CODEX ALIMENTARIUS
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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

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1. INTRODUCTION

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 haemolytic uremic syndrome (HUS) with kidney failure and death. STEC have occasionally been linked with neurological symptoms, including epileptic seizures and cognitive dysfunction. 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. STEC strains are a diverse group which can cause disease in humans. STEC strains that can cause haemorrhagic colitis may be referred to as enterohaemorrhagic *E. coli* (EHEC). The most well-studied and documented STEC serotype is *E. coli* O157:H7. The burden of the disease is significant, with substantial outbreaks associated with diverse food commodities. Thus, STEC have a serious impact on public health.

Clinical symptoms of the disease in humans arise as a consequence of consuming food contaminated with *E. coli* that produces Shiga toxin type 1 (Stx1, encoded by the gene *stx1*) and/or Shiga toxin type 2 (Stx2, encoded by the gene *stx2*). Historically, the term verotoxin has also been used for the Shiga toxins of *E. coli* and the term verotoxigenic *E. coli* (VTEC) used synonymously 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* and produce Stx. STEC are pathogenic to humans after ingestion and attachment to the intestinal epithelial cells where production of Stx occurs. Attachment to intestinal epithelial cells is the result of other proteins, including the principal adherence protein intimin, encoded by *eae.* The aggregative adherence fimbrial adhesins commonly associated with enteroaggregative *E. coli*, regulated by the *aggR* gene, when found in isolated strains with *stx,* have also been linked to severe illness and have been used as predictors of pathogenicity. Combinations of virulence genes and their association with disease severity that can be used for risk management purposes are described in these guidelines. There may be additional genes involved in pathogenicity that have not yet been identified. 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 the virulence 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 these guidelines.

Direct contact with animals and person-to-person transmission have been identified as important routes of transmission. Historically, foodborne illnesses caused by STEC have been linked to the consumption of raw or undercooked ground/minced or tenderized (i.e. non-intact) beef; however, fresh leafy vegetables, sprouts, and dairy products (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.

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 faeces is the most likely initial source of STEC in most foods. For example, STEC outbreaks have been associated with raw beef contaminated with STEC during the slaughtering process, field-grown fresh leafy vegetables have been linked to STEC-contaminated irrigation water, and STEC illnesses from sprouts have resulted from contamination during seed production enhanced during sprouting. Raw milk is most commonly contaminated as a result of soiled udders and teats, as well as poor hygiene during milking.

The large degree of variation exhibited by STEC in their biological properties, host preferences, and environmental survival presents a challenge for managing the presence of STEC in animal and plant production. In practice, this means that there is no “one size fits all” solution, and different production systems may require different approaches to control STEC (such as approaches based on pathogenicity and ability to cause severe illness). In most instances, control measures will reduce STEC but not eliminate them.

These 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.

Examples of control measures in each commodity-specific annex have been subjected to a scientific evaluation by the Joint FAO/WHO Expert Meetings on Microbiological Risk Assessment (JEMRA) in development of the guidelines. Such examples are illustrative only; their use and approval may vary among Member Countries.

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; and
- assists in assessing the equivalence (*Guidelines on the Judgement of Equivalence of sanitary Measures Associated with Food Inspection and Certification Systems* [CXG 53-2003])[1] of control measures for raw beef, fresh leafy vegetables, raw milk and raw milk cheeses, and sprouts applied in different countries.

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

2. OBJECTIVES

These guidelines provide information to governments and food business operators (FBOs) on the control of STEC that aims to reduce foodborne disease from raw beef, fresh leafy vegetables, raw milk and raw milk cheeses, and sprouts. They provide a science-based and practical tool for the effective control of STEC in raw beef, fresh leafy vegetables, raw milk intended for drinking 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.

These guidelines do not set quantitative limits as described in the *Principles and Guidelines for the Establishment and Application of Microbiological Criteria Related to Foods* (CXG 21-1997)[2] 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]

3. SCOPE AND USE OF THE GUIDELINES

3.1 Scope

These guidelines are applicable to STEC that may contaminate raw beef, fresh leafy vegetables, raw milk[i] 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

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* (CXC 1-1969),[4] the *Code of Hygienic Practice for Meat* (CXC 58-2005),[5] the *Code of Hygienic Practice for Fresh Fruits and Vegetables* (CXC 53-2003),[6] the *Code of Hygienic Practice for Milk and Milk Products* (CXC 57-2004),[7] the *Guidelines for the Validation of Food Safety Control Measures* (CXG 69-2008),[8] the *Principles and Guidelines for the Conduct of Microbiological Risk Management (MRM)* (CXG 63-2007)[3] and the *Principles and Guidelines for the Establishment and Application of Microbiological Criteria Related to Foods* (CXG 21-1997).[2] These general and overarching provisions are mentioned as appropriate, and their content is not duplicated in these guidelines.

[i] These guidelines present specific guidance for control of STEC related to raw milk intended for drinking and for production of raw milk cheeses.

The guidelines present a number of control measures. These control measures will likely vary at the national level and therefore these guidelines only provide examples of them. Examples of control measures are limited to those that have been scientifically demonstrated as effective in a commercial setting. Countries should note that these 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 hazard reduction. Governments and FBOs can choose hazard-based control measures to inform decisions on critical control points (CCPs) when applying HACCP principles to a particular food process.

Several 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 decontamination of 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 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.

The provision of flexibility in the application of the guidelines is an important attribute. They are primarily intended for use by government risk managers and FBOs in the design and implementation of food hygiene systems.

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

For the purposes of these guidelines, the following terms are defined as below:

Control measure: Any action or activity that can be used to prevent or eliminate a hazard or reduce it to an acceptable level (*General Principles of Food Hygiene* [CXC 1-1969]).[4]

Fresh leafy vegetables: Vegetables of a leafy nature where the leaf is intended for raw consumption**,** 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.

Indicator microorganisms: Microorganisms used as an indicator of quality, process efficacy, or hygienic status of food, water, or the environment, commonly used to suggest conditions that would allow the potential presence or proliferation of pathogens, a failure in process hygiene or in food processing. Examples of indicator microorganisms include mesophilic aerobic bacteria, coliforms or faecal coliforms, *E. coli* and Enterobacteriaceae.

Monitor: The act of conducting a planned sequence of observations or measurements of control parameters to assess whether a control measure is under control (*General Principles of Food Hygiene* [CXC 1-1969]).[4]

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

Raw milk: Milk (as defined in the *General Standard for the Use of Dairy Terms* [CXS 206-1999])[9] which has not been heated beyond 40 ºC or undergone any treatment that has an equivalent effect.[iii, iv] See also the *Code of Hygienic Practice for Milk and Milk Products* (CXC 57-2004).[7]

Raw milk cheeses: Cheeses made from raw milk.

Shiga toxin-producing *E. coli* (STEC): A diverse group of pathogenic bacterial strains of *Escherichia coli* that are demonstrated to carry Shiga toxin genes (*stx*) and produce Shiga toxin protein (Stx).

[ii] 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.
[iii] Temperatures between 40 ºC and pasteurization temperatures are generally considered to be insufficient to consistently kill STEC in raw milk. Heat treatment beyond 40 ºC results in changes such that the structure of the resultant product is no longer the same as that of raw milk.
[iv] Milk that has been subject to processing techniques such as microfiltration and/or bactofugation is no longer considered raw milk because these processes require milk to be heated above 40 °C.

Sprouts: Sprouted seeds or beans harvested when the cotyledons (or seed leaves) are still un- or underdeveloped and true leaves have not begun to emerge. They can be grown in water, soil or substrate and can be harvested with or without the root (cut sprouts) (FAO and WHO. 2022. *Prevention and control of microbiological hazards in fresh fruits and vegetables – Part 3, sprouts.* Meeting report. Microbiological Risk Assessment Series No. 43. Rome).[10]

Validation of control measures: Obtaining evidence that a control measure or combination of control measures, if properly implemented, is capable of controlling the hazard to a specified outcome (*General Principles of Food Hygiene* [CXC 1-1969]).[4]

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 (*General Principles of Food Hygiene* [CXC 1-1969]).[4]

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

Overarching principles for good hygienic practice for meat production are presented in the *Code of Hygienic Practice for Meat* (CXC 58-2005),[5] 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),[6] Annex I on Ready-to-eat fresh pre-cut fruits and vegetables, Annex II on Sprouts and Annex III on Fresh leafy vegetables. Additionally, see the *Code of Hygienic Practice for Milk and Milk Products* (CXC 57-2004)[7] 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[11] 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[3] 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

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.

Good hygiene practices (GHPs) and other prerequisite programmes provide the foundation for most food hygiene systems. Where possible and practicable, food safety control measures for STEC should incorporate hazard analysis activities and appropriate 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)* (CXC 63-2007).[3] 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. FBOs can select the risk-based measures to facilitate the effective application of process control systems and comply with the requirements of the competent authority. When no microbiological criteria or food safety objectives have been established by competent authorities, FBOs are also able to propose control measures based on risk assessment. These control measures need to be validated.

Specific control measures for STEC are described in each commodity-specific annex, where appropriate: Annex I – Raw beef; Annex II – Fresh leafy vegetables; Annex III – Raw milk and raw milk cheeses; Annex IV – Sprouts.

6.1 Development of risk-based control measures

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

Risk-modelling tools can be developed[12] to assess the impact of control measures on the prevention, reduction, or elimination of the hazard. The capability and limitations, including the need for quantitative data, of the tools should be clearly specified and understood by the risk manager.

Competent authorities formulating risk management metrics[3] as regulatory control measures should apply a methodology that is scientifically robust and transparent.

7. PRIMARY PRODUCTION CONTROL MEASURES

Controls in the primary production phase of the process flow are focused on decreasing the number of animals that are carrying STEC, and the degree of shedding by those that are, as well as preventing or reducing contamination of crops/plants 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

Appropriate controls to prevent and/or reduce the contamination and cross-contamination by STEC of commodities during processing are important. Control measures during post-processing handling and storage are also important to prevent growth of and cross-contamination with STEC.

9. FOOD DISTRIBUTION CONTROL MEASURES

Control measures during distribution to ensure product is stored at an appropriate temperature to prevent growth of STEC, when present, to higher levels and to minimize cross-contamination by STEC are important.

10. VALIDATION, IMPLEMENTATION, AND VERIFICATION OF CONTROL MEASURES

Implementation 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 (Section 7 of the *Principles and Guidelines for the Conduct of Microbiological Risk Management (MRM)* [CXG 63-2007]).[3]

10.1 Prior to validation

Prior to validation of the control measures for STEC, the following tasks should be completed:

a) 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.

b) Identification of any existing food safety objective or target established by the competent authority or FBOs. In order to comply with the target set by the competent authority, FBOs may set stricter targets than those set by the competent authority.

10.2 Validation

Validation of control measures may be carried out by FBOs and/or the competent authority.

Where validation is undertaken for a measure to control 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)[8] (Section VI) provides detailed advice on the validation process.

10.3 Implementation of validated control measures

Refer to Section 9.2 of the *Code of Hygienic Practice for Meat* (CXC 58-2005),[5] the *Code of Hygienic Practice for Fresh Fruits and Vegetables* (CXC 53-2003),[6] and the *Code of Hygienic Practice for Milk and Milk Products* (CXC 57-2004).[7]

10.4 Food business operators' responsibility

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

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

10.5 Regulatory systems

The competent authority, working with the relevant food sector, may provide guidelines and other implementation tools to FBOs, as appropriate, for the development of the food hygiene systems.

The competent authority should assess the documented process control systems to ensure they are science based and establish verification frequencies. Microbiological testing programmes, or molecular testing programmes, should be established to verify the effectiveness of control measures for STEC.

10.6 Verification of control measures

Refer to Section 9.2 of the *Code of Hygienic Practice for Meat* (CXC 58-2005),[5] the *Code of Hygienic Practice for Fresh Fruits and Vegetables* (CXC 53-2003),[6] the *Code of Hygienic Practice for Milk and Milk Products* (CXC 57-2004),[7] and Section IV of the *Guidelines for the Validation of Food Safety Control Measures* (CXC 69-2008).[8]

10.7 Food business operators

FBOs may use testing information on indicator microorganisms for verification of STEC control measures due to the high cost of testing for detection of STEC and its low prevalence in food. FBO 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 an employee with overall responsibility for monitoring activities observe the person conducting a monitoring activity at a specified frequency), reviewing monitoring, corrective action and verification records, and sampling and testing for indicator microorganisms and STEC where appropriate.

Due to typically low numbers and low prevalence of STEC in food, quantitative monitoring of STEC is impractical and the utility of presence/absence testing in monitoring process performance is also limited.[13] 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 or growing environment. The hygiene indicator microorganisms used should be those that are the most informative for the specific processing or growing environment. An increase in the number of the indicator microorganisms above established control values indicates a loss of control and the need for corrective action. Additionally, with the increase in the frequency of verification, there is also an increase in the speed of detecting a loss of control of manufacturing hygiene. Verification at multiple points in the processing chain can assist in rapid identification of the specific process step where corrective action should be taken. Monitoring of hygiene indicator microorganisms can be supplemented by periodic testing for STEC, where appropriate and as needed, to make risk-based decisions. If testing results are linked to requirements for corrective action, then STEC testing can contribute to reducing contamination rates, improving food safety, and promoting continuous process improvement.

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.

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

10.8 Regulatory systems

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

11. MONITORING AND REVIEW

Monitoring and review of food hygiene systems is an essential component of the application of a risk management framework.[v] It contributes to verification of process control and demonstrating progress towards achievement of public health goals. Effective monitoring programmes are essential to verify the effectiveness of STEC control processes throughout the food chain.

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 regulatory goals for STEC control, 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 hygiene systems and make improvements where necessary.

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

11.1 Monitoring

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

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

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

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

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

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

11.2 Laboratory analysis criteria for detection of STEC

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

- product batch or lot acceptance;
- process performance control to meet domestic food regulation;
- to verify controls to meet market access requirements (e.g. to meet microbiological criteria of another country); and
- public health investigations.

The number of foods identified as a vehicle 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 strains considered to be a country's highest priority (e.g. those strains with virulence factors capable of causing severe illness or considered to cause significant illness in that country). Analytical methods that are fit for purpose, that will provide answers to risk management questions, and that are within the resources of governments and FBOs should be chosen.[13] In the event that a laboratory does not have the resources and technology to characterize the isolate, it could be sent to a reference centre/laboratory.

The risk of severe illness due to STEC infection can be predicted to a large extent 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 virulence potential of STEC strains recovered from food samples. Based on current scientific knowledge, all STEC strains are pathogenic for humans and capable of causing illness. However, STEC strains with *stx2a* and adherence genes, *eae* or *aggR,* have the greatest association with severe illness such as bloody diarrhoea (BD), HUS and hospitalizations. 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.

The severity of STEC illness and the potential to cause diarrhoea, BD and HUS, hence the degree of public health relevance, can be defined to a large extent 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[13] as criteria (Table 1) for developing risk management goals that prioritize:

- 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.

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. Knowledge on virulence factors and their combination and their public health importance is evolving rapidly, and it is therefore important to continually monitor new scientific evidence.

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

The determination of virulence and other salient marker genes for testing purposes may be achieved by using, for example, polymerase chain reaction (PCR) methods or whole genome sequencing (WGS) analysis on isolated strains. Special consideration should be given to the efficacy of sample collection techniques to maximize portions of product most likely to be contaminated. The choice of enrichment culture techniques used to recover STEC from foods is also important, as STEC strains are physiologically diverse, with variable growth characteristics. Selective conditions which are permissive to specific subpopulations of STEC, such as *E. coli* serotype O157:H7, can be used, but this poses the risk of inhibiting the multiplication of other STEC strains, preventing their detection.

In addition, bacteria other than STEC may contain the same virulence genes and the detection of these genes alone may not fully reflect health risks due to differential or lack of gene expression. It is therefore important to confirm that the defining genes are within a single STEC isolate, following isolation by traditional culture, with or without immunomagnetic separation (IMS), or other validated methods (e.g. molecular techniques). An isolate may also be required for characterization of STEC (e.g. molecular sequencing for epidemiological investigation) and a better estimation of food safety risk.

The virulence genes carried by STEC isolates should be considered when deciding how STEC will be managed in food commodities, including the actions to be taken when STEC is detected in the food. As shown in Table 1, different combinations of virulence genes differ in the risk for severe illness, but other factors also play a role. Both strains carrying particular virulence genes and other factors associated with a greater risk of severe illness, or number of illnesses, may vary regionally. Countries may identify factors to differentiate STEC that are considered to be a higher priority (e.g. those strains with virulence factors capable of causing severe illness or considered to cause significant numbers of illness in that country) from those that are a lower priority. In general, more stringent corrective actions would be applied in response to the presence of high priority STEC strains.

11.3 Review

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

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

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

Competent authorities should consider the results of monitoring and review when re-evaluating and updating public health goals for control of STEC in foods, and when evaluating progress. Monitoring of food chain information in combination with data on food source attribution and human health surveillance 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.

ANNEX I

RAW BEEF

1. INTRODUCTION

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

STEC can be part of the normal 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. STEC shedding by individual cattle is transient and episodic. In addition, STEC can be found within the farm environment, and it is therefore likely that cattle arriving for slaughter have STEC on their hides. Individual feedlot cattle studies have reported high prevalence of STEC on cattle hides presenting for slaughter.

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

STEC carried by cattle could be spread to carcasses during slaughter. Prior to slaughter, the muscle tissue of healthy cattle is free of STEC. STEC can be transferred to carcass surfaces from the contents of the gastrointestinal tract or hide during the operations of de-hiding, head removal, bunging and evisceration. Generally, contamination is confined to the carcass surface and is not found in deep muscle tissues of intact raw beef.

STEC contamination has historically been detected in non-intact raw beef products. Practices including grinding/mincing, and mechanical tenderization 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.

Mixing of tissues from one or multiple animals/herds can increase the likelihood of spreading and diluting STEC contamination of ground/minced raw beef. Distribution and level of STEC in non-intact raw beef, such as 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.

2. SCOPE

These guidelines apply to control of STEC in raw beef, including non-intact products such as raw ground/minced or tenderized beef.

These guidelines do not apply to raw beef meat preparations (raw beef meat which has had foodstuffs, seasonings or additives added to it).

3. DEFINITIONS

For the purpose of these guidelines, the following definitions apply:

Minced beef: Boneless beef which has been comminuted, i.e. reduced into fragments.[i]

Non-intact raw beef:[ii] Comminuted beef products such as those that are ground or minced, as well as those that have been mechanically tenderized.

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

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[i] Adapted from the *Code of Hygienic Practice for Meat* (CXC 58-2005).

[ii] Non-intact raw beef products can also include raw beef that has been injected/enhanced with solutions or reconstructed into formed entrees (e.g. beef that has been scored to incorporate a marinade, beef that has a solution of proteolytic enzymes applied to or injected into the cut of meat, or a formed and shaped product such as beef gyros), but these non-intact beef products are out of scope for this document.

[iii] 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.

Tenderized raw beef:[iv] Cuts of beef that have gone through a technological process for the rupture of muscle fibres by mechanical action with small blades or needles which penetrate the muscle surface thereby resulting in tenderizing.

4. PRIMARY PRODUCTION TO CONSUMPTION APPROACH TO CONTROL MEASURES

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. Some of the control measures of this document may be subject to approval by competent authorities.

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 the application of control measures individually or in combination. This is particularly important as individual countries use different primary production and processing systems. Risk managers need the flexibility to choose risk management options that are appropriate to their national context.

STEC have a wide range of potential hosts, and STEC cells can potentially persist for over a year in the natural environment; therefore, effective control strategies based on preventing STEC infection of cattle or contamination of their environment can be difficult to implement.

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 hygienic practices during slaughter, processing, and distribution. Conversely, there is evidence that the adoption of good hygienic practices during slaughter and processing can minimize carcass contamination with STEC. Consequently, the adoption of best practices for pre-harvest management of cattle can support hygienic slaughter and processing.

Operations to decontaminate carcasses or raw beef cuts will be of limited effectiveness if poor hygienic 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 the type of treatment, duration, method of application, operator training, temperature, etc.

5. GENERIC FLOW DIAGRAM FOR APPLICATION OF CONTROL MEASURES

Figure 1 provides an example of a process flow diagram for primary production to consumption of beef.

These process steps are generic, and not all the steps may occur during processing, in the order shown, or at the same establishment. Grinding/mincing, for example, can be done at sites other than the slaughter or fabrication site, and carcass washing with or without biocides is not performed in all countries or slaughterhouses. 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.

[iv] Tenderizing processes that include the injection of solutions with or without a vacuum are out of scope.

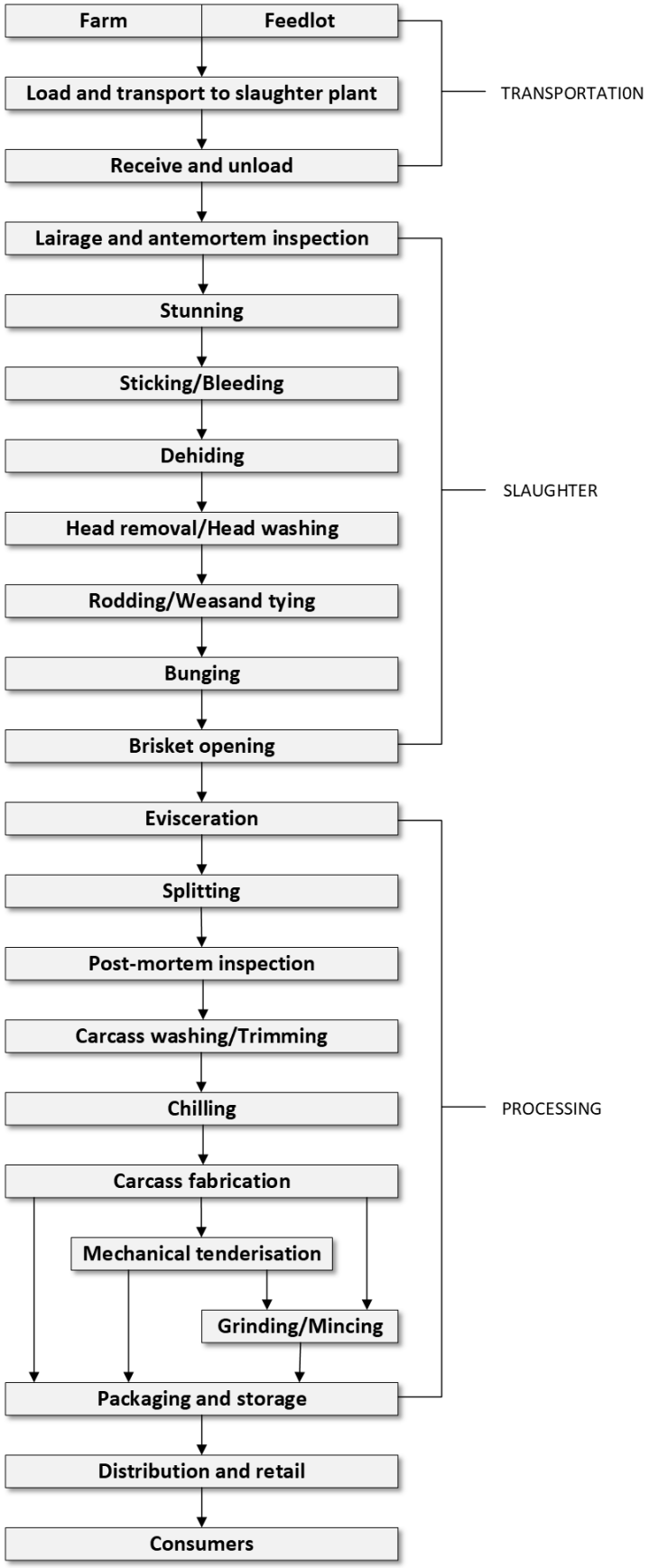

Figure1. Example flow diagram of raw beef primary production and processing

6. PRIMARY PRODUCTION

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

6.1 Specific control measures for primary production

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 used in water and feeds, manipulation of animal feeds, and primary production management practices, as explained below.

Many of these proposed pre-slaughter control methods have not been demonstrated to effectively 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).

6.2 Diet components

A wide variety of cattle diets have been investigated for their impact on STEC serotype O157:H7 prevalence and/or level of shedding, including hay, barley, distillers and brewers' grains, sage brush, millet, alfalfa. 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 serotype O157:H7. Some diets that have been proposed increase STEC serotype O157:H7 shedding.

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, but the effect of forage diets on faecal shedding of STEC serotype O157:H7 is inconclusive.

6.3 Use of direct-fed microbials

Faecal shedding of STEC serotype O157:H7 by cattle can be reduced using direct-fed microbials (DFM) such as *Lactobacillus acidophilus* and *Propionibacterium freudenreichii*. The impact of DFM against STEC is highly specific, thus STEC reduction with one probiotic product cannot necessarily be extrapolated to another product. To be effective, the component strains in the product should be consistent and the products should be administered at the recommended CFU/g doses in feed.

6.4 Vaccination

Some vaccines have been shown to reduce faecal shedding of STEC serotype O157:H7 but their efficacy at individual level is dependent on type of vaccine and the number of doses administered; most vaccines will need more than one application in order to be effective. The impact of reducing STEC serotype O157:H7 in raw beef is dependent on the extent of adoption of vaccination. The use of vaccines should consider feasibility of application regimes to ensure their efficacy at individual and herd level.

6.5 Good management practices at primary production

The following good management practices for cattle are recommended for minimizing 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 dehiding 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 (including sudden changes to diet) and feed deprivation).
- Minimize exposure between herds to avoid or reduce horizontal transmission of STEC across herds.
- Reducing animal density to reduce direct animal-to-animal transmission (e.g. maintain ample space for animals to move to reduce defecation directly onto one another).
- To the extent of possible, maintain clean living conditions (e.g. clean holding areas, remove gross contamination, and maintain clean and dry bedding) to prevent potential transmission from the living environment (e.g. animals resting in STEC-contaminated materials). Use of slatted housing requires careful attention to stocking density to avoid hide soiling.
- Reduce the potential for STEC transmission through consumption of contaminated feed and water by the following:

- Design feed and water delivery systems (tanks, trough, bins, etc.) in a way to reduce the potential for animal entrance and defecation.
- Ensure water is fit for purpose and of a microbiological quality that minimizes animal contamination. If there is doubt, treat the water to render it both microbiologically and chemically safe.
- Clean water troughs and, when possible, use materials in water troughs that facilitate the cleaning process.

7. TRANSPORTATION

7.1 Specific control measures for transport to slaughterhouse

Transportation can be a major contributor to the increasing occurrence of pathogens in cattle 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.

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

- Handle animals so that they are not unduly stressed.
- As much as practical, minimize the distance over which cattle are transported to slaughter; longer transportation distances have been shown to increase the risk of having STEC-positive hides at slaughter compared to cattle that travel a shorter distance.
- Ensure animals are as clean as possible to reduce the risk for pathogen cross-contamination from hides onto carcasses 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 the top level to bottom level in multi-level trailers to the extent possible, and do not overcrowd the vehicle.

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 may include:

- When possible, 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 inspection and controls, when needed, for soiled animals, transportation vehicles and lairage pens for visible faecal contamination.

7.2 Specific control measures at receive and unload

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.

Adequate training of the operators on procedures that can minimize stress at this step (which could increase shedding of STEC) is recommended.

8. SLAUGHTER AND DRESSING

Good hygiene practices (GHPs) and emphasis on good manufacturing practices (GMPs) at slaughter 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, clipping the weasand, bunging and evisceration, as these operations are the initial sources of microbiota that contaminate meat surfaces. Other measures may 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 carcass.

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.

Interventions aimed at removing STEC from the surface of beef carcasses should consider that tolerance to 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 validated (e.g. by conducting experimental trials with surrogate microorganisms that have similar or greater resistance to individual treatments than STEC. Careful consideration is needed when determining suitable strains for validation of interventions, since surrogates may not necessarily be equivalent to wild-type strains isolated from raw beef).

Interventions should be safe and application feasible along the production process and should not change the organoleptic properties of beef.

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

Operators should be effectively and appropriately trained to perform their operation in the slaughter process in order to minimize the potential for STEC contamination.

8.1 Specific control measures at lairage and antemortem inspection

In this stage, the condition of the animals should be evaluated; animals should be as clean and dry as possible to minimize the initial load of microorganisms, which potentially includes STEC, on their hide. STEC is harboured on the hide, not only in faecal material, but also in dried-on dust. The level of both on the hide should therefore be minimized. Where practical, dirty, or wet animals should be segregated to prevent cross-contamination.

The lairage area should be cleaned to the extent possible for each lot of animals using fit-for-purpose water under appropriate pressure to remove gross contamination on the floor. Cleaning and disinfection should be applied according to GHP and manufacturer's instructions. The lairage area should be designed to be well-drained in order to facilitate drying. A dry bedding area is preferable where possible (e.g. the use of straw-bedded pens may be considered). Whenever possible, waiting time at the lairage should be minimized.

GHP such as washing dirty live animals (e.g. spray, mist, rinse, or wash), specifically the animal´s hide, with different substances (e.g. fit-for-purpose water, bacteriophage) to reduce contamination has been investigated. However, in general, the evidence for washing in reducing the transfer of STEC from hide to carcass is low.

When feasible, at lairage cattle should not be comingled with other herds/lots to prevent cross-contamination between herds/lots.

8.2 Specific control measures at stunning, sticking and bleeding

Prior to stunning, animals may be sprayed in the accessway using low volume water jets at appropriate pressure. Similarly, the perianal region may be washed, but sparingly and only to remove faeces (the source of STEC) released during the stunning process. Washes should be designed to reduce faecal and STEC contamination and not stress the animal or inhibit the stunning, sticking or bleeding effectiveness. Where water is applied, consideration should be given to removal of excess water prior to hanging of the carcass.

The stunning box and sticking table should be kept as clean as possible with frequent removal of faeces and ingesta to avoid contamination of animal hides in the fall after the stunning process.

Any stunning method (e.g. self-contained bolt, firearm, electrical stunning) should be evaluated and used in a manner that minimizes STEC transfer into the head meat.

Sticking and bleeding should be done in a manner to reduce transfer of hide contamination to the carcass. This includes cleaning and disinfection of knives. Preparing the penetration or cut sites (e.g. with steam/vacuum treatment or a mechanical process like scraping the hide surface) can reduce the likelihood of contamination.

Allow an adequate distance between carcasses (i.e. avoid carcass-to-carcass contact), walls and equipment to minimize cross-contamination during processing.

8.3 Specific control measures at dehiding

Dehiding is the systematic process for separating the hide from the carcass and is perhaps one of the most critical operations 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 appropriately trained to perform this operation to maximize hygienic dressing.

To prevent transfer of contamination from the hide to the carcass during hide opening (opening cuts), 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.

The number of workers, their training requirements, and the role of their rotation in cross-contamination during the dehiding process, needs to be considered.

The dehiding operation should be performed in a manner to avoid contact of the hide with the already exposed parts of carcass (i.e. dehiding the entire peri-anal region and bending the hide, making it stay above the tail). Using non-absorbent paper to protect specific areas of the carcass such as the brisket and bagging of the tail may also be useful practices for reduction of STEC contamination due to contact with hide during dehiding. Remove the hide from the top down rather from the bottom up to prevent contaminating the carcass with dust and hair that may be contaminated with STEC. Care should also be taken to avoid cross-contamination in other operations carried out simultaneously with dehiding, such as the removal of the pizzle, the skinning of the shank tendons, the removal of the udder or scrotum, and transfers by overhead rail.

Measures should be taken to prevent tail flapping and its contact to the carcass when hide pullers are used.

8.4 Specific control measures at rodding

The rodding operation consists of using a metal rod to free the oesophagus (weasand) from the trachea and surrounding tissues. In some countries, 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.

To prevent cross-contamination of the carcass from the weasand/oesophagus during the rodding operation, procedures can include:

- Avoid a delay in tying the weasand to minimize contamination of neck meat with STEC.
- Hanging the carcass vertically, to cut the muscle and tissue to expose the oesophagus.
- Using ties, clips, or bungs to close the weasand hygienically to prevent rumen spillage.
- “Dropping” heads by cutting the oesophagus below the tie or clip.
- Changing or cleaning and disinfecting the weasand rod between each carcass.

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, such as separating the carcass immediately from the others.

When appropriately applied, these procedures will reduce contamination with gut microorganisms, but their specific effect on contamination by STEC remains unknown. Nevertheless, procedures that reduce faecal contamination are most likely to have an impact on STEC contamination.

8.5 Specific control measures at bunging

Bunging is the point in the slaughter process where a cut is made around the rectum to free it from the carcass. Then it is tied off and bagged to prevent spillage of faecal material.

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.

The use of separate clean knives for dehiding and rectum removal is recommended to avoid cross-contamination of the rest of the carcass.

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

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

8.6 Specific control measures at brisket opening

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

To prevent introduction of contamination into the carcass during brisket opening, procedures 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, such as separating the carcass immediately from the others.

9. PROCESSING

STEC on the carcass can remain on meat cuts or be transferred to previously uncontaminated meat cuts as the carcass is further processed, especially via hands and meat processing equipment.

9.1 Specific control measures at evisceration

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.

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

- 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.
- Use belly spreaders, when possible.

To prevent contamination of the carcass by employees during evisceration, techniques can include:

- The appropriate use of knives and equipment 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. Cleaning of the environment as well as operator protective equipment and tools being used at the time of the contamination event should be undertaken as needed, to prevent cross-contamination of leading and trailing carcasses.

9.2 Specific control measures at carcass splitting and trimming

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

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

- Removing visible carcass defects that may contaminate the saw or cleaver (e.g. faeces, milk, ingesta, abscesses) 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 split half carcasses and between different carcasses (i.e. avoid carcass-to-carcass contact), walls and equipment.

Targeted removal of visible contamination on carcasses by trimming may be applied, but trimming may also contribute to possible redistribution of contamination on the carcass or cross-contamination of other carcasses from knives (if not using a knife-switching disinfection protocol in-between cuts) and personnel hands/gloves. Removal of visible faecal material from carcasses is a GHP; there is published evidence of efficacy for reducing STEC in raw beef, although the efficacy of this intervention is dependent on the workers' skill level.

Carcass trimming should be done in an area designated for that purpose and should result in trimmed carcasses that are free of visible contaminants.

9.3 Specific control measures at post-mortem inspection

Post-mortem inspection is useful to detect faecal contamination and some GHP-based measures at this step that could prevent contamination with STEC are:

- Ensuring the line speed and the amount of light are appropriate for effective post-mortem inspection of carcasses and visualization of physical contaminants (e.g. faecal material, bone dust, and hair).
- Minimizing touching the carcasses with hands, tools or garments during post-mortem inspection palpation and incision to reduce cross-contamination. Hands-free inspection should be encouraged, where possible.

9.4 Specific control measures at carcass washing

Carcass washing with potable water alone may remove visible soiling and reduce overall bacterial counts on beef carcasses. However, care must be taken when washing carcasses to prevent splashing and spread of contamination.

The effectiveness of validated biocidal carcass washing depends on factors such as concentration, temperature, method of application, operator competency and the initial load of STEC on the carcass.

9.5 Carcass washing with biocides

Carcass washing with biocides, such as organic acids (e.g. citric acid, lactic acid, acetic acid), oxidizing agents (e.g. chlorine, peroxides, ozone) or other agents, in accordance with label directions, may be effective in reducing STEC. Some biocidal treatments may be applied with hot water to have a combined thermal impact. Individual STEC strains may vary in their sensitivity to such treatments. Organic acids alone can reduce but not completely eliminate STEC serotype O157:H7.

9.6 Carcass surface pasteurization

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. 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 reductions in total *E. coli* in commercial slaughter operations.

9.7 Steam and vacuum

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 82–95 °C on the surface of the carcass. The process is effective at removing visible contamination on the carcasses.

9.8 Specific control measures at chilling

Rapid chilling minimizes the potential for bacterial growth; STEC can only replicate at temperatures of 7 °C and above. The potential for bacterial growth is also dependent upon the water activity at the carcass surface, and if water activity (a_w) 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.

9.9 Specific control measures at carcass fabrication (mechanical tenderization, grinding/mincing)

Mechanical tenderizers and associated processing equipment should be cleaned and disinfected 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 among lots of production.

Manufacturers of tenderized beef should consider supplier assurances that require the incoming meat to be produced in accordance with good agricultural practices (GAPs) and GHPs to reduce STEC or, in the absence of these assurances, treat the beef prior to mechanical tenderization.

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

To minimize STEC contamination of, and/or the spread of contamination throughout ground/minced beef, measures may include, where appropriate (e.g. supported by a risk assessment and context in the country of production or end use):

- Storing products to prevent the growth of STEC. Multiplication of STEC is inhibited below 7 °C, but low temperatures do not significantly reduce STEC. Establishments need to control STEC, using adequate time/temperature combinations.
- Cleaning/disinfection of equipment and the environment on a regular basis and ensuring employees follow GHPs to avoid cross-contamination.
- Treating the outer surfaces of the beef with organic acid sprays or other validated treatments.
- Appropriately chilling raw meat during production to reduce possible multiplication of STEC if they are present.

When appropriate and indicated by conditions (e.g. to validate a process or intervention, or monitor effectiveness of a control system or process; when a deviation, disruption or change to a process, has been identified or suspected), manufacturers could specify that beef that will be used for grinding or already minced beef should be pretested according to a defined sampling plan and samples found negative (i.e. not detected) for specific strains of STEC, e.g. *E. coli* serotype O157:H7.

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

9.10 Specific control measures at packaging and storage

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 include 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.

During packaging and storage, temperature control should minimize the potential for bacterial growth; STEC can only replicate at temperatures of 7 °C and above.

10. DISTRIBUTION/RETAIL

10.1 Specific control measures at distribution and retail

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.

Raw beef should be stored and prepared separately from cooked or ready to eat food to prevent cross-contamination. 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.

10.2 Packaging conditions

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.

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, should be included to provide consumers and food service workers the essential information to safely prepare the product.

11. CONSUMERS

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 made aware of the proper cooking and handling of raw beef.

Since non-intact raw beef products may pose an increased risk for consumers, appropriate consumer guidance on safe handling, including cooking temperatures, may be needed.

Consumers should apply the general principles for safer food to ensure safety of raw beef when handling, preparing and consuming beef. These are:

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

12. VALIDATION OF CONTROL MEASURES

Refer to the general section of these guidelines.

13. MONITORING OF CONTROL MEASURES

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.

Process performance monitoring may be accomplished more effectively and efficiently by quantitatively monitoring indicator microorganisms. These indicator microorganisms 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 the STEC strains considered to be a country's highest priority (e.g. those strains with virulence factors capable of causing severe illness or considered to cause significant illness in that country country) may also be conducted for verification of process performance.

Some raw beef will need more control measures and monitoring than others (e.g. non-intact raw beef).

14. VERIFICATION OF CONTROL MEASURES AND REVIEW OF CONTROL MEASURES

STEC testing may be an important part of verification of process performance. However, STEC are generally present at very low levels and are characterized by heterogeneous distribution (including in ground/minced products), making STEC detection challenging. This means that there may be a significant delay in identifying loss of process control based on STEC detection. Consequently, verification programmes should focus on quantitative monitoring of indicator microorganisms. Hygiene indicators used should be those that are the most informative for the specific processing environment. An increase in the numbers of the selected indicator microorganisms indicates decreasing process control and corrective action should be taken. The speed in detecting a loss of control 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.

Regular testing for STEC strains considered to be a country's highest priority (e.g. those strains with virulence factors capable of causing severe illness or demonstrated to cause significant illness in that country) can also be conducted for verification of process performance. Lot testing may be 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.

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.

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

Intact raw beef cuts used for purposes other than the manufacture of finished ground or blade tenderized raw beef products do not present the same level of risk, since STEC will be on the external surfaces that will receive the most heat in cooking; testing for STEC therefore offers limited value. However, when the final intended use of raw beef cuts is not known, sampling could be implemented for STEC strains demonstrated to be a country's highest priority for verification and if supported by in-country risk assessment. In general, the occurrence of STEC in meat products is lower for intact meat products than in trim or ground/minced beef. However, the overall occurrence of STEC in these products can vary considerably due to differences in primary processing and post-processing conditions and interventions.

ANNEX II

FRESH LEAFY VEGETABLES (under development)

ANNEX III

RAW MILK AND RAW MILK CHEESES

1. INTRODUCTION

Although most milk for drinking is either pasteurized or sterilized by ultra-high temperature (UHT) processing, raw drinking milk is 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 large-scale industry and cheese makers. Specific combinations of ingredients and cheese-making processes are used by manufacturers to obtain a wide variety of cheeses with desired characteristics that meet consumer expectations.

Raw milk and raw milk cheeses have been associated with foodborne infections in humans from different countries caused by Shiga toxin-producing *Escherichia coli* (STEC). Consuming raw drinking milk or raw milk cheeses without any control measures is associated with a higher risk of illness than drinking pasteurized milk or eating cheeses made from milk subject to heating such as thermization[i] in conjunction with other control measures or pasteurization to reduce the risk from foodborne pathogens. The infectious dose for STEC in raw milk or raw milk cheese is low. A comprehensive approach, considering all the aspects of raw milk and raw milk cheeses from production to consumption, is necessary to reduce the presence of STEC in these products.

Cattle are a main source of STEC. Infected cattle can carry the bacteria in their gastrointestinal tract without any symptoms of disease and shed them in their faeces. STEC have also been isolated from the faeces of other species of animals, including buffalo, goat, camel, yak and sheep, which are commonly milked for human consumption. Detailed investigations have shown that without observance of appropriate cleaning and disinfecting steps and udder good hygiene practices, faecal matter can contaminate the cow's teats and udders, which can increase the risk of microbial contamination of the milk during the milking process. 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 in the resulting cheeses.

Raw milk cheeses are made from raw milk coagulated through the action of rennet, selected microbiological organisms or other suitable coagulating agents, and then partially or completely draining the whey resulting from the coagulation. This process results in a concentration of milk protein and milk fat. Following this step, various processing techniques are applied to produce 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 fresh, blue, semi-soft, semi-hard, hard, or extra-hard product, which may be ripened, coated, cooked or pressed. The different processing steps applied, and the raw milks used from different species (e.g. cow, buffalo, goat, sheep, yak) can influence the behaviour (survival, growth or inactivation) of STEC strains.

This document is intended for use by a variety of food business operators (FBOs) using diverse milk production systems and cheese-making processes. Therefore, flexibility has been included throughout it to allow different systems of control and prevention of contamination considering cultural matters and different processing practices and conditions.

These guidelines describe prerequisite programmes, including good hygiene practices (GHPs), that can contribute to control 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 concentration of STEC in raw milk and raw milk cheeses has not been quantified. Consequently, it will be up to competent authorities and each operator (farmer, dairy, or FBO) to define and implement appropriate risk-based monitoring and control measures, considering relevant scientific and technical information.

2. OBJECTIVE

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 (cow, buffalo, goat, camel, yak and sheep), raw milk cheese making, storage, and distribution to consumers.

[i] Thermization: the application to milk of a heat treatment of a lower intensity than pasteurization that aims at reducing the number of microorganisms.

3. SCOPE AND DEFINITIONS

3.1 Scope

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

3.2 Definitions

- Refer to the *General Standard for the Use of Dairy Terms* (CXS 206-1999),[9] and the *Code of Hygienic Practice for Milk and Milk Products* (CXC 57-2004),[7] 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)[4] and the *General Standard for Cheese* (CXS 283-1978).[14]
- **Milk:** Milk is the normal mammary secretion of milking animals obtained from one or more milkings without either addition to it or extraction from it, intended for consumption as liquid milk or for further processing.[9]
- **Raw milk:** Milk (as defined in the *General Standard for the Use of Dairy Terms* [CXS 206-1999])[9] which has not been heated beyond 40 ºC or undergone any treatment that has an equivalent effect.[ii, iii,7]
- **Raw milk cheeses:** Cheeses made from raw milk.

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

Figure 1 and Figure 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.

Raw milk should come from healthy animals, be obtained by hygienic milking practices and be free of colostrum. 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, as it does not undergo a microbial reduction treatment prior to packaging for drinking milk or before making raw milk cheeses.

The application of combined control measures throughout the food chain particularly at the farm, transport and processing 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.

[ii] Temperatures between 40 ºC and pasteurization temperatures, are generally considered to be insufficient to consistently kill STEC in raw milk. Heat treatment beyond 40 ºC results in changes such that the structure of the resultant product is no longer the same as that of raw milk.

[iii] Milk that has been subject to processing techniques such as microfiltration and/or bactofugation is no longer considered raw milk because these processes require milk to be heated above 40 °C.

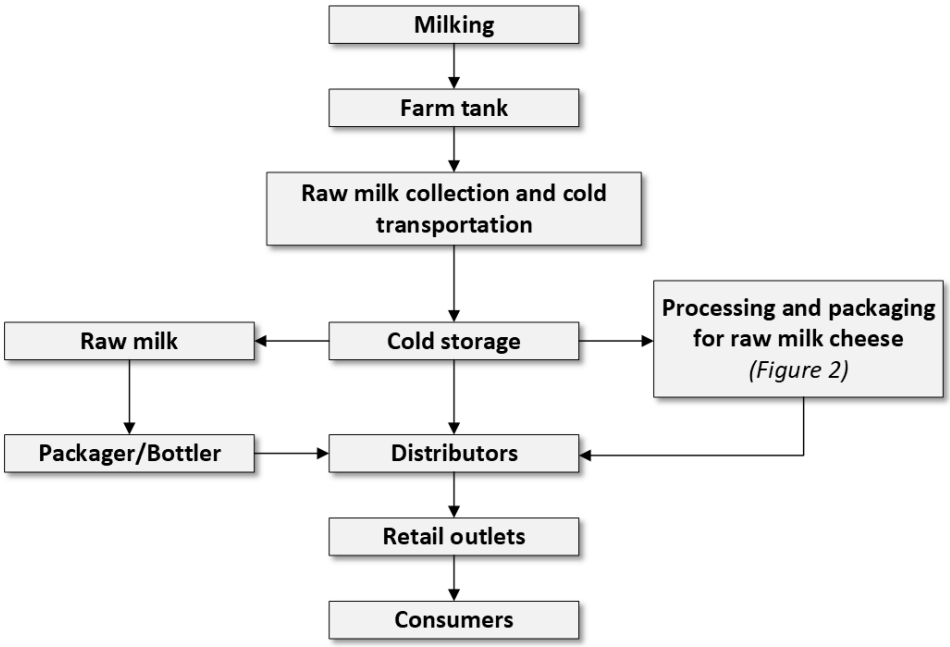

Figure 1. Process flow diagram for raw milk production, distribution and sale

The diagram illustrates a generalized process flow for raw milk for illustrative purposes only. Steps may not occur in all operations and may not occur in the order presented in the flow diagram.

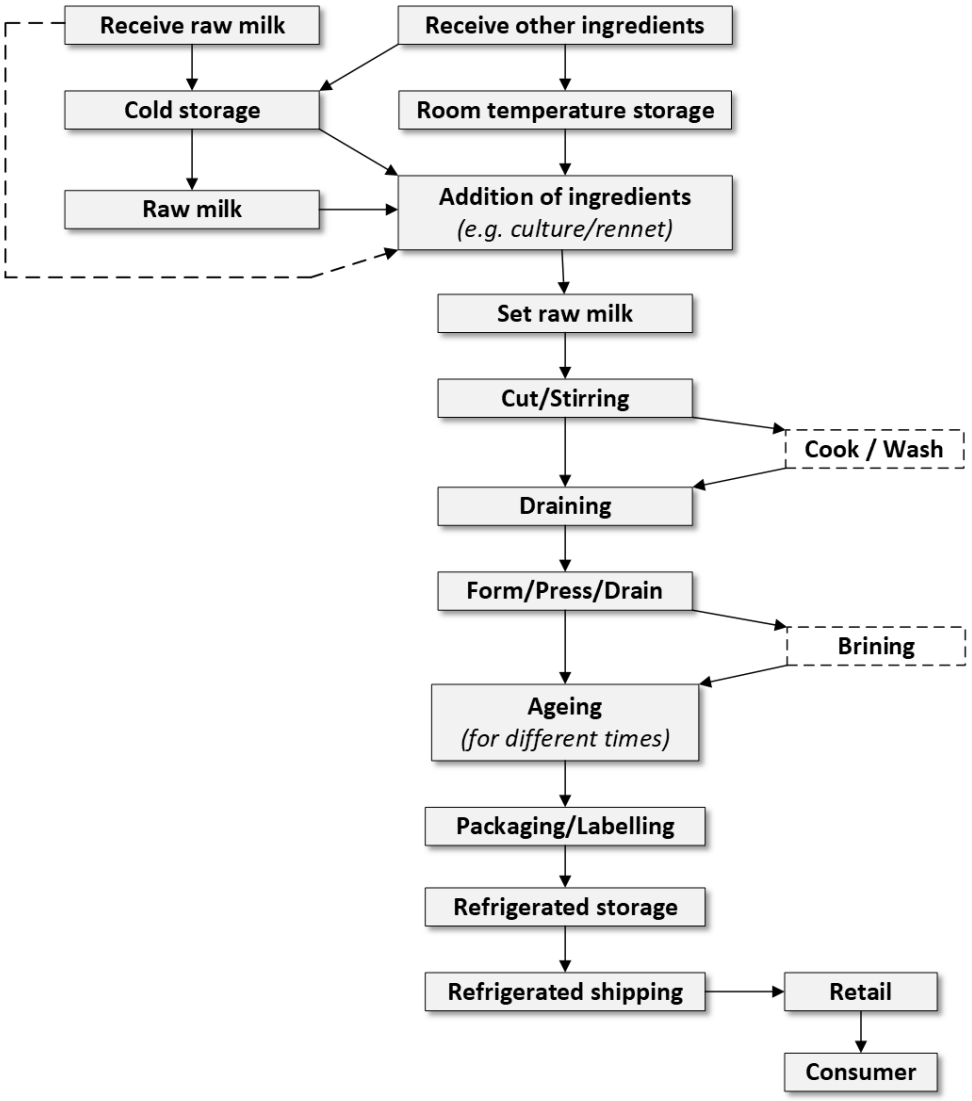

Figure 2. Making cheese from raw milk

The diagram illustrates a generalized process flow for raw milk cheese for illustrative purposes only. Steps may not occur in all operations and may not occur in the order presented in the flow diagram.

5. PRIMARY PRODUCTION – MILK PRODUCTION AT DAIRY FARM

5.1 Control measures for STEC for dairy herds at the dairy farm

STEC are commonly present in the microbiota of milk-producing animals, and it is not possible to eradicate them. The excretion of STEC by ruminants seems to be sporadic but may also be persistent over several months. Studies have shown that excretion varies according to the season, peaking in warmer months. Excretion also varies among individual cows, with some individuals considered to be “high shedders” (a high-level excretion of STEC), and excretion levels may even differ between cow droppings of the same animal. 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 milk from sheep and goats occurs but is less likely than from cows, because of anatomical differences as their faeces tend to be more solid and thus are less likely to cross-contaminate. There are no established methods to prevent STEC carriage or ensure reduced shedding by ruminants. 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 minimize animal stress.
- Maintain the hygienic condition of bedding and remove it when it becomes soiled with manure in a manner that increases the likelihood of contamination of the milk.

Other wildlife or livestock, pests, and birds can also carry STEC and thus contribute to their circulation in milking herds. It may be useful to manage each of these potential sources, using scientifically validated methods, and thus reduce or minimize the risk of transmission from these sources.

Animal-to-animal transmission via faecal-oral transmission is a likely contamination route of STEC within the herd. In addition, the introduction of new animals to a herd may introduce STEC. The following are examples of measures that may be useful:

- Segregate and limit faecal cross-contamination between newborn or young animals and mature animals.
- Keep young animals in the same groups throughout rearing without introducing new animals.

Environmental transmission has also been demonstrated due to poor housing conditions or to the survival of STEC (potentially more than a year) in effluent and the environment (soil, plants, crops, grain and water). Pastures can also maintain bacterial circulation by faeces deposited onto the ground and/or spreading of effluent. Good agricultural practices (GAPs) for managing manure and slurry include frequent removal from the milking herd environment, maintaining necessary intervals between spreading on pasture and the reintroduction of animals for grazing.

When appropriate, other validated control measures at primary production, such as diet, vaccination, administration of probiotics and additional good management practices (as described in the raw beef Annex) may be helpful in minimizing the shedding of STEC and, thus, contamination of raw milk.

Contaminated feed and water (surface water, roofing water, contaminated drinking water) can contribute to the introduction or circulation of STEC, following direct or indirect contamination. The presence of STEC in feed 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]).[15] 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.

5.2 Control measures for STEC during preparation of animals for milking, milking, and then transfer of milk to bulk containers/tanks

The major route of raw milk contamination is from faecal sources (directly or indirectly). Faeces can soil the teats, and the milk can subsequently become contaminated during the milking process. Therefore, limiting faecal contamination during milking is of key importance to manage STEC on the farm. For this, it is important to apply good hygiene practices during milking, to keep animals clean, and most importantly to prevent contamination with faeces.

Minimizing faecal contamination before and during milking:

- Ensure 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 after each milking and allowed to dry when possible.
- 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 need to be properly cleaned.

STEC can also potentially persist on milking equipment and pipelines if these are not adequately cleaned and disinfected (Annex I on Guidelines for the primary production of milk from the *Code of Hygienic Practice for Milk and Milk Products* [CXC 57-2004]).[7] Cleaning and disinfecting are 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 STEC serotypes O157:H7 and non-O157 STEC with increased tolerance to sanitizers commonly used in the food processing environment, particularly if cleaning is not done effectively (resulting in biofilm formation in which the sanitizer cannot reach microorganisms) or the unintended application of a sanitizer at sublethal concentrations. 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 (*Code of Hygienic Practice for Milk and Milk Products* [CXC 57-2004]).[7] In line with the *General Principles of Food Hygiene* (CXC 1-1969),[4] 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 (see the *Code of Hygienic Practice for Milk and Milk Products* [CXC 57- 2004]).[7]

6. CONTROLS DURING MILK COLLECTION, STORAGE AND TRANSPORTATION

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

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 cleaning, 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.

STEC can rapidly replicate in raw milk if the milk is at the temperature of STEC growth. Therefore temperature control of the milk post-harvest is crucial, including during its storage at the farm and throughout the collection route to prevent microbial growth (see the *Code of Hygienic Practice for Milk and Milk Products* [CXC 57-2004],[7] Annex I on Guidelines for primary milk production). Temperatures ≥ 6 °C, extended storage of raw milk, and high initial bacterial counts in raw milk during collection, storage and transportation have been associated with increased counts of *E. coli* in raw milk. Milk temperature should be monitored during storage and checked before it is unloaded, when possible.

Transport has not been identified as a step likely to contaminate the milk with STEC, if good hygiene practices are followed. However, transport is identified as a stage where growth of STEC may occur if the temperature of the milk is not properly maintained.

7. CONTROL DURING PROCESSING

The contamination of dairy products with STEC during processing in the manufacturing plants is rare if appropriate hygiene practices are followed. 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),[4] the *Code of Hygienic Practice for Milk and Milk Products* (CXC 57-2004)[7] and other relevant Codex texts such as codes of hygienic practice and codes of practice.

At the initial stages of cheese making, the temperature (ranging from 27 °C–35 °C), water activity (a_w) value and nutrients in 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 can be observed in some cheese making processes. 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. However, “cooking” of cheese curd, as well as rapid acidification (when pH decreases to under 4.3), coupled with the increase of non-dissociated lactic acid, have been associated with STEC or *E. coli* log reductions. During the ripening step, the microbial stability of cheeses is determined by the combined application of different hurdle factors (pH, a_w, titratable acidity, sodium chloride, non-dissociated lactic acid, amount of starter cultures (such as lactic acid bacteria)) still active in the cheese, brining of the cheese, as well as the temperature and length of time for ripening. These hurdles create an increasingly challenging environment for STEC during the manufacturing process and ripening. The FBO should analyse the risks associated with its manufacturing process with respect to the potential for 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.

“Cooking” of cheese curd (heating to increase separation of whey from the 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 appropriate control measures should be identified and applied. For example, testing raw milk for the presence of STEC can be established, as well as an audit programme of milk suppliers to assess their hygienic practices.

Nevertheless, these procedures have the potential to reduce STEC, but they cannot ensure the safety of the product if the raw milk is contaminated with STEC. Consequently, the microbiological quality of raw milk used in cheese making is crucial for reduction of the risk associated with the end products.

8. PRODUCT INFORMATION FOR CONSUMERS

In line with the *Code of Hygienic Practice for Milk and Milk Products* (CXC 57-2004)[7] (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

Although STEC can be isolated from raw milk and raw milk cheeses, STEC testing is uncommon and most sampling and testing protocols target indicator microorganisms such as *E. coli*, whose level can be used as an indicator of raw milk sanitary quality prior to raw milk cheeses production. Microbiological criteria (refer to the *Principles and Guidelines for the Establishment and Application of Microbiological Criteria Related to Foods* [CXG 21-1997])[2] based on process and hygiene indicator microorganisms (e.g. *E. coli*/Enterobacteriaceae) may also prove a useful tool for validation, monitoring and verification of control measures.

Even if they are useful hygienic markers of the quality of raw milk, the presence or concentration of generic *E. coli* or other indicator microorganisms in raw milk does not necessarily indicate the presence of STEC. More specific analyses are needed to detect and confirm by strain isolation the presence of STEC. Periodic testing for STEC strains considered to be a country's highest priority (e.g. those strains with virulence factors capable of causing severe illness or considered to cause significant illness in that country) may also be conducted for verification of hygienic practices.

Testing raw milk for the presence of STEC strains considered to be a country's highest priority can be established, but testing may not be effective on its own: because of the low prevalence of STEC, samples tested may not contain STEC despite their presence in the food. Thus, such testing should be used in combination with other control measures, including an audit programme of milk suppliers to assess hygienic practices on the farm.

9.2 Validation and monitoring of control measures

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 organization which may gather, analyse, and interpret data in order to establish alternative or improved measures, for example by writing GHP and/or HACCP (e.g. fast acidification or long ripening) guidelines adapted to the local context or to the traditional steps of raw milk harvesting and processing.

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

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

Enhanced monitoring should be implemented when STEC strains have been detected in raw milk; and production and sale of the product that have not undergone effective treatment should be ceased until the contamination issue has been resolved. In such situations, 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 results.

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

Milk collection at the dairy establishment: Routine monitoring of the quality of the raw milk received by the dairy establishment (indicator microorganisms or/and STEC) conducted by the dairy establishment can be based on samples collected periodically or even for each load. Sampling milk filters may be a more suitable monitoring point for STEC than sampling raw milk from the bulk tank, considering dilution due to pooling and sporadic contamination issues. Milk filter sample analysis can also be useful in investigating the source of contaminated cheese.

Enhanced monitoring 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.

During processing, the FBO or industry association generally defines its sampling plan in line with an acceptable hygiene level. A milk safety 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 safety checks can be performed based on *E. coli*, to verify the application of GHPs.

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 programme are successful. Accurate safety and quality test results are crucial and depend on appropriate sampling and sample handling, the type of representative samples, and proper analytical methods. For routine monitoring, FBOs should consider analysing cheese during the early stages of manufacturing (e.g. after coagulation), 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 ageing and storing contaminated products. Analysis could also be done during ripening and/or before placing the cheese on the market.

When STEC is detected in raw milk, it has been found at very low levels in cheeses. This contamination is characterized by heterogeneous distribution, making STEC difficult to detect. Sampling plans should therefore be designed according to the *General Guidelines on Sampling* (CXG 50-2004)[16] and the *Principles and Guidelines for the Establishment and Application of Microbiological Criteria Related to Foods* (CXG 21-1997).[2] In addition, sampling plans should be adapted over the entire production chain (number of samples, nature of the samples (i.e. milk, cheese at the start of coagulation, during ripening, etc.), quantity analysed, frequency of analysis, etc.).

Enhanced monitoring 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, other batches of cheeses can be screened in greater detail for STEC to assess the magnitude of contamination. In addition, it is important to identify the remaining contaminated milk, if any, and stop using it for production of raw milk cheese.

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. 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) on the capacity for growth or survival of the microorganisms considered.

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

Application of prerequisite programmes, including GHPs, 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.

ANNEX IV

SPROUTS (under development)

NOTES

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