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Bacteriological examination in place in five European countries to assess carcass fitness for consumption during meat inspection

Riikka Laukkanen-Ninios [a,*], Nina Langkabel [b], Sergio Ghidini [c], Mariel Pikkemaat [d], Elisabeth G. Biesta-Peters [e], Kees van der Ark [f], Lis Alban [g,h]

[a] Department of Food Hygiene and Environmental Health, Faculty of Veterinary Medicine, University of Helsinki, P.O. Box 66, 00014, University of Helsinki, Finland
[b] Institute of Food Safety and Food Hygiene, School of Veterinary Medicine, Freie Universität, Berlin, Germany
[c] Department of Food and Drug, University of Parma, Italy
[d] Wageningen Food Safety Research (WFSR), Wageningen University & Research, Wageningen, the Netherlands
[e] Netherlands Food and Consumer Product Safety Authority (NVWA), Utrecht, the Netherlands
[f] Centre of Infectious Disease Control of the National Institute for Public Health and the Environment (Cib-RIVM), Bilthoven, the Netherlands
[g] Danish Agriculture & Food Council, Copenhagen, Denmark
[h] Department of Veterinary and Animal Sciences, University of Copenhagen, Denmark

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ABSTRACT

In the European Union, bacteriological examination (BE) can be used as a decision support tool for an individual slaughter animal, if a clear decision regarding fitness for human consumption cannot be reached after performing the post-mortem meat inspection at the abattoir. The mandatory use of BE started already in the beginning of 20th century and the methods have since evolved in the different countries using it. Although still in use, discussions have taken place on whether BE is still a useful part of meat inspection. Currently, there is no European consensus regarding how to set up the methods or how to interpret the results. Still, there is a need to avoid unnecessary food waste, while at the same time guaranteeing food safety. In this descriptive study, we mapped the BE methods currently used in five European countries, namely Denmark, Finland, Germany, Italy and the Netherlands. The results show there is considerable variation between the countries regarding the specific analyses, sample matrices and media used. There is also variation in the indications when BE should be performed as well as when the results lead to condemnation. Although the results will be interpreted together with the pathological findings in the carcass, clearly written instructions should be available on how to interpret the results and when to perform condemnation. BE is used more often for cattle than for pigs, and e.g., in Denmark, BE is not used for pigs due to costs. Although BE can still be used to detect animals with a generalised infection at the time of slaughter, other methods that would be easier to standardise and accredit should be developed.

1. Introduction

In the European Union (EU), laboratory analyses, such as bacteriological examination (BE), can be used to support a meat inspection decision for an individual slaughter animal, if a clear decision regarding fitness for human consumption cannot be reached after performing the post-mortem inspection at the abattoir (EU 2019/627). Visibly sick animals should not be transported to an abattoir or if they enter an abattoir, they should be rejected in ante-mortem inspection (EC No 853/2004; EU 2019/627). A BE is indicated if, e.g., post-mortem findings point to a prior systemic infection (Kogka et al., 2021). Moreover, emergency slaughter has historically been one of the primary reasons for performing a BE (Alban et al., 2020; Edelmann, 1920) and on-farm casualty slaughtered animals have been shown to contain higher bacterial counts inside meat, liver and spleen than healthy cattle (Coello et al., 2007). The use of BE helps to reach a decision on whether to accept or condemn a carcass and the organs, whereby unnecessary food waste is prevented in a safe way.

In the late 19th century, the idea that disease in animals will lead to food poisoning in humans after consumption of meat from the diseased animals was first proven in Germany. In 1894, the Dutch Basenau suggested standardised examination with laboratory methods of animals that underwent emergency slaughter. The BE method was set, and it became popular in Germany in the early years of the 20th century after

* Corresponding author.
E-mail address: riikka.laukkanen-ninios@helsinki.fi (R. Laukkanen-Ninios).

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the first meat inspection law in Europe came into force in 1903. After implementing BE stepwise in different regions of Germany, a detailed description of the samples to be taken and the methods to be used was introduced into the German legislation in 1921 (Edelmann, 1920; Standfuβ, 1922; Von Ostertag, 1922). Similarly, BE was introduced into the countries surrounding Germany (Christiansen, 1921-1922). For more details about the historical development, see the Supplementary text.

During recent decades, discussions have taken place in some countries on whether BE is still a useful part of meat inspection (Engel et al., 1987; Kogka et al., 2021; Schalch et al., 1998). The bacteriological methods in place have been considered dated, and they are difficult to accredit (Kogka et al., 2021). In addition, since BE only detects bacteraemia, it is impossible to detect generalised disease caused by viruses or parasites. In response, some countries, e.g., Norway in 2014 (Norwegian Ministry of Health and Care Services, 2014), have stopped using BE, whereas others continue using it.

In Germany (DE), BE is still being used as decision support in meat inspection for individual slaughter animals, and there have only been slight modifications and adaptions in the methods since the 1920s. The use of BE has always been connected to the national legislation on meat inspection, with detailed descriptions of when to use the method, which samples to take and which tests to perform. In contrast to former regulations, detailed descriptions on the condition describing when to use BE are missing in the current national Administrative Regulation (AVV Lebensmittelhygiene, 2009).

In Denmark (DK), BE was introduced in 1923. Since then, the details have been updated several times, including indications for the use of BE, choice of matrices and specifications regarding how to pack and transport samples. As in DE, the use is closely connected to the national legislation specifying when and how to undertake a BE. Moreover, decision criteria have been defined to interpret the outcome of the BE. The validity of BE was recently assessed, and the conclusion was that it is still considered as a useful part in the decision making in specific cases (Kogka et al., 2021).

In Finland (FI), the requirement for a BE in meat inspection was introduced in 1922 (Finnish Government Decision 202/1922), but there was no description of the required method. A common method was first described in 1933, and it has subsequently been modernised and simplified over the years, for the last time in 2000 (Finnish Ministry of Agriculture and Forestry, 1982, 2000; Finnish Ministry of Agriculture, 1933, 1937, Finnish Ministry of Agriculture, 1961). During these years, the number of sample matrices and performed analyses has been reduced and the media used in the analyses have been updated. In addition, the indication for BE and assessment of the results have been simplified. In 2021, the method for BE was removed from the legislation, but BE can still be used (Finnish Governmental Bill 3/2021).

In Italy (IT), BE after emergency slaughter was made mandatory in 1928 (Italian Royal Decree, 1928) and this obligation is still in force all over the country. Today, the obligation is fulfilled in different ways in the different regions, because the National Health Service has been organised by the regions since 1992 (Legislative Decree No 502, 1992). For instance, in Emilia-Romagna and Lombardy, which are the most relevant regions in terms of animal production in IT, similar guidelines have been implemented, specifying the use of BE and the sampling procedures to be adopted (Emilia-Romagna, 2014; Lombardia, 2012).

In the Netherlands (NL), a law on meat inspection was introduced in 1919, after which a detailed method for implementation for BE was described in 1923 (Dutch Meat Inspection Act, 1919; Dutch Ministry of Labor, Trade and Industry, 1923). The law has been updated multiple times during the last century, for the last time in 2002 (Dutch Inspection Regulations, 1994; Dutch Regulations on Examinations, 2002).

Hence, the laboratory methods used for BE are based on methods established several decades ago, and there is no European consensus regarding how to set up the methods and how to interpret the results. As a consequence, the BEs differ between the countries (Schalch et al., 1998; Von Ostertag & Schönberg, 1955). However, the differences in the methodology and matrices can affect the result of the BE and, therefore, the result of meat inspection. Still, in a larger perspective, BE continues to be used and it reduces the number of unnecessary condemnations, and hence, food losses are kept low in a safe and defensible way.

The purposes of this descriptive article are to 1) map the BE methods currently used in five European countries participating in the study, 2) evaluate the extents to which the laboratory approach, interpretation of results and the frequency of their use differ between countries, 3) discuss how any differences can affect the uniformity of meat inspection performed in different countries, and 4) discuss alternative approaches.

2. Materials and methods

We collected information on BE methods used in support of meat inspection, data on how often BE is used in meat inspection, and the proportion of cases where the outcome of laboratory analysis has led to condemnation of carcasses. This work was done under the COST Action 18105 RIBMINS. For this study, data were collected for cattle and pigs from five European countries participating in the RIBMINS network and that were known to use BE in support of meat inspection. The countries included were Germany (DE), Denmark (DK), Finland (FI), Italy (IT) and the Netherlands (NL). For IT, only data from the regions Emilia-Romagna and Lombardy were included. For reasons of simplicity, these were defined as "IT" in this article.

We collected descriptions of the laboratory methods used for BE for cattle and pigs. Based on the descriptions, flowcharts of the laboratory procedures were created using Microsoft PowerPoint. In addition, the following data were extracted:

1) Indications for the use of BE method
2) Samples used (matrix, weight, other information)
3) Sample pre-treatment
4) Short description of the laboratory procedure (e.g., culture media, incubation conditions and duration, interpretation of the results)
5) Description of how the results are used for a meat inspection decision.

To compare the frequency of use and the outcome of the BE, the following data from 2019 for cattle and pigs were collected from each of the five countries:

1) Number of slaughtered animals in total
2) Number of animals examined for BE
3) Outcome of the BE

This study focuses only on the BE methods used in meat inspection and how BE supports the meat inspection decision. Possible links to or effects on, e.g., surveillance programmes, are outside the scope of this study.

3. Results and discussion

The five countries included in this study were selected from the RIBIMINS network and represent ca. 27% of slaughtered bovine animals and ca. 39% of slaughtered pigs in the EU in 2019 (Eurostat, 2023). It is likely that more countries may be using BE in one form or another. Moreover, not all methods used for BE in the EU are likely to be represented here. Nonetheless, the five countries selected for the present study provided pertinent data on the considerable variety of BE methods used today, as described below.

3.1. Indication for the use of bacteriological examination

The indication for the use of BE differs between the five countries, although in general, it is used to check for presence of a bacterial

systemic infection in the slaughtered animal at the time of slaughter. In DK, BE is in principle only used if endocarditis and/or endophlebitis are observed during post-mortem meat inspection, as these lesions are indications of a systemic condition, and where it is necessary to rule out that the disease stage is still generalised at the time of slaughter (Kogka et al., 2021). In NL, BE is performed if the inspecting veterinarian suspects bacteriaemia based on meat inspection findings (Dutch Regulations on Examinations, 2002). In FI, BE is indicated if suspicion arises of a zoonosis transmitted via meat and in cases where a systemic infection in the slaughter animal is thought to be caused by bacteria (Finnish Ministry of Agriculture and Forestry, 2000). In IT, BE is always performed in cases of emergency slaughter and in cases when the official veterinarian requests it to inform a final decision on carcass destination (Italian Royal Decree, 1928). In DE, after the implementation of the EU Regulations of the Hygiene Package and the adoption of the new national Administrative Regulation (AVV Lebensmittelhygiene, 2009), no clear case definition is mentioned any longer. Still, BE in the context of meat inspection must be undertaken to serve as an aid for the assessment of individual carcasses regarding fitness for human consumption when bacteria are a concern. Therefore, the indications stated in the former German regulation for meat hygiene can still be applied (FlHV, 2001) – namely, i) generalised condition, ii) acute inflammation without signs of generalised condition, iii) pathological alterations with potential risk to humans due to bacteria, iv) animals from *Salmonella*-positive herds, v) evisceration later than 1 h post stunning, vi) the carcass parts necessary for post-mortem meat inspection are missing, vii) emergency slaughter, viii) additional information is present that makes BE necessary. Hence, these indications can and are applied in DE for deciding whether a BE should be performed or not.

Indications for when not to use a BE are equally as important as the indications for when to use a BE (Jepsen, 1960). If intoxication or viraemia is suspected, then BE is not indicated, as it will not be able to detect the toxin or virus. Additionally, BE is not intended to be used in cases where decisions regarding the condemnation can be made without any test, e.g., if multiple lesions present in the carcass point to the need for total condemnation. If a systemic disease is evident prior slaughter, the animal should not be transported to the slaughterhouse or it should be rejected in ante-mortem inspection (EC No 853/2004; EU 2019/627).

3.2. Collected samples and sample pre-treatment

The sample matrices analysed from each carcass for BE differ between countries (Table 1): in NL, only the spleen is sampled. In DK and FI, muscle and the spleen and in IT, muscle and liver are sampled. In DE, it is mandatory to analyse five different matrices: muscle, spleen, liver, lymph node and kidney. The requirements for size and condition for each sample matrix type are similar in all five countries (Table 1). According to the instructions, the muscle samples need to be covered by fascia and spleen samples need to be undamaged. The integrity of the sample matrix is vital in the prevention of false positive results due to sample contamination (Smit, 1988).

The samples taken at the abattoir must be cooled immediately and stored and transported under chilled conditions (DE: <10 °C, DK: 0–5 °C,FI: 0–4 °C, NL: 0–4 °C) (Agriculture and Forestry, 2000; AVV Lebensmittelhygiene, 2009; Danish Ministry for Food, Agriculture and Fisheries, 2021; Emilia-Romagna, 2014; Finnish Ministry of Agriculture and Forestry, 2000; Lombardia, 2012). Similarly, to the integrity of sample matrix, the storage and transportation temperature are vital to prevent false positive results (Marx & Reuter, 1974; Skovgaard & Kirk Andersen, 1990; Smit, 1988; von Stuker, Schällibaum, & Schweizer, 1977).

The number of sample matrices used for BE has been reduced in many countries over the years, but there is little research published on the suitability of different sample matrices for BE. Schoenmakers (1977)

Table 1
Matrices collected in the abattoir for bacteriological examination in five European counties (DE, Germany; DK, Denmark; FI, Finland; IT, Italy; NL, the Netherlands).

	Muscle	Spleen	Liver	Lymph node	Kidney	Other	Reference
DE	Whole muscle encased in fascia or a coherent muscle piece (approx. 6–8 cm side length) from the lower limb of the fore or hind extremity from one carcass half	Whole, or for large animals or if hyperplasia is present, a hand-sized portion of spleen	Fist-sized piece of liver tissue from the portal area or the Spigelian flap	From the other carcass half than the one from which the muscle was collected: superficial cervical lymph node or large internal iliac lymph node with the surrounding fat or connective tissue	One kidney	Based on case-by-case scenario: - altered parts with associated lymph nodes depending on suspicion (e.g. heart or heart valves in case of suspected erysipelas) - for *Salmonella* excretory herds, additionally to other samples, a 10 cm piece of small intestine with associated lymph nodes	AVV Lebensmittelhygiene (2009)
DK	Extensor muscle from the foreleg, surrounded by fascia	Undamaged spleen				Any additional relevant material	(Danish Ministry for Food, Agriculture and Fisheries, 2021)
FI	Ca. 250 g of muscle surrounded by an intact fascia from the extensors or flexors of the front or back leg	Ca. 250 g of undamaged spleen. If undamaged spleen is impossible to obtain, a 250 g of liver (not from the portal area)	Ca. 250 g of liver from the portal area including the lymph node and the mucous membrane of the gallbladder			Other necessary samples	Finnish Ministry of Agriculture and Forestry (2000)
IT[a]	Muscle cube of at least 10 × 10 × 10 cm from the shoulder		Ca. 200 g of liver				(MP 01/056 IZSLER, 2023)
NL		Undamaged spleen					(Dutch Regulations on Examinations, 2002; Wageningen Food Safety Research, 2016)

[a] Lombardy and Emilia-Romagna.

mentioned that kidney and liver are not suitable matrices to use for the study of aerobic bacteria in BE. A current study in DK investigated, whether the kidneys should be part of a BE to distinguish between so-called slaughter bleeding and septicaemia, in cases when petechiae are found on the kidneys. The conclusion is that the kidney is not ideal as a matrix, because many bacteria can be found also in kidneys that are unconditionally approved at post-mortem inspection (Abildgaard et al., 2023).

The surfaces of the sampled matrices are pre-treated in the bacteriological laboratory by either searing with a hot iron (DK, FI, and IT) or a gas burner (DE and NL), except for the liver sample in FI (Table 2). Although commonly used, searing does not always destroy all external contaminants (Gill, 1979). Care should be taken that the surface is properly seared before sampling to avoid contamination of the sample and the possibility of external contamination should be kept in mind when assessing the results.

After searing, a deep cut with sterile instruments is made into the sample to take smaller samples, which will be used for the analyses. The quantity of the inoculated sample varies between countries between rubbing with an inoculation loop to 2 g (Table 2). The quantity of inoculated sample can affect the sensitivity of the method.

3.3. Analyses performed as part of bacteriological examination

Seven different analyses are routinely conducted as part of BE: 1) aerobic bacteria; 2) anaerobic bacteria; 3) obligatory anaerobic Gram-positive rods or sulphite reducing anaerobic bacteria (*Clostridia*); 4) *Salmonella*; 5) *Listeria*; 6) pathogenic Enterobacteriaceae and; 7) *Erysipelothrix rhusiopathiae* (only for pigs) (Table 3). Aerobic bacteria are analysed in all countries; anaerobic bacteria in DK, FI, and IT; *Clostridia* in DE and DK; *Salmonella* in DE, FI (if needed) and IT; *Listeria* and pathogenic Enterobacteriaceae only in IT; *E. rhusiopathiae* in pigs only in DE. In DE, additional samples can be taken if animals from *Salmonella*-positive farms are slaughtered. Additional analyses can be performed in cases of suspected specific pathogens in DE, DK and FI (Tables 2 and 3).

Among the five countries, the BE in DE is the most elaborate, with four different examinations (aerobic bacteria, *Clostridia, Salmonella*, and *E. rhusiopathiae* for pigs) and five different sample matrices. The BE in NL is the simplest, with only direct culture on a blood agar plate using a spleen sample, incubated aerobically (Table 3, Fig. 1, Suppl. Figs. 1–4).

3.3.1. Analyses for aerobic and anaerobic bacteria

Examination for aerobic bacteria is most often conducted using muscle (in 4/5 countries) and/or spleen (in 4/5 countries) with cultivation on blood agar plates (Table 3). Examination for aerobic bacteria is performed from muscle in IT, from spleen in NL, from muscle and spleen in DK and FI, and from muscle, spleen, lymph node, liver, and kidney in DE (Fig. 1 and Suppl. Figs. 1–4). Direct plating onto blood agar plates is the most used method.

Examination for anaerobic bacteria is conducted from muscle in IT, from muscle and spleen in DK and FI, while in DE, this is performed from muscle and only in cases in which *Clostridia* is suspected (Fig. 1 and Table 2). Similarly, to the examinations for aerobic bacteria, direct plating onto blood agar plates is the most commonly used method.

3.3.2. Analyses for other bacteria

There are differences regarding the type of samples required for *Salmonella* analysis, but all include liver (Table 3). Also, the *Salmonella*

Table 2
Overview of the bacteriological examination methods used in 2019 in five European countries (DE, Germany; DK, Denmark; FI, Finland; IT, Italy; NL, the Netherlands).

Parts of BE method/Sample details	DE	DK	FI	IT[a]	NL
Non-selective examination	Aerobic bacteria	Aerobic and anaerobic bacteria	Aerobic and anaerobic bacteria	Aerobic	Aerobic
Matrix	Muscle, spleen, lymph node, liver kidney	Muscle, spleen	Muscle, spleen	Muscle	Spleen
Pre-treatment	Searing	Searing	Searing	Searing	Searing
Size	Hazelnut sized sample	1 g	Rubbing with an inoculation loop	1 g	0.07–0.1 g
Clostridia[b]	Yes	Yes	No	No	No
Matrix	Muscle	Muscle, spleen			
Pre-treatment	Searing	Searing			
Size	Bean sized sample	1g			
Salmonella	Yes	No	Only if suspected carrier	Yes	No
Matrix	Muscle, spleen, liver, lymph node, kidney		Liver	Liver	
Pre-treatment	Searing		No	Searing	
Size	Aggregated sample consisting of 5 × ca. 2 g amounts (coarsely crushed)		25 g	1 g	
Other specific pathogens	*Erysipelothrix rhusiopathiae*[c]	Haemolytic bacteria are interpreted as specific infection	No	*Listeria,* pathogenic Enterobacteriaceae	No
Matrix	Spleen, lymph node, liver, kidney, additional sample (heart or heart valve)			Liver	
Pre-treatment	Searing			Searing	
Size	Bean sized sample			Streak plating	
Additional pathogens	If suspected	If suspected	If suspected	No	No
Other examinations done together with BE	Antimicrobial residues	Antimicrobial residues	Antimicrobial residues, pH and cooking test	Antimicrobial residues	Antimicrobial residues

[a] Lombardy and Emilia-Romagna.
[b] Defined as Gram-positive anaerobic rods in DE and sulphite-reducing anaerobic bacteria in DK.
[c] Only for pigs.

Table 3
Sample matrices and media[a] used for the different analysis in the bacteriological examination in five European countries (DE, Germany; DK, Denmark; FI, Finland; IT, Italy; NL, the Netherlands).

Analysis/Sample	DE	DK	FIN	IT[b]	NL
Examination for aerobic bacteria					
Muscle	PCA and BA	BA	BA	CMM + BA	
Spleen	PCA and BA	BA	BA		BA
Lymph node	PCA and BA				
Liver	PCA and BA				
Kidney	PCA and BA				
Examination for anaerobic bacteria					
Muscle		BA	BA	CMM + BA	
Spleen		BA	BA		
Examination for *Clostridia*[c]					
Muscle	BA[d], LLB/BHI + BA (aerobic & anaerobic)	IS			
Spleen		IS			
Examination for *Salmonella*					
Muscle	Aggregate sample [f]:				
Spleen	TT/SEL + BPLS+2nd				
Lymph node	plate[g] + biochemical				
Liver	reaction + serotyping of suspected colony		X[e]	RVS + Hektoen	
Kidney					
Examination for *Listeria*					
Spleen				(TSYE)[h]	
Liver				TSYE	
Kidney				(TSYE)[h]	
Examination for pathogenic Enterobacteriaceae					
Muscle				CMM + Gassner	
Spleen				(Gassner)[h]	
Liver				Gassner	
Kidney				Gassner)[h]	
Examination for *Erysipelothrix rhusiopathiae*					
Spleen	BA, Na-azide + BA + Gram-staining				
Lymph node	BA, Na-azide + BA + Gram-staining				
Liver	BA, Na-azide + BA + Gram-staining				
Kidney	BA, Na-azide + BA + Gram-staining				
Additional sample (heart or heart valve)	BA, Na-azide + BA + Gram-staining				

[a] BA, blood agar; BHI, brain heart infusion broth; BPLS, brilliant-green phenol-red lactose sucrose; CMM, cooked meat medium; IS, Iron-sulphite agar, LLB, liver liver broth; Na-azide, sodium azide enrichment; PCA, plate count agar; RVS, Rappaport Vassiliadis soy; SEL, selenite broth; TSYE, tryptic soy yeast extract agar; TT, tetrathionate broth.
[b] Lombardy and Emilia-Romagna.
[c] Defined as obligatory anaerobic Gram-positive rods in DE and as sulphite reducing anaerobic bacteria in DK.
[d] Done if *Clostridia* are suspected.
[e] ISO 6579, NMKL 71 or other method approved by Finnish Food Authority. Performed only, if an animal is a suspected carrier of *Salmonella*.
[f] An aggregated sample of muscle, lymph node, spleen, liver and kidney.
[g] Another agar plate selective for *Salmonella* used by the laboratory.
[h] Done if samples from spleen and kidney are collected.

analysis methods are different. In FI and IT, only a liver sample is used, whereas in DE, an aggregated sample of muscle, spleen, lymph node, liver and kidney is used. In DE and FI, the liver sample is specified as to be taken from the portal area, and in FI part of the lymph node and mucosa of the gallbladder need to be included in the sample. In IT, the liver sample is not specified in detail. The sample size for *Salmonella* analysis varies between 1 g and 25 g. The liver is thought to be infected with *Salmonella* via the portal vein from the intestines (Buxton, 1957), and traditionally, the gallbladder has been considered a common site of *Salmonella* in carrier animals (Buxton, 1957; Field, 1948; Hoedemaker et al., 2014). Particularly *Salmonella* Dublin in cattle has been detected from the gallbladders (Buxton, 1957; Field, 1948). In Finland, *Salmonella* is examined from a liver sample that also includes a part of the lymph node and mucosa of the gallbladder when an animal is a suspected *Salmonella* carrier (Finnish Ministry of Agriculture and Forestry, 2000). However, two recent studies questions how likely *Salmonella* carriage in the gallbladder is in modern pig production, where *Salmonella* is associated with subclinical infection (Alban, Poulsen, et al., 2022; Just et al., 2023).

Clostridia are examined from muscle in DE and from muscle and spleen in DK (Table 3), and *Clostridia* are examined and defined differently in these countries: In DE, *Clostridia* are defined as obligatory anaerobic Gram-positive rods and analysed as such, while in DK, sulphite reducing anaerobic bacteria are in focus. Therefore, the analyses conducted to detect *Clostridia* differ in these two countries.

3.4. Examinations performed together with the bacteriological examination

In all five countries, the presence of antimicrobial residues (the most frequent component of inhibitory substances) is required to be analysed together with BE (Table 2), although FI presently has no legislation stating which examinations are required. In FI, the legislation up until 2020 stated that in addition to antimicrobial residues, pH and cooking tests were required to be done together with BE (Finnish Ministry of Agriculture and Forestry, 2014). There can be at least two reasons for the antimicrobial residue testing. Firstly, diseased animals could have been treated with antimicrobials before being sent to slaughter. Therefore, the carcass and organs could contain residues over the maximum residue limits. Secondly, this possible treatment, similarly to the situation with clinical samples (Markey, 2013; Scheer et al., 2019), could interfere with the BE, resulting in false negative results, because the residues could inhibit the growth of bacteria (Jepsen, 1960). However, in DK, antimicrobial residues in 269 bovine kidney samples collected between 2015 and 2019 and investigated together with BE were all below EU maximum residue limits (Kogka et al., 2021).

3.5. Result of bacteriological examination and judgement criteria

As a general rule, the meat inspection decision is made by the official veterinarian taking into account the laboratory results together with post-mortem findings from the carcass and organs. However, there is substantial variation between the countries in the written instructions that the official veterinarians can rely on when interpreting the laboratory results in support of the meat inspection decision.

3.5.1. Aerobic and anaerobic bacteria

The interpretation of aerobic and anaerobic bacteria differs considerably between the countries.

In DE, the number of colonies on blood agar is interpreted semi-quantitatively (0 = negative, 1–20 colony forming units (cfu)/plate = weakly positive, >20 cfu/plate = strongly positive). The laboratory must report if bacteriaemia is present or if high bacterial loads are assumed to be present due to contamination. The results of the cultivation of the muscle, spleen, lymph node, liver and kidney are first assessed individually and then as a whole. Finally, the laboratory results are interpreted together with the findings on the carcass.

In DK, the presence of haemolysis, the number of colonies on blood agar and the type of organ (muscle or spleen) all affect the interpretation of the results. Haemolytic bacteria are perceived as more pathogenic than non-haemolytic bacteria. Therefore, the limit of acceptance is lower for haemolytic bacteria than for non-haemolytic bacteria. In general, the presence of bacteria cultivated from the muscle is perceived as more serious than presence of bacteria cultivated from the spleen, so the limit of acceptance is lower for muscle samples. Some laboratory results lead to partial condemnation, i.e., condemnation of the just the

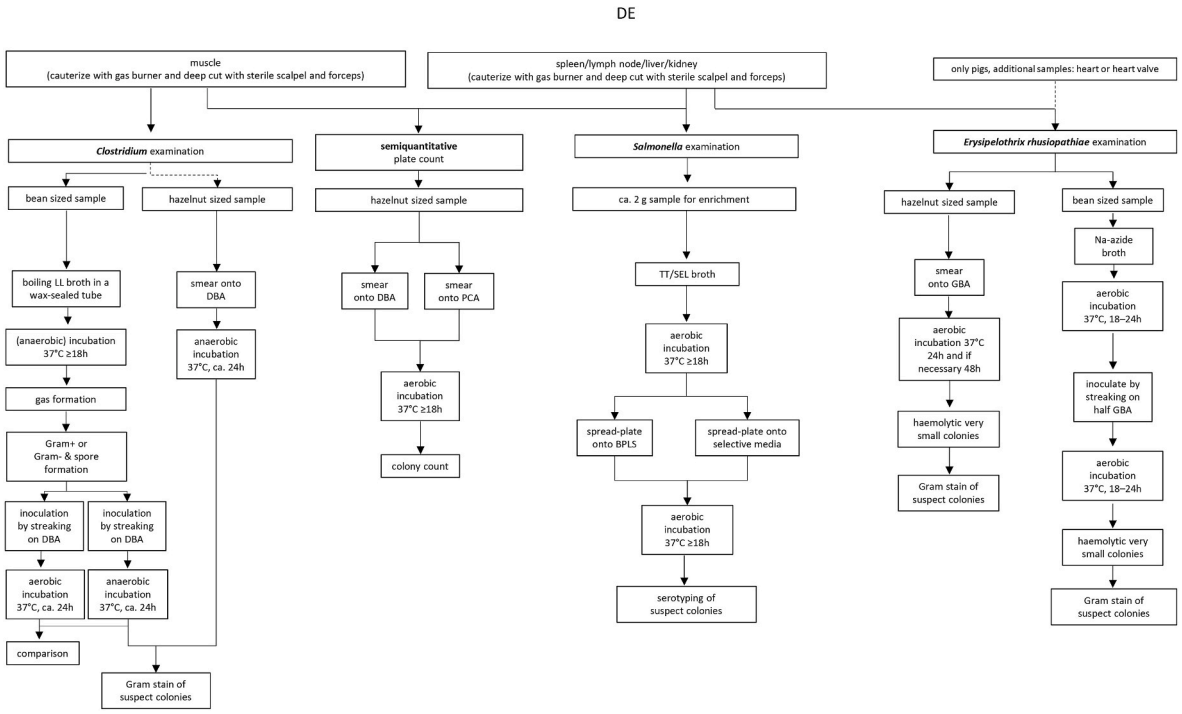

Fig. 1. Flowchart of the bacteriological examination method used in Germany. Dashed arrow; path used only in special cases, if a carcass is suspected to harbour *Clostridia* and as additional samples only for pigs for the examination for *Erysipelothrix rhusiopathiae.* BPLS, brilliant-green phenol-red lactose sucrose agar; DBA, dextrose blood agar; GBA, glucose blood agar; LL broth, liver liver broth; PCA, plate count agar; SEL broth; selenite broth; TT broth, tetrathionate broth.

liver, lungs and kidneys. In contrast, other laboratory results result in total condemnation of the carcass and organs. The cut-off points for total condemnation based on haemolytic bacteria are >3 cfu/plate from muscle and >10 cfu/plate from spleen. Over 10 non-haemolytic colonies per plate from muscle or from spleen as well as 5–10 haemolytic colonies per plate from spleen lead to condemnation of all organs (Kogka et al., 2021).

In NL, unlike in DK, the presence of haemolysis does not affect the interpretation of the results. Colony count limits on blood agar are prescribed, so > 10 cfu per sample leads to total condemnation.

In FI, there is no description of what is regarded as a positive result in BE indicating systemic infection. According to FI method, if the cultivated bacteria are suspected to be pathogenic, culture purification and further examinations are done to determine the bacterial species, if needed.

In IT, the detection of typical colonies on a plate are a requirement for positive outcome of BE indicating systemic infection.

Little research can be found on the reasoning behind the limits for numbers of bacterial colonies in BE. However, Schoenmakers (1977) studied the connection between the bacterial counts in spleen and in muscle and concluded that with a colony count of ten or fewer from spleen, no bacteria are expected from the muscle. In any case, if both muscle and spleen are tested, bacterial findings from muscle should be interpreted more strictly than those from spleen, since spleen is part of reticuloendothelial system (Bohnsack & Brown, 1986; Schoenmakers, 1977). In contrast, the muscle of healthy animals is virtually sterile (Gill, 1979), although a low number of bacteria can be found occasionally, possibly being transported passively with the blood (Kruse et al., 2015).

As the above shows, there are some conceptual differences in the methods applied in the five countries. The method in DK includes the concepts of specific infection and non-specific infection, represented by haemolytic and non-haemolytic bacteria, which are not used in the other four countries. The results of the analyses are also interpreted differently in the countries.

3.5.2. Analyses for other bacteria

As previously mentioned, *Clostridia* are defined slightly differently in DE and DK, and therefore, the definition of a positive laboratory result is accordingly different. In DE, the examination for obligatory anaerobic Gram-positive rods is determined as positive or negative depending on growth and gas production in brain heart infusion or liver-liver broth, on growth on anaerobically incubated blood agar and on Gram reaction. The result must be reported as positive or negative with indication of the cultivation method (direct plating or after enrichment).

In DK, the presence of more than one colony together with blackening on iron sulphite agar leads to total condemnation of the carcass.

There are some differences between the countries concerning when a sample is considered as positive for *Salmonella.*

In DE, the sample is determined as *Salmonella*-positive after biochemical and serological tests. If serotyping does not result in a definite result, the isolates are sent to a reference laboratory.

In FI, all *Salmonella* isolates must be sent to the reference laboratory for confirmation (Finnish Ministry of Agriculture and Forestry, 2000), and *Salmonella* detected in BE from other samples than from muscle sample after searing leads to the requirement to heat-treat the carcass (Finnish Ministry of Agriculture and Forestry, 2021).

In IT, detection of typical *Salmonella* colonies on selective agar plates are considered positive. There are no definite guidelines for the judgement of the carcass and organs, and the results must be reported to the official veterinarian who decides while taking into account the post-mortem findings of the carcass.

Listeria, pathogenic Enterobacteriaceae and *E. rhusiopathiae* are used only in DE and IT. In DE, after selective Na-azide enrichment, the presence of typical colonies on blood agar, based on haemolysis and Gram-staining, are considered as positive for *E. rhusiopathiae.*

In IT, typical colonies on the selective agar plate are considered positive for *Listeria* and pathogenic Enterobacteriaceae.

In general, assessment of a positive result for a pathogen could be considered as a more straightforward process than the analyses for aerobic or anaerobic bacteria without further identification of the

bacteria. Indeed, detection of a pathogen inside a muscle or organ sample could be considered to be a positive laboratory result that should lead to condemnation. However, the quantitative number of bacteria would also play a role regarding food safety risk. We observed that there are differences in the level of pathogen identification. In some cases, the pathogen needs to be identified to the subtype level (e.g. *Salmonella* in DE), while in other cases, it is enough to have typical colonies on a selective agar (e.g. *Listeria, Salmonella* and pathogenic Enterobacteriaceae in IT), or even something in between (*E. rhusiopathiae* in DE).

3.5.3. Written instructions for interpretation of results and condemnation of carcasses

The level of detail in the description of a positive result enables the required interpretation, but the descriptions differ between the countries from no written description to detailed and specific limits for action. Although difficult to define, the official national guidelines for interpretation of laboratory results, where they exist, seem appropriate. E.g., in NL, the cut-off point of 10 cfu/sample was set to avoid misinterpretation of the results due to technical reasons and to rule out (i.e., to disregard) the sporadic occurrence from spleen of bacteria not caused by bacteraemia (Schoenmakers, 1977). As there are no harmonised guidelines and, due to considerable differences in the methods, it does not seem possible to create them. Still, it would be helpful to create national guidelines on how to interpret laboratory results and when they lead to condemnation. It would also be beneficial if the countries using BE co-operated in making these guidelines. This would enable the official veterinarians to have more support for their meat inspection decisions based on BE, and meat inspection decisions could be more harmonised.

3.6. The current use and the outcome of bacteriological examination

BE can be used for both pigs and cattle in all countries. However, it is not in practice used for pigs in DK or NL (Table 4). In addition, BE is more frequently used for cattle than for pigs in the three countries where the method is used for both species. BE is also more frequently used for finishing pigs than for sows or boars, and more often for cows than for other cattle types (Table 4).

The frequency of BE being required by an official veterinarian differs considerably between the countries: For cattle, BE is most frequently used in IT (3.4%), followed by DE (0.17% of all cattle slaughtered), DK (0.02%), FI (0.01%), and is least used in the NL (0.003%). If applied to finishing pigs, BE is most frequently used in DE (0.01%), followed by FI (0.001%), and is least used in IT (0.0004%). An investigation of the reasons for this variation in BE frequency was outside the remits of our study. However, various reasons could affect how often BE is used, such as the cost of analyses, the value of each carcass, the prevalence of diseases in the animal population, the reason for slaughter (animals removed from production vs. meat production), the indication in the national legislation for the examination, laboratory availability, ease of sending samples and speed of results. The costs of the analyses differ between the countries, at least between those countries from which data were found. In IT, where the use of BE is most frequent, the cost of BE and antimicrobial (AM) residue testing is approximately € 16 (MP 01/056 IZSLER, 2023; MP 02/003 IZSLER, 2023), whereas in DK, the total cost of the BE including the antimicrobial residue testing is € 122 for small and € 500 for large abattoirs (Danish Ministry of Food, Agriculture and Fisheries, 2023; Maybritt Kiel Poulsen, Personal communication). In DK, BE is not used for pigs due to the costs (Kogka et al., 2021).

As also previously shown, the data recorded from meat inspection differs between countries (Alban, Vieira-Pinto, et al., 2022): The outcome of the BE is centrally collected in DK, FI and NL, but not in DE and IT. In 2019, a total of 6%, 8% and 18% of the beef carcasses subjected to BE were subsequently totally condemned due to the outcome of BE in FI, NL and DK, respectively (Table 4). The outcome of BE of finishing pigs was available only from FI, where 21% of 14 carcasses with BE were condemned. Due to the differences in the data recording and the low number of BEs performed in DK, FI, and NL, where the outcome of the BE is available, it was not possible to compare the frequency of condemned carcasses due to BE in the different countries.

Table 4
Use of bacteriological examination (BE) in bovine and pig meat inspection in 2019.[a].

Country	Animal population	N animals slaughtered in total	Bacteriological examination	
			N tests (% of slaughtered animals)	N totally condemned[b] (% of BE tests conducted)
DE	Cattle (all)	3 406 984	5874 (0.17)	ND[c]
	Calves	323 932	125 (0.04)	ND[c]
	Cows	1 209 125	3759 (0.31)	ND[c]
	Heifer	578 803	690 (0.12)	ND[c]
	Bulls, steers	1 295 124	1300 (0.1)	ND[c]
	Finishing pigs	53 561 424	6568 (0.01)	ND[c]
	Breeding pigs (sows, boars)	955 386	53 (0.005)	ND[c]
	Suckling pigs	803 789	0 (0)	ND[c]
DK	Cattle	468 000	77 (0.02)	14 (18.1)
	Finishing pigs	16 178 602	0	0
	Sows	534 210	0	0
FI	Cattle	267 408	33 (0.01)	2 (6.1)
	Finishing pigs	1 788 634	14 (0.001)	3 (21.0)
	Sows	33 391	0	0
IT[d]	Cattle	960 178	32 427 (3.4)	ND[c]
	Pigs	8 276 183	28 (0.0004)	ND[c]
NL	Cattle	2 065 685	63 (0.003)	5 (7.9)
	Pigs	15 686 570	0	0

[a] DE, Germany; DK, Denmark; FI, Finland; IT, Italy; NL, the Netherlands.
[b] Based on the outcome of the bacteriological examination, acceptance, a partial or a total condemnation of the carcass can take place.
[c] No data, data not centrally collected.
[d] Lombardy and Emilia-Romagna.

3.7. Future prospects

The original aim of using BE was to assist the meat inspector in judging meat fitness, whereby unnecessary condemnations could be avoided in a safe way. Around 100 years later, this aim is still relevant and in line with the European Green Deal (European Commission, 2019): minimise food loss and food waste when possible. However, BE has its limitations, and concerns have been raised regarding the method's ability to separate carcasses that are fit for consumption from carcasses that are not fit for consumption in an objective way as described by Kogka et al. (2021).

If we go back in history to the intention of why BE was implemented, it was to identify animals that harboured a generalised infection at time of slaughter and to identify ill animals. Because the overall goal was to prevent consumption of meat from infected animals, due to zoonotic disease risks. Today, apart from bacteriological methods in modern laboratories, also other possibilities exist. One of the promising methods is testing for acute phase proteins (APPs) that are indicative of a recent or ongoing infection seems a promising tool for modernised BE. APPs are produced by the animal, and some forms appear in the blood soon after infection, whereas others appear later during an infection (Eckersall & Bell, 2010; Heegaard et al., 2000; Karreman et al., 2000; Schrödl et al., 2016). The cause of infection is impossible to infer from APP measurements only, but they could be used as an indicator of generalised disease, including bacteraemia (Gutiérrez et al., 2015; Tourlomoussis et al.,

2004). To investigate the utility of APPs, it is important to identify the specific APPs that are relevant for BE cases specifically, or generalised disease in general, ascertain the normal protein range, and determine the species-specific animal-to-animal variation. Measuring APPs could be possible using on-site tests, reducing the costs and time needed for a test. Therefore, joint efforts and future studies focusing on this topic are needed before APPs measurements can be considered as a potential replacement of BE in post mortem meat inspection (Gutiérrez et al., 2015; Tourlomoussis et al., 2004).

4. Conclusions

Based on our study, there are differences between the five European countries studied in BE procedures: indications, matrices, samples, laboratory methods, interpretations of the results, and outcomes of BE. Among the bacterial groups analysed, examination for aerobic bacteria was conducted in all five countries and most often direct plating onto blood agar is used. However, the interpretation of the results differed between the countries. The frequency of use of BE and the prevalence of condemned carcasses based on the BE also differed between countries. However, the reasons for these differences are not known. There were not always clearly written instructions on how to interpret the laboratory results or on when to condemn the carcass. Guidelines to interpret the laboratory results and instructions for condemnation decisions should be developed to help the official veterinarians and to reduce variation in the meat inspection decisions within each country. The countries using BE could cooperate in drafting such guidelines for the use of BE in meat inspection. However, full harmonisation of the methods does not seem to be feasible due to the considerable variation observed. Other methods that would be easier to standardise and accredit should be examined and developed.

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CRediT authorship contribution statement

Riikka Laukkanen-Ninios: Conceptualization, Methodology, Investigation, Visualization, Writing - original draft, Writing - review & editing. **Nina Langkabel:** Visualization, Investigation, Writing - original draft, Writing - review & editing. **Sergio Ghidini:** Visualization, Investigation, Writing - review & editing. **Mariel Pikkemaat:** Investigation, Writing - review & editing. **Elisabeth G. Biesta-Peters:** Investigation, Writing - review & editing. **Kees van der Ark:** Visualization, Investigation, Writing - review & editing. **Lis Alban:** Conceptualization, Visualization, Supervision, Investigation, Writing - original draft, Writing - review & editing.

Declaration of competing interest

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Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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