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PROPOSED DRAFT GUIDELINES FOR THE CONTROL OF SHIGA TOXIN-PRODUCING *ESCHERICHIA COLI* (STEC) IN RAW BEEF, FRESH LEAFY VEGETABLES, RAW MILK AND RAW-MILK CHEESES, AND SPROUTS: GENERAL SECTION

(*Prepared by the Electronic Working Group co-chaired by Chile, France, New Zealand and the United States of America*)

SUMMARY

1. The proposed draft Guidelines for the Control of Shiga Toxin-Producing *Escherichia coli* (STEC) in Raw Beef, Fresh Leafy Vegetables, Raw Milk and Raw-Milk Cheeses, and Sprouts was circulated for comments at Step 3 in December 2021. Changes to the General Section in the document have been made based on comments received (CX/FH 22/52/7 Add.1), as considered appropriate by the co-chairs. Generally, changes were for clarification. Some suggestions were not accepted because they conflicted with other changes, were not clear or would be confusing, or specific language was not provided. Some proposed changes have been placed in square brackets for Committee input.

2. A request to specify in paragraph 6 that the control measures were for STEC strains of public health relevance in the specified commodities was not accepted; paragraph 6 had previously been modified to refer to control measures specific for STEC strains in general, instead of for STEC strains of public health relevance, since the STEC strains relevant to public health can vary among countries.

3. A request to add text after paragraph 9 about STEC transmission from other sources such as animals and person-to-person spread, as well as information about sampling at different stages of processing being used in a quantitative risk assessment simulating meat treatment scenarios that reduce risk and improve existing controls, was not considered appropriate at that location in the document or necessary.

4. As explained in CX/FH 22/52/7, the paragraphs that described GHP-based and hazard-based control measures had been deleted, since the control measures in the annexes are not presented as either GHP-based or hazard-based. However, there were still places in the text that referred to "GHP-based" or "hazard-based" control measures. Some countries have indicated these should be deleted, which has been done except in paragraph 28, which refers to incorporation of "hazard analysis activities and hazard-based control measures."

5. Several definitions were modified based on the comments. For the definition of "Fresh leafy vegetables" two options to indicate that these were to be eaten without cooking had been presented in square brackets; the first option ("where the leaf is intended for consumption without cooking") was generally preferred. Two options were presented in square brackets for a definition of "Indicator microorganisms;" the second option was preferred, with a number of modifications. The definition of "Raw beef" was modified to refer to meat from slaughtered cattle, to match the definition in the raw beef annex. One comment indicated that the definition should be clarified as to whether meat preparations (such as meat tenderized with injection of brine) are included in the definition, since it is included in the scope of the raw bee annex but is not mentioned in the definition. However, while appropriate for the scope of the raw been annex, this did not seem necessary for the definition.

6. Revisions to the definition of "Raw milk" were made based on comments. A revision to the definition of "Sprouts" was not included; the suggestion and its rationale should be considered in the development of the Sprouts Annex.

7. It was suggested that the definitions for the commodities not be duplicated in the annexes; the Committee had previously indicated that the definitions should appear in both places – in the general section

to understand the commodities to which the text applied and again in the annex for the specific commodity, so the annex could be read without referring to the General Section.

8.   A recommendation was made to delete Section 6.1 on Development of Risk-Based Control Measures (since the paragraphs did not contain any specific information related to the control of STEC) or to replace paragraphs 30-33 with a cross-reference to the *Principles and Guidelines for the conduct of Microbiological Risk Management* (MRM) (CXG 63-2007). If the paragraphs are kept, there is text in square brackets in paragraph 31 to choose from. Paragraph 32 has been deleted due to lack of quantitative examples of the likely level of control.

9.   Paragraph 61 was deleted, as it was repetitive of paragraph 60.

10.   With respect to paragraphs 62-68 on Laboratory Analysis Criteria for Detection of STEC, a suggestion was made to develop the section to better explain how virulence genes can be taken into account in corrective actions, considering in addition other elements such as whether food is ready to-eat (e.g., raw milk and raw dairy products), eating/cooking habits, etc. However, specific text was not provided.

11.   A recommendation was made to delete paragraphs 69-71 (since the paragraphs did not contain any specific information related to the control of STEC) or to replace the paragraphs with a cross-reference to the *Principles and Guidelines for the conduct of Microbiological Risk Management* (MRM) (CXG 63-2007). Instead, it was clarified that the review was for monitoring data for STEC.

RECOMMENDATIONS

12.   CCFH is invited to consider the revised General Section provided in Appendix I with tracked changes; in particular, whether to:

   i.   delete or retain terms such as "GHP-based" and "hazard-based" control measures as indicated (see paragraphs 10, 14, 15, 28, 39, 54);

   ii.   accept the revised definitions;

   iii.   reaffirm the decision to retain commodity definitions in both the General Section and in the specific commodity annexes;

   iv.   delete Section 6.1 on Development of Risk-Based Control Measures, replace paragraphs 30-33 with a cross-reference to the *Principles and Guidelines for the conduct of Microbiological Risk Management (MRM)* (CXG 63-2007), or revise the text based in the revisions as indicated (selecting the desired square-bracketed text in paragraph 31); and

   v.   whether to explain how virulence genes can be taken into account in corrective actions.

APPENDIX I

GUIDELINES FOR THE CONTROL OF SHIGA TOXIN-PRODUCING *E. COLI* (STEC) IN RAW BEEF, FRESH LEAFY VEGETABLES, RAW MILK AND RAW MILK CHEESES, AND SPROUTS

INTRODUCTION

1.   Shiga toxin-producing *Escherichia coli* (STEC) are recognized as foodborne pathogens, causing human illnesses with a wide range of mild to severe gastrointestinal presentations from asymptomatic to diarrhoea to bloody diarrhoea, occasionally leading to severe hemolytic uremic syndrome with kidney failure and death. Strains of *E. coli* that are pathogenic to humans have been classified into several groups, and STEC are defined by the potential to produce one or more Shiga toxins. ~~The most well-known STEC pathogen is *E. coli* O157:H7, and STEC strains with genomic and pathogenic features similar to *E. coli* O157:H7 may be referred to as enterohemorrhagic E. coli (EHEC). Although the group is quite diverse, *E. coli* O157:H7 is considered the most well-documented.~~ STEC strains are a diverse group which can cause disease in humans. These strains may be referred to as enterohemorrhagic *E. coli* (EHEC). The most well-studied and documented STEC strain is *E. coli* O157:H7. The burden of the disease and the cost of control measures are significant; STEC outbreaks have been associated with diverse food commodities, and thus STEC have the potential to have a serious impact on public health.

2.   Clinical symptoms of the disease in humans arise as a consequence of consuming food contaminated with *E. coli* that produces ~~protein toxins~~ Shiga- toxin type 1 (Stx-1) (encoded by the gene *stx1*)~~,~~ and/or Shiga- toxin type 2 (Stx-2, encoded by the gene *stx2*)~~ or protein toxins from a combination of these genes~~. Historically, the term verotoxin has also been used for the Shiga toxins of *E. coli* and the term verotoxigenic *E. coli* (VTEC) used as synonymous with STEC. In this document, the term Shiga toxin (Stx) is used to indicate the protein toxin, *stx* to indicate the toxin gene, and STEC to indicate the *E. coli* strains demonstrated to carry *stx* or produce Stx. STEC are pathogenic to humans by entry into the human gut and attachment to the intestinal epithelial cells where production of Stx occurs. Attachment to intestinal epithelial cells is the result of other ~~genes~~proteins, including the principal adherence ~~gene for a~~ protein~~,~~ i~~I~~ntimin, encoded by *eae.* The aggregative adherence fimbriae adhesins commonly associated with enteroaggregative *E. coli,* regulated by the *aggR* gene, when found with *stx* have also been linked to severe illness and have been used as predictors of pathogenicity ~~are also effective adherence factors. These genes, in addition to genes encoding Stx, are considered predictors of the pathogenicity of strains~~. (~~This document provides a~~ Table 1 show~~sing~~ combinations of virulence genes and their association with disease severity that can be used for risk management purposes.) There may be additional genes involved that have not been identified yet. Some of these virulence genes are located on mobile genetic elements (e.g., plasmids, bacteriophages, pathogenicity islands) and can be horizontally transmitted to related microorganisms or be lost. Symptoms and their severity are determined by the variability in these genes, among other factors such as gene expression, dose, host susceptibility, and age. Because STEC are primarily a genotype-based hazard, this has implications for hazard identification and characterization, which will be discussed in this guidance document.

3.   Historically STEC illnesses have been linked to the consumption of raw or undercooked ground/minced or tenderized beef; however fresh leafy vegetables, sprouts, and dairy products (~~in particular~~ raw milk and raw milk cheeses) have been increasingly recognized as commodities that pose a risk of illness from STEC. Sources of STEC in these foods can vary, as does the ability of the organism to survive and multiply within them. The association of specific food categories with STEC illness reflects the historical and current practices of food production, distribution and consumption. Changes in food production, distribution and consumption can cause changes in STEC exposure. Consequently, microbial risk management should be informed by an awareness of current local sources of STEC exposure. This guidance document will identify commodity-specific intervention practices based on known source attribution in these different foods, and practices for monitoring STEC in food products, including the utility of indicator microorganisms.

4.   It is generally accepted that animals, in particular ruminants, are the primary reservoir/source of STEC. STEC-positive ruminants are typically asymptomatic. Contamination with intestinal content or feces is the ~~likeliest ultimate~~most likely initial source of STEC in most foods. For example, STEC outbreaks have been associated with raw beef contaminated with STEC during the slaughtering process, field-grown fresh leafy vegetables have been linked to STEC-contaminated irrigation water, and STEC illnesses from sprouts have resulted from contamination during seed production enhanced during sprouting. Raw milk is most commonly contaminated as a result of soiled udders and teats, as well as poor hygiene during milking.

5.   The large degree of variation exhibited by STEC in their biological properties, host preferences, and environmental survival presents a challenge for ~~controlling~~ managing the presence of STEC in animal and plant production. In practice, this means that there is no "one size fits all" solution, and different production systems may require different approaches to control the various ~~serovars~~ serotypes of STEC. In most instances, control measures will reduce STEC but not eliminate them.

6.   The Guidelines build on general food hygiene provisions already established in the Codex system and propose potential control measures specific for STEC strains in raw beef, fresh leafy vegetables, raw milk and raw milk cheeses, and sprouts.

7.   Examples of control measures in each commodity-specific annex have been subjected to a scientific evaluation by JEMRA in development of the Guidelines. Such examples are illustrative only and their use and approval may vary among member countries.

8.   The format of this document:

- Provides an opening general section with STEC guidance applicable to all commodities.

- Demonstrates the range of the approaches of control measures for STEC.

- Facilitates development of hazard analysis and critical control points (HACCP) plans at individual establishments and at national levels.

- Assists in assessing the equivalence[1] of control measures for raw beef, fresh leafy vegetables, raw milk and raw milk cheeses, and sprouts applied in different countries.

9. The Guidelines provide flexibility for use at the national (and individual processing) level.

2. OBJECTIVES

10. These Guidelines provide information to governments and industry on the control of STEC in raw beef, fresh leafy vegetables, raw milk and cheeses produced from raw milk, and sprouts that aims to reduce foodborne disease. The Guidelines provide a scientific tool for the effective application of GHP- and hazard-based approaches for control of STEC in raw beef, fresh leafy vegetables, raw milk and raw milk cheeses, and sprouts according to national risk management decisions. The control measures that are selected can vary among countries and production systems.

11. These Guidelines do not set quantitative limits as described in the *Principles and Guidelines for the Establishment and Application of Microbiological Criteria Related to Food*s (CXG 21-1997) for STEC in raw beef, fresh leafy vegetables, raw milk and raw milk cheeses, and sprouts. Rather, the Guidelines describe control measures that countries can establish as appropriate to their national situation as described in the *Principles and Guidelines for the Conduct of Microbiological Risk Management (MRM)* (CXG 63-2007).

3. SCOPE AND USE OF THE GUIDELINES

3.1. Scope

12. These Guidelines are applicable to STEC that may contaminate raw beef, fresh leafy vegetables, raw milk and raw milk cheeses, and sprouts and cause foodborne disease. The primary focus is to provide information on scientifically validated practices that may be used to prevent, reduce, or eliminate STEC contamination of raw beef, fresh leafy vegetables, raw milk and raw milk cheeses, and sprouts.

3.2. Use

13. The Guidelines provide specific control measures for STEC in raw beef, fresh leafy vegetables, raw milk and raw milk cheeses, and sprouts according to a primary production-to-consumption food chain approach, with potential control measures being identified at applicable steps in the process flow. The Guidelines are supplementary to and should be used in conjunction with the *General Principles of Food Hygiene* (CXG 1-1969), the *Code of Hygienic Practice for Meat* (CXC 58-2005), the *Code of Practice on Good Animal Feeding (CXC 54-2004)*, the *Code of Hygienic Practice for Fresh Fruits and Vegetables* (CXC 53-2003), the *Code of Hygienic Practice for Milk and Milk Products* (CXC 57-2004), and the *Guidelines for the Validation of Food Safety Control Measures* (CXG 69-2008) and *Principles and Guidelines for the Conduct of Microbiological Risk Management (MRM)* (CXG 63-2007). These general and overarching provisions are mentioned as appropriate and their content is not duplicated in these Guidelines.

14. The Guidelines present a number of GHP-based control measures. GHPs are prerequisites to making choices on hazard-based control measures. Hazard-based These control measures will likely vary at the national level and therefore these Guidelines only provide examples of hazard-based controlsthem. Examples of hazard-based control measures are limited to those that have been scientifically demonstrated as effective in a commercial setting. Countries should note that these hazard-based control measures are indicative only. The quantifiable outcomes reported for control measures are specific to the conditions of particular studies and the control measures would need to be validated under local commercial conditions to provide an estimate

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[1] *Guidelines on the Judgement of Equivalence of sanitary Measures Associated with Food Inspection and Certification Systems* (CXG 53-2003)

of hazard reduction[2]. Government and industry can use choices on ~~hazard-based~~ control measures to inform decisions on critical control points (CCPs) when applying HACCP principles to a particular food process.

15. Several ~~hazard-based~~ control measures as presented in these Guidelines are based on the use of physical, chemical and biological decontamination processes to reduce the prevalence and/or concentration of STEC-positive commodities, for example beef carcasses from slaughtered cattle (i.e. beef from animals of the species of *Bos indicus*, *Bos taurus*, and *Bubalus bubalis)*. The use of these control measures is subject to approval by the competent authority, where appropriate, and varies based upon the type of product being produced. Also, these Guidelines do not preclude the choice of any other ~~hazard-based~~ control measure that is not included in the examples provided herein, and that may have been scientifically validated as being effective in a commercial setting.

16. A provision of flexibility in application of the Guidelines is an important attribute. They are primarily intended for use by government risk managers and industry in the design and implementation of food safety control systems.

17. The Guidelines should be useful when assessing whether different food safety measures for raw beef, fresh leafy vegetables, raw milk and raw milk cheeses, and sprouts in different countries are appropriate.

4. DEFINITIONS

18. For the purposes of this Code, the following terms are defined as below:

19. Fresh leafy vegetables - Vegetables of a leafy nature ~~[where the leaf is intended for consumption]~~ ~~[that may be consumed]~~ without cooking, including, but not limited to, all varieties of lettuce, spinach, cabbage, chicory, endive, kale, radicchio, and fresh herbs such as coriander, cilantro, basil, curry leaf, colocasia leaves and parsley, among other local products for foliar consumption.

20. ~~[Indicator microorganisms - microorganisms that are used to evaluate the microbiological status of food production and food control systems, including the evaluation of the quality or safety of raw or processed food products and the validation of the efficacy of microbiological control measures. Some hygiene indicator microorganisms are total bacterial counts, coliform or faecal coliform counts, total *E. coli* counts and counts of Enterobacteriaceae.]~~ [Indicator microorganisms - microorganisms used as ~~a sign~~an indicator of quality, process efficacy, or hygienic status ~~in~~ of food, water, or the environment, ~~often~~ commonly used to ~~signify suggest~~ the potential presence of pathogens, a lapse in ~~sanitation~~ process hygiene or a process failure. ~~Common~~ Examples of indicator microorganisms include counts of total ~~bacterial counts~~mesophilic aerobic bacteria, coliform~~s~~ or faecal coliform~~s~~ ~~counts~~, total *E. coli* ~~counts~~ and ~~counts of~~ Enterobacteriaceae.]

21. Raw beef – Skeletal muscle meat from slaughtered cattle, including primal cuts[3], sub-primal cuts, and trimmings.

22. Raw milk: Milk (as defined in Codex General Standard for the Use of Dairy Terms (CXS 206-1999)) that is intended for direct consumption or a primary input for dairy products and which has not been heated beyond 40ºC or undergone any treatment that has an equivalent effect.[4] This definition excludes product that has been subjected to processing techniques used for microbiological control (e.g. heat treatment above 40 °C, ~~as well as~~ microfiltration and bactofugation ~~which lead to a decrease in the microbiota equivalent to heating.~~).

23. Raw Milk Cheeses: Cheeses made from raw milk[4].

24. Shiga Toxin-Producing *E. coli (STEC)*: A large, highly diverse group of bacterial strains of *Escherichia coli* that are demonstrated to carry Shiga toxin genes (*stx*) and produce Shiga toxin protein (Stx).

25. Sprouts: Products obtained from the germination of seeds collected before the development of true leaves. The final product contains the seed.

5. PRINCIPLES APPLYING TO CONTROL OF STEC IN RAW BEEF, FRESH LEAFY VEGETABLES, RAW MILK AND RAW MILK CHEESES, AND SPROUTS

26. Overarching principles for good hygienic practice for meat production are presented in the *Code of Hygienic Practice for Meat* (CXC 58-2005), Section 4: General Principles of Meat Hygiene. For fresh leafy vegetables and sprouts, overarching principles for good hygienic practice are presented in the *Code of*

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[2] FAO/WHO 2009. Risk characterization of microbiological hazards in food. Microbiological risk assessment series 17. Available at http://www.fao.org/docrep/012/i1134e/i1134e00.htm and http://www.who.int/foodsafety/publications/riskcharacterization/en/

[3] A primal cut is a piece of meat on the bone initially separated from the carcass of an animal during butchering. Primal cuts are then divided into sub-primal cuts. These are basic sections from which steaks and other subdivisions are made

[4] For technical purposes, cheese curd might be "cooked" (i.e., by application of heat at temperatures below 40°C to expel water from the curds). The heat stresses microorganisms, making them more susceptible to other microbiological control measures. *Code of Hygienic Practice for Milk and Milk Products* (CXC 57-2004), Annex II, Appendix B, p. 43.

Hygienic Practice for Fresh Fruits and Vegetables (CXC 53-2003), Annex I on Ready-To-Eat Fresh Pre-Cut Fruits and Vegetables and Annex III on Fresh Leafy Vegetables. Additionally, see the *Code of Hygienic Practice for Milk and Milk Products* (CXC 57-2004) for dairy products. Two overarching food safety principles that have particularly been taken into account in these Guidelines are:

a) The principles of food safety risk analysis [5] should be incorporated wherever possible and appropriate in the control of STEC in raw beef, fresh leafy vegetables, raw milk and raw milk cheeses, and sprouts from primary production-to-consumption.

b) Wherever possible and practical, competent authorities should formulate risk management metrics[6] so as to objectively express the level of control of STEC in raw beef, fresh leafy vegetables, raw milk and raw milk cheeses, and sprouts that is required to meet public health goals (including focusing on subtypes of particular concern where appropriate).

6. PRIMARY PRODUCTION-TO-CONSUMPTION APPROACH TO CONTROL MEASURES

27. These guidelines incorporate a "primary production-to-consumption" flow approach that identifies the main steps in the food chain where control measures for STEC can potentially be applied in the production of each commodity. The systematic approach to the identification and evaluation of potential control measures allows consideration of the use of controls in the food chain and allows different combinations of control measures to be developed and implemented. This is particularly important where differences occur in primary production and processing systems among countries. Risk managers need the flexibility to choose risk management options that are appropriate to their national context.

28. GHPs provide the foundation for most food safety control systems. Where possible and practicable, food safety control measures for STEC should incorporate hazard analysis activities and hazard-based control measures. Identification and implementation of risk-based control measures based on risk assessment can be elaborated by application of a risk management framework process as advocated in the *Principles and Guidelines for the Conduct of Microbiological Risk Management (MRM)* (CXG 63-2007).

29. While these Guidelines provide generic guidance on development of control measures for STEC, development of risk-based control measures for application at a single step or at multiple steps in the food chain are primarily the domain of competent authorities at the national level. Industry can select the risk-based measures to facilitate the effective application of process control systems and comply with the requirements of the competent authority. [When no microbiological criterion or food safety objectives have been established by competent authorities, industry is also able to propose control measures based on risk assessment. Validation should be performed based on the capacity of the control measures to decrease the risk for public health.]

[6.1 Development of risk-based control measures

30. Competent authorities operating at the national level should, working with the relevant food sector, develop risk-based control measures for STEC where possible and practical.

31. [*Option 1:* Risk modelling tools can be developed to assess the impact of control measures on the reduction or elimination of the hazard. Capability and limitations should be clearly specified.] [*Option 2:* When risk-modelling tools are developed[7], the risk manager needs to understand the capability and limitations.] [*An option that combines the two:* Risk modelling tools can be developed to assess the impact of control measures on the reduction or elimination of the hazard. The capability and limitations of the tools should be clearly specified and understood by the risk manager.]

32. When developing risk-based control measures, competent authorities may use the quantitative examples of the likely level of control of a hazard in this document.

33. Competent authorities formulating risk management metrics[8] as regulatory control measures should apply a methodology that is scientifically robust and transparent.]

7. PRIMARY PRODUCTION CONTROL MEASURES

34. Controls in the primary production phase of the process flow are focused on decreasing the number of animals that are carrying and/or shedding STEC, as well as preventing or reducing plants being contaminated with STEC on the farm. In addition, Good Agricultural Practices (GAPs) and animal husbandry practices related to water, worker hygiene, appropriate use of fertilizers and biosolids, appropriate handling during transport,

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[5] *Working Principles for Risk Analysis for Food Safety for Application by Governments* (CXG 62-2007)
[6] *Principles and Guidelines for the Conduct of Microbiological Risk Management (MRM)* (CXG 63-2007)
[7] *Principles and Guidelines for the Conduct of Microbiological Risk Assessment* (CXG 30-1999)
[8] *Principles and Guidelines for the Conduct of Microbiological Risk Management (MRM)* (CXG 63-2007)

temperature control, and cleanliness of contact surfaces can reduce the incidence of STEC at primary production.

8. PROCESSING CONTROL MEASURES

35. Appropriate controls to prevent and/or reduce the contamination and cross contamination by STEC of commodities during processing are important.

9. **FOOD** DISTRIBUTION ~~CHANNEL~~ CONTROL MEASURES

36. Control measures during distribution to ensure product is stored at an appropriate temperature to prevent growth of STEC beyond a detectable level and to minimize cross contamination by STEC are important.

37. Specific control measures for STEC are described in each commodity-specific annex, where appropriate. The raw beef specific control measures are found in Annex I; the fresh leafy vegetables specific control measures are found in Annex II, the raw milk and raw milk cheeses specific control measures are found in Annex III, and the sprouts specific control measures are found in Annex IV.

10. IMPLEMENTATION OF CONTROL MEASURES

38. Implementation [9] involves giving effect to the selected control measure(s), development of an implementation plan, communication of the decision on control measure(s), ensuring a regulatory framework and infrastructure for implementation exists, and a monitoring and evaluation process to assess whether the control measure(s) have been properly implemented.

10.1 Prior to Validation

39. Prior to validation of the ~~hazard-based~~ control measures for STEC, the following tasks should be completed:

- Identification of the specific measure or measures to be validated. This would include analysis of any measures agreed to by the competent authority and whether any measure has already been validated in a way that is applicable and appropriate to specific commercial use, such that further validation is not necessary.

- Identification of any existing food safety outcome or target established by the competent authority or industry. In order to comply with the target set by the competent authority, industry may set stricter targets than those set by the competent authority.

10.2 Validation

40. Validation of measures may be carried out by industry and/or the competent authority.

41. Where validation is undertaken for a measure based on hazard control for STEC, evidence will need to be obtained to show that the measure is capable of controlling STEC to a specified target or outcome. This may be achieved by use of a single measure or a combination of control measures. The *Guidelines for the Validation of Food Safety Control Measures* (CXG 69-2008) (Section VI) provides detailed advice on the validation process.

10.3 Implementation of validated control measures

42. Refer to the Section 9.2 of the *Code of Hygienic Practice for Meat* (CXC 58-2005), the *Code of Hygienic Practice for Fresh Fruits and Vegetables* (CXC 53-2003), and the *Code of Hygienic Practice for Milk and Milk Products* (CXC 57-2004).

10.3.1 Industry responsibility

43. Industry has the [primary] responsibility for implementing, documenting, validating, verifying and supervising process control systems to ensure the safety and suitability of raw beef, fresh leafy vegetables, raw milk and raw milk cheeses, and sprouts. These should incorporate measures for control of STEC as appropriate to national government requirements and industry's specific circumstances, and where applicable the measures should be applied in accordance with manufacturer's instructions.

44. The documented ~~process~~ control ~~systems~~ measures should describe the activities applied, including any sampling procedures, specified targets (e.g. performance objectives or performance criteria) set for STEC, industry verification activities, and corrective actions.

10.3.2 Regulatory systems

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[9] See Section 7 of the *Principles and Guidelines for the Conduct of Microbiological Risk Management (MRM)* (CXG 63-2007).

45. The competent authority, working with the relevant food sector, ~~[should] [could]~~may provide guidelines and other implementation tools to industry, as appropriate, for the development of the process control systems.

46. The competent authority may assess the documented process control systems to ensure they are science based and establish verification frequencies. Microbiological testing programmes should be established ~~for verification~~to verify the effectiveness of control measures ~~of HACCP systems when specific targets~~ for ~~control of~~ STEC ~~have been identified~~.

10.4 Verification of control measures

47. Refer to Section 9.2 of the *Code of Hygienic Practice for Meat* (CXC 58-2005), the *Code of Hygienic Practice for Fresh Fruits and Vegetables* (CXC 53-2003), the *Code of Hygienic Practice for Milk and Milk Products* (CXC 57-2004), and Section IV of the *Guidelines for the Validation of Food Safety Control Measures* (CXG 69 -2008).

10.4.1 Industry

48. Industry may use testing information on indicator microorganisms for verification of STEC control measures due to the high cost of testing for detection of STEC and its low prevalence in food. Industry verification activities should verify that all control measures for STEC have been implemented as intended. Verification should include observation of monitoring activities (such as having a~~n program~~ employee with overall responsibility for monitoring activities observe the person conducting a monitoring activity ~~perform monitoring procedures~~ at a specified frequency), ~~document verification by~~ reviewing monitoring, corrective action and verification records, and sampling and testing for indicator microorganisms and STEC where appropriate.

49. Due to typically low levels and low prevalence of STEC in food, ~~enumerative~~ quantitative monitoring of STEC is impractical and the utility of presence/absence testing in monitoring process performance is also limited (FAO/WHO 2018). Process performance monitoring may be accomplished more effectively and efficiently by quantitatively monitoring sanitary and hygiene indicator microorganisms. These indicator microorganisms do not indicate pathogen presence or absence; instead, they provide a quantitative measure of the control of general microbial contamination in the product and processing environment. The hygiene indicator microorganisms used should be those that are the most informative for the specific processing environment. Examples of potential hygiene indicator~~s~~ microorganisms include counts of total mesophilic aerobic~~bacterial (mesophilic aerobes) counts~~ bacteria, ~~counts of~~ coliforms or fecal coliforms, ~~counts of~~ total *E. coli,* and ~~counts of~~ Enterobacteriaceae. An increase in the number of the indicator microorganism above established control values indicates a loss of control and the need for corrective action. ~~An increase in the numbers of the selected indicator microorganism indicates decreasing control and the need for corrective action~~. Additionally, with the increase in the frequency of verification, there is also an increase in the speed of detecting a loss of control of manufacturing hygiene. Verification at multiple points in the processing chain can assist in rapid identification of the specific process step where corrective action should be taken. Monitoring of hygiene indicator microorganisms can be supplemented by periodic testing for STEC where appropriate and as needed to make risk-based decisions. STEC testing can contribute to reducing contamination rates, improving food safety, and promoting continuous process improvement, if testing results are linked to requirements for corrective action.

50. Verification frequency could vary according to the operational aspects of process control, the historical performance of the establishment, and the results of verification activity itself.

51. Record keeping is important to facilitate verification and for traceability purposes.

10.4.2 Regulatory systems

52. The competent authority should verify that all regulatory control measures implemented by industry comply with regulatory requirements, as appropriate, for control of STEC.

11. MONITORING AND REVIEW

53. Monitoring and review of food safety control systems is an essential component of application of a risk management framework[10]. It contributes to verification of process control and demonstrating progress towards achievement of public health goals. Effective monitoring includes verifying the effectiveness of STEC control processes throughout the food chain.

54. Information on the level of control of STEC at appropriate points in the food chain can be used for several purposes, e.g. to validate and/or verify outcomes of food control measures, to monitor compliance with ~~hazard-based and risk-based~~ regulatory goals for STEC control, and to help prioritize regulatory efforts to reduce

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[10] See Section 8 of the *Principles and Guidelines for the Conduct of Microbiological Risk Management (MRM)* (CXG 63-2007).

foodborne illness. Systematic review of monitoring information allows the competent authority and relevant stakeholders to make decisions in terms of the overall effectiveness of the food safety control systems and make improvements where necessary.

11.1 Monitoring

55. Monitoring via sampling and testing should be carried out at appropriate steps throughout the food chain using a validated diagnostic test and randomized or targeted sampling as appropriate.

56. For instance, the monitoring systems programmes for STEC and/or indicator microorganisms, when appropriate, in raw beef, fresh leafy vegetables, raw milk and raw milk cheeses, and sprouts may include testing at the farm (e.g. for fresh leafy vegetables), in the slaughter and processing establishments, and the retail distribution chains where appropriate and according to the monitoring objective.

57. Competent authority regulatory monitoring programmes should be designed in consultation with relevant stakeholders, where appropriate, taking into accountand should consider the sampling plan, including the number, location,most cost-efficient resourcing option for collection and testing of samples and resource constraints. Given the importance of monitoring data for risk management activities, sampling and testing components of regulatory monitoring programmes should be standardized on a national basis and be subject to quality assurance.

58. The type of samples and data collected in monitoring systems should be appropriate for the outcomes sought. Enumeration and further characterization of microorganisms generally provides more information for risk assessment and risk management purposes than presence/ absence testing. Where the regulatory monitoring program is to be carried out by industry, there should be flexibility with respect to the procedures used, as long as the industry procedures provide equivalent performance to regulatory procedures.

59. Monitoring information should be made available to relevant stakeholders in a timely manner (e.g. where appropriate, to producers, the processing industry, competent authorities, the public health sector, and consumers).

60. Monitoring information collected from throughout the food chain should be used to affirm achievement of risk management goals. Wherever possible, such information should be combined with human health surveillance data and foodborne illness source attribution data to validate risk-based control measures and verify progress towards risk-reduction goals.

61. Activities that may provide new information to consider in the monitoring include:

  • Surveillance of clinical illness from STEC in humans and

  • Epidemiological investigations, including outbreaks and sporadic cases.

11.2 Laboratory Analysis Criteria for Detection of STEC

62. The choice of analytical method should reflect both the type of sample to be tested and the purpose for which the data collected will be used. The purpose of analysis for bacterial foodborne pathogens, including STEC, can be divided into the following categories:

    • product batch or lot acceptance;

    • process performance control to meet domestic food regulation;

    • to verify controls to meet market access requirements; and

    • public health investigations.

63. The risk of severe illness due to STEC infection can be predicted according to virulence factors (encoded by genes) present in an STEC strain, and testing for such factors should be used as complementary data to assess and predict the pathogenic potential of STEC strains recovered from food samples. Based on current scientific knowledge, all STEC strains are strains are pathogenic for humans and capable of causing illness. However, STEC strains with *stx2a* and adherence genes, *eae* or *aggR* have the greatest association with diarrhoea, severe illness such as bloody diarrhoea (BD), and haemolytic uremic syndrome (HUS) and hospitalisations. Strains of STEC with other *stx* subtypes may cause diarrhoea, but their association with HUS is less certain and can be highly variable. Thus, to appropriately manage the risk of STEC in commodities discussed in this guidance document, tests that detect virulence factors such as these should be used. The risk of severe illness may also depend on virulence gene combinations and gene expression, the dose ingested, and the susceptibility of the human host, so a risk management framework should also be applied when laboratory methodologies for STEC detection are selected by countries.

64. The determination of virulence and other salient marker genes for testing purposes may be achieved by using, for example, polymerase chain reaction (PCR) methods or whole genome sequencing (WGS) analysis

on isolated strains. Special consideration should be given to the efficacy of sample collection techniques to maximize portions of product most likely to be contaminated. The choice of enrichment culture techniques used to recover STEC from foods is also important, as STEC strains are physiologically diverse, with variable growth characteristics. Selective conditions can be used which are permissive to specific sub-populations of STEC, such as *E. coli* O157:H7, but this risks inhibiting the multiplication of other STEC strains, preventing their detection.

65. In addition, bacteria other than STEC may harbor contain the same virulence genes and the detection of these genes alone may not fully reflect health risk due to differential or lack of gene expression. It is also very important to characterize STEC isolates. Indeed, tThe isolation of STEC by immunomagnetic separation (IMS) or by traditional culture-based methods is essential to confirm presumptive PCR positive samples.

66. The number of foods identified as a vector for STEC transmission has increased over time. Baseline studies and targeted surveys are conducted to provide prevalence data and identify risk factors along the food chain. These data, together with public health surveillance data, are used in risk assessments and risk profiles of STEC /food combinations to prioritize foods and STEC of the highest public health relevance. Analytical methods should be chosen that are fit for purpose, that will provide answers to risk management questions, and that are within the resources of governments and industry (FAO/WHO STEC Expert Report, 2018). In the event that a laboratory does not have the resources and technology to characterize the isolate, it could be sent to a reference centre/laboratory.

67. The severity of STEC illness and the potential to cause diarrhoea, bloody diarrhoea and haemolytic uremic syndrome, hence the degree of public health relevance, can be defined by the combination of virulence genes within an isolated strain of STEC. These combinations can be ranked from the most severe (1) to least severe (5), and are recommended by JEMRA[11] as criteria (Table 1) for developing risk management goals that prioritise:

- the STECs of greatest public health relevance,
- the design of monitoring and surveillance programmes by competent authorities, and
- resourcing public health investigations and recalls in response to a positive test.

68. The JEMRA report notes that the association of Stx subtypes other than Stx2 with HUS is less conclusive and varies depending on other factors, for example host susceptibility, pathogen load, and antibiotic treatment.

Table 1. STEC virulence genes in isolated strains and the potential to cause diarrhoea (D), bloody diarrhoea (BD) and haemolytic uremic syndrome (HUS) (where 1 is the highest risk level). * [11]

LEVEL	TRAIT (GENE)	POTENTIAL FOR
1	stx_{2a} + *eae* or *aggR*	D/BD/HUS
2	stx_{2d}	D/BD/HUS**
3	stx_{2c} + *eae*	D/BD^
4	stx_{1a} + *eae*	D/BD^
5	Other *stx* subtypes	D^

* depending on host susceptibility or other factors; e.g. antibiotic treatment
**association with HUS dependent on stx_{2d} variant and strain background
^ some subtypes have been reported to cause BD, and on rare occasions HUS

11.3 Review

69. Periodic review of monitoring data for STEC at relevant process steps should be used to inform the effectiveness of risk management decisions and actions, as well as future decisions on the selection of specific control measures for STEC and provide a basis for their validation and verification.

70. Information gained from monitoring for STEC in the food chain should be integrated with human health foodborne disease surveillance, food source attribution data, and withdrawal and recall data, where available to evaluate and review the effectiveness of STEC control measures from primary production to consumption.

71. Where monitoring of hazards or risks indicates that regulatory performance goals are not being met, risk management strategies and/or control measures should be reviewed.

11.4 Public health goals

72. Countries should consider the results of monitoring and review when reevaluating and updating public health goals for control of STEC in foods, and when evaluating progress. Monitoring of food chain information

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[11] FAO/WHO. 2018. Shiga toxin-producing *Escherichia coli* (STEC) and food: attribution, characterization, and monitoring. Microbiological Risk Assessment Series No. 31. Rome. Available at http://www.fao.org/3/ca0032en/ca0032en.pdf.

in combination with food source attribution data and human health surveillance data is an important component. The surveillance and application of controls for the proper functioning of the STEC control systems need to ensure that the food chain is sufficiently safe for human health.

STEC Guidance General Section References

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