Open Access

Berl Münch Tierärztl Wochenschr (135)
DOI 10.2376/1439-0299-2022-9
1–10

ISSN 1439-0299

Korrespondenzadresse:
cara.foerster@tiho-hannover.de

Eingegangen: 02.05.2022
Angenommen: 06.10.2022
Veröffentlicht: 07.11.2022

https://www.vetline.de/berliner-und-muenchener-tieraerztliche-wochenschrift-open-access

Department of Biometry, Epidemiology and Information Processing, WHO Collaborating Centre for Research and Training for Health in the Human-Animal-Environment Interface, University of Veterinary Medicine Hannover, Hannover, Germany[1]; Task Force Consumer Protection, Lower Saxony State Office for Consumer Protection and Food Safety, Oldenburg, Germany[2]; Veterinary Service Osnabrück, Osnabrück, Germany[3]

A Framework for Study Planning in Food Safety Investigations

Ein Handlungsrahmen für die Studienplanung bei Untersuchungen zur Lebensmittelsicherheit

Cara Förster[1], Katja Nordhoff[2], Jörg Fritzemeier[3], Jutta Breuer[3], Lothar Kreienbrock[1]

Summary

In investigations related to foodborne outbreaks and evaluation of food safety, the responsible veterinary authorities are usually required to make rapid decisions and take related action. These steps are based on an assessment based on a representative sampling procedure with sufficient precision.
However, a variety of complex factors need to be considered to properly set up the investigation, including the characteristics of the pathogenic agent, the homogeneity of the distribution, and the matrix. Furthermore, to calculate its sample size, information on the epidemiological method used (e.g., prevalence estimation and freedom from contamination) and its related statistical parameters (e.g., prevalence, alpha, and delta) must be considered.
These considerations and decisions are associated with far-reaching legal and economic consequences and must often be made immediately. This presents a great challenge for veterinary administration and creates the need for a smart and manageable solution. To achieve the best outcome, cooperation of practice and science is necessary.
To broach this complex topic, an approach is described in which use cases from administrative practice are presented that link all the essential components for determining the correct sample design and size. These practical examples incorporate the perspective of the veterinary authority and lead to the creation of specific concepts for individual pathogen-matrix combinations. The use cases serve as cornerstones for the development of a concept that can also be applied to other pathogens and matrices.

Keywords: sample size, foodborne outbreak, zoonotic pathogens, veterinary administration, measurement uncertainty

Zusammenfassung

Bei Untersuchungen im Zusammenhang mit lebensmittelbedingten Krankheitsausbrüchen und der Beurteilung der Lebensmittelsicherheit müssen die zuständigen Veterinärbehörden schnelle Entscheidungen treffen und Maßnahmen ergreifen. Diese Schritte erfolgen nach einer Bewertung, welche auf Basis eines repräsentativen Probenahmeverfahrens mit ausreichender Genauigkeit erfolgt.
Für die korrekte Durchführung der Untersuchung muss jedoch eine Vielzahl komplexer Faktoren berücksichtigt werden, darunter die Eigenschaften des Krankheitserregers, die Homogenität der Verteilung und die Matrix. Außerdem müssen zur Berechnung des Stichprobenumfangs Informationen über die verwendete epidemiologische Methode (z. B. Prävalenzschätzung und Nachweis der Freiheit von einem Erreger) und die entsprechenden statistischen Parameter (z. B. Prävalenz, Alpha und Delta) berücksichtigt werden.
Diese Überlegungen und Entscheidungen sind mit weitreichenden rechtlichen und wirtschaftlichen Konsequenzen verbunden und müssen oft unmittelbar getroffen werden. Dies stellt eine große Herausforderung für die Veterinärverwaltung dar und zeigt den Bedarf einer intelligenten und anwendungsbezogenen

Lösung auf. Zur Entwicklung eines solchen Konzepts ist eine Zusammenarbeit von Praxis und Wissenschaft zwingend notwendig.
Zur Erschließung dieses komplexen Themas wird ein Ansatz beschrieben, in dem Anwendungsfälle aus der Verwaltungspraxis vorgestellt werden, welche alle wesentlichen Komponenten zur Bestimmung des geeigneten Stichprobenplans und -umfangs verknüpfen. Diese Praxisbeispiele beziehen die Perspektive der Veterinärbehörde mit ein und führen zur Erstellung von spezifischen Konzepten für einzelne Erreger-Matrix-Kombinationen. Die Anwendungsfälle dienen als Eckpfeiler für die Entwicklung eines Konzepts, welches auch auf andere Erreger und Matrices übertragen werden kann.

Schlüsselwörter: Stichprobenumfang, lebensmittelbedingte Krankheitsausbrüche, Zoonoseerreger, Veterinärverwaltung, Messunsicherheit

Introduction

Generally, there are two sectors to ensure safe food and consumer protection. The fundamental responsibility lies with the respective producers, importers, transporters and traders. The veterinary authorities control this duty of care of food businesses. These responsibilities and their related tasks are implemented in various European Union (EU) regulations and national legislation on food safety and food hygiene. For example, Directive 2003/99/EC applies to the special monitoring of zoonotic agents. It states that each member state of the EU is obliged to report on the development of its zoonoses once a year (EU 2003). The zoonosis monitoring data collected are published annually by the European Food Safety Authority (EFSA).

In addition to such monitoring programmes, which are performed by the veterinary authorities, there are guidelines and procedures for producers to prevent food contamination, such as Good Hygiene Practices (GHP) and Hazard Analysis and Critical Control Points (HACCP) (EU 2016).

Nevertheless, foodborne zoonotic infections and contamination of food with the corresponding pathogens are frequently detected.

In Germany, the most common zoonotic pathogens in humans are *Campylobacter* spp. and *Salmonella* spp. For 2019, the Robert Koch Institute (RKI) reported 61,526 cases of campylobacteriosis and 13,693 cases of salmonellosis in humans (RKI 2020). In addition to the total number of cases, the RKI and the Federal Office of Consumer Protection and Food Safety (BVL) reported 402 foodborne outbreaks (41% caused by *Campylobacter* spp. and 32% caused by *Salmonella* spp.) for 2019 (BVL 2020). The number of cases and foodborne outbreaks in 2020 have already been published by the RKI (RKI 2021) and by the BVL (2021a). However, the figures for 2020 are noticeably lower than those for 2017–2019 (RKI 2018, 2019, 2020). The RKI states that „The COVID-19 pandemic placed not only an enormous burden on the public health system in Germany, it has also affected the incidence and detection of other notifiable infectious diseases" (RKI 2021). For this reason, the numbers for 2019 are discussed here, which gives an indication of the figures without the influence of the COVID-19 pandemic.

The veterinary authorities are regularly facing a wide variety of questions in relation to food safety. For example, it has to be decided whether a food item is safe, determine whether single or multiple batches are affected, or identify possible foodborne outbreak sources.

It is essential for the veterinary administration to investigate and assess the level of hazard and make assumptions about the safety of the food with sufficient precision. To meet these requirements, precise sample plans and accurate sample sizes are needed for a given population of interest, such as farms, stables or abattoirs. There are various components to consider. The most important ones are the food laws in force, method of sampling, laboratory method, characteristics of the agent, time and way of contamination and homogeneity of the distribution in the contaminated matrix.

This requirement is often challenging for the veterinary authority, especially in the face of a public health service that is already stretched to its limits. The questions are scientifically complex and require immediate response and rapid action. These challenges make it difficult to accurately describe the population of interest, design customised sampling scenarios and integrate the scientific work into daily work.

To support the authorities in these tasks, various manuals, standards and guidelines already exist, such as the following:

- Codex Alimentarius (CAC 2022)
- Guidelines from German Institutions (BfR 2016, LAVES and NLGA 2019, Conraths et al. 2020, ML and LAVES 2021a, b)
- Handbook from Lower Saxony State (ML and LAVES 2021a)

For the calculation of sample sizes, various tools are available as open source. These include, among others, the web list „Epitools" by Ausvet (Sergeant 2018), the „Shiny server" by the Friedrich-Loeffler-Institut (FLI 2022), the „Shiny server" by the Federal Institute for Risk Assessment (BfR 2022a) and the „R4EU platform" by EFSA (EFSA 2022).

However, we are not yet aware of any tool that combines the existing collection of information material and the calculation of an actual sample size in one integrated approach. Therefore, our goal is to develop a concept that combines existing knowledge into a framework of action and the possibility of calculating a sample size embedded in a robust, feasible and biometrically accepted sampling scenario. The concept should be implemented in an application-oriented tool in the future and is intended to support the user with a practical guide in carrying out their investigation.

In this study, we describe the development of the concept and framework with its challenges and limitations. Due to the need for the concept to be practical and

manageable for the veterinary authority, the study unites partners from different fields of science and practice to consider as many perspectives and needs as possible. The study partners are the Lower Saxony State Office for Consumer Protection and Food Safety (LAVES) as a state authority, the Veterinary Service Osnabrück (LKOS) as a local authority and the University of Veterinary Medicine Hannover, Foundation (TiHo) as a scientific member.

Material and Methods/Concept

Methodological approach

As the process of finding an appropriate sample plan and sampling size is challenging, the methodological approach described here is to build and work with selected, prestructured examples (so-called „use cases"). A use case defines a specific scenario that sets requirements for a concept and thus serves as the basis for its development. In our work, the use cases contain the description of potential contaminated pathogen-matrix combinations. By defining these, we are able to identify the relevant components that vary from one specific case to another. They are processed step by step from the practical perspective of a veterinary authority. This procedure identifies the relevant areas for sample planning and builds a framework of action. The examples open up the opportunity to maintain a practical relationship for the concept, to identify all relevant components and to determine the complexity of this process. The necessary cornerstones to develop the concept are as follows:

1. defining relevant and current pathogen-matrix combinations, i.e., identifying pathogens and food that appear to have a substantial burden on human health
2. building use cases with these selected combinations, i.e., creating possible scenarios to work with
3. determining the necessary steps for processing the use cases and identifying the relevant components of the different areas, i.e., collecting the parameters of the different areas needed
4. implementing checklists with all relevant aspects to develop the sampling scenario, i.e., inspecting and combining all needed areas and components for the use case
5. linking the collected information to create a toolbox, i.e., building the framework
6. developing an application-oriented tool.

Cornerstones (1.) and (2.) form the basis for the framework through use-case design. For the development of the framework, one selected use case is applied, which has a high relevance for human health. Further use cases will be added for future development.

The use of different use cases allows the implementation of a concept by providing the opportunity to develop a fundamental framework but also considers the different inputs and outputs that each use scenario requires. By combining the generic core with the possibility of flexibly adapting the concept to different situations, a hybrid approach is created. Users can apply the basic framework and adapt it to their current case by specifically selecting the areas that are relevant to them.

Concept proposal

To develop a hybrid concept for entire sample planning, all necessary aspects that are relevant for sample planning and the associated sample calculation are combined in a toolbox (Table 1). The process described in Figure 1 describes a framework of action for an investigation, which emerged from processing the use cases as the generally applicable procedure.

Seven areas, A to G, were identified that are particularly relevant and generic for study planning.

Area A forms the basis for the study. The fundamental consideration is the definition of the general study design and the study question (A1) as well as the determination of the statistical hypotheses to be tested (A2). By defining the population to be investigated (A3), which parts of the population will actually be investigated and what the investigation should focus on are decided. If necessary, stratification of the population, including groups of units of the same likelihood to be contaminated (A4), must be considered. These steps are crucial because they influence the validity and conclusiveness of the study. Based on this, information is collected on the food item (B) and pathogen (C).

Area B concentrates on the characteristics of the matrix (B1) and background information on the production technique (B2). This information is particularly important for planning an appropriate sampling. Traceability (B3) and business information (B4) are important in the case of possible interventions.

In **area C**, the characteristics of the pathogen (C1), its distribution (C2) and infectivity (C3) are as important as its epidemiology (C4) and microbiology (C5). Knowledge of these areas provides initial indications of the necessary procedure that should be used in sampling and possible following actions.

In particular, B1, C1 and C2 are important for the selection of the sample. Depending on the pathogen in

TABLE 1: *Toolbox: Extraction of relevant components to develop a representative sampling scenario*

Generic areas		Components	
A	Population of interest	1	General study design and study questions
		2	Statistical hypothesis to be tested
		3	Unit to be investigated (batch/lot)
		4	Stratification of the population (including groups of units of the same likelihood to be contaminated)
B	Food item/ Matrix	1	Characteristics
		2	Production technique
		3	Traceability
		4	Business information
C	Pathogen	1	Characteristics
		2	Distribution in the matrix
		3	Contagiousness
		4	Epidemiology
		5	Microbiology
D	Laboratory analysis/Detection method	1	Method
		2	Sensitivity
		3	Specificity
E	Legal regulations	1	Responsibilities
		2	Detection method
		3	Limit values
F	Veterinary administration	1	Staff capacity
		2	Financial capacity
G	Statistical parameters	1	Calculation method
		2	Statistical errors (alpha, beta)
		3	Absolute error delta (or effect size)
		4	Measures of uncertainty (or effect size)

focus, its detection is considered in **area D**. Here, the detection method (D1), with its sensitivity (D2) and specificity (D3), has to be specified.

In **area E**, the legal requirements are considered. Their consideration is essential to complete the sample design. This area includes responsibilities (E1) as well as prescribed detection methods (E2) and limit values (E3).

The available resources of the organisation executing the sampling process must also be considered. This is done in **area F** with the consideration of staff (F1) and financial capacity (F2).

Finally, the required statistical parameters are collected in **area G**. First, the calculation method previously selected for calculating the sample size has to be decided (G1). In an investigation addressing food safety from the perspective of the target population, two main objectives must be considered here: (i) is there a positive subject in the target population and (ii) if positive results may occur, what prevalence can be assumed? These approaches yield two statistical methods for sampling, namely, the concept of state freedom from an event and the concept of calculating a confidence interval for an expected prevalence, which are briefly summarised in the following section. Depending on this method, these include the probabilities of statistical errors alpha and beta (G2), the (absolute) measure of uncertainty (delta, G3), the parameters of uncertainty (G4), or combinations such as effect sizes.

Concept of state freedom from an event

In many cases, zero tolerance for contamination is needed. However, a statement about the freedom of a population from a pathogen requires the examination of each statistical unit of the population, which is not feasible in most situations. Therefore, the concept of state freedom from an event is usually proposed and used in different concepts (Cannon and Roe 1982, Glaser and Kreienbrock 2011). For example, the concept is included in the Codex Alimentarius rules (CAC 2004). Here, using a statement for an acceptance number d in the batch (this corresponds to a [small] prevalence limit $P_0 = d/N$, i.e., the Acceptable Quality Level AQL [CAC 2004]) is

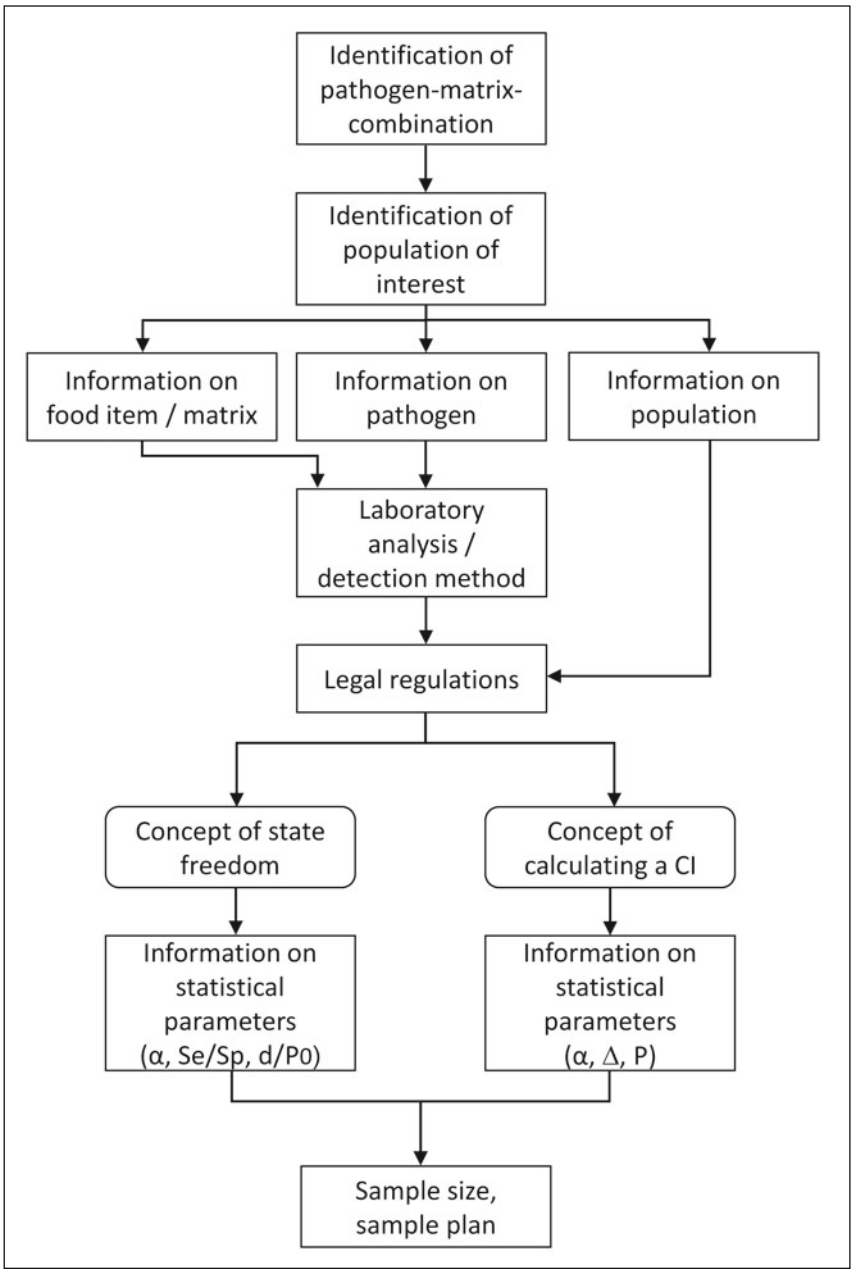

FIGURE 1: *Framework for food safety investigations (Source: Cara Förster)*

suggested. This term refers to the Codex Alimentarius definition, according to which the acceptance number is the maximum number of nonconforming units or the maximum number of nonconformities allowed in the sample (CAC 2004).

A general formula for the calculation of sample size does not exist due to the hypergeometric structure of the probability of obtaining (true- and false-) positive results. Therefore, an algorithmic solution obtained by special programming routines (see, e.g., Ausvet) or prepared tables (see, e.g., Glaser and Kreienbrock 2011) must be used.

To use these procedures, the following input measures are crucial. First, the number of units N in the batch is explicitly needed. This requires the knowledge and accurate definition of the batch itself. In addition, the acceptance number d must be determined. If for various reasons not all units can be examined, a certain number of possibly missed positive units and thus a certain uncertainty must be accepted. This does not mean that a positive finding is accepted. The parameter d is theoretically selected by professional assessment and could be based on the literature, experience or the opinion of an expert, in practice, this is often a complicated task. Another challenge is to make a statement about the sensitivity Se and specificity Sp of the laboratory method. Because no laboratory method is perfect, the use of the abovementioned process is recommended to account for diagnostic failures. If it is not possible to specify sensitivity and specificity, a simplified formula may be used. This refers to an infinite population and only requires the specification of an error probability alpha and the prevalence limit P_0 and describes a „lower threshold" for the sample size only (for details, please follow Glaser and Kreienbrock 2011).

Concept of calculating a confidence interval (CI)

If a pathogen is definitely present in a population and it can be assumed that freedom from this pathogen is unusual, it is recommended to determine the prevalence in the population within accepted bounds of uncertainty, i.e., to calculate a confidence interval (CI) with a certain width as an accepted absolute error of the investigation. In this situation, a popular proposal for the sample size is (Glaser and Kreienbrock 2011)

$$n = \frac{P\,(1 - P)}{\Delta^2}\, u^2_{1-\frac{\alpha}{2}},$$

which includes a value for the absolute (prevalence) error Δ, which can be an absolute statement or statement relative to the prevalence P. This prevalence P represents the relative amount of potentially contaminated samples and is set by professional assessment, the literature or estimation. As always, the statistical precision is set with an appropriate error α with its $\left(1-\frac{\alpha}{2}\right)$-quantile of the standard normal distribution $\left(u_{1-\frac{\alpha}{2}}\right)$.

The required parameters for these basic two methods are briefly summarised in Table 2.

The described generic areas A to G represent the developed toolbox, which is shown in Table 1. The points identified in Table 1 are translated in detail into checklists for the users, which help them work in a structured and comprehensive manner. As an example, a developed generic checklist is attached in the supplements (see supplement 1). For every new case, the users inspect the pathogen-matrix combination they want to work on. Based on that, they choose the relevant components for the case in question and fill in the required information.

TABLE 2: *Necessary parameters for sample size calculation*

Parameter	Explanation	Determination
n	Number of sample units in the sample	Sample size calculation
N	Size of population or lot to be investigated	Observation
α	Error probability	Professional assessment
Δ	Accuracy	Professional assessment
Sensitivity	True positive proportion	Literature, estimation
Specificity	True negative proportion	Literature, estimation
d	Acceptance number	Professional assessment, literature, and estimation
P/P_0	Prevalence/prevalence limit	Professional assessment, literature, and estimation

Results/Use case

The development of the concept is demonstrated exemplarily on one use case.

Example use case „Salmonella Derby in table eggs"

Although no positive samples were found in zoonosis monitoring 2020 by the BVL (2021b), the consumption of eggs is still considered a risk factor for foodborne diseases. To fulfil its responsibility to produce safe food, an egg-producing farm monitors its production internally. The laying flocks are kept in two separated stables, but the eggs are transported to the packing station via the same conveyor. In the packing station, the eggs cannot be traced back to one of the two stables. In this setting, eggs in the egg packing station are detected to be *Salmonella* Derby positive.

The laboratory reports test results to the producer and to the veterinary authority seven days after the sampling process on a Saturday. The veterinary authority decides to take official egg samples to investigate the safety status of the eggs. They organise an official control appointment and the sampling on Tuesday. Sampling takes place eleven days after self-monitoring and three days after the laboratory announcement of the positive sampling result. Because of the weekend between communication and sampling, the producer has to store his eggs for three days. After sampling, no eggs leave the production as table eggs.

In this example scenario, the question for the veterinary authority arises what the adequate sampling plan and sample size in this case is if the farm produces 12,000 eggs a day.

Processing of the use case: „Salmonella Derby in table eggs"

With the use of the framework of action, the areas necessary for processing the use case can be filled with information. To process the case, a specific checklist can be used (see supplement 2).

- **Identification of the pathogen-matrix combination:** The combination to be investigated is *Salmonella* Derby in table eggs.
- **Identification of the population of interest/information on population:** The farm produces 12,000

eggs per day. Thus, within the scenario described, the collected eggs of three days are in the warehouse, and therefore, the size of the population N is 36,000.

- **Information on food item/matrix:** Eggs can be contaminated with *Salmonella* spp. externally on the shell or internally. They may be infected with *Salmonella* spp. through the faeces of the laying hens, by penetration of the eggshell or by direct contamination of the egg contents prior to egg laying as a result of infection of the hen's reproductive organs with *Salmonella* spp. Both contamination of the egg content and contamination of the shell are hazards to human health. Likewise, cross-contamination to other foodstuffs used cannot be excluded (RKI 2016).
- **Information on pathogen:** The pathogen *Salmonella* spp. is a worldwide spread, gram-negative bacterium that forms a heat-sensitive toxin and is considered a zoonosis. Based on their antigenic formula, *Salmonella* spp. can be differentiated into more than 2500 serotypes (RKI 2016). *Salmonella Enteritidis* and *Salmonella Typhimurium* are considered particularly relevant. However, in principle, each serotype has the potential to be a human pathogen (WHO and FAO 2002). The Codex Alimentarius classifies *Salmonella* spp. as „risk groups" or „micro-organisms with severe hazard or with moderate direct health hazard of potentially extensive spread in food" (CAC 2004). Salmonellosis is a classic foodborne infection. The disease is one of the most common infectious diseases. However, the proportion of reported cases is estimated at only 10 to 20% of the actual illnesses (BfR 2022b).
- **Laboratory analysis/detection method:** In this case, the ISO standard DIN EN ISO 6579-1 (DIN 2020) is used.
- **Legal regulation:** There is no regulation that prescribes specific measures to be taken in case of *Salmonella* spp. occurrence in eggs; likewise, no specific sampling numbers are specified. The detection of *Salmonella* spp. in eggs results in an assessment as harmful to health according to Art. 14 (2a) of Regulation (EC) No. 178/2002, subject to an examination according to Art. 14 (3) of Regulation (EC) No. 178/2002 (AFFL 2018).
- **Calculation method:** For *Salmonella* spp. in food, the presence of a single pathogen is a potential hazard for humans. This leads to the assumption that a zero tolerance of *Salmonella* spp. is needed. Therefore, the „concept of state freedom from an event" should be used.
- **Information on statistical parameters:** The size of population N is 36,000. For α, we assume 5% as a first approach. The chosen value of d or P_0 differs depending on the particular requirements of the veterinary administration. In the use case „*Salmonella* spp. in table eggs", the ISO standard DIN EN ISO 6579-1 is the regulation used to select the values for sensitivity and specificity. The regulation provides no specific information on *Salmonella* spp. in (table) eggs. The product „dried egg powder" is the most similar product to eggs, which leads to values of 98% for sensitivity and 100% for specificity. All chosen values are summarised in Table 3.

TABLE 3: *Chosen values in the use case „Salmonella Derby in table eggs"*

Parameter	Explanation	Chosen value
N	Size of the population (lot)	36,000
α	Error probability	0.05 (5%)
Sensitivity	True positive proportion	0.98 (98%)
Specificity	True negative proportion	1 (100%)
d or P_0	Acceptance number or prevalence limit	Professional assessment

The sample size in this use case depends on the chosen value for d or P_0. This consideration results from professional assessment, the literature and estimation but also needs to consider the time, capacity, staff and financial resources.

Discussion

Generally, zoonotic infections in humans and contaminated food are common events. To prevent and control cases of human infections, it is crucial to monitor food with conclusive sampling.

Hybrid concept

This proposal for a structured guide for science-based study planning in food safety investigations was developed as part of a project to support legal administrative services in veterinary medicine. The initial approach was to develop a generic concept that could be directly applied to other use cases. Throughout the development of the concept, the partners faced challenges and difficulties that proved to be limiting factors for the development and usage of a generic concept.

The largest limiting factors are, on the one hand, the need for a sampling scenario with a minor error probability and an extended accuracy, which usually leads to an increased sample size and, on the other hand, a flexible, practical and affordable concept. To optimise the concept, much information and background on the specific pathogen-matrix combination is needed. For this reason, a hybrid approach seems more fitting than a generic approach. The hybrid approach uses a mixture of the generic core (framework of action) and extends it with the flexible toolbox. This allows the user to respond to a wider range of application situations and to consider the diversity of their requirements. This concept can provide important information and statistical aspects for pathogen-matrix combinations and can be used as guidance for similar cases, offering numerous possibilities. In addition, the toolbox opens up the possibility to gradually expand and improve the concept with each application by adding more components and expanding the checklists. Such a structured method of food safety testing allows the user to make a statement about reliability and to influence it by choosing different parameters.

Fundamentals

Before planning and conducting an investigation, some basic points need to be considered. Primarily, legal regulations need to be observed that specify the existing requirements. Some legal acts and their related regulations prescribe specific rules and sample sizes. These demands are clearly the goal of the responsible veterinary authorities to be fulfilled. However, within the

regulations applied, a general statement is often made about the sample size without addressing any specific parameters, such as the population under study or the error bounds accepted. In such cases, the question arises whether such a general approach disregards possible uncertainties. The concept developed should help reduce or clarify uncertainties, which generally enriches the conclusiveness of the entire sampling process.

Furthermore, the study design and drawing of the sample play a crucial role. To ensure the representativeness of its sample, random sampling should be performed whenever possible. Otherwise, risk-based sampling is common to increase the chance of positive findings. With this method, statistical units are selected that seem to be particularly exposed to the characteristics to be investigated. Therefore, this procedure is not representative of the population, and the setting of P_0 must be discussed in the context of these assumptions.

Another factor to consider is the distribution of the pathogen in the matrix itself (Jongenburger et al. 2015). In cases of the heterogeneous distribution of the pathogen in the matrix, simple random sampling may not be appropriate. Then, it may be useful to draw a stratified random sample (CAC 2004). In this case, the population will be divided into homogeneous strata, from which simple random samples will be drawn. The groups stratify based on similar characteristics, which can be different criteria, for example, different stables or risk-based structures. Sampling plans that consider possible heterogeneous contamination suggest a larger sample size than plans for homogeneous distribution (e.g., CAC 2004). This is a challenge for a practical and manageable sampling solution. In the described use case, the veterinary office directly collects the samples in the egg-packing station. Stratified or risk-based sampling in this case is possible but not necessary.

Choice of parameters

As mentioned in area A of the concept, the specification of the population to be investigated is essential. The target population describes the totality of all statistical units, which at the same time represent the smallest sampling point. The determination of the statistical unit is particularly crucial for drawing the sample and influences the definition of the population itself. The definition of the population of interest should be based on an epidemiological point of view. For this, it is particularly important whether the same production conditions are given. As a consequence, this does not necessarily match the producer's definition. Therefore, an exact definition is crucial.

When considering the exemplary use case „*Salmonella* Derby in table eggs", it must be defined for sampling whether the statistical unit represents, here, a single egg, an egg carton or an egg pallet. The distribution of the pathogen and possible transmissibility between the individual units must also be considered. In the use case discussed, the questions of whether the pathogen is evenly distributed over all eggs and how the eggs are divided into the cartons arise. This involves collecting the eggs from the stables, transporting them to the packing station, sorting them into the cartons and stacking and storing the cartons in the packing station.

Based on the study design chosen, its necessary parameters must be specified. Depending on the pathogen-matrix combination, it may be difficult to find satisfactory answers to the information needed in the scientific literature and in practice by colleagues in the immediate study planning phase. This will potentially weaken the process, which demonstrates the necessity of preparing common use cases beforehand.

By defining the study question and the hypothesis to be investigated, this process will be specified more precisely. In the present use case, it was assumed that the eggs to be examined could be contaminated with *Salmonella* Derby. Therefore, sampling is supposed to prove the statistical hypothesis that the eggs are free of *Salmonella* spp. with a given certainty. For this, the error probability α was set at 5%. This is, on the one hand, a commonly accepted value in sample planning, although on the other hand, a 5% error level does not guarantee an error-free decision. Overall, the choice of the error probability influences the sample size directly, which might make it attractive to choose different values for alpha. Hence, there should be an awareness of the consequence of its choice in all directions.

Determining the value for the acceptance number d or the prevalence limit P_0 presents another challenge. Choosing a small value for d means that fewer potentially contaminated units are accepted, but at the same time, it increases the number of samples needed. Often, this cannot be expected and afforded by the veterinary authorities. However, the veterinary authority must also be aware that these considerations imply that not even a negative test result definitely proves the freedom of a population from a pathogen. This requires a balanced assessment of the conclusiveness of the investigation result and the actions to be followed.

Furthermore, various epidemiological factors, the characteristics of the pathogen, its distribution and infectivity must be considered in the determination. For example, if a high contamination of eggs with *Salmonella* spp. can be assumed based on the time courses and other investigation results, it is possible to select a high value for d while at the same time having a high conclusiveness. The Codex Alimentarius recommends evaluating the pathogen-matrix combinations according to their degree of seriousness and making an assessment for d based on this. Here, a division into CA-class A („Those nonconformities considered to be of the highest concern in terms of the quality and/or safety of the product") and CA-class B („Those nonconformities considered to be less important than the Class A nonconformities") is assumed (CAC 2004). Thus, it is recommended to use a lower value for d for potentially more dangerous cases (CA-class A). For less dangerous cases (CA-class B), a larger number should be accepted (CAC 2004). This would imply for the use case addressed here that a low value of d would be recommended for the calculation, which is followed by a higher number of samples. If this is not possible for the veterinary authority, another approach is to determine for itself what resources are available and what number of samples can be taken and to choose the value of d based on that. This approach does not gain the same conclusiveness, but it is nevertheless possible to make a statement about the general precision of the sample results under these restrictions.

The considerations above assume perfect diagnostics, which are usually not given. Therefore, information on sensitivity and specificity is needed. For the pathogen-matrix combination in question, there is no definite

information on the sensitivity and specificity of the laboratory method available. This seems to be a common problem for many pathogen-matrix combination.

However, the reliability of the chosen laboratory method usually has a major impact on the validity of the sampling procedure and the sample size (FAO 2014, Conraths et al. 2020). As an example for the use case addressed here, several assumptions on the diagnostic precision may demonstrate the consequences. Using perfect diagnostics, alpha = 0.05 and P_0 = 0.1, would result in a sample size of n = 29. However, if an extended formula with Se = 100% and Sp = 97.5% is used, a sample size of n = 73 would result, and for Se = 97.5% and Sp = 95%, it is n = 107 (see Glaser and Kreienbrock 2011 for the base formula).

These examples may indicate that the uncertainty in diagnostics in combination with the given thresholds of P_0 leads to a wide range of possible sample sizes. However, omitting the test quality will weaken the validity of the sampling result. Therefore, as a matter of principle, information on sensitivity and specificity seems to be crucial. If it is not possible to include information on these measures of accuracy, it is necessary to be aware of the possible consequences stated above.

Use of concept

In this paper, the development of the concept has been exemplarily described using one use case. However, in the course of the work, further use cases were utilised, which can also be implemented in this form.

The need for structured approaches and, thus, such concepts is generally accepted, e.g., by the WHO (WHO 2017). To be able to perform an investigation and to use the concept, it seems helpful if the addressed authorities prepare themselves by defining the relevant use cases for themselves and use the framework and toolbox to perform basic research work on the required parameters.

Even if such preparation seems useful, many of the veterinary authorities are already under a heavy workload. Therefore, such a reappraisal of individual cases is certainly not to be expected as a standard. In this situation, it is necessary to be aware of one's uncertainties in the investigation and to communicate them in the necessary places. This also adds conclusiveness to the statement drawn.

Outlook

Generally, the hybrid concept will be helpful for study planning in food safety investigations. Therefore, the concept developed will be further implemented in an application-oriented tool in the future. The usability of the tool, which does not require any prior knowledge of programming, should act as a system for directly assisting the veterinary authority. In addition, suggestions for the required parameters should also be made. This supports the users if detailed research of the parameters is not possible in advance.

Abbreviations

AFFL	Arbeitsgruppe Fleisch- und Geflügelfleischhygiene und fachspezifische Fragen von Lebensmitteln tierischer Herkunft der Länderarbeitsgemeinschaft gesundheitlicher Verbraucherschutz
BfR	Federal Institute for Risk Assessment (Bundesinstitut für Risikobewertung)
BVL	Federal Office of Consumer Protection and Food Safety (Bundesamt für Verbraucherschutz und Lebensmittelsicherheit)
CAC	Joint FAO/WHO Codex Alimentarius Commission (Codex Alimentarius-Kommission)
DIN	German Institute for Standardization (Deutsches Institut für Normung e. V.)
EFSA	European Food Safety Authority
EU	European Union (Europäische Union)
FAO	Food and Agriculture Organization of the United Nations (Ernährungs- und Landwirtschaftsorganisation der Vereinten Nationen)
FLI	Friedrich-Loeffler-Institut
GHP	Good Hygiene Practice (Gute Hygiene-Praxis)
HACCP	Hazard Analysis and Critical Control Points (Gefahrenanalyse und kritische Kontrollpunkte)
LAVES	Lower Saxony State Office for Consumer Protection and Food Safety (Niedersächsisches Landesamt für Verbraucherschutz)
LKOS	Veterinary Service Osnabrück (Veterinärdienst für Stadt und Landkreis Osnabrück)
ML	Lower Saxony Ministry of Food, Agriculture and Consumer Protection (Niedersächsisches Ministerium für Ernährung, Landwirtschaft und Verbraucherschutz)
NLGA	Public Health Agency of Lower Saxony (Niedersächsisches Landesgesundheitsamt)
RKI	Robert Koch Institute (Robert Koch-Institut)
TiHo	University of Veterinary Medicine Hannover, Foundation (Stiftung Tierärztliche Hochschule Hannover)
WHO	World Health Organization (Weltgesundheitsorganisation)

Ethical approval

The authors hereby declare that they have followed the universally accepted guidelines of good scientific practice while preparing the present paper.

Conflict of interest

The authors hereby declare that they have no proprietary, professional or other personal interests in any product, service and/or company that could have influenced the contents or opinions expressed in this publication.

Funding

This study was supported by the Federal Ministry of Education and Research (BMBF) (Project No. 01KI1813) as part of the Research Network Zoonotic Infectious Diseases. The funders had no role in the study design, decision to publish or preparation of the manuscript. The authors hereby agree to provide the details of such funding upon reasonable request. This Open Access publication was funded by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) – 491094227 "Open Access Publication Funding" and the University of Veterinary Medicine Hannover, Foundation.

Authors contribution

Study conception and design: CF, KN, JF, JB, LK.
Drafting of the article: CF.
Critical revision of the article: KN, JF, JB, LK.
Final approval of the version to be published: CF, KN, JF, JB, LK.

References

Arbeitsgruppe Fleisch- und Geflügelfleischhygiene und fachspezifische Fragen von Lebensmitteln tierischer Herkunft der Länderarbeitsgemeinschaft gesundheitlicher Verbraucherschutz (AFFL) (2018): Protokoll der 32. Sitzung der Arbeitsgruppe Fleisch- und Geflügelfleischhygiene und fachspezifische Fragen von Lebensmitteln tierischer Herkunft der Länderarbeitsgemeinschaft gesundheitlicher Verbraucherschutz (AFFL) vom 06. und 07. November 2018.

Bundesinstitut für Risikobewertung (BfR) (2016): Ausbruchsaufklärung entlang der Lebensmittelkette, Leitfaden einer am BfR eingerichteten Bund-Länder-Arbeitsgruppe zur Erarbeitung eines Leitfadens zur Ausbruchsaufklärung entlang der Lebensmittelkette.

Bundesinstitut für Risikobewertung (BfR) (2022a): Portal der statistischen Web-Apps des Bundesinstitut für Risikobewertung. http://shiny.bfr.bund.de/ (accessed 10.07.2022).

Bundesinstitut für Risikobewertung (BfR) (2022b): Bedeutung der Salmonellen als Krankheitserreger. https://www.bfr.bund.de/de/bedeutung_der_salmonellen_als_krankheitserreger-537.html#attachments (accessed 10.07.2022).

Bundesamt für Verbraucherschutz und Lebensmittelsicherheit (BVL) (2020): Gemeinsamer nationaler Bericht des BVL und RKI zu lebensmittelbedingten Krankheitsausbrüchen in Deutschland 2019.

Bundesamt für Verbraucherschutz und Lebensmittelsicherheit (BVL) (2021a): Gemeinsamer nationaler Bericht des BVL und RKI zu lebensmittelbedingten Krankheitsausbrüchen in Deutschland 2020.

Bundesamt für Verbraucherschutz und Lebensmittelsicherheit (BVL) (2021b): Zoonosen-Monitoring 2020. Gemeinsamer Bericht des Bundes und der Länder.

Cannon RM, Roe ET (1982): Livestock Disease Surveys, A Field Manual for Veterinarians, Bureau of Resource Science. Department of Primary Industry. Aust Gov Publ Serv Canberra.

Conraths FJ, Gethmann J, Schulz J, Amler S, Fröhlich A, Ziller M, Denzin N (2020): Epidemiologische Untersuchungen in Tierpopulationen: Ein Leitfaden zur Bestimmung von Stichprobenumfängen/erstellt auf der Grundlage von Texten und Tabellen von Dr. R. Lorenz, Tübingen (Krankheitsüberwachung in Tierbeständen. Ein Leitfaden zur Bestimmung von Stichprobenumfängen. AID, Bonn, 1990). Friedrich-Loeffler-Institut, 3. Auflage.

Deutsches Institut für Normung e. V. (DIN) (2020): DIN EN ISO 6579-1:2020-08. Microbiology of the food chain – Horizontal method for the detection, enumeration and serotyping of Salmonella - Part 1: Detection of Salmonella spp. (ISO 6579-1:2017 + Amd.1:2020); German version EN ISO 6579-1:2017 + A1:2020.

European Food Safety Authority (EFSA) (2022): EFSA R4EU platform. https://r4eu.efsa.europa.eu (accessed 12.07.2022).

European Union (2003): Directive 2003/99/EC of the European Parliament and of the Council of 17 November 2003 on the monitoring of zoonoses and zoonotic agents, amending Council Decision 90/424/EEC and repealing Council Directive 92/117/EEC. https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:02003L0099-20130701&from=EN (accessed 18.06.2022).

European Union (2016): European Commission, Commission Notice on the implementation of food safety management systems covering prerequisite programs (PRPs) and procedures based on the HACCP principles, including the facilitation/flexibility of the implementation in certain food businesses. https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:52016XC0730(01)&from=EN (accessed 18.06.2022).

Food and Agriculture Organization of the United Nations (FAO) (2014): Risk-based disease surveillance – A manual for veterinarians on the design and analysis of surveillance for demonstration of freedom from disease. FAO Animal Production and Health manual 17. https://www.fao.org/publications/card/en/c/1440fee4-be47-4d38-8571-4dad3f3036d6/ (accessed 20.06.2022).

Friedrich-Loeffler-Institut (FLI) (2022): FLI Shiny Server. https://shiny.fli.de/home/ (accessed 10.07.2022).

Glaser S, Kreienbrock L (2011): Stichprobenplanung bei veterinärmedizinischen Studien. Ein Leitfaden zur Bestimmung des Untersuchungsumfangs. Schlütersche, Hannover.

Joint FAO/WHO Codex Alimentarius Commission (CAC) (2004): General Guidelines on Sampling CAC/GL 50-2004. https://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCXG%2B50-2004%252FCXG_050e.pdf (accessed 12.07.2022).

Joint FAO/WHO Codex Alimentarius Commission (CAC) (2022): Codex Alimentarius. Rome: Food and Agriculture Organization of the United Nations. https://www.fao.org/fao-who-codexalimentarius/home/en/ (accessed 08.07.2022).

Jongenburger I, den Besten HMW, Zwietering MH (2015): Statistical Aspects of Food Safety Sampling. Annu Rev Food Sci Technol 6(1): 479–503.

Niedersächsisches Landesamt für Verbraucherschutz und Lebensmittelsicherheit, Niedersächsisches Landesgesundheitsamt (LAVES/NLGA) (2019): Lebensmittelbedingte Krankheitsausbrüche in Niedersachsen, Leitfaden für Gesundheitsämter und Lebensmittelüberwachungsbehörden.

Niedersächsisches Ministerium für Ernährung, Landwirtschaft und Verbraucherschutz, Niedersächsisches Landesamt für Verbraucherschutz und Lebensmittelsicherheit (ML/LAVES) (2021a): Krisenmanagment Handbuch Lebensmittel/Futtermittel.

Niedersächsisches Ministerium für Ernährung, Landwirtschaft und Verbraucherschutz, Niedersächsisches, Landesamt für Verbraucherschutz und Lebensmittelsicherheit (ML/LAVES) (2021b): Niedersächsischer Leitfaden zu Salmonellen in der Lebensmittelkette Geflügel.

Robert Koch-Institut (RKI) (2016): Salmonellose. RKI Ratgeber. https://www.rki.de/DE/Content/Infekt/EpidBull/Merkblaetter/Ratgeber_Salmonellose.html (accessed 10.07.2022).

Robert Koch-Institut (RKI) (2018): Infektionsepidemiologisches Jahrbuch meldepflichtiger Krankheiten für 2017.

Robert Koch-Institut (RKI) (2019): Infektionsepidemiologisches Jahrbuch meldepflichtiger Krankheiten für 2018.

Robert Koch-Institut (RKI) (2020): Infektionsepidemiologisches Jahrbuch meldepflichtiger Krankheiten für 2019.

Robert Koch-Institut (RKI) (2021): Infektionsepidemiologisches Jahrbuch meldepflichtiger Krankheiten für 2020.

Sergeant ESG (2018): Epitools Epidemiological Calculators. Ausvet. Available at: http://epitools.ausvet.com.au (accessed 10.07.2022).

World Health Organization, Food and Agriculture Organization of the United Nations (WHO/FAO) (2002): Risk assessments of Salmonella in eggs and broiler chickens.

World Health Organization (2017): Strengthening surveillance of and response to foodborne diseases: a practical manual. Stage 1. Investigating foodborne disease outbreaks.

Address for correspondence
Cara Förster
Department of Biometry, Epidemiology and Information Processing
WHO Collaborating Centre for Research and Training for Health in the Human-Animal-Environment Interface
University of Veterinary Medicine Hannover
Bünteweg 2
30559 Hannover, Germany
cara.foerster@tiho-hannover.de