
<b>HCPC</b>	Hierarchical clustering on principal components

<b>KPIs</b>	Key performance indicators
<b>MCA</b>	Multiple correspondence analysis
<b>MD</b>	Madrid, Autonomous Community of
<b>MSEP</b>	Mean square error of prediction
<b>OVS</b>	Official veterinary services
<b>PI</b>	Persistently infected
<b>UK</b>	United Kingdom
<b>WOAH</b>	World Organisation for Animal Health

# Summary

The overall objective of this doctoral thesis was to evaluate farm biosecurity from multiple perspectives, considering both the measures applied during cattle transport and the assessment methods used on farms, as well as how improvements in biosecurity may translate into economic benefits for livestock operations.

In the first study, a biosecurity survey was conducted with 82 cattle transport drivers in Spain to characterise the biosecurity practices applied during animal transport. The questionnaire included items related to general driver characteristics, implemented biosecurity measures, and hygiene practices. To explore potential response patterns, a multiple correspondence analysis (MCA) followed by hierarchical clustering on principal components (HCPC) was performed. The results revealed poor compliance with biosecurity measures during transport, including visits to multiple farms in a single journey, entry into farm perimeters, and the failure to clean and disinfect vehicles after each operation. Four response clusters were identified. Clusters 1 and 4 comprised most of drivers and were mainly distinguished by their loading/unloading practices and the frequency of vehicle disinfection. Clusters 2 and 3, which were smaller, included drivers whose routes were primarily to slaughterhouses and those who used dedicated work clothing, respectively. These findings underscore the need to raise awareness of the role animal transport plays in the spread of pathogens between cattle farms and the importance of biosecurity in preventing such transmission.

The second study aimed to identify and characterize biosecurity assessment methods (BAMs) that are used in practice at farms. A structured questionnaire was developed, addressing various aspects such as animal species, BAM objectives, legal requirements, output of the assessment, and whether feedback was provided. The questionnaire was distributed across 28 countries and translated into 23 languages. A descriptive analysis of the responses was conducted, along with an MCA and HCPC. In total, 74 BAMs were identified, most of which were used in specific

countries and for some animal species. Four BAM clusters were distinguished based on their objective, evaluator type, and feedback mechanisms. Cluster 1 included voluntary methods aimed at general biosecurity improvement, while cluster 4 comprised legally mandated methods targeting both general biosecurity enhancement and control of specific diseases. Cluster 2 encompassed quality assurance schemes with additional data collection, whereas cluster 3 grouped methods primarily focused on reducing antibiotic use or the voluntary control of specific diseases. These results highlight the wide diversity of approaches used to assess farm biosecurity and point to the lack of a harmonised assessment protocol.

The third study aimed to estimate the most cost-effective decision to improve biosecurity on dairy cattle farms. A biosecurity cost calculator was developed, which considered the measures currently implemented on each farm and potential improvement scenarios. The cost and probability of bovine viral diarrhoea (BVD) introduction were estimated using stochastic models that incorporated the specific characteristics of each farm. Data integration was carried out through decision analysis, with the objective of identifying the most profitable strategy for each case. The annual cost of the biosecurity measures currently in place ranged from €27.58 to €72.11 per animal, while the cost of a BVD outbreak represented a loss of approximately 6% of the gross margin. The estimated probability of BVD introduction ranged from 0.36% to 15.7%. According to the results of the decision analysis, the most cost-effective measure across all three farms studied was the provision of dedicated boots for cattle transport drivers.

# Resumen

El objetivo general de esta tesis doctoral fue evaluar la bioseguridad en las explotaciones ganaderas desde múltiples perspectivas, considerando tanto las medidas aplicadas durante el transporte de ganado bovino como los métodos de evaluación utilizados en las granjas, y cómo las mejoras en bioseguridad pueden traducirse en beneficios económicos para las explotaciones.

En el primer estudio, se realizó una encuesta sobre bioseguridad a 82 conductores que transportaban ganado bovino en España, con el fin de caracterizar las prácticas de bioseguridad aplicadas durante el transporte. El cuestionario incluyó preguntas relacionadas con las características generales de los conductores, las medidas de bioseguridad implementadas y las prácticas de higiene. Para explorar posibles patrones de respuesta, se llevó a cabo un análisis de correspondencias múltiples (MCA) seguido de una clasificación jerárquica sobre componentes principales (HCPC). Los resultados revelaron un bajo cumplimiento de las medidas de bioseguridad durante el transporte, incluyendo visitas a múltiples granjas en un mismo viaje, entrada en los perímetros de las explotaciones y la falta de limpieza y desinfección de los vehículos tras cada operación. Se identificaron cuatro clústeres de respuestas. Los clústeres 1 y 4 incluyeron a la mayoría de los conductores y se diferenciaron principalmente por sus prácticas de carga/descarga y la frecuencia de desinfección del vehículo. Los clústeres 2 y 3, más reducidos, incluyeron conductores cuyas rutas eran principalmente hacia mataderos y aquellos que utilizaban ropa de trabajo específica, respectivamente. Estos hallazgos subrayan la necesidad de sensibilizar sobre el papel que desempeña el transporte animal en la diseminación de patógenos entre granjas bovinas y la importancia de la bioseguridad para prevenir dicha transmisión.

El segundo estudio tuvo como objetivo identificar y caracterizar los métodos de evaluación de la bioseguridad (BAMs) que se utilizan en la práctica en las explotaciones. Se elaboró un

questionario estructurado que abordaba diversos aspectos, como la especie animal, los objetivos de los BAMs, los requisitos legales, los resultados de la evaluación y si se proporcionaba retroalimentación. El cuestionario se distribuyó en 28 países y se tradujo a 23 idiomas. Se realizó un análisis descriptivo de las respuestas, junto con un MCA y un HCPC. En total, se identificaron 74 BAMs, la mayoría de los cuales se aplicaban en países específicos y para ciertas especies animales. Se distinguieron cuatro clústeres de BAMs en función de su objetivo, el tipo de evaluador y los mecanismos de retroalimentación. El clúster 1 incluía métodos voluntarios orientados a la mejora general de la bioseguridad, mientras que el clúster 4 abarcaba métodos obligatorios por ley destinados tanto a mejorar la bioseguridad general como a controlar enfermedades específicas. El clúster 2 incluía esquemas de aseguramiento de la calidad con recogida adicional de datos, y el clúster 3 agrupaba métodos centrados principalmente en la reducción del uso de antibióticos o el control voluntario de enfermedades concretas. Estos resultados destacan la amplia diversidad de enfoques utilizados para evaluar la bioseguridad en las granjas y evidencian la falta de un protocolo de evaluación armonizado.

El tercer estudio tuvo como objetivo estimar la decisión más coste-efectiva para mejorar la bioseguridad en explotaciones lecheras. Se desarrolló una calculadora de costes de bioseguridad que consideraba las medidas actualmente implementadas en cada granja y distintos escenarios de mejora potencial. Los costes y la probabilidad de introducción del virus de la diarrea viral bovina (BVD) se estimaron mediante modelos estocásticos que incorporaban las características específicas de cada granja. La integración de datos se realizó a través de un análisis de decisión, con el objetivo de identificar la estrategia más rentable en cada caso. El coste anual de las medidas de bioseguridad actualmente implementadas oscilaba entre 27,58 y 72,11 euros por animal, mientras que el coste de un brote de BVD representaba una pérdida de aproximadamente el 6 % del margen bruto. La probabilidad estimada de introducción del BVD oscilaba entre el 0,36 % y el 15,7 %. Según los resultados del análisis de decisión, la medida más

coste-efectiva en las tres granjas estudiadas fue el suministro de botas específicas para los conductores de transporte de ganado.



# Resum

L'objectiu general d'aquesta tesi doctoral va ser avaluar la bioseguretat a les explotacions ramaderes des de múltiples perspectives, tenint en compte tant les mesures aplicades durant el transport de bestiar boví com els mètodes d'avaluació utilitzats a les granges, així com de quina manera les millors en bioseguretat poden traduir-se en beneficis econòmics per a les explotacions.

En el primer estudi, es va dur a terme una enquesta sobre bioseguretat a 82 conductors que transportaven bestiar boví a Espanya, amb l'objectiu de caracteritzar les pràctiques de bioseguretat aplicades durant el transport. El qüestionari incloïa preguntes sobre les característiques generals dels conductors, les mesures de bioseguretat aplicades i les pràctiques d'higiene. Per explorar possibles patrons de resposta, es va realitzar una anàlisi de correspondències múltiples (MCA) seguida d'una classificació jeràrquica sobre components principals (HCPC). Els resultats van revelar un baix compliment de les mesures de bioseguretat durant el transport, incloent visites a diverses granges en un mateix trajecte, entrada als perímetres de les explotacions i la manca de neteja i desinfecció dels vehicles després de cada operació. Es van identificar quatre clústers de resposta. Els clústers 1 i 4 comprenen la majoria dels conductors i es diferenciaven principalment per les pràctiques de càrrega/descàrrega i la freqüència de desinfecció dels vehicles. Els clústers 2 i 3, més petits, incloïen conductors amb rutes principalment cap a escorxadors i aquells que utilitzaven roba de treball específica, respectivament. Aquests resultats posen de manifest la necessitat d'augmentar la consciència sobre el paper que juga el transport animal en la propagació de patògens entre granges bovines i la importància de la bioseguretat per evitar aquesta transmissió.

El segon estudi va tenir com a objectiu identificar i caracteritzar els mètodes d'avaluació de la bioseguretat (BAMs) que s'utilitzen en la pràctica a les explotacions. Es va desenvolupar un qüestionari estructurat que abordava diversos aspectes com l'espècie animal, els objectius dels

BAMs, els requisits legals, els resultats de l'avaluació i si es proporcionava retroacció. El qüestionari es va distribuir en 28 països i es va traduir a 23 idiomes. Es va dur a terme una anàlisi descriptiva de les respostes, juntament amb una MCA i una HCPC. En total, es van identificar 74 BAMs, la majoria dels quals s'utilitzaven en països específics i per a algunes espècies animals. Es van distingir quatre clústers de BAMs segons el seu objectiu, el tipus d'avaluador i els mecanismes de retroacció. El clúster 1 incloïa mètodes voluntaris dirigits a la millora general de la bioseguretat, mentre que el clúster 4 comprenia mètodes exigits legalment orientats tant a la millora general com al control de malalties específiques. El clúster 2 incloïa esquemes d'assegurament de la qualitat amb recollida addicional de dades, mentre que el clúster 3 agrupava mètodes centrats principalment en la reducció de l'ús d'antibiòtics o el control voluntari de malalties específiques. Aquests resultats destaquen la gran diversitat d'enfocaments utilitzats per avaluar la bioseguretat a les explotacions i evidencien la manca d'un protocol harmonitzat d'avaluació.

El tercer estudi va tenir com a objectiu estimar la decisió més rendible per millorar la bioseguretat a les explotacions lleteres. Es va desenvolupar una calculadora de costos de bioseguretat que tenia en compte les mesures ja implementades a cada granja i diversos escenaris de millora potencial. Els costos i la probabilitat d'introducció del virus de la diarrea vírica bovina (BVD) es van estimar mitjançant models estocàstics que incorporaven les característiques específiques de cada explotació. La integració de dades es va dur a terme mitjançant una anàlisi de decisions, amb l'objectiu d'identificar l'estratègia més rendible per a cada cas. El cost anual de les mesures de bioseguretat implementades oscil·lava entre 27,58 i 72,11 euros per animal, mentre que el cost d'un brot de BVD representava una pèrdua d'aproximadament el 6 % del marge brut. La probabilitat estimada d'introducció de la BVD oscil·lava entre el 0,36 % i el 15,7 %. Segons els resultats de l'anàlisi de decisions, la mesura més rendible en les tres granges estudiades va ser el subministrament de botes específiques per als conductors de transport de bestiar.

# Publications

## **Study I: Characterization of biosecurity practices among cattle transport drivers in Spain.**

Fernando Duarte, Alberto Allepuz, Jordi Casal, Ramon Armengol, Enric Mateu, Joaquim Castellà, Javier Heras, Giovanna Ciaravino

Preventive Veterinary Medicine, 28 January 2024

Volume 224, March 2024, 106138 | <https://doi.org/10.1016/j.prevetmed.2024.106138>

## **Study II: Methods to assess on-farm biosecurity in Europe and beyond.**

Fernando Duarte, Lena-Mari Tamminen, Miroslav Kjosevski, Giovanna Ciaravino, Mattias Delpont, Carla Correia-Gomes, Bart H.P. van den Borne, Ilias Chantzias, Laura Valeria Alarcón, Line Svennesen, Ina Toppari, Alessandra Piccirillo, Reze M. Gecaj, Artur Zbikowski, Telmo Nunes, Jasna Prodanov-Radulović, Marco De Nardi, Vitalii Nedosekov, Amelie Desvars-Larrive, Alberto Allepuz

Preventive Veterinary Medicine, 19 February 2025

Volume 239, June 2025, 106486 | <https://doi.org/10.1016/j.prevetmed.2025.106486>

## **Study III: Estimate the most cost-effective option for improving biosecurity on dairy cattle farms to support informed decision making**

Fernando Duarte, Alberto Allepuz, Arnau Àlvarez, Natalia Ciria, Bodil Højlund, Jehan Ettema, Giovanna Ciaravino

This manuscript has been submitted to *Preventive Veterinary Medicine* for publication as original research



# Chapter I: Introduction



# 1 Introduction

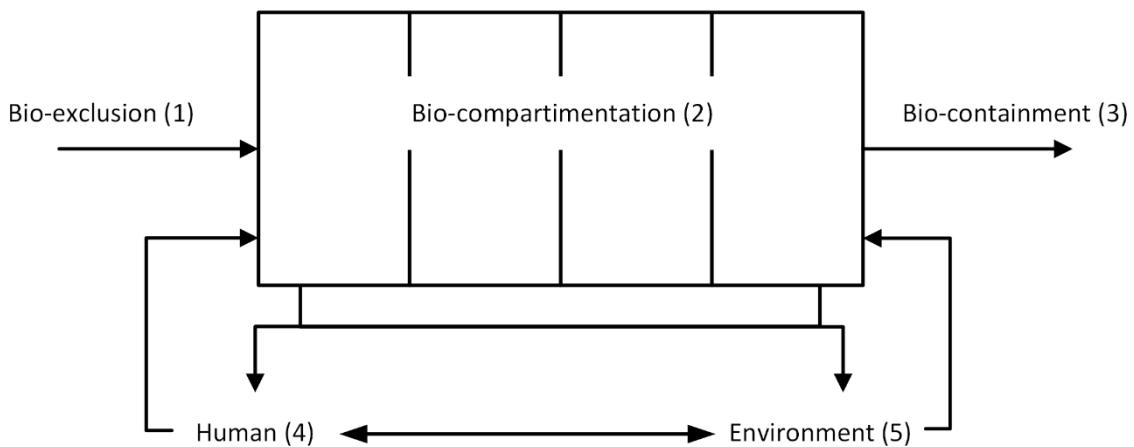
## 1.1 Definition and importance of biosecurity on animal farms

Biosecurity aims to reduce the likelihood of pathogen transmission and is widely recognised as the foundational pillar of any disease control programme. When effectively implemented, it can significantly improve animal health and welfare, reduce the need for curative treatments for infectious diseases, increase farm profitability, and protect public health (Dewulf and Immerseel, 2019; Dhaka et al., 2023; Kuster et al., 2015a). The definition of the concept of biosecurity has evolved over time, shaped by diverse perspectives across countries, academic disciplines, professional fields and operational contexts (Food and Agriculture Organization of the United Nations (FAO), 2016; Rappert, 2009; Renault et al., 2021b). Traditionally, in the field of animal health, biosecurity refers to practices and measures aimed at preventing or managing infectious diseases. This commonly accepted definition has been adopted in international initiatives (FAO, 2011, 2010), national and regional diseases control programmes (Defra, 2003; Stokstad et al., 2020), and in international regulations and official standard, as outlined in the Terrestrial Code of the World Organisation for Animal Health (WOAH) (WOAH, 2023a).

Over the past decades, while general principles remained the same, biosecurity definition and applications have progressively evolved toward a broader, integrated vision encompassing plant, animal, and human health. This integrated approach has been endorsed at the international level, through its adoption in guidelines (FAO and WOAH, 2009) and regulatory frameworks aimed at supporting national competent authorities in the field of animal health (FAO, 2016, 2007; Manzella et al., 2007). In alignment with this, the scientific literature has emphasised the One Health approach by expanding the definition of biosecurity to include additional dimensions, particularly the prevention of risks to human and environmental health, while promoting interdisciplinary cooperation to address complex socio-ecological challenges (Hulme, 2020;

Meyerson et al., 2002; Rappert, 2009; Saegerman et al., 2012). Moreover, within this framework, strengthening on-farm biosecurity contributes not only to improved animal health, but also to a reduced risk of zoonotic transmission and decreased reliance on antimicrobials (Bellini, 2018; Dhaka et al., 2023; Pinto Jimenez et al., 2023). This evolution has led to frameworks applicable at both national and local scales, such as the One Biosecurity concept (Hulme, 2020) and the “5B rule” (Saegerman et al., 2012), which defines biosecurity on animal farms through five key components (Figure 1): (1) reduction of the risk of pathogen introduction (bio-exclusion); (2) control of the spread beyond the facility (bio-compartmentation); (3) control of the spread within a facility (bio-containment); (4) prevention of the risk of human infection (bio-prevention); (5) prevention of environmental contamination (bio-preservation). In a recent survey evaluating preferences for biosecurity definitions in the context of animal farming the “5B rule” received the highest level of acceptance among the academic and research community, ranking first out of nine proposed definitions (Saegerman et al., 2023).

*Figure 1. Schematic representation of the five components (“5 B rule”) of the on-farm biosecurity approach that contribute to reducing the risk of pathogen introduction to, and dissemination from, the farm. Source: Saegerman et al., 2012.*



Nevertheless, and despite the different existing definitions on biosecurity in animal farms, the newest and probably most accepted definition describes this concept as the application of a set of **management, behavioural** and **physical** measures designed to reduce the risk of **introduction, establishment** and **spread** of pathogenic agents **to, within and from** an animal population. This definition, build-up from a previous one provided by World Organisation for Animal Health (WOAH, 2023), has been proposed by an ad-hoc group appointed by the WOAH for the creation of a new chapter on biosecurity and is currently being reviewed by representatives from all countries belonging to this organization (i.e., 183 countries). Therefore, still must be considered as a draft as it is subjected to further modifications by country members.

In line with previous WOAH definition, the European Union legislation on animal health also defines biosecurity as a combination of physical and management measures intended to minimize the risk of introduction, development, and spread of diseases into, from, and within: a) an animal population, or b) any establishment, zone, compartment, means of transport, or other premises, facilities, or locations (European Parliament and Council of the European Union, 2016).

Biosecurity is essential for achieving and maintaining a defined animal health status in specific geographical areas, particularly in the establishment of disease-free zones and compartments.

In the case of infectious diseases that are present in a territory the role of biosecurity extends beyond individual farms, as regional or national biosecurity standards are directly linked to the broader animal health status of a given area. Within the European Union, this applies to diseases classified under categories B and C of the Animal Health Law (AHL) such as bovine tuberculosis and bovine viral diarrhoea (BVD), which are subject to voluntary and compulsory eradication programmes, respectively. As a matter of fact, epidemiological studies conducted in Spain in the context of the tuberculosis eradication programme suggest that several outbreaks could have been prevented with enhanced biosecurity measures (Ciaravino et al., 2021).

The importance of biosecurity becomes even more evident in the context of diseases not normally present in a given territory, as for example transboundary animal diseases (TADs), which are characterised by their rapid spread across borders (Kompas 2022; Amass et al., 2004; Elbers et al., 2001) and, within the European Union, all the diseases of category A under the AHL. From the moment such a disease enters a territory to the point it is detected by surveillance systems, a considerable amount of time may pass. During this time-window, no disease-specific control measures can be applied, and only the general farm-level biosecurity protocols already in place can act as the first line of defence (Cameron et al., 2020; Léger et al., 2017; Martin et al., 2015).

Finally, the implementation of biosecurity measures contributes not only to prevent pathogens transmission among animals but also to reduce the zoonotic risks (LeJeune and Kersting, 2010; Klous et al., 2016; Layton et al., 2017; Msimang et al., 2022). Under this view, farm personnel hygiene and behavioural biosecurity measures are considered essential for mitigating the risk of zoonotic transmission to humans (Layton et al., 2017). Recently, a systematic review conducted by Youssef et al. (2021) showed that the implementation of improved biosecurity practices, such as hygiene protocols and personal protective equipment, can significantly decrease the transmission of zoonotic bacteria at farm level, although stronger empirical evidence is still needed. However, it is worth to consider that technical measures alone are insufficient without ensuring stakeholder engagement. Consequently, the adoption of preventive measures should be supported by effective communication strategies and appropriate training of all stakeholders, including farm personnel (Layton et al., 2017; Msimang et al., 2022; Saegerman et al., 2012).

### 1.1.1 Assessing on-farm biosecurity

A higher level of biosecurity is generally associated with a reduced risk of pathogens introduction and transmission. Moreover, on-farm biosecurity comprises a range of elements that differ in

terms of complexity and feasibility of implementation. For this reason, the Animal Health Law highlights the need for biosecurity measures to be both practical and adaptable, and the level of implementation to be subject to monitoring (European Parliament and Council of the European Union, 2016). Consequently, the capacity to assess biosecurity implementation on farms is of critical importance (Hagenaars et al., 2018; Serafini Poeta Silva et al., 2023).

Methods for assessing on-farm biosecurity serve to evaluate efforts made at the farm level to prevent pathogen introduction via various routes. These tools are also useful for identifying potential improvements, prioritising interventions, detecting weaknesses, and comparing the biosecurity status between farms (i.e., benchmarking). Given the often-limited availability of resources, prioritisation of biosecurity actions represents a strategic approach to maximising the effectiveness of interventions (Alarcón et al., 2021; Dewulf and van Immerseel, 2019). For these assessments to be effective, data collection is typically recommended to be carried out in person and directly on site. Assessors should be appropriately trained and maintain objectivity throughout the process (Shapiro and Stewart-Brown, 2008).

Although various methods for assessing biosecurity have been developed and described (Gelaude et al., 2014; Pedersen et al., 2023), the scientific literature remains limited in terms of their practical application and validation (Alarcón et al., 2021; Vouga Ngom et al., 2024). Moreover, a comprehensive overview that categorises on-farm biosecurity assessment methods across different contexts (e.g., farm type or animal species) is still lacking. Such information could be valuable when interpreting the results of assessed farms, particularly when considering the specific characteristics of the methods employed.

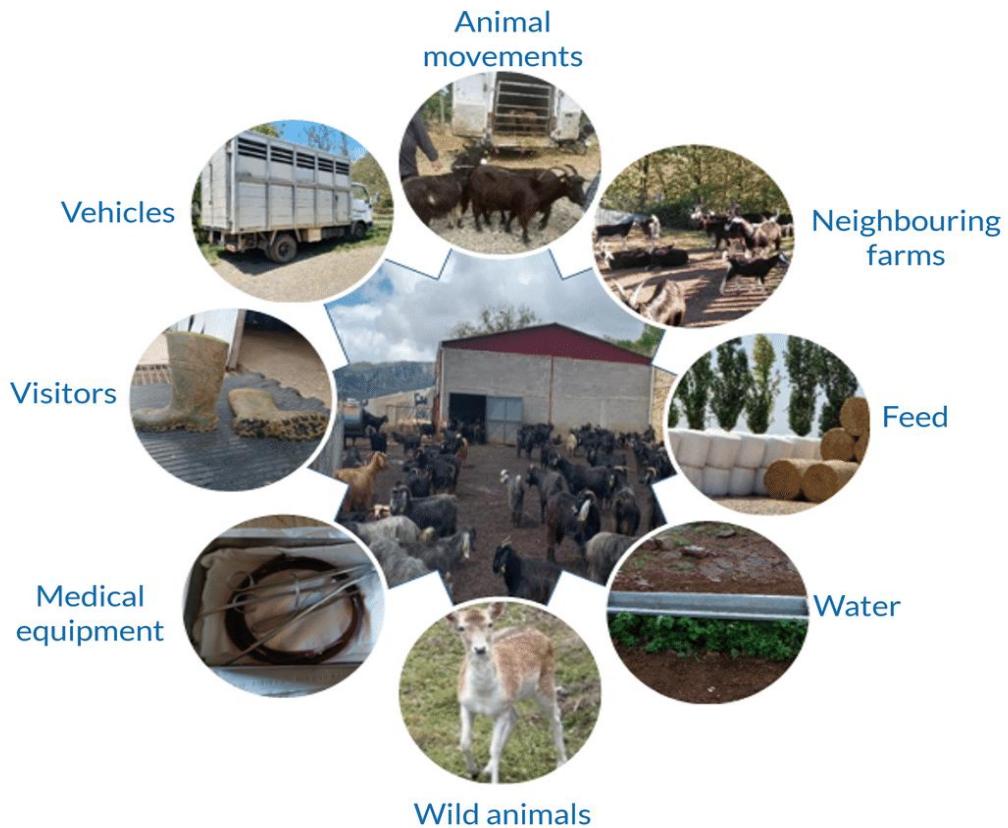
## 1.2 Biosecurity in animal farms

The risk of disease transmission among animals can be mitigated and controlled through the implementation of appropriate biosecurity measures tailored to each transmission route (Alarcón et al., 2021; Dewulf and van Immerseel, 2019; Fèvre et al., 2006). Moreover, biosecurity on farms can be broadly divided into external and internal biosecurity. External biosecurity focuses on reducing the risk of pathogen introduction into a farm (Erling Kristensen and Jakobsen, 2011a; Lewerin et al., 2015; Sayers et al., 2013), while internal biosecurity aims to mitigate the risk of pathogen spread within a farm (Alarcón et al., 2021; Dewulf and van Immerseel, 2019; Sayers et al., 2013).

### 1.2.1 External biosecurity

The introduction of new animals to the farm, as well as of workers, visitors, vehicles, feed and water management, and environmental sources, are considered the main potential routes through which pathogens can enter the farm (Figure 2).

*Figure 2. Schematic representation of the potential routes through which pathogens can enter the farm. Source: FAO Improving ruminant biosecurity course, Virtual Learning Centers.*



#### 1.2.1.1 Animal introduction

The introduction of new animals into a herd represents a significant risk for the entry of pathogens to the farm. While some farms operate under production systems that minimise the need for external animal purchases, acquiring animals from other farms, sometimes with unknown health statuses, is occasionally unavoidable (Ezanno et al., 2006; Santman-Berends et al., 2017; Smith et al., 2010; Waldeck et al., 2021). At present, the widespread and professional use of artificial insemination in dairy cattle farms has notably reduced the risk of pathogen transmission via the purchase of animals, reducing the need to introduce bulls and cows onto the farm (De Ruigh et al., 2006; Givens, 2018; Parkinson and Morrell, 2019; Wentink et al., 2000).

Measures aimed at preventing direct contact between new and resident animals should follow a risk-based approach. Knowing the health status of the source farm is essential. Cattle introduced into the herd should come from holdings of equivalent or better health status (Bazeley, 2009; Raspert et al., 2008). Upon arrival at the farm, it is recommended that incoming animals undergo a quarantine (i.e., segregation) before being mixed with the herd. This quarantine period should last long enough to allow for the identification of diseases of concern to the receiving farm. Additional measures, such as disease testing during quarantine, can further reduce the risk of pathogen introduction (Dewulf and van Immerseel, 2019; Knight-Jones et al., 2014; Sarrazin et al., 2014). A clear example of the effectiveness of quarantine can be seen in the case of diseases with short incubation periods and acute clinical signs, such as bovine respiratory syncytial virus (BRSV). For diseases that do not typically present acute symptom, such as brucellosis, leptospirosis, neosporosis, salmonellosis, or BVD, among others, quarantine should be complemented with appropriate diagnostic testing to identify asymptomatic carriers (Sanderson, 2009). Quarantine facilities must meet minimum biosecurity standards to be effective. They should be physically separated from the main herd to prevent both direct and indirect contact. Tools used within the quarantine area must either be dedicated exclusively to that area or thoroughly cleaned and disinfected before use elsewhere on the farm (Bernaerd et al., 2021; Nöremark et al., 2010; Sarrazin et al., 2014). It is recommended that dedicated clothing and boots be made available for use in the quarantine area, including a designated space where visitors can change into farm-specific clothing. Visitors and farm workers should enter the quarantine area only after visiting the main farm facilities (Brennan and Christley, 2012; Nöremark and Sternberg-Lewerin, 2014; Renault et al., 2021a). The farm's workflow must align with the biosecurity measures implemented in the quarantine zone to ensure their effectiveness is not compromised (Nöremark et al., 2010).

Animals that leave the farm and later return (such as replacement heifers raised off-site and shared with other farms) should be subjected to similar protocols upon re-entry (Dewulf and van Immerseel, 2019; McCarthy et al., 2021).

#### 1.2.1.2 *Cattle grazing outdoors*

Some farms implement management systems that involve outdoor grazing. This practice may present biosecurity risks, particularly when neighbouring farms share grazing areas or when boundary separation is insufficient, allowing for direct contact between herds. In such cases, fencing is recommended to prevent between-herd contact. Ideally, double fencing should be employed, creating a buffer zone between adjacent grazing herds (Dewulf and van Immerseel, 2019; M.C. Gates et al., 2013; Sarrazin et al., 2014; Wolff et al., 2017). A study conducted on dairy farms in Denmark demonstrated that farms with a higher number of neighbouring holdings exhibited a greater likelihood of testing positive for BVD. This increased risk was associated with the potential for direct transmission through fences separating adjacent farms, among other factors (Ersbøll et al., 2010; M. C. Gates et al., 2013). Potential interactions with wildlife that may act as disease reservoirs should also be considered from a biosecurity standpoint. Certain diseases, such as bovine tuberculosis, can be transmitted by wild hosts. The use of feeding and watering systems that are protected from the access to wildlife can reduce the likelihood of wildlife interaction in high-risk areas. Additionally, effective fencing that deters wildlife, the use of deterrents such as guardian dogs, and environmental management strategies that discourage wildlife presence, such as avoiding attractive habitats, have all been reported as preventive measures (Balseiro et al., 2019; Barasona et al., 2013; Jori et al., 2021; Judge et al., 2011; Pozo et al., 2024; Sanderson, 2009).

#### 1.2.1.3 *Visits*

Visits are very common on dairy farms; professionals and commercial agents frequently visit these farms as part of their normal operations. The perimeter of the farm can be delineated with fencing and access gates to separate the "clean" area (the farm) from the "dirty" area (the external environment). Some visitors may come into direct contact with the farm animals, including veterinarians, hoof trimmers or reproduction specialists, among others. It is recommended to minimise the number of visits as much as possible and to provide visitors with work clothing, boots, and hygiene measures, along with the necessary facilities for their proper implementation (Brennan and Christley, 2013; Damiaans et al., 2018; Oliveira et al., 2018; Sarrazin et al., 2014; Villaamil et al., 2020). For instance, farms that were regularly visited by hoof trimmers using equipment shared with other farms showed a higher likelihood of testing positive for digital dermatitis. This equipment was frequently contaminated with the pathogen after being used on cattle affected by the disease (Oliveira et al., 2017; Wells et al., 1999). A recommended measure is to limit farm access to a single-entry point, where visitors can be supplied with the necessary equipment and information regarding biosecurity measures (Damiaans et al., 2018). Providing designated parking areas for vehicles that should not approach farm facilities can also help prevent cars from parking near animal enclosures (Baraitareanu and Vidu, 2021; Ferreira et al., 2024).

#### 1.2.1.4 *Vehicles entering the farm*

Dairy farms typically experience significant vehicular traffic, with a variety of vehicles entering and leaving the premises. Examples include animal transport trucks, milk and carcass vehicles, and feed and grain delivery trucks. These vehicles often visit multiple farms in a single day, posing a considerable risk for the transmission of infectious diseases. Both structural and management-based measures can be implemented to mitigate these risks. Ideally, vehicle entry to the farm

should be avoided altogether. Where this is not feasible, optimising vehicle movement routes within the premises can serve as a risk-reduction strategy. It is recommended that the carcass disposal area be located at the periphery of the farm, close to an external road, to prevent such vehicles from crossing into clean zones (Alarcón et al., 2021; Brennan and Christley, 2012; Dewey et al., 2014; Dewulf and van Immerseel, 2019). The same principle applies to other types of vehicles: farm management and workflow should be organised to prevent both direct and indirect contact between these vehicles and the animal enclosures. Strategic infrastructure planning can help to minimise vehicle proximity to critical areas, for instance, by situating feed silos near the farm perimeter (Alarcón et al., 2021; Lee et al., 2019). Drivers of external vehicles should not enter farm facilities, and farm personnel should likewise refrain from boarding vehicles that travel between farms, as such contact may facilitate disease transmission (Alarcón et al., 2021; Nöremark and Sternberg-Lewerin, 2014; Troutt et al., 2008).

#### 1.2.1.5 *Feed and water*

Feed can become contaminated with pathogens or toxins either during production or storage. Special attention should be provided when fields are fertilised with manure from other farms or when feed is sourced externally (Dewulf and van Immerseel, 2019). Feeding equipment should be cleaned regularly and should not be shared between farms (Brandt et al., 2008). Feeders and equipment used in quarantine areas should either be designated for that area or thoroughly cleaned and disinfected after each use if shared with the rest of the farm. Young animals should be kept from contacting with feed or manure from older animals. Feed storage areas must be enclosed and inaccessible to wildlife and pests to minimise the risk of contamination (Sanderson, 2009; Wells et al., 2002).

Effective water management is essential to prevent potential sources of contamination, requiring regular inspection and maintenance of water sources, storage systems, and distribution

pipelines (Dewulf and van Immerseel, 2019). Drinking water should be regularly tested for relevant bacterial contaminants, and measures should be taken to prevent access by wild or non-farm animals (Bayne et al., 2025; Damiaans et al., 2020; Wells et al., 2002). Where possible, water purification systems should be used to remove or inactivate potential pathogens in drinking water. Common water treatment methods include chlorine, chlorine dioxide, chloramines, and ozone (Olkowski, 2019; Pinto Jimenez et al., 2023).

### 1.2.2 Internal biosecurity

Measures aimed at reducing the risk of disease transmission within the farm, such as those related to calf management, handling of sick animals, workflow organisation, milking routines, age-based segregation of animals, vermin and other animals present at the farm, are considered part of internal biosecurity (Figure 3).

*Figure 3. Schematic representation of the potential routes of pathogen transmission within the farm. Source: FAO Improving ruminant biosecurity course, Virtual Learning Centers.*



### 1.2.2.1 Farm management

Calving and calf management, particularly in neonates with underdeveloped immune systems, must be carried out with care to prevent the transmission of pathogens. The designated calving area should be physically isolated from other animals. After each birth, the site must be cleaned and disinfected, with all tissues or membranes removed from the pen. Keeping the calving area dry helps reduce the survival of potential pathogens and limits neonatal exposure. Calving should never take place in the hospital pens (i.e., the area used to house sick animals). All equipment used in these facilities must either be dedicated to the area or thoroughly cleaned and disinfected between each use (Dewulf and van Immerseel, 2019; McCarthy et al., 2021; Sanderson, 2009).

Calves should be placed in shelters as soon as possible after birth. These shelters should be easy to clean and disinfect and physically separated from other animals on the farm. In the calf area, each calf should have its own designated feeding bucket, which must be cleaned after every use. It is recommended that separate equipment and tools be used for each age group on the farm (Barnhardt and Raabis, 2025; Dewulf and van Immerseel, 2019; McCarthy et al., 2021).

In the milking routine, several practices should be followed to ensure optimal machine performance and cow health. Maintenance should be carried out at intervals recommended by the manufacturer, including timely replacement of teat cup liners. Milking personnel must practise appropriate hygiene, including handwashing and disinfection, or the use of gloves. Cow teats should first be dry-cleaned, then disinfected and dried. Single-use paper towels are recommended for cleaning and drying. After milking, teats should be disinfected again. A thorough cleaning of the milking system should be conducted after each milking session (Dewulf and van Immerseel, 2019; Hoe and Ruegg, 2006; Page Dinsmore, 2002).

#### *1.2.2.2 Disease management*

Hospital pens should be physically separated and located at a distance from other pens to minimise potential direct and indirect contact. These pens must be used exclusively for sick animals, including all equipment and materials associated with them. Where possible, lactating cows should be milked within these dedicated facilities; if this is not feasible, they should be milked last. Following milking, special attention must be given to the cleaning and disinfection of the milking machine and teat cup liners. Chronically infected animals may be kept in isolation until they are sent to slaughter to eliminate them as a source of infection. Regarding workflow routines, the hospital pen should be the last to be visited during daily activities. Exclusive clothing, hygiene measures, and dedicated equipment should be used when handling animals in this area (Dewulf and van Immerseel, 2019; Emanuelson et al., 2018; Villarroel et al., 2007).

#### *1.2.2.3 Flies, rodents, dogs and cats*

An effective approach to biosecurity requires a comprehensive and integrated pest management programme (Agunbiade et al., 2025; Smith et al., 2022), alongside the exclusion of non-livestock domestic animals from farm environments (Moran et al., 2018).

Flies can act as mechanical or biological vectors of infectious diseases in livestock, contributing to the transmission of pathogens that may cause significant economic losses and adversely affecting animal welfare (Smith et al., 2022). This has been documented, for example, in the case of lumpy skin disease, where flies play a role in disease spread. Fly control may be even more challenging on organic farms due to regulatory restrictions (Meerburg et al., 2007). In terms of biosecurity, the objective of fly control is to reduce population density through both chemical and non-chemical methods. Examples include maintaining hygiene in animal pens and feed storage areas, as well as using electric or adhesive traps. These measures should be accompanied by continuous monitoring of fly populations to assess the effectiveness of control strategies and

determine when corrective action is necessary (Dewulf and van Immerseel, 2019; Meerburg et al., 2007; Wells et al., 2002).

Rodents are recognised vectors and reservoirs of pathogens that affect cattle (Damiaans et al., 2018; Judge et al., 2011). An example of such pathogens is *Leptospira*, for which rodents act as maintenance hosts and can transmit the infection to cattle, potentially causing significant losses due to its impact on reproduction, among other effects (Bayne et al., 2025). Control strategies should focus both on preventing rodent access to farm facilities and managing the internal population. This involves eliminating potential nesting sites and maintaining cleanliness in areas that could serve as shelters (Dewulf and van Immerseel, 2019). Environmentally safe chemical and mechanical control methods, in conjunction with hygienic practices, have been shown to be effective tools for rodent management (Djedovic et al., 2014). Proper feed storage systems are essential to prevent rodent interaction with livestock feed, which could pose a contamination risk (Bayne et al., 2025). Bird control follows similar principles to avoid contamination of feed and drinking water through contact with bird faeces (Jori et al., 2021; Judge et al., 2011; Wells et al., 2002).

Regarding dogs and cats, these animals should not be allowed access to farm facilities or contact with livestock (Wells et al., 2002). They may act as carriers of some pathogens such as *Neospora*, among others (Moran et al., 2018; Sarrazin et al., 2014).

### 1.3 Live cattle transport

The transport of live animals between farms is a common practice in livestock production. Large numbers of animals are moved daily by road, as well as through ports and airports (Dahl-Pedersen and Herskin, 2023; Noordhuizen et al., 2013). The transmission of pathogens during animal transport is a matter of particular concern, as several external factors, such as inadequate

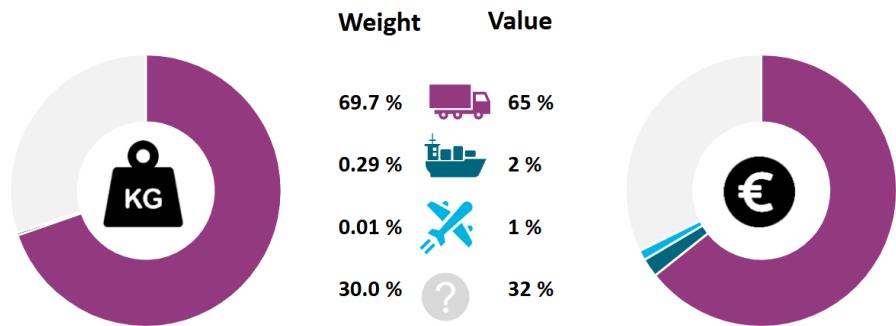
hygiene measures during transport, stress induced by travel, or the mixing of animals of different ages, can further increase the risk of the spread of pathogens (Alarcón et al., 2021; Belk et al., 2019; Fike and Spire, 2006; Greger, 2007; Weber and Meemken, 2018). Therefore, animal transport constitutes a vast and complex network that operates at multiple scales, making legal regulation essential to promote practices that minimise associated risks (Dahl-Pedersen and Herskin, 2023; Fèvre et al., 2006).

Each year, millions of animals are raised within the European Union. By the end of 2023, the EU was home to approximately 133 million pigs, 74 million cattle, and 68 million sheep and goats (European Commission, 2024). During the production cycle, these animals are transported live from one farm to another or to slaughterhouses. These movements may occur within the country of origin or involve cross-border transport. Between 2017 and 2021, 86% of international live animal transport originating from EU member states took place within the EU itself. In total, around 1.6 billion live animals were transported during this period (European Court of Auditors, 2023).

Specifically, in 2018 alone, 4.3 million cattle, 3.5 million small ruminants (sheep and goats), 33.4 million pigs, and 1 billion poultry were moved within the EU (European Parliamentary Research Service, 2020). Approximately 70% of this intra-EU transport (Figure 4) is carried out by road (European Court of Auditors, 2023).

*Figure 4. Modes of live animal transport within the European Union (EU) between 2017 and 2021. Source: Extracted from European Court of Auditors (2023). Transport of Live Animals in the EU: Challenges and Opportunities. Luxembourg: European Court of Auditors.*

[https://www.eca.europa.eu/Lists/ECADocuments/RV-2023-03/RV-2023-03\\_EN.pdf](https://www.eca.europa.eu/Lists/ECADocuments/RV-2023-03/RV-2023-03_EN.pdf)



Given the volume of live animal movements taking place annually, such transport routes represent a potential pathway for the spread of infectious diseases (Fèvre et al., 2006).

For example, in the case of bovine tuberculosis (bTB), long-distance animal movements have been shown to play a key role in the disease's spread within the United Kingdom (Fèvre et al., 2006). Another example is Foot and Mouth Disease (FMD): outbreaks detected initially on farms in the UK and subsequently in France and the Netherlands highlighted the risks associated with large-scale animal movements (Ellis-Iversen et al., 2010). Similarly, the spread of Classical Swine Fever (CSF) demonstrated that the movement of pigs between European countries was a critical factor in the dissemination of the disease among commercial farms (Allepuz et al., 2007; Elbers et al., 1999; Mintiens et al., 2001).

### 1.3.1 Biosecurity measures applied during the transport of animals

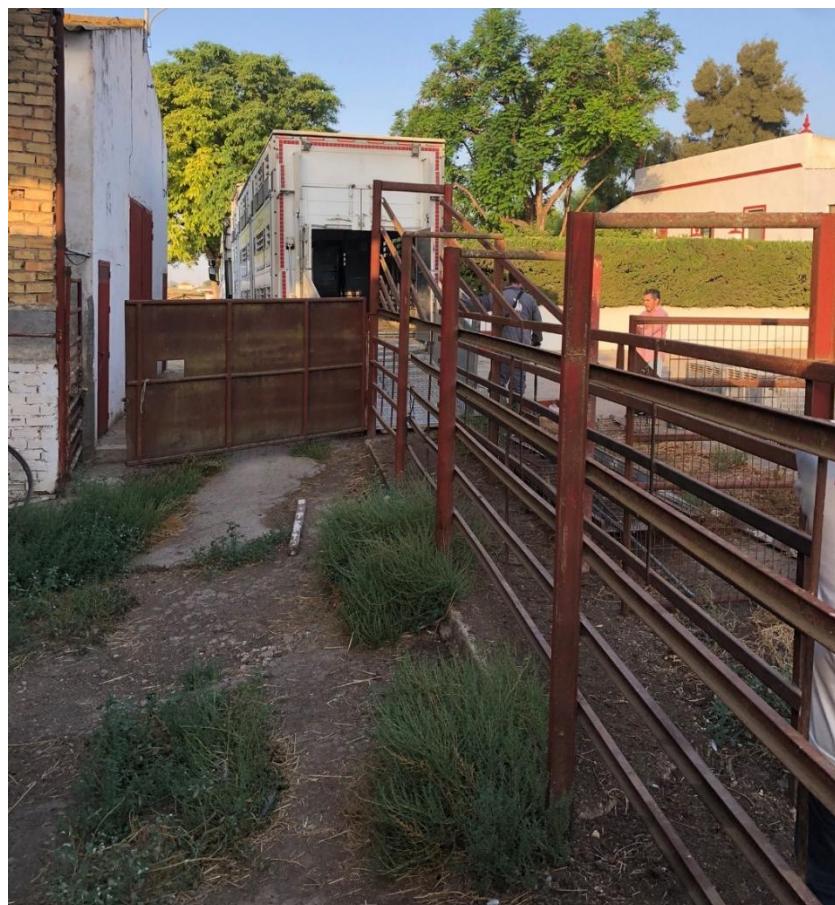
The transport of live animals constitutes a critical point of potential disease transmission. Inadequate biosecurity during this phase may contribute to the introduction and spread of

pathogens within and between farms. Therefore, a set of biosecurity measures is essential to mitigate these risks (Alarcón et al., 2021; Belk et al., 2019; Dewulf and van Immerseel, 2019; Fike and Spire, 2006).

#### 1.3.1.1 *Loading and unloading procedures*

In the ideal scenario, a clear separation between clean and dirty zones must be established. Vehicles transporting animals should remain outside the clean perimeter of the farm. A dedicated loading dock adjacent to the animal pen is recommended to facilitate loading and unloading without allowing the vehicle to the clean areas of a farm (Figure 5).

*Figure 5. Cattle loading and unloading dock located adjacent to the farm perimeter, preventing cross-contamination between dirty and clean zones. Source: Author's own elaboration.*



Additionally, access routes should be designed to avoid the intersection of vehicle and personnel movements. Where feasible, separate paths for vehicles and staff should be implemented.

Drivers and assistants should not enter the clean zone. If entry is unavoidable, they must perform the following actions before crossing the perimeter:

- Remove visible organic matter from boots using water and detergents.
- Apply disinfectant.
- Alternatively, the use of farm-specific boots or disposable boot covers to improve hygiene and practicality.

These measures are essential to minimise the introduction of infectious agents into the farm premises (Alarcón et al., 2021; Amass et al., 2000; Seedorf and Schmidt, 2017).

#### *1.3.1.2 Cleaning and disinfection of transport vehicles*

Regardless of whether a vehicle is farm-owned or outsourced, it must arrive clean and empty at the farm. Following every transport operation, thorough cleaning and disinfection are mandatory. However, evidence indicates that this process is often poorly executed (Weber and Meemken, 2018). The literature describes that pathogens have been detected on vehicles even after supposed cleaning and disinfection, posing a risk to both animal and public health (Belk et al., 2019; Dewulf and van Immerseel, 2019; Wrathall et al., 2004).

To enhance the efficacy of disinfection, a multi-step cleaning protocol is recommended (Alarcón et al., 2021; Barrington et al., 2002; Dewulf and van Immerseel, 2019; Fike and Spire, 2006; Newell et al., 2011):

1. Dry cleaning: Removal of organic material such as manure, bedding, or feed.
2. Washing: Application of water, preferably hot, with detergents to remove residual contaminants (Figure 6).
3. Drying: Drying of surfaces to improve disinfectant action.

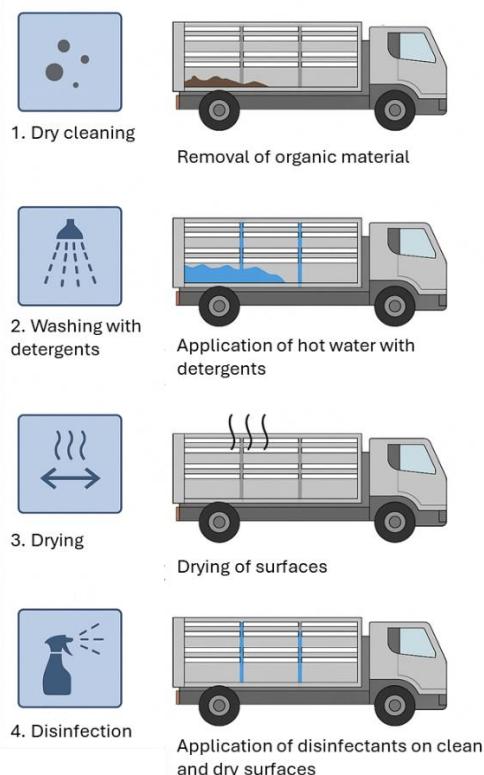
4. Disinfection: Application of disinfectants only after surfaces are visibly clean and dry.

An illustration of the steps involved in the cleaning and disinfection of transport vehicles is presented in Figure 7.

*Figure 6. Washing of a cattle transport vehicle with water and detergent at an officially approved vehicle cleaning and disinfection facility. Source: Author's own elaboration.*



*Figure 7. Schematic representation of the cleaning and disinfection steps for vehicles transporting animals to farms. Source: Author's own elaboration.*



Due to the challenges involved in thoroughly cleaning and disinfecting transport vehicles, alternative methods have been explored. For example, heat-assisted systems, where the interior temperature of the vehicle is raised, have been tested experimentally. However, their commercial application remains limited, and their economic feasibility has not yet been demonstrated (Dee et al., 2005).

#### *1.3.1.3 Vehicle assignment and transport policies*

Assigning vehicles to specific transport tasks is another useful measure. For instance, a vehicle used to bring in replacement stock should not be used to transport animals to feedlots or slaughterhouses. However, this distinction is not always feasible, especially when transport services are outsourced.

In such cases, several practices are recommended to minimise biosecurity risks. One important measure is to avoid sharing transport vehicles between farms, thereby reducing the likelihood of indirect contact with animals of unknown health status. In addition, animals of different age groups should not be transported together during the same journey, as this practice has been associated with an increased risk of disease transmission (Ferreira et al., 2024; Filippitzi et al., 2018).

#### **1.3.2 Legislation on biosecurity in the transport of live animals**

At European level, the Animal Health Law (Regulation (EU) 2016/429) acknowledges the role of transport vehicles as potential vectors for pathogen transmission and outlines the animal health requirements that vehicles must meet. It mandates the cleaning and disinfection of vehicles and recommends the use of biocides to improve hygiene standards during transport. Moreover, it encourages member states to implement systems for the registration and traceability of

transporters and their vehicles, to enhance the response capacity during animal health emergencies (European Parliament and Council of the European Union, 2016).

In addition, Council Regulation (EC) No 1/2005 of 22 December 2004 on the protection of animals during transport and related operations establishes specific biosecurity measures that must be applied during the transport of live animals. The regulation sets minimum training requirements for all personnel involved in the transport of live animals, particularly in relation to animal welfare, and requires that transporters hold valid authorisation for carrying animals. It explicitly requires that vehicles must be cleaned and disinfected after every journey and stipulates that these procedures be carried out at officially approved facilities. Transporters must also carry documentation certifying the completion of such procedures. Furthermore, the regulation outlines biosecurity measures to be enforced at control posts, which are tasked with verifying compliance. These posts must be equipped to carry out vehicle cleaning and disinfection under all weather conditions, and the cleaning areas themselves must also be disinfected after use. In addition, staff at these facilities must be provided with suitable clothing and equipment to prevent the spread of pathogens (Council of the European Union, 2005). This legislation is directly applicable in all EU member states and allows for national adaptations to suit local contexts (Council of the European Union, 2005; European Parliament and Council of the European Union, 2016).

Another relevant legislative instrument is Regulation (EC) No 561/2006 of the European Parliament and of the Council of 15 March 2006, which harmonises certain aspects of social legislation relating to road transport. This regulation sets limits on drivers' working hours, including time spent on activities such as transport, loading, unloading, and vehicle sanitation. These time constraints may impact the effective implementation of cleaning and disinfection protocols (European Commission, 2006).

In Spain, as an EU member state, the national implementation of these regulations is set out in Royal Decree 990/2022 of 29 November, which governs animal health and protection during transport. This decree incorporates the provisions of Regulation (EC) No 1/2005 and outlines the registration requirements for transporters, training content for drivers, and responsibilities during the transport of live animals. It also requires personnel to wear clothing and footwear that ensure biosecurity and mandates that cleaning and disinfection certificates be obtained from authorised facilities (Ministerio de Agricultura Pesca y Alimentación, 2016; Ministerio de la Presidencia Relaciones con las Cortes y Memoria Democrática, 2022).

## 1.4 Economic costs and benefits of biosecurity on farms

Biosecurity measures implemented on farms, whether recommended through assessments or mandated by regulation, require both initial investments and ongoing maintenance. The economic costs of the measures already implemented or planned are highly relevant for those responsible for their implementation. However, these costs have rarely been studied in depth (Rushton, 2008; Siekkinen et al., 2012).

Biosecurity is frequently perceived by farmers as an additional cost, with its benefits not always being immediately evident (Fasina et al., 2012b; Fraser et al., 2010; Renault et al., 2021a; Souillard et al., 2024; Toma et al., 2013). The economics of biosecurity measures may be particularly problematic in small-scale farms, where the required investments can represent a proportionally greater burden in relation to overall turnover (Siekkinen et al., 2012; Can and Altug, 2014).

Implementing effective biosecurity on farms entails multiple cost components. Direct costs might include investments in infrastructure, such as perimeter fencing, sanitation points, and dedicated loading/unloading docks, as well as equipment for disinfection systems, surface

cleaning, and pest control. Labour costs also constitute a significant cost, particularly the salaries of personnel responsible for implementing biosecurity measures (Fasina et al., 2012b; Fountain et al., 2022; Niemi et al., 2016; Osawe et al., 2022). Nonetheless, not all biosecurity measures require costly investments; several actions, such as restricting access to high-risk visitors, can substantially reduce disease risk with minimal or no cost (Horrillo et al., 2022).

Estimating biosecurity costs is a prerequisite for understanding their cost-effectiveness in relation to expected benefits. Access to cost-benefit information can support more informed decisions by farm managers and policymakers. Presenting clear cost estimates may also facilitate greater uptake of biosecurity measures (Niemi et al., 2016; Toma et al., 2013).

Examples of biosecurity cost estimation can be found in the literature. For instance, in a study conducted on poultry farms in Finland, which assessed biosecurity costs using standardised surveys to farmers and telephone interviews, the authors concluded that farms with better economic returns were more likely to invest in and sustain rigorous biosecurity practices, and that the average cost of implementing basic biosecurity measures (e.g., cleaning and disinfection, protective clothing) decreased on a per-bird basis as farm size increased, making biosecurity economically feasible for large-scale commercial broiler producers (Siekkinen et al., 2012).

In Australia, Fountain et al. (2022) estimated the costs of several biosecurity measures, such as fencing, quarantine protocols, and vaccination (considered a biosecurity practice), on beef cattle farms. Cost data were collected directly from suppliers and personal contacts and supplemented with information from the literature. The objective was to evaluate the impact of Bovine Viral Diarrhoea (BVD) on farms with different health statuses and to quantify both the economic and non-economic benefits of biosecurity implementation. Their findings provided evidence of the cost-effectiveness of targeted strategies, particularly under scenarios of elevated disease risk.

Another poultry study explored farmers' perceptions of biosecurity costs in conjunction with their willingness to implement measures. In this case, costs were categorised as "low", "medium" or "high", but no specific monetary values were provided, finding that the willingness to implement biosecurity measures was inversely related to their estimated cost (Fraser et al., 2010). In the context of pig and cattle production, Niemi et al. (2016) conducted a study in which participants were asked to estimate biosecurity costs in quantitative terms, rather than referring to actual market prices. The aim was to understand how perceived costs might influence the implementation of biosecurity practices. The study also highlighted a high degree of variability in responses, both across and within production systems. Similarly to what was observed by Fraser et al. (2010), it was found that the higher the perceived cost, the lower the likelihood that producers would implement the biosecurity measure.

The economic benefits of biosecurity are closely tied to the losses it helps to avoid. These include declines in productivity due to disease, loss of access to markets because of suboptimal health status, and the cost of disease control interventions such as antimicrobial use. Healthier animals are generally more productive (Dewulf and van Immerseel, 2019; Laanen et al., 2013; Postma et al., 2016; van Schaik et al., 2001). A recent review evaluating the impact of farm-level biosecurity on antibiotic use in animal production systems reported that approximately 78% of the studies analysed identified a positive association between the implementation or improvement of biosecurity measures and a reduction in antimicrobial use (Dhaka et al., 2023).

Several studies have estimated the economic benefits of biosecurity using different approaches. For example, van Schaik et al. (2001) applied partial budgeting to Dutch dairy farms under different hypothetical scenarios, demonstrating that in many cases, the implementation of biosecurity measures was economically viable. Similarly, Fasina et al. (2012a) evaluated three scenarios in domestic poultry flocks using partial budgeting and cost–benefit analysis, finding

benefit–cost ratios of 8.45, 4.88 and 1.49 depending on the type and severity of the disease considered.

In pig production, a study in Nigeria assessed the economic feasibility of measures such as segregation, cleaning, and disinfection to prevent African Swine Fever. Assuming 100% effectiveness, the benefit–cost ratio reached 29.14 (Fasina et al., 2012b). In the context of beef cattle production in Australia, the economic evaluation conducted by Fountain et al. (2022) assessed the cost-effectiveness of various combinations of biosecurity strategies, taking into account the disease status of the herd. Using a simulation model, the study identified specific strategies, particularly the combination of quarantine and double fencing, as the most effective in reducing economic losses associated with Bovine Viral Diarrhoea (BVD)

The relationship between biosecurity levels and both productive and health parameters has also been evaluated. Dairy farms with higher levels of biosecurity were associated with freedom from Bovine Viral Diarrhoea (BVD) and lower mortality rates, suggesting a potential economic benefit of implementing biosecurity measures (Renault et al., 2020). Similarly, a study conducted on Irish dairy farms, using both parametric and non-parametric estimation methods, found a positive impact on gross margin resulting from the implementation of vaccination and bulk tank milk testing (Osawe et al., 2022).

Not all avoided costs derived from biosecurity are strictly monetary: the potential outbreak of a disease with high expected mortality could render farm infrastructure redundant or unusable, and may also lead to psychosocial stress, among other consequences (Fasina et al., 2012b). Moreover, the benefits of biosecurity may vary depending on the disease and production context. When cost-benefit analyses consider only one or a few diseases, the overall value of biosecurity may be underestimated. If additional diseases are considered, the economic benefit would likely be much greater (van Schaik et al., 2001).

Farmers are more likely to be motivated to implement new biosecurity measures when they can perceive tangible benefits from doing so. This is logical when we consider that implementing biosecurity usually requires an investment and carries a cost, while the benefit lies in avoiding a loss that depends on the probability of disease introduction to the farm (Laanen et al., 2014; Postma et al., 2016). Furthermore, farmers may not be aware of the economic impact of diseases that present subclinical symptoms, which can make it more difficult to raise awareness of the need to apply biosecurity (Osawe et al., 2022). Moreover, awareness of the benefits of biosecurity should not be limited to farmers alone; other actors in the production chain, industry, and government should also be informed, to enhance cooperation and enable the progressive improvement of biosecurity standards through clearly defined roles and responsibilities (Subasinghe et al., 2023).

Communicating the benefits of biosecurity to farmers and stakeholders involved in animal production is a challenge. Training and educating veterinarians, farm advisors and veterinary service workers on how to effectively communicate the advantages of biosecurity could support more successful implementation at the farm level (Fasina et al., 2012b; Laanen et al., 2014).

Despite the overall economic benefits of biosecurity, it is important to note that recommended measures should be tailored to the specific requirements of each farm (Fountain et al., 2022). Furthermore, economic benefits are only one component of the decision-making process. For instance, investment in hospital pens is often not prioritised by farmers, despite evidence of its benefit (Damiaans et al., 2019). This highlights the need to consider the human and behavioural dimensions of decision-making in biosecurity adoption (Mankad, 2016; Moya et al., 2020).

A variety of approaches have been used to estimate the economic benefit of biosecurity (Rushton, 2008). One such approach is partial budgeting, which has been specifically used in the context of biosecurity. This technique is employed to evaluate specific changes within farms or livestock systems by accounting for the costs and benefits that appear or disappear following a

given intervention. A limitation of this method is that it does not incorporate time dynamics or uncertainty (Fasina et al., 2012a; Rushton, 2008; van Schaik et al., 2001).

Cost-benefit analysis is another method described in the literature within the context of biosecurity. This approach comprehensively evaluates the many costs and benefits associated with implementing biosecurity and, consequently, avoiding the introduction of diseases onto the farm. It is typically applied to assess interventions over time and/or at national or regional scales. The results are usually presented in terms of net present value, internal rate of return, and benefit-cost ratio. It is important to note that, since these models rely heavily on local input data, their results must be interpreted with caution when extrapolating to other contexts (Fasina et al., 2012b, 2012a; Rushton, 2008).

In cases where decision-making involves multiple options and uncertainty, decision analysis is an appropriate tool. Whether using payoff tables or decision trees, this approach considers the economic outcomes of each option analysed alongside the probabilities of their occurrence. Data inputs can also be derived from partial budgets or cost-benefit analyses, with probabilities added to reflect the uncertainty (Rushton, 2008).

There is still a limited amount of literature that estimates the economic benefits of biosecurity, and therefore, future studies are encouraged to focus more explicitly on the economic dimension of biosecurity (Renault et al., 2020; Youssef et al., 2021).

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## Chapter II: Objectives



## 2 Objectives

The overarching objective of this doctoral thesis was to provide information that could be used to reduce the risk of pathogen transmission in the cattle sector, encourage the adoption of biosecurity practices, and support stakeholders in making informed decisions. With that purpose, we tackled biosecurity from different perspectives: live animal transport, on-farm biosecurity assessment methods, and the cost-effectiveness of adopting biosecurity.

The specific objectives of this PhD were:

- To understand how biosecurity practices are implemented during cattle live transport.
- To identify and characterise existing methods for assessing biosecurity.
- To evaluate the expected economic benefit of implementing biosecurity at farm level.



## Chapter III: Research articles



### 3 Study I: Characterization of biosecurity practices among cattle transport drivers in Spain

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### 3.1 Abstract

Transmission of pathogens between farms via animal transport vehicles is a potential concern; however, the available information on driver routines and biosecurity measures implemented during transport is limited. Given the above, the aim of this study was to describe and characterize the prevailing practices and biosecurity measures adopted by cattle transport drivers in Spain. Eighty-two drivers were surveyed via face-to-face or remotely. The survey included questions on general characteristics of the drivers (type of journeys and vehicles) together with biosecurity practices implemented during cattle transport and vehicle hygiene practices. Results showed that several risky practices are performed quite frequently such as visiting different premises with different levels of risk (e.g., breeder and fattening farms); entering the farm premises to load/unload animals, passing by several farms to load and unload animals, or not always cleaning and disinfecting the vehicle between travels, among others. To explore similarities among the drivers and identify groups sharing specific practices, hierarchical clustering on principal components (HCPC) was computed on the results of multiple correspondence analysis (MCA). The first three MCA dimensions (out of 13) were retained in the agglomerative clustering and four different clusters were identified. Clusters 1 and Cluster 4 accounted for 39.5% and 29.6% of respondents, respectively. The clusters were mainly differentiated by practices in the loading/unloading of cattle, such as the frequency of contact with animals remaining on the farm, and the frequency of the vehicle's disinfection between farms. Cluster 2 and Cluster 3 were of similar size, about 15% of respondents each. Cluster 2 consisted of drivers who mainly made journeys to slaughterhouse, while drivers in Cluster 3 were characterised by the use of working clothes and boots. Based on these findings, it is advisable to increase awareness on the role that animal transport can have in the spread of pathogens between cattle farms and the importance of biosecurity in preventing such transmission. There is also a need to support animal transport professionals in such task, not only through the development of initiatives to increase awareness,

but also through the investment in improving cleaning and disinfection facilities and to consider the economic cost associated with some practices to not compromise the economic viability of the sector.

### 3.2 Introduction

The transport of live animals is necessary during the production cycle of animals for different reasons such as the purchase of replacements or sending animals to fatten or slaughter. A high number of animals are moved every day and almost all cattle are transported at some moment of their lives. According to the available data, about 4 million cattle are moved every year between different countries within the European Union (Dahl-Pedersen and Herskin, 2023). In the case of other species these number of movements can be even much higher. For example, in 2021 Denmark exported 14.5 million of piglets (Gao et al., 2023b).

Vehicles used for animal transport can play a significant role in the spread of pathogens, either through direct contact when animals from different farms are loaded in the same truck or, by indirect contact, through contaminated vehicles that have not been properly cleaned and disinfected between transports (Dee et al., 2005, Benavides et al., 2020, Alarcón et al., 2021, Gao et al., 2023a). Moreover, due to stress during transport, shedding of some pathogens may be exacerbated (Barham et al., 2002). To reduce this risk, several biosecurity practices are recommended, such as limiting the entry of vehicles into the farm premises, avoiding mixing animals from different farms in the same transport, cleaning boots between loading and unloading, wearing exclusive or clean work clothes, and following vehicle cleaning and disinfection protocols, among others (Barrington et al., 2002, Wrathall et al., 2004, Fike and Spire, 2006, Newell et al., 2011, Dewulf and Immerseel, 2019, Alarcón et al., 2021). However, only a few studies described which biosecurity practices are applied during transports and all of them concluded that there is large room for improvement (Greger, 2007, Brennan and Christley, 2012,

Schnyder et al., 2019, Nielsen et al., 2022). Indeed, only a few livestock drivers clean and disinfect vehicles, allowing pathogens to persist in vehicles and thus increasing the risk of pathogen spread between farms (Greger, 2007). Moreover, often compliance with biosecurity practices during transport is out of the control of the farmers as frequently transport vehicles are not owned by them (Brennan and Christley, 2012). Therefore, as it is common for farmers to use professional transport companies, these companies should implement appropriate biosecurity measures and drivers should follow hygiene recommendations to minimise the risk contamination on the farm.

Animal transport vehicles have been related to different disease outbreaks. For example, in Germany and Belgium in 1997, vehicles that had not been properly cleaned and disinfected were traced as the most likely source of classical swine fever virus in these countries (Elbers et al., 1999, Mintiens et al., 2001). In Spain, in 2001, contaminated transport vehicles were identified as the likely source of classical swine fever virus for almost 10% of the infected farms (Allepuz et al., 2007). Similarly, animal transport vehicles have also been identified as an important element for the transmission of foot and mouth disease between farms in England (Ellis-Iversen et al., 2011).

The objective of this study was to characterize which biosecurity practices are regularly implemented during the transport of cattle in Spain and to identify profiles of drivers applying similar biosecurity practices in the transport of animals to inform strategically the development of awareness campaigns.

### 3.3 Material and methods

#### 3.3.1 Sampling design and sample selection

To estimate the proportion of drivers implementing the different biosecurity practices, we used the sample size formula to estimate a proportion (Dohoo et al., 2003). Using a worst-case assumption, we assumed that 50% of the drivers would apply each measure and the desired precision for the estimate was set to 10% with a level of confidence of 95%. With this starting

hypothesis, 97 drivers were expected to be surveyed. In Spain, live animal transport is regulated by law (European Union, 2005). Drivers and vehicles must be authorized to perform animal transport. In the case of the vehicles and their characteristics, this authorization may be for journeys of less than 8 h, more than 8 h, or up to 12 h. All authorized drivers and/or companies are registered in a national system named SIRENTRA (Anonymous, 2016). As this register is not publicly accessible, it was not possible to have access to a complete sampling frame to do a random sampling. Therefore, a convenience sampling was followed by the snowball sampling. In our study, snowball sampling consisted of surveying the first drivers contacted and then asking them to recommend other drivers, who might be willing to participate in the study.

Different channels were used to contact the first drivers: a) through personal networks from the authors of this article, b) the agri-food cooperatives in Spain disseminated information about the project among their members and asked for volunteers to participate (<https://www.agro-alimentarias.coop/>), c) visiting the cleaning and disinfection centres of several cattle slaughterhouses from Catalonia (Northeast of Spain) and asking the drivers to participate before or after the cleaning and disinfection of their vehicles, and d) every time that a driver was interviewed, we asked about the possibility of performing a similar interview with a colleague.

### 3.3.2 Survey

The survey covered the following aspects:

- i. Characteristics of the drivers and the type of vehicle they usually used: years of experience, self-employed or employees in a company, number of animals that were allowed to be transported or maximum time allowed to travel with their vehicle (Anonymous, 2022).
- ii. Characteristics of their journeys: national/international movements, production systems visited (i.e., dairy and/or beef), the purpose of animals transported (e.g., for replacement,

for fattening or for slaughter) and for each purpose, number of journeys per day, the number of farms from which the cattle were loaded/unloaded, and the time spent on the journeys.

- iii. Biosecurity practices during loading and unloading of cattle: whether they had to enter the farm premises and/or the stables where animals are located, and their practices related to the use of boots and clothes.
- iv. Cleaning and disinfection practices of the vehicle: time spent cleaning and disinfecting each vehicle, frequency of cleaning and disinfecting, and routines during the process.

The survey was piloted with four drivers using a face-to-face interview to test it for clarity and adequacy of the questions. Modifications and amendments were included in the survey where needed. The original survey, in Spanish, can be found in the Supplementary material 1 (Figure 1).

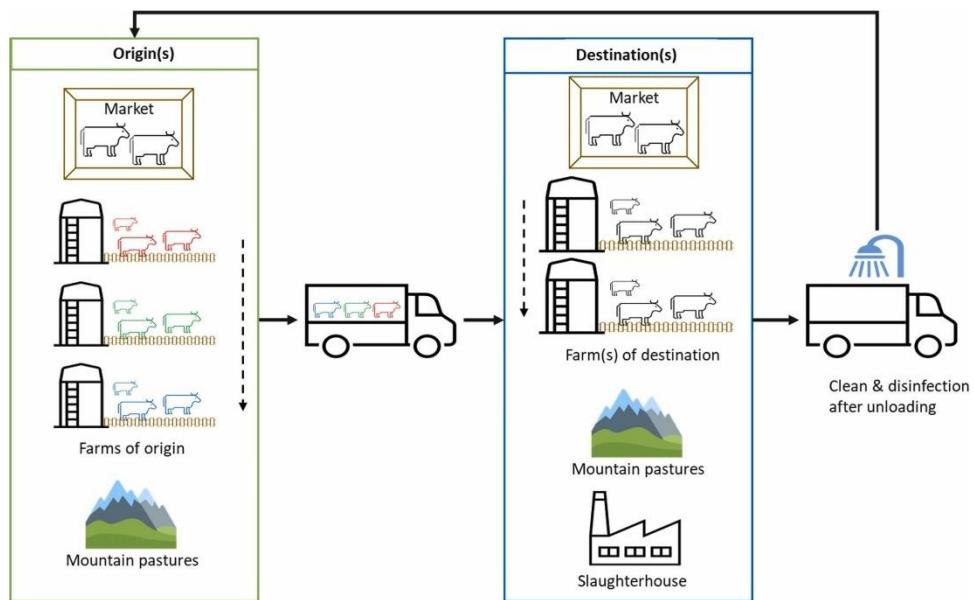
### 3.3.3 Definitions used in this study

#### 3.3.3.1 *Journey*

A journey was defined as the itinerary between the loading of cattle onto a vehicle at one point and the moment when the last animal was unloaded, regardless of the number of stops within that journey and whether new animals were loaded. Therefore, a journey began when the first animal was loaded in the vehicle and ended when the last animal was unloaded from the vehicle.

A graphical depiction of the definition of a journey, including the different possible loading/unloading points, is shown in Figure 1.

*Figure 1. Representation of journey. According to the definition in this study, one journey would begin and end with the vehicle empty, regardless of the number of loading and unloading points during the journey. The dotted arrow represents the optional stops between farms for loading or unloading and the solid arrow represents the mandatory flow of events.*



### 3.3.3.2 Categories of transported animals

Seven types of journey were identified according to their destination or the category of animal being transported: i) rearing: weaned calves to be raised in another farm as future breeders; ii) replacement: heifers to be used as part of the reproductive stock; iii) fattening: rearing of calves for meat production; iv) slaughterhouse: the slaughter of fattened calves; v) culling: movement to the slaughterhouse because the animal is no more productive or because other reasons (e.g., has some injury from which cannot be recovered); vi) pastures: movement to a seasonal pasture and vii) bulls: males to be used in breeding.

### 3.3.3.3 Shared journey

If during one journey, the driver made a stop to load or unload animals from other farms, this was considered a shared movement.

### 3.3.4 Data analysis

The surveys responses were coded and tabulated using MS Excel. The R software version 4.2.2 (R Core Team, 2023) was used for data processing and descriptive analysis.

Patterns of biosecurity and hygiene practices implemented by the drivers during cattle transport (i.e., the respondents' profiles) were identified by performing a hierarchical clustering on principal components (HCPC) on the results of a multiple correspondence analysis (MCA) (Husson et al., 2010) using the "FactoMineR" (Lê et al., 2008) and "factoextra" (Kassambara and Mundt, 2020) packages in R Statistical Software.

Responses for which less than 50% of the questions were answered were excluded from the analysis. Only the questions related to practices and biosecurity were included for analysis using multiple correspondence analysis (MCA). Variables were coded with the letter "q", where "q" means question, and a sequential number to simplify illustration. As a previous step of the MCA, a comparison between pairs of variables was carried out. Only variables with a correlation coefficient between -0.4 and 0.4 (95% confidence level,  $P < 0.05$ ) were retained. In addition, categories of active variables with less than 10% (at least eight drivers selecting the category) were also not considered for the analysis. Missing values were imputed using the regularised iterative algorithm from the "missMDA" package (Josse and Husson, 2016).

MCA was performed on the indicator matrix, and the number of dimensions to retain was determined by examining the eigenvalues (a measure of inertia, or variance, accounted for by a dimension). Assuming randomness in the data, those dimensions with eigenvalues  $> 1 / [(No. active variables) - 1]$  were considered in the results (Bendixen, 1995).

HCPC was then performed on the selected MCA dimensions to cluster individuals based on similar patterns in survey responses, thus identify groups of drivers that share specific biosecurity and hygiene practices. Ward's method with the Euclidean distance metric was used to aggregate

individuals into homogeneous groups and build the HCPC tree (Kassambara and Mundt, 2020).

The number of clusters was defined using the automatic cut-off point of the "FactoMineR" package (i.e., based on the inertia of the partitions).

## 3.4 Results

### 3.4.1 Descriptive analysis

Between November 2021 and November 2022, 82 drivers transporting cattle were interviewed: 26 face-to-face and 56 by phone call. Drivers from different regions of Spain were included in the study, despite most of them worked in Catalonia (north-eastern Spain), followed by Andalusia and Extremadura (south and south-central Spain, respectively). Further details on the home location of the driver can be found in supplementary material 2 (Table 1.S2).

In Table 1, the characteristics of the drivers and the type of vehicle they usually used are described. Forty-eight percent (40/82) were self-employed and 74.3% of the drivers had more than 10 years of experience transporting live animals. The most common practice was to use one and the same vehicle on a regular basis. Most of the drivers used trucks (i.e., the smallest type of vehicle) and journeys were done mainly by vehicles authorized to transport for less than 8 h a day. Fifty-two percent of the drivers (i.e., 43 out of 82) reported visiting both dairy and beef farms, while the rest exclusively visited one type of production system. From this last group, two drivers reported visiting only dairy farms and the other 37, only beef farms. International journeys were done by just 23% of the drivers (19/82).

Table 1. Characteristics of the 82 surveyed drivers.

Variable	N <sup>a</sup>	%
<b>Affiliation of the drivers</b>	<b>82</b>	
Self-employed	40	48.8%
Transport company	34	41.5%
Production company or cooperative	8	9.8%
<b>Professional experience (years)</b>	<b>82</b>	
≤10	21	25.6%
>10 - ≤20	28	34.1%
>20	33	40.2%
<b>Number of vehicles regularly used</b>	<b>82</b>	
One	64	78.0%
Two or more	18	22.0%
<b>Type of vehicle regularly used</b>	<b>82</b>	
Truck	38	46.3%
Semi-trailer	21	25.6%
Full trailer	23	28.0%
<b>Transport authorization</b>	<b>81</b>	
Less than 8 h	48	59.3%
Until 12 h	12	14.8%
More than 8 h	21	25.9%
<b>Type of farm visited</b>	<b>82</b>	
Mixed	43	52.4%
Single type <sup>b</sup>	39	47.6%
<b>International routes</b>	<b>82</b>	
Yes	19	23.2%
No	63	76.8%

<sup>a</sup> Not all the variables sum 82, as response rate was not 100% for all questions.

<sup>b</sup> Only two drivers worked only with dairy farms.

According to the definition of a journey, most of the drivers reported that in their daily work they made, on average, one journey per day, with a maximum of two journeys in a day. However, some drivers reported that on some days with a high demand of work, they could make a maximum of three, and even one of them reported to make up to four journeys in one single day (Supplementary material 2 (Table 2.S2)).

The number of different types of journeys made by each driver is described in Table 2. The most common were movements to the slaughterhouse, followed by fattening farms, and the least

frequent were journeys with bulls and to seasonal pastures. Only 13 drivers indicated that they exclusively made one type of journey, which was mostly to the slaughterhouse, while two drivers only moved animals for replacement and the remaining driver, only for culling.

The most common was to combine two different types of journeys in their working routines. As a matter of fact, a total of 35 drivers combined movements to the slaughterhouse with the transport of breeders (i.e., rearing, replacement, bulls, and pastures). In addition, one driver reported that he routinely made five different types (Table 3).

Table 4 shows the number of journeys per week made by the drivers. Shared journeys were reported within all the different types of movements, except for movements to seasonal pastures. In these shared journeys, it was common to load or unload animals in between one and six other farms (see details in

Table 5).

In Table 6, practices during the loading and unloading of animals are described. Even though most of the drivers (65.8%) reported frequent use of a loading dock, access into the perimeter of the farm (62.0%) or the cattle stables (60.8%) were frequent practices. In addition, having contact with animals remaining in the farm (e.g., for sorting animals that will be loaded with a batch) was a frequent practice for 55.7% of drivers.

Regarding work clothes, most of the drivers used their own boots, which were commonly cleaned with cold water between different farms. Only one driver reported cleaning and disinfecting the boots between different farms. Also, a non-negligible proportion of drivers (19.8%) cleaned the boots only at the end of the day (not between farms) and four drivers did not routinely clean their boots. Several drivers (48/79) mentioned to have a compartment in their vehicle specifically designed for separating clean and dirty clothes. Finally, a high proportion of drivers (45.5%) reported entering the truck cabin with their working clothes.

Table 2. Number of different types of journeys made by the specific type of journey.

Type of journey	Total	1 type (N=13)		2 types (N=45)		3 types (N=21)		4 types (N=2)		5 types (N=1)	
	No. of drivers	No. of drivers	%	No. of drivers	%						
Rearing	17	0	0%	9	53%	7	41%	0	0%	1	6%
Replacement	13	2	15%	3	23%	5	38%	2	15%	1	8%
Fattening	47	0	0%	28	60%	16	34%	2	4%	1	2%
Slaughterhouse	76	10	13%	42	55%	21	28%	2	3%	1	1%
Culling	14	1	7%	2	14%	8	57%	2	14%	1	7%
Pastures	10	0	0%	5	50%	5	50%	0	0%	0	0%
Bulls	2	0	0%	1	50%	1	50%	0	0%	0	0%

Table 3. Frequency of combinations of different types of journeys made by those drivers that combine more than one type of movement in their daily practice (N = 68).

Type of journey	N	%
Slaughterhouse and fattening	28	41.2%
Slaughterhouse and "breeders" <sup>a</sup>	19	27.9%
Slaughterhouse, fattening and "breeders" <sup>a</sup>	11	16.2%
Slaughterhouse, fattening and culling	5	7.4%
Slaughterhouse, fattening, culling and "breeders" <sup>a</sup>	3	4.4%
Culling and "breeders" <sup>a</sup>	2	2.9%

<sup>a</sup> "Breeders" include rearing, replacement, bulls, and movements to/from pastures.

*Table 4. Number of journeys per week and proportion of drivers loading/unloading animals at multiple farms during a single journey.*

Type of journey	Number of journeys per week				Journey-sharing drivers <sup>b</sup>	
	N	Min	Median	Max	N	% of drivers
Rearing	17	0.25	1	4	7	41.2%
Replacement	13	0.25	1	4	4	30.8%
Fattening	47	0.2	2	6	29	61.7%
Slaughterhouse	76	0.25	4	20	52	68.4%
Culling	14	1	2	8	12	85.7%
Pastures <sup>a</sup>	10	0.25	1.5	5	0	0.0%
Bulls	2	3	3.5	4	1	50.0%

<sup>a</sup>During the corresponding period.<sup>b</sup>Number of drivers that shared journeys divided by the total number of drivers performing each type of journey.*Table 5. Number of farms where animals are loaded/unloaded in shared journeys by farm type.*

Type of journey	No. of farms from where animals are loaded in shared journeys				No. of farms where animals are unloaded			
	N	Min	Median	Max	N	Min	Median	Max
Rearing	7	2	2	3	1	2	2	2
Replacement	4	2	2	3	5	2	3	4
Fattening	29	2	3	6	3	2	2	2
Slaughterhouse	52	2	2	5	n/a			
Culling	12	2	3	5	n/a			
Pastures	0				0			
Bulls	1	2	2	2	0			

Table 6. Activities carried out during the loading of animals.

Activities during the loading	N <sup>a</sup>	%
<b>q132. Access to the farm premises<sup>b</sup></b>	<b>79</b>	
Frequently	49	62.0%
Occasionally	25	31.6%
Rarely	5	6.3%
<b>q135. Use of loading dock<sup>b</sup></b>	<b>79</b>	
Frequently	52	65.8%
Occasionally	26	32.9%
Rarely	1	1.3%
<b>q137. Access to the stables where animals are kept<sup>b</sup></b>	<b>79</b>	
Frequently	48	60.8%
Occasionally	25	31.6%
Rarely	6	7.6%
<b>q139. Have contact with animals that remain on the farm<sup>b</sup></b>	<b>79</b>	
Frequently	44	55.7%
Occasionally	29	36.7%
Rarely	6	7.6%
<b>q142. Boots used by the driver</b>	<b>81</b>	
Property of the driver	68	84.0%
Property of the farm	2	2.5%
Use the farm's or the driver's boots depending on the destination	11	13.6%
<b>q148. Enter the truck cab with boots and overall<sup>b</sup></b>	<b>79</b>	
Frequently	14	17.7%
Occasionally	22	27.8%
Rarely	43	54.4%
<b>q150. Frequency of boot cleaning</b>	<b>81</b>	
Between farms	61	75.3%
Every day	16	19.8%
Rarely	4	4.9%
<b>q156. Practices during boot cleaning</b>	<b>81</b>	
Cleaning and disinfection	1	1.2%
Only disinfection without cleaning	9	11.1%
Only cleaning with cold water	71	87.7%

<sup>a</sup> Not all the variables sum 82, as response rate was not 100% for all questions.

<sup>b</sup> Frequently = more than 60% of the journeys; Occasionally = between 20 - 60% of journeys; Rarely = less than 20% of journeys.

Table 7 shows the practices for cleaning and disinfection of the vehicles. Most of the drivers reported washing and disinfecting their vehicle between journeys, and 56.8% of them used

detergent and disinfectant. Notably, 32.1% of them used only disinfectant without prior use of detergent. Moreover, 46.9% of the drivers stated that they had to drive more than 30 km to reach a cleaning and disinfection centre. Additionally, drivers mentioned that farmers rarely request a certificate of cleaning and disinfection of the vehicle (43.2%).

*Table 7. Practices during cleaning and disinfection of vehicles.*

Activities and measures	N <sup>a</sup>	%
<b>q162. Cleaning and disinfection (C&amp;D) after a journey of vehicles between farms<sup>b</sup></b>	<b>79</b>	
Frequently C&D	70	88.6%
Occasionally C&D	5	6.3%
Frequently cleaning and occasionally disinfection	3	3.8%
Occasionally cleaning and no disinfection	1	1.3%
<b>q166. Average distance between farms and C&amp;D centre</b>	<b>81</b>	
≤30 km	43	53.1%
>30km - ≤60km	28	34.6%
>60 km	10	12.3%
<b>q178. Practices during C&amp;D of trucks</b>	<b>81</b>	
Use of detergent and disinfectant	46	56.8%
Only use detergent	5	6.2%
Only use disinfectant	26	32.1%
Only use cold water	4	4.9%
<b>q182. Clothes used during C&amp;D of trucks</b>	<b>81</b>	
Overall and raincoat	45	55.6%
Only overall	30	37.0%
Only raincoat	3	3.7%
No work clothes	3	3.7%
<b>q187. Separation between clean and soiled work clothes</b>	<b>79</b>	
Use of clothes drawer without separation	17	21.5%
Use of clothes drawer and separation	48	60.8%
Without clothes drawer	14	17.7%
<b>q190. Requirement of C&amp;D certificate by farms<sup>b</sup></b>	<b>81</b>	
Frequently	24	29.6%
Occasionally	22	27.2%
Rarely	35	43.2%

<sup>a</sup> Not all the variables sum 82, as response rate was not 100% for all questions.

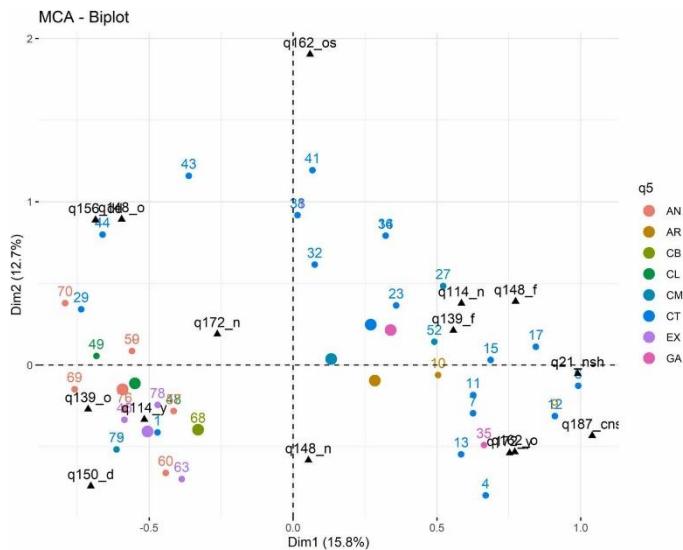
<sup>b</sup> Frequently = more than 60% of the journeys; Occasionally = between 20 - 60% of journeys; Rarely = less than 20% of journeys.

### 3.4.2 Multiple correspondence analysis

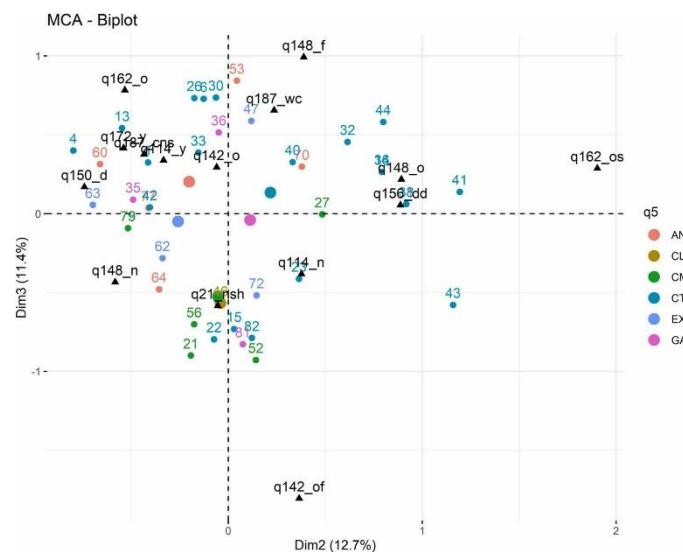
Data from 81 surveys were included in the multiple correspondence analysis (MCA) which was performed using 10 active variables and five supplementary categorical variables. The complete list of variables and their categories analysed can be found in Supplementary material 3 (Table 1.S3). The ten active variables contained 23 active categories in total. The categories of variables were also abbreviated and linked to their corresponded code of variable.

For the MCA interpretation, three dimensions were chosen from the 13 generated (i.e., 23 active categories - 10 active variables), accounting for 39.8% of the cumulative variance (see Supplementary material 3 (Figure 1.S3). The correlogram with the most contributing variables for each retained dimension and a table with detailed MCA results can be found in Supplementary material 3 Figure 2.S3 and Table 2.S3, respectively. Briefly, the first dimension was characterized by the practices of the drivers regarding the loading and unloading of animals (Figure 1), including: making shared journeys (q21), mixing cattle of different age within a journey (q114), and the frequency of contact with animals not being loaded (q139). The second dimension was linked to the hygiene measures adopted by the drivers (Figure 1 and Figure 2), mainly the frequency of disinfecting the vehicle between farms (q162), the use of working clothes entering the vehicle's cab (q148), and the frequency of cleaning the boots (q150). The third dimension (Figure 2) mainly separated drivers who had their own boots from those who used the farm's boots depending on the occasion (q142).

**Figure 1. Graph of the categories of variables and individuals according to dimensions 1 and 2.**  
 The categories of variables are shown in black, and the colours represent the Autonomous Community (AN: Andalusia; AR: Aragon; CB: Cantabria; CL: Castile and Leon; CM: Castilla-La Mancha; CT: Catalonia; EX: Extremadura; GA: Galicia) to which the drivers belong (q5). The graph shows the top 15 categories of variables and the top 40 contributing drivers. In Supplementary material 3 (Table 1.S3) contains a list of all the variables and their corresponding categories.



**Figure 2. Graph of the categories of variables and individuals according to dimensions 2 and 3.**  
 The categories of variables are shown in black, and the colours represent the Autonomous community (AN: Andalusia; CL: Castile and Leon; CM: Castilla-La Mancha; CT: Catalonia; EX: Extremadura; GA: Galicia) to which the drivers belong (q5). The graph shows the top 15 categories of variables and the top 40 contributing drivers. In Supplementary material 3 (Table 1) contains a list of all the variables and their corresponding categories.



### 3.4.3 Hierarchical clustering on principal components

The outcomes of the MCA were used to perform a Hierarchical Clustering on Principal Components (HCPC). The analysis resulted in the identification of four distinct clusters (see Supplementary material 3, Figure 3.S3). The size of each cluster was 32, 12, 13, and 24 drivers, respectively. More details on the characteristics of each cluster can be found in Table 8 and Table 9.

Cluster 1 and Cluster 4 showed the greatest differentiation concerning biosecurity practices (Table 9) and encompassed 56 drivers (69.1%) across these two clusters. In Cluster 1, all drivers went to the slaughterhouse at least once per week and loaded cattle at several farms during the same journey. Most of them mixed animals of different ages in the same load. They had occasionally contact with animals that were not loaded into the vehicle (i.e., remained on the farm). These drivers mainly used their personal boots, and they frequently disinfected their vehicles after each journey between breeder farms. In contrast to the previous cluster, the drivers grouped in Cluster 4 mainly transported “rearing” and “replacements animals” (i.e., breeder farms). During the animal loading procedures, they frequently had contact with animals that remained on the farm, although they did not usually mix animals of different ages in the same load. They reported cleaning the vehicle only occasionally between journeys (most often with hot water), entering in the cabin of the vehicle wearing work clothes, and washing their boots after each journey, but only with water. Cluster 2 and Cluster 3 were smaller in size (14.8% and 16.1% respectively) and had fewer distinguishing characteristics than the previous clusters.

Cluster 2 consisted of drivers who almost exclusively drove to slaughterhouses, which influenced the frequency of vehicle disinfection (required by law after each journey to the slaughterhouse) and their hygiene practices regarding the use of detergents and/or disinfectants when cleaning the boots.

Drivers in Cluster 3 travelled to both slaughterhouse and breeder farms. They usually loaded cattle of homogenous ages from only one farm per journey. Moreover, they did not enter in the vehicle's cab wearing work clothes and tended to use the farm's boots if available.

*Table 8. Clusters identified by HCPC. The number of drivers included in each cluster is detailed by geographical location, affiliation, experience, and journey frequencies.*

Variables	Cluster 1 (N=32)		Cluster 2 (N=12)		Cluster 3 (N=13)		Cluster 4 (N=24)	
	N <sup>a</sup>	%						
<b>Autonomous community of the driver</b>								
AN: Andalusia	9	28.1%	2	16.7%	2	15.4%	3	12.5%
AR: Aragon	1	3.1%					3	12.5%
CB: Cantabria	2	6.3%						
CL: Castile and Leon	3	9.4%			1	7.7%		
CM: Castile-La Mancha	1	3.1%	1	8.3%	4	30.8%		
CT: Catalonia	8	25.0%	8	66.7%	4	30.8%	15	62.5%
EX: Extremadura	7	21.9%			1	7.7%	1	4.2%
GA: Galicia			1	8.3%	1	7.7%	2	8.3%
MD: Madrid	1	3.1%						
<b>Affiliation of the driver</b>								
Self-employed	16	50.0%	4	33.3%	8	61.5%	11	45.8%
Production company	2	6.3%	3	25.0%			3	12.5%
Transport company	14	43.8%	5	41.7%	5	38.5%	10	41.7%
<b>Year of experience of the driver</b>								
≤10 years	8	25.0%	6	50.0%	5	38.5%	2	8.3%
>10 - ≤20 years	11	34.4%	3	25.0%	5	38.5%	8	33.3%
>20 years	13	40.6%	3	25.0%	3	23.1%	14	58.3%
<b>Frequency of rearing and replacement journeys</b>								
0 journeys	25	78.1%	11	91.7%	9	69.2%	8	33.3%
Up to 1 journey per week	3	9.4%			1	7.7%	5	20.8%
More than 1 journeys per week	4	12.5%	1	8.3%	3	23.1%	11	45.8%
<b>Frequency of journeys to slaughterhouse</b>								
0 journeys					1	7.7%	2	8.3%
Up to 1 journey per week	6	18.8%	1	8.3%	5	38.5%	1	4.2%
Between 2-6 journeys per week	17	53.1%	5	41.7%	7	53.8%	14	58.3%
More than 6 journeys per week	9	28.1%	6	50.0%			7	29.2%

<sup>a</sup> Not all the variables sum the total of the cluster, as some drivers did not answer all questions.

Table 9. Clusters identified by HCPC. Biosecurity-related practices carried out by drivers.

Variable	Cluster 1 (N=32)		Cluster 2 (N=12)		Cluster 3 (N=13)		Cluster 4 (N=24)	
	N <sup>a</sup>	%	N <sup>a</sup>	%	N <sup>a</sup>	%	N <sup>a</sup>	%
<b>Shared journeys with different origins</b>								
No	3	25.0%	6	46.2%	9	37.5%		
Yes	32	100.0%	9	75.0%	7	53.8%	15	62.5%
<b>Mixing animals of different ages in the vehicle</b>								
No	4	12.5%	8	66.7%	10	76.9%	16	66.7%
Yes	28	87.5%	4	33.3%	3	23.1%	8	33.3%
<b>Contact with animals that will not be loaded in the vehicle</b>								
Frequently	7	21.9%	9	75.0%	6	54.5%	22	91.7%
Occasional	25	78.1%	3	25.0%	5	45.5%	2	8.3%
<b>Ownership of the boots used during the transport</b>								
Own	32	100.0%	10	90.9%	3	23.1%	23	100.0%
Own and farm			1	9.1%	10	76.9%		
<b>Enter into the cabin with the working clothes</b>								
Frequently	1	3.2%	3	25.0%	1	7.7%	9	39.1%
Occasionally	12	38.7%	8	66.7%			2	8.7%
Rarely	18	58.1%	1	8.3%	12	92.3%	12	52.2%
<b>Disinfection of the vehicle on journeys between different farms</b>								
Frequently	29	93.5%	2	16.7%	11	91.7%	16	69.6%
Occasionally	2	6.5%			1	8.3%	7	30.4%
Only journeys to slaughterhouse (slaughterhouse protocols)			10	83.3%				
<b>Use of hot water to wash the vehicle</b>								
No	27	84.4%	11	91.7%	12	92.3%	10	41.7%
Yes	5	15.6%	1	8.3%	1	7.7%	14	58.3%
<b>Clothes drawer with clean and dirty area</b>								
Clothes drawer and no separation	2	6.5%	2	16.7%	1	7.7%	12	52.2%
Clothes drawer with separation	23	74.2%	7	58.3%	11	84.6%	7	30.4%
Without clothes drawer	6	19.4%	3	25.0%	1	7.7%	4	17.4%

<sup>a</sup> Not all the variables sum the total of the cluster, as some drivers did not answer all questions.

### 3.5 Discussion

Results from this study show an inadequate adherence to biosecurity protocols. Risk practices are common such as entering the farm premises to load/unload animals, passing by several farms to load and unload animals, combining journeys with different levels of risk or not always cleaning and disinfecting the vehicle between journeys, among others. Therefore, biosecurity

practices related to cattle transport in Spain have a large room for improvement, and the question is how to achieve improvements.

An important barrier might be the number of existing cleaning and disinfection centres and the conditions they offer to the drivers to perform an adequate cleaning and disinfection of their vehicles. According to the results of this study, a high proportion of drivers had to drive more than 30 km to arrive at one of these centres, and several of them had the nearest centre located more than 60 km away. This long distance might hamper an adequate cleaning and disinfection of the vehicles and make it difficult for them to reach the cleaning and disinfection centres. Furthermore, considering the working time regulations, which establish limits for drivers' driving and working time (European Union, 2006), leaving less time for cleaning and disinfection of vehicles.

The efficacy of cleaning and disinfection (C&D) of the vehicles relies on adherence to proper C&D protocols and the conditions offered by the C&D centres. For instance, the effectiveness of these processes can be significantly influenced by the time available to the drivers, the availability of hot water with sufficient pressure and disinfectant, driver training and the correct use of detergent and disinfect products. Indeed, using only one of the products (e.g., solely disinfectant) may decrease the efficiency of the C&D process (Dee et al., 2004). Currently, the existing legislation does not mandate supervision during the C&D process. As a matter of fact, in 2018, a Danish study identified that 42% of the pig transport vehicles were not properly cleaned and disinfected (Gao et al., 2023b). Poorly washed, and empty animal transport vehicles pose a risk to the next load of animals, even after several days, as was evidenced by Gao et al. (2023a) for African Swine Fever in pig live animal transport. Based on their results, without an efficient C&D, the probability of pigs getting infected from the contaminated vehicle remained non-negligible after several days at 10 °C due to a slow decay of virus at that temperature.

The costs associated with vehicle washing in Spain can also impact the situation, as prices can vary from being free of charge to several tens of euros, depending on the geographical location. It is evident that given these factors, there may be inconsistencies in the execution and adherence to proper cleaning and disinfection practices. The same holds true for cleaning boots. It was common practice to clean boots either between farm visits or daily. However, the effectiveness of solely using water may be limited (Amass et al., 2000).

Other barriers can be linked to the cost of some biosecurity practices. For example, from the perspective of reducing the probability of spreading pathogens, journeys should not be shared. The ideal would be to not mix animals from different farms in the vehicle and not to unload animals in different farms. However, the cost associated with transport together with the size of the farms, may be associated with the incentive to shared journeys (Villaamil et al., 2020, Muñoz-Ulecia et al., 2021). These circumstances could pose financial challenges for some producers. Additionally, it was noted that there is a lack of specialization among drivers regarding the type of journey or specific productive stages, likely influenced by low demand or seasonality of certain types of journeys (e.g., mountain pastures movements in spring and autumn).

Finally, other barriers might be linked to social aspects. For example, the significant number of drivers entering the farm premises, entering the stables, and directly interacting with animals that would remain on the farm could be attributed to the methods applied on some traditional farms (e.g., animal loading and unloading practices). According to the drivers' responses in the survey, it is customary for the driver to organize the load based on the animals' weight and even assist the farmer in selecting the animals. This practice aims to optimize the carrying capacity of the vehicle. Furthermore, due to the limited availability of farm personnel for loading and unloading animals, drivers often assist in separating the animals from the stables. Most of the surveyed drivers had been involved in animal transportation for 10 years or more. Their experience may lead them to

engage in certain longstanding habits or behaviours that could not be aligned with current regulations and may be difficult to change (Moya et al., 2021).

Due to the difficulty in finding drivers, 82 instead of 97 drivers were finally surveyed, resulting in a precision of around 11%, with a proportion of 50%, a confidence level of 95% and an unknown population size. The multiple correspondence analysis (MCA) method is commonly used for exploratory and descriptive analyses. In adherence to the method's recommendations, categories with low frequency and anomalous cases were eliminated (Further details can be found in Supplementary material 3). Despite its advantages, this technique is highly sensitive to the dataset used, thus it is recommended to have a sample size with approximately 20 observations for each active category (Di Franco, 2016). In our study, the MCA was conducted with 23 active categories and 81 drivers, being a relatively small sample size (representing only 17.6% of the recommended number). For this reason, the results of these profiles should therefore be approached with caution.

All the drivers who took part in this survey did so voluntarily and without receiving any form of financial compensation or incentives. Consequently, it is plausible that the respondents were more knowledgeable about disease transmission, biosecurity measures, and current regulations, and that they might have provided information that is biased towards the most accurate answer rather than reflecting their actual practices or behaviours. Additionally, due to the snowball sampling, we maximized participation, as participants were already aware that they were going to be contacted for this study, which facilitated their participation. Therefore, interviewed drivers might have recommended individuals from their own work areas or those with similar practices (Sedgwick, 2013, Etikan, 2016).

Although the study has its limitations, and results might not be representative of the situation in Spain, it still can provide a good picture about the present condition of cattle transportation. It has also been able to identify some barriers that could be useful in developing guidelines for

future driver training. Furthermore, it highlighted the need to invest in infrastructures to assure an adequate cleaning and disinfection of vehicles. Moreover, identified profiles of drivers could be utilized to strategically enhance awareness campaigns for biosecurity, considering the type of transportation they engage in and the geographical location of the drivers (Beltrán-Alcrudo et al., 2018). In terms of the risk of disease spread between farms and according to our exploratory profiling, Cluster 4 may pose a greater risk than the others. In this cluster, drivers regularly drove both to the slaughterhouse and between farms, coupled with the fact that these drivers often have contact with cattle remaining on farms and do not always clean/disinfect the vehicle between farms. However, the results did not suggest any clear patterns associated with the clusters. None of the four clusters adequately implemented all the recommended biosecurity measures.

It might be desirable to conduct future research on evaluating the effectiveness of implementing specific biosecurity measures and understanding the factors that drive the implementation of these measures. Such studies would be valuable in enhancing understanding on how to reduce the risk associated with the spread of pathogens during transportation. Further efforts to improve cleaning and disinfection facilities for cattle transport vehicles in collaboration between the public and private sectors are desirable.

### 3.6 Conclusions

This study showed that biosecurity practices in vehicles used for cattle transport should be reinforced. Results highlighted the need of investment in cleaning and disinfection centres to enable drivers with adequate infrastructures to improve biosecurity without compromising the economic viability of the sector, and the need of further training or awareness campaigns to increase biosecurity in cattle transport.

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### **3.9 Ethics statement**

This work has been approved by the Ethics Committee of the Autonomous University of Barcelona – approval number CEEAH 6188.

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## 3.11 Supplementary material

### 3.11.1 Supplementary material 1: Survey

*Figure 1.S1. Survey (in Spanish) used during the study. This survey was completed in paper or digital format depending on whether it was completed face-to-face or by phone call. Each survey was tabulated and coded in an excel file database.*

ENCUESTA DE BIOSEGURIDAD EN TRANSPORTES DE BOVINOS		Nº					
Fecha:	Nombre encuestado:						
Comarca:	Municipio:	C.A:					
¿Pertenece a alguna de las siguientes afiliaciones?							
<input type="checkbox"/> Empresa de producción / Cooperativa		<input type="checkbox"/> Empresa de transportes	<input type="checkbox"/> Autónomo				
Granjas del <b>mismo</b> cliente solamente		<input type="checkbox"/> Si	<input type="checkbox"/> No				
Años de trabajo en el sector del transporte de animales: <input type="text"/> años							
¿ Cuántas cajas/camiones utiliza de manera regular? <input type="text"/> cajas/camiones							
¿Qué tipo de vehículo utiliza más frecuentemente? <input type="checkbox"/> Camión <input type="checkbox"/> Semi-remolque <input type="checkbox"/> Tren de carretera							
¿Para qué viajes está certificado su vehículo más utilizado? <input type="checkbox"/> <8 hrs <input type="checkbox"/> Hasta 12 hrs <input type="checkbox"/> >8 hrs							
¿Qué granjas visita frecuentemente? <input type="text"/> % Granjas lecheras <input type="text"/> % Granjas de carne							
Rellene la tabla con el valor más frecuente de viajes:							
	Out. Terneras a recría	In Repos: terneras ya recriadas para madres	Animales a cebo	Animales a matadero	Desvieje (toros y vacas)	Viaje a pastos de montaña	Toro(s)
<b>Peso</b> promedio (kg)							
<b>Capacidad</b> del camión (nº animales)							
¿Durante una semana, cuántos transportes realiza?							
Y sobre estos, ¿Cuántos se completa la carga en mas de una granja?							
¿Y de cuántas granjas se <b>carga</b> animales?							
¿En cuántas granjas <b>descarga</b> animales?							
¿Realiza transportes transfronterizos? <input type="checkbox"/> Si <input type="checkbox"/> No				Si es Si, Señale:			
Último mes	Out. Terneras a recría	In Repos: terneras ya recriadas para madres	Animales a cebo	Animales a matadero	Desvieje (toros y vacas)	Viaje a pastos de montaña	Toro(s)
Nº de viajes <b>desde</b> España							
País de <b>destino</b> (s) si corresponde							
Nº de viajes <b>hacia</b> España							
Origen(es) si corresponde							

¿En un mismo viaje puede transportar animales de **diferentes** grupos de edad [1] en el mismo camión? (especifique los tipos de animales).

Si ¿Qué tipos?   
 ¿Con qué frecuencia?  (al mes)  
 No

¿En un mismo día **cuantos** viajes realiza (en el mismo camión)?

Más frecuente  y máximo   
 ¿De qué edades (grupos de la preg. Anterior)?

¿Cuántas horas **transcurren** entre dos destinos? (dentro de una jornada de trabajo, no superior a 9 horas).

Min:  Media:  Max:   
 Señale los destinos mas frecuentes:

Si realiza transportes entre **granjas**, señale:

De las granjas que visita, cual es la frecuencia (marque con una x) [2]:

N	R	O	F	MF

Veces que entra dentro del perímetro de la granja:

Utilizan un muelle de carga/descarga:

Entra en los patios donde están los animales:

Tiene contacto con los animales que se quedan en la granja:

N	R	O	F	MF

Utiliza botas propias que lleva en el camión

Utiliza botas proporcionadas por la explotación

Utiliza cubrecalzado/cubrezapatos

Cambia de calzado cuando realiza el viaje

Cambia de ropa cuando realiza el viaje

Sube a la cabina con las botas y mono

En caso de hacerlo en determinadas granjas, indique cuando lo hace (tipo de granjas, vacas de carne, frecuencia...)

En caso de utilizar botas propias, responda:

¿Cuál es la frecuencia de limpieza de las botas?

Entre granjas  Cada día  Cada 2-4 días  Cada semana  No realiza Limpieza

[1] Por grupos de edad, considere: 0-2 meses; 2-6 meses; 6-12 meses; 12 a 24 meses y >24 meses.

[2] N: Nunca ; R: Raro ; O: Ocasional ; F: Frecuente ; MF: Muy frecuente.

## Biosecurity in cattle production

¿Cómo realiza la limpieza de las botas propias?

- Con agua fría y cepillado
- Con detergente
- Con desinfectante
- Otras opciones, especificar:

Si realiza transportes de animales vivos entre granjas, señale la frecuencia si aplica [2]:

N	R	O	F	MF

¿Limpia el camión entre dos transportes a granja de distinto cliente?

¿Limpia el camión entre dos transportes a granja del mismo cliente?

¿Desinfecta el camión entre dos transportes a granja de distinto cliente?

¿Desinfecta el camión entre dos transportes a granja del mismo cliente?

¿Limpia la cabina entre dos transportes a granja?

¿Cuál es la distancia media entre el trayecto y la estación de lavado?

km      ¿Y la mayor distancia?  km

En caso de limpiar y desinfectar el camión, ¿Cuánto tarda en realizar la limpieza y desinfección del camión? (condición estándar, día de invierno):

Minutos

En caso afirmativo, ¿cómo se realiza la limpieza y desinfección del camión?

- Agua fría a presión       Uso de detergente ;      Nombre del detergente
- Agua caliente a presión       Uso de desinfectante ;      Nombre del desinfectante
- Cepillado

¿Cuándo limpia el camión utiliza ropa apropiada?

- Si, un mono.      ¿También se usa para otras actividades?  Si       No      ¿Cuál?
- Si, una prenda impermeable
- No

¿Cuenta con una caja o contenedor para dejar la ropa de trabajo?

- Si      ¿Separa ropa para L&D del camión de la de carga/descarga de animales?  Si       No
- No

Proporción de granjas que le piden certificado de limpieza y desinfección o revisan si se ha aplicado:  %

¿Ha tenido alguna vez algún problema relacionado a la entrada de alguna enfermedad en granjas?

- Si      Describa brevemente
- No

[2] N: Nunca ; R: Raro ; O: Ocasional ; F: Frecuente ; MF: Muy frecuente.

### 3.11.2 Supplementary material 2

Table 1.S2. Location of the surveyed drivers.

Spanish autonomous community	N	%
Catalunya	35	42.7%
Andalucía	16	19.5%
Extremadura	10	12.2%
Castilla - La Mancha	6	7.3%
Aragón	4	4.9%
Castilla y Leon	4	4.9%
Galicia	4	4.9%
Cantabria	2	2.4%
Madrid	1	1.2%
<b>Total</b>	<b>82</b>	<b>100.0%</b>

Table 2.S2. Number of daily journeys performed by the surveyed drivers.

Daily journeys	N	%
<b>Average</b>	<b>82</b>	
One	62	75.6%
Two	20	24.4%
<b>Maximum</b>	<b>82</b>	
One	19	23.2%
Two	42	51.2%
Three	20	24.4%
Four	1	1.2%

### 3.11.3 Supplementary material 3

#### Data preparation for conducting Multiple Correspondence Analysis

1. Data frame with 21 variables in totals.
2. 15 variables after excluding variables with correlation.
3. 10 variables on biosecurity practices (active variables) and 5 variables on characteristics of the drivers and their journeys (supplementary variables).
4. 23 categories of active variables remaining after excluding 4 categories with less than 8 responses (q142, q150, q156 and 162). In the case of supplementary variables, categories with less than 8 responses were not excluded (not analysed by MCA).

*Table 1.S3. List of variables used in the MCA and HCPC (final dataset). Complete list of variables, their code and corresponding level (categories). The table is divided into active variables and supplementary variables.*

Code of the variable	Variable	Levels
<b>Active variables</b>		
q21	Shared journeys with different origins	Sh: Shared journeys Nsh: Not shared journeys
q114	Mixing animals of different ages in the vehicle	Y: Yes N: No
q139	Contact with animals that will not be loaded in the vehicle	F: Frequently O: Occasionally
q142	Ownership of the boots used during the transport	O: own F: farm Of: own and the farm
q148	Enter into the cabin with the working clothes	F: Frequently O: Occasionally N: Rarely
q150	Cleaning frequency of the boots	Bf: Between farms D: Daily N: no cleaning
q156	Products used for cleaning and disinfection of the boots	Dd: detergent and/or disinfectant W: Only water
q162	Disinfection of the vehicle on journeys between different farms	F: Frequently O: Occasionally

		Os: Only journey to slaughterhouse
<b>q172</b>	Use of hot water to wash the vehicle	Y: Yes N: No
<b>q187</b>	Clothes drawer with clean and dirty area	Cs: Clothes drawer with separation Cns: Clothes drawer and no separation Wc: Without clothes drawer
<b>Supplementary variables</b>		
<b>q5</b>	Autonomous community of the driver	AN: Andalusia AR: Aragon CB: Cantabria CL: Castile and Leon CM: Castile-La Mancha CT: Catalonia EX: Extremadura GA: Galicia MD: Madrid
<b>q6</b>	Affiliation of the driver	Pc: Production company A: Self-employed Tc: Transport company
<b>q9</b>	Year of experience of the driver	1: ≤10 years 2: >10 - ≤20 years 3: >20 years
<b>q193</b>	Frequency of journeys to slaughterhouse	N: 0 journeys S1: up to 1 journey per week S2: between 2-6 journeys per week S6: more than 6 journeys per week
<b>q194</b>	Frequency of rearing and replacement journeys	N: 0 journeys S1: up to 1 journey per week S2: more than 1 journey per week

*Figure 1.S3. Histogram of the explained variance by dimension. Histogram showing the variance of the top 10 dimensions from the result of multiple component analysis. The first three dimensions accounted for 39.8% of the cumulative variance. Three dimensions were retained following the criterion eigenvalues > 1 / (No. of variables - 1)) = 11.1%. These dimensions were selected because their eigenvalues were higher than expected in the case of random data.*

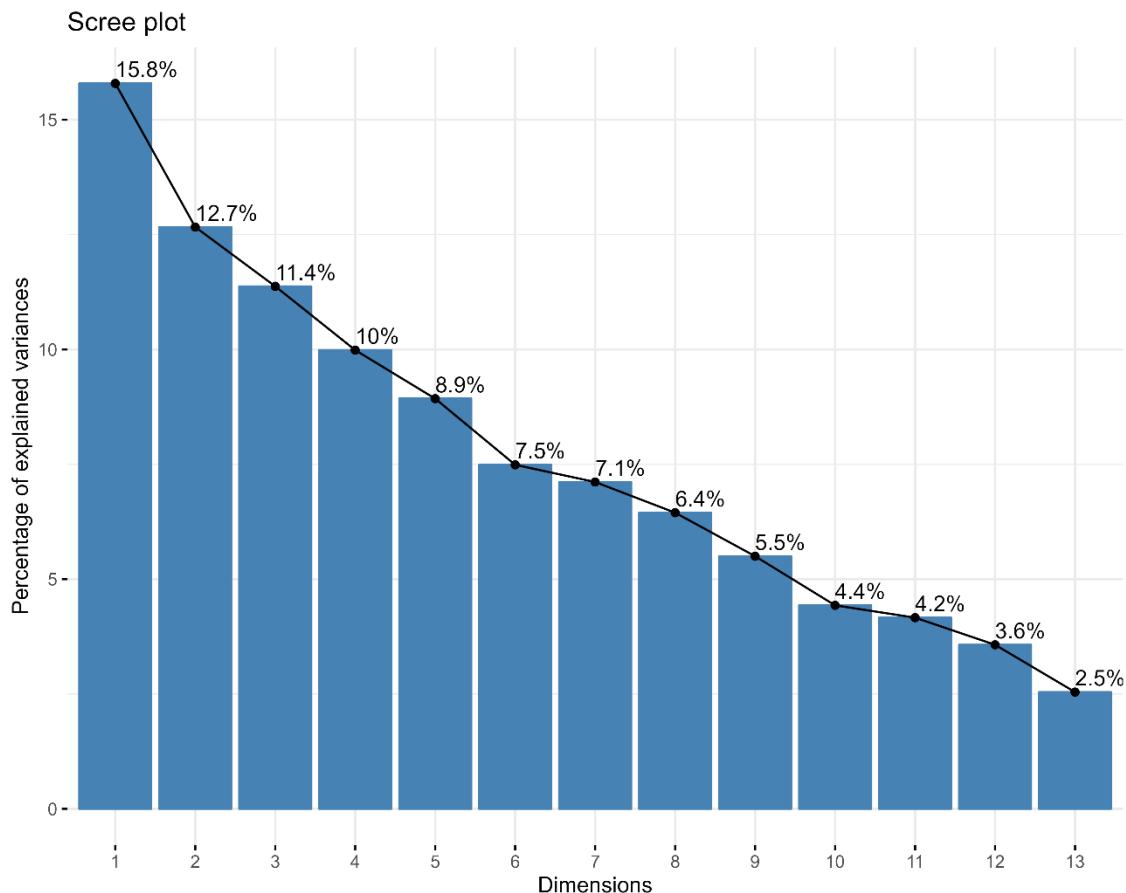
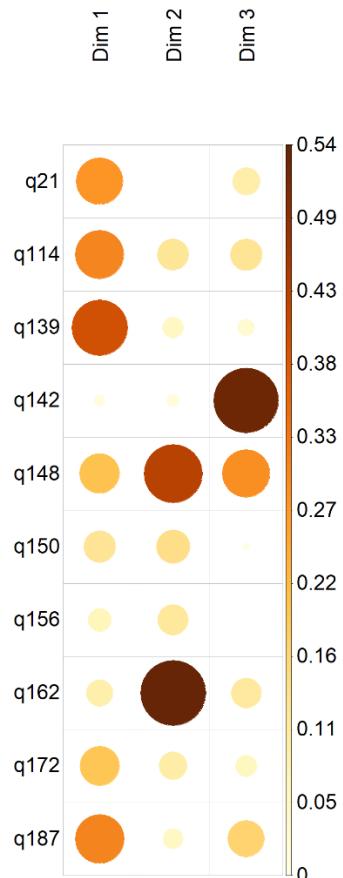


Figure 2.S3. Correlation plot. Correlogram showing the most contributing variables per dimension one, two and three. On the left the variable code and, on the right the correlation ratio (eta-squared).



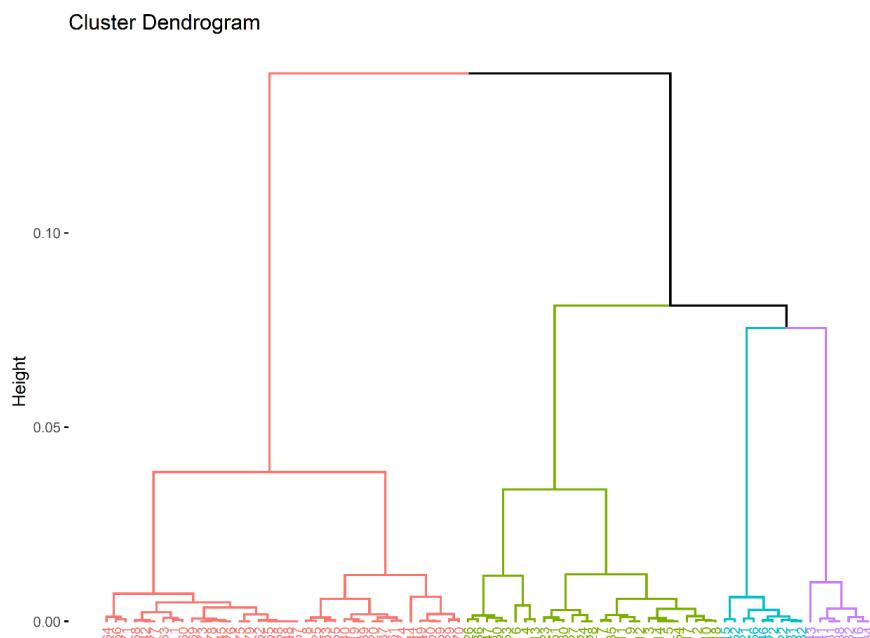
*Table 2.S3. List of categories of variables (the first five) related to the first three dimensions obtained by a Multiple Component Analysis.*

<b>Categories of the categorical variables<sup>1</sup></b>	<b>Estimate</b>	<b>P value</b>
<b>Dimension 1</b>		
q139=q139_f	0.177	0.000
q114=q114_n	0.247	0.000
q187=q187_cns	0.431	0.000
q21=q21_nsh	0.285	0.000
q172=q172_y	0.227	0.000
<b>Dimension 2</b>		
q162=q162_os	0.689	0.000
q148=q148_o	0.349	0.000
q156=q156_dd	0.240	0.001
q114=q114_n	0.142	0.001
q150=q150_bf	0.121	0.003
<b>Dimension 3</b>		
q142=q142_o	0.347	0.000
q148=q148_f	0.237	0.000
q114=q114_y	0.137	0.001
q187=q187_wc	0.111	0.007
q139=q139_f	0.381	0.012

<sup>1</sup>Complete list of variables, their code and corresponding level (categories) are in Table 1.S3.

*Figure 3.S3. Dendrogram of the Hierarchical Clustering on Principal Components*

*Cluster dendrogram representing the outcomes of the analysis conducted using Hierarchical Clustering on Principal Components (HCPC). The x-axis represents the drivers, while the initial partitioning is determined by cutting the dendrogram based on the inertia gains between partitions. The hierarchical classification resulted in four clusters, represented in the figure by the colours red, green, light blue, and purple.*





## 4 Study II: Methods to assess on-farm biosecurity in Europe and beyond

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## 4.1 Abstract

The aim of this study was to identify which biosecurity assessment methods (BAMs) are currently used in practice in animal farms. To address this, a structured questionnaire was developed to gather information such as the animal species, main objectives, type of enforcement, output generated and feedback of the result. In the context of the BETTER Cost Action project, country representatives identified in each of their countries which BAMs were used and completed an online survey. The survey was prepared and translated in 23 languages. Besides a descriptive analysis, clusters of BAMs were determined using a multiple correspondence analysis. Responses, collected between December 2022 and July 2023, included 74 BAMs used in 28 countries. Most of them were used in a single country while three were used in multiple countries. This study provides a comprehensive picture of existing BAMs and insights into their diversity, such as variations in objectives, implementation, evaluators, respondents, feedback, or assessment outputs. Moreover, we identified four BAMs clusters differentiated by their objective, evaluator and type of feedback provided. This study might also represent the basis for future research on strengths and weaknesses of different BAMs.

## 4.2 Introduction

Biosecurity on farms, as defined by the World Organization for Animal Health (WOAH), are a set of management and physical measures designed to reduce the risk of introduction, establishment and spread of animal diseases, infections or infestations to, from and within an animal population (World Organization for Animal Health, 2023). Despite in the last years, a broader definition for biosecurity in livestock farms has also been proposed, named the 5Bs, which considers not only measures to prevent the introduction and spread of pathogens, but also to prevent zoonotic pathogens and environmental contamination (Saegerman et al., 2023).

Assessing biosecurity includes an evaluation on which and how biosecurity measures are implemented on the farm. Outputs from these assessments might be used to determine strengths and weaknesses, provide recommendations, monitor farmers' compliance, compare it with other farms (benchmarking), and/or to develop or improve a biosecurity plan for the farm (Alarcón et al., 2021, Sayers et al., 2013). Moreover, they can be used to raise awareness among farmers and veterinarians to improve their perception on disease risk and to promote education and responsibility against the prevention and control of diseases (Alarcón et al., 2021, Nöremark et al., 2009). In addition, they might be useful for monitoring national biosecurity strategies allowing countries to demonstrate their capacity to prevent, control and eradicate diseases (Hastein et al., 2008).

Several approaches for assessing on-farm biosecurity exist varying in their purpose, implementation, and outputs (Alarcón et al., 2021, Benavides et al., 2020, Gelaude et al., 2014, Martínez-Guijosa et al., 2021, Sasaki et al., 2020, Tilli et al., 2022) but they have not been described comprehensively. In addition, there is no comprehensive overview that maps and describes how biosecurity is assessed on farms in different countries. Therefore, the aim of this study was to identify and characterize the different biosecurity assessment methods (BAMs) that are used in practice in different countries and farming systems.

## 4.3 Material and methods

### 4.3.1 Survey design and data collection

For the purpose of the study, a BAM at farm level was defined as a standardized process (i.e., performed in a similar way in each farm) through which the status of biosecurity at the farm is evaluated.

To identify and characterize the different BAMs, a survey was developed by experts from the Cost Action CA20103 “Biosecurity Enhanced Through Training Evaluation and Raising Awareness” (BETTER, 2021), which is a collaborative EU-founded network of farm biosecurity experts.

Several online and in-person meetings were held to establish the content and structure of the questionnaire. The survey was designed to collect information on BAMs across a range of countries. The final version of the survey (Supplementary material 1, Figure 1.S1) covered (i) characteristics of the method used (e.g., animal species, objective, regulatory requirement, developer, and cost); (ii) how the assessment was done (e.g., evaluator, respondent, and process for data collection) and (iii) output of the assessment (i.e., descriptive, scores or probability estimates).

Before the survey collection process, pilot tests were conducted in three European countries, and the feedback gathered was used to refine the survey. Through BETTER, a call was made to identify volunteer focal points from the participating countries. Once these country focal points (CFPs) expressed their interest, a training session was organized. Two training sessions were held with CFPs to guide them on how to conduct the survey and to answer any questions. CFPs were responsible for identifying potential stakeholders using BAMs in their respective countries and completing the survey with them. To ensure a structured approach to data entry, it was agreed that a single survey would be completed for each BAM used in each country.

The survey was uploaded in EUSurvey online survey management system (<https://ec.europa.eu/eusurvey>) and translated into 23 languages. After the data collection phase, between October and November 2023, the first author conducted semi-structured interviews lasting 15–30 min with each CFP or a country expert suggested by the CFP to validate the responses submitted. Prior to the validation meeting, the submitted responses were checked for omissions, inconsistencies, or ambiguities. Where available, the legislation referred to, often in the language of the respondent country, was translated to provide a brief overview of the issue.

Subsequently, the issues identified by the first author in the questionnaire were discussed in detail with CFPs. If necessary, changes were made, and once all responses were clear and both the CFP or expert and the first author agreed, the final dataset for the country was considered validated.

#### 4.3.2 Data analysis

The survey contained thirteen animal categories corresponding to the production types of poultry, ruminants, pigs, and "other species" (e.g., lagomorph, guinea fowl, wild boar farms). These categories were aggregated within their respective species to facilitate the description of the results. Only poultry, ruminants, and pigs were analysed as only one answer was obtained from other species. As the same BAM could be used in different countries, but its implementation may vary per country, some variables were analysed per BAM while others were described by number of answers received. For example, for each biosecurity method, data on developer or type of output of the assessment were described per unique method while other variables such as objective, species, evaluator, or time spent during the on-site assessment, were analysed per number of surveys received. Data processing and description were performed in R software version 4.2.2 (R Core Team, 2023).

As an exploratory approach, using the responses received, a hierarchical clustering on principal components (HCPC) was conducted based on the results of a multiple correspondence analysis (MCA) (Husson et al., 2010). Questions related to objectives (n = 6), evaluators (n = 5), extra-data collection (n = 1), feedback (n = 1) and method of calculation of the BAMs (n = 1) were included for analysis using multiple correspondence analysis (MCA).

To avoid analysing variables shared across all BAMs, those with a correlation coefficient of  $\pm 0.4$  or higher were considered for elimination. Variables with response rates below 10 % were also excluded from the analysis. MCA was performed using the indicator matrix method. The optimal

number of dimensions to retain was determined by the lowest mean square error of prediction (MSEP). Ward's method with the Euclidean distance metric was used to aggregate individuals into homogeneous groups and build the HCPC tree. All other MCA and HCPC settings were kept at their default values from the “factoextra” and “FactoMineR” packages (Husson et al., 2010, Kassambara and Mundt, 2020).

## 4.4 Results

A total of 115 responses were received between December 2022 and July 2023. Following the validation process, 84 responses, covering 28 countries (21 countries from Europe, 4 in America, 2 in Asia and 1 in northern Africa), were validated and included in the analysis. During the validation, doubts and inconsistencies, if any, were clarified.

A total of 74 unique BAMs were identified. Seventy-one BAMs were used in a single country while three were used in more than one country. Among these three, Biocheck.UGent™ (Gelaude et al., 2014) was reported in seven countries, while 1000 points biosecurity assessment (Pig Improvement Company, 2020) and Combat (Boehringer Ingelheim, 2018) were used in four and two countries, respectively. Further details on the countries can be found in Supplementary material 2 (Table 1.S2). Thirteen out of 28 countries reported using more than one BAM. For example, 15 different methods were described for Spain, of which, 11 were used in the private industry, to assess biosecurity in pig (12/15), poultry (3/15) and ruminant farms (1/15).

### 4.4.1 General characteristics of the BAMs

Most of the methods (61 out of 74) were species-specific and therefore assessed only one type of animals (i.e., pigs, poultry, or ruminants). The number of methods varied by species, with pigs reporting most methods (35/74), followed by poultry (33/74) and ruminants (27/74). Methods used across multiple species (13/74) were predominantly in pig, ruminant, and poultry farms, as well as methods used in both poultry and ruminant farms (2/74), or poultry and pig farms (2/74).

Only one method was applied in both pig and ruminant farms. Supplementary material 2 provides further details on the animal species targeted by these biosecurity methods (Table 2.S2 and Table 3.S2).

The regulatory requirements and the main objectives of the methods are presented in Table 1. The main objective varied according to the animal species. The most frequently mentioned objective was a voluntary assessment to improve biosecurity followed by assessment focusing on the prevention/control of a specific disease. Diseases reported from these methods were, salmonellosis (9/84), African swine fever (5/84), bovine tuberculosis (4/84), brucellosis (4/84), porcine reproductive and respiratory syndrome (4/84), among others. Several assessments were implemented following both compulsory and voluntary requirements. For example, in Spain farm biosecurity assessment in cattle is mandatory in high-risk areas for tuberculosis while it is voluntary in the rest of the territory.

There was little collaboration between governmental agencies, industry and veterinarians when designing BAMs since most methods were developed by single entities (

Figure 1). In the case of methods focused on pig and poultry production, the most frequent developers were producer associations and private companies (40 % and 42 %, respectively) while for ruminants, the official veterinary service was the most reported developer, with 33 % (9/27). Although several combinations of developers were reported, the most common combinations were the official veterinary services together with producer/farmer associations and the combination of university and producer/farmer associations. More details in Supplementary Material Table 4.S2.

Approximately half of the BAMs were provided without requiring payment from farmers at the time of use. None of the legally mandatory methods required payment from farmers across all three animal categories. In contrast, voluntary methods requiring payment by the end-user (e.g.,

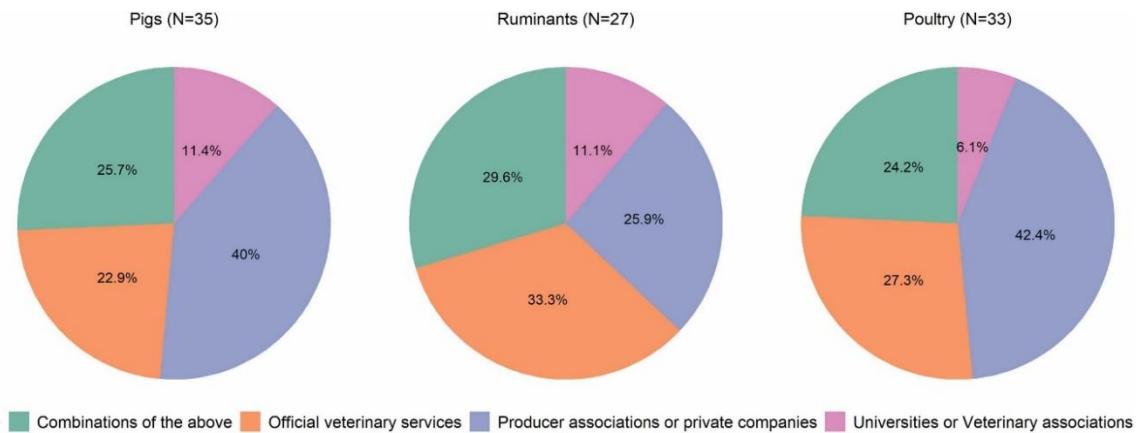
veterinarian or farmer) constituted 16 % (7/45), 17 % (5/30) and 19 % (7/37) of each respective category.

*Table 1. Regulatory requirements and main objectives of the biosecurity assessment methods (BAMs) by animal production type in 28 countries reported between October and November 2023. The reported N represents the number of responses received for each type of animal production, with the percentage (%) calculated within each respective production type.*

<b>Main objective</b>	<b>Pigs N=45</b>		<b>Ruminants N=30</b>		<b>Poultry N=37</b>	
	<b>N</b>	<b>%</b>	<b>N</b>	<b>%</b>	<b>N</b>	<b>%</b>
<b>Certification for quality assurance</b>						
Mandatory	2	4%	1	3%	3	8%
Mandatory; Voluntary					1	3%
Voluntary	4	9%	2	7%	6	16%
<b>To improve biosecurity of the farm</b>						
Mandatory	12	27%	7	23%	8	22%
Mandatory; Voluntary			1	3%	3	8%
Voluntary	26	58%	14	47%	16	43%
<b>To control/prevent a specific disease</b>						
Mandatory	6	13%	4	13%	4	11%
Mandatory; Voluntary			1	3%	2	5%
Voluntary	8	18%	10	33%	7	19%
<b>“Other” objective</b>						
Mandatory	2	4%	1	3%		
Mandatory; Voluntary					1	3%
Voluntary			1	3%		

*Figure 1. Developers of the biosecurity assessment methods (BAMs) by animal production type in 28 countries reported between October and November 2023. The reported N represents the*

*number of unique methods received for each type of animal production, with the percentage (%) calculated within each respective production type.*



#### 4.4.2 How the assessment was done

In legally mandatory assessments, veterinarians from the official veterinary services, veterinary consultants or private veterinarians paid by the official veterinary services (OVS), were the most frequent professionals involved (Table 2). On the other hand, voluntary assessments were primarily conducted by veterinary consultants and farm managers. Researchers, farm managers and external auditors were only involved in voluntary assessments.

Assessments were conducted "on-site" (i.e., visiting the production units of the farm) for 89 % (40 out of 45) of pig farms assessments and 100 % of ruminant (N = 30) and poultry (N = 37) farms assessments. The duration of on-farm assessment visits varied by production type, with the most common duration being up to two hours across all three types of production (i.e., 17/40, 15/30 and 22/37 for pigs, ruminants, and poultry, respectively). In all three production sectors, most of the assessment data were collected on paper, 23/45, 18/30 and 20/37 for pig, ruminant, and poultry farms, respectively. The rest of the BAMs were collected using a digital system (e.g. app or website).

The person in charge of answering to the farm-assessment was mostly the farm manager (41/45, 25/30 and 32/37, for pigs, ruminants, and poultry). Nevertheless, some assessments involved

multiple respondents (e.g. farm owner, veterinary consultant). More details in how the assessments were done are available in Supplementary material (Table 5, 6, 7 and 8.S2).

Between 44 % and 65 % of the BAMs (Table 3), on top of collecting of collecting biosecurity practices at the farm, also collected extra data to assess biosecurity in a systematic manner. The most collected extra-data was to evaluate farm-specific written protocols (e.g., standard operating procedures) or to inspect farm records (e.g., antimicrobial use).

*Table 2. Evaluator (person in charge of doing the assessment) and the regulatory requirement of the biosecurity assessment methods (BAMs) by animal production type in 28 countries reported between October and November 2023. The reported N represents the number of responses received for each type of animal production, with the percentage (%) calculated within each respective production type.*

<b>Evaluator</b>	<b>Pigs N = 45</b>		<b>Ruminants N = 30</b>		<b>Poultry N = 37</b>	
	<b>N</b>	<b>%</b>	<b>N</b>	<b>%</b>	<b>N</b>	<b>%</b>
<b>Official veterinary service (OVS)</b>						
Mandatory	11	24 %	7	23 %	6	16 %
Mandatory & voluntary			1	3 %	2	5 %
Voluntary	1	2 %	1	3 %		
<b>Farm veterinary advisor</b>						
Mandatory	6	13 %	4	13 %	4	11 %
Mandatory & voluntary					2	5 %
Voluntary	20	44 %	9	30 %	11	30 %
<b>Researchers</b>						
Mandatory						
Mandatory & voluntary						
Voluntary	5	11 %	5	17 %	3	8 %
<b>Farm manager</b>						
Mandatory						
Mandatory & voluntary						
Voluntary	14	31 %	6	20 %	9	24 %
<b>External auditor</b>						
Mandatory						
Mandatory & voluntary						
Voluntary	5	11 %	5	17 %	5	14 %
<b>Veterinarian paid by OVS</b>						
Mandatory	5	11 %	4	13 %	2	5 %

Mandatory & voluntary		1	3 %	1	3 %	
Voluntary	4	9 %	4	13 %	2	5 %
<b>“Other” evaluator<sup>a</sup></b>						
Mandatory						
Mandatory & voluntary						
Voluntary	2	4 %	1	3 %	3	8 %

<sup>a</sup> i.e. veterinarian working in the pharmaceutical industry or advisor of the integrator company.

*Table 3. Extra-data collection of the biosecurity assessment methods (BAMs) by animal production type in 28 countries reported between October and November 2023. The reported N represents the number of responses received for each type of animal production, with the percentage (%) calculated within each respective production type.*

	Pigs N = 45		Ruminants N = 30		Poultry N = 37	
	N	%	N	%	N	%
<b>Extra-data collection</b>						
Yes	20	44 %	18	60 %	24	65 %
No	25	56 %	12	40 %	13	35 %
<b>Type of extra-data collected (only methods with extra-data)</b>						
Environmental sampling	5	25 %	1	6 %	3	13 %
Data from national authority's databases	5	25 %	9	50 %	7	29 %
Inspection of farm records	15	75 %	13	72 %	18	75 %
Written protocols	17	85 %	14	78 %	20	83 %
Animal welfare status	6	30 %	11	61 %	16	67 %
Animal sampling	10	50 %	5	28 %	7	29 %
Other extra-data <sup>a</sup>	1	5 %	2	11 %	1	4 %

<sup>a</sup>e.g Geographical data, camera trap imaging, chlorine testing of water.

It is noteworthy that, among methods considering additional data collection, 30 % (6/20) of those for pigs included a welfare assessment. For methods used in ruminant and poultry farms, more than 60 % of them included a welfare component.

#### 4.4.3 Output of the biosecurity assessment

Most of the methods used in pig production yielded a quantitative score based on the relative weight of the biosecurity measures applied on the farm (19 out of 35). In the case of ruminant and poultry production, the most common was a descriptive output, while 12/27 and 13/33 of BAMs, respectively, also provided a score reflecting the farm biosecurity level. Only one assessment in pig and poultry farms provided an output based on probability estimates (e.g., based on risk models or machine learning) while another was based on key performance indicators (KPIs) related to the use of antibiotics in pig farms (details in Supplementary material Table 9.S2).

Most of the BAMs involved feedback on biosecurity implementation level provided to farmers after the assessment (Table 4). Reports were mostly provided in written format or in a combination of written and verbal formats (35/45, 22/30 and 32/27, for pig, ruminant, and poultry farms, respectively). Benchmarking (i.e., comparative assessment) in terms of biosecurity level of farm results was provided mostly in relation to pig farms (29/45) either at an aggregate level or by breaking down individual biosecurity measures. In the case of ruminant and poultry farms, 12/30 and 18/37 of the methods had benchmarking, respectively. More details can be found in Supplementary material Table 10.S2.

*Table 4. Types of feedback provided after the use of biosecurity assessment methods (BAMs) by animal production type in 28 countries reported between October and November 2023. The reported N represents the number of responses received for each type of animal production, with the percentage (%) calculated within each respective production type.*

	Pigs N = 45		Ruminants N = 30		Poultry N = 37	
	N	%	N	%	N	%
<b>Feedback</b>						
No	2	4 %	5	17 %	2	5 %
Yes	43	96 %	25	83 %	35	95 %
<b>Feedback details</b>						
Verbal report	2	4 %	5	17 %	2	5 %
Written report	43	96 %	25	83 %	35	95 %
Verbal & written report	2	4 %	5	17 %	2	5 %
Other	43	96 %	25	83 %	35	95 %

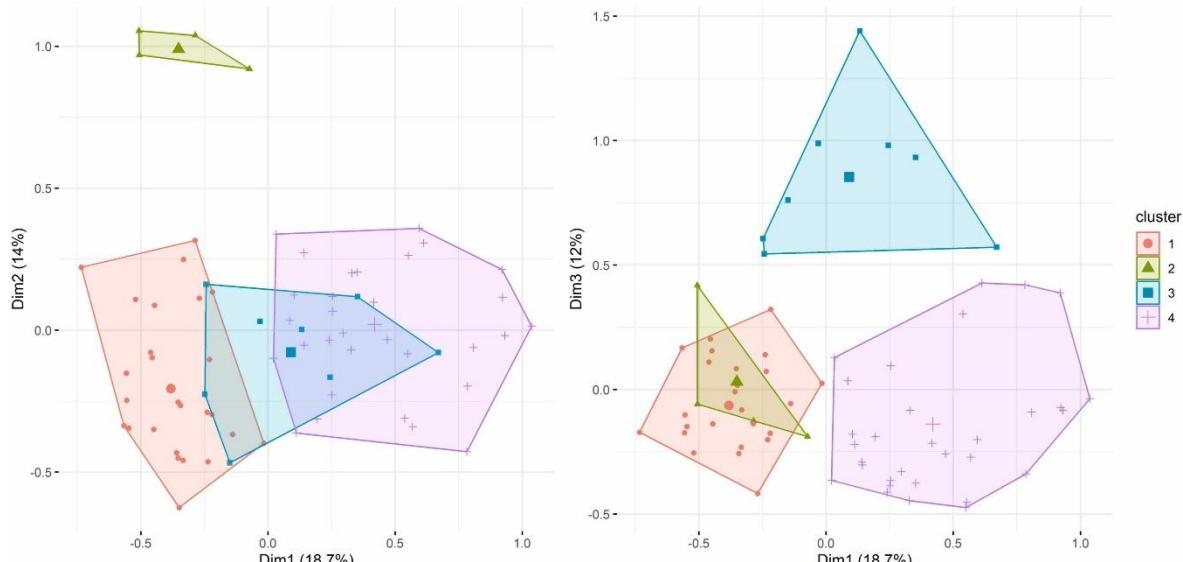
#### 4.4.4 Exploratory clustering

To enhance the exploration of the results, HCPC was applied based on the MCA outcomes. The MCA included 13 active variables and one supplementary variable (in this case, the type of BAM calculation), resulting in 27 active variable categories. A total of 84 responses were analysed. Four dimensions were retained, collectively explaining 54.3 % of the cumulative variance (Supplementary material Figure 1.S2).

Four clusters were identified (Figure 2), consisting of 34, 7, 8, and 35 BAMs in clusters one, two, three, and four, respectively. Cluster one was characterized by voluntary BAMs aimed at improving overall farm biosecurity, typically implemented by the farm veterinary consultant or farm manager, with feedback provided both verbally and in writing. In contrast, cluster four comprised methods mandated by law, conducted by official veterinary services (OVS), targeting both general farm biosecurity and specific diseases, and incorporating systematic collection of additional data.

*Figure 2. Visualisation of the four clusters resulting from the Hierarchical Clustering on Principal Components (HCPC) analysis on the results of the Multiple Correspondence Analysis (MCA). The*

Cluster plots of the biosecurity assessment methods (BAMs)



*plots show the first and second dimensions on the left, and the first and third dimensions on the right. The percentage given for each of the first three dimensions refers to the amount of inertia that they explain, which together account for 44.8 % of the variability in the data frame analysed. The points represent the biosecurity assessment methods (BAMs) clustered using Ward's method with the Euclidean distance metric.*

Clusters two and three, which included a smaller number of BAMs, were characterized by a few variables. Cluster two consisted mainly of BAMs carried out by external auditors and farm managers, focusing on quality assurance and the collection of additional data. BAMs in cluster three aimed to reduce the use of antibiotics on farms or to voluntarily tackle a specific disease. They are usually carried out by external veterinarians paid by the OVSs and the results are communicated verbally.

More details on clustering in Supplementary material Figure 2.S2.

## 4.5 Discussion

Our results demonstrate the wide range of methods used to assess farm biosecurity and that there is not a uniform biosecurity assessment protocol. Countries and production systems use different approaches to assess biosecurity, varying in terms of objectives, professionals involved in the evaluation, data collection methods, whether on-farm visit is required, time spent, or types of feedback, among others. The heterogeneity of methods found can be a challenge to have comparable outputs among countries. On the other hand, this diversity might reflect inter- and intra-countries differences in relation to the epidemiological context or characteristics of the livestock production systems. Pros and cons of having standardized biosecurity assessments among countries, might deserve further research.

Most of the methods identified in this study are being used in Europe, reflecting the regions where country focal points were situated. The recent implementation of the Animal Health Law strongly emphasizes biosecurity and its assessment is becoming compulsory. Moreover, the growing

interest in biosecurity across Europe (Chantziras et al., 2020, Filippitzi et al., 2018) due to various health threats (e.g., African Swine Fever or Avian Influenza) may explain the large number of existing BAMs. In addition, there were variations in the use of BAMs across countries. However, it should also be considered that CFPs might have differed in their effort in identifying all methods used in their respective countries. Thus, the number of methods reported here might be an underestimate of the real number of methods being used.

The most common evaluator in voluntary methods, was the veterinary consultant. This is consistent with findings by Delpont et al., 2023, Sayers et al., 2014, indicating that clinicians and veterinary consultants play a central role in providing information on matters related to animal health. Training in biosecurity for private veterinarians, veterinary services, and farmers is a crucial component in promoting the proper implementation of measures and practices related to biosecurity. Therefore, better biosecurity training, considering their needs and expectations (Saegerman et al., 2024) could help in the accurate application of BAMs, resulting in more reliable and repeatable assessments (Alarcón et al., 2021, Robertson, 2020).

More than half of the methods used a paper-based survey system to be filled out during the visit. This process could paradoxically be risky for disease transmission, as the assessor may have visited other farms and used the same materials on multiple farms without disinfection (Kim et al., 2017, Mee et al., 2012, Ssematimba et al., 2013). In addition, if the data on paper require transcription, this process may contribute to the entry of data with errors (Barchard and Pace, 2011).

Most BAMs stored data in a database, but only a few of them were publicly accessible and therefore the quality of the data and the nature of the information collected could not be verified. The advantage of digital storage is that it facilitates the review of existing information, avoiding double work and using more efficient analytical tools (Delpont et al., 2023). It might be beneficial to develop user-friendly digital tools for farm biosecurity assessments. These tools should allow

easy and accessible on-farm assessments without requiring external materials or additional tools.

Results showed that in some BAMs other evaluations were also done, such as for example animal welfare, as this component was evaluated in several BAMs in ruminant and poultry farms, while almost a third of the methods used in pigs also had this item involved. A good level of welfare and health is associated with enhanced production and health performances (Diana et al., 2020, Fusi et al., 2021, Pandolfi et al., 2018, Stygar et al., 2020). Therefore, integrating different assessments in the same visit will have practical and cost-effective advantages, despite it might also offer some challenges. For example, there might be conflicts between welfare and biosecurity that might impact the practical implementation of biosecurity (Alarcón et al., 2021) and which need to be considered when proposing recommendations after the assessment. Further studies on how to integrate different assessments might be desirable.

Furthermore, while a detailed description of the biosecurity components of each BAM was beyond the scope of this study, it is recognized that such an analysis would not have been feasible, as many BAMs are not publicly accessible. In most cases, we did not have access to the full protocols used for farm biosecurity assessments, which limited the ability to provide detailed descriptions. Further efforts, evaluating how different methods differ in terms of biosecurity practices assessed might be of interest. Also, the actual frequency of use or the number of farms where the BAM is systematically used was not requested. Given this, it is possible that in our results, methods that are applied very intensively coexist with others that have a more limited and regional application.

## 4.6 Conclusions

This study provides an overview of the main methods that are currently used to perform farm-biosecurity assessments in ruminant, poultry and pig farms showing that there is a high diversity.

Assessments differed in terms of who performs the evaluation, how the evaluation is done, how biosecurity practices are implemented, the type of feedback provided, and outputs generated after the assessment.

## 4.7 Acknowledgments

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## 4.8 Financial disclosure statement

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## 4.9 Ethics statement

This work has been approved by the Ethics Committee of the Autonomous University of Barcelona – approval number CEEAH 6168.

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## 4.11 Supplementary material

### 4.11.1 Supplementary material 1: Survey

Figure 1. S1. Survey (English version) used during the study. This survey was completed through EUSurvey system (<https://ec.europa.eu/eusurvey>).



COST Action CA20103: Biosecurity Enhanced Through Training Evaluation and Raising Awareness

### Survey to identify methods used to assess biosecurity

#### Considerations

- The objective of this survey is to identify existing methods for **evaluation of biosecurity at farm level**. At present, methodologies are diverse and have not been compared comprehensively.
- A **method to assess biosecurity at farm level** is a standardised process (i.e., it is performed in a similar way in each farm) through which the level of biosecurity and/or risk of introduction of (a) disease(s) is evaluated.
- One survey **must be completed for each existing method** in the country in which you are working.

If you have any question on how to complete this survey, please do not hesitate to contact: Fernando Duarte ([fernando.duarte@autonoma.cat](mailto:fernando.duarte@autonoma.cat)) / Alberto Allepuz ([alberto.allepuz@uab.cat](mailto:alberto.allepuz@uab.cat)).

Your data will be treated in a confidential manner and will be used exclusively for scientific purposes. If the information obtained is published, it will remain anonymous, that is, no data will appear with which they can identify you. The data collected will be accessible only by the research team during the development of the project. You can submit claims to the Catalan Data Protection Authority (apdcat.gencat.cat/ca/inici/) and make any queries you deem necessary to the Autonomous University of Barcelona (UAB) Data Protection Officer ([proteccio.dades@uab.cat](mailto:proteccio.dades@uab.cat)). If you wish, you will be able to learn about the results of this research through the dissemination activities planned in the BETTER COST Action project. This project has been evaluated by the Ethics Committee of the Autonomous University of Barcelona (CEEAH).

This survey has been approved by the Ethics committee of the Autonomous University of Barcelona – approval number CEEAH 6168

This survey is anonymous.

**\* I wish to participate:**

If you select "No", the survey will end.

Yes  
 No

**1 Identification of respondent**

---

\* Country in which you are working:

\* Which is your field (s) of work?

- Official control veterinary services (i.e., from the government)
- Private sector (e.g., veterinarian, agronomist, etc)
- Academia
- Other (specify):

\* Specify your field(s) of work

## 2 Mapping the diversity in applied biosecurity methods

State the name of the method to assess biosecurity (if any):

If the method has no name, type "none" or "unnamed".

In which animal category(ies) is it used?

Mandatory: If the method is a **legal obligation** in this/these animal category(ies).

Voluntary: Not a legal obligation but frequently carried out in this/these animal category(ies).

	Mandatory	Voluntary
Breeding pigs	<input type="checkbox"/>	<input type="checkbox"/>
Fattening pigs	<input type="checkbox"/>	<input type="checkbox"/>
Beef cattle	<input type="checkbox"/>	<input type="checkbox"/>
Dairy cattle	<input type="checkbox"/>	<input type="checkbox"/>
Veal calves	<input type="checkbox"/>	<input type="checkbox"/>
Mixed (beef and dairy)	<input type="checkbox"/>	<input type="checkbox"/>
Small ruminants	<input type="checkbox"/>	<input type="checkbox"/>
Pullets units	<input type="checkbox"/>	<input type="checkbox"/>
Layer units	<input type="checkbox"/>	<input type="checkbox"/>
Broilers	<input type="checkbox"/>	<input type="checkbox"/>
Breeders (turkeys, layers, broilers...)	<input type="checkbox"/>	<input type="checkbox"/>
Turkeys	<input type="checkbox"/>	<input type="checkbox"/>
Ducks	<input type="checkbox"/>	<input type="checkbox"/>

Other	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
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**Please specify the "Other animal category"**

--

**Open comment:**

--

**3 Main objectives details**

## What are the main objectives of the biosecurity assessment?

	Certification for quality assurance scheme	Compulsory audits for improving biosecurity	Voluntary assessment for improving biosecurity	Compulsory method focusing on a specific disease	Voluntary method focusing on a specific disease	To decrease antimicrobial use	Other
• Breeding pigs	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
• Fattening pigs	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
• Beef cattle	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
• Dairy cattle	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
• Veal calves	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
• Mixed (beef and dairy)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
• Small ruminants	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
• Pullet units	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
• Layer units	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
• Broilers	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
*							

5

Breeders (turkeys, layers, broilers...)	<input type="checkbox"/>						
• Turkeys	<input type="checkbox"/>						
• Ducks	<input type="checkbox"/>						
• Other animal categories (the same as you indicated above)	<input type="checkbox"/>						

6

Specify disease in each animal category if apply

Specify the reason (e.g., compliance of specific biosecurity measures, etc)

Specify other objective

Open comment:

#### 4 Biosecurity assessment method details

\* Who developed the biosecurity assessment method?

- University
- Veterinary associations
- Official veterinary services
- Producer associations
- Private company
- Other:

Specify other:

Open comment:

\* Does the End-User (e.g. veterinarian, farmer, etc) pay for using the biosecurity assessment method?

- Yes
- No
- Free for basic use
- Other:

Specify other:

**\* Is a protocol/instruction/guide/manual of the biosecurity assessment method available on a link? If possible, import the document.**

- Yes, please import
- Yes, but it is confidential
- No

**If possible, paste a link of the protocol/instruction/guide/manual of the biosecurity assessment method or attach it below:**

**Please upload your file(s) - Protocol/instruction/guide/manual of the biosecurity assessment method**

## 5 About the biosecurity assessment, please indicate

## Biosecurity in cattle production

In which production system(s) is it used and by whom?

	Official veterinary services or public health authorities (veterinarians or technicians)	Farm veterinary advisor	Researchers	Farm manager or farm worker	External auditor from industry or similar (for certification)	Private veterinarians or technicians paid by the official veterinary services for doing the assessment	Other
• Breeding pigs	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
• Fattening pigs	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
• Beef cattle	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
• Dairy cattle	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
• Veal calves	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
• Mixed (beef and dairy)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
• Small ruminants	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
• Pullet units	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
*							

9

Layer units	<input type="checkbox"/>						
• Broilers	<input type="checkbox"/>						
• Breeders (turkeys, layers, broilers...)	<input type="checkbox"/>						
• Turkeys	<input type="checkbox"/>						
• Ducks	<input type="checkbox"/>						
• Other animal categories (the same as you indicated above)	<input type="checkbox"/>						

10

Specify other:

Open comment:

**\* How is the biosecurity assessment conducted?**

- Remotely (e.g., telephone, email, postal survey, online survey...)
- Face-to-face without visiting the production units (e.g., just going to farm office)
- Observation on-site by direct inspection of production units
- Other

Specify other:

**6 (2) About the biosecurity assessment, please indicate**

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If observation on-site is included, how long does it usually take to perform the on-site visit? (Do not consider the duration of the travel to the holding, if applicable).

	<1 hour	1-2 hours	3-4 hours	>4 hours
* Breeding pigs	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
* Fattening pigs	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
* Beef cattle	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
* Dairy cattle	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
* Veal calves	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
* Mixed (beef and dairy)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
* Small ruminants	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
* Pullet units	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
* Layer units	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
* Broilers	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
* Breeders (turkeys, layers, broilers...)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
* Turkeys	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
* Ducks	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>

* Other animal categories (the same as you indicated above)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
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Open comment:

## 7 About the biosecurity assessment, please indicate (2)

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\* How is the data on biosecurity practices collected during the assessment?

- On paper
- With an app, website or similar
- Other

If you select an app, website or similar, please name it

Specify other:

---

\* How is the data stored/saved?

- Paper records
- Database

\* Who is being interviewed during the evaluation?

- Farm owner
- Farm manager
- Farm worker
- Farm veterinarian
- Farm veterinary advisor
- Private veterinarian
- Other

Specify other:

Open comment:

**\* Does the method to assess biosecurity include the collection of extra-data different from biosecurity practices?**

- Yes
- No

**\* If yes, what extra-data is usually collected in the different evaluations?**

- Environmental sampling such as Adenosine Triphosphate (ATP), colony forming unit (CFU) measures, etc.
- Data from national authority's databases (e.g., animal movements, location, etc)
- Inspection of farm records (e.g., animal movements, treatment protocols, antimicrobial use, health status, production, last water analysis, etc)
- Written protocols (e.g., SOPs, Standards, HACCP, etc)
- Animal welfare status
- Animal sampling
- Other(s)

Specify other(s)

Open comment:

**\* How is the farm biosecurity level derived from the data collected?**

- Scoring method based on weighting biosecurity measures or group of measures
- Probability estimates based on risk models, machine learning, etc
- None (it is only a descriptive evaluation)
- Do not know
- Other

Specify other(s)

Open comment:

**\* Is there a publication/report/manual of the method used to analyse the data (in case it applies) available on a website/paper/manual/etc? If possible, import the document**

- Yes, attach it below if possible
- Yes, but is confidential
- No

## Biosecurity in cattle production

There is no method of data analysis

If possible, paste a link of the publication/report/manual of the method used to analyse the data or attach it below:

Please upload your file(s) - Publication/report/manual of the method used to analyse the data

---

\* Is there feedback of the results to the producer/farmer?

Yes  
 No

\* If yes, how is this feedback provided?

Written report to the interested party with recommendations/suggestions on how to improve farm biosecurity  
 Verbal in-person or via telephone with recommendations/suggestions on how to improve farm biosecurity  
 Other

Specify other(s)

Open comment:

---

\* Is benchmarking (i.e., comparison in relation to other participating farms) done?

Yes, with individual biosecurity practices  
 Yes, at general aggregated level  
 Yes, at individual and general aggregated level  
 No  
 I do not know

\* Is the database or report with results available?

Yes  
 Yes, under certain conditions or request  
 No  
 I do not know

If possible, paste a link of the database/report or attach it below:

---

Please upload your file(s) - results database.

---

## Biosecurity in cattle production

Which institution(s) is/are responsible for storing and managing the data? *Leave blank if you do not know.*

	Institution	Website
1		
2		
3		

\* Do you routinely perform biosecurity assessment with this method in the field?

- Yes
- No
- Sometimes

General comment about the opinion on the method (e.g., easy to perform, practicality, represents correctly the level of biosecurity of the farm, etc):

## 8 COST ACTION BETTER

---

\* Do you want to provide your email contact to receive further feedback on the results and/or to become part of the network?

- Yes
- No

\* Email address

## 4.11.2 Supplementary material 2

*Table 1. S2. List of participating countries and the number of different methods to assess biosecurity on farm reported through the online survey between October and November 2023.*

<b>Country</b>	<b>No. of methods</b>
Spain	15
France	9
Netherlands	9
Ukraine	7
Argentina	4
Kosovo	4
Poland	4
Portugal	4
Sweden	4
Chile	3
Denmark	2
Finland	2
Serbia	2
Albania	1
Belgium	1
Bosnia and Herzegovina	1
Croatia	1
Estonia	1
Hungary	1
Ireland	1
Israel	1
Italy	1
Jordan	1
Mexico	1
North Macedonia	1
Slovenia	1
Tunisia	1
Venezuela	1

*Table 2.S2. Number of methods reported to assess biosecurity on farms by number of types of animal production for which the method was designed.*

No. of animal production type	N	No. of methods %
<b>One species</b>	61	82%
<b>Two species</b>	5	7%
<b>Three species</b>	8	11%
<b>Total</b>	74	100%

*Table 3. S2. Number of methods to assess biosecurity on farms (N=74) animal production type in 28 countries reported between October and November 2023. The reported N represents the number of unique methods received for each type of animal production, with the percentage (%) calculated within each respective production type.*

Animal production type	No. of methods (N=74)	
	N	%
<b>Pigs</b>	35	47%
<b>Ruminants</b>	27	36%
<b>Poultry</b>	33	45%

*Table 4.S2. Developers of the biosecurity assessment methods (BAMs) by animal production type in 28 countries reported between October and November 2023. The reported N represents the number of unique methods received for each type of animal production, with the percentage (%) calculated within each respective production type.*

Developer	Pigs N=35		Ruminants N=27		Poultry N=33	
	N	%	N	%	N	%
<b>Universities</b>	2	6%	2	7%	1	3%
<b>Veterinary associations</b>	2	6%	1	4%	1	3%
<b>Official veterinary services</b>	8	23%	9	33%	9	27%
<b>Producer associations</b>	2	6%	4	15%	7	21%
<b>Private companies</b>	12	34%	3	11%	7	21%
<b>Combinations of the above</b>	9	26%	8	30%	8	24%

*Table 5.S2. Implementation of the assessment to collect data from the farm by animal production type in 28 countries reported between October and November 2023. The reported N represents the number of responses received for each type of animal production, with the percentage (%) calculated within each respective production type.*

Implementation	Pigs N=45		Ruminants N=30		Poultry N=37	
	N	%	N	%	N	%
<b>On-site</b>	40	89%	30	100%	37	100%
<b>Without visit</b>	5	11%	0	0%	0	0%

*Table 6.S2. Duration of on-farm assessment by animal production type in 28 countries reported between October and November 2023. The reported N represents the number of responses received for each type of animal production, with the percentage (%) calculated within each respective production type.*

Duration	Pigs N=40 <sup>a</sup>		Ruminants N=30		Poultry N=37	
	N	%	N	%	N	%
<b>Up to 1 hour</b>	3	8%	6	20%	4	11%
<b>Up to 2 hours</b>	17	43%	15	50%	22	59%
<b>Up to 3 hours</b>	10	25%	4	13%	4	11%
<b>Up to 4 hours</b>	6	15%	2	7%	1	3%
<b>No data</b>	4	10%	3	10%	6	16%

<sup>a</sup>On-site assessments only.

*Table 7.S2. Data collection and data storage of the methods by animal production type in 28 countries reported between October and November 2023. The reported N represents the number of responses received for each type of animal production, with the percentage (%) calculated within each respective production type.*

	Pigs N=45		Ruminants N=30		Poultry N=37	
	N	%	N	%	N	%
<b>Data collection</b>						
<b>On paper</b>	23	51%	18	60%	20	54%
<b>With an app, website or similar</b>	17	38%	6	20%	12	32%
<b>On paper and / or an app or similar</b>	5	11%	5	17%	3	8%
<b>Other</b>			1	3%	2	5%
<b>Data storage</b>						
<b>Paper records</b>	7	16%	7	23%	6	16%
<b>Database</b>	25	56%	11	37%	17	46%
<b>Paper records &amp; database</b>	13	29%	12	40%	14	38%

*Table 8.S2. Respondents who can respond to the on-farm assessment by animal production type in 28 countries reported between October and November 2023. The reported N represents the number of responses received for each type of animal production, with the percentage (%) calculated within each respective production type.*

Respondent	Pigs N=45		Ruminants N=30		Poultry N=37	
	N	%	N	%	N	%
<b>Farm owner</b>	20	44%	20	63%	26	67%
<b>Farm manager</b>	41	77%	25	78%	32	82%
<b>Farm worker</b>	18	34%	13	41%	16	41%
<b>Farm veterinarian</b>	26	49%	11	34%	12	31%
<b>Farm veterinarian advisor</b>	5	9%	2	6%	4	10%
<b>Private veterinarian</b>	5	9%	3	9%	3	8%
<b>Other respondent</b>	1	2%	0	0%	1	3%

*Table 9.S2. Outputs of the different of the biosecurity assessment methods (BAMs) by animal production type in 28 countries reported between October and November 2023. The reported N represents the number of unique methods received for each type of animal production, with the percentage (%) calculated within each respective production type.*

Output	Pigs N=35		Ruminants N=27		Poultry N=33	
	N	%	N	%	N	%
<b>Descriptive output</b>	14	40%	15	56%	19	58%
<b>Scoring based on weighting biosecurity measures</b>	19	54%	12	44%	13	39%
<b>Probability estimates (e.g., based on risk models or machine learning)</b>	1	3%			1	3%
<b>Other methods of calculation <sup>a</sup></b>	1	3%				

<sup>a</sup> KPIs related to antibiotic use.

*Table 10.S2. Benchmarking provided by the method by animal production type in 28 countries reported between October and November 2023. The reported N represents the number of responses received for each type of animal production, with the percentage (%) calculated within each respective production type.*

Benchmarking	Pigs N=45		Ruminants N=30		Poultry N=37	
	N	%	N	%	N	%
<b>Do not know</b>	2	4%	4	13%	2	5%
<b>No</b>	14	31%	14	47%	17	46%
<b>At general or individual level</b>	29	64%	12	40%	18	49%

*Figure 1.S2. Bar plot of the explained variance by dimension obtained by multiple correspondence analysis.*

*Four dimensions were retained, determined by the number of dimensions yielding the lowest mean square error of prediction (MSEP), collectively explaining 54.3% of the cumulative variance.*

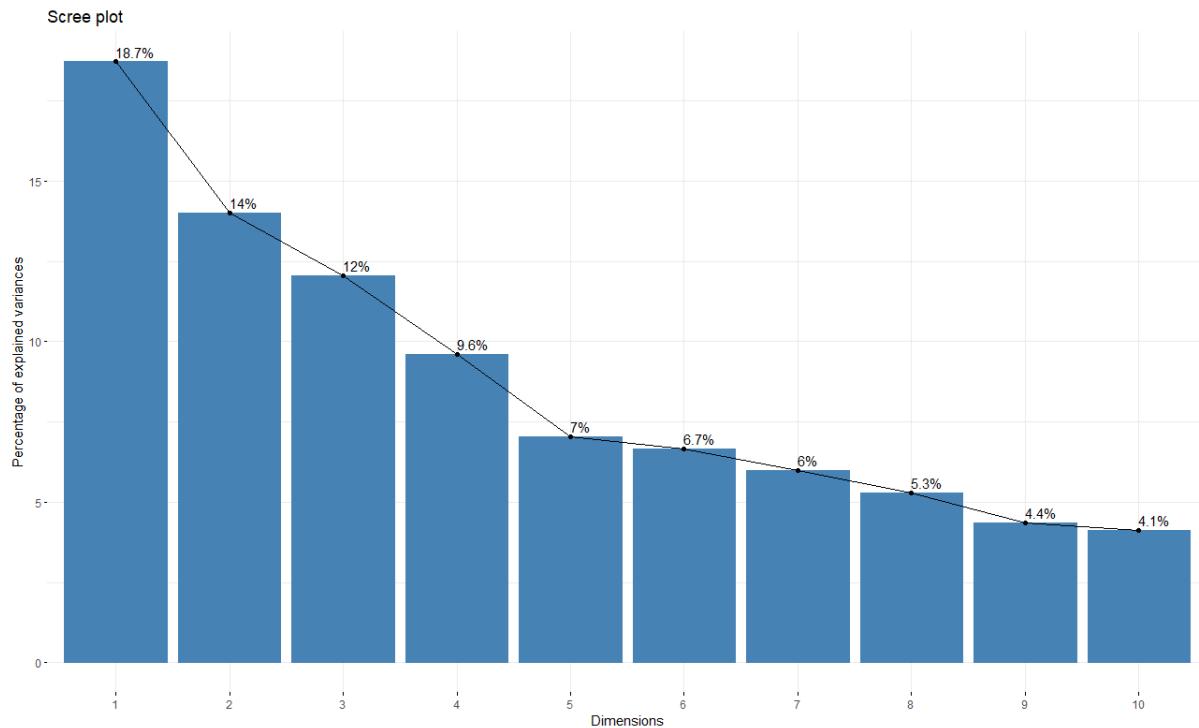
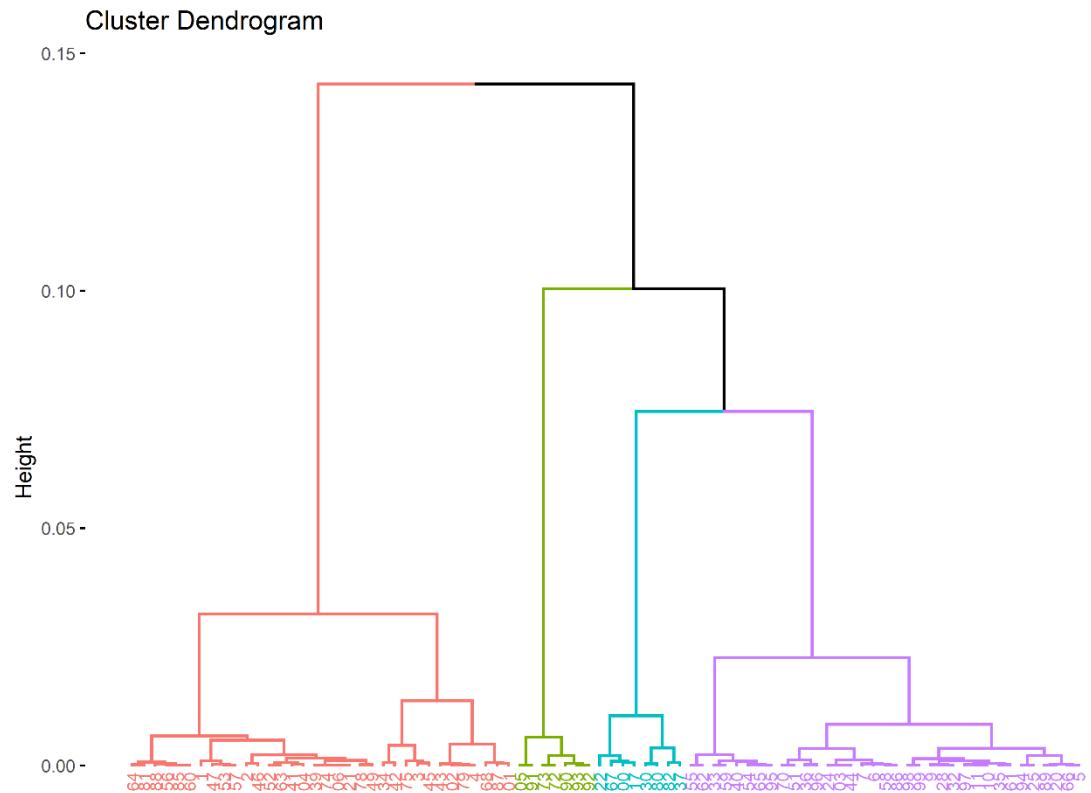


Figure 2. S2. Dendrogram of the Hierarchical Clustering on Principal Components.

Dendrogram illustrating the results of the Hierarchical Clustering on Principal Components (HCPC) analysis. The x-axis displays the various BAMs received, with the initial partitioning determined by cutting the dendrogram according to inertia gains between partitions. The hierarchical classification identified four distinct clusters.





## 5 Study III: Estimate the most cost-effective option for improving biosecurity on dairy cattle farms to support informed decision making

This manuscript has been submitted to *Preventive Veterinary Medicine* for publication as original research

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## 5.1 Abstract

The application of farm biosecurity measures depends on experience, risk perception, social pressure, epidemiological factors, as well as economic profitability. The objective of the study was to estimate the most profitable decision for the farm to improve biosecurity.

A cost calculator was developed to estimate the expenses associated with various biosecurity measures. This calculator was fed by a database created with prices of items that made up the biosecurity measures. To estimate the probability of pathogen introduction a quantitative risk assessment model was used. The cost of an outbreak was estimated using the dynamic stochastic model developed by SimHerd. Bovine Viral Diarrhoea (BVD) was selected as the case study due to its well-documented impact on dairy cattle. This methodology was tested on three dairy farms in northeastern Spain, where biosecurity and production data were collected.

The annual biosecurity cost per cow currently implemented on the farm was €27.49, €44.50, and €71.94 for farms A, B, and C, respectively. The risk of BVD introduction under the existing biosecurity measures was estimated at 2.66%, 15.70%, and 0.36% for farms A, B, and C, respectively. In the outbreak scenario, the average farm gross margin was reduced by approximately 6% over the first five years. Given the specific context of each farm, the most cost-effective measure across all three was “providing boots to cattle drivers”.

Using this methodology, it was possible to identify which biosecurity measure offered the best combination of risk reduction and cost efficiency. These findings may encourage farmers to adopt biosecurity practices by clearly showing both the economic and epidemiological benefits.

## 5.2 Introduction

The introduction of new pathogens in a dairy cattle farm can impact animal health and welfare while also posing significant economic consequences for the farm. As a matter of fact, diseases such as neosporosis, paratuberculosis, bovine viral diarrhoea or infectious bovine rhinotracheitis, among others, can have substantial effects on farm's finances (Garcia and Shalloo, 2015; Iscaro et al., 2021; Reichel et al., 2013; Stott et al., 2003). In this context, the implementation of measures aimed at preventing the transmission of pathogens, referred to as biosecurity (Saegerman et al., 2023), plays a key role in reducing the impact of disease outbreaks (De Vos et al., 2005), while also improving animal health and reducing the need for antimicrobial treatments (Plummer and Fajt, 2025).

However, the implementation of biosecurity in animal farms can be challenging as it depends on several factors, including the farmer's previous experience, risk perception, social pressure and epidemiological aspects, among others (Moya et al., 2020; Oliveira et al., 2018). In addition, the cost of implementing or improving existing biosecurity measures have been highlighted as an important barrier, as farm resources are limited and making optimal resource allocation is crucial (Amenu et al., 2023; Rushton, 2008). However, the cost of biosecurity might not be perceived as a barrier when considered in relation to its expected benefits (Renault et al., 2021a; Souillard et al., 2024). Therefore, estimating the cost-benefit of biosecurity is of paramount importance to enhance biosecurity adoption and contribute to effective communication by the farm advisor (Amenu et al., 2023; E Kristensen and Jakobsen, 2011; Moya et al., 2020; Rushton, 2008). Nevertheless, and despite its importance, there is limited research on this area. Some studies have provided valuable insights by estimating the cost of specific measures across different livestock systems, including swine farms (Fasina et al., 2012b), cattle farms (Fountain et al., 2022; Han et al., 2020), and poultry production (Siekkinen et al., 2012). These studies have shown

the economic advantages of biosecurity, but they tend to focus on a limited set of interventions and on species or production systems other than dairy cattle.

Making informed decisions on farm biosecurity requires access to comprehensive information about the risk of introduction of different pathogens, preventive measures that can be implemented to reduce such risk and their expected benefits (Ellis-Iversen et al., 2010; Valeeva et al., 2011). Therefore, the objective of this study was to evaluate the most cost-effective biosecurity strategy for dairy cattle farms by applying an integrated, farm-level analysis that combines biosecurity implementation costs, cost of disease introduction, and the risk of pathogen introduction into a comprehensive economic evaluation. The framework is designed to support informed decision-making and to encourage the adoption of biosecurity measures by farmers and their veterinarians, by showing both the economic benefits and the potential for risk reduction. A key component of this framework is the newly developed Biosecurity Cost Calculator, which estimates the costs associated with implementing biosecurity measures. Both the framework and the tool are described in detail in this article.

### 5.3 Material and methods

To identify the most cost-effective biosecurity strategy on a farm, a decision analysis methodology was applied. The analysis incorporated three main inputs: i) the estimated cost of farm-level biosecurity implementation, ii) the potential economic loss from a disease outbreak, and iii) the assessed risk of introduction of a pathogen.

To achieve this, a new tool, biosecurity cost calculator, was developed and its outputs were combined with those from two existing models, *SimHerd* and *FarmRisk*, within a decision tree analysis framework. This integrated approach enabled the economic evaluation of the current farm situation and comparison of alternative biosecurity scenarios.

To show the practical application and validate the proposed approach, Bovine Viral Diarrhoea (BVD) was selected as the target disease, and data were gathered from three dairy farms in Catalonia. The following subsections describe each component of the analysis in detail.

### 5.3.1 Estimation of biosecurity cost

A biosecurity cost calculator, based on a deterministic model, was developed using Excel.

The cost calculator was structured around different pathogen entry routes (i.e., pathway of introduction). These routes included: (i) animal introduction, (ii) farm access by visitors and vehicles, (iii) Feed storage and water treatment, and (iv) pest control.

For each route, different biosecurity measures were considered. For the animal introduction route, these included quarantine protocols for incoming animals, quarantine facilities and management, exclusive farm transport (i.e. vehicles transporting only animals destined for that specific farm), and disease testing. The farm access route covered perimeter fencing, vehicle and visitor control measures (e.g. vehicle disinfection arches, provision of dedicated boots and clothing for drivers and visitors), on-farm mortality management, and hygiene protocols for farm workers. The water and feed route addressed feed storage and the treatment of drinking water to ensure potability. Finally, the pest control route encompassed the management of rodents, flying insects, and crawling insects within the farm environment.

Using these four routes, an inventory of specific biosecurity measures typically employed on dairy farms was compiled. A summary of the biosecurity measures included in each route is presented in Table 1.

To estimate the cost of applying each listed biosecurity measure, the calculator utilized inputs from three main data sources: (1) a price database, (2) fixed parameters, and (3) data collected from farms. The first two sources were pre-defined within the tool, meaning users were not required to modify or input this information. To operate the calculator, users simply needed to

provide the relevant information about their farm through a standardised questionnaire included in the calculator tool. The full survey is available in Supplementary material 1, Figure 1.S1.

*Table 1. Biosecurity measures by pathway of introduction included in the cost calculator.*

Pathway of introduction	Biosecurity measure	Number of farm-level inputs <sup>a</sup>
<b>Animal introduction</b>	Quarantine	16
	Transport of incoming animals	13
	Diagnostic testing for incoming animals	8
<b>Farm access by visitors and vehicles</b>	Farm perimeter fencing	19
	Visitor control	6
	Transport of outgoing animals	27
	Mortality management	3
	Worker hygiene protocols	3
<b>Feed storage and water treatment</b>	Feed storage	7
	Drinking water treatment	2
<b>Pest control</b>	Rodent control	4
	Flying and crawling insect control	9

<sup>a</sup> Farm-level inputs refer to the number of parameters required for the cost estimation of the corresponding biosecurity measure gathered through the farm questionnaire.

i) Price database

After listing the biosecurity measures, each was broken down into specific cost components (e.g. materials, equipment, and labour time). This process resulted in the identification of 75 cost items, which formed the basis of the price database (full list available in Supplementary material 1, Table 1.S1). Multiple items could correspond to a single biosecurity measure, offering users the flexibility to select the option(s) most aligned with their specific context. For each item, between one and three price points were obtained from public sources such as supplier websites. Only suppliers operating within or distributing to Spain were considered. The final unit cost was calculated as the average of the collected prices. All values were expressed in euros. Price sources and update dates were documented in the price database.

ii) Fixed parameters

The fixed parameters used for cost estimation were derived from literature, good practice guidelines, and consultations with service providers. These parameters included: the time required for veterinarians and farm workers to sample incoming animals, time for changing clothing and footwear, time for pest trap inspections, water consumption per animal, dosage and quantity of disinfectants used, and transport costs for animals.

iii) Farm-specific data.

A questionnaire was embedded within one of the Excel worksheets (Supplementary Material 1, Figure 1.S1) to gather general farm information along with details relevant to the four pathways of pathogen introduction described above. Each section included a series of questions necessary for cost estimation. General farm data included the number of workers, wages, working hours, and herd size, allowing for a more accurate estimation of costs. The overall number of items collected by pathway is shown in Table 1, and the full list of parameters can be found in Supplementary Material 1, Table 2.S1.

iv) Integration of data

The integration of inputs from the three data sources (i.e., prices, fixed parameters and farm-specific data) is performed in a separate Excel worksheet. Biosecurity costs were calculated in two ways: first, by considering the total investment in biosecurity measures, and second, by accounting for the useful life of the components, thereby obtaining an annualized cost. This approach allowed for the estimation of the total biosecurity cost implemented on the farm, the annualized biosecurity cost, and, based on the number of lactating and dry cows, the annualized cost per cow. Results were presented both in aggregate and disaggregated by entry route.

Additionally, the calculator allowed for the inclusion of additional biosecurity measures beyond those already implemented on the farm. These additional costs were also calculated as total and

annualized costs. A new summary of results was generated, presenting the updated biosecurity costs for different scenarios, both in aggregate and in detail across the same sections mentioned above.

### 5.3.2 Estimating the cost of a disease outbreak

To estimate the cost of a disease outbreak, the model developed by SimHerd (Østergaard et al., 2005) was used. SimHerd is a mechanistic, dynamic, and stochastic model of a dairy herd including youngstock. It has been used in several scientific studies to estimate the cost of changes in production management and production-related diseases (Clasen et al., 2024; Ettema et al., 2017; Østergaard et al., 2005).

Reductions in productivity and health parameters resulting from a BVD outbreak were obtained from the literature. Specifically, the parameters considered in the cost estimation included an increase in cow-level risk of mastitis, a decrease in conception rate, and a reduction in milk yield. The parameters used for estimating the cost of a BVD outbreak are presented in Table 2.

*Table 2. Proportional changes in cow-level parameters due to BVD outbreak used for herd-level cost estimation.*

Parameter	Proportional change (%)	Reference
Mastitis risk	7.1% (+)	(Waage, 2000)
Conception rate	26.0% (-) <sup>a</sup>	(Han et al., 2020; Houe et al., 1993)
Milk yield	2.1% (-) <sup>a</sup>	(Tschoop et al., 2017; Yue et al., 2021)

<sup>a</sup> Mean value of the parameter change reported in literature.

The parameter changes were applied to the production data of the farms included in the study using SimHerd. In addition, to avoid double counting of losses, the effects of increased mastitis risk and reduced conception rate were each simulated separately to quantify their respective impacts on milk yield. It was concluded that the observed reduction in milk yield partially accounted for the 2.1% decrease shown in Table 2. Accordingly, the assumed reduction in milk

yield was adjusted to reflect the mediating effects of mastitis and conception rate. Based on this, the contribution of each parameter was discounted to derive the reported mean value for the change in parameters.

The main economic output of SimHerd is the Gross Margin (GM). It is the difference between revenues from milk, cows for slaughter and livestock sales on the one side, and variable expenses of feed, breeding, veterinary costs and other variable costs like bedding. As a result, the GM of the farm under current conditions was compared to the GM in a scenario where the modified parameters reflected a BVD outbreak. All monetary values were expressed in euros.

### 5.3.3 Estimating the probability of BVD introduction

A quantitative risk analysis model was used to estimate the probability of BVD introduction into the studied farms (Ciria et al., 2024). The model estimated the risk of diseases (BVD and other pathogens) introduction through different pathways using Monte Carlo simulations, accounting for parameter uncertainty. Additionally, the model allowed for the prioritization of biosecurity measures based on their effectiveness reducing the risk of introduction.

The model's output included both the annual probability of BVD introduction at a global level and the probability associated with specific entry pathways: i) animal movements, including purchases, external rearing, pastures and animal markets, ii) vehicle and personnel entering the farm, and iii) proximity to neighbouring farms.

### 5.3.4 Estimating the cost-effectiveness of farm biosecurity

Economic evaluation was conducted using decision analysis methodology with decision trees. This approach was chosen because it considers multiple scenarios, the potential cost of each node, and its respective probability of occurrence (Huirne and Dijkhuizen, 1997; Rushton, 2008). The expected monetary value (EMV) was calculated for each branch of the decision tree and compared among the branches, with the option yielding the highest EMV being selected.

Each branch of the tree (Figure 1) represented the probability of BVD introduction based on the biosecurity measures implemented on the farm.

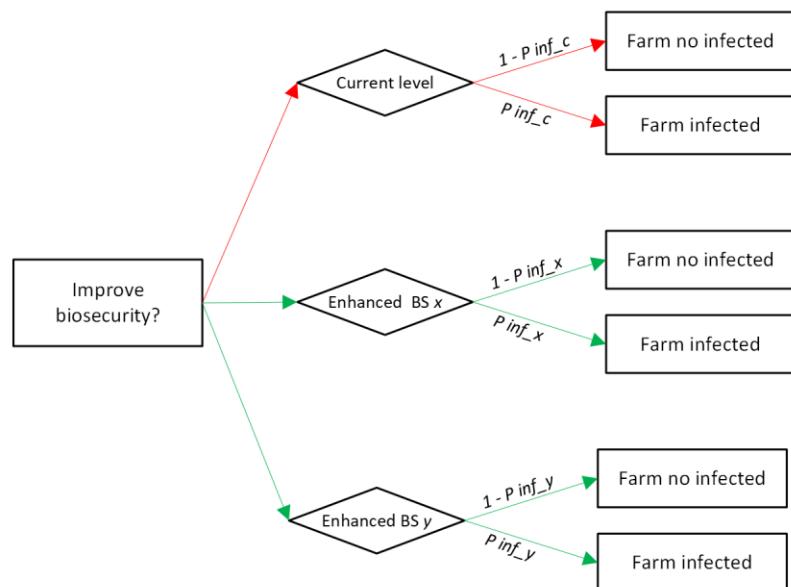
The EMV of each scenario was calculated as:

$$EMV_{(i)} = Pinf_{(i)} * [Cost\_EBS_{(i)} + Cost\_outbreak] + (1 - Pinf_{(i)}) * Cost\_EBS_{(i)}$$

Where:

- “i” is a subscript indicating each scenario (i.e., the implementation of each selected biosecurity measure).
- $Pinf_{(i)}$ : probability of BVD introduction under each scenario “i”.
- $Cost\_EBS_{(i)}$ : Cost of biosecurity if implementing the selected biosecurity measure.
- $Cost\_outbreak$ : Cost of Bovine Viral Diarrhoea outbreak at farm level.

*Figure 1. Decision tree used to assess the cost-benefit of biosecurity implementation in dairy cattle farms. Three biosecurity scenarios were considered: the farm’s current level ("Current level") and two enhanced biosecurity strategies, referred to as "Enhanced BS x" and "Enhanced BS y". The probability of infection depends on the level of biosecurity in place. For the current level, the probability of infection is denoted as  $Pinf_c$ , and the probability of remaining infection-free is  $1 - Pinf_c$ . The same logic applies to the enhanced scenarios, using  $Pinf_x$  and  $Pinf_y$  respectively.*



#### *5.3.4.1 Sensitivity analysis*

A sensitivity analysis of the decision analysis model was conducted by varying the three main input parameters using the data from one of the farms described in the next section (Farm A). First, the probability of BVD infection was progressively increased up to 50% for the "current situation" branch of the decision tree. Correspondingly, the relative reduction in the probability of BVD introduction associated with each additional biosecurity measure was adjusted proportionally (details on the proportional reduction are shown in Supplementary Material 3, Table 4.S3). Second, the cost of a BVD outbreak was progressively increased, up to twice the initially estimated outbreak cost, in 10% increments. Similarly, the on-farm biosecurity implementation cost was increased in steps of 10% up to a maximum of double the original estimate. For each change in the parameters, the difference in Expected Monetary Values (EMVs) between the current situation and each biosecurity improvement strategy was calculated and compared, in order to assess the impact of each parameter on the model's outcomes.

#### *5.3.4.2 Data collection*

Through the professional network of one of the authors, dairy farms willing to participate voluntarily in the study were sought. To facilitate subsequent estimations, it was required that the farms be negative for BVD. Three dairy cattle farms were visited between October and November 2024. These farms were located in Catalonia, Spain (northeastern Iberian Peninsula).

During these visits, production and economic data together with biosecurity practices implemented in the farm needed to feed the risk assessment model, the biosecurity cost calculator and the risk analysis model was collected. Details of the farms included in the study are provided in Table 3.

*Table 3. General description of the three visited dairy farms in Catalonia between October and November 2024.*

<b>Variable</b>	<b>Farm A</b>	<b>Farm B</b>	<b>Farm C</b>
Number of cows	724	247	79
Kg ECM per cow-year	13,141	13,602	9,567
Number of calvings	646	214	60
Cow mortality	5%	11%	9%
Mastitis incidence	60%	20%	26%
Conception rate - cows	36%	49%	31%
Conception rate - heifers	41%	43%	24%

## 5.4 Results

### 5.4.1 Farm biosecurity cost

The annual biosecurity cost per cow currently implemented on the farm was €27.49, €44.50, and €71.94 for farms A, B, and C, respectively. The section with the highest annual expenditure for farms A and C was pest control. In contrast, for farm B, which had a quarantine system in place, the highest annual expenditure was related to the introduction of animals. The difference between the total biosecurity cost and the amortised cost, ranging from 56% to 87% of the total, was primarily attributed to measures involving substantial initial investments, such as farm fencing, access control, and feed storage infrastructure, which typically have long useful lifespans, ranging from 10 to 20 years. More details on the current cost of biosecurity in Table 4.

*Table 4. Cost of implemented biosecurity in the three dairy farms studied. The current biosecurity cost is presented both in aggregate form and by section.*

	Farm A	Farm B	Farm C
<b>Current biosecurity cost</b>			
Total cost	74,680 €	25,238 €	42,481 €
Amortized cost	19,969 €	11,015 €	5,697 €
Amortized cost per cow <sup>a</sup>	27.58 €	44.59 €	72.11 €
<b>Biosecurity section (with amortization)</b>			
Animal introduction	2,880 €	4,524 €	- €
Farm access by visitors and vehicles	3,128 €	461 €	1,583 €
Feed storage and water treatment	5,493 €	3,706 €	1,711 €
Pest control	8,467 €	2,325 €	2,403 €

<sup>a</sup>Both lactating and dry cows were considered.

#### 5.4.2 Risk of BVD introduction and cost of an outbreak

The risk of BVD introduction under the currently implemented biosecurity measures was estimated at 2.66% (95% CI: 0.99–5.67), 15.70% (95% CI: 10.1–21.5), and 0.36% (95% CI: 0.15–0.73) for farms A, B, and C, respectively. The highest-risk introduction pathways were those associated with vehicles and visitors to the farms. Based on these findings, the model's recommendations focused on mitigating risks related to these specific pathways.

The cost of a potential BVD outbreak was calculated for each of the three farms, using the average from the first five years of the simulation. The outbreak scenario led to a reduction in the average GM over the first five years of -6.0%, -5.7%, and -5.9% for farms A, B, and C, respectively. Further details on the calculations obtained from SimHerd are provided in Supplementary material 2 (Tables 1.S2 to 4.S2).

Detailed results on the risk of BVD introduction and outbreak costs are presented in Table 5.

*Table 5. Risk of BVD introduction under different biosecurity scenarios and farm-level costs of a potential BVD outbreak.*

Farm	Scenarios	Probability of introduction of BVD (%) <sup>a</sup>	BVD outbreak cost at farm level
<b>Farm A</b>	Current level	2.66 (0.99–5.67)	
	Provide boots to all drivers	1.55 (0.58–3.31)	
	Provide clothing to visitors	2.54 (0.94–5.41)	-153,871 €
	Provide boots to all visitors	2.55 (0.95–5.43)	
<b>Farm B</b>	Current level	15.7 (10.1–21.5)	
	Exclusive animal transport	3.67 (2.36–5.03)	
	Provide boots to all drivers	14.4 (9.27–19.73)	-46,386 €
	Provide clothing to visitors	15.57 (10.02–21.32)	
<b>Farm C</b>	Current level	0.36 (0.15–0.73)	
	Provide boots to all drivers	0.22 (0.09–0.45)	
	Provide boots to all visitors	0.33 (0.14–0.67)	-8,977 €
	Provide clothing to visitors	0.33 (0.14–0.67)	

<sup>a</sup>Monte Carlo simulations are provided as median (50<sup>th</sup> percentile) with 95% confidence interval.

#### 5.4.3 Decision analysis on biosecurity improvements

Decision trees were constructed for each farm to evaluate the most cost-effective biosecurity improvements. The analysis compared the expected monetary values (EMVs) of the current biosecurity practices with scenarios in which risk was reduced through the implementation of additional measures recommended by the risk analysis model. Table 6 summarises the differences in EMVs across all branches of the decision trees for the three farms included in the study.

For Farm A, the recommended measures included: providing boots for all drivers entering the animal enclosures; supplying farm-specific clothing to visitors in contact with animals; and providing boots for those visitors. Among these, the most cost-effective option (i.e., the one associated with the greatest reduction in losses) was supplying boots to drivers, resulting in an EMV improvement of €1,680.

Using the same approach, new EMVs were calculated for Farms B and C, considering three proposed biosecurity measures alongside the current practices. For Farm B, the most cost-

effective intervention was again the provision of boots to drivers, yielding an EMV gain of €576. In contrast, for Farm C, none of the suggested measures offered a cost-effective improvement over the biosecurity measures already in place. The full details of the calculated pay-off tables can be found in Supplementary material 3 (Tables 1.S3 to 3.S3).

*Table 6. Summary of the three pay-off table resulting from the decision analysis for the three farms in the study.*

Farm	Scenario	Risk reduction	Differences in EMV relative to the current biosecurity level <sup>a</sup>	
			Absolute change in EMV (€)	Relative change in EMV (%)
<b>Farm A</b>	Provide boots to all drivers <sup>b</sup>	41,6%	1,680 (608; 3,607)	+7.0%
	Provide clothing to visitors	4,6%	162 (44; 375)	+0.7%
	Provide boots to all visitors	4,3%	152 (41; 351)	+0.6%
<b>Farm B</b>	No shared transport	76,6%	381 (-1,609; 2,442)	+2.1%
	Provide boots to all drivers <sup>b</sup>	8,3%	576 (362; 798)	+3.1%
	Provide clothing to visitors	0,8%	33 (12; 55)	+0.2%
<b>Farm C</b>	Provide boots to all drivers	38,2%	0 (-7; 13)	0%
	Provide boots to all visitors	8,9%	-22 (-23; -19)	-0.4%
	Provide clothing to visitors	8,6%	-24 (-25; -21)	-0.4%

<sup>a</sup>The values presented correspond to the difference in EMV compared to the current biosecurity level, considering the average, minimum, and maximum risk of BVD introduction on the farm calculated by the risk analysis model.

<sup>b</sup> Most cost-effective biosecurity improvement option for the farm.

#### 5.4.3.1 Sensitivity analysis

The decision tree model showed consistent results across the tested input ranges. Despite progressive increases in the three key input parameters, probability of BVD infection, cost of a BVD outbreak, and cost of implementing biosecurity, the optimal decision (i.e., the scenario with the highest Expected Monetary Value, EMV) remained unchanged for the farm. Specifically, the strategy of providing boots to drivers consistently yielded the highest EMV across all sensitivity scenarios.

When the probability of BVD introduction was increased to its upper limit (50%), the EMV of the “Provide boots to all drivers” strategy was 33% higher than that of the current situation. The second-best option, providing protective clothing to visitors, showed a 3.6% improvement in EMV compared to the current scenario, indicating that both measures would be economically beneficial under increased risk. Regarding the cost of a BVD outbreak, doubling the outbreak cost resulted in a 12% higher EMV for the “Provide boots to all drivers” strategy compared to the current situation. This reflects a 5-percentage point increase from the original difference of 7% under baseline outbreak costs. Similarly, when the cost of implementing biosecurity measures was doubled, the EMV of the “Provide boots to all drivers” strategy remained higher than that of the current scenario, although the difference was reduced to 3.8%. This represents a decrease of 3.2-percentage points compared to the initial 7% advantage under the original biosecurity cost.

These results show that, even under less favourable economic conditions, the strategy of providing boots to drivers remains the most cost-effective option among those evaluated. Detailed results of the sensitivity analysis are presented in Supplementary material 3 (Tables 4.S3 to 6.S3).

## 5.5 Discussion

This study highlights the economic benefits of implementing biosecurity measures, particularly in reducing the risk of BVD introduction. BVD was selected as a model disease in this study to estimate the cost of an outbreak, due to the availability of robust quantitative data on its impact on productive parameters in dairy cattle (Han et al., 2020; Houe, 1993; Tschopp et al., 2017; Waage, 2000; Yue et al., 2021).

As emphasised in previous research, making these benefits more visible is crucial to encouraging the adoption of biosecurity practices by farmers, who often struggle to perceive a clear return on investment (Moya et al., 2020; Renault et al., 2020). This type of information can also support

veterinarians in advising, motivating, and educating farmers, as they are frequently regarded as one of the most trusted and influential sources of information (Kuster et al., 2015b; Laanen et al., 2014).

While existing literature typically approaches this topic through case studies, to the authors' knowledge, there are no available tools that integrate farm-level productive and economic aspects, pathogen introduction risk, outbreak impact, and the cost or investment in biosecurity. The tool developed in this study attempts to fill that gap. Although it is based on simulation models that involve a degree of uncertainty and may not fully reflect real farm conditions (Allepuz et al., 2018; Lewerin et al., 2015; Pouillot and Delignette-Muller, 2010), the results provide a valuable foundation for reflecting on the benefits of biosecurity at the farm level and might serve as a useful educational resource.

It is important to note that the parameters used to calibrate the model are specific to each farm and its local context. This feature enhances the adaptability of the tool, allowing it to be used in other countries or production systems. Moreover, the model's modular structure allows users to focus on specific components of interest, without requiring the use of the full process. Moreover, the use of decision analysis as a framework for identifying the most cost-effective biosecurity strategy emerges as a quick and accessible way to obtain results, and it has also been successfully applied in previous studies on farm animal health (Berry et al., 2004; Pinzón-Sánchez et al., 2011).

In the case of the decision tree results, it was observed that lower-cost biosecurity measures, such as providing farm-specific boots or clothing, yielded the most cost-effective outcomes for the specific context of the farms. For Farm B, although hiring exclusive transport was the top recommended measure in terms of risk reduction (with an estimated 77% decrease in the risk of BVD introduction), its high cost made it less cost-effective. As a result, providing boots to drivers ranked higher in terms of expected monetary value (EMV), making it the preferred strategy from a

cost-effectiveness perspective. It is also important to highlight that both the biosecurity cost estimation and the risk analysis model assume that the recommended biosecurity measures are properly implemented. For instance, in this case, the recommendation to provide boots is accompanied by the assumption that they are used correctly to achieve the intended level of effectiveness.

Despite the ability to show farmers and advisors the potential economic benefits of implementing biosecurity measures, it is important to recognize that economic factors are not the sole drivers of decision-making. Other barriers may limit the adoption of improved biosecurity practices, such as practicality or the farmer's personal beliefs (Damiaans et al., 2018; Moya et al., 2020; Oliveira et al., 2018). In some contexts, the economic rationale may be sufficient to trigger implementation, but in others, additional factors must be considered, which is a limitation of this study.

The cost of biosecurity among the farms included in the study varied significantly, ranging from €27.6 to €72.1 per cow (amortized cost). Notably, the smallest farm had the highest cost per cow, indicating a greater economic effort required to achieve a given level of biosecurity. To improve biosecurity levels in farms with lower financial capacity, it may be valuable to explore support mechanisms such as subsidies for the implementation of biosecurity measures, or incentives aimed at improving biosecurity standards (Can and Altuğ, 2014b; Erling Kristensen and Jakobsen, 2011b). Additionally, comparing the cost of biosecurity implementation across farms is challenging, as the existing literature estimates the cost of different biosecurity measures under varying geographic and production contexts (Fountain et al., 2022; Smith et al., 2014).

For the purposes of this study, only the risk of BVD introduction and the potential cost of a BVD outbreak were estimated. The estimated economic impact of a BVD outbreak per cow ranged from €114 to €213, considering increased mastitis risk, reduced conception rates, and decreased milk production. These values fall within the range reported in a review on the economic impact

of BVD (Richter et al., 2017). Furthermore, biosecurity measures implemented on a farm can also help prevent the introduction and spread of other endemic and exotic diseases (Sibley, 2010; Wells, 2000). Therefore, the benefits observed here could be even greater if the model were extended to include additional pathogens. In some cases, biosecurity measures suggested by the model were not more cost-effective than the current practices, likely due to the specific production context or disease impact at the farm. However, if additional diseases were considered, the differences in expected monetary values (EMVs) between strategies would likely become more significant.

The biosecurity cost calculator used in this study does not currently include certain structural or management-related measures, due to the difficulty and uncertainty in quantifying them. Furthermore, some biosecurity items included in the calculator also serve broader purposes related to food production quality (e.g., silage storage or water sanitisation), beyond strictly biosecurity concerns (Pinto Jimenez et al., 2023). Most of the measures considered fall under the category of external biosecurity. Therefore, future improvements to the tool should aim to incorporate internal biosecurity measures and assess their impact on within-farm pathogen transmission. Lastly, the items included in the biosecurity cost calculator need to be updated over time or if applied to other geographical areas (i.e., only prices from suppliers operating in Spain were included). However, the structure of the tool allows adjusting prices and materials as needed, and future improvement could explore automatic update of prices.

Future research should focus on improving the estimation of economic benefits from biosecurity investments at the farm level, and on exploring the impact of communicating the economic benefits and effectiveness of biosecurity measures to farmers.

## 5.6 Conclusions

The proposed methodology enabled the generation of a ranking of biosecurity measures based on their cost-effectiveness, allowing the identification of the most cost-effective measures within the specific risk context of each farm. The tool developed in this study, along with the results obtained, will support farmers and their advisors in increasing awareness about the benefits of investing in farm biosecurity, by clearly showing the advantages of reducing the risk of disease introduction.

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## 5.8 Financial disclosure statement

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## 5.9 Ethics statement

This work has been approved by the Ethics Committee of the Autonomous University of Barcelona – approval number CEEAH 6188.

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## 5.11 Supplementary material

### 5.11.1 Supplementary material 1: Cost estimation of biosecurity measures.

Figure 1.S1. Survey used for data collection.

INTENSIVE DAIRY FARM	
Survey date: <input type="text"/>	
General farm information	
<b>Question</b>	<b>Specify</b>
Farm name	
Farm ID	
Number of lactating cows	
Number of dry cows	
Number of heifers	
Total herd size	
Number of farm workers (including the owner if working on the farm)	
Worker wage (per hour)	
Veterinarian wage (per hour)	
Animal introduction	
<b>Question</b>	<b>Specify</b>
Number of animal entries (batches per year)	
Number of animals entering per year (external source)	
Does the farm have a quarantine facility?	
Is the quarantine area within the farm perimeter?	
Duration of quarantine (days)	
Perimeter of quarantine area (m)	
Frequency of access to quarantine area (daily)	
Time spent daily in the quarantine pen (hours)	
Number of workers entering the quarantine area	
Farm characteristics	
<b>Type of fencing around quarantine (single or mixed)</b>	<b>Implemented on the farm?</b>
Concrete block wall, 2.5 m	
Brick wall, 2.5 m	
Reinforced concrete wall, 2.5 m	
Chain-link mesh, 2 m (3 cm mesh spacing)	
Proportion (totals 100%)	
<b>Is there double fencing around the quarantine area?</b>	
<b>Entrance to the corral with clean footwear</b>	
Dedicated boots for quarantine	
Dedicated overalls for quarantine	
Footbath with disinfectant solution for quarantine	
Disposable protective equipment for quarantine staff (gloves + shoe covers + overalls)	
<b>Transport of animals from off-site quarantine to farm (entries)</b>	
Type of transport from off-site quarantine to replacement facility (own or hired)	
<b>Type of vehicle used for incoming replacements</b>	
Is the transport shared with other farms?	
Shoe covers provided to drivers (off-site quarantine to farm trip)	
Boots provided to drivers (off-site quarantine to farm trip)	
Distance from off-site quarantine to the farm (km)	
<b>Transport of replacement animals to farm (entries)</b>	
Type of transport from replacement facility to the farm (own or hired)	
Type of vehicle used for transport to the farm	
Is the transport to the farm shared with other farms?	
Shoe covers provided to drivers (farm entry)	
Boots provided to drivers (replacement to farm entry)	
Distance from origin to farm (km)	
Does the same journey bringing replacements also transport animals to the replacement unit?	
<b>Diagnostic testing on quarantine</b>	
BVD test during quarantine	
IBR test during quarantine	
Neospora test during quarantine	
Paratuberculosis test during quarantine	
<b>Diagnostic testing at origin</b>	
BVD test at replacement origin	
IBR test at replacement origin	
Neospora test at replacement origin	

Paratuberculosis test at replacement origin

## Feed storage and water treatment

Farm characteristics	Implemented on the farm?
<b>Feed storage</b>	
Silos	
Number of 7,000 kg silos	
Number of 10,000 kg silos	
Number of 17,000 kg silos	
Lidded feed bins	
Number of lidded feed bins	
Pallets used to keep buckets off the ground	
Number of pallets	
<b>Drinking water treatment</b>	
Chlorine water treatment	
Hydrogen peroxide water treatment	

## Farm access by visitors and vehicles

Question	Specify
Farm perimeter (m)	
Perimeter fencing of the farm	
Number of farm access points	
Are access points controlled?	
Number of different visitors in contact with animals per year	
Total frequency of visits involving animal contact per month (excluding drivers)	
Number of footbaths	
Frequency of vehicle entries (per month)	

Farm characteristics	Implemented on the farm?	Proportion (totals 100%)
<b>Type of fencing (single or mixed)</b>		
<b>Material of perimeter fencing</b>		
Concrete block wall, 2.5 m		
Brick wall, 2.5 m		
Reinforced concrete wall, 2.5 m		
Chain-link mesh, 2 m (3 cm mesh spacing)		
<b>Is there double fencing around the farm perimeter?</b>		
<b>Access (gates)</b>		
Lifting barrier		
Number of lifting barriers		
Manual swing gate		
Number of manual swing gates		
Automatic swing gate		
Number of automatic swing gates		
Manual sliding gate		
Number of manual sliding gates		
Automatic sliding gate		
Number of automatic sliding gates		
<b>Vehicles entry point</b>		
Disinfection arch		
<b>Transport of outgoing animals</b>		
Shoe covers provided to drivers (outgoing trips)		
Boots provided to drivers		
Type of transport to slaughterhouse (own or hired)		
Type of vehicle for slaughterhouse trips		
Is transport shared with other farms?		
Number of animal shipments to slaughterhouse (annual)		
Number of animals sent to slaughterhouse (annual)		
Distance from farm to slaughterhouse (km)		
Are animals separated before transport to slaughterhouse?		

## Biosecurity in cattle production

Does the driver come into contact with animals remaining on the farm?	
Type of transport to fattening unit (own or hired)	
Type of vehicle for fattening transport	
Is transport shared with other farms?	
Number of animal shipments to fattening (annual)	
Number of animals sent to fattening (annual)	
Distance from farm to fattening unit (km)	
Are animals separated before transport to fattening?	
Does the driver come into contact with animals remaining on the farm?	
Type of transport to rearing unit (own or hired)	
Type of vehicle for rearing transport	
Is transport shared with other farms?	
Number of animal shipments to rearing (annual)	
Number of animals sent to rearing (annual)	
Distance from farm to rearing unit (km)	
Are animals separated before transport to rearing or slaughter?	
Does the driver come into contact with animals remaining on the farm?	
<b>Mortality management</b>	
Paved area for mortality disposal	
Fencing around mortality disposal area	
Disposal service (annual fee)	
<b>Clothing area</b>	
Farm-dedicated boots for workers	
Farm-provided boots for regular visitors (in contact with animals)	
Overalls for workers	
Farm-provided overalls for regular visitors (in contact with animals)	
Footbath with disinfectant solution (for visitors only)	
Disposable protective equipment for workers (gloves + shoe covers + overalls)	
Disposable protective equipment for visitors (gloves + shoe covers + overalls)	

### Pest control

Farm characteristics	Implemented on the farm?
Rodent control measures	
Control of flying/biting insects	
Control of crawling insects/cockroaches	
<b>Number of control points installed</b>	
Number of physical rodent traps	
Number of bait stations	
Number of traps for flying/biting insects	
Chemical control of flying/biting insects	
Annual cost of chemical control of flying/biting insects	
Monthly time dedicated to flying insect control (hours)	
Number of cockroach traps	
<b>Monthly cost external company (indicate corresponding plans)</b>	
Overall cost of pest control	
Cost of rodent control only	
Cost of fly control only	
Cost of cockroach control only	
<b>Extra cost for pest control</b>	

Table 1.S1. List of items used to estimate the cost of biosecurity measures considered in the cost estimation.

Data price	Cost type	Useful life	Units	Annual frequency	Quantity	Price 1	Price 2	Price 3	Mean/ Total	Source 1	Source 2	Source 3
<b>Perimeter fencing</b>												
Concrete block wall 2.5m materials	Structural	40	Meter						69.54 €			
Materials	Variable		Meter			23.27 €			23.27 €			
Labour + tools	Variable		Hour/meter			46.27 €			46.27 €	<a href="#">Source1</a>		
Brick wall 2.5m materials	Structural	40	Meter						131.90 €			
Materials	Variable		Meter			48.45 €			48.45 €			
Labour + tools	Variable		Hour/meter			83.45 €			83.45 €	<a href="#">Source1</a>		
Concrete wall 2.5m materials	Structural	40	Meter						167.84 €			
Materials	Variable		Meter			150.69 €			150.69 €			
Labour + tools	Variable		Hour/meter			17.15 €			17.15 €	<a href="#">Source1</a>		
Twisted wire mesh 2m (3cm mesh spacing)	Structural	20							31.24 €			
Materials	Variable		Meter			25.40 €			25.40 €	<a href="#">Source1</a>		
Labour + tools	Variable		Hour/meter			5.84 €			5.84 €			
Double fencing (multiplier by quantity)	Multiplier				2							
Fence maintenance/ inspection		% initial investment over useful life	1	0.1								
<b>Vehicle entry</b>												

## Biosecurity in cattle production

Data price	Cost type	Useful life	Units	Annual frequency	Quantity	Price 1	Price 2	Price 3	Mean/ Total	Source 1	Source 2	Source 3
Disinfection arch	Fixed	10	Un		1	984.54 €	4,407.48 €		2,696.01 €	<a href="#">Source1</a>	<a href="#">Source2</a>	
Disinfectant solution for footbath	Variable	1	Lt		17.94	145.56 €	498.33 €		321.95 €	<a href="#">Source1</a>	<a href="#">Source2</a>	
<b>Visitors change area</b>												
Latex gloves	Variable		Un	260	100	6.34 €	8.47 €	9.14 €	7.98 €	<a href="#">Source1</a>	<a href="#">Source2</a>	<a href="#">Source3</a>
Disposable polypropylene overall	Variable		Un	260	1	3.36 €	1.54 €	2.36 €	2.42 €	<a href="#">Source1</a>	<a href="#">Source2</a>	<a href="#">Source3</a>
Disposable plastic boot covers	Variable		Un	260	50	10.05 €	13.35 €	13.31 €	12.24 €	<a href="#">Source1</a>	<a href="#">Source2</a>	<a href="#">Source3</a>
Time in change area per day per worker (hours)	Variable											
Plastic tub	Fixed	2	Un		1	9.55 €	22.91 €		16.23 €	<a href="#">Source1</a>	<a href="#">Source2</a>	
Disinfection mat	Fixed	2	Un		1	57.91 €	47.01 €	51.97 €	52.30 €	<a href="#">Source1</a>	<a href="#">Source2</a>	<a href="#">Source3</a>
Disinfectant solution	Variable		Lt		17.94	145.56 €			145.56 €	<a href="#">Source1</a>		
Concentrated disinfectant for footbath	Variable		Lt		20	99.00 €			99.00 €	<a href="#">Source1</a>		
Automatic boot cleaner	Fixed	5	Un		1	120.00 €	148.00 €	293.03 €	187.01 €	<a href="#">Source1</a>	<a href="#">Source2</a>	<a href="#">Source3</a>
Farm-specific boots	Variable	2	Pair		1	38.48 €	22.54 €	12.90 €	24.64 €	<a href="#">Source1</a>	<a href="#">Source2</a>	<a href="#">Source3</a>
Time in change area per day (per worker) in hours	Variable	1	Hour	260	0.17							
<b>Signage</b>												

Data price	Cost type	Useful life	Units	Annual frequency	Quantity	Price 1	Price 2	Price 3	Mean/ Total	Source 1	Source 2	Source 3
No entry sign	Fixed	3	Un		1	14.75 €			14.75 €	<a href="#">Source1</a>		
<b>Access (gates)</b>												
Lifting barrier	Fixed	10	Un		1	1,014.89 €			1,014.89 €	<a href="#">Source1</a>		
Manual swing gate	Fixed	20	Un		1	2,495.41 €			2,495.41 €	<a href="#">Source1</a>		
Automatic swing gate	Fixed	20	Un		1	3,667.06 €			3,667.06 €	<a href="#">Source1</a>		
Manual sliding gate	Fixed	20	Un		1	2,962.39 €			2,962.39 €	<a href="#">Source1</a>		
Automatic sliding gate	Fixed	20	Un		1	3,949.02 €			3,949.02 €	<a href="#">Source1</a>		
<b>Mortality disposal</b>												
Concrete surface 9m <sup>2</sup>	Structural	20	Un		1	217.05 €			217.05 €	<a href="#">Source1</a>		
Mortality fencing (9m <sup>2</sup> = 14.4 linear metres)	Structural	20	Un		1	449.86 €			449.86 €			
Mortality handling per cow/year (collection company)	Variable		Cow/year			6.70 €			6.70 €	<a href="#">Source1</a>		
<b>Feed storage</b>												
Silo – 7,000 kg	Fixed	10	Un		1	1,531.09 €	2,317.15 €		1,924.12 €	<a href="#">Source1</a>	<a href="#">Source2</a>	
Silo – 10,000 kg	Fixed	10	Un		1	1,780.80 €	1,914.22 €		1,847.51 €	<a href="#">Source1</a>	<a href="#">Source2</a>	

## Biosecurity in cattle production

Data price	Cost type	Useful life	Units	Annual frequency	Quantity	Price 1	Price 2	Price 3	Mean/ Total	Source 1	Source 2	Source 3
Silo – 17,000 kg	Fixed	10	Un		1	2,227.20 €	2,524.06 €		2,375.63 €	<a href="#">Source1</a>	<a href="#">Source2</a>	
Feed bin with lid (500/600L)	Fixed	10	Un		1	390.00 €	784.96 €		587.48 €	<a href="#">Source1</a>	<a href="#">Source2</a>	
Pallets (up to 750 kg)	Fixed	3	Un		1	16.32 €	14.51 €		15.42 €	<a href="#">Source1</a>	<a href="#">Source2</a>	
<b>Water Treatment</b>												
Hydrogen peroxide 50%	Variable		Lt		1195	2,977.81 €			2,977.81 €	<a href="#">Source1</a>		
220V dosing pump	Fixed	5	Un		1	325.00 €			325.00 €	<a href="#">Source1</a>		
Hydrogen peroxide test strips	Variable		Un		100	64.98 €	51.02 €		58.00 €	<a href="#">Source1</a>	<a href="#">Source2</a>	
Sodium hypochlorite	Variable		Lt		1036	963.48 €			963.48 €	<a href="#">Source1</a>		
Chlorine test strips	Variable		Un		50	23.00 €			23.00 €	<a href="#">Source1</a>		
<b>Pest Control –</b>												
<b>Physical Measures</b>												
Mouse trap clamp	Fixed	1	Un	1	1	2.48 €	1.52 €	2.99 €	2.33 €	<a href="#">Source1</a>	<a href="#">Source2</a>	
Sticky trap for rats and mice (33 x 21 cm / 135 g glue)	Fixed	1	Un	1	1	2.91 €	3.91 €		3.41 €	<a href="#">Source1</a>	<a href="#">Source2</a>	
Adhesive fly and mosquito tape (10 m x 25 cm / 30 sticky sheets)	Fixed	1	Un	12	1	14.06 €	28.36 €		21.21 €	<a href="#">Source1</a>	<a href="#">Source2</a>	
Cockroach glue trap	Variable		Un	12	5	6.53 €	8.32 €		7.43 €	<a href="#">Source1</a>	<a href="#">Source2</a>	
Fly and wasp trap	Fixed	1	Un	12	1	14.84 €	14.21 €		14.53 €	<a href="#">Source1</a>	<a href="#">Source2</a>	
Sealant paste for openings	Variable		mL	1	300	13.07 €	12.33 €		12.70 €	<a href="#">Source1</a>	<a href="#">Source2</a>	

Data price	Cost type	Useful life	Units	Annual frequency	Quantity	Price 1	Price 2	Price 3	Mean/ Total	Source 1	Source 2	Source 3
<b>Pest Control – Repellents</b>												
Animal and rodent repellent (500 ml, with 2 dispensers) / ultrasonic coverage up to 500 m <sup>2</sup>												
Variable	Un	1	88.95 €	94.16 €	91.56 €	<a href="#">Source1</a>	<a href="#">Source2</a>					
Ultrasonic bird and pigeon repellent (500 m <sup>2</sup> coverage)	Variable	Un	118.10 €		118.10 €	<a href="#">Source1</a>						
Wild boar repellent	Variable	Un	40	29.61 €	29.61 €	<a href="#">Source1</a>						
<b>Pest Control – Chemical Rodent Control</b>												
Rodenticide bait	Variable	Un	6	17.07 €	17.07 €	<a href="#">Source1</a>						
Rodent bait station (for rats and mice)	Variable	Un	2	1	4.55 €	6.86 €	5.71 €	<a href="#">Source1</a>	<a href="#">Source2</a>			
5L sprayer for phytosanitary products	Variable	Un	1	49.00 €	49.00 €	<a href="#">Source1</a>						
Time spent checking traps/bait (hours)	Variable	Hour	12	2								
<b>Perimeter and Wildlife Control</b>												
Electric fence energiser	Fixed	5	Un	1	263.57 €	277.09 €	270.33 €	<a href="#">Source1</a>	<a href="#">Source2</a>			
Conductive tape	Variable	Mt	200	35.00 €	31.00 €	25.95 €	30.65 €	<a href="#">Source1</a>	<a href="#">Source2</a>	<a href="#">Source3</a>		

Data price	Cost type	Useful life	Units	Annual frequency	Quantity	Price 1	Price 2	Price 3	Mean/ Total	Source 1	Source 2	Source 3
<b>Feed and Water</b>												
<b>Supply for Extensive Systems</b>												
Elevated drinkers	Fixed	10	Un		1	392.18 €	174.02 €		283.10 €	<a href="#">Source1</a>	<a href="#">Source2</a>	
Covered raised feeder with protective bars	Fixed	10	Un		1	800.00 €	1.386.72 €	621.32 €	936.01 €	<a href="#">Source1</a>	<a href="#">Source2</a>	<a href="#">Source3</a>
Separate drinkers for wildlife	Fixed	10	Un		1	74.05 €			74.05 €	<a href="#">Source1</a>		
Concrete surface for drinkers (per m <sup>2</sup> )	Structural	15	m <sup>2</sup>		1	11.45 €			11.45 €	<a href="#">Source1</a>		
<b>Hand Washing Station</b>												
Hands-free sink (knee- or foot-operated)	Fixed	10	Un		1	464.80 €	343.99 €		404.40 €	<a href="#">Source1</a>		
Soap dispenser (1.8 L)	Variable		Un		1	25.44 €	17.85 €		21.65 €	<a href="#">Source1</a>	<a href="#">Source2</a>	
Hand sanitiser	Variable		Lt		1	8.96 €	17.85 €		13.41 €	<a href="#">Source1</a>	<a href="#">Source2</a>	
Pre-cut paper roll (180 m)	Variable		Un		6	56.92 €			56.92 €	<a href="#">Source1</a>	<a href="#">Source2</a>	
<b>Protective Clothing and Footwear</b>												
Latex gloves	Variable		Un		100	6.34 €	8.47 €	9.14 €	7.98 €	<a href="#">Source1</a>		
Disposable polypropylene overall	Variable		Un		1	3.36 €	1.54 €	2.36 €	2.42 €	<a href="#">Source1</a>	<a href="#">Source2</a>	<a href="#">Source3</a>

Data price	Cost type	Useful life	Units	Annual frequency	Quantity	Price 1	Price 2	Price 3	Mean/ Total	Source 1	Source 2	Source 3
Disposable plastic boot covers	Variable		Un		50	10.05 €	13.35 €	13.31 €	12.24 €	<a href="#">Source1</a>	<a href="#">Source2</a>	<a href="#">Source3</a>
Plastic tub	Fixed	3	Un		1	9.55 €	22.91 €		16.23 €	<a href="#">Source1</a>	<a href="#">Source2</a>	<a href="#">Source3</a>
Disinfection mat	Fixed	2	Un		1	57.91 €	47.01 €	51.97 €	52.30 €	<a href="#">Source1</a>	<a href="#">Source2</a>	
Automatic boot cleaner	Fixed	5	Un		1	120.00 €	148.00 €	293.03 €	187.01 €	<a href="#">Source1</a>	<a href="#">Source2</a>	<a href="#">Source3</a>
Disinfectant solution	Variable		Kg		17.94	145.56 €			145.56 €	<a href="#">Source1</a>	<a href="#">Source2</a>	<a href="#">Source3</a>
Reusable overall	Fixed	2	Un		1	27.92 €	24.75 €		26.34 €	<a href="#">Source1</a>	<a href="#">Source2</a>	
<b>Transport</b>												
<b>Disinfection</b>												
Foaming cleaning product	Variable		Kg		17.94	145.56 €			145.56 €	<a href="#">Source1</a>		
Foam application gun	Fixed	3	Un		1	27.92 €	24.75 €		26.34 €	<a href="#">Source1</a>	<a href="#">Source2</a>	
<b>Diagnostic Testing</b>												
BVD (Bovine Viral Diarrhoea)	Variable		Un		1	7.00 €	9.50 €	12.60 €	9.70 €	<a href="#">Source1</a>	<a href="#">Source2</a>	<a href="#">Source3</a>
IBR (Infectious Bovine Rhinotracheitis)	Variable		Un		1	7.00 €	9.50 €	4.40 €	6.97 €	<a href="#">Source1</a>	<a href="#">Source2</a>	<a href="#">Source3</a>
Neospora	Variable		Un		1	7.00 €	11.00 €	4.40 €	7.47 €	<a href="#">Source1</a>	<a href="#">Source2</a>	<a href="#">Source3</a>
Paratuberculosis	Variable		Un		1	7.00 €	8.00 €		7.50 €	<a href="#">Source1</a>	<a href="#">Source2</a>	
<b>Sampling</b>												
Time spent by vet and farmer on quarantine testing (hours)	Variable		Hour/ animal		0.0167							

Table 2.S1. List of biosecurity measures included in the cost estimation.

<b>Pathway of introduction</b>	<b>Biosecurity measure</b>	<b>Number of farm-level inputs</b>
<b>Animal introduction</b>	Quarantine	Number of animal entries (batches per year) Number of animals entering per year (external source) Does the farm have a quarantine facility? Is the quarantine area within the farm perimeter? Duration of quarantine (days) Perimeter of quarantine area (m) Frequency of access to quarantine area (daily) Time spent daily in the quarantine pen (hours) Number of workers entering the quarantine area Type of fencing around quarantine (single or mixed) Material of quarantine perimeter fencing Is there double fencing around the quarantine area? Dedicated boots for quarantine Dedicated overalls for quarantine Footbath with disinfectant solution for quarantine Disposable protective equipment for quarantine staff (gloves + shoe covers + overalls)
	Transport of incoming animals	Type of transport from off-site quarantine to replacement facility (own or hired)
		Type of vehicle used for incoming replacements
		Is the transport shared with other farms?
		Shoe covers provided to drivers (off-site quarantine to farm trip)
		Boots provided to drivers (off-site quarantine to farm trip)
		Distance from off-site quarantine to the farm (km)
		Type of transport from replacement facility to the farm (own or hired)
		Type of vehicle used for transport to the farm

Pathway of introduction	Biosecurity measure	Number of farm-level inputs
		Is the transport to the farm shared with other farms?
		Shoe covers provided to drivers (farm entry)
		Boots provided to drivers (replacement to farm entry)
		Distance from origin to farm (km)
		Does the same journey bringing replacements also transport animals to the replacement unit?
		BVD test during quarantine
		IBR test during quarantine
		Neospora test during quarantine
	Diagnostic testing for incoming animals	Paratuberculosis test during quarantine
		BVD test at replacement origin
		IBR test at replacement origin
		Neospora test at replacement origin
		Paratuberculosis test at replacement origin
		Farm perimeter (m)
		Perimeter fencing of the farm
		Number of farm access points
		Are access points controlled?
		Number of footbaths
		Type of fencing (single or mixed)
		Material of perimeter fencing
		Is there double fencing around the farm perimeter?
		Lifting barrier
		Number of lifting barriers
		Manual swing gate
		Number of manual swing gates

<b>Pathway of introduction</b>	<b>Biosecurity measure</b>	<b>Number of farm-level inputs</b>
Visitor control	Automatic swing gate	
	Number of automatic swing gates	
	Manual sliding gate	
	Number of manual sliding gates	
	Automatic sliding gate	
	Number of automatic sliding gates	
	Disinfection arch	
	Number of different visitors in contact with animals per year	
	Total frequency of visits involving animal contact per month (excluding drivers)	
	Farm-provided boots for regular visitors (in contact with animals)	
Transport of outgoing animals	Farm-provided overalls for regular visitors (in contact with animals)	
	Footbath with disinfectant solution (for visitors only)	
	Disposable protective equipment for visitors (gloves + shoe covers + overalls)	
	Frequency of vehicle entries (per month)	
	Shoe covers provided to drivers (outgoing trips)	
	Boots provided to drivers	
	Type of transport to slaughterhouse (own or hired)	
	Type of vehicle for slaughterhouse trips	
	Is transport shared with other farms?	
	Number of animal shipments to slaughterhouse (annual)	
	Number of animals sent to slaughterhouse (annual)	
	Distance from farm to slaughterhouse (km)	
	Are animals separated before transport to slaughterhouse?	
	Does the driver come into contact with animals remaining on the farm?	
	Type of transport to fattening unit (own or hired)	

Pathway of introduction	Biosecurity measure	Number of farm-level inputs
		Type of vehicle for fattening transport
		Is transport shared with other farms?
		Number of animal shipments to fattening (annual)
		Number of animals sent to fattening (annual)
		Distance from farm to fattening unit (km)
		Are animals separated before transport to fattening?
		Does the driver come into contact with animals remaining on the farm?
		Type of transport to rearing unit (own or hired)
		Type of vehicle for rearing transport
		Is transport shared with other farms?
		Number of animal shipments to rearing (annual)
		Number of animals sent to rearing (annual)
		Distance from farm to rearing unit (km)
		Are animals separated before transport to rearing or slaughter?
		Does the driver come into contact with animals remaining on the farm?
		Paved area for mortality disposal
Mortality management		Fencing around mortality disposal area
		Disposal service (annual fee)
Worker hygiene protocols		Farm-dedicated boots for workers
		Overalls for workers
		Disposable protective equipment for workers (gloves + shoe covers + overalls)
Feed storage and water treatment	Feed storage	Number of 7,000 kg silos
		Number of 10,000 kg silos
		Number of 17,000 kg silos
		Lidded feed bins

<b>Pathway of introduction</b>	<b>Biosecurity measure</b>	<b>Number of farm-level inputs</b>
<b>Pest control</b>	Drinking water treatment	Number of lidded feed bins
		Pallets used to keep buckets off the ground
		Number of pallets
	Rodent control	Chlorine water treatment
		Hydrogen peroxide water treatment
		Rodent control measures
		Number of physical rodent traps
	Flying and crawling insect control	Number of bait stations
		Cost of rodent control only
		Control of flying/biting insects
		Control of crawling insects/cockroaches
		Number of traps for flying/biting insects
		Chemical control of flying/biting insects
		Annual cost of chemical control of flying/biting insects
		Monthly time dedicated to flying insect control (hours)
		Number of cockroach traps
		Cost of fly control only
		Cost of cockroach control only

## 5.11.2 Supplementary material 2: Outputs obtained from the SimHerd simulation

*Table 1.S2. Contribution margin across the first 5 years of simulation for Farm A. The table presents the gross margin (GM) obtained from the simulation using the farm's original parameters (standard), as well as the GM under the scenario in which the farm's productivity parameters were modified (scenario).*

Year of simulation	GM Standard	GM Scenario
Year 1	2,558,391 €	2,404,019 €
Year 2	2,537,029 €	2,360,193 €
Year 3	2,567,494 €	2,417,324 €
Year 4	2,561,577 €	2,416,841 €
Year 5	2,558,255 €	2,415,014 €

*Table 2.S2. Contribution margin across the first 5 years of simulation for Farm B. The table presents the gross margin (GM) obtained from the simulation using the farm's original parameters (standard), as well as the GM under the scenario in which the farm's productivity parameters were modified (scenario).*

Year of simulation	GM Standard	GM Scenario
Year 1	804,074 €	754,233 €
Year 2	811,393 €	764,063 €
Year 3	808,975 €	758,756 €
Year 4	804,473 €	763,533 €
Year 5	804,251 €	760,650 €

*Table 3.S2. Contribution margin across the first 5 years of simulation for Farm C. The table presents the gross margin (GM) obtained from the simulation using the farm's original parameters (standard), as well as the GM under the scenario in which the farm's productivity parameters were modified (scenario).*

Year of simulation	GM Standard	GM Scenario
Year 1	157,259 €	156,868 €
Year 2	150,172 €	135,197 €
Year 3	152,593 €	140,590 €
Year 4	151,172 €	142,852 €
Year 5	150,096 €	140,901 €

*Table 4.S2. Selected parameter values resulting from the SimHerd simulation for the study farms. The table presents a selection of outputs (not all parameters simulated by SimHerd), showing the average values over five simulation years for both the “Standard” scenario (using the farm’s original parameters) and the “Scenario” with modified productivity parameters.*

Parameter of the farm	Farm A		Farm B		Farm C	
	Standard	Scenario	Standard	Scenario	Standard	Scenario
<b>Milk yield per cow-year, kg ECM</b>	13,232	12,998	13,287	13,056	8,685	8,565
<b>Feed intake in FE per cow-year</b>	8,262	8,150	8,299	8,192	6,170	6,103
<b>Number of cow-years</b>	725	724	234	234	72	71
<b>Number of calvings</b>	756	739	255	251	66	60
<b>Replacement rate</b>	30.5	36	30.6	36.1	42.8	45.8
<b>Inseminations per cow-year (# on cows + heifers)</b>	3.5	4.3	3.1	3.9	3.4	3.9
<b>Number of first parity cows</b>	226	272	70	84	36	41
<b>Number of second parity cows</b>	167	182	53	58	19	18
<b>Number of third and older parity cows</b>	332	270	112	93	17	12
<b>Revenues milk</b>	4,512,975 €	4,431,685 €	1,480,622 €	1,454,332 €	296,753 €	287,531 €
<b>Total revenues</b>	4,873,978 €	4,725,906 €	1,616,598 €	1,571,222 €	356,356 €	351,227 €
<b>Expenses Feed, cows</b>	1,672,104 €	1,649,304 €	592,527 €	584,789 €	129,015 €	125,388 €
<b>Total expenses</b>	2,317,438 €	2,313,478 €	812,464 €	810,580 €	206,892 €	210,278 €

## 5.11.3 Supplementary material 3 : Decision analysis on the most cost-effective biosecurity improvements

Table 1.S3. Results from the decision analysis for Farm A.

Scenarios	Probability of disease introduction								
	Median value (50th percentile)			Minimum value (2,5% percentile)			Maximum value (97,5% percentile)		
	Disease	No Disease	EMV <sup>a</sup>	Disease	No Disease	EMV <sup>a</sup>	Disease	No Disease	EMV <sup>a</sup>
Current level	Euro€	-173,840 €	-19,969 €		-173,840 €	-19,969 €		-173,840 €	-19,969 €
	p	2.66%	97.34%	-24,064 €	0.99%	99.01%	-21,489 €	5.67%	94.33%
	Euro€*p	-4,627 €	-19,437 €		-1,718 €	-19,771 €		-9,857 €	-18,836 €
Provide boots to all drivers	Euro€	-173,864 €	-19,993 €		-173,864 €	-19,993 €		-173,864 €	-19,993 €
	p	1.55%	98.45%	-22,384 €	0.58%	99.42%	-20,881 €	3.31%	96.69%
	Euro€*p	-2,702 €	-19,683 €		-1,003 €	-19,878 €		-5,755 €	-19,331 €
Do not share equipment with other farms	Euro€	-173,866 €	-19,995 €		-173,866 €	-19,995 €		-173,866 €	-19,995 €
	p	2.54%	97.46%	-23,902 €	0.94%	99.06%	-21,445 €	5.41%	94.59%
	Euro€*p	-4,415 €	-19,487 €		-1,639 €	-19,806 €		-9,404 €	-18,913 €
Provide boots to all visitors	Euro€	-173,864 €	-19,993 €		-173,864 €	-19,993 €		-173,864 €	-19,993 €
	p	2.55%	97.45%	-23,912 €	0.95%	99.05%	-21,448 €	5.43%	94.57%
	Euro€*p	-4,428 €	-19,484 €		-1,644 €	-19,804 €		-9,433 €	-18,908 €

<sup>a</sup>EMV= Expected monetary value.

Table 2.S3. Results from the decision analysis for Farm B.

Scenarios	Probability of disease introduction									
	Median value (50th percentile)			Minimum value (2,5% percentile)			Maximum value (97,5% percentile)			
	Disease	No	EMVa	Disease	No	EMVa	Disease	No Disease	EMVa	
Disease										
Current level	Euro€	-57,401 €	-11,015 €		-57,401 €	-11,015 €		-57,401 €	-11,015 €	
	p	15.70%	84.30%	-18,297 €	10.10%	89.90%	-15,700 €	21.50%	78.50%	-20,988 €
	Euro€*p	-9,012 €	-9,286 €		-5,798 €	-9,902 €		-12,341 €	-8,647 €	
Provide boots to all drivers	Euro€	-62,599 €	-16,213 €		-62,599 €	-16,213 €		-62,599 €	-16,213 €	
	p	3.67%	96.33%	-17,917 €	2.36%	97.64%	-17,309 €	5.03%	94.97%	-18,546 €
	Euro€*p	-2,299 €	-15,617 €		-1,479 €	-15,830 €		-3,149 €	-15,397 €	
Do not share equipment with other farms	Euro€	-57,426 €	-11,039 €		-57,426 €	-11,039 €		-57,426 €	-11,039 €	
	p	14.40%	85.60%		9.27%	90.73%		19.73%	80.27%	
			-17,721 €				-15,338 €			-20,189 €
Provide boots to all visitors	Euro€*p	-8,272 €	-9,449 €		-5,321 €	-10,017 €		-11,328 €	-8,862 €	
	Euro€	-57,427 €	-11,041 €		-57,427 €	-11,041 €		-57,427 €	-11,041 €	
	p	15.57%	84.43%	-18,264 €	10.02%	89.98%	-15,688 €	21.32%	78.68%	-20,933 €
	Euro€*p	-8,942 €	-9,322 €		-5,753 €	-9,935 €		-12,246 €	-8,687 €	

<sup>a</sup>EMV= Expected monetary value.

Table 3.S3. Results from the decision analysis for Farm C.

Scenarios	Probability of disease introduction									
	Median value (50th percentile)			Minimum value (2,5% percentile)			Maximum value (97,5% percentile)			
	Disease	No	EMVa	Disease	No	EMVa	Disease	No	EMVa	
Disease										Disease
Current level	Euro€	-14,674 €	-5,697 €		-14,674 €	-5,697 €		-14,674 €	-5,697 €	
	p	0.36%	99.64%		-5,729 €	0.15%	99.85%	-5,710 €	0.73%	99.27%
Provide boots to all drivers	Euro€*p	-53 €	-5,676 €		-22 €	-5,688 €		-107 €	-5,655 €	
	Euro€	-14,686 €	-5,709 €		-14,686 €	-5,709 €		-14,686 €	-5,709 €	
	p	0.22%	99.78%		0.09%	99.91%		-5,717 €	0.45%	99.55%
Do not share equipment with other farms	Euro€*p	-33 €	-5,696 €		-14 €	-5,704 €		-66 €	-5,683 €	
	Euro€	-14,698 €	-5,721 €		-14,698 €	-5,721 €		-14,698 €	-5,721 €	
	p	0.33%	99.67%		-5,751 €	0.14%	99.86%	-5,734 €	0.67%	99.33%
Provide boots to all visitors	Euro€*p	-48 €	-5,703 €		-20 €	-5,714 €		-98 €	-5,683 €	
	Euro€	-14,700 €	-5,723 €		-14,700 €	-5,723 €		-14,700 €	-5,723 €	
	p	0.33%	99.67%		-5,753 €	0.14%	99.86%	-5,736 €	0.67%	99.33%
	Euro€*p	-49 €	-5,704 €		-20 €	-5,715 €		-98 €	-5,685 €	

<sup>a</sup>EMV= Expected monetary value.

Table 4.S3. Sensitivity analysis of the probability of Bovine Viral Diarrhoea (BVD) introduction on the outcomes of the decision analysis.

Probability of introduction (Pintro)	EMV <sup>a</sup> of current level	Provide boots to all drivers			Do not share equipment with other farms			Provide boots to all visitors			Relative change in EMV <sup>a</sup> scenario vs current level (%)	
		Reduced Pintro	EMV <sup>a</sup> (€)	Relative change in EMV <sup>a</sup> scenario vs current level (%)	Reduced Pintro	EMV <sup>a</sup> (€)	Relative change in EMV <sup>a</sup> scenario vs current level (%)	Reduced Pintro	EMV <sup>a</sup> (€)			
2%	- 23,046.01 €	0.00%	- 21,789.76 €	5.5%	0.00%	- 22,930.64 €	0.5%	0.00%	- 22,938.09 €	0.5%		
4%	- 26,123.43 €	1.17%	- 23,586.30 €	9.7%	1.91%	- 25,866.36 €	1.0%	1.91%	- 25,882.96 €	0.9%		
6%	- 29,200.85 €	2.34%	- 25,382.83 €	13.1%	3.82%	- 28,802.07 €	1.4%	3.83%	- 28,827.82 €	1.3%		
8%	- 32,278.27 €	3.50%	- 27,179.36 €	15.8%	5.72%	- 31,737.79 €	1.7%	5.74%	- 31,772.68 €	1.6%		
10%	- 35,355.69 €	4.67%	- 28,975.90 €	18.0%	7.63%	- 34,673.50 €	1.9%	7.66%	- 34,717.55 €	1.8%		
12%	- 38,433.11 €	5.84%	- 30,772.43 €	19.9%	9.54%	- 37,609.21 €	2.1%	9.57%	- 37,662.41 €	2.0%		
14%	- 41,510.53 €	7.01%	- 32,568.96 €	21.5%	11.45%	- 40,544.93 €	2.3%	11.48%	- 40,607.27 €	2.2%		
16%	- 44,587.95 €	8.17%	- 34,365.49 €	22.9%	13.36%	- 43,480.64 €	2.5%	13.40%	- 43,552.14 €	2.3%		
18%	- 47,665.37 €	9.34%	- 36,162.03 €	24.1%	15.26%	- 46,416.35 €	2.6%	15.31%	- 46,497.00 €	2.5%		
20%	- 50,742.79 €	10.51%	- 37,958.56 €	25.2%	17.17%	- 49,352.07 €	2.7%	17.22%	- 49,441.86 €	2.6%		
22%	- 53,820.21 €	11.68%	- 39,755.09 €	26.1%	19.08%	- 52,287.78 €	2.8%	19.14%	- 52,386.73 €	2.7%		
24%	- 56,897.63 €	12.84%	- 41,551.63 €	27.0%	20.99%	- 55,223.50 €	2.9%	21.05%	- 55,331.59 €	2.8%		
26%	- 59,975.05 €	14.01%	- 43,348.16 €	27.7%	22.89%	- 58,159.21 €	3.0%	22.97%	- 58,276.45 €	2.8%		
28%	- 63,052.47 €	15.18%	- 45,144.69 €	28.4%	24.80%	- 61,094.92 €	3.1%	24.88%	- 61,221.32 €	2.9%		
30%	- 66,129.89 €	16.35%	- 46,941.23 €	29.0%	26.71%	- 64,030.64 €	3.2%	26.79%	- 64,166.18 €	3.0%		
32%	- 69,207.31 €	17.51%	- 48,737.76 €	29.6%	28.62%	- 66,966.35 €	3.2%	28.71%	- 67,111.04 €	3.0%		
34%	- 72,284.73 €	18.68%	- 50,534.29 €	30.1%	30.53%	- 69,902.07 €	3.3%	30.62%	- 70,055.91 €	3.1%		

Probability of introduction (Pintro)	EMV <sup>a</sup> of current level	Provide boots to all drivers			Do not share equipment with other farms			Provide boots to all visitors			Relative change in EMV <sup>a</sup> scenario vs current level (%)	
		Reduced Pintro	EMV <sup>a</sup> (€)	Relative change in EMV <sup>a</sup> scenario vs current level (%)	Reduced Pintro	EMV <sup>a</sup> (€)	Relative change in EMV <sup>a</sup> scenario vs current level (%)	Reduced Pintro	EMV <sup>a</sup> (€)			
36%	- 75,362.15 €	19.85%	- 52,330.82 €	30.6%	32.43%	- 72,837.78 €	3.3%	32.54%	- 73,000.77 €	3.1%		
38%	- 78,439.57 €	21.02%	- 54,127.36 €	31.0%	34.34%	- 75,773.49 €	3.4%	34.45%	- 75,945.63 €	3.2%		
40%	- 81,516.99 €	22.18%	- 55,923.89 €	31.4%	36.25%	- 78,709.21 €	3.4%	36.36%	- 78,890.50 €	3.2%		
42%	- 84,594.41 €	23.35%	- 57,720.42 €	31.8%	38.16%	- 81,644.92 €	3.5%	38.28%	- 81,835.36 €	3.3%		
44%	- 87,671.83 €	24.52%	- 59,516.96 €	32.1%	40.07%	- 84,580.63 €	3.5%	40.19%	- 84,780.23 €	3.3%		
46%	- 90,749.25 €	25.69%	- 61,313.49 €	32.4%	41.97%	- 87,516.35 €	3.6%	42.10%	- 87,725.09 €	3.3%		
48%	- 93,826.67 €	26.85%	- 63,110.02 €	32.7%	43.88%	- 90,452.06 €	3.6%	44.02%	- 90,669.95 €	3.4%		
50%	- 96,904.09 €	28.02%	- 64,906.56 €	33.0%	45.79%	- 93,387.78 €	3.6%	45.93%	- 93,614.82 €	3.4%		

<sup>a</sup> Expected monetary value.

Table 5.S3. Sensitivity analysis of the cost of a Bovine Viral Diarrhoea (BVD) outbreak on the outcomes of the decision analysis.

BVD outbreak cost	EMV a of current level	Provide boots to all drivers		Do not share equipment with other farms		Provide boots to all visitors	
		EMV a (€)	Relative change in EMV a scenario vs current level (%)	EMV a (€)	Relative change in EMV scenario vs current level (%)	EMV a (€)	Relative change in EMV a scenario vs current level (%)
<b>-153,871.00 €</b>	- 24,064.11 €	- 22,384.11 €	7.0%	-23,901.87 €	0.7%	- 23,912.34 €	0.6%
<b>-169,258.10 €</b>	- 24,473.66 €	- 22,623.20 €	7.6%	-24,292.56 €	0.7%	- 24,304.25 €	0.7%
<b>-184,645.20 €</b>	- 24,883.22 €	- 22,862.29 €	8.1%	-24,683.25 €	0.8%	- 24,696.16 €	0.8%
<b>-200,032.30 €</b>	- 25,292.77 €	- 23,101.37 €	8.7%	-25,073.95 €	0.9%	- 25,088.08 €	0.8%
<b>-215,419.40 €</b>	- 25,702.32 €	- 23,340.46 €	9.2%	-25,464.64 €	0.9%	- 25,479.99 €	0.9%
<b>-230,806.50 €</b>	- 26,111.87 €	- 23,579.55 €	9.7%	-25,855.33 €	1.0%	- 25,871.90 €	0.9%
<b>-246,193.60 €</b>	- 26,521.43 €	- 23,818.64 €	10.2%	-26,246.03 €	1.0%	- 26,263.81 €	1.0%
<b>-261,580.70 €</b>	- 26,930.98 €	- 24,057.73 €	10.7%	-26,636.72 €	1.1%	- 26,655.72 €	1.0%
<b>-276,967.80 €</b>	- 27,340.53 €	- 24,296.81 €	11.1%	-27,027.41 €	1.1%	- 27,047.63 €	1.1%
<b>-292,354.90 €</b>	- 27,750.08 €	- 24,535.90 €	11.6%	-27,418.11 €	1.2%	- 27,439.54 €	1.1%
<b>-307,742.00 €</b>	- 28,159.64 €	- 24,774.99 €	12.0%	-27,808.80 €	1.2%	- 27,831.45 €	1.2%

<sup>a</sup> Expected monetary value.

Table 6.S3. Sensitivity analysis of biosecurity costs on the outcomes of the decision analysis.

Biosecurity cost	EMV a of current level	Provide boots to all drivers		Do not share equipment with other farms		Provide boots to all visitors	
		EMV a (€)	Relative change in EMV a scenario vs current level (%)	EMV a (€)	Relative change in EMV scenario vs current level (%)	EMV a (€)	Relative change in EMV a scenario vs current level (%)
-19,968.59 €	- 24,064.11 €	- 22,384.11 €	7.0%	-23,901.87 €	0.7%	- 23,912.34 €	0.6%
-21,965.45 €	- 26,060.97 €	- 24,383.43 €	6.4%	-25,901.36 €	0.6%	- 25,911.67 €	0.6%
-23,962.31 €	- 28,057.83 €	- 26,382.76 €	6.0%	-27,900.85 €	0.6%	- 27,910.99 €	0.5%
-25,959.17 €	- 30,054.69 €	- 28,382.08 €	5.6%	-29,900.34 €	0.5%	- 29,910.31 €	0.5%
-27,956.03 €	- 32,051.55 €	- 30,381.40 €	5.2%	-31,899.84 €	0.5%	- 31,909.63 €	0.4%
-29,952.89 €	- 34,048.41 €	- 32,380.72 €	4.9%	-33,899.33 €	0.4%	- 33,908.96 €	0.4%
-31,949.74 €	- 36,045.27 €	- 34,380.05 €	4.6%	-35,898.82 €	0.4%	- 35,908.28 €	0.4%
-33,946.60 €	- 38,042.13 €	- 36,379.37 €	4.4%	-37,898.32 €	0.4%	- 37,907.60 €	0.4%
-35,943.46 €	- 40,038.98 €	- 38,378.69 €	4.1%	-39,897.81 €	0.4%	- 39,906.93 €	0.3%
-37,940.32 €	- 42,035.84 €	- 40,378.02 €	3.9%	-41,897.30 €	0.3%	- 41,906.25 €	0.3%
-39,937.18 €	- 44,032.70 €	- 42,377.34 €	3.8%	-43,896.80 €	0.3%	- 43,905.57 €	0.3%

<sup>a</sup> Expected monetary value.



## Chapter IV: General discussion



## 6 General discussion

At the farm-level, biosecurity involves multiple stakeholders, including veterinary services and external contractors such as animal transporters, among others. Different actors may influence the implementation and the effectiveness of biosecurity measures throughout the production chain (FAO and WOAH, 2009; Hernández-Jover et al., 2012; Maye et al., 2017; Subasinghe et al., 2023). As a matter of fact, recognising the role of all stakeholders influencing on-farm biosecurity is essential for promoting the adoption of preventive measures and strengthening protection against pathogen introduction (Hernández-Jover et al., 2012; Reed and Curzon, 2015).

This thesis has explored biosecurity from multiple angles, including live animal transport, methods for assessing biosecurity, and the cost-effectiveness analysis of its implementation. By adopting this multifaceted approach, the research has provided valuable insights to enhance biosecurity strategies across different stages of the production chain.

It is also important to acknowledge that many factors influencing biosecurity implementation lie outside farmers' direct control. Indeed, several aspects of external biosecurity are carried out by individuals not employed on the farm, limiting the farmer's ability to intervene in certain measures (Amalraj et al., 2024). Consequently, without adequate enforcement of biosecurity across all production stages of the production chain, such as during livestock transport, on-farm efforts may be insufficient. For instance, transporting animals from multiple farms during the same journey, or failing to consistently clean and disinfect vehicles between farm visits, may create critical breaches in farm-level biosecurity, which are outside the farmers' control, thus posing farms at significant risk of infection. In this regard, findings from the first study highlighted significant gaps in current transport-related biosecurity practices, indicating considerable room for improvement.

Further research should also consider other stakeholders, such as animal traders, livestock markets, feed companies, and carcass collection companies, among others. Improving engagement in biosecurity among different stakeholders, together with their coordination and communication across the supply chain, would support more effective implementation of biosecurity measures, facilitate necessary processes, and foster stronger commitment to biosecurity (Lipovšek et al., 2024).

As highlighted in the introduction to this thesis, on-farm biosecurity is of utmost importance for the prevention and control of animal diseases. This is evidenced by the fact that most European countries have legislation in place regarding biosecurity (Biebaut et al., 2025; Mahmood et al., 2025), with veterinary services often actively involved in the process. Partly due to growing awareness of the importance of biosecurity, assessing farm biosecurity is becoming increasingly common. Indeed, the Animal Health Law (European Parliament and Council of the European Union, 2016) establishes that animal farms must receive an animal health visit during which, among other aspects, biosecurity must be assessed.

As revealed by the second study, a wide variety of approaches to assessing farm biosecurity coexist in Europe. These approaches differ in their objectives, who conducts the assessment, how it is conducted, and the type of outcomes produced. However, such comprehensive assessments can place a significant time burden on farmers, adding to existing evaluations such as welfare quality checks, among others. Therefore, the time required from farmers to complete these assessments must be carefully considered.

Farmers frequently report experiencing administrative overload and excessive paperwork during disease control or eradication programmes mandated by veterinary authorities. Technology may help alleviate this workload. The integration of new technologies and methodologies for evaluating and reviewing compliance may support more effective biosecurity assessments on farms. Findings from Study II showed that more than half of the methods still relied on paper-

based data collection, highlighting the limited adoption of digital technology in biosecurity evaluation systems. For instance, the use of real-time monitoring systems with immediate feedback could help enhance biosecurity compliance at the farm level. Additionally, digital visitor registration systems, capable of identifying high-risk visitors based on the farms they have previously entered, can reduce the burden on farmers, who are often responsible for managing this aspect. With the support of technology, these processes might become faster and more efficient (Racicot et al., 2022; Soriano et al., 2024).

The practicality, efficiency, and cost-effectiveness of biosecurity interventions must be evaluated to ensure their suitability for implementation in rural farming contexts (Jaye et al., 2021; Moya et al., 2020). Future research should prioritise the assessment of such interventions across the animal production chain, with a particular focus on identifying optimal strategies for monitoring biosecurity compliance without overburdening farmers or the broader system.

As highlighted in several studies, personalised recommendations have been shown to improve the implementation of on-farm biosecurity compared to generic advice advocating for overall improvement (Cardwell et al., 2016; Levallois et al., 2023; Scollo et al., 2023). For instance, regular audit-style visits accompanied by progressive improvement plans have been shown to enhance biosecurity compliance at the farm level (Racicot et al., 2012). However, communication between veterinarians or advisors and farmers is not always straightforward and can hinder the adoption of additional biosecurity measures (Moya et al., 2025; Sayers et al., 2014). Therefore, any support aimed at motivating improvements in on-farm biosecurity, such as providing relevant technical information to farmers, is highly valuable. This technical support can be enhanced by equipping veterinarians and advisors with practical tools (Dhaka et al., 2023; Fraser et al., 2010; Laanen et al., 2014), as demonstrated by the decision analysis developed in Study III.

The framework developed in the third study is intended to support and facilitate effective communication with farmers by clearly demonstrating the expected benefits of implementing specific biosecurity measures. One of the key findings of this study was that each farm presents a different level of biosecurity, associated with varying costs and degrees of risk of disease introduction. Furthermore, it was shown that the potential impact of a disease outbreak depends on factors such as herd size and production level. The availability of a tool that allows consideration of farm-specific characteristics can support veterinarians, advisors, and farmers in making informed decisions about the most appropriate measures to reduce the risk of pathogen introduction.

Across the three studies presented in this thesis, a recurring theme was the heterogeneity observed in biosecurity, whether in its level of implementation, the variety of assessment frameworks employed, or the identification of the most appropriate measures to recommend. In fact, differences were observed in how truck drivers applied biosecurity practices depending on their profiles (Study I), in the methods used to assess farm-level biosecurity (Study II), and in the identification of the most cost-effective biosecurity measure across farms (Study III). Although the development of standardised frameworks can provide clearer guidance for both implementation and evaluation, it is essential that critical control points are not overlooked (Delpont et al., 2023). The findings of this thesis highlight the challenges associated with developing such standardised biosecurity protocols and underscore the importance of tailoring biosecurity improvement programmes and their assessment tools to the specific risk profiles of each stakeholder, farm, and region. Tools and methodologies should be context-sensitive, considering species, production systems, and geographical specificities (O Donovan et al., 2024; Schembri et al., 2015; Scollo et al., 2023).

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# Chapter V: Conclusions



## 7 Conclusions

1. The evaluation of biosecurity practices among live cattle transport drivers in Spain underscored the need to raise awareness of disease risks associated with transport activities among stakeholders and to promote strategies to reduce such risk without compromising the economic viability of the transport sector.
2. Limited access to authorised disinfection facilities, time constraints, and implementation costs were identified as major barriers to effective implementation of biosecurity in livestock transport. Addressing these challenges requires targeted communication and institutional support through infrastructure investment.
3. There are many different methods that are used in practice to assess biosecurity in farming systems. These methods differ in their objectives, employed methodologies and output generated, reflecting differences in regional priorities, species-specific needs, and resource availability. Further research is needed to assess if harmonised frameworks are needed to improve comparability and practical use, or if this heterogeneity is beneficial.
4. A new biosecurity cost calculator was developed and applied to dairy farms to assess current and potential costs associated with biosecurity. Structured around main disease introduction pathways, it supports farm-specific evaluations and provides a practical tool to communicate with farmers about biosecurity improvements.
5. The integration of farm-specific data on biosecurity costs, outbreak costs, and the probability of disease introduction through decision analysis enables the identification and prioritisation of the most profitable biosecurity interventions tailored on farms, supporting evidence-based decision-making for more efficient and targeted prevention strategies, and promoting farmers' engagement toward biosecurity.