INFORMATION PACKAGE

FOR THE

Working group on the proposed draft "guidelines for the control of Shiga toxin producing *Escherichia coli* (STEC) in raw beef, fresh leafy vegetables, raw milk and raw milk cheeses and sprouts: annexes"

Sunday 27th February 2022

8:00-11:00 EST / 14:00-17:00 CET

Dear Delegates to the STEC Working Group,

To facilitate your participation in and engagement with this working group the presentations will be made available to you by email (see Compiled STEC Annexes CCFH52 final.pdf) and on the CCFH52 webpage.

In addition to facilitate the discussions at the co-chairs of the electronic working group have made some initial proposed changes to the Annexes which will be referenced during the presentations. The three annexes with these proposed changes are attached below and all delegates are asked to bring these to the working group on Sunday 27th February.

The agenda for the working group on STEC is available on the CCFH52 webpage here.

We look forward to seeing you .

Codex Secretariat

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

ANNEXES

with changes for discussion in Tracked Changes

SUMMARY

The proposed draft Guidelines for the Control of Shiga Toxin-Producing *Escherichia coli* (STEC) in Raw Beef, Fresh Leafy Vegetables, Raw Milk and Raw-Milk Cheeses, and Sprouts was circulated for comments at Step 3 in December 2021. Changes in the document have been made based on comments received through the OCS, as considered appropriate by the co-chairs. Later comments will be taken into consideration after CCFH 52 in the preparation of a new draft for consideration by the eWG.

Generally, comments and changes were editorial aiming for clarification of the text, avoid repetition and to improve English language, also swords and phrases like “may”, “where feasible”, “when possible” and “when practical” were included to provided flexibility to the recommendations. Some suggestions were not accepted because they conflicted with other changes, were not clear or would be confusing, or specific language was not provided. Meanwhile others will be pending to the publication of the JEMRA report from the 2020 meeting. Comments with recommendations with no scientific basis where no consider at this point.

ANNEX 1: RAW BEEF

1. INTRODUCTION

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

2. STEC can be ~~are a common~~ 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 (Hussein and Bollinger; 2005, Callaway et al 2013). STEC shedding by individual cattle is transient and episodic. ~~, with almost all cattle carrying and shedding STEC at some time during their life~~ (Williams et al., 2014; Williams et al., 2015). In addition, STEC can be found ~~are widespread~~ within the farm environment **and it is therefore likely**. ~~It should be expected~~ that ~~the majority of~~ cattle arriving for slaughter **have STEC on their hides** ~~could have hides contaminated to some extent with STEC~~. Individual **feedlot cattle** studies have reported the prevalence of STEC O157 on cattle hides presenting for slaughter as high as 94.5% (Arthur et al., 2007), and as high as 74.5% for other STEC (Stromberg et al., 2018).

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

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

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

2. SCOPE

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

3. DEFINITIONS

For the purpose of this guideline the following definitions apply:

Raw Beef: Skeletal muscle meat from slaughtered cattle, including primal cuts[1], sub-primal cuts, and trimmings.

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

7. These Guidelines incorporate a "primary production-to-consumption" flow diagram that identifies the main steps in the food chain and identifies where control measures for STEC may potentially be applied in the production of raw beef. While control in the primary production phase can decrease the number of animals carrying and/or shedding STEC, controls after primary production are important to prevent the contamination and cross-contamination of carcasses and, in particular, raw ground/minced beef. The systematic approach to the identification and evaluation of potential control measures allows consideration of the use of controls in the food chain and allows the application of ~~different combinations of~~ control measures individually or in combination ~~to be developed~~. This is particularly important as individual countries use different primary production and processing systems. ~~where differences occur in primary production and processing systems among countries~~. Risk managers need the flexibility to choose risk management options that are appropriate to their national context.

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

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

10. Similarly, operations to decontaminate carcasses or raw beef cuts will be of limited effectiveness if poor hygienic~~e~~ 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, application, temperature, etc.

4.1 GENERIC FLOW DIAGRAM FOR APPLICATION OF CONTROL MEASURES

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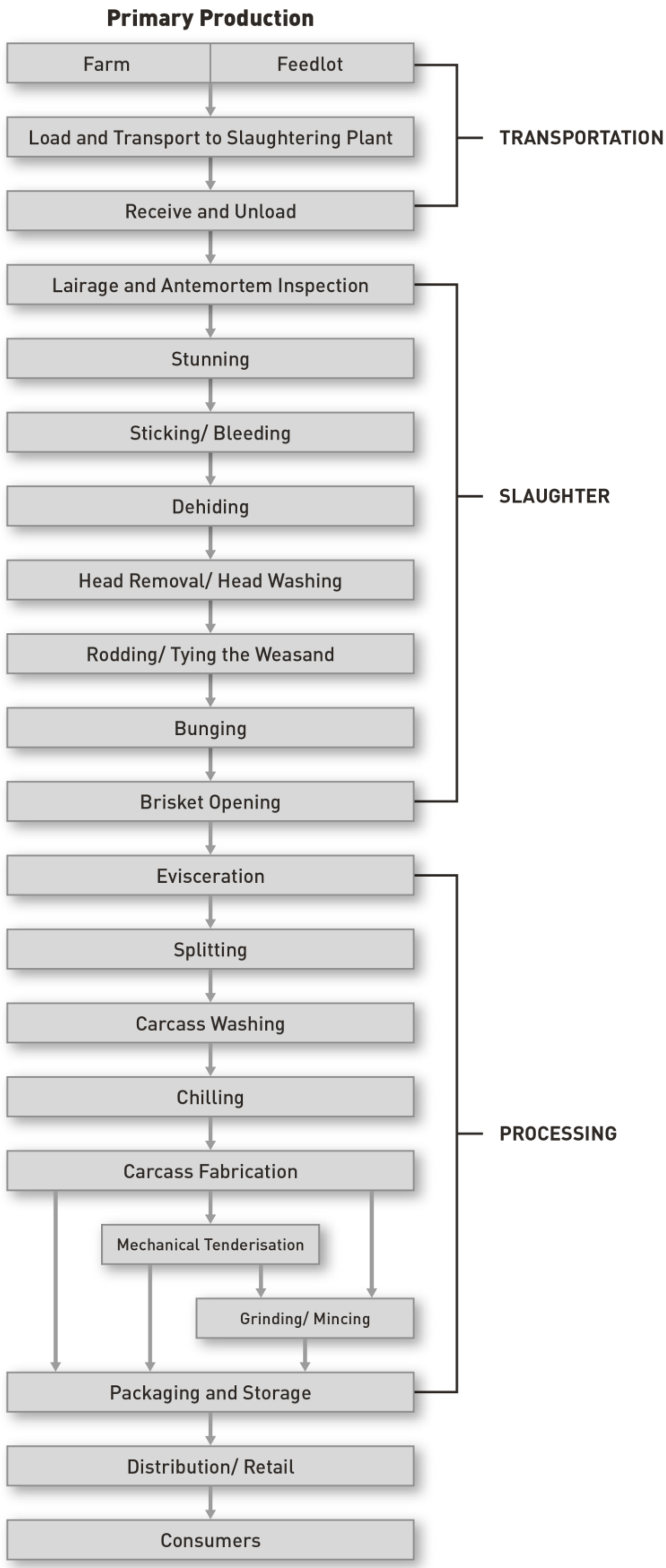
[1] A primal cut is a piece of meat on the bone initially separated from the carcass of an animal during butchering. Primal cuts are then divided into sub-primal cuts. These are basic sections from which steaks and other subdivisions are made.

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

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



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

4.2 PRIMARY PRODUCTION

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

4.2.1 Specific Control Measures for Primary Production

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

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

4.2.1.1 Diet Ingredients

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

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

Use of Direct-Fed Microbials

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

Use of other feed additives

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

4.2.1.2 Vaccination

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

4.2.1.3 Good management practices at primary production

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

- Stressful situations should be minimized wherever possible, because increased stress increases shedding of pathogens (e.g. poor animal husbandry, rough handling, dietary stress and food deprivation (Stein and Katz, 2017; Venegas-Vargas et al 2016)).
- Minimize exposure between herds to avoid or reduce horizontal transmission of STEC across herds (Callaway et al 2009).
- Maximize living space to reduce direct animal-to-animal transmission (e.g. maintain ample space for animals to move to reduce defecation directly onto one another).
- To the extent of possible, Mmaintain clean living conditions (e.g. clean holding areas, remove gross contamination to the extent possible, and maintain clean and dry bedding) to prevent potential transmission from the living environment (e.g. animals resting in STEC-contaminated materials).
- Reduce the potential for STEC transmission through consumption of contaminated food and water by the following:
- Design food and water delivery systems (tanks, trough, bins, etc.) in a way to reduce the potential for animal entrance and defecation.
- Ensure water is of a microbiological quality that minimises animal contamination and, if there is doubt, treat the water.
- Clean water troughs frequently to reduce replication and/or survival of these foodborne pathogens (Lejeune et al 2001).
- Use materials in water troughs that facilitate the cleaning process; E. coli O157:H7 counts have been shown to be lower in metal troughs had lower E. coli O157:H7 counts compared with troughs that were manufactured from concrete or plastic (Lejeune, 2001).

4.3 Transportation

4.3.1 Specific Control Measures for Transport to Slaughterhouse

21.Transportation can be a major contributor to the increasing occurrence of pathogens in 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 (Norrung et al., 2008; Dewell et al. 2008, Stein and Katz, 2017).

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

- Improve truck design, allowing for separation of animal lots.
- When possible, ~~S~~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.

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

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

4.3.2 Specific Control Measures at Receive and Unload

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

25. ~~The unloading should be carried out in a way that minimizes the stress caused by the action that could increase shedding of STEC, with A~~Adequate training of the operators on procedures that can minimize stress at this step is recommended.~~.~~

4.4 SLAUGHTER AND DRESSING

26. Interventions **during primary processing** at the slaughterhouse include physical, chemical or biological interventions that can be applied alone or in combination; these are likely to reduce the number of STEC microorganisms but should not be considered to eliminate STEC on every ~~animal~~ carcass. Strict good hygiene practices (GHP) and emphasis on good manufacturing practices (GMP) at slaughter~~ing~~ 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 ~~transfer to~~ meat surfaces (Gill and Gill, 2010).

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

28. Interventions aimed at removing STEC from the surface of beef carcasses should consider that tolerance to ~~heat~~, salt and acid has been observed in some STEC strains. Determining the effectiveness of interventions to reduce microbial pathogens is complex, particularly as multiple interventions may be applied simultaneously or in sequence~~,~~. The impact of interventions should be quantified by conducting experimental trials with surrogate organisms that have similar or greater resistance to individual treatments than STEC. Careful consideration is needed when determining suitable strains for validation of interventions since surrogates may not necessarily equivalent to wild type strains isolated from raw beef.

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

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

30bis. Operators should be effectively and appropriately trained to perform their operation in the slaughter process.

4.4.1 Specific Control Measures at Lairage and Antemortem Inspection

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

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

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

34. When feasible, at lairage cattle should not be comingled with other herds/lots be maintained in closed herds to reduce social stress and prevent cross-contamination between herds/lots.

4.4.2 Specific Control Measures at Stunning, Sticking and Bleeding

35. Prior In the access to the stunning box, animals may be sprayed in the accessway using low volume or following the stunning box, the animals can be treated with water jets at appropriate pressure, Similarly, the rectum may be spray washed but sparingly and only to remove faeces (the source of STEC) released during the stunning process. aiming at the hygiene of the rectum for possible elimination of faeces and STEC shed due to stress in leading the animal to slaughter. Use of any water or rinses should be designed to reduce fecal and STEC contamination and not stress the animal or inhibit the stunning, stick or bleeding effectiveness. Where water is applied, consideration should be given to removal of excess water prior to hanging of the

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

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

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

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

4.4.3 Specific Control Measures at Dehiding

40. Dehiding is the systematic process for separating the hide from the carcass and is perhaps the most critical operation in determining the level of STEC transferred to the carcass. To prevent transfer of contamination from the hide to the freshly exposed carcass, operators working at this stage should be effectively appropriately trained to perform this operation to maximize hygienic dressing.

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

be removed (i.e. vacuumed) from the hide to avoid contamination of the carcass with liquid that could easily run onto the carcass when the hide is opened (Bosilevac et al 2005, Wang et al 2013).

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

42.bis 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 role of worker rotation in cross-contamination during the dehiding process needs to be considered and procedures used to prevent contamination.

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

44. Measures should be taken to prevent tail flapping and contacting the carcass ~~or splattering~~ when hide pullers are used ~~.~~

4.4.4 Specific Control Measures at Rodding

45. The rodding operation consists of using a metal rod to free the esophagus (weasand) from the trachea and surrounding tissues. 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.

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

- Hanging the carcass vertically, to cut the muscle and tissue to expose the esophagus.
- The weasand should be closed (i.e., tied) hygienically to prevent rumen spillage; ties or clips can be used to prevent ingesta ~~digestive track material~~ movement.
- Heads can be "dropped" by cutting the esophagus below the tie or clip.
- Changing or disinfecting the weasand rod between each carcass.

- ~~Cleaning the weasand to minimize cross-contamination~~.
- If the gastrointestinal tract has been punctured, causing a major contamination, the carcass should be identified and additional procedures to avoid cross-contamination of other carcasses should be performed, such as, separated immediately from the others.

47. 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 .

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

4.4.5 Specific Control Measures at Bunging

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

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

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

4.4.6 Specific Control Measures at Brisket Opening.

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

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

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

4.5 PROCESSING

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

4.5.1 Specific Control Measures at Evisceration

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

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

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

]4.5.2 Specific Control Measures at Carcass Splitting

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

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

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

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

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

4.5.3 Specific Control Measures at Carcass Washing/Treatment

Carcass washing with antimicrobial agents.

59. Carcass washing may remove visible soiling and reduce overall bacterial counts on beef carcasses by up to 1 log unit (Gill and Landers, 2003).

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

Carcass surface pasteurisation.

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

Steam and vacuum

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

4.5.4 Specific Control Measures at Chilling

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

4.5.5 Specific Control Measures at Mechanical Tenderization, Grinding/Mincing

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

64. Manufacturers should ensure that mechanical tenderizers and associated processing equipment are cleaned 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 between ~~within~~ and among lots of production. Manufacturers should also consider purchase specifications that require that incoming beef to be tenderised has been treated to eliminate or reduce STEC such as E. coli O157:H7 to an undetectable level or should apply such treatments prior to mechanical tenderization.

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

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

~~:~~

- Storing products to prevent the growth of STEC. Multiplication of STEC is inhibited below 7°C ,but low temperatures do ~~would~~ not significantly reduce STEC. Establishments need to control STEC, using adequate time/temperature combinations.
- Cleaning equipment and the environment on a regular basis and ensuring employees follow good ~~personal~~ hygiene practices in order to avoid ~~cross-~~contamination.
- Specifying that all beef used for grinding be pretested and found negative for specific strains of STEC, e.g. E. coli O157:H7.
- Treating the outer surfaces of the meat with organic acid sprays or other approved treatments before grinding/mincing.
- Appropriately chilling raw meat during production to reduce possible multiplication of STEC if they are present.

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

4.5.6 Specific Control Measures at Packaging and Storage

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

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

4.6. DISTRIBUTION/ RETAIL

4.6.1 Specific Control Measures at Distribution and Retail

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

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

Packaging conditions

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

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

4.7. CONSUMERS

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

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

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

5. VALIDATION OF CONTROL MEASURES

Refer to the general section of this guidance.

6. MONITORING OF CONTROL MEASURES

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

77. Process performance monitoring may be accomplished more effectively and efficiently by quantitatively monitoring hygiene indicator microorganismsorganisms. These indicator microorganisms organisms do not indicate pathogen presence; instead they provide a quantitative measure of the control of microbial contamination in the product and processing environment. Periodic testing for "high risk"[2] STEC may also be conducted for verification of process performance (FAO/WHO, 2018).

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

7. VERIFICATION OF CONTROL MEASURES AND REVIEW OF CONTROL MEASURES

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

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

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

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

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

82. Intact raw beef cuts used for purposes other than the manufacture of finished ground or blade tenderized -raw beef products do not present the same level of risk as STEC will be on the external cooked surfaces. and therefore may require lesssamplingand laboratory testing for STEC therefore offers little value. However, when the final intended use of raw beef cuts is not known, sampling should be implemented for "high risk"[2] STEC verification.

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

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

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

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SUMMARY

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

In Paragraph 2 a footnote was added to explain "soil amendments."

In the definition of Fresh Leafy Vegetables, the first wording option with respect to cooking was chosen, i.e., Vegetables of a leafy nature <u>where the leaf is intended for consumption without cooking</u>…

A request to replace "cannot be controlled" in paragraph 11 with "may need to be evaluated" was not accepted, however the sentence was modified to include that point ("The effects of some environmental events cannot be controlled and may need to be evaluated.")

In paragraph 15 on water for primary production, a request to add "Under no circumstances should contaminated water be used" was not accepted. It and would seem to preclude the use of contaminated water that has been treated and did not seem necessary in light of the statement that corrective actions should be taken to ensure that the water is suitable for its intended use.

A request to add a point around "sewage management" in section 3.2.3 Personnel health, hygiene and sanitary facilities was not accepted since specific text was not provided. Similarly, adding "something around the placement of a "buffer zone" around animal waste" in paragraph 19 for Harvesting was not done; specific text was not provided and it is not clear whether there is a scientific basis for defining such a buffer zone.

A sentence was added in paragraph 21 in the section on transit from the field about effective cleaning between loads.

A request to delete "time" from paragraph 29 in the section on "Time and Temperature Control" was not accepted because both are considerations in minimizing growth of STEC and thereby reducing risk.

In paragraph 43, a bullet to thoroughly wash fresh leafy vegetables prior to use was added to measures for Retail and Foodservice. A request to provide examples of appropriate measures to prevent cross-contamination, maintain appropriate storage temperature, and ensure proper cleaning of tools and surfaces was not accepted, as these are common GHP measures.

A footnote was added to the flow diagram to reemphasize that it is for illustrative purposes only and that steps may not occur in all operations and may not occur in the order presented in the flow diagram (as indicated in paragraph 3).

Issues related to water for primary production and for packing (given the ongoing work to develop guidelines on safe use of water), reference to "hygiene" indicator microorganisms, recommendations for a temperature of 7°C or below, retaining the section on Retail and Foodservice, and modifications to the flow diagram are presented in PowerPoint and will be discussed in the working group meeting on the annexes.

RECOMMENDATIONS

CCFH is invited to review the revised annex on Fresh Leafy Vegetables with tracked changes along with the PowerPoint presentation and draft recommendations in preparation for the virtual working group meeting.

ANNEX 2. FRESH LEAFY VEGETABLES

INTRODUCTION

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

2. Outbreaks of illness caused by a broad range of microbial pathogens, including Shiga toxin-producing *Escherichia coli* (STEC), have been linked to the consumption of fresh leafy vegetables (Bottichio et al., 2019; CDC, 2006, 2012, 2020; Gobin et al., 2018; Herman et al., 2015; Kintz et al., 2019; Kinnula et al., 2018; Marden et al., 2014; Sharapov et al., 2006). Epidemiological evidence, outbreak investigations, research, and risk assessments have identified several possible contamination sources of fresh leafy vegetables with STEC, including water, domestic and wild animals, workers and manure-based soil amendments[1] (Berry et al., 2015; Gelting et al., 2011; Islam et al., 2004; Jay-Russell et al., 2014; Jongman and Korsten, 2018; Olaimat and Hoolley, 2012; Soderstrom et al. 2008). Fresh leafy vegetables are typically grown and harvested in large volumes, increasingly in places where harvest and distribution of fresh leafy vegetables is efficient and rapid. Fresh leafy vegetables are packed in diverse ways, including: field packed direct for market; field cored and prepared for later processing; and as pre-cut fresh leafy vegetable mixtures and blends with other vegetables. Control measures such as antimicrobial washes to minimize cross-contamination may be applied prior to packaging and/or shipment to market. As fresh leafy vegetables move through the supply chain, there is also the potential for the introduction and growth of pathogens, including STEC. The increasing worldwide use of pre-packaged fresh-cut leafy vegetables to expand the supply chain might increase the potential for the presence of contaminated product in the marketplace through cross-contamination with STEC, and ~~their~~ STEC replication during distribution and storage if fresh-cut leafy vegetables are improperly handled. There is no processing treatment applied that would eliminate or inactivate STEC, although contamination can be reduced by washing in water containing antimicrobials. Examples of field level control measures provided in this document are illustrative only and their use and approval may vary by country.

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

1. OBJECTIVE

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

2. SCOPE AND DEFINITIONS

2.1 Scope

5. This Annex covers specific guidance for the control of STEC related to fresh leafy vegetables that are intended to be consumed without cooking. Fresh leafy vegetables for the purposes of this Annex include all vegetables of a leafy nature where the leaf is intended for consumption without cooking, and include, but are not limited to, all varieties of lettuce, spinach, cabbage, chicory, endive, kale, radicchio, and fresh herbs such as coriander, cilantro, basil, curry leaf, colocasia leaves and parsley. The Annex is applicable to fresh leafy vegetables grown in open fields or in fully or partially protected facilities (hydroponic systems, greenhouses/controlled environments, tunnels etc.).

2.2 Definitions

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

Fresh leafy vegetables - Vegetables of a leafy nature ~~[~~where the leaf is intended for consumption ~~] [that may be consumed]~~ without cooking, including, but not limited to, all varieties of lettuce, spinach, cabbage, chicory,

[1] "Soil amendments" are fertilizers soil improvers, conditioners, or other material added to a soil to improve nutrients or the soil's physical properties, such as water retention, permeability, water infiltration, and drainage.

endive, kale, radicchio, and fresh herbs such as coriander, cilantro, basil, curry leaf, colocasia leaves and parsley, among other local products for foliar consumption.

3. PRIMARY PRODUCTION

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

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

3.1 Environmental Conditions

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

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

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

3.1.1 Location of the Production Site

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

3.1.2 Animal activity

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

- Appropriate methods should be used in order to exclude animals from the primary production and handling areas to the extent practicable. Possible methods include the use of physical barriers (e.g. fences) and active deterrents (e.g. noise makers, scarecrows, images of owls, foil strips).

- Primary production and handling areas should be properly designed and maintained to reduce the likelihood of attracting animals that can contaminate fresh leafy vegetables with STEC. Possible methods include minimizing standing water in fields, restricting animal access to water sources, and maintaining production sites and handling areas free of waste and clutter.
- Fresh leafy vegetable primary production areas should be regularly checked for evidence of the presence of wildlife or domestic animal activity (e.g. presence of animal faeces, bird nests, hairs/fur, large areas of animal tracks, burrowing, decomposing remains, crop damage from grazing), particularly near the time of harvesting. Where such evidence exists, growers should evaluate the risks to determine whether the fresh leafy vegetables in the affected area of the production site should be harvested for consumption without cooking (Wells et al., 2019).

3.2 Hygienic primary production of fresh leafy vegetables

3.2.1 Water for primary production

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

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

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

3.2.2 Manure, biosolids and other natural fertilizers

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

3.2.3 Personnel health, hygiene and sanitary facilities

18. Hygiene and health requirements should be followed to ensure that personnel who come into direct contact with fresh leafy vegetables prior to, during or after harvesting will not contaminate them with STEC. Adequate access to, and use of, hygienic and sanitary facilities, including adequate means for hygienically washing and drying hands, are critical to minimize the potential for workers to contaminate fresh leafy vegetables. People known or suspected to be suffering from illness due to STEC should not be allowed to enter any area handling

leafy vegetables, including the harvest area. Refer to section 3.2.3 of the *Code of Hygienic Practice for Fresh Fruits and Vegetables* (CXC 53-2003) for practices to minimize microbial pathogens such as STEC.

3.2.4 Harvesting

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

3.2.5 Field packing

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

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

21. Fresh leafy vegetables should be stored and transported under conditions that will minimize the potential for STEC contamination and/or growth. Fresh leafy vegetables should not be transported in vehicles previously used to carry heavily soiled root vegetables, live animals, animal manure, compost, or biosolids. When vehicle receptacles or containers have been used for the transport of products other than fresh leafy vegetables, effective cleaning should be carried out between loads to avoid the risk of contamination.

4. PACKING OPERATIONS

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

4.1 Time and temperature control

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

4.2 Cooling fresh leafy vegetables

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

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

4.3 Washing fresh leafy vegetables

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

5. PROCESSING OPERATIONS

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

28. It is recommended that unprocessed fresh leafy vegetable handling areas be physically separated from processing areas to minimize contamination with STEC. Processing, with some exceptions (e.g. cooking) cannot fully eliminate STEC contamination that may have occurred during primary production of fresh leafy vegetables. Processors should ensure that growers, harvesters, packers and distributors have implemented

measures to minimize the contamination during primary production of the fresh leafy vegetables and also during subsequent handling in accordance with the provisions in the *Code of Hygienic Practice for Fresh Fruits and Vegetables* (CXC 53-2003).

5.1 Time and temperature control

29. Refer to the *General Principles of Food Hygiene* (CXC 1-1969). Time and temperature control during pre-processing storage, processing and post-processing storage is essential to prevent growth of any STEC that may be present, since an increase in numbers will increase the risk of consumer illnesses. A temperature of 7°C or below will prevent growth of STEC and is appropriate for those fresh leafy vegetables that are not subject to cold injury.

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

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

5.3 Washing and dewatering/drying cut fresh leafy vegetables

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

5.4 Cold storage

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

5.5 Microbiological and other specifications

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

5.6 Documentation and records

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

6. ESTABLISHMENT: MAINTENANCE AND SANITATION

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

7. ESTABLISHMENT: PERSONAL HYGIENE

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

8. TRANSPORTATION

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

9. PRODUCT INFORMATION AND CONSUMER AWARENESS

9.1 Lot identification

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

9.2 Product information

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

9.3 Labelling

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

9.4 Consumer education

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

10. TRAINING

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

11. RETAIL AND FOODSERVICE

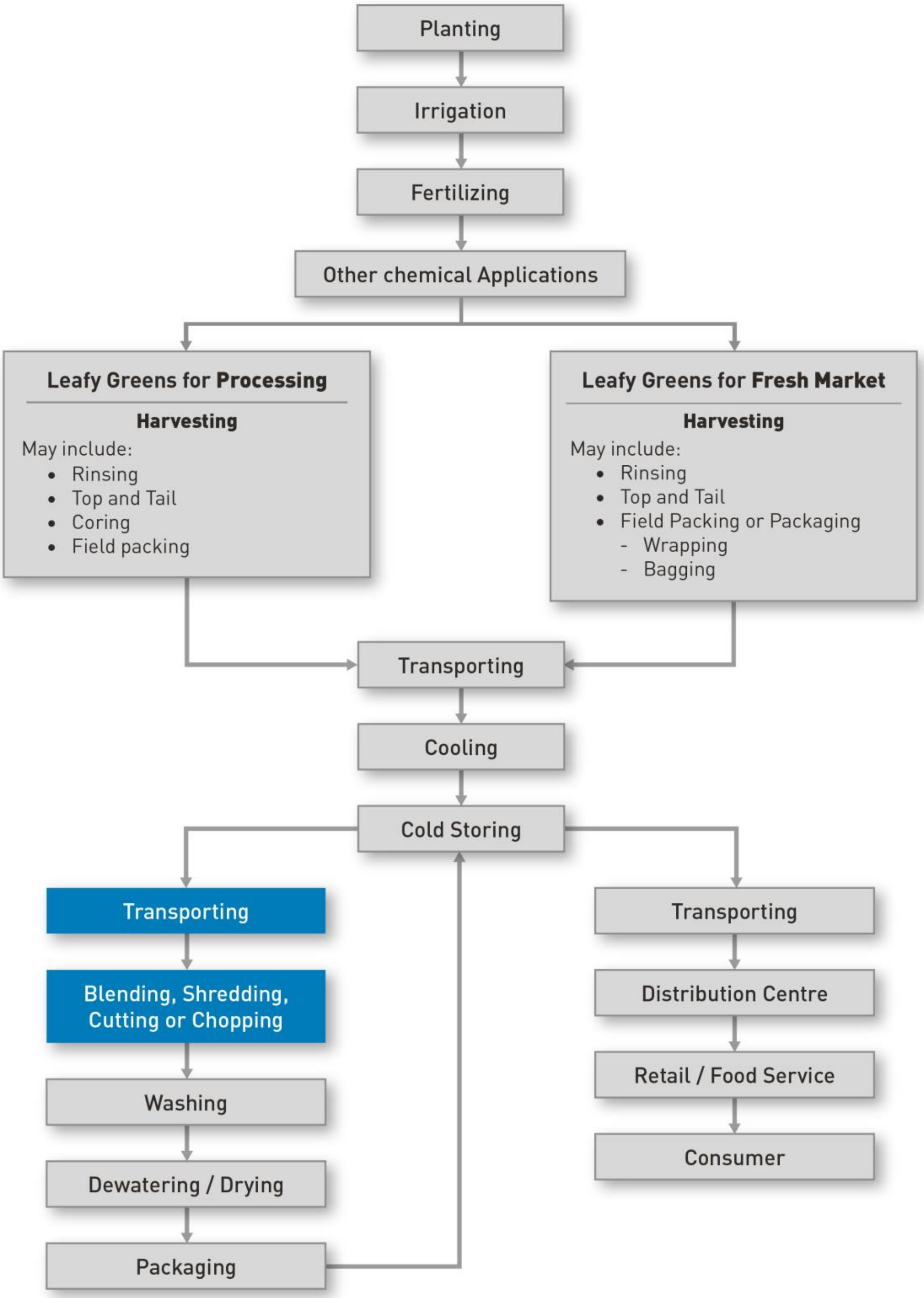
43. Fresh leafy vegetables (intact and pre-cut) should be held at an appropriate temperature ~~that prevents~~to minimize growth of STEC ~~[i.e., 7°C or below]~~. Cross-contamination from or to other food items should be prevented. Food business operators serving fresh leafy vegetables for consumption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

- prevent cross-contamination,
- maintain appropriate storage temperature,
- thoroughly wash fresh leafy vegetables prior to use, and
- ensure proper cleaning of tools and surfaces that may come in contact with these products.

12. CONSUMER

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

Figure1: Fresh Leafy Vegetables Flow Diagram[2,3]



[2] The diagram illustrates a generalised process flow for fresh leafy vegetables for illustrative purposes only. Steps may not occur in all operations (as shown with dotted lines) and may not occur in the order presented in the flow diagram.

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

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

ANNEX ON RAW MILK AND RAW MILK CHEESES

SUMMARY

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

A request to specify comprehensive risk-based approach in paragraph 2. This addition would have consequences throughout the document as to how a "risk-based" determination applies. The change was not made at this point. But we can discuss it in the PWG meeting.

Revisions to the definition of "Raw milk" were made based on comments. These modifications were homogenized with the general part.

A request to move the definitions of validation, monitoring and verifications to the general part as they are used in all annexes was received. Change was made.

A request to clarify why control measures included in Annex I, 4.2 primary production, are not mentioned here (Diet ingredients, microbials, feed additives, vaccination, good management practices at primary production). A new paragraph 16bis with a cross-reference to the raw beef annex was added. It indicates: *Other control measures at primary production, such as diet ingredients, vaccination and additional good management practices (as described in the Raw Beef Annex) may be helpful in minimizing shedding and thus, contamination of raw milk, but more research on efficacy is needed.*

We received the suggestion of deleting the paragraph 22 on acid treatment on the milking machine following or during disinfecting of the equipment. Application of acids is normally done to remove mineral salts prior to disinfecting. The paragraph was deleted because it was confusing

A request to modify paragraph 33 on "testing the raw milk for the presence of STEC can is unlikely to be effective" was issued. The specific change was not made since the flow of the sentence would not reflect at all what we wanted to say. But we inserted a new paragraph 36 bis in section 9: *Testing raw milk for the presence of STEC can be established, but since it is unlikely to be effective on its own because of low prevalence of STEC, it should be used in combination with other control measures including an audit program of milk suppliers to assess hygienic practices on the fam.*

A request in paragraph 36 to indicate: Periodic testing for ~~"high risk~~" STEC virulence genes may also be conducted for verification of hygienic practices (FAO/WHO, 2018). The change was not made since it is confusing because we have never talked about gene detection. Moreover, the term "high risk STEC" has been already discussed and accepted by the EWG: High risk STEC are generally isolates that present pathogenic virulence factors that are responsible for significant numbers of illness and/or that cause the most severe illnesses. The point about virulence genes is captured by the footnote. We must keep in mind that this may vary by country. And finally, this term is used in the other annexes, it is important to keep this homogeneity.

A request in paragraph 45 was to insert “Collection of filters from the milking machine at the time of milking, could constituted a sample library at the cheese factory for use in any possible investigation according to the results of the analyses of the cheeses, customer complaints, or during enhanced surveillance”. The change as is was not made as suggesting since it was due to be consider unpractical for farmers but we added at the end of the paragraph “Milk filter samples can also be useful in investigating the source of contaminated cheese.”

Figure 2 has been modified because there was an error in one of the box: “set raw milk” instead of “receive raw milk” in the box before “cutting/stirring.”

ANNEX 3. RAW MILK AND RAW MILK CHEESES

SPECIFIC CONTROL MEASURES FOR RAW MILK AND RAW MILK CHEESES

1. INTRODUCTION

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

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

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

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

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

2. OBJECTIVE

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

3. SCOPE AND DEFINITIONS

3.1. Scope

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

3.2. Definitions

8. Refer to the *General Standard for the Use of Dairy Terms* (CXS 206-1999), and the *Code of Hygienic Practice for Milk and Milk Products* (CXC 57-2004) Annex I (Guidelines for the Primary Production of Milk) and

Annex II (Guidelines for the Management of Control Measures During and After Processing). Also refer to *the General Principles of Food Hygiene* (CXC 1-1969).

- Milk: milk is the normal mammary secretion of milking animals obtained from one or more milking without either addition to it or extraction from it, intended for consumption as liquid milk or for further processing[1].
- Raw milk –Milk (as defined in *Codex General Standard for the Use of Dairy Terms* (CXS 206-1999)) that is intended for direct consumption or a primary input for dairy products and which has not been heated beyond 40ºC or undergone any treatment that has an equivalent effect.[2] This definition excludes processing techniques used for microbiological control (e.g. heat treatment above 40 °C, ~~as well as~~ microfiltration and bactofugation)~~, which lead to a decrease in the microbiota equivalent to heating.~~)
- Raw milk cheeses: cheeses made from raw milk.
- ~~Validation: Obtaining evidence that a control measure or combination of control measures, if properly implemented, is capable of controlling the hazard to a specified outcome.[3]~~
- ~~Monitoring: The act of conducting a planned sequence of observations or measurements of control parameters to assess whether a control measure is under control[18].~~
- ~~Verification: The application of methods, procedures, tests, and other evaluations, in addition to monitoring, to determine whether a control measure is or has been operating as intended.[18]~~

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

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

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

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

5. PRIMARY PRODUCTION – MILK PRODUCTION AT DAIRY FARM

5.1. STEC at the dairy farm.

5.1.1. Scientific Knowledge

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

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

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

~~[3] *Guidelines for the Validation of Food Safety Control Measures* (CXG 69 - 2008)~~

of STEC ecology indicate that control strategies based on denying STEC access to hosts or habitat will be highly challenging to implement in a manner which reliably prevents exposure of ruminants to STEC.

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

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

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

5.1.2. Control measures for STEC at the dairy farm

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

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

16 bis. Other control measures at primary production, such as diet ingredients, vaccination and additional good management practices (as described in the Raw Beef Annex) may be helpful in minimizing STEC shedding and, thus, contamination of raw milk, but more research on efficacy is needed.

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

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

5.2.1. Scientific Knowledge

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

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

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

20. Reducing faecal contamination before and during milking:

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

21. STEC can also potentially persist on milking equipment and pipelines if these are not adequately cleaned and disinfected (Annex I Guidelines for the primary production of milk from CXC 57-2004). Cleaning and disinfecting is more challenging if equipment is not well designed for cleaning, and/or not well maintained. STEC can form biofilms in milking machines if they are improperly designed, poorly maintained and/or poorly cleaned. Studies have shown biofilm formation by O157:H7 STEC and non-O157 ~~strains~~ STEC with increased tolerance to sanitizers commonly used in the food processing environment (Wang *et al.,* 2012) particularly if cleaning is not done effectively prior to the application of a sanitizer or if a sanitizer is used at sub-lethal 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 (Schets et al., 2005; Lascowski et al., 2013) (CXC 57-2004). In line with the *General Principles of Food Hygiene* (CXC 1-1969), only water fit for purpose (i.e. it does not cause contamination of the milk) should be used. If recycled water is used, it should be treated and maintained under conditions ensuring that its use does not impact the safety of the milk (CXC 57-2004). Well water regularly tested for indicators microorganisms and/or STEC could also be used.

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

6. CONTROLS DURING MILK COLLECTION, STORAGE AND TRANSPORTATION

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

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

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

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

7. CONTROL DURING PROCESSING

7.1. Scientific Knowledge

27. Raw milk cheeses are made from raw milk coagulating through the action of rennet or other suitable coagulating agents, and by partially draining the whey resulting from the coagulation, while adhering to the principle that cheese-making results in a concentration of milk protein. ~~Then~~Following this step, different processing techniques ~~can be~~are applied to ~~give~~ generate the end-products. Different microbiota and very diverse enzymatic reactions play a complex role during processing and maturation. This results in very different

cheese types, including ripened or unripened soft, semi-hard, hard, or extra-hard product, which may be coated, uncooked or cooked pressed cheeses (with short or long ripening), blue-type cheeses, lactic cheeses, and white mould cheeses. The different processing steps applied, and the raw milks used from different species (e.g. cow, buffalo, goat, sheep) can influence the behaviour and survival of STEC strains (Miszczycha et al., 2013). The behaviour of STEC (survival, growth or inactivation) can also be influenced by temperature, by the intrinsic physico-chemical properties (pH, a_w, % lactic acid) and by other microflora present specific to different cheeses during their manufacture.

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

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

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

7.2. Measures for preventing contamination of milk and milk products

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

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

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

8. PRODUCT INFORMATION FOR CONSUMERS

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

9. VALIDATION, MONITORING AND VERIFICATION OF CONTROL MEASURES

9.1 *E. coli* enumeration and STEC testing

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

36. Even if they are useful hygienic markers of the quality of raw milk, the presence or concentration of generic *E. coli* or other indicator microorganisms in raw milk does not ~~indicate~~ prove the presence of STEC. More specific analyses are needed ~~in cases such as food alerts~~to detect and confirm by strain isolation the presence

of STEC). Periodic testing for "high risk"[4] STEC may also be conducted for verification of hygienic practices (FAO/WHO, 2018).

36 bis. Testing raw milk for the presence of STEC can be established, but since it is unlikely to be effective on its own because of low prevalence of STEC, it should be used in combination with other control measures including an audit program of milk suppliers to assess hygienic practices on the fam.

9.2. Validation and monitoring of control measures

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

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

9.3. Verification of control measures

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

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

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

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

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

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

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

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

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

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

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

49. **Quantitative risk assessment:** Several sampling plans may be applied at different steps (milk harvested at the farm, milk delivered at the dairy establishment, curds, final products). Their combination in a quantitative risk assessment (QRA) model can help assess the efficacy of this sampling plan, using simulation, in terms of risk reduction of illness and percentage of batches rejected. Specific QRA models for STEC in several raw milk cheeses matrices have been developed (Perrin 20154; see also the opinion of ANSES 2018 STEC (saisine n°2018-SA-0164)). QRA models can also be built based on databases obtained when combining results of microbiological analyses performed regularly on the milk at different levels (farm and tank) and on cheeses (during the process and on the final product), values on technological process parameters and physico-chemical values (e.g., pH, a_w, acid resistance) on the capacity for growth or survival of the microorganisms considered.

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

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

Figure 1. Process Flow Diagram for Raw milk P~~p~~roduction, Didistribution and S~~s~~ale

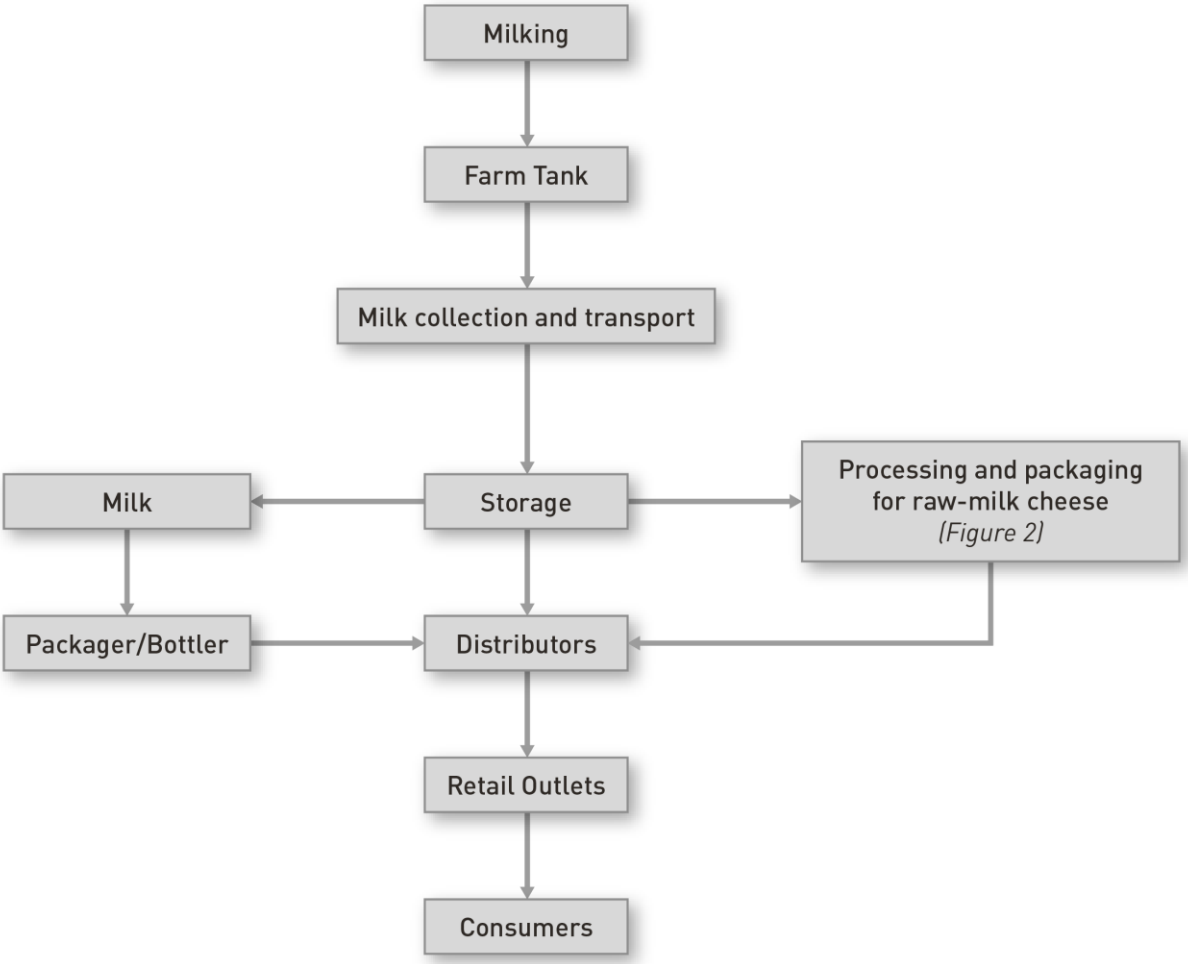
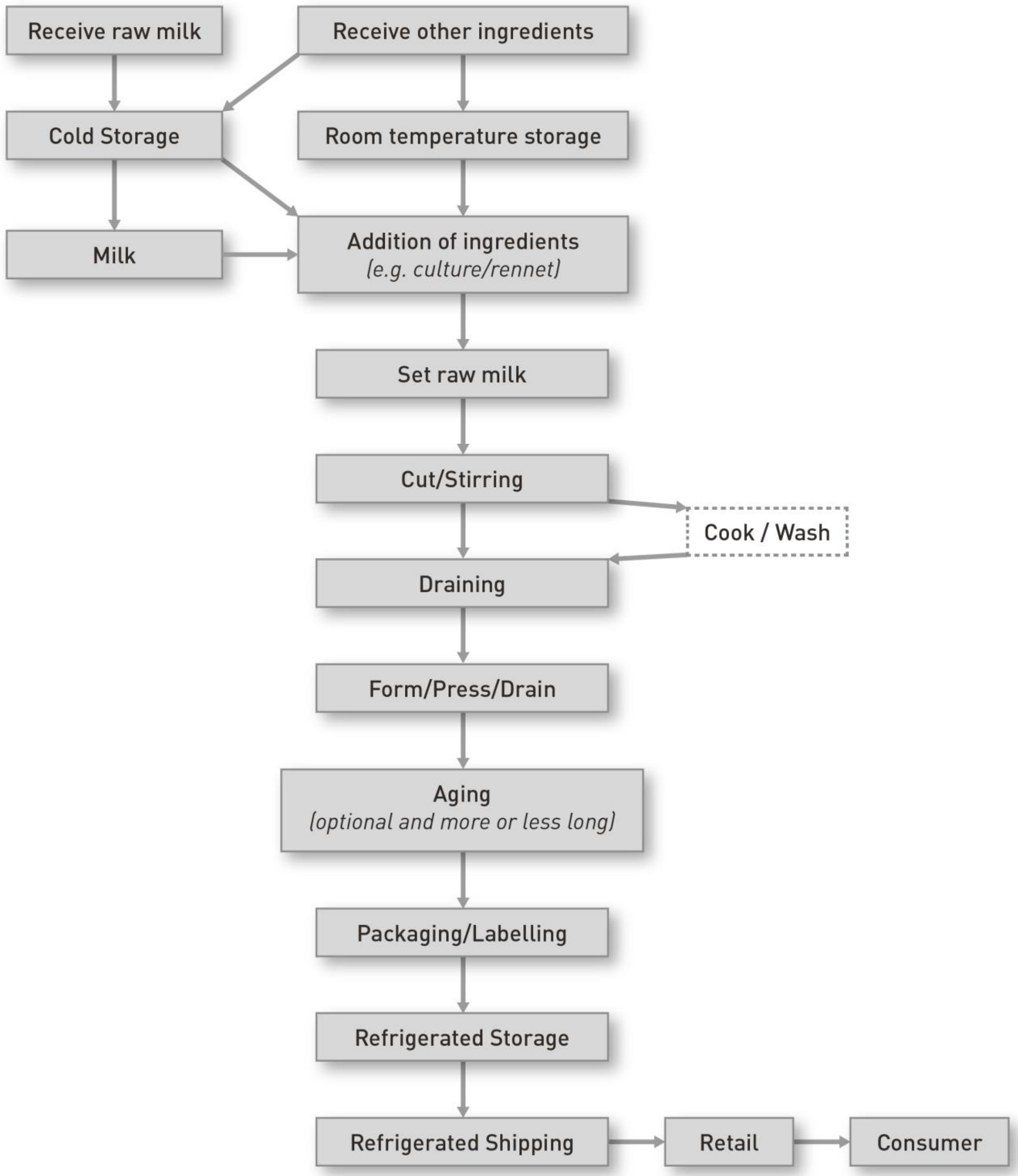


Figure 2: Making Cheese from Raw Milk



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