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Methods to assess on-farm biosecurity in Europe and beyond

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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.

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1. 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.

2. Material and methods

2.1. 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 (Annex 1, Fig. 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.

2.2. 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 board 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 spend 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).

3. 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 Annex 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).

3.1. 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 Annex 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 (Fig. 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.

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.

	Pigs N = 45		Ruminants N = 30		Poultry N = 37	
Main objective	N	%	N	%	N	%
Certification for quality assurance						
Mandatory	2	4 %	1	3 %	3	8 %
Mandatory; Voluntary					1	3 %
Voluntary	4	9 %	2	7 %	6	16 %
To improve biosecurity of the farm						
Mandatory	12	27 %	7	23 %	8	22 %
Mandatory; Voluntary			1	3 %	3	8 %
Voluntary	26	58 %	14	47 %	16	43 %
To control/prevent a specific disease						
Mandatory	6	13 %	4	13 %	4	11 %
Mandatory; Voluntary			1	3 %	2	5 %
Voluntary	8	18 %	10	33 %	7	19 %
To decrease antibiotic use						
Mandatory	3	7 %	1	3 %	1	3 %
Mandatory; Voluntary						
Voluntary	5	11 %	6	20 %	4	11 %
“Other” objective						
Mandatory	2	4 %	1	3 %		
Mandatory; Voluntary					1	3 %
Voluntary			1	3 %		

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.

3.2. 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).

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.

3.3. 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 sup. mat. 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 sup. mat. Table 10.S2.

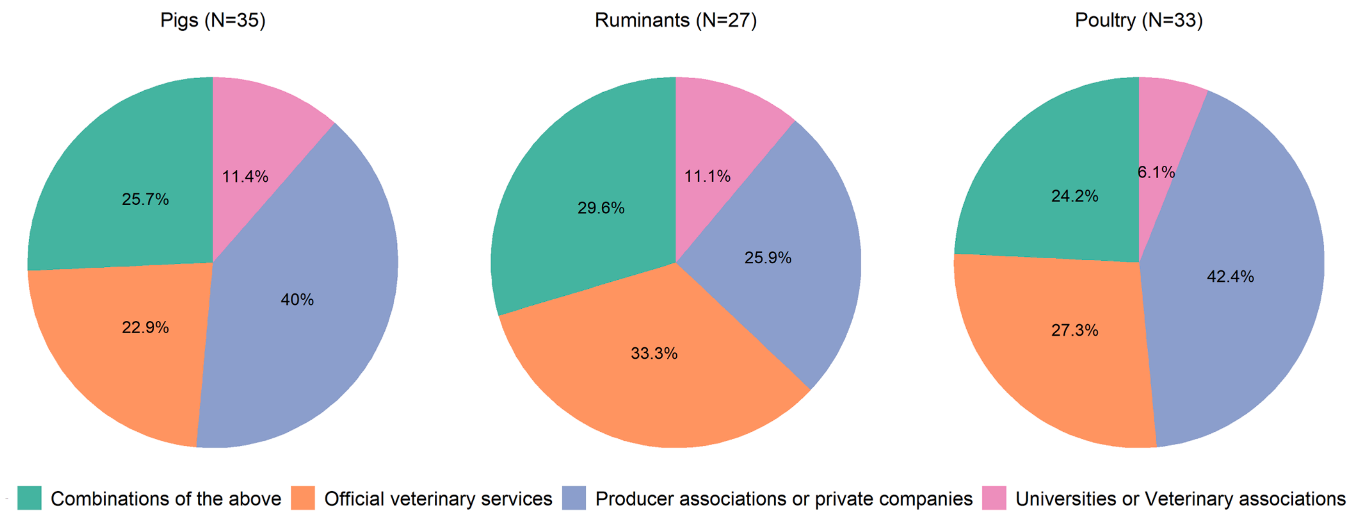

Fig. 1. Developers of the biosecurity assessment methods (BAMs) by animal production type in 28 countries reported between October and November 2023. The reported N represents the number of unique methods received for each type of animal production, with the percentage (%) calculated within each respective production type.

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.

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

[a] i.e. veterinarian working in the pharmaceutical industry or advisor of the integrator company.

3.4. *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 (sup. mat. Fig. 1.S2).

Four clusters were identified (Fig. 2), consisting of 34, 7, 8, and 35

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	%
Extra-data collection						
Yes	20	44 %	18	60 %	24	65 %
No	25	56 %	12	40 %	13	35 %
Type of extra-data collected (only methods with extra-data)						
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[a]	1	5 %	2	11 %	1	4 %

[a] e.g Geographical data, camera trap imaging, chlorine testing of water.

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	%
Feedback						
No	2	4 %	5	17 %	2	5 %
Yes	43	96 %	25	83 %	35	95 %
Feedback details						
Verbal report	6	14 %	2	8 %	1	3 %
Written report	23	53 %	15	60 %	19	54 %
Verbal & written report	12	28 %	7	28 %	13	37 %
Other	2	5 %	1	4 %	2	6 %

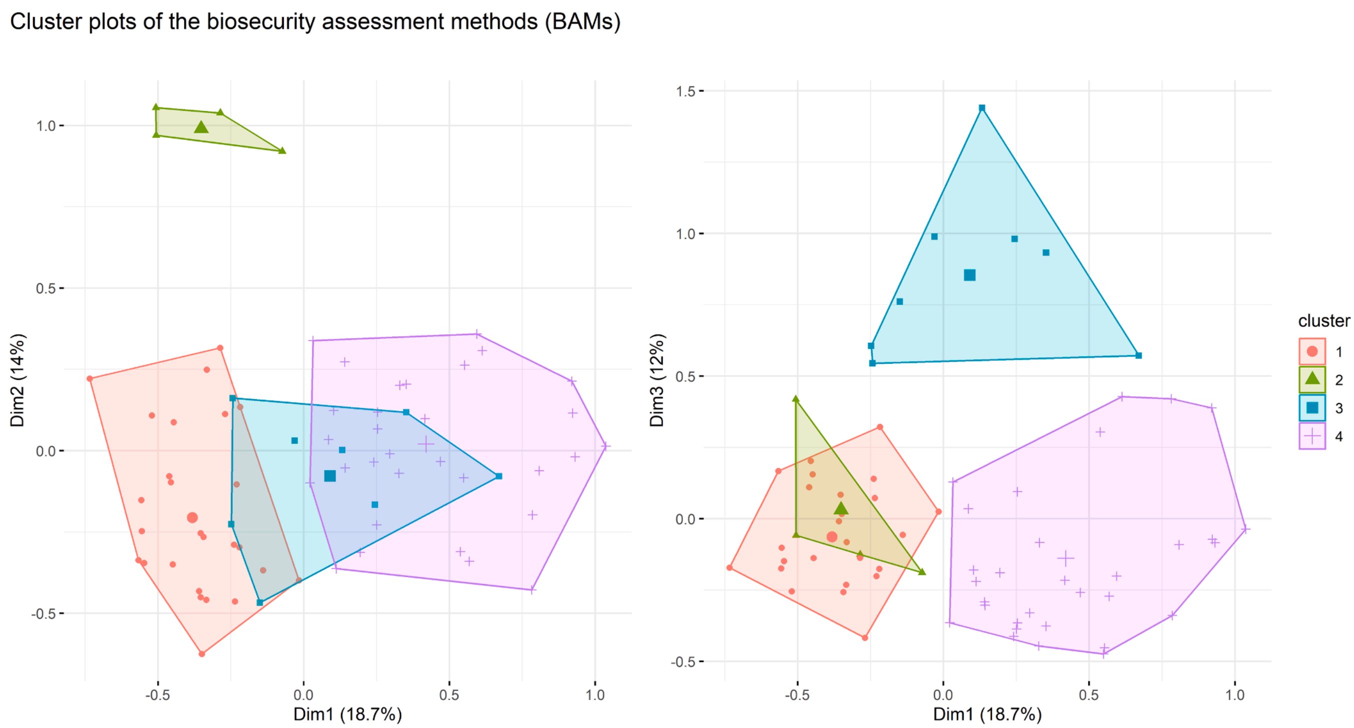

Fig. 2. Visualisation of the four clusters resulting from the Hierarchical Clustering on Principal Components (HCPC) analysis on the results of the Multiple Correspondence Analysis (MCA). The plots show the first and second dimensions on the left, and the first and third dimensions on the right. The 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.

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.

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 Fig. 2.S2.

4. 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 (Chantziaras 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.

5. 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.

Ethics statement

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

CRediT authorship contribution statement

Duarte Godoy Fernando Javier: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Kjosevski Miroslav:** Writing – original draft, Validation, Supervision, Conceptualization. **Tamminen Lena-Mari:** Writing – review & editing, Writing – original draft, Validation, Supervision, Methodology. **Delpont Mattias:** Writing – original draft, Validation, Conceptualization. **Ciaravino Giovanna:** Writing – original draft, Validation, Supervision. **van den Borne Bart H.P.:** Writing – original draft, Validation, Conceptualization. **Correia-Gomes Carla:** Writing – original draft, Validation, Conceptualization. **Allepuz Alberto:** Writing – original draft, Visualization, Validation, Supervision, Methodology, Investigation, Funding acquisition, Conceptualization. **Alarcón Laura Valeria:** Writing – original draft, Validation. **Desvars-Larrive Amelie:** Writing – original draft, Visualization, Validation, Conceptualization. **Chantziaras Ilias:** Writing – original draft, Validation. **Toppari Ina:** Writing – original draft, Validation. **Svennesen Line:** Writing – original draft, Validation. **Gecaj Rreze M:** Writing – original draft, Validation. **Piccirillo Alessandra:** Writing – original draft, Validation. **Nunes Telmo:** Writing – original draft, Validation. **Zbikowski Artur:** Writing – original draft, Validation. **De Nardi Marco:** Writing – original draft, Validation, Conceptualization. **Prodanov-Radulović Jasna:** Writing – original draft, Validation. **Nedosekov Vitalii:** Writing – original draft, Validation.

Declaration of Competing Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could represent potential competing interests.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.prevetmed.2025.106486.

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