CODEX ALIMENTARIUS COMMISSION

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World Health Organization

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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

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

Codex members and Observers wishing to submit comments at Step 3 on this draft should do so as instructed in CL 2021/63-FH available on the Codex webpage/Circular Letters 2021: http://www.fao.org/fao-who-codexalimentarius/circular-letters/en/

SUMMARY

1. CCFH51 established an electronic working group (EWG) to continue the work on both the General sections and the annexes of the 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. Participants from 34 Codex members and 5 observers registered for the EWG. The EWG redrafted the texts based on the feedback from CCFH as well as the output from the relevant Joint FAO/WHO Expert Meetings on Risk Assessment of Microbiological Hazards in Foods (JEMRA). As CCFH52 was postponed due to the COVID19 pandemic, the revised texts were also distributed by Circular Letter, CL 2021/35-FH, for comments by members and observers, and then further revised.

2. The General section has been revised to improve clarity and to address the comments received in the EWG and from the Circular Letter. A few outstanding issues remain which have been placed in square brackets, including: definitions for indicator organisms and how the responsibility of industry and competent authorities are characterized (e.g. whether it should be indicated that the primary responsibility lies with industry and how strongly that of competent authorities should be stated i.e. should vs could).

3. The annexes have been revised based on discussions within the EWG, comments from Members and Observers and JEMRA scientific advice where available.

4. The list of references included in the Guidelines is for information purposes only to assist in the development of the Guidelines and will not be retained once the Guidelines have been finalised.

5. A full report of the work of the EWG, including a summary of the discussions and decisions, is presented in Appendix I.

RECOMMENDATIONS

6. CCFH52 is invited to consider the proposed draft Guidelines as presented in Appendix II, including the General Section, Annex 1 (Raw Beef), Annex 2 (Fresh Leafy Vegetables), and Annex 3 (Raw Milk and Raw Milk Cheeses), taking into account the report of the EWG as presented in Appendix I, and in particular to:

i. Review the General section for completeness, address, in particular, the abovementioned outstanding issues in square brackets and provide a recommendation on whether it is ready for progression in the step procedure; and

ii. Review the annexes with a view to providing overarching comments to facilitate their completion, in particular on the completeness of Annex 1 on Raw Beef; the definition of vegetables of a leafy nature; and the retention of certain elements (e.g. Section 11 Retail and Food service and Flow charts) in Annex 2 on Fresh Leafy Vegetables; and on the structure and format of the annexes, in particular Annex 3 on Raw Milk and Raw Milk Cheeses.

APPENDIX I

REPORT OF THE EWG

INTRODUCTION

1. The 50th Session of the Codex Committee on Food Hygiene (CCFH50) agreed to start new work on 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. An electronic working group (EWG) was established, co-chaired by Chile and the United States of America, and working via the Codex Forum; an invitation was sent to all Codex members and observers to participate in the EWG.

2. CCFH51 considered the report of the EWG on the guidelines for the control of STEC and focused on giving guidance on the terminology to be used for each of the commodities covered by the Guidelines, as well as the request to JEMRA for scientific advice. CCFH51 agreed to return the draft to Step 2/3 for redrafting and to establish an EWG, chaired by Chile and co-chaired by France, New Zealand and the United States of America.

TERMS OF REFERENCE

3. The EWG was given the following terms of reference:

- redraft the General Section, the Raw Beef Annex, and the Fresh Leafy Vegetables Annex based on written comments submitted to CCH51;
- update the Raw Beef Annex with any additional information on interventions relevant to control of STEC in raw beef and submit the annex to JEMRA prior to June 2020;
- draft an annex on Raw Milk and Raw Milk Cheeses describing interventions relevant to control of STEC in these foods and submit the annex to JEMRA prior to June 2020; and,
- based on JEMRA feedback, revise the Annexes, as necessary. The report of the EWG was to be made available to the Codex Secretariat at least three months before CCFH52 for circulation for comments at Step 3.

PARTICIPATION AND METHODOLOGY

4. An invitation was sent to all Codex members and observers to participate in the EWG; participants from 34 Codex members and 5 observer organizations registered for the EWG. The list of participants is attached as Appendix III.

5. The EWG redrafted the General Section, the Raw Beef Annex, and Fresh Leafy Vegetables Annex based on written comments submitted to CCH51; developed an annex on Raw Milk and Raw Milk Cheeses describing interventions relevant to control of STEC in these foods; provided the documents on the forum for EWG input; revised the documents based on EWG input; and submitted the Raw Beef Annex and the Raw Milk and Raw Milk Cheeses Annex to JEMRA. The Joint FAO/WHO Expert Meeting on Shiga toxin-producing *Escherichia coli* (STEC) associated with Meat and Dairy Products was convened virtually from 1 to 26 June 2020 to review relevant measures for pre- and post-harvest control of STEC in animals and foods of animal origin. The co-chairs from Chile and the United States observed the JEMRA sessions to clarify questions about CCFH needs. After receiving the executive summary of the JEMRA meeting, the General Section and the annexes were further revised by the co-chairs.

6. In light of the one-year postponement of CCFH52, in order to facilitate progress on the Guidelines, the report of the EWG containing the revised draft Guidelines was made available (via Circular Letter) for comments to guide further revision by the EWG. The draft Guidelines were revised based on comments received through the Codex online comment system. The revised draft Guidelines are attached as Appendix I.

SUMMARY OF DISCUSSION

7. The EWG had requested input on the format of the annexes, specifically whether the annexes should be harmonized and, if so, in what way. Although some countries indicated they would prefer a standard format for all the annexes, with several countries recommending that annexes follow the *General Principles of Food Hygiene* (CXC 1-1969), others felt that this was not necessary, since there were different production and manufacturing processes that apply to the commodities and STEC control needed to be managed differently from one food chain sector to another.

8. Input was also requested on whether, after revisions based on country comments, work should be suspended pending input from JEMRA on STEC-specific control measures for fresh leafy vegetables. Comments supported this approach.

General Section

9. The General Section of the document was revised in numerous places to improve clarity. Paragraphs have been renumbered as a result of these changes.

10. Paragraph 6 was modified to refer to control measures specific for STEC strains in general, instead of for STEC strains of public health relevance, since the STECs relevant to public health can vary among countries. The paragraphs that described GHP-based and hazard-based control measures were deleted, since the control measures in the annexes are not presented as either GHP-based or hazard-based. There was a consequential change to delete “GHP-based and hazard-based” before “control measures” in paragraph 29.

11. In the Objective, the reference to fair trade was deleted in the text indicating that the Guidelines aim to reduce foodborne disease while ensuring fair practices in international food trade, based on a comment that the Guidelines focus on the reduction of foodborne illness and it is not clear how they would contribute to fair practices in international trade.

12. In the Scope, the sentence that the Guidelines apply in conjunction with the relevant OIE (World Organisation for Animal Health) standards with respect to raw beef was deleted because OIE indicated they are not planning on developing any standards.

13. The definition of fresh leafy vegetables was modified (as described in the discussion of the Fresh Leafy Vegetables Annex); two phrases remain in square brackets for the Committee to consider. The definition of sprouts was revised to “Products obtained from the germination of seeds collected before the development of true leaves. The final product contains the seed.” This definition will distinguish sprouts from microgreens and can serve as a starting point for the working group developing the sprouts annex. The definitions of raw milk and raw milk cheeses in the General Section were made consistent with the definitions in the annex for raw milk and raw milk cheeses. Two definitions for “indicator microorganisms,” both based on information in the *Encyclopedia of Food Microbiology* (Second Edition), have been added in square brackets for the committee to consider.

14. In section 10.3.1 Industry responsibility, paragraph 43 states that “industry has the primary responsibility” for the specified activities to ensure the safety and suitability of the food; “validating” was added to the activities. One country recommended deleting “primary,” as the activities listed are industry responsibilities. However, since government also has a responsibility for oversight of food safety systems, the term has been put in square brackets for the Committee to consider.

15. In 10.3.2 Regulatory Systems, paragraph 45 refers to the competent authority providing guidelines and other implementation tools to industry, as appropriate, for the development of the process control systems. One country requested changing the “should” to “could” because FBOs are primarily responsible for food safety; however, other countries disagreed with the change. These terms have been put in square brackets for the Committee to consider.

16. In section 10.4 verification of control measures, several changes were made for clarification, such as adding “mesophilic aerobes” in parentheses in “total bacterial counts” and changes to better indicate that increasing the frequency of verification increases the speed with which a loss of control is detected. A change was made to indicate that verification frequency “could” vary (rather than “should”) based on several factors.

17. In section 11.1 on monitoring, there was an issue on whether to make monitoring information available to “relevant stakeholders” or to “food business operators” and, if the former, who the stakeholders would be. Some comments supported “relevant stakeholders” to avoid limitations on information dissemination and to include regulatory authorities, while others preferred to limit the information to FBOs. Paragraph 59 was modified to retain “relevant stakeholders” and to indicate that these would include “where appropriate” producers, the processing industry, competent authorities, the public health sector, and consumers.

18. In section 11.2 Laboratory Analysis Criteria for Detection of STEC, “validated” was deleted in paragraph 64 with respect to polymerase chain reaction methods based on a comment that the validated PCR techniques do not yet include those for all the *stx* variants. A sentence was added in paragraph 66 about sending an isolate to a reference centre if a laboratory does not have the resources and technology to characterize an isolate.

19. In section 11.4 Public Health Goals, a sentence was added to indicate that 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. This addition is intended to emphasize the control of risks through the control of the data (records) that are generated during the process.

Raw Beef Annex

20. Recommendations from the JEMRA meeting draft report were included and others were removed from the document for not having enough scientific support as an STEC control measure.
21. The word "undercooked" was deleted from the scope, since it was noted by one member country that the annex was about raw beef.
22. Specific parameters for chemical disinfectants were deleted when only mentioned in one scientific publication.
23. In paragraph 66, the third bullet was redrafted, since there was some confusion about the requirement of pretesting for STEC, and to give clarity about this not being a competent authority requirement, but rather a supplier one.
24. One member suggested to bracket some of the recommendations until the JEMRA meeting report becomes available, but this recommendation was not taken up since the whole document is at Step 3.
25. One member suggested deleting the paragraph where recommendations for slaughter without stunning were provided, but it wasn´t taken into consideration since the paragraph aims to give advice to slaughter for religious purpose.
26. Other recommendations about deleting or changing words without further explanation or justification were not considered.

Fresh Leafy Vegetables Annex

27. It was noted that most control measures in the Fresh Leafy Vegetable Annex were not specific for STEC. Limited information was provided on control measures that have been studied scientifically with respect to control of STEC. A draft annex with the information that had been identified, along with specific questions related to information in the annex, was provided to JEMRA for its consultation on microbial safety of fruits and vegetables.
28. There was support from several countries to revise this annex to more closely follow the *Code of Hygienic Practice for Fresh Fruits and Vegetables* (CXC 53-2003). However, because CCFH recently revised the *General Principles of Food Hygiene* (CXC 1-1969) (GPFH) and revisions may be needed in documents that are based on the GPFH, including CXC 53-2003, the consensus was to not reorganize the annex until we get input from JEMRA on whether there is sufficient STEC-specific control information for an annex and there is a decision on restructuring CXC 53-2003.
29. The definition of fresh leafy vegetables was modified to add "among other local products for foliar consumption" at the end to capture additional leafy vegetables such as quintonil, watercress, and papaloquelite that may be limited to certain regions. In the definition there was no consensus on whether the vegetables of a leafy nature should be described as "where the leaf is intended for consumption" or "that may be consumed" without cooking, so the two phrases remain in square brackets for the Committee to consider.
30. It had been suggested that the guidelines should indicate whether GHPs are sufficient at specific steps of production to control STEC, and, if not, provide examples of applicable Critical Control Points (CCPs). Although there is some support for identifying GHPs and CCPs, in general countries believe that GAPs and GHPs are adequate at primary production. Input on this was requested of JEMRA.
31. In section 3.1.1 Location of the Production Site, it has been proposed that growers should consider a minimal distance, if possible, between fields and nearby animal operations based on recent scientific studies and publications. The consensus was to seek input from JEMRA on control measures related to distance from adjacent animal operations.
32. In section 3.1.2 Animal activity, the consensus was to indicate that fresh leafy vegetables should not be harvested where animal faeces are found, but not say more, since it is not practical to define the size of the area impacted, as many factors would determine this.
33. JEMRA was asked to provide advice on the role of testing of water to control STEC in fresh leafy vegetables, including appropriate indicator organisms and levels, as well as whether testing for STEC is warranted and under what circumstances. Thus, the information on testing water in section 3.2.1 Water for primary production was not modified at this time.
34. In section 4.1 Time and temperature control, in section 5.4 Cold storage, and in Section 11 Retail and Foodservice, the committee should consider whether to include a recommendation for storage of fresh leafy vegetables at a temperature below 7°C. It has been added in square brackets in these sections. JEMRA was requested to provide input on whether this would be an appropriate temperature for preventing growth of STEC in fresh leafy vegetables.

35. In section 5.5 Microbiological and other specifications, the first sentence was revised to say that "Microbiological testing of fresh leafy vegetables and of water for primary production for STEC is currently of limited use due to difficulty in detecting STEC because of low prevalence and low numbers of the organism in fresh leafy vegetables and in water." JEMRA was requested to address this issue.

36. There are different opinions on whether to keep Section 11 – Retail and Foodservice as a separate section or include the measures elsewhere. This section is not in the revised GPFH or in CXC 53-2003, but it has been retained for consideration after it has been determined whether CXC-53-2003 will be rearranged for consistency with a revised *General Principles of Food Hygiene* (CXC 1-1969).

37. The flow diagram was revised by adding steps such as planting, irrigation, fertilizing and other chemical applications, and field packing at the production site. One country questioned the usefulness of the flow diagram and recommended deleting it. Whether the flow diagram should be retained should be addressed by CCFH.

<u>Raw Milk and Raw Milk Cheeses Annex</u>

38. The raw milk and raw milk cheeses annex was revised in numerous places to improve clarity. As an example, "faecal transmission" was changed to "faecal-oral transmission". In paragraph 37, a confusion about financial matter (and not laboratory skills) appeared; the text has been modified to eliminate any risk of misunderstanding. Paragraphs have been renumbered as a result of changes. In addition, some sentences have been edited to improve the language. A number of changes in the Annex are based on information provided in the JEMRA Executive Summary.

39. The definition of raw milk has been clarified for consistency with the Executive Summary of JEMRA (which notes that both bactofugation and microfiltration require raw milk to be heated to 50-60°C to reduce viscosity before treatment).

40. Additional scientific references such as those related to STEC in buffalo and on newly purchased animals. were added in the text.

41. Based on the JEMRA Executive Summary, additional information on STEC circulation on the farm, which may depend on farm size and farm practices, was added in the text.

42. The JEMRA expert consultation considered the issue of factors likely to have an impact in STEC excretion (age, diet, housing, stress, herd size, animal health, geographical area, and previous contamination with STEC strains) with respect to "high shedders." JEMRA indicated that the Identification of a specific animal as a super shedder was impractical and unreliable due to the highly intermittent nature of shedding and as such is not recommended as an STEC intervention strategy. No specific control measure on "high shedders" was added in the text.

43. Based on the JEMRA Executive summary, an additional control measure was added in the "at dairy farm part" and at the "collection, storage and transportation" part.

44. Information was added in paragraph 25 following a response from JEMRA on control during milk storage and collection: All equipment that may come in contact with milk such as tubes and pipes used for transferring milk to larger containers, pumps, valves, storage containers and tanks, etc. should be thoroughly cleaned and disinfected before every use. Although not a standard practice, a full cleaned-in-place, once per 24 h tanker cleaning approach, with the use of a between load water rinse with or without a disinfecting treatment reduced the presence of surface bacteria in the tanker.

45. JEMRA evaluated the impact of the milk cooling process at the farm on the growth of STEC and this information was used to complete the text.

46. JEMRA provided an evaluation of the scientific literature with respect to reduction of STEC by acidification, long ripening and curd warming. The text was modified consequently: As STEC elimination is not always achieved, the quality of the raw milk used in the cheese making is crucial to reduce the risk associated with the end products (paragraphs 29 and 30).

47. JEMRA was asked to provide an assessment of testing raw milk as a means of controlling STEC in raw milk for drinking and for production of raw milk cheeses. Sampling and testing plans are very dependent on consumption practices, scale of production and local regional regulations. The text was modified based on information in the JEMRA Executive Summary.

48. The 2 processing flow diagrams have been reworked.

49. With respect to the format of the raw milk and raw milk cheeses annex, there was no consensus from the EWG on whether to harmonize it with the *General Principles of Food Hygiene* (CXC 1-1969) or the *Code of Hygienic Practice for Milk* (CXC 57-2004) or to retain the current format, which provides "scientific knowledge" followed

by “recommended good hygiene practices.” It is important to highlight that the control measures proposed in this annex are justified by the data described in the "scientific knowledge" sections. It is therefore considered necessary to keep this information in this annex so that it is understood independently. Moreover, the control of STEC must be managed differently from one sector of the food chain to another, and therefore, homogeneity in the annexes is by design impossible. We request input from CCFH on this format.

CONCLUSIONS

50. The EWG completed the tasks identified in its Terms of Reference; specifically, the EWG:

- redrafted the General Section, the Raw Beef Annex, and the Fresh Leafy Vegetables Annex based on written comments submitted to CCH51;
- updated the Raw Beef Annex with additional information on interventions relevant to control of STEC in raw beef and submitted the Annex to JEMRA prior to June 2020;
- drafted an annex on Raw Milk and Raw Milk Cheeses describing interventions relevant to control of STEC in these foods and submitted the Annex to JEMRA prior to June 2020; and,
- based on JEMRA feedback, revised the Annexes, as necessary.

51. Taking advantage of the additional time available due to the postponement of CCFH, the draft Guidelines were circulated for comments and revised based on comments received.

RECOMMENDATIONS

52. The EWG recommends that CCFH52 consider the proposed draft Guidelines as presented in Appendix I, including the General Section, Annex 1 (Raw Beef), Annex 2 (Fresh Leafy Green Vegetables), and Annex 3 (Raw Milk and Raw Milk Cheeses) and, in particular:

- Review the General section for completeness, address the abovementioned outstanding issues in square brackets and provide a recommendation on whether it is ready for progression in the step procedure; and
- Review the annexes with a view to providing overarching comments to facilitate their completion, in particular on the completeness of Annex 1 on Raw Meat; the definition of vegetables of a leafy nature, and the retention of certain elements (e.g. Section 11 Retail and Food service and Flow charts) in Annex 2 on Fresh Leafy Vegetables; and on the structure of Annex 3 on Raw Milk and Raw Milk Cheeses.

APPENDIX II

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

(For comments at Step 3 through CL 2021/63-FH)

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. 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 *stx*1), Shiga-toxin type 2 (Stx-2, encoded by the gene *stx*2) 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, including the principal adherence gene for a protein, Intimin, encoded by *eae.* The aggregative adherence fimbriae adhesins regulated by the *aggR* gene 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 showing 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 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 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 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 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). 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 control measures will likely vary at the national level and therefore these Guidelines only provide examples of hazard-based controls. 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 of

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

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 of quality or hygienic status in food, water, or the environment, often used to signify the potential presence of pathogens, a lapse in sanitation or a process failure. Common indicator microorganisms include total bacterial counts, coliform or faecal coliform counts, total *E. coli* counts and counts of Enterobacteriaceae.]

21. Raw beef – Skeletal muscle meat from 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 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 Hygienic Practice for Fresh Fruits and Vegetables* (CXC 53-2003), Annex I on Ready-To-Eat Fresh Pre-Cut

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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.

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.

6.1 Development of risk-based control measures

30. Competent authorities operating at the national level should develop risk-based control measures for STEC where possible and practical.

31. When risk-modelling tools are developed[7], the risk manager needs to understand the capability and limitations.

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, 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.

[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)

9. 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, 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 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

45. The competent authority [should] [could] 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 of HACCP systems when specific targets for control of STEC have been identified.

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

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. 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 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 and verification records, and sampling and testing for indictor organisms and STEC where appropriate.

49. Due to typically low levels and low prevalence of STEC in food, enumerative 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 indicators include total bacterial (mesophilic aerobes) counts, counts of coliforms or fecal coliforms, counts of total *E. coli,* and counts of Enterobacteriaceae. 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 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 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.

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, and to help prioritize regulatory efforts to reduce 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 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 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.

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

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 account the most cost-efficient resourcing option for collection and testing of samples. 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 from 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 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, STEC strains with *stx2a* and adherence genes, *eae* or *aggR* have the greatest association with diarrhoea, bloody diarrhoea (BD), and haemolytic uremic syndrome (HUS). 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 polymerase chain reaction methods or whole genome sequencing analysis. 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 the same virulence genes and the detection of 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, the 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.

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). *

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 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 and provide a basis for their validation and verification.

70. Information gained from monitoring in the food chain should be integrated with human health surveillance, food source attribution data, and withdrawal and recall data, where available to evaluate and review the effectiveness of 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 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.

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ANNEX 1: RAW BEEF

1. **INTRODUCTION**

1. Foodborne outbreaks of Shiga toxin-producing *Escherichia coli* (STEC) have been linked to a wide variety of foods, including meat products (FAO/WHO, 2018). Beef is one of the most significant sources of foodborne STEC outbreaks, with raw or undercooked non-intact beef products (e.g., ground/minced or tenderized beef) recognised as posing an elevated risk to consumers.

2. STEC are a common part of the intestinal microbiota of cattle, with the reported prevalence in cattle faeces varying greatly, depending on factors such as animal age, herd type, season, geographic location and production type (Hussein and Bollinger; 2005, Callaway et al 2013). STEC shedding by individual cattle is transient and episodic, with almost all cattle carrying and shedding STEC at some time during their life (Williams et al., 2014; Williams et al., 2015). In addition, STEC are widespread within the farm environment. It should be expected that the majority of cattle arriving for slaughter could have hides contaminated to some extent with STEC. Individual studies have reported the prevalence of STEC O157 on cattle hides presenting for slaughter as high as 94.5% (Arthur et al., 2007), and as high as 74.5% for other STEC (Stromberg et al., 2018).

3. The sporadic nature of STEC and common movement and comingling of cattle prior to slaughter through means such as feedlots, lairage, and livestock markets can allow STEC to spread. The transient nature of STEC in cattle and the impracticality of testing all cattle for STEC prior to slaughter demonstrate the need for slaughter operations to treat all incoming cattle as if they could have STEC on the hide or could be shedding STEC.

4. Zoonotic pathogens such as STEC carried by cattle could be spread to carcasses during slaughter. Prior to slaughter, the muscle tissue of healthy cattle is essentially sterile. STEC can be transferred to carcass surfaces from the contents of the gastrointestinal tract or hide during the operations of dehiding, head removal, bunging and evisceration (Gill and Gill, 2010). Generally, contamination is confined to the carcass surface and is not found in deep muscle tissues of intact raw beef.

5. STEC contamination has historically occurred in raw beef. The purpose of this guidance is to provide information on measures that can reduce contamination of raw beef with STEC and guidance on when raw beef contaminated with STEC should be considered fit for human consumption to minimize the potential for disputes and facilitate global trade.

2. SCOPE

6. This guidance applies to control of STEC in raw beef, including cuts such as steaks and raw ground/minced or tenderized beef.

3. DEFINITIONS

For the purpose of this guideline the following definitions apply:

Raw Beef: Skeletal muscle meat from slaughtered cattle, including primal cuts[12], sub-primal cuts, and trimmings.

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

7. These Guidelines incorporate a “primary production-to-consumption” flow diagram that identifies the main steps in the food chain and identifies where control measures for STEC may potentially be applied in the production of raw beef. While control in the primary production phase can decrease the number of animals carrying and/or shedding STEC, controls after primary production are important to prevent the contamination and cross-contamination of carcasses and, in particular, raw ground/minced beef. 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. 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.

8. STEC have a wide range of potential hosts (Persad and LeJeune, 2014), and STEC cells can potentially persist for over a year in the natural environment (Jiang et al., 2017; Nyberg et al., 2019). These features of the ecology of STEC indicate that control strategies based on denying STEC access to hosts or habitat will be highly challenging to implement in a manner which reliably prevents exposure of cattle to STEC.

[12] 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.

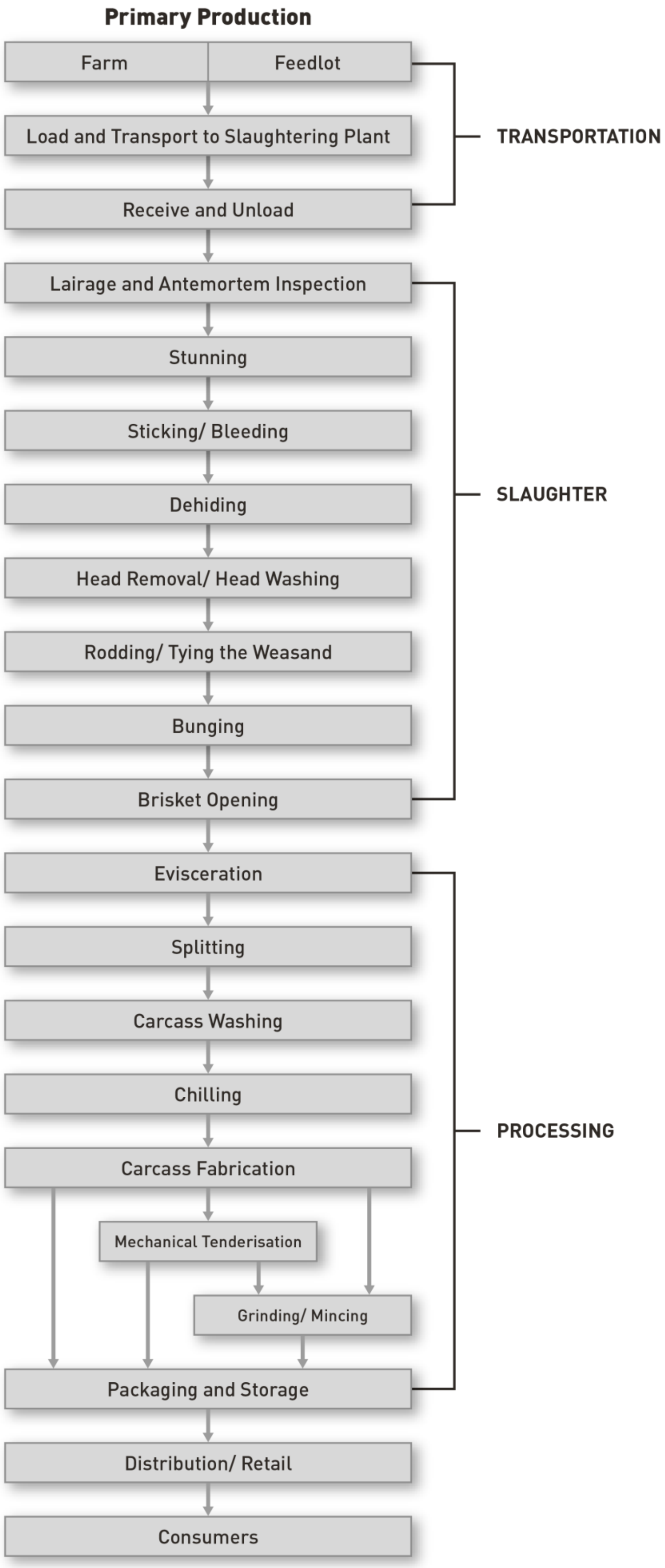
9. Interventions to control enteric pathogens should always be part of an integrated food safety system that includes all the stages from primary production to consumption. Measures to reduce STEC shedding or hide contamination prior to slaughter have the potential to reduce environmental exposure to STEC and may improve raw beef safety, but they cannot prevent STEC contamination or compensate for poor hygiene practices during slaughter, processing and distribution. Conversely, there is evidence that the adoption of the best hygienic practices during slaughter and processing can minimise contamination with STEC (Brichta-Harhay et al., 2008; Pollari et al., 2017). Consequently, the adoption of best practices for preharvest management of cattle should be promoted as a support to hygienic slaughter and processing.

10. Similarly, operations to decontaminate carcasses or raw beef cuts will be of limited effectiveness if poor hygiene practices during subsequent processing and distribution permit recontamination or if the initial contamination load is high. Decontamination only reduces STEC by a certain amount, which can be quite variable depending on type of treatment, duration, application, temperature, etc.

4.1 GENERIC FLOW DIAGRAM FOR APPLICATION OF CONTROL MEASURES

Process Flow Diagram 1: Primary Production-to-Consumption of Beef

11. These process steps are generic, all the steps may not occur, and the order may be varied as appropriate; it should be noted that not all steps may be completed within the same establishment. Grinding/mincing, for example, can be done at sites other that the slaughter or fabrication site. This flow diagram is for illustrative purposes only. For application of control measures in a specific country or an establishment, a complete and comprehensive flow diagram should be drawn up for each situation.


Primary Production
Farm
Feedlot
Load and Transport to Slaughtering Plant
Receive and Unload
TRANSPORTATION
Lairage and Antemortem Inspection
Stunning
Sticking/ Bleeding
Dehiding
Head Removal/ Head Washing
Rodding/ Tying the Weasand
Bunging
Brisket Opening
SLAUGHTER
Evisceration
Splitting
Carcass Washing
Chilling
Carcass Fabrication
Mechanical Tenderisation
Grinding/ Mincing
Packaging and Storage
PROCESSING
Distribution/ Retail
Consumers

4.2 PRIMARY PRODUCTION

12. Control measures to reduce the carriage of STEC in cattle prior to slaughter that have the potential to reduce the prevalence of STEC are described in this section.

4.2.1 Specific Control Measures for Primary Production

13. The prevalence of STEC shedding in a herd and the individual animal shedding status for STEC is generally unpredictable, although factors have been identified that may influence STEC shedding. Interventions proposed to reduce the prevalence of STEC shedding or numbers of STEC shed by cattle include animal vaccination, dietary additives and manipulation of animal feeds, and primary production practices.

14. Many of these proposed pre-harvest control methods have not been demonstrated to reliably reduce the prevalence or the level of STEC shedding from cattle in a commercial setting. Research into pre-harvest control of STEC in cattle has focused on the serotypes O157:H7 and O157:NM and so there is often limited data available on the impact on other STEC serotypes. Additionally, some of the proposed methods are focused on specific subpopulations of STEC (e.g. vaccines, bacteriophage).

4.2.1.1 Diet Ingredients

15. A wide variety of cattle diets have been investigated for their impact on STEC serotype O157:H7 prevalence and/or shedding, including hay, barley, distillers and brewers' grains, sage brush, millet, alfalfa, (Callaway et al., 2009). Both STEC serotype O157:H7 and generic *E. coli* populations have been demonstrated to respond to changes in diet, but replication of results indicating STEC serotype O157:H7 reduction has been poor and no dietary composition has been identified that reliably reduces STEC O157:H7. Some diets that have been proposed increase STEC serotype O157:H7 shedding (Thomas and Elliott, 2013).

16. In general, research supports that cattle on grain-based diets appear to shed higher levels of generic *E. coli* in their faeces than cattle on forage diets (Callaway et al 2003), but the effect of forage diets on faecal shedding of STEC serotype O157:H7 is inconclusive.

Use of Direct-Fed Microbials

17. Use of probiotics or direct-fed microbials, involves feeding animals with viable microorganisms which are antagonistic toward pathogens, either by modifying environmental factors in the gut or producing antimicrobial compounds. There is evidence that specific direct-fed microbial treatments, such as *Lactobacillus acidophilus* (NP51) and *Propionibacterium freudenreichii* (NP24), can reduce STEC serotype O157:H7 shedding by cattle (Wisener et al., 2015, Venegas-Vargas et al 2016). The addition of viable microorganisms to feed should be assessed with respect to whether these microorganisms pose a risk for emergence of antimicrobial resistance in pathogens in the gut.

Use of other feed additives

18. The seaweed *Ascophyllum nodosum* (Tasco-14) is marketed as a supplement for cattle feed. It has been reported to reduce faecal and hide prevalence of STEC O157:H7 when added to corn feed (Braden et al., 2004).

4.2.1.2 Vaccination

19. Various vaccines have been designed and tested for preventing colonisation and/or reducing faecal shedding of STEC O157:H7. Some vaccines have been shown to reduce faecal shedding of STEC O157:H7 but their efficacy is dependent on the type of vaccine and the number of doses administered. Only a few vaccines have been tested under production conditions, and the duration of immunity after vaccination is unknown because the evaluation period in feedlot studies has been relatively short. The use of vaccination in cattle has not been commercially adopted due to the lack of evidence to support the reduction of STEC in beef following vaccination and the lack of farm-level incentives to cover additional cost associated with vaccines and their administration (JEMRA, 2020).

4.2.1.3 Good management practices at primary production

20. The following good management practices for animals are recommended for minimising STEC shedding and hide contamination on animals presented for slaughter. Of particular concern is preventing the formation of faecal accumulation on animal hides, as this can interfere with hygienic skinning and evisceration.

- Stressful situations should be minimized wherever possible, because increased stress increases shedding of pathogens (e.g. poor animal husbandry, rough handling, dietary stress and food deprivation (Stein and Katz, 2017; Venegas-Vargas et al 2016)).
- Minimize exposure between herds to avoid or reduce horizontal transmission of STEC across herds (Callaway et al 2009).

- Maximize living space to reduce direct animal-to-animal transmission (e.g. maintain ample space for animals to move to reduce defecation directly onto one another).
- Maintain clean living conditions (e.g. clean holding areas, remove gross contamination to the extent possible, and maintain clean and dry bedding) to prevent transmission from the living environment (e.g. animals resting in STEC-contaminated materials).
- Reduce the potential for STEC transmission through consumption of contaminated food and water by the following:
    - Design food and water delivery systems (tanks, trough, bins, etc.) in a way to reduce the potential for animal entrance and defecation.
    - Ensure water is of a microbiological quality that minimises animal contamination and, if there is doubt, treat the water.
    - Clean water troughs frequently to reduce replication and/or survival of these foodborne pathogens (Lejeune et al 2001).
    - Use materials in water troughs that facilitate the cleaning process; metal troughs had lower *E. coli* O157:H7 counts compared with troughs that were manufactured from concrete or plastic (Lejeune, 2001).

4.3 Transportation

4.3.1 Specific Control Measures for Transport to Slaughterhouse

21.Transportation can be a major contributor to the increasing occurrence of pathogens in animals and a source of hide contamination. Contributing factors include mixing of animals of different origin, increased stress, increased exposure to STEC during extended duration of transportation, and cleanliness of transport vehicles (Norrung et al., 2008; Dewell et al. 2008, Stein and Katz, 2017).

22. Cross-contamination among animals from different farms during transportation to the slaughter facility and at lairage (holding pens) can be an important source of hide contamination. Therefore, appropriate controls should be in place to minimize hide contamination. Controls include:

- Improve truck design, allowing for separation of animal lots.
- Separate lots of animals from different farms, use holding pens of an appropriate size for the number of animals, avoid overpopulation and stress of the animals.
- Appropriately clean holding pens between lots of cattle.
- Implement visual controls for soiled animals, transportation vehicles and lairage pens for visible faecal contamination.

23. Transportation practices should minimize any condition that could affect contamination of the meat. Control measures implemented prior to travel include:

- Gather and handle animals so that they are not unduly stressed.
- Transport animals from the same herd in the same truck where possible to avoid social stress.
- Minimize distance over which slaughter cattle should be transported. One study noted that transporting cattle more than 100 miles doubled the risk of having positive hides at slaughter compared to cattle that traveled a shorter distance (Dewell et al, 2008).
- Ensure animals are as clean as possible to decrease the opportunity for pathogen contamination onto carcasses or hides during the slaughter and dressing processes. The likelihood of STEC contaminating the meat increases where levels of faecal contamination on the hide are high.
- Load the animals onto clean vehicles, prevent faecal transfer from top level to bottom level (in multi-level trailers) to the extent possible, and do not overcrowd the vehicle.

4.3.2 Specific Control Measures at Receive and Unload

24. Maintain herd integrity during load assembly and transport through unloading and placing in holding pens. To minimize STEC shedding, stress levels should be minimized using good animal handling practices; minimize or eliminate the use of electric prods and avoid overcrowding.

25. The unloading should be carried out in a way that minimizes the stress caused by the action that could increase shedding of STEC, with adequate training of the operators on procedures that can minimize stress.

4.4 SLAUGHTER

26. Interventions at the slaughterhouse include physical, chemical or biological interventions that can be applied alone or in combination; these are likely to reduce the number of STEC microorganisms but should not be considered to eliminate STEC on every animal. Strict hygiene practices and good manufacturing practices at slaughtering are necessary to prevent transfer of STEC from the hide and digestive tract to the carcass. Particular focus should be given to ensuring best practice in the operations of dehiding, head removal, bunging and evisceration, as these operations are the initial sources of microbiota transfer to meat surfaces (Gill and Gill, 2010).

27. The specific control measures during this stage are intervention techniques aimed at preventing transfer of contamination to the carcass, as well as cross-contamination to other carcasses. Interventions selected should be validated for their effectiveness.

28. Interventions aimed at removing STEC from the surface of beef carcasses should consider that tolerance to heat, salt and acid has been observed in some STEC strains. Determining the effectiveness of interventions to reduce microbial pathogens is complex, particularly as multiple interventions may be applied simultaneously or in sequence, The impact of interventions should be quantified by conducting experimental trials with surrogate organisms that have similar or greater resistance to individual treatments than STEC.

29. Specific control measures should be safe and feasible along the production process and should not change the organoleptic properties of beef meat.

30. The interventions described for the following steps may reduce the level of microbiota, including STEC, on carcasses and raw beef surfaces. Many operations can be performed manually or with automated equipment. Automation offers the advantage of greater consistency of application but needs proper adjustment (Signorini et al., 2018).

4.4.1 Specific Control Measures at Lairage and Antemortem Inspection

31. In this stage the hygiene condition of the animals should be evaluated; animals should be as clean as possible to minimize the initial load count of microorganisms, which potentially includes STEC, on their hide. Dirty or wet animals should be segregated to prevent cross-contamination.

32. The lairage area should be cleaned as much as possible for each lot of animals, with the removal of gross contamination and residues with application of chlorinated water under pressure on the floor. Cleaning and disinfection should be applied according to good hygiene practices and manufacturer's instructions. The lairage area should be designed to be well-drained in order to facilitate drying.

33. Practices such as washing animals (e.g., spray, mist, rinse or wash), specifically the animal´s hide, with different substances (e.g. tap water, bacteriophage) to reduce contamination has been investigated (Byrne et al.,2000; Arthur et al., 2007; Arthur et al., 2011; LeJeune and Wetzel 2007). However, in general, the evidence for washing in reducing the transfer of STEC from hide to carcass is low.

34. When feasible, at lairage cattle should be maintained in closed herds to reduce social stress and prevent cross-contamination between herds.

4.4.2 Specific Control Measures at Stunning, Sticking and Bleeding

35. In the access to the stunning box, or following the stunning box, the animals can be treated with water jets at appropriate pressure, aiming at the hygiene of the rectum for possible elimination of faeces and STEC shed due to stress in leading the animal to slaughter. Use of any water or rinses should be designed to reduce STEC contamination and not stress the animal or inhibit the stunning, stick or bleeding effectiveness.

36. The stunning box should be kept as clean as possible to avoid contamination of the animal's hide in the fall after the stunning process.

37. The stunning method employed (self-contained bolt, firearm, alternative) can have different effects on STEC transfer into the skull.

38. In slaughter where there is no stunning, special attention should be paid to avoid a delay in clipping the weasand to minimize contamination with STEC of neck meat, when STEC is present in the ingesta.

39. Sticking and bleeding should be done in a manner to reduce transfer of hide contamination to the carcass. Preparing the penetration or cut sites (e.g. with steam/vacuum treatment) can reduce the likelihood of contamination.

4.4.3 Specific Control Measures at Dehiding

40. Dehiding is the systematic process for separating the hide from the carcass and is perhaps the most critical operation in determining the level of STEC transferred to the carcass. To prevent transfer of contamination

from the hide to the freshly exposed carcass, operators working at this stage should be effectively trained to perform this operation.

41. Slaughterhouses may consider, when feasible, a pre-hide removal carcass decontamination procedure to reduce visible hide contamination. Prior to dehiding, applying a process that decontaminates the hides (such as washes, hair removal, the application of bacteriophage cocktails or the application of steam and vacuum at the hide incision sites) may lower carcass microbial contamination. However, in general, the evidence on their role in reducing the transfer of STEC from hide to carcass is low. The excess liquid from the decontamination procedure should be vacuumed from the hide to avoid contamination of the carcass with liquid that could easily run onto the carcass when the hide is opened (Bosilevac et al 2005, Wang et al 2013).

42. Rinsing of the rectum and disinfection of the perianal hide should be performed in order to reduce or eliminate contamination prior to dehiding. Hide-on carcass washes are frequently used for that purpose (Yang et al., 2015). To prevent transfer of contamination from the hide to the carcass, techniques can include:

- Using clean and disinfected knives to cut through the hide.
- Cleaning and disinfecting the knife (or tool) each instance the hide is penetrated, or using different knives, one to cut through the hide and the other to remove the hide.
- Using a systematic trimming pattern, to work outward from a single hide opening site.
- Using one hand to hold, pull and control the hide while separating/cutting the hide away from the carcass using the other hand.
- Washing hands and aprons as often as needed to prevent cross-contamination of carcasses.

43. The dehiding operation should be performed in a manner to avoid contact of the hide with the part of carcass that is already dehided (i.e. dehiding the entire perianal region and bending the hide, making it stay above the tail). Using paper to protect specific areas of the carcass such as brisket and bagging of the tail may also be useful practices for reduction of STEC contamination due to contact with hide during dehiding.

44. Measures should be taken to prevent tail flapping or splattering when hide pullers are used.

4.4.4 Specific Control Measures at Rodding

45. The rodding operation consists of using a metal rod to free the esophagus (weasand) from the trachea and surrounding tissues. Weasand meat may be recovered from the gastrointestinal tract for use in raw ground/minced beef production. The rodding operations should be performed in a manner to avoid contamination of the weasand and of the carcass interior from the exterior. If during the rodding operation the gastrointestinal tract is punctured, it can cause contamination of the carcass interior and exterior with ingesta.

46. To prevent cross-contamination of the carcass from the weasand/esophagus during the rodding operation, techniques can include:

- Hanging the carcass vertically, to cut the muscle and tissue to expose the esophagus.
- The weasand should be closed (i.e., tied) hygienically to prevent rumen spillage; ties or clips can be used to prevent digestive track material movement.
- Heads can be “dropped” by cutting the esophagus below the tie or clip.
- Changing or disinfecting the weasand rod between each carcass.
- Cleaning the weasand to minimize cross-contamination.
- If the gastrointestinal tract has been punctured, causing a major contamination, the carcass should be identified and additional procedures to avoid cross-contamination of other carcasses should be performed.

47. When appropriately applied, these techniques will reduce contamination with gut microorganisms generally, and these may include pathogens; however, insufficient evidence was found specifically for their effects on STEC.

4.4.5 Specific Control Measures at Bunging

48. Rectum occlusion should be performed hygienically in order to avoid contamination of the carcass and tools with the gastrointestinal contents or the hide, if the dehiding was not already done.

49. To prevent transfer of contamination from the bung to the carcass, techniques can include:

- Rinsing or washing the bung area before cutting.

- Stuffing the bung with physical materials (e.g. paper towels) to push faecal material into the bung and reduce fecal movement out of the bung.
- Bag the bung by wrapping the bung in a bag to contain any incidental leakage that may occur during the evisceration process.

4.4.6 Specific Control Measures at Brisket Opening.

50. Brisket opening should be performed hygienically in order to avoid contamination of the carcass and tools, especially if dehiding has not been done.

51. To prevent introduction of contamination into the carcass during brisket opening, techniques can include:

- Cleaning and disinfecting the brisket saw and knife between each carcass and ensuring that the gastrointestinal tract is not punctured.
- If the gastrointestinal tract has been punctured causing a major contamination, the carcass should be identified and additional procedures to avoid cross-contamination of other carcasses should be performed.

4.5 PROCESSING

52. STEC on the carcass can be transferred to meat cuts as the animal is further processed and can also be transferred between meat cuts via meat processing equipment (ICMSF, 2005).

4.5.1 Specific Control Measures at Evisceration

53. Evisceration includes procedures to remove the digestive track and organs from the carcass. The evisceration should be done avoiding contamination with gastrointestinal contents due to a cut in the gastrointestinal tract.

54. To prevent contamination of the carcass by the viscera during removal, techniques can include:

- Removing visible contamination from the area to be cut (e.g. by trimming, by using air knives, or by steam vacuuming) before the cut is made. This should be done in a timely manner and in accordance with commonly accepted reconditioning procedures.
- If the animal is pregnant, removing the uterus in a manner that prevents contamination of the carcass and viscera.
- Cutting through tonsils should be avoided.
- To prevent contamination of the carcass by employees during evisceration, techniques can include:
    - The appropriate use of knives to prevent damage (i.e., puncturing) to the rumen and intestines.
    - Using footbaths or separate footwear by employees on moving from evisceration lines to prevent contaminating other parts of the operation.
    - Using trained and experienced individuals to perform the evisceration; this is particularly important at higher line speeds.
    - If the gastrointestinal tract has been punctured causing a major contamination, no further work should be carried out on the carcass until it has been removed from the slaughter line.

4.5.2 Specific Control Measures at Carcass Splitting

55. Carcass Splitting is the point in the process where carcasses are split vertically into two halves.

56. To prevent the split carcass from becoming contaminated, techniques can include:

- Removing defects that may contaminate the saw or cleaver (e.g. faeces, milk, ingesta, abscesses, etc.) in a sanitary manner before splitting the carcass.
- Cleaning to remove organic material and disinfecting the saws and knives between each carcass.
- Allowing adequate distance between carcasses (i.e., avoid carcass-to-carcass contact), walls and equipment.

57. Targeted removal of visible contamination by trimming may be applied to carcasses, but the disadvantage of manual methods is potential cross-contamination from dirty knives (if not using a knife-switching disinfection protocol in-between cuts), aprons, mesh gloves, and waste. Also, even though practices may be effective at removing visible defects, the effectiveness of these practices to reduce pathogen contamination, including STEC, is limited (Gill and Landers, 2003; Gill and Baker et al 1998).

58. Carcass trimming should be done in an area designated for that purpose and should result in trimmed carcasses that are free of stick wounds, blood clots, bruised tissue, pathological defects, visible contaminants, and dressing defects. After trimming, all carcasses should be washed to remove blood and bone dust.

4.5.3 Specific Control Measures at Carcass Washing/Treatment

- *Carcass washing with antimicrobial agents.*

59. Carcass washing may remove visible soiling and reduce overall bacterial counts on beef carcasses by up to 1 log unit (Gill and Landers, 2003). Carcass washing with antimicrobial agents, such as organic acids (e.g. citric acid, lactic acid, acetic acid), oxidising agents (e.g. chlorine, peroxides, ozone) or other antimicrobial agents may be effective in reducing STEC (Gill and Gill, 2010). Such antimicrobial treatments may be applied with hot water to have a combined thermal impact. Factors determining the effectiveness of such treatments include the concentration of the agent, uniformity of surface coverage, the temperature of the solution, and the contact period. Individual STEC strains may vary in their sensitivity to such treatments (Berry and Cutter, 2000; A. Gill et al., 2019). Organic acids alone can reduce but not completely eliminate STEC O157:H7 (Hussein and Sakuma, 2005).

- *Carcass surface pasteurisation.*

60. This form of treatment is most commonly applied to carcass sides at the end of dressing. Water at >85 °C may be applied as a spray, a sheet or as steam (Gill and Bryant, 2000; Retzlaff et al., 2005). Treatment is most effective when applied to clean, dry carcass sides as large drops or sheets of water; when applied under such conditions the treatment can achieve >2 log reductions in total *E. coli* in commercial slaughter operations (Gill and Jones, 2006). The specific impact on STEC is not known.

- *Steam and vacuum*

61. The carcasses are sprayed with steam and then an aspiration is performed, which fulfils a double function of eliminating and / or inactivating surface contamination. The manual device includes a vacuum tube with a hot water spray nozzle, which delivers water at approximately 82-88 ° C on the surface of the carcass. The process is effective in removing visible contamination in the carcasses (Huffman, 2002; Dorsa et al. 1996,1997; Koohmaraie, 2005; Kochevar et al., 1997). The specific impact on STEC is not known.

4.5.4 Specific Control Measures at Chilling

62. Rapid chilling minimizes the potential for bacteria to replicate; STEC, can only replicate at temperatures of 7 °C and above. The potential for bacterial replication is also dependent upon the water activity at the carcass surface, and if water activity is low enough (less than a_w 0.95), a decline in bacterial numbers will occur. Thus, controlling the humidity of the chilling process can impact STEC levels on the carcass. Alternatively, spray chilling with antimicrobial agents may reduce STEC survival (Liu Y et al., 2016, Kocharunchitt, et al., 2020).

4.5.5 Specific Control Measures at Mechanical Tenderization, Grinding/Mincing

63. Studies have shown that processes such as marinating, in combination with knife scoring, proteolytic enzymes, or vacuum brine injection, and mechanical tenderisation in which blades or needles penetrate the muscle surface, create a potential for increased food safety risks due to the transfer of pathogens from the surface to the interior, resulting in internalization of STEC into previously intact raw beef (Johns et al., 2011; CDC, 2010; Lewis et al., 2013). Such products should be considered as “non-intact” raw beef, and appropriate consumer guidance on safe handling, including cooking temperatures, may be needed (USDA FSIS, 2019; Health Canada, 2019), since these products may pose an increased risk for consumers.

64. Manufacturers should ensure that mechanical tenderizers and associated processing equipment are cleaned on a regular basis to minimize the potential for translocating STEC from the exterior surface of the product to the interior and to minimize the potential for cross-contamination within and among lots of production. Manufacturers should also consider purchase specifications that require that incoming beef to be tenderised has been treated to eliminate or reduce STEC such as *E. coli* O157:H7 to an undetectable level or should apply such treatments prior to mechanical tenderization.

65. Antimicrobial washes, such as lactic acid, peroxyacetic acid and acidified sodium chlorite have been shown to reduce *E. coli* O157:H7 and other STEC concentrations on beef (i.e., carcasses, primal cuts or other cuts) and could be used to minimize contamination of materials used to manufacture ground/minced beef.

66. To minimize STEC contamination and/or the spread contamination of ground/minced beef with STEC, measures may include:

- [311]Storing products to prevent the growth of STEC. Multiplication of STEC is inhibited below 7°C, but low temperatures would not significantly reduce STEC. Establishments need to control STEC, using adequate time/temperature combinations.

- Cleaning equipment and the environment on a regular basis and ensuring employees follow good personal hygiene practices in order to avoid cross-contamination.
- Specifying that all beef used for grinding be pretested and found negative for specific strains of STEC, e.g. *E. coli* O157:H7.
- Treating the outer surfaces of the meat with organic acid sprays or other approved treatments before grinding/mincing.
- Appropriately chilling raw meat during production to reduce possible multiplication of STEC if they are present.

67. Since processes such as grinding/mincing may potentially spread contamination in the meat, there should be increased awareness when handling the meat throughout the rest of the food chain.

4.5.6 Specific Control Measures at Packaging and Storage

68. A range of non-thermal preservation technologies (e.g. pulsed light, natural bio-preservatives, high hydrostatic pressure, ionizing radiation) and thermal preservation technologies (e.g. microwave and radiofrequency tunnels, Ohmic heating or steam pasteurization) have been investigated for meat decontamination either during processing or after final packaging. The practical use of these methods is dependent upon the impact on the organoleptic properties of the meat and its final use. Factors determining the effectiveness of such treatments includes the sensitivity of the microorganism, the temperature of the environment, the intrinsic characteristics of the food (e.g., fat content, salt, additives, pH) and the level of initial contamination (Aymerich et al., 2008; Gill and Gill, 2010).

69. During packaging and storage, the time/temperature combination should be such that one generation of bacterial growth cannot occur.

4.6. DISTRIBUTION / RETAIL

4.6.1 Specific Control Measures at Distribution and Retail

70. Control of refrigeration temperatures should be maintained during transport and storage of the carcasses, beef cuts, or minced/ground beef along the distribution chain until the product reaches the consumer.

71. If product is removed from the original package for further processing or re-portioning, appropriate good hygienic practices should be observed to avoid recontamination with STEC.

Packaging conditions

72. Ground/minced products should have sufficient information so that the recipient can safely handle and prepare the product e.g. use-by dates and the need for thorough cooking on the label.

73. Since not all tenderized products are readily distinguishable from non-tenderized products, labelling to state that the product is tenderized, along with validated cooking instructions, may be needed to provide consumers and food service workers the essential information to safely prepare the product (USDA FSIS, 2015).

4.7. CONSUMERS

74. The consumer has an important role in the prevention of foodborne illness from STEC during the manipulation of raw beef at home and should be aware of the proper cooking and handling of raw beef.

75. Consumers should apply the general principles for safer food to ensure safety of raw beef when handling, prepare and consuming; these are.

- Keep the food preparation and consuming sites clean,
- Separate raw and cooked food to avoid/prevent cross-contamination.
- Cook thoroughly.
- Keep food at safe temperatures.
- Use safe water and raw materials for food preparations.

5. VALIDATION OF CONTROL MEASURES

Refer to the general section of this guidance.

6. MONITORING OF CONTROL MEASURES

76. Monitoring data are used to measure the effectiveness of any control measure put in place, to establish alternative or improved measures, and to identify trends and emerging STEC hazards, food vehicles, and food chain practices (FAO/WHO, 2018).

77. Process performance monitoring may be accomplished more effectively and efficiently by quantitatively monitoring hygiene indicator organisms. These indicator organisms do not indicate pathogen presence; instead, they provide a quantitative measure of the control of microbial contamination in the product and processing environment. Periodic testing for “high risk”[13] STEC may also be conducted for verification of process performance (FAO/WHO, 2018).

78. Some raw beef will need more control measures and monitoring than others (e.g. non- intact raw beef, ground/minced raw beef, trim).

7. VERIFICATION OF CONTROL MEASURES AND REVIEW OF CONTROL MEASURES

79. STEC testing is an important part of verification of process performance. However, STEC are generally present at very low levels and are characterised by heterogeneous distribution (including in ground/minced products), making STEC detection challenging. This means that there may be a significant delay between loss of process control and STEC detection. Consequently, verification programs should also include quantitative monitoring of hygiene indicator organisms. Hygiene indicators used should be those that are the most informative for the specific processing environment. Examples of potential hygiene indicators include total bacterial counts, counts of faecal coliforms, and counts of total *E. coli.* An increase in the numbers of the selected indicator indicates decreasing control and corrective action should be taken. The speed in detecting a loss of control of manufacturing hygiene increases with the verification frequency. Verification at multiple points in the processing chain can assist in rapid identification of the specific process where corrective action should be taken.

80. Regular testing for “high risk” STEC can also be conducted for verification of process performance (FAO/WHO, 2018). lot testing is of significant utility, particularly in raw beef that is intended for further processing into ground/minced beef and contributes to directly reducing contamination rates in retail ground/minced beef and promoting continuous process improvement.

81. Verification of other control measures, e.g. concentration of organic acid, temperature of a steam/vacuum or hot water treatment, etc., should be routinely conducted in addition to appropriate microbiological testing.

8. CONSIDERATIONS FOR LABORATORY TESTING FOR DETECTION OF STEC IN RAW BEEF

82. Intact raw beef cuts used for purposes other than the manufacture of finished raw beef products do not present the same level of risk and therefore may require less sampling and laboratory testing.

83. In general, the occurrence of STEC in meat products is lower for intact meat products than in trim or ground / minced beef (Kintz et al., 2017; Develeesschauwer et al., 2019). However, the overall occurrence of STEC in these products can vary considerably due to differences in primary processing and post-processing conditions and interventions.

84. Levels of STEC in non-intact and ground/ minced products are often higher than in intact beef because ground or disrupted tissue presents an environment that is more conducive for bacterial growth. In addition, many of the processing and post-processing interventions are more efficacious if the targeted pathogen is exposed on the surface of the meat as opposed to embedded within a tissue matrix.

85. in large scale processing plants, trim and ground / minced beef originate from the tissues of multiple carcasses, whereas intact raw beef mostly originates from the cuts obtained from a single carcass. The process of amalgamation of tissues from multiple animals can increase the risk of contamination of ground / minced beef.

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[340][13] “High risk” STEC are generally those that present pathogenic virulence factors that are responsible for significant numbers of illness and/or that cause the most severe illnesses, and this may vary by country.

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ANNEX 2. FRESH LEAFY VEGETABLES

INTRODUCTION

1. Fresh leafy vegetables are grown, processed and consumed throughout the world. They are grown on farms of varying size; distributed and marketed locally and globally, providing year-round availability to consumers; and sold as fresh, fresh pre-cut or other ready-to-eat (RTE) products such as pre-packaged salads.

2. Outbreaks of illness caused by a broad range of microbial pathogens, including Shiga toxin-producing *Escherichia coli* (STEC), have been linked to the consumption of fresh leafy vegetables (Bottichio et al., 2019; CDC, 2006, 2012, 2020; Gobin et al., 2018; Herman et al., 2015; Kintz et al., 2019; Kinnula et al., 2018; Marden et al., 2014; Sharapov et al., 2006). Epidemiological evidence, outbreak investigations, research, and risk assessments have identified several possible contamination sources of fresh leafy vegetables with STEC, including water, domestic and wild animals, workers and manure-based soil amendments (Berry et al., 2015; Gelting et al., 2011; Islam et al., 2004; Jay-Russell et al., 2014; Jongman and Korsten, 2018; Olaimat and Hoolley, 2012; Soderstrom et al. 2008). Fresh leafy vegetables are typically grown and harvested in large volumes, increasingly in places where harvest and distribution of fresh leafy vegetables is efficient and rapid. Fresh leafy vegetables are packed in diverse ways, including: field packed direct for market; field cored and prepared for later processing; and as pre-cut fresh leafy vegetable mixtures and blends with other vegetables. Control measures such as antimicrobial washes may be applied prior to packaging and/or shipment to market. As fresh leafy vegetables move through the supply chain, there is also the potential for the introduction and growth of pathogens, including STEC. The increasing worldwide use of pre-packaged fresh-cut leafy vegetables to expand the supply chain might increase the potential for cross-contamination with STEC, and their replication during distribution and storage. There is no processing treatment applied that would eliminate or inactivate STEC, although contamination can be reduced by washing in water containing antimicrobials. Examples of field level control measures provided in this document are illustrative only and their use and approval may vary by country.

3. It is recognized that some of the provisions in this Annex may be difficult to implement in areas where primary production is conducted in smallholdings, whether in developed or developing countries, and in areas where traditional farming is practiced. The Annex is, therefore, a flexible one, to allow for diverse systems of control and prevention of contamination for different cultural practices and growing conditions. Figure 1 provides a flow diagram illustrating a generalized process flow for fresh leafy vegetables. This flow diagram is for illustrative purposes only. Steps may not occur in all operations (as shown with dotted lines) and may not occur in the order presented in the flow diagram.

1. OBJECTIVE

4. The objective of this Annex is to provide guidance to reduce, during production, harvesting, packing, processing, storage, distribution, marketing and consumer use, the risk of foodborne illness from STEC associated with fresh leafy vegetables intended for human consumption without cooking.

2. SCOPE AND DEFINITIONS

2.1 Scope

5. This Annex covers specific guidance for the control of STEC related to fresh leafy vegetables that are intended to be consumed without cooking. Fresh leafy vegetables for the purposes of this Annex include all vegetables of a leafy nature where the leaf is intended for consumption without cooking, and include, but are 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. The Annex is applicable to fresh leafy vegetables grown in open fields or in fully or partially protected facilities (hydroponic systems, greenhouses/controlled environments, tunnels etc.).

2.2 Definitions

6. Refer to the *General Principles of Food Hygiene* (CXC 1-1969) and the *Code of Hygienic Practice for Fresh Fruits and Vegetables* (CXC 53-2003), including Annex I for Ready-to-Eat Fresh, Pre-cut Fruits and Vegetables and Scope of Annex III for Fresh Leafy Vegetables.

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.

3. PRIMARY PRODUCTION

7. Refer to the *General Principles of Food Hygiene* (CXC 1-1969) and the *Code of Hygienic Practice for Fresh Fruits and Vegetables* (CXC 53-2003). As noted in CXC 1-1969, some of the principles of HACCP can be applied at primary production and may be incorporated into Good Agricultural Practices for the production of fresh leafy vegetables to minimize contamination with STEC.

8. Most contamination of fresh leafy vegetables with STEC is thought to occur during primary production (FAO/WHO, 2008; Julien-Javaux, 2019; Mogren et al., 2018; Monaghan et al., 2016). Fresh leafy vegetables are grown and harvested under a diverse range of climatic and geographical conditions. They can be grown in production sites indoors (e.g., greenhouses) and outdoors, harvested, and either field-packed or transported to a packing establishment, using various agricultural inputs and technologies, and on farms of varying sizes. In each primary production area, it is necessary to consider the agricultural practices and procedures that could minimize the potential for contamination of fresh leafy vegetables with STEC, taking into account the conditions specific to the primary production area, type of products, and growing (including irrigating) and harvesting methods used.

3.1 Environmental Conditions

9. Potential sources of STEC contamination should be identified prior to primary production activities. Where possible, growers should evaluate present and previous uses of both indoor and outdoor fresh leafy vegetable primary production sites and the nearby and adjacent land (e.g. animal production, sewage treatment site) in order to identify potential sources of STEC. The assessment of environmental conditions is particularly important because subsequent interventions would not be sufficient to fully remove STEC contamination that occurs during primary production, and in some cases, conditions may enable the growth of STEC, thereby increasing the risk of illness for consumers.

10. If the environment presents a likelihood of contamination of the primary production site with STEC, measures should be implemented to minimize the potential for contamination of fresh leafy vegetables at the site. When such possibilities exist and cannot be minimized, the production site should not be used for fresh leafy vegetable production.

11. The effects of some environmental events cannot be controlled. For example, heavy rains may increase the exposure of fresh leafy vegetables to STEC if soil contaminated with STEC splashes onto them. When heavy rains occur, growers should evaluate the need to postpone harvesting fresh leafy vegetables for consumption without cooking and/or to subject them to a treatment that will minimize consumer exposure to STEC. If fresh leafy vegetables that contact flood waters are not subjected to any measure to mitigate risks from STEC to consumers, they should not be consumed raw. This does not include flooding of furrows for irrigation purposes, where the source of water is known and appropriate quality and is not the result of a weather event.

3.1.1 Location of the Production Site

12. Animal production facilities located in proximity to sites where fresh leafy vegetables are grown and access to the growing site by wildlife can pose a significant likelihood of contamination of production fields or water sources with STEC. Concentrated animal feeding operations and cattle grazing lands present a significant risk of contamination of leafy greens in the field (FDA, 2020; Berry et al., 2015; Yanamala et al, 2011); although guidelines exist for the distance between fields and nearby animal operations (California Leafy Green Products Handler Marketing Agreement (CA-LGMA), 2019), the safe distance depends on factors that can increase or decrease the risk of contamination, such as topography of the land and opportunity for water runoff through or from such operations (CA-LGMA, 2019). Growers should evaluate the potential for such contamination and take measures to mitigate the risk of STEC contamination associated with runoff and flooding (e.g. terracing, digging a shallow ditch to prevent runoff from entering the field).

3.1.2 Animal activity

13. Some wild and domestic animals present in the primary production environment are known to be potential carriers of STEC. Wild animals represent a particularly difficult risk to manage because their presence is intermittent. The following are particularly important to minimize the potential for animal contamination of fresh leafy vegetables with STEC:

- Appropriate methods should be used in order to exclude animals from the primary production and handling areas to the extent practicable. Possible methods include the use of physical barriers (e.g. fences) and active deterrents (e.g. noise makers, scarecrows, images of owls, foil strips).
- Primary production and handling areas should be properly designed and maintained to reduce the likelihood of attracting animals that can contaminate fresh leafy vegetables with STEC. Possible

methods include minimizing standing water in fields, restricting animal access to water sources, and maintaining production sites and handling areas free of waste and clutter.

- Fresh leafy vegetable primary production areas should be regularly checked for evidence of the presence of wildlife or domestic animal activity (e.g. presence of animal faeces, bird nests, hairs/fur, large areas of animal tracks, burrowing, decomposing remains, crop damage from grazing), particularly near the time of harvesting. Where such evidence exists, growers should evaluate the risks to determine whether the fresh leafy vegetables in the affected area of the production site should be harvested for consumption without cooking (Wells et al., 2019).

3.2 Hygienic primary production of fresh leafy vegetables

3.2.1 Water for primary production

14. Several parameters may influence the likelihood of contamination of fresh leafy vegetables with STEC: the source of water used for irrigation and the application of fertilizers and pesticides, the type of irrigation (e.g. drip, sprinkler, overhead), whether the edible portions of fresh leafy vegetables have direct contact with irrigation or other water, the timing of irrigation in relation to harvesting and, most importantly, the occurrence of STEC in the irrigation water. Growers should evaluate the sources of water used on the farm for the likelihood of contamination with STEC and identify corrective actions to prevent or minimize STEC contamination (e.g. from livestock, wildlife, sewage treatment, human habitation, manure and composting operations, or other intermittent or temporary environmental contamination, such as heavy rain or flooding). (Refer to section 3.2.1.1 of the *Code of Hygienic Practice for Fresh Fruits and Vegetables* (CXC 53-2003).)

15. Where necessary, growers should test the water they use for appropriate indicator organisms and, where necessary, STEC, according to the risk associated with the production. The frequency of testing will depend on the water source (i.e. lower for adequately maintained deep wells, higher for surface waters), the risks of environmental contamination, including intermittent or temporary contamination (e.g. heavy rain, flooding), or the implementation of a new water treatment process by growers. If the water source is found to contain unacceptable levels of indicator organisms or is contaminated with STEC, corrective actions should be taken to ensure that the water is suitable for its intended use. Possible corrective actions to prevent or minimize contamination of water for primary production may include the installation of fencing to prevent large animal contact, the proper maintenance of wells, water filtering, chemical water treatment, the prevention of the stirring of the sediment when drawing water, the construction of settling or holding ponds or water treatment facilities. The effectiveness of corrective actions should be verified by periodic water testing. Where possible, growers should have a contingency plan in place that identifies an alternative source of water fit for purpose.

16. It is especially critical in hydroponic operations to maintain the quality of water used as a growth medium for fresh leafy vegetables to reduce the likelihood of contamination and survival of STEC; the nutrient solution used may enhance the survival or growth of STEC. (Refer to section 3.2.1.1.3 of the *Code of Hygienic Practice for Fresh Fruits and Vegetables* (CXC 53-2003).)

3.2.2 Manure, biosolids and other natural fertilizers

17. The use of manure, biosolids and other natural fertilizers in the production of fresh leafy vegetables should be managed to limit the potential for contamination with STEC, which can persist in manure, biosolids and other natural fertilizers for weeks or even months, if the treatment of these materials is inadequate (Shepherd et al. 2007; Gurtler et al., 2018). Composting can be effective in controlling STEC in manure, depending on factors that include time, temperature, indigenous microorganisms, moisture, composition of the compost, pile size, and turning of the pile (Jiang et al., 2003; Shepherd et al., 2007; Gurtler et al., 2018, Gonçalves and Marin, 2007; Rigobelo et al., 2016). Another manure treatment method involves anaerobic digestion (Alegbeleye and Sant'Ana, 2020; Martens and Böhm, 2009). Treatment methods should be validated to inactivate STEC. Refer to section 3.2.1.2 of the *Code of Hygienic Practice for Fresh Fruits and Vegetables* (CXC 53-2003) for practices to minimize microbial pathogens such as STEC in manure, biosolids and other natural fertilizers.

3.2.3 Personnel health, hygiene and sanitary facilities

18. Hygiene and health requirements should be followed to ensure that personnel who come into direct contact with fresh leafy vegetables during or after harvesting will not contaminate them with STEC. Adequate access to, and use of, hygienic and sanitary facilities, including adequate means for hygienically washing and drying hands, are critical to minimize the potential for workers to contaminate fresh leafy vegetables. People known or suspected to be suffering from illness due to STEC should not be allowed to enter any area handling leafy vegetables, including the harvest area. Refer to section 3.2.3 of the *Code of Hygienic Practice for Fresh Fruits and Vegetables* (CXC 53-2003) for practices to minimize microbial pathogens such as STEC.

3.2.4 Harvesting

19. The field should be evaluated for animal intrusion, the presence of faecal deposits, or other sources of STEC contamination prior to harvest to determine if the field or portions thereof should not be harvested. Growers should avoid moving harvesting equipment across fields where manure or compost was applied. Harvesting equipment should be cleaned and disinfected as needed to avoid the contamination of fresh leafy vegetables (e.g., if the equipment runs over an area with animal intrusion and faecal deposits). Containers stored outside should be cleaned and, as appropriate, disinfected before being used to transport fresh leafy vegetables.

3.2.5 Field packing

20. When packing fresh leafy vegetables in the field, care should be taken to avoid contaminating containers or bins by exposure to manure or other contamination sources. When fresh leafy vegetables are trimmed or cored in the field, knives and cutting edges should be cleaned and disinfected frequently to minimize the potential for cross-contamination with STEC.

3.2.6 Storage and transport from the field to the packing or processing facility

21. Fresh leafy vegetables should be stored and transported under conditions that will minimize the potential for STEC contamination and/or growth. Fresh leafy vegetables should not be transported in vehicles previously used to carry heavily soiled root vegetables, live animals, animal manure, compost, or biosolids.

4. PACKING OPERATIONS

22. Refer to the *General Principles of Food Hygiene* (CXC 1-1969) and the *Code of Hygienic Practice for Fresh Fruits and Vegetables* (CXC 53-2003).

4.1 Time and temperature control

23. Refer to the *General Principles of Food Hygiene* (CXC 1-1969). Time and temperature [i.e., 7°C or below] control during packing and storage is essential to prevent growth of any STEC that may be present, since an increase in numbers of STEC will increase the risk of illness.

4.2 Cooling fresh leafy vegetables

24. As far as possible, the cooling of fresh leafy vegetables should take place as rapidly as possible to minimize growth of any STEC that may be present and in a manner that does not contribute to contamination of product with STEC. For example, fresh leafy vegetables can be cooled immediately after harvest by using ice (e.g. for parsley), forced-air cooling, vacuum cooling (e.g. for iceberg lettuce), hydrocooling or spray-vacuum (hydro-vac) cooling.

25. If water used for cooling comes into direct contact with the fresh leafy vegetables, it should be controlled, monitored and recorded to ensure that the concentration of biocides is sufficient to minimize the likelihood of cross-contamination.

4.3 Washing fresh leafy vegetables

26. Packers washing fresh leafy vegetables should follow good hygienic practices (GHPs) to prevent or minimize the potential for the introduction or spread of STEC in wash water. Where used, biocides should be added to wash water as per GHPs, with their levels monitored, controlled and recorded regularly during production to ensure the maintenance of effective concentrations (Zhang, et al. 2009; Nou et al., 2011; Lou et al., 2012; López-Gálvez et al., 2019; Tudela et al., 2019(a), 2019(b)). The characteristics of post-harvest water that may impact the efficacy of the biocidal treatments (e.g. the pH, turbidity and water hardness) should be controlled, monitored and recorded (Gombas, et al. 2017).

5. PROCESSING OPERATIONS

27. Refer to the *General Principles of Food Hygiene* (CXC 1-1969) and the *Code of Hygienic Practice for Fresh Fruits and Vegetables* (CXC 53-2003), including Annex III on Fresh Leafy Vegetables and Annex I on Ready-to-Eat, Fresh, Pre-Cut Fruits and Vegetables.

28. It is recommended that unprocessed fresh leafy vegetable handling areas be physically separated from processing areas to minimize contamination with STEC. Processing, with some exceptions (e.g. cooking) cannot fully eliminate STEC contamination that may have occurred during primary production of fresh leafy vegetables. Processors should ensure that growers, harvesters, packers and distributors have implemented measures to minimize the contamination during primary production of the fresh leafy vegetables and also during subsequent handling in accordance with the provisions in the *Code of Hygienic Practice for Fresh Fruits and Vegetables* (CXC 53-2003).

5.1 Time and temperature control

29. Refer to the *General Principles of Food Hygiene* (CXC 1-1969). Time and temperature control during pre-processing storage, processing and post-processing storage is essential to prevent growth of any STEC that may be present, since an increase in numbers will increase the risk of consumer illnesses.

5.2 Trimming, coring, cutting and shredding of fresh leafy vegetables

30. Cutting knives and other cutting tools, equipment and any other contact surfaces, should be cleaned and disinfected frequently to minimize the potential for transfer of STEC.

5.3 Washing and dewatering/drying cut fresh leafy vegetables

31. Washing and drying are important steps in the control of STEC for fresh-cut leafy vegetables. See Section 4.3 above and section 5.2.2.5.1 of Annex I on Ready-to-Eat, Fresh, Pre-Cut Fruits and Vegetables of the *Code of Hygienic Practice for Fresh Fruits and Vegetables* (CXC 53-2003),

5.4 Cold storage

32. Fresh leafy vegetables should be maintained at appropriate temperatures [i.e., 7°C or below] after cooling to minimize growth of any STEC that may be present. The temperature of the cold storage should be controlled, monitored and recorded.

5.5 Microbiological and other specifications

33. Microbiological testing of fresh leafy vegetables and of water for primary production for STEC is currently of limited use due to difficulty in detecting STEC because of low prevalence and low numbers of the organism in fresh leafy vegetables and in water. Testing of fresh leafy vegetables for indicator organisms, supplemented, where appropriate, by periodic testing for STEC, can be a useful tool to evaluate and verify the safety of the product and the effectiveness of the control measures and to provide information about an environment, a process or even a specific product lot when sampling plans and testing methodology are properly designed and performed. Measures to be undertaken in case of positive results for STEC (or when indicator organisms reach a pre-defined threshold) need to be established and defined. Refer to the *Principles and Guidelines for the Establishment and Application of Microbiological Criteria Related to Foods* (CXG 21-1997).

5.6 Documentation and records

34. It is recommended that harvesting, processing, production and distribution records should be retained long enough to facilitate STEC illness investigation and recalls if needed. This period may significantly exceed the shelf-life of fresh leafy vegetables. Refer to section 5.7 of the *Code of Hygienic Practice for Fresh Fruits and Vegetables* (CXC 53-2003) for the types of records that should be maintained by growers, harvesters and packers that may be important when investigating foodborne illness outbreaks due to STEC.

6. ESTABLISHMENT: MAINTENANCE AND SANITATION

35. Refer to the *General Principles of Food Hygiene* (CXC 1-1969) and the *Code of Hygienic Practice for Fresh Fruits and Vegetables* (CXC 53-2003).

7. ESTABLISHMENT: PERSONAL HYGIENE

36. Refer to the *General Principles of Food Hygiene* (CXC 1-1969).

8. TRANSPORTATION

37. Refer to the *General Principles of Food Hygiene* (CXC 1-1969), the *Code of Hygienic Practice for the Transport of Food in Bulk and Semi-Packed Food* (CXC 47-2001) and the *Code of Practice for the Packaging and Transport of Fresh Fruits and Vegetables* (CXC 44-1995).

9. PRODUCT INFORMATION AND CONSUMER AWARENESS

9.1 Lot identification

38. Refer to the *General Principles of Food Hygiene* (CXC 1-1969).

9.2 Product information

39. Refer to the *General Principles of Food Hygiene* (CXC 1-1969).

9.3 Labelling

40. Refer to the *General Standard for the Labelling of Pre-packaged Foods* (CXS 1-1985) and the *Code of Hygienic Practice for Fresh Fruits and Vegetables* (CXC 53-2003).

9.4 Consumer education

41. Refer to the *Code of Hygienic Practice for Fresh Fruits and Vegetables* (CXC 53-2003).

10. TRAINING

42. Refer to the *General Principles of Food Hygiene* (CXC 1-1969) and the *Code of Hygienic Practice for Fresh Fruits and Vegetables* (CXC 53-2003).

11. RETAIL AND FOODSERVICE

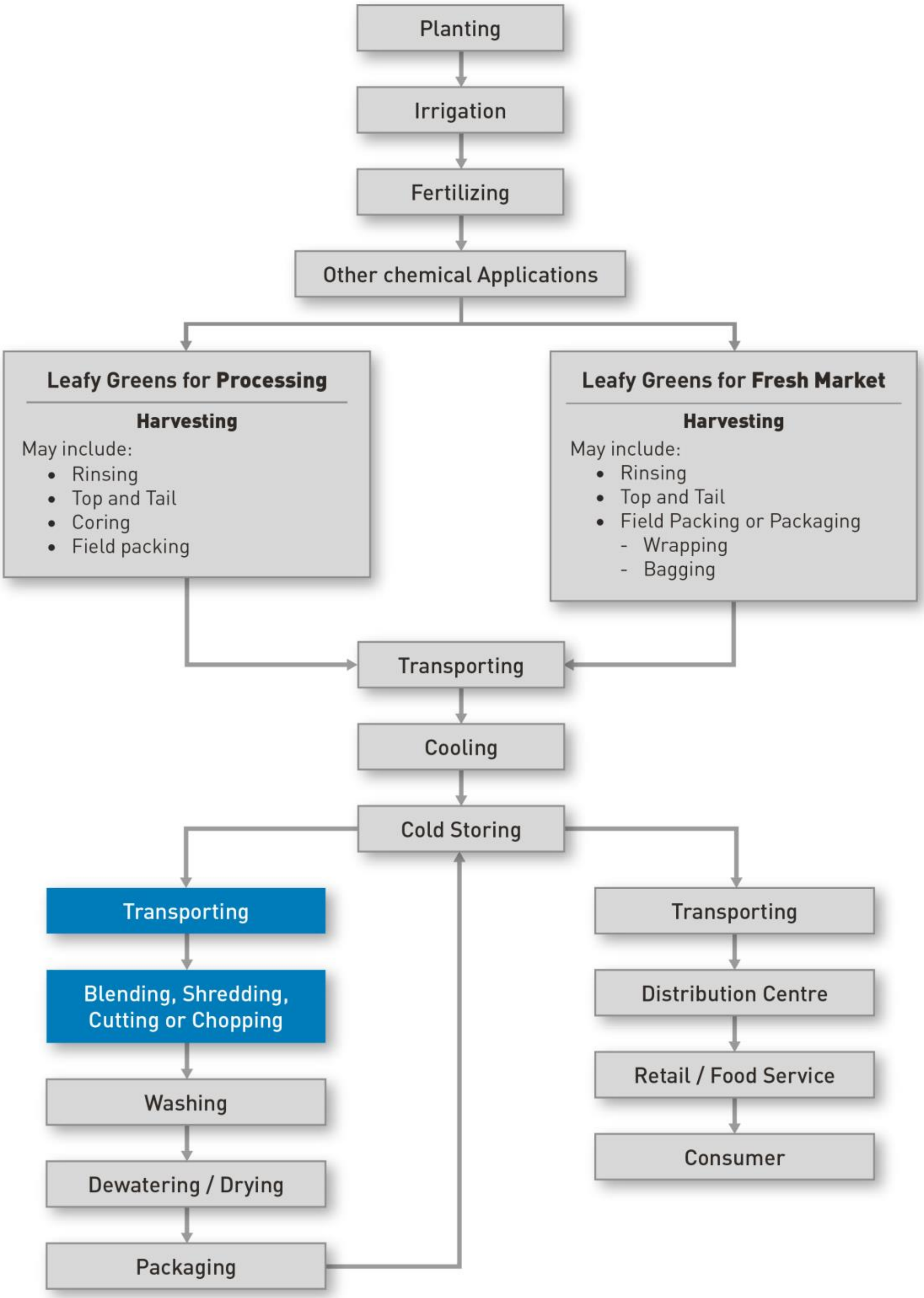
43. Fresh leafy vegetables (intact and pre-cut) should be held at a temperature that prevents growth of STEC [i.e., 7°C or below]. Cross-contamination from or to other food items should be prevented. Food business operators serving fresh leafy vegetables for consumption without cooking to consumers should take appropriate measures to

- prevent cross-contamination,
- maintain appropriate storage temperature, and
- ensure proper cleaning of tools and surfaces that may come in contact with these products.

12. CONSUMER

44. See section 9.4 in the *Code of Hygienic Practice for Fresh Fruits and Vegetables* (CXC 53-2003).

Figure1: Fresh Leafy Vegetables Flow Diagram[14]



[14] Blue boxes indicate steps that may not be included, depending in part on the commodity

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ANNEX 3. RAW MILK AND RAW MILK CHEESES

SPECIFIC CONTROL MEASURES FOR RAW MILK AND RAW MILK CHEESES

1. INTRODUCTION

1. Although most milk for drinking is pasteurized or sterilized UHT milk, raw milk products are consumed in many countries. Raw milk cheeses are fermented products made from raw milk that are consumed in a variety of countries around the world. Cheeses are produced by both large manufacturers and small factories such as farm cheese producers, artisanal cheese producers or industrial cheese makers. Specific combinations of ingredients and technologies are used by manufacturers to obtain a wide variety of cheeses with desired characteristics and meet consumer expectations.

2. Raw milk and raw milk cheeses have been associated with foodborne infections associated with Shiga toxin-producing *Escherichia coli* (STEC) in humans from different countries (FAO/WHO, 2019; Baylis, 2009; Perrin et al., 2015; Honish et al., 2005; Espie et al., 2006; Mungai et al. 2015, Currie et al., 2018; Treacy et al., 2019,). A comprehensive approach, considering all the aspects of raw milk and raw milk cheeses production and consumption, is necessary to reduce the presence of STEC in these products.

3. Cattle are the main reservoir of STEC (Karmali et al., 2010; Salaheen et al., 2019 Rhades et al., 2019). Infected cattle can carry the bacteria in their gastrointestinal tract without any symptoms of disease and shed them in their faeces (Chapman *et al*., 2001; Sarimehmetoglu *et al*., 2009; Brown *et al.,* 1997). STEC have also been isolated from the faeces of other species of animals, including buffaloes, goats and sheep, that are commonly milked for human consumption (Vu-Khac et al., 2008; McCarthy *et al*., 2019; Álvarez-Suárez *et al.*, 2019). Detailed investigations have shown that without observance of appropriate cleaning steps and udder hygiene practices, faecal matter can contaminate the cow's teats and udders, which in turn can contaminate the milk during the milking process (Ruegg 2003). For this reason, STEC can potentially be found in raw milk. When STEC-contaminated milk is used to produce raw milk cheeses, STEC may survive and be isolated from some resulting raw milk cheeses.

4. It is recognized that some of the provisions in this Annex may be difficult to implement in areas where primary production (milk production) and processing (sometimes traditional) are conducted in small establishments. It is also important to emphasize that this document is intended for use by a variety of operators utilizing diverse farming and milk product processing systems. This Annex is therefore intentionally flexible, to allow for different systems of control and prevention of contamination for different cultural and to different processing practices and conditions.

5. This guidance describes the surveillance and the good practices that can contribute to control of STEC in raw milk and raw milk cheeses at different steps in the production chain and, when implemented correctly, can help reduce the risk of contamination and resulting illness. Effectiveness of interventions of different production practices to control STEC based on published data is variable. This is due to the significant differences in experimental design and manufacturing practice among studies. In particular, the efficacy of control measures at multiple steps in the food chain on the overall reduction of STEC in raw milk and raw milk cheeses has not been quantified. Consequently, it will be up to competent authorities and to each operator (farmer and/or dairy) and / or cheese industry to define appropriate risk-based monitoring and control measures, considering relevant scientific and technical information.

2. OBJECTIVE

6. The objective of this annex is to provide science-based guidance for the control of STEC related to raw drinking milk and raw milk cheeses. This guidance focuses on control of STEC during raw milk production (cows, buffaloes, goats and sheep), raw milk cheese making, storage, distribution and consumer use of these products.

3. SCOPE AND DEFINITIONS

3.1. Scope

7. This annex presents specific guidance for control of STEC related to raw milk intended to be drunk and raw milk cheeses.

3.2. Definitions

8. Refer to the *General Standard for the Use of Dairy Terms* (CXS 206-1999), and the *Code of Hygienic Practice for Milk and Milk Products* (CXC 57-2004) Annex I (Guidelines for the Primary Production of Milk) and Annex II (Guidelines for the Management of Control Measures During and After Processing). Also refer to *the General Principles of Food Hygiene* (CXC 1-1969).

- Milk: milk is the normal mammary secretion of milking animals obtained from one or more milking without either addition to it or extraction from it, intended for consumption as liquid milk or for further processing[15].
- 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.[16] This definition excludes 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.)
- Raw milk cheeses: cheeses made from raw milk.
- Validation: Obtaining evidence that a control measure or combination of control measures, if properly implemented, is capable of controlling the hazard to a specified outcome.[17]
- Monitoring: The act of conducting a planned sequence of observations or measurements of control parameters to assess whether a control measure is under control[18].
- Verification: The application of methods, procedures, tests, and other evaluations, in addition to monitoring, to determine whether a control measure is or has been operating as intended.[18]

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

9. Figures 1 and 2 provide flow diagrams describing key steps of raw milk and raw milk cheeses production. Not all steps occur in all operations, there may be other steps, and steps may occur in a different order than shown in the Figures.

10. Raw milk can be a potential source of microbial pathogens, including STEC. It is of major importance to ensure the sanitary quality of the raw milk, which does not undergo a microbial reduction treatment prior to bottling for drinking milk or before the cheese making.

11. The application of combined control measures throughout the food chain is necessary for the control of STEC in the end-products. However, these measures and flow diagrams can vary according to different dairy farming practices and cheese-making processes.

5. PRIMARY PRODUCTION – MILK PRODUCTION AT DAIRY FARM

5.1. STEC at the dairy farm.

5.1.1. Scientific Knowledge

12. STEC contamination on the farm: healthy cattle and other ruminants commonly host and shed STEC. (Karmali et al., 2010, Salaheen et al., 2019; Rhades et al., 2019) (see additional data in the Raw Beef Annex). Most of the available data concern cattle. However, there are a number of scientific articles on the presence of STEC in goat, sheep and buffalo, as well as the environment on these farms (Jacob et al., 2013; Otero et al., 2017; Vu-Khac et al., 2008). Animal-to-animal transmission via faecal-oral transmission is a likely contamination route of STEC within the herd (Chase-Topping et al., 2008). In addition, the introduction of newly purchased animals may be a relevant introduction of a new STEC source (Sanderson et al. 2006; Ellis-Iversen et al. 2008). Environmental transmission has also been demonstrated due to poor housing conditions or to a long survival period of STEC (potentially more than a year) in effluent and the environment (soil, plants, crops, grain and water) (Jang et al., 2017; Nyberg et al., 2019; Haymaker et al., 2019). Pastures can also maintain bacterial circulation by direct faeces deposited onto the ground and/or spreading of effluent (Fremaux et al., 2008; Jang et al., 2017; Nyberg et al., 2019). Risks for STEC contamination on farm are varied and include many factors such as animal health status, animal age, stage of lactation, geography, climate, exposure to wildlife, farm practices and farm practices. Other factors (such as major cleansing in the barn and culling) were associated with a lower *stx* (gene) detection in milk. Other wildlife or livestock, pests, and birds can also carry STEC and thus contribute to their circulation in livestock (Berry et al., 2010; Puri-Giri et al., 2017). These environmental factors and the features of STEC ecology indicate that control strategies based on denying STEC access to hosts or habitat will be highly challenging to implement in a manner which reliably prevents exposure of ruminants to STEC.

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[15] Codex *General Standard for the Use of Dairy Terms* (CXS 206-1999)

[16] 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

[17] *Guidelines for the Validation of Food Safety Control Measures* (CXG 69 - 2008)

13. Feed and drinking water: Contamination of feed with STEC is unusual (Berry and Wells, 2010). Nevertheless, water (surface water, roofing water, contaminated drinking water) can contribute to introduction or circulation of STEC, following direct or indirect contamination (Schets et al., 2005; Lascowski et al., 2013; Saxena et al., 2015).

14. STEC excretion by dairy ruminants: Ruminants are the main reservoir of STEC. A review (Hussein and Sakuma, 2005) has indicated a wide range of estimates for the prevalence of healthy carriage of STEC in dairy cattle. Different studies reported prevalence in faeces varying greatly depending on animal factors, geographic location and production type (Karmali et al., 2010, Salaheen et al., 2019; Rhades et al., 2019). Studies have reported that sheep and goats are also asymptomatic carriers of STEC (Schilling et al., 2012; Pinaka et al., 2013; Bosilevac et al., 2015; Vu-Khac et al.; 2008; Zaheri et al., 2020).

15. The excretion of STEC by ruminants seems to be sporadic but may also be persistent over several months (Rahn et al., 1997; Widiashi et al., 2004). Studies have shown that excretion varies according to the season, peaking in warmer months (Berry and Wells, 2010; Jaakkonen et al., 2019). Excretion also varies among individual cows, with some individuals considered to be "high shedders" (a high-level excretion of STEC) (Chase-Topping et al., 2008), and excretion levels may even differ between cow droppings of the same animal (Berry and Wells, 2010). Other factors proposed to contribute to changes in STEC excretion include age, diet, housing, stress, herd size, animal health, geographical area, and previous contamination with STEC strains. Faecal contamination of sheep and goat milks exist but is less likely than for cows, as their faeces tend to be more solid and thus are less likely to easily cross-contaminate (Otero et al., 2017).

5.1.2. Control measures for STEC at the dairy farm

16. There are no interventions shown to be consistently efficacious in significantly reducing or eliminating STEC in ruminant intestines. In addition, no interventions specific for small ruminants are suggested. Control measures should be implemented to minimize spread between animals and their environments. The following are examples of measures that may be useful:

- maintain animal health and, where possible, minimize animal stress,
- keep litter and bedding as dry as possible,
- apply pest control practices,
- if possible, limit faecal contact with newborn or young animals,
- keep young cattle in the same groups throughout rearing without introducing new animals,
- apply hygienic practices for manure and slurry management, with the maintenance of necessary intervals between spreading on pasture and the reintroduction of animals for grazing (Fremaux et al., 2008).

17. As previously noted, contamination of feed with STEC is uncommon. The presence can be minimized by application of good manufacturing practices and appropriate manure and slurry management when the feed is produced on the farm (*Code of Practice on Good Animal Feeding* (CXC 54-2004)). Secure storage of feed is important to prevent STEC contamination from runoff water, pests and birds. In addition, it is important to limit water contamination for watering animals by adequate maintenance of water troughs (LeJeune et al., 2001).

5.2. STEC during prepping animals for milking, milking, and then transfer of milk to bulk containers/tanks.

5.2.1. Scientific Knowledge

18. STEC are commonly present in the microbiota of milk-producing animals, and it is not possible to eradicate them. There are no established methods to prevent STEC carriage or ensure reduced shedding by ruminants. The major route of raw milk contamination is from faecal sources (directly or indirectly). This in turn soils the teats, and consequently the milk can be subsequently contaminated during the milking process. Therefore, limiting faecal contamination during milking is a major key to manage STEC on the farm (Farrokh *et al*., 2013).

5.2.2. Specific control measures during prepping animals for milking, milking, and then transfer of milk to bulk containers/tanks

19. The implementation of control measures aims primarily at avoiding contamination of the raw milk with STEC during milking and storage on the farm. For this it is important to apply good hygiene practices during milking, to keep animals clean, and to reduce cross-contamination with faeces.

20. Reducing faecal contamination before and during milking:

- Manage a clean and hygienic environment for the milking animals to reduce faecal contamination. For example, the area where milking will be performed should be cleaned.
- Clean and disinfect all milking materials, utensils and equipment.
- Udders and teats should be properly cleaned before the milking process to minimize the risk of contamination of milk with STEC.
- In the case of manual milking, in addition to udder and teats, the operator's hands should be properly cleaned.

21. STEC can also potentially persist on milking equipment and pipelines if these are not adequately cleaned (Annex I Guidelines for the primary production of milk from CXC 57-2004). Cleaning is more challenging if equipment is not well designed for cleaning, and/or not well maintained. STEC can form biofilms in milking machines if they are improperly designed, poorly maintained and/or poorly cleaned. Studies have shown biofilm formation by O157:H7 STEC and non-O157 strains with increased tolerance to sanitizers commonly used in the food processing environment (Wang *et al.,* 2012). All equipment that may come in contact with milking animal teats and milk as it is collected, such as milk collecting buckets, should be thoroughly cleaned and disinfected before every use. The hygienic quality of the water used for the last rinse is very important to prevent contamination of the milking machine (Schets et al., 2005; Lascowski et al., 2013) (CXC 57-2004). In line with the *General Principles of Food Hygiene* (CXC 1-1969), only water fit for purpose (i.e. it does not cause contamination of the milk) should be used. If recycled water is used, it should be treated and maintained under conditions ensuring that its use does not impact the safety of the milk (CXC 57-2004). Well water regularly tested for indicators and/or STEC could also be used.

22. If necessary, carry out an acid treatment based on the milking machine, possibly following or during disinfecting of the equipment (Trząskowska et al. 2018; Sabillon et al, 2020).

6. CONTROLS DURING MILK COLLECTION, STORAGE AND TRANSPORTATION

23. If milk is processed immediately after milking, cooling is not necessary.

24. All equipment that may come in contact with milk, such as tubes and pipes used for transferring milk to larger containers, pumps, valves, storage containers and tanks, etc., should be thoroughly cleaned and disinfected before every use. Although not a standard practice, a full cleaned-in-place, once per 24 h, tanker cleaning approach, with the use of a between-load water rinse with or without a disinfecting treatment has been shown to reduce the presence of surface bacteria in the tanker, and thus may provide some risk reduction.

25. STEC can rapidly multiply in raw milk if the milk is at the temperature of STEC growth (Wang et al, 1997), so temperature control of the milk post-harvest is crucial. Milk should be maintained cold during its storage in the farm and throughout the collection route (Wang et al. 1997, Kim et al. 2014) to prevent microbial growth. Temperatures ≥ 6°C, extended storage of raw milk, and initial bacterial counts in raw milk during collection, storage and transportation have been associated with increased counts of *E. coli* in raw milk. In contrast, deep cooling (2°C) significantly extended the storage life for quality. Milk temperature should be monitored during storage and checked before it is unloaded, when possible.

26. The stage of transport has not been identified as a step likely to contaminate the milk with STEC, if good practices are followed.

7. CONTROL DURING PROCESSING

7.1. Scientific Knowledge

27. Raw milk cheeses are made from raw milk coagulating through the action of rennet or other suitable coagulating agents, and by partially draining the whey resulting from the coagulation, while adhering to the principle that cheese-making results in a concentration of milk protein. Then, different processing techniques can be applied to give the end-products. Different microbiota and very diverse enzymatic reactions play a complex role during processing and maturation. This results in very different cheese types, including ripened or unripened soft, semi-hard, hard, or extra-hard product, which may be coated, uncooked or cooked pressed cheeses (with short or long ripening), blue-type cheeses, lactic cheeses, and white mould cheeses. The different processing steps applied, and the raw milks used from different species (e.g. cow, buffalo, goat, sheep) can influence the behaviour and survival of STEC strains (Miszczycha et al., 2013). The behaviour of STEC (survival, growth or inactivation) can also be influenced by temperature, by the intrinsic physico-chemical properties (pH, a_w, % lactic acid) and by other microflora present specific to different cheeses during their manufacture.

28. At the initial stages of cheese-making, the temperature (around 30 °C) and a_w value of milk provide favourable conditions for the growth of STEC. During the first hours of cheese-making (transition from milk to curd), an increase in STEC level by 1-3 log can be observed for some cheese-making technologies. This increase in number is due to the multiplication of the cells in the liquid milk and then in the curd where cells are entrapped (Miszczycha et al., 2013; Peláez et al., 2019).

29. “Cooking” of cheese curd, as well as rapid acidification (when pH decreases to under 4.3) coupled to the increase of non-dissociated lactic acid, were associated with a range in STEC or *E. coli* log reductions (from 1 to 4 log CFU/g) (Miszczycha et al., 2013; Donnelly and al., 2018). However, the magnitude of reduction varied by STEC serotype and type of cheeses, depending on their intrinsic physico-chemical characteristics (Miszczycha et al., 2013).

30. During the ripening step, the microbial stability of cheeses is determined by the combined application of different hurdle factors (low pH, a_w values, NaCl, non-dissociated lactic acid, starter cultures (such as lactic acid bacteria, *Penicillium* mould)). These hurdles make the cheese become an increasingly challenging environment for STEC during the manufacturing process and ripening (Montel et al., 2014). Various studies have shown that when the ripening is long and therefore the a_w low, the STEC numbers will decrease (Miszczycha et al., 2013). However, if the drying is not long enough, the a_w remains high and a significant reduction of STEC does not occur in the products (Miszczycha et al., 2013 and 2015). Nevertheless, these procedures reduce the number of STEC, but they cannot ensure the safety of the product if the raw milk is contaminated with STEC (Gill and Oudit, 2015). Consequently, the quality of raw milk used in cheese making is crucial to reduce the risk associated with the end products.

7.2. Measures for preventing contamination of milk and milk products

31. The contamination of dairy products with STEC during processing in the manufacturing plants is rare if appropriate hygiene practices are followed (Kousta et al., 2010). It is recommended that the products should be prepared and handled in accordance with the appropriate sections of the *General Principles of Food Hygiene* (CXC 1-1969), the *Code of Hygienic Practice for Milk and Milk Products* (CXC 57-2004) and other relevant Codex texts such as Codes of Hygienic Practice and Codes of Practice.

32. The food business operator (FBO) should analyze the risks associated with its manufacturing process regarding the potential growth or decline of STEC. Based on this assessment, the FBO should adapt the process and/or implement controls to reduce any identified risks for STEC contamination and growth.

33. “Cooking” of cheese curd, rapid acidification or long ripening may not be compatible with some traditional production practices, as they may impact the sensory characteristics of the cheese. In such cases other control measures should be identified and applied. For example, testing the raw milk for the presence of STEC can be established, as well as an audit program of milk suppliers to assess their hygienic practices.

8. PRODUCT INFORMATION FOR CONSUMERS

34. In line with the *Code of Hygienic Practice for Milk and Milk Products* (CXC 57-2004, section 9.1), raw milk products should be labelled to indicate they are made from raw milk according to national requirements in the country of retail sale.

9. VALIDATION, MONITORING AND VERIFICATION OF CONTROL MEASURES

9.1 *E. coli* enumeration and STEC testing

35. Although STEC can be isolated from raw milk and raw milk cheeses, STEC testing is uncommon and most sampling and testing protocols target indicator organisms such as *E. coli*, whose level can be used as an indicator of raw milk quality prior to raw milk cheeses production. Microbiological criteria (refer to the *Principles and Guidelines for the Establishment and Application of Microbiological Criteria Relating to Food* (CXG 21-1997)) based on process and hygiene indicators (*E. coli* / Enterobacteriaceae) may also prove a useful tool for validation, monitoring and verification of control measures.

36. Even if they are useful hygienic markers of the quality of raw milk, the presence or concentration of generic *E. coli* or other indicator organisms in raw milk does not indicate presence of STEC. More specific analyses are needed in cases such as food alerts. Periodic testing for “high risk”[18] STEC may also be conducted for verification of hygienic practices (FAO/WHO, 2018).

[18] “High risk” STEC are generally those that present pathogenic virulence factors that are responsible for significant numbers of illness and/or that cause the most severe illnesses, and this may vary by country.

9.2. Validation and monitoring of control measures

37. Control measures should be validated before being implemented. To limit the cost of this important step, it can be shared by several FBOs and a professional association which may gather, analyse and interpret data in order to establish alternative or improved measures, for example by writing GHP guidelines adapted to the local context or to the traditional steps of processing.

38. The description of control measures may also include the procedures for monitoring their implementation to ensure the control measures are carried out as intended.

9.3. Verification of control measures

39**. At the dairy farm:** Indicator organism testing for faecal contamination can be implemented periodically using indicators of hygiene in milk. For example, routine analysis of milk at the point of production for microbial quality indicators (*E. coli*, coliform levels or total aerobic plate counts) can provide information on the hygiene of the operation. Nevertheless, low levels of microbial quality indicators do not confirm the absence of STEC nor other pathogens.

40. Enhanced monitoring should be implemented when STEC strains have been detected in milk or in cheeses. In such situations an input from technical experts or professional association guidance, as well as guidance from competent authorities, can help to identify the risk factors for milk contamination. Finally, a criterion should be defined for when to return to routine monitoring. This criterion should be based on experience and statistical evaluation of the history of microbiological analyses.

41. General hygiene audits can be useful to check periodically that the GHPs are effectively implemented at each farm where the milk is collected. They might be conducted by the dairy establishment or by a local professional association.

42**. Milk collection to the dairy establishment:** Routine surveillance of the quality of the raw milk received by the dairy establishment (indicators or/and STEC) can be based on samples collected periodically ~~regularly~~ or even for each load. Sampling milk filters may be a more suitable monitoring point for STEC than raw milk from the bulk tank, considering dilution due to pooling and sporadic contamination issues.

43. Enhanced surveillance of all the suppliers can be set up when STEC strains have been detected in mixed milk unloaded at the processing plant. In such a situation, another measure could be to increase the frequency of sampling and STEC analysis in order to assess the milk origin of the strain, the magnitude of contamination and the persistence of the strains in the processing plant. Then, criteria to return to routine monitoring should be defined.

44. **During processing:** A milk quality check based on STEC detection is an option that some FBOs may consider for raw milk (STEC negative milks). This approach can nevertheless be difficult because of the complexity, the time taken and the cost to analyse for STECs in milk. Alternatively, milk quality checks can be performed based on *E. coli*, to verify the application of good hygienic practices.

45. Sampling and testing of raw milk cheeses are an important part of verification plans, to confirm that practices and procedures described in the food safety program are successful. Accurate quality and compositional test results are crucial and depend on appropriate sampling and sample handling, the type of representative samples and proper methods. For routine surveillance, FBOs should consider analysing cheese during the early stages of manufacturing, when the peak of STEC growth is likely to take place. Testing at this time would have a greater sensitivity than end product testing and would save producers the expense of aging and storing contaminated product. Analysis could also be done during ripening and / or before placing the cheese on the market.

46. When STEC are accidentally present in raw milk, it has been found at very low levels in cheeses (Strachan et al., 2001; Buvens et al., 2011; Miszczycha et al., 2013; Gill and Oudit, 2015). This contamination is characterized by heterogeneous distribution (Autry et al.; 2005), making STEC difficult to detect. Sampling plans should therefore be designed according to the *General Guidelines on Sampling* (CXG 50-2004). In addition, sampling plans should be adapted over the entire production chain (number of samples, nature of the samples (for example: milk, cheese at the start of coagulation, during ripening, etc.), quantity analyzed, frequency of analysis, etc.).

47. The FBO defines its sampling plan in line with its own acceptable quality level.

48. Enhanced surveillance can be put in place when STEC are detected in curds or in cheeses or in the case of a public health risk. For example, STEC can be screened in greater detail in other batches of cheeses to assess the magnitude of contamination. In addition, it is important to identify the remaining contaminated milk, if any, and stop using it.

49. **Quantitative risk assessment:** Several sampling plans may be applied at different steps (milk harvested at the farm, milk delivered at the dairy establishment, curds, final products). Their combination in a quantitative risk assessment (QRA) model can help assess the efficacy of this sampling plan, using simulation, in terms of risk reduction of illness and percentage of batches rejected. Specific QRA models for STEC in several raw milk cheeses matrices have been developed (Perrin 2014; see also the opinion of ANSES 2018 STEC (saisine n°2018-SA-0164)). QRA models can also be built based on databases obtained when combining results of microbiological analyses performed regularly on the milk at different levels (farm and tank) and on cheeses (during the process and on the final product), values on technological process parameters and physico-chemical values (e.g., pH, a_w, acid resistance) on the capacity for growth or survival of the microorganisms considered.

50. QRA models can help compare sampling plans to determine which one provides better protection.

51. **Application of prerequisite programmes, including good hygiene practices, and HACCP principles**: Given the low frequency and low level of contamination by STEC strains and the limits of the sampling plans, it is the combination of control measures (including GHPs and HACCP, when applicable) throughout the dairy chain that will reduce the risk of STEC contamination of the products put on the market.

Figure 1. Process Flow Diagram for Raw milk

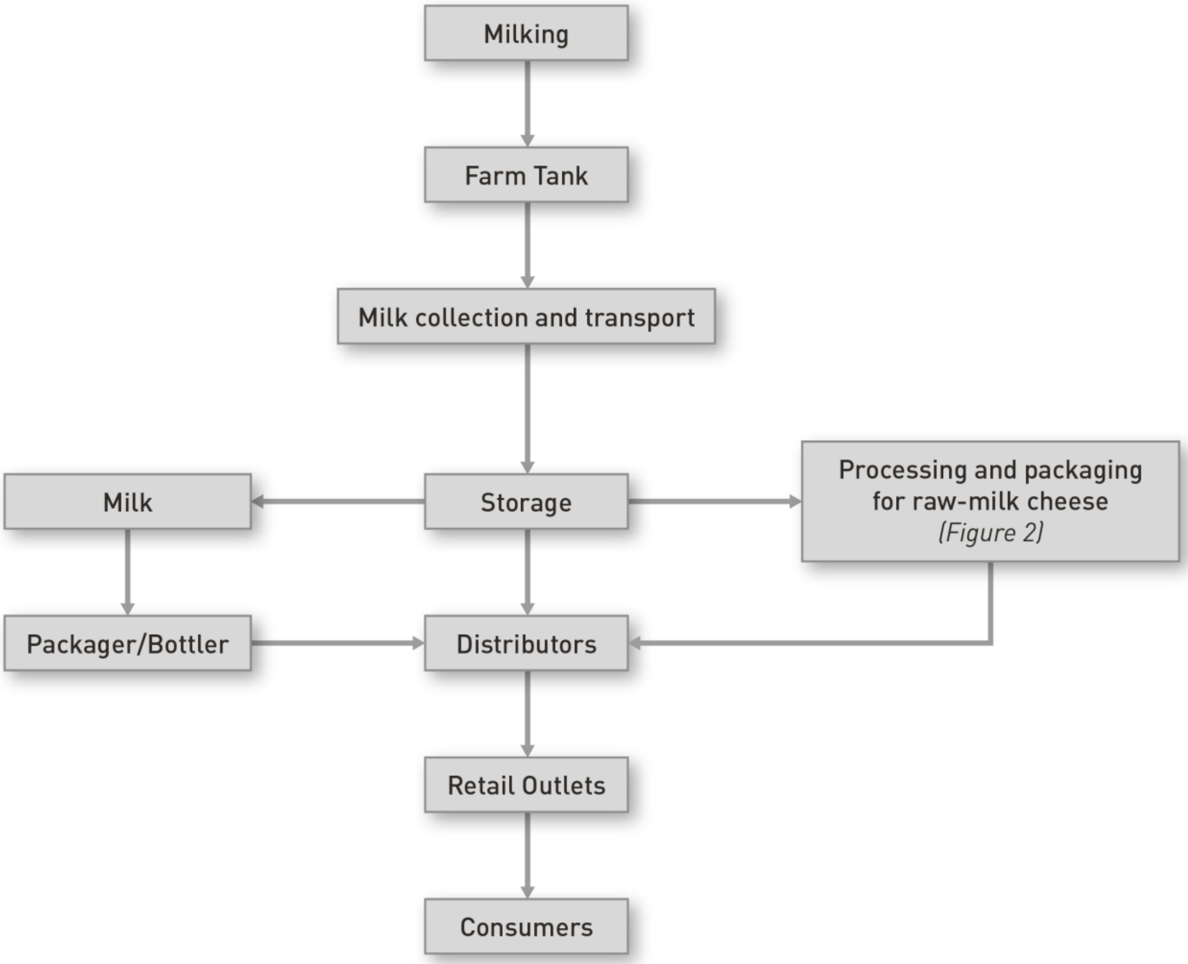
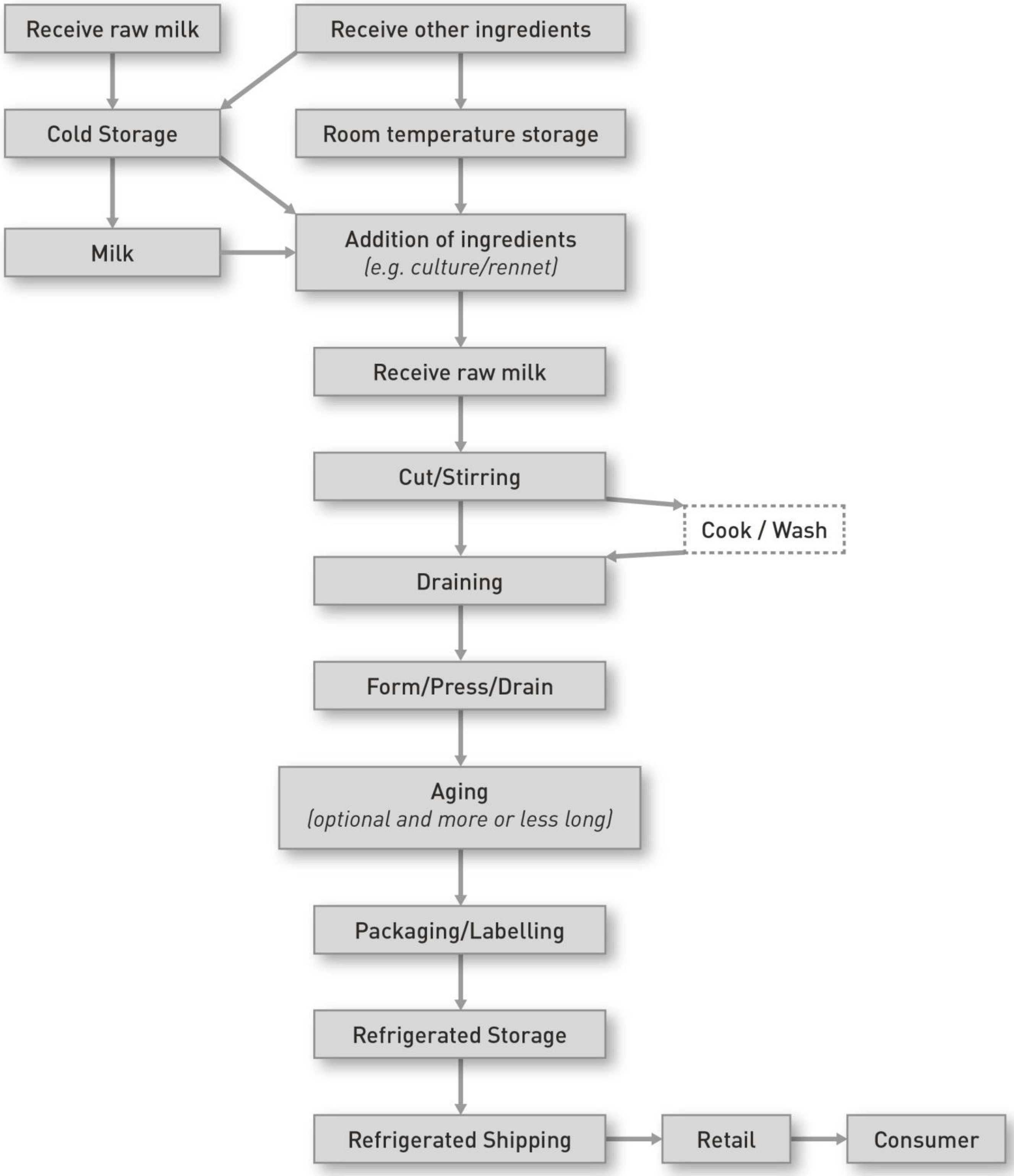


Figure 2: Making Cheese from Raw Milk



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APPENDIX III

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