Food and Agriculture Organization of the United Nations

World Health Organization

Joint FAO/WHO Expert Meeting on Microbiological Risk Assessment (JEMRA) on Shiga toxin-producing *Escherichia coli* (STEC) associated with meat and dairy products

(Virtual meeting, 1 to 26 June 2020)

Executive Summary

Issued on 22 March 2022

A virtual JEMRA meeting on Shiga toxin-producing *Escherichia coli* (STEC) associated with meat and dairy products was convened from 1 to 26 June 2020 to provide scientific advice on measures for pre- and post-harvest control of STEC in animals and foods of animal origin. The meeting participants are listed in Annex 1 of this summary report. This document summarizes the conclusions of the meeting. The full report will be published in due course as part of the FAO and WHO Microbiological Risk Assessment (MRA) Series. If conditions had permitted, this meeting would have been held at FAO headquarters in Rome, Italy. Because of the travel restrictions and lockdowns due to the COVID-19 pandemic in many countries, the joint FAO/WHO secretariat was unable to convene a physical meeting. Therefore, the meeting was held as a videoconference using a virtual online platform.

More information on this work is available at:

http://www.fao.org/food-safety/en/

and

https://www.who.int/foodsafety/en/

1. Background and approach

Shiga toxin-producing *Escherichia coli* (STEC) are an important cause of food-borne disease. Infections can result in a wide range of disease symptoms from mild intestinal discomfort and hemorrhagic diarrhea to severe conditions including haemolytic uremic syndrome (HUS), end-stage renal disease and death. In its report on the global burden of food-borne disease, WHO estimated that in 2010 food-borne STEC caused more than 1.2 million illnesses, 128 deaths, and nearly 13 000 Disability Adjusted Life Years (DALYs) (WHO, 2015).

The Codex Committee on Food Hygiene (CCFH) has highlighted the importance of STEC in foods since its 32nd Session in 1999, when it prioritized their presence in beef and sprouts as significant public health problems in Member countries (FAO and WHO, 2000). Following a request from the 47th Session in November 2015 (FAO and WHO, 2016), the FAO and WHO published the report *Shiga toxin-producing Escherichia coli (STEC) and food: attribution, characterization and monitoring* in 2018 (FAO and WHO, 2018). As part of the 50th session of CCFH in November 2018, the FAO/WHO further updated the committee with additional information on STEC that was subsequently published in the report *Attributing illness caused by Shiga toxin-producing Escherichia coli (STEC) to specific foods* (FAO and WHO, 2019a). The Codex Alimentarius Commission (CAC) at the 42nd Session, July 2019, approved new work on the development of guidelines for the control of STEC in beef, raw milk and cheese produced from raw milk, leafy greens and sprouts (FAO and WHO, 2019b). To support this work, the Joint FAO/WHO Expert Meeting on Shiga toxin-producing *Escherichia coli* (STEC) associated with Meat and Dairy Products was convened virtually from 1 to 26 June 2020 to review relevant measures for pre- and post-harvest control of STEC in animals and foods of animal origins.

The scientific literature describing physical, chemical and biological control measures (and their combinations) against STEC during primary production, processing and post-processing of raw meat, raw milk and raw milk cheeses was reviewed. The efficacy and utility of reported control measures were scored as high, medium or low, based on expert opinion informed by systematic reviews and/or meta-analyses, when available.

2. Control of STEC in meat

2.1 Primary production control measures

In meat production and processing systems, many approaches to support control of STEC are based on Good Agricultural Practice (GAP) and/or Good Hygiene Practice (GHP) that aim to generally reduce the spread of pathogens and are not specifically focused on STEC. On-farm, these include managing the hygienic conditions of housing, bedding and drinking water hygiene, appropriate animal density and biosecurity measures, effective sanitation of facilities and proper disposal of manure, all of which have been shown to contribute to generally reduce the faecal-oral transmission of pathogens, among livestock.

2.1.1 Cattle

On-farm, several dietary and herd management strategies with varying levels of impact on STEC populations in beef and dairy animals have been explored. Evidence to support cattle demographics, animal density, exposure to other animals, and sanitation of bedding were rated as having medium or high confidence to impact STEC. Interventions including feeding of forage versus concentrate rations, specific grain types, and the inclusion of citrus products and essential oils in feed were supported at lower

levels of confidence in their efficacy. There was limited evidence supporting the implementation of other reported interventions for the control of STEC in cattle.

A range of feed additive options to manage STEC has been explored. Although several products, product combinations and doses have shown varying efficacies, studies have shown that probiotics may be useful when administered in feed. Evidence of consistent reductions of STEC using bacteriophages *in vivo* was not identified. Additions of colicins or sodium chlorate to cattle feed remain premature for consideration for the control of STEC in cattle herds due to limited available data, but the confidence in the evidence was medium.

Some vaccines have been shown to reduce faecal excretion of STEC O157:H7, but their efficacy is variable and is dependent on the type of vaccine and the number of doses administered. The use of vaccination in cattle has not been commercially adopted, likely due to the lack of incentives for the producer (farm-level) to cover the additional costs associated with vaccines and their administration; however, support for vaccine efficacy and confidence ranged from low to high depending upon the specific vaccine.

The use of antimicrobials to reduce STEC colonization or excretion remains controversial, due to the valid concerns about dissemination of antimicrobial resistance. Regardless of efficacy, the use of antimicrobials in cattle feed was not recommended for the control of STEC in cattle herds based on the available evidence.

Long distance transport and the stress of interim unloading/loading have been shown to increase faecal excretion of STEC that can lead to cross-contamination between animals. Evidence supports that, whenever possible, transport distance from farm to slaughter should be minimized in accordance with best practices for animal welfare, and the evidence for this was supported with a low/medium level of confidence.

2.1.2 Small Ruminants

In general, most GAP and GHP that are used in cattle production are thought to have similar efficacy on reduction of STEC in goats and sheep. No interventions against STEC specific to small ruminant production are suggested due to the limited evidence supporting their adoption. Studies pertaining to vaccination in goats experimentally challenged with STEC O157:H7 showed promising results, but included only a small number of animals. Use of probiotic lactic acid bacteria in lambs and sheep reduced STEC (O157:H7 and non-O157) excretion and prevalence. Bacteriophage therapy reduced STEC O157:H7 counts in the lower intestinal tract of sheep. Lactoferrin, an immunomodulatory protein, when administered orally reduced STEC O157:H7 faecal concentration and duration of shedding in sheep. Supplementation of sodium chlorate in water provided to the sheep before transport to slaughter reduced STEC O157:H7 throughout the intestine. Vaccination, probiotics, bacteriophage, lactoferrin, and sodium chlorate can reduce STEC levels in small ruminants, but are not recommended for implementation due to the limited evidence supporting their efficacy.

2.1.3 Swine

Domestic swine can carry and shed STEC, prevalently non-O157 STEC, but includes serogroups associated with severe disease in humans. No interventions for the control of STEC in live pigs were evaluated.

2.1.4 Other animals

Several other ruminant animals are also commonly raised or hunted for food and may be a source of STEC. These include, but is not limited to, reindeer (*Rangifer tarandus*), yak (*Poephagus grunniens* or *Bos grunniens*), camelids, bison (*Bison bison* or *B. bonasus),* and buffalo (*Bubalus bubalis*). Pre-harvest

intervention is not possible for wild-harvested animals. As for domesticated meat-producing animals, GHP can be used for pathogen reduction and control in farm-raised wild animal species. Specific interventions targeted at STEC in these species have not been reported, but controls used in cattle and small ruminants may have similar efficacy in these animal species.

2.2 Meat processing control measures

During processing, GHP measures including lairage hygiene and optimized dressing and evisceration procedures to minimize carcass microbial contamination from the hide or the gastro-intestinal tract, trimming to remove visible carcass contamination, minimizing cross-contamination during handling, and the use of effective cooling systems can minimize microbial growth on carcasses. Avoiding contamination of the carcass through contact with hides, gut content or faeces during slaughter is an accepted management practice for controlling bacterial contamination, including food-borne pathogens, but is not specific to controlling STEC. However, evidence supporting the effectiveness and reliability of these GHP measures for the specific control of STEC was limited.

2.2.1 Beef

The exact role of lairage in the spread of STEC is unclear and it is likely dependent on facility design, holding time, animal stress response, animal density and cleanliness. The efficacy of cleaning and disinfection of lairage pens can vary and are dependent on the implemented protocols. In lairage, hide scoring can be used to support targeted management of dirty animals, but the association between clean animal hide scores and reduced prevalence of pathogens on carcasses, including STEC, is not well understood.

Hide decontamination treatments including washes, dehairing and bacteriophage can be applied before or after stunning. Within the research studies, there were high levels of variability in the reported efficacy of these procedures, combined with practical issues, regarding their in-plant application. In general, the evidence on their role in reducing the transfer of STEC from hide to carcass was low.

Processing measures where evidence supported a high efficacy in reduction in the prevalence of STEC included steam vacuuming of visible faecal contamination on carcasses, use of a hot potable water carcass wash, steam pasteurization followed by 24 hour air chilling and combinations of these. The use of knife trimming to remove carcass tissue contaminated with faecal material is common and is supported by a medium confidence level in the evidence. In-plant studies demonstrated significant reductions in STEC prevalence derived from these processing control measures individually. Results of using bacteriophages have been more promising in processing and post-processing stages than in live animals.

Despite the widespread commercial use of pre-chill carcass decontamination treatments using organic acids and other chemical agents, there is a wide variation in the reported reductions in STEC levels and prevalence in both research and commercial applications depending on trial parameters and therefore, the confidence in the evidence was low.

The comparative efficacy of available and putative control measures (e.g. bacteriophage, lactic acid, irradiation) for reducing or eliminating STEC on primal cuts, trim and cheek meats was widely varied, ranging from <1 to >5 $\log_{10}$. Comparative studies under relevant pilot scale or production conditions were not identified; so, these studies were generally laboratory-based rather than at the scale or conditions used in commercial beef or veal production. Yet, chemical antimicrobial dips may prove to have a good efficacy for the control of STEC on primals and trims in establishments of all sizes and the data were supported at a medium level of confidence.

The comparative efficacy of available and putative control measures for reducing or eliminating STEC on ground beef and in retail packs was varied, ranging from no effect to >5 $\log_{10}$, with only high-pressure

processing (HPP), gamma irradiation and electron beam sterilization (eBeam) producing significant reductions (>4 $\log_{10}$).

Several additional control measures including the commercial use of visualization technologies for faecal/ingesta contamination, blast freezing and storage of packed beef, innovative processing aides and packaging technologies, along with inclusion of post-packaging intervention strategies such as the use of microbial competition, colicins, and lactoferricin B, are currently being evaluated and may be important in the future.

Some studies showed that combinations of interventions during processing are more efficacious than individual treatments in reducing STEC levels, but the results were inconsistent and varied depending on study parameters.

2.2.2 Small Ruminants

Carcass processing in sheep and goats is broadly similar to beef carcass processing. Despite the widespread use of pre-chill carcass decontamination treatments using organic acids and other agents in beef processing, their evidence supporting STEC reduction in sheep and goat processing in commercial settings was considered low. Hot water, lactic acid, and other organic acids have been used as sprays and applied on small ruminant carcasses, with STEC reductions broadly similar to those observed in cattle. No recommendations or interventions were available specifically targeting the control STEC in the processing of small ruminants.

2.2.3 Pork

Pork products have occasionally been confirmed as vehicles of STEC transmission, but it remains unknown whether STEC contamination occurred during processing or via cross-contamination from other foods or animals during slaughter. In pig processing and post-processing, there are no interventions or practices that are specific for STEC, however, interventions described for pigs in the FAO/WHO report; "Interventions for the control of non-typhoidal *Salmonella* spp. in beef and pork" published in 2016, are expected to be broadly effective against STEC.

2.2.4 Meat derived from other animals

Wild game harvested for meat may be processed at facilities specifically designed for that purpose or species (e.g. reindeer slaughter and processing facilities). However, a large portion of wild game is dressed using traditional field methods which often lack water or other sanitary facilities, making compliance with GMPs difficult. Meat from these species could be treated post-harvest in a similar fashion as beef to reduce STEC, but reports of the efficacy of these interventions in non-beef matrices is not reported.

3. Control of STEC in raw dairy products

3.1 Control in primary production

Contamination of milk with pathogens, including STEC, mainly occurs from contact of the product with faecal materials directly from the animal during milking or via milking equipment, milking personnel, and from the farm environment. Thus, factors affecting the carriage of STEC in live animals and those practices surrounding milking hygiene can reduce, but not assure the absence of contamination of raw milk for consumption or further processing.

3.2 Control in dairy processing

3.2.1 Control measures during harvest, storage and transport

Contamination can also occur during processing and post-processing. Hence, application of GHP and GMPs are essential to minimize contaminated milk.

Most of the interventions against STEC during the production of raw milk and raw milk cheeses were evaluated in challenge studies in a laboratory setting or implemented in pilot plants rather than under commercial or production-scale conditions. Furthermore, it was noted that the efficacy of interventions against STEC in raw milk cheeses varied greatly depending on the animal origin of the raw milk, manufacturing practices, the scale of production, the baseline microbial load, composition of the raw milk, and the STEC serotype used in the study.

Temperature control and hygiene within the harvest environments as well as during transportation of raw milk from the farm are critical parts of the milk supply chain and are part of GHP. Temperature can significantly affect the microbiological load of raw milk prior to processing, packaging and sale of milk intended for drinking or for manufacturing of raw milk cheeses. Although these interventions can mitigate the growth of *E. coli* and other indicator organisms, confidence in the evidence in support for these interventions for the control of STEC ranged from medium (udder hygiene) to low (milking environment hygiene and milk storage).

3.2.2 Control measures during processing

Apart from pasteurization, which is very effective, several technologies have been evaluated to mitigate the presence of STEC in raw milk. Most processing interventions require costly equipment and some are time consuming, thus the volume of raw milk that can be efficiently processed in a plant can be negatively impacted. Both bactofugation (centrifugation to remove bacterial spores) and microfiltration require raw milk to be heated to between 50°C and 60°C to reduce viscosity before treatment. Furthermore, as microfiltration only works with skim milk, the cream needs to be separated, hence its use in treating milk intended for cheese manufacturing is uncommon. Bactofugation is more efficient for spore removal, whereas microfiltration is more effective in removing bacterial cells, but it may be susceptible to clogging problems. Some studies on the effectiveness of these interventions examined generic *E. coli,* but none have evaluated their effect against STEC.

Bacteriophages specific to *E. coli* and STEC have been evaluated to control STEC in raw milk. Bacteriophage treatments have shown some reductions in STEC levels during refrigerated storage. However, strain-to-strain variations in STEC reduction efficiency, and possible emergence of phage-resistant *E. coli* strains, have been observed. The effect of adding bacteriophage to control *E. coli* during milk fermentation in the making of cheeses has also been examined. Research demonstrated that a phage cocktail was able to completely inactivate *E. coli* and STEC O157:H7 strains without affecting the lactic acid starter strain, whilst for other STEC serovars, only small reductions (<1 $\log_{10)}$ CFU/mL were observed. However, there are STEC strain to strain variations in response to the phages, variations in phage specificity and the possible emergence of phage-resistant strains. Confidence in the evidence of bacteriophage usage during fermentation of milk was evaluated as medium.

Gamma or eBeam irradiation are very effective at reducing bacterial levels in milk. Although studies showed that 1 to 2 kGy doses did not seem to affect sensory qualities, off-flavors are often reported to be associated with irradiation, especially in dairy products. Confidence in the evidence was rated as medium. HPP has also been shown to be effective at reducing STEC O157:H7 levels in inoculated ultra-high

temperature (UHT) treated milk. However, strain-to-strain variations in STEC response to high pressure have been observed and its effect in raw milk at low temperatures is unknown.

3.2.4 Post-manufacturing steps - packaging

Active packaging is used in the food industry primarily to extend shelf-life, but also to prevent growth of pathogens. Although not as effective against STEC O157:H7, the use of technology such as modified atmosphere packaging (MAP) can slow the growth of other bacterial pathogens (e.g. *Listeria monocytogenes* and *Staphylococcus aureus*) in hard cheeses made from raw sheep's milk. However, the use of MAP in the cheese industry is still being explored using different combinations of gases for different cheese types.

In a limited study, bacteriophages added to cheese slices were not very efficacious at reducing STEC O157:H7 levels. The ineffectiveness of phage treatment was thought to be due to the low pH of the product, or a result of other undetermined factors. Irradiation via eBeam treatment was very effective in controlling pathogens on cheese surfaces, and the confidence in the evidence was rated as medium.

4. STEC monitoring and detection

Sampling and testing of beef and raw milk products are an important part of verification plans, to confirm that practices and procedures described in the food safety programs are successful. Accurate quality and compositional test results are crucial and depend on appropriate sampling and sample handling, the type of representative samples (e.g., bulk tank, raw milk, milk filters, cheese) and proper methods and analysis. If GHP are implemented during primary production, the microbial load can be maintained at low levels in the raw materials and in the processed products. Once at the processing facility, testing at critical control points are required to assure food safety and wholesomeness of meat, raw milk, and raw milk cheese products.

Because STEC are often present only at low levels in foods, culture enrichment of food samples is an indispensable and critical step in detecting STEC in meat, dairy and other foods. Improved selective media formulations that facilitate STEC enrichment coupled with rapid screening assays (e.g. Shiga toxin Enzyme-linked Immunosorbent Assay [EIA], Polymerase chain reaction [PCR], Loop-mediated isothermal amplification [LAMP]) can decrease the sample-to-result time and improve sensitivity. Further work is required to develop and validate STEC virulence traits and/or serogroup-specific enrichment procedures. Since STEC testing is complex, the quantitative detection of generic *E. coli* has been proposed as an alternative hygienic indicator during processing and post-processing stages, although it is not an absolute indicator of the microbial ecology of STEC. Furthermore, indicator counts can vary considerably depending on detection methodologies and practices, making it challenging to develop specific recommendations for all production conditions. The decision-making criteria based on *E. coli* indicator levels vary depending on the pre-defined acceptable limits and sampling plan, but can be useful for measuring trends and estimating baseline levels of STEC.

The use of molecular techniques, such as PCR, that target STEC virulence genes are highly sensitive and specific for STEC detection. Most PCR methods that have been validated for STEC testing are fast, low cost and commercially available. Presently, assays that target Shiga toxin genes (*stx*) and the *E. coli* attaching and effacing gene (*eae)* use different technologies, including real time-PCR, LAMP-PCR and digital droplet PCR (ddPCR). However, bacteria other than STEC may harbour the same virulence genes and the detection of genes alone may not fully reflect health risks due to differential or lack of gene expression. It is also

important to characterize the stx_1 and stx_2 subtypes of STEC isolates, as some subtypes are more often associated with severe human disease.

The isolation of STEC by traditional culture-based methods or by immunomagnetic separation (IMS), are valuable to confirm presumptive PCR positive samples. Methods are needed that enable the efficient and specific isolation of STEC O157:H7 and non-O157 STEC. IMS techniques have greatly improved the isolation of STEC from enrichment cultures. However, IMS is only available for the major STEC serogroups that are commonly *eae* positive (O157, O145, O121, O111, O103, O26, O45). IMS assays can also lack specificity or fail to identify certain serogroups and additional PCR analyses for identifying *stx* genes for confirming STEC are required.

4.1 Testing in live animals

The implementation of monitoring plans at the farm level to measure the impact of STEC prevalence on raw beef is considered impractical. Some concerns include: the highly intermittent nature of STEC excretion; the fact that super shedder cattle cannot be reliably identified; the detection of all STEC serogroups and strains is often not possible; the application of methodologies on farm would be costly, and the value of test outcomes would be uncertain.

4.2 Testing in meats

Monitoring and laboratory testing for STEC O157:H7 and non-O157 in beef at the processing and post-processing stages, is usually a stepwise approach which involves screening the sample for essential STEC virulence genes (*stx* and *eae*), followed by testing for the major STEC serogroups based on epidemiological evidence or as required for market access of export products. At the JEMRA meeting on STEC in 2017, a risk-based strategy was recommended for testing of a STEC isolate from food to discern the level of health risk based on STEC virulence genes, namely, specific *stx* subtypes, *eae*, and *aggR* (FAO and WHO, 2018).

STEC prevalence are generally lower in intact meat products than in trim or ground/minced beef. STEC presence and levels in meat and meat products can vary considerably due to differences in primary, processing and post-processing conditions and interventions.

4.3 Testing in raw milk and raw milk cheese

Although STEC are isolated from raw milk and raw milk cheeses, STEC testing is not common in the dairy industry for many countries and most sampling and testing protocols target indicator organisms such as *E. coli.* Manufacturers often use indicator levels to select for raw milk of good quality for raw milk cheese production. Though the presence or concentration of generic *E. coli* or other indicator organisms in raw milk does not necessarily indicate the presence of STEC, they remain useful hygienic markers of the quality of raw milk.

5. Limitations and gaps

Strength of the evidence on the efficacy of the evaluated interventions and their perceived utility/practicality varied greatly based on the study design. Some studies were conducted in meat or dairy plants and others in laboratory settings. There were also differences in the quantitative and the qualitative approaches, the analytical methods used, the serotype and strains of STEC used and their inoculum concentrations.

Scientific evaluations of interventions and treatments to control STEC throughout raw beef, raw milk and raw milk cheese production chains are as representative as possible to the scenario in which they would

be applied. Conducting these studies in-plant are frequently prohibited due to health risks associated with the potential introduction of pathogens into the food supply and the budgetary constraints associated with testing large number of samples required for detecting STEC in food matrices. Consequently, surrogate bacteria, such as generic *E. coli*, are used as substitutes and the results extrapolated, meaning that evidence of intervention effects specifically for STEC may not be available currently or in the future. Therefore, there is doubt and uncertainty as to whether the detection and reduction levels observed in surrogate studies are truly representative of STEC or of commercial production and processing.

As part of the review, it was acknowledged that there is a need to bridge the evidence gap between studies that demonstrated efficacy on a small scale (laboratory) versus those demonstrating efficacy at a large scale (pilot plant or commercial processing). Most STEC interventions have been evaluated using laboratory-based challenge studies rather than under commercial or production scale conditions. Many of the challenge studies examining the impact of interventions used artificially high starting inoculum levels of one or more STEC strains and with limited serotypes, and only a few studies assessed the impact of high versus low inoculum levels. Low inoculum levels are likely to be more representative of natural contamination situations, and were, in general, associated with minimal reductions of STEC by the interventions used. Many studies evaluated the inherent effect of the control measure in the animal host or meat matrices on the efficacy of STEC reduction, however these results are equivocal, as it is difficult to quantify such effects in actual situations.

Many studies focused on the impact of an individual control measure at a specific stage in the food chain, rather than in the context of a total food chain or of the safety of the food available to the consumer. Many food businesses have implemented multiple control measures concurrently or sequentially on farms and in processing facilities, but the overall efficacy of multiple "hurdles" in the total chain remains difficult to quantify. It is uncertain that observed reductions associated with each individual control will be cumulative when combined. Furthermore, the efficacy of an intervention may vary from establishment to establishment. This report has therefore considered each control measure in isolation, unless there were scientific data supporting a reduction when used in combination with others.

It was recognized that with advances in analytical methods, including increasing use of molecular tools, the evaluation of evidence concerning some STEC control measures and interventions may need to be revised in the future.

6. References

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Annex 1. List of participants

EXPERTS

Frederic Auvray, France

Li Bai, China

Todd Callaway, the United States of America (Chairperson)

Natalia Cernicchiaro, the United States of America

Isabel Chinen, Argentina

Paul Cook, the United Kingdom

Roger Cook, New Zealand

Patricia Desmarchelier, Australia

Geraldine Duffy, Ireland

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