Norwegian Scientific Committee for Food Safety

A risk assessment of shiga toxin-producing *Escherichia coli* (STEC) in the Norwegian meat chain with emphasis on dry-cured sausages

Norwegian Scientific Committee for Food Safety

Panel on Biological Hazards

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Contents

Abbreviations used in this report

CCP	Critical Control point
CIP	Cleaning-In-Place
CFU	Colony Forming Unit
eae	Gene encoding intimin
EHEC	Enterohaemorrhagic *Escherichia coli*
EAEC or EAggEC	Enteroaggregative *Escherichia coli* (EAEC or EAggEC)
EIEC	Enteroinvasive *Escherichia coli* (EIEC)
ETEC	Enterotoxigenic *Escherichia coli* (ETEC)
EPEC	Enteropathogenic *Escherichia coli*
GHP	Good Hygiene Practice
HACCP	Hazard Analysis of Critical Control Point
H antigen	Flagellar structure antigen
IMS	Immunomagnetic separation
LEF	enterocyte effacement
MLVA	Multilocus Variable Number Tandem Repeat Analysis
O antigen	Somatic structure antigen, surface antigens of *E. coli*
RH	Relative Humidity
Serogroup	O-group, after O variant present
Serotype	O:H-type
STEC	Shiga toxin-producing *Escherichia coli*
stx	Shiga toxin gene, encoding Stx
Stx	Shiga toxin
VTEC	Vero toxin-producing *Escherichia coli*

Summary

E. coli is part of the normal gastrointestinal microbial flora of humans and animals. *E. coli* bacteria causing enteric/diarrhoeal disease are categorized into different groups based on their virulence properties and pathogenic features in humans. Enterohaemorrhagic *E. coli* (EHEC) are *E. coli* strains that cause bloody diarrhoea and haemolytic uraemic syndrome (HUS) in humans, and have a defined zoonotic association. The major virulence factor of EHEC (and the actual cause of HUS) is the ability to produce Shiga toxins (Stx), thus the name Shiga Toxin Producing *E. coli* (STEC). With enteropathogenic *Escherichia coli* (EPEC), the diarrhoea in these patients is due to attaching and effacing (A/E) lesions in the enteric epithelium.
This risk assessment was conducted after a human outbreak of STEC O103 in 2006, associated with contaminated dry-fermented sausages.

The Norwegian Scientific Committee for Food Safety (Vitenskapskomitéen for mattrygghet), Panel on Biological Hazards, was asked by the Norwegian Food Safety Authority (Mattilsynet) for a risk assessment regarding shiga toxin-producing *E. coli* (STEC) in the Norwegian meat chain, with emphasis on dry-cured sausages. In response, an *ad hoc* Working Group of experts was appointed with the mandate to draft a risk assessment regarding this issue.

The current report approaches the task by following and analysing the entire process, from the origin of the meats at farm level, to the final production and storage of dry-cured sausages. An overall aim of the report has been to identify and describe potential intervention options in various parts of this chain.

The main conclusions from the risk assessment are as follows:

1. It is not possible to give any reliable quantitative estimates of the current risk associated with consumption of dry-cured sausages.
2. There are no clear indications of any general change in the epidemiology of STEC infections in humans in Norway over the last decade.
3. There is no documentation that there has been any change in the occurrence of various STEC in the domestic animal reservoir during the last decade.

4. The combination of proper slaughter hygiene and use of thermal decontamination of sheep, cattle and pig carcasses represents an efficient way to reduce STEC contamination. This approach would not only cause a reduction in the contamination level of STEC, but also provide a general beneficial effect on the level of other enteric pathogens, such as *Salmonella* and *Yersinia enterocolitica.*
5. Proper use of starter cultures in fermentation, combined with higher fermentation temperatures, will reduce the probability of growth of STEC in contaminated dry-cured sausages.
6. A combination of higher fermentation temperatures, a lower pH during the process, and heat-treatment of the final product should effectively eliminate the potential risk for transmission of STEC infections from consumption of dry-cured sausages. A 5 log reduction is possible.
7. Technological options are available to reduce significantly the transfer of potential pathogens through meats in general, and specifically through dry-cured sausages.
8. The most important data gap is the lack of information about the actual occurrence of STEC infections in humans in Norway. Improved laboratory diagnostic procedures and epidemiological surveillance, combined with better reporting and tracing in the health care system are necessary.
9. The implementation of properly designed base-line studies of various domestic animals, to provide data on the occurrence of various serotypes and their virulence factors present is recommended. Also, this would provide a better basis for comparison with human isolates.

Sammendrag

E. coli er en del av den naturlige mikroflora hos mennesker og dyr. *E. coli* som forårsaker tarmsjukdom/ diaré er gruppert etter deres virulensegenskaper og evne til å gi sjukdom hos mennesker. Enterohaemorrhagisk *E. coli* (EHEC) er stammer av *E. coli* som forårsaker blodig diaré og haemolytisk uremisk syndrom (HUS) hos mennesker. Slike stammer antas å ha sin opprinnelse hos dyr. Den viktigste virulensegenskapen hos EHEC er evnen til å produsere shigatoksin (Stx), derav navnet shigatoksinproduserende *E. coli* (STEC). Diaréen hos pasienter med infeksjon med enteropatogen *Escherichia coli* (EPEC) er på grunn av deres evne til å feste seg til tarmen og gi spesielle epitelskader i tarmen. STEC og EPEC kan være svært like og det kan være vanskelig å skille mellom dem ved bruk av laboratoriemetoder.

Denne risikovurderingen ble gjennomført etter et utbrudd hos mennesker forårsaket av STEC O103 i 2006, et utbrudd assosiert med konsum av en spesiell spekepølse. Vitenskapskomiteen for mattrygghet ble etter utbruddet i 2006 spurt om å lage en risikovurdering omkring STEC i den norske kjøttkjeden, med vekt på spekepølser. På grunnlag av denne henvendelsen ble en ad hoc arbeidsgruppe nedsatt for å gjennomføre oppdraget.

Den framlagte rapporten tilnærmer seg tema ved å følge og analysere hele prosessen fra kjøttets opprinnelse på gården til den endelige produksjon og lagring av spekepølse. Et overordnet mål for rapporten har vært å beskrive mulige intervensjoner i forskjellige deler av denne kjøttkjeden.

Hovedkonklusjonene i risikovurderingen er som følger:

1. Det er ikke mulig å gi et pålitelig kvantitativt estimat av nåværende risiko forbundet med konsum av spekepølse.
2. Det er ingen klare indikasjoner på noen vesentlig endring i det epidemiologiske mønsteret for STEC-infeksjoner hos mennesker i Norge det siste tiåret.
3. Det er ikke dokumentert noen endring i forekomsten av forskjellige STEC i husdyr-reservoaret det siste tiåret.
4. Kombinasjonen av en bedret slaktehygiene og bruk av dekontaminering av slakteskrotter (varme) ved slakting av sau, storfe og gris representerer en effektiv måte å redusere graden av kontaminering av skrotter med STEC. Denne tilnærmingen vil ikke bare gi en reduksjon når det gjelder STEC, men også gi en generell effekt når det

gjelder forekomst av tarmpatogener på kjøtt – som *Salmonella* og *Yersinia enterocolitica.*

5. Riktig bruk av startkultur, kombinert med en noe høyere fermenteringstemperatur vil redusere sannsynligheten for vekst av STEC under produksjon av spekepølse.
6. En kombinasjon av høyere fermenteringstemperatur, lavere pH under prosessen og en mild varmebehandling i slutten av prosessen vil i praksis kunne eliminere risikoen for overføring av STEC via spekepølse. En reduksjon i nivået av STEC på 5 log-enheter er mulig.
7. Overføringen av mulige patogener fra kjøtt generelt og spesifikt via spekepølse kan reduseres dramatisk ved bruk av styrbar teknologi på slakteri og/eller i spekepølseproduksjon og lagring.
8. Den viktigste kunnskapsmangelen er mangelen på informasjon omkring den faktiske forekomsten av STEC-infeksjon hos mennesker i Norge. En bedret diagnostikk og epidemiologisk overvåkning samt bedre rapportering og sporing av infeksjoner er nødvendig for å komplettere bildet.
9. Det anbefales å bruke godt planlagte baselinestudier for å skaffe bedre oversikt over forekomst av forskjellige serotyper og virulensfaktorer hos husdyr. Dette vil også gi tilgang på flere isolater som kan sammenlignes med isolater fra mennesker.

Background

There are a wide variety of traditional Norwegian cured products that contain meat from various domestic animals. The production processes for these products differ from those used for similar products in other countries in a variety of aspects. Therefore, a scientific update on the risk for transmission of Shiga toxin-producing *E. coli* (STEC) to humans, through consumption of Norwegian dry-cured sausages was considered necessary. To comprehend fully the complexity of the production, description and assessment of all steps in the process, from live animals, through slaughter, and to the final industrial production processes, was necessary.

Risk assessments have been conducted for *E. coli* O157 transmitted by meat and meat products in other countries, while limited information is available regarding other O-groups of *E. coli*.

The Norwegian Scientific Committee for Food Safety (Vitenskapskomiteen for mattrygghet), Panel on Biological Hazards was asked by the Norwegian Food Safety Authority (Mattilsynet) to present a risk assessment on the transmission of STEC to humans from consumption of dry-cured sausages.

Terms of reference

The terms of reference for the risk assessment were agreed upon through a process including written correspondence and meetings between the Committee and the Norwegian Food Safety Authority (FSA), and a meeting where representatives from the FSA were present.

Qualitative aspects to be addressed in the risk assessment

1. Have there been any changes in the distribution of STEC and enteropathogenic *Escherichia coli* (EPEC) in the domestic animal reservoirs (e.g. cattle, sheep, and pigs) in recent years?
2. Have there been any changes in the epidemiological pattern of enterohaemorrhagic *Escherichia coli* (EHEC) infections in the human population in Norway in recent years?
3. Identify the groups at risk from EHEC infections.

4. Describe the variations in occurrence of virulence factors in the different STEC-serotypes (and atypical EPEC) isolated from:
   a. Animals,
   b. Food,
   c. Humans

   and the relevance for pathogenicity in humans.
5. Are current laboratory techniques (including indicator organisms) sufficient for providing reliable results regarding STEC and their pathogenicity factors (e.g. *stx*$_1$/*stx*$_2$ *genes, eae* gene)?

Quantitative aspects to be addressed in the risk assessment

1. What magnitudes of risk are associated with consumption of dry-cured sausages with the current production process?
2. Describe, and if possible quantify, the effects of interventions in the meat production line on the level of STEC on carcasses or in the processing of meat by:
   a. Pre-harvest intervention
   b. At slaughter
      i. General slaughter hygiene,
      ii. Decontamination procedures.
3. Describe critical control points, and if possible quantify, the effects of different interventions during the production of dry-cured sausages regarding:
   a. Raw material quality (meats, sugar, spices, etc.),
   b. Production parameters (temperatures, recipes, maturation times, etc.).
4. Describe and quantify the risks associated with consumption of dry-cured sausages?

FSA would like questions 1, 2 and 4 (qualitative aspects) and question 2 (quantitative aspects) to be prioritised.

General introduction

E. coli; pathogenic variability, nomenclature and definitions

E. coli is part of the normal gastrointestinal microbial flora of humans and animals. Based on the main surface antigens, the O- (somatic), and the H- (flagellar), sub-groups of *E. coli* can be serologically differentiated from each other, the O antigen defining the “serogroup” and

the combination of O and H antigens defining the "serotype" of an isolate. Some strains of *E. coli* are pathogenic and may cause a wide variety of infections in humans (41,56). *E. coli* bacteria causing enteric/diarrhoeal disease are further categorized into the following groups, based on their virulence properties and their pathogenic features in humans:

1. **Enterotoxigenic *E. coli* (ETEC)** are the most common cause of travellers' diarrhoea, as well as diarrhoea among children in developing countries. ETEC is defined as *E. coli* strains that produce specific heat-labile and/or heat-stable toxins.
2. **Enteroaggregative *E. coli* (EAEC or EAggEC)** are the second most common cause of travellers' diarrhoea. This group of *E. coli* adheres to enteric cells with a diffuse adherence pattern.
3. **Enteroinvasive *E. coli* (EIEC)** are pathogenetically related to *Shigella* spp., and like *Shigella* spp. invade the enteric cells, causing diarrhoea. EIEC are uncommon in industrialised countries.
4. **Enteropathogenic *E. coli* (EPEC)** are considered a major cause of infant bacterial diarrhoea in developing countries. The central mechanism of EPEC pathogenesis is the ability to cause attaching and effacing (A/E) lesions in the enteric epithelium, a virulence characteristic shared with the next pathogroup; EHEC. EPEC can be further grouped into typical and atypical EPEC, by differences in adherence patterns. The majority of typical EPEC fall into certain well-recognized O:H serotypes and possess a virulence plasmid known as the EPEC adherence factor (EAF) plasmid (40).. The reservoir of typical EPEC is the human bowel. Atypical EPEC do not possess the EAF plasmid, but frequently express EAST1, an enteroaggregative heat stable toxin, encoded by *astA*. Atypical EPEC have been shown to be prevalent among children in both developing and developed countries, but only a few studies have reported an association with diarrhoea, possibly prolonged diarrhoea in particular (1,2,59,76), and the significance that they may have for human health remains unknown. In recent years it has become clear that atypical EPEC not only has a human reservoir, but also an animal reservoir. Atypical EPEC is considered to be genetically and epidemiologically related to the Shiga toxin-producing *E. coli* (STEC) (84), of which EHEC is a subgroup. Whilst atypical EPEC has been discussed as a possible emerging pathogen, its health importance still remains unclear.
5. **Enterohaemorrhagic *E. coli* (EHEC)** are *E. coli* strains that cause bloody diarrhoea and haemolytic uraemic syndrome (HUS) in humans, and the only group that has a defined zoonotic association. As with EPEC, the diarrhoea in the patients infected with this

pathogen is due to attaching and effacing (A/E) lesions in the enteric epithelium. In addition, the major virulence factor of EHEC (and the actual cause of HUS) is the ability to produce Shiga toxins (Stx).

EHEC constitutes a subset of **Shiga toxin-producing *E. coli* (STEC)**. The major virulence factors of STEC, which also define the STEC group, are Stx, a name that reflects the close genetic relationship to the Stx produced by *Shigella dysenteriae.* STEC are also known as verocytotoxin producing *E. coli* (VTEC), as the toxins produced by these organisms are toxic to African Green Monkey Kidney (Vero) cells (56). The Stx family comprises Stx_1 and Stx_2, with their respective subtypes (56). The structural genes for Stx (*stx*) are carried by bacteriophages, but incorporated in the bacterial host chromosome of STEC. However, depending on the bacteriophages and their bacterial hosts, these incorporated bacteriophages may vary in stability and as a result the bacteriophages may leave the bacteria, and the isolates lose their genes for Stx (34,50,76). This may also happen during isolation or sub-cultivation and was first seen among strains belonging to serotypes O2:H5, O26:H11, O73:H34 and O100:H32 (42), but was later observed among strains belonging to O157:H7 (77). This has also been suspected to have occurred in *E. coli* O103:H25 isolates from human patients during the 2006 outbreak. However, data is lacking on how frequently such genetic loss of *stx* occurs. There is also a lack of data on the relationship and ratio between *stx* positive and *stx* negative *E. coli* of the same serotype, as well as their relationships and ratio to *eae* positive *E. coli* of the same serotype (atypical EPEC).

There is no international consensus on *stx* nomenclature. However, based on sequence variation, stx_1 has been further subtyped into stx_1, stx_{1c} and stx_{1d}, while stx_2 can be further subtyped into stx_2, stx_{2c}, stx_{2d}, stx_{2e}, stx_{2f} and stx_{2g} (and further groups within these subtypes) (6,15,22,86,106). *E. coli* bacteria carrying any of these *stx* variants are, by definition, STEC. However, not all these *stx* variants are regarded as pathogenic to humans (17). Among subtypes of stx_1, stx_1 is regarded as the most pathogenic and most frequently associated with strains isolated from patients with HUS, while stx_{1c} is associated with common strains from sheep and has seldom been isolated from human patients. stx_{1d} has not been associated with cases among humans. stx_2 is regarded as more pathogenic than stx_1. Among stx_2, subtypes of stx_2 and stx_{2c} have been frequently found in strains from patients with HUS, while stx_{2d} carrying strains have been isolated from patients with mild diarrhoea. stx_{2e} and stx_{2f} are associated with STEC in pigs and pigeons,

respectively, and are not regarded as pathogenic to humans and stx_{2g} has only been described from wastewater (30,79).

In addition to variations in pathogenicity due to *stx* variation, many STEC do not have the ability to cause A/E lesions in the human enteric epithelium, while STEC associated with bloody diarrhoea and HUS in human patients typically have this virulence property. The ability of EPEC and EHEC to attach to the human enteric epithelium and cause A/E lesions is due to the presence of a membrane protein, intimin, which is encoded by *eae* and located on the locus of enterocyte effacement (LEE) (56). LEE also encodes for other genes important for adherence. As with *stx*, *eae* can be subtyped by sequence variations in the structural genes (105). Almost 20 subtypes of *eae* have been described to date, and named *eae-α, eae-ß, eae-γ, eae-δ, eae-ε, eae-θ* etc. Typical and atypical EPEC, as well as EHEC, have been reported to differ with regard to *eae* subtypes (84). The chromosomal location of LEE is also reported to differ among EPEC and EHEC strains according to their evolutionary lineage, and it has therefore been suggested that it may have been acquired at different stages during the evolution of these groups (21,102). However, data on differences in *eae* subtypes and the chromosomal location of LEE in typical and atypical EPEC and EHEC is sparse and therefore for the purposes of this report, the term *eae* will include all subtypes.

Terminology used in the report

Although many STEC are not associated with human disease and do not necessarily have the ability to cause A/E lesions, EHEC is often used as a synonym of STEC[1]. Others use the term EHEC for the five most common serotypes associated with human disease, whether virulence factors are present or not; O26:H11, O111:H8, O103:H2, O145:H21 and O157:H7.

In this report, the term STEC will be used for *E. coli* carrying *stx* (irrespective of possible loss of the stx gene during storage or cultivation). Serotypes will be specified as required in specific contexts. Presence of *eae* will be specified as *eae* positive or *eae* negative STEC. If not further specified, the use of stx_1 in this report is synonymous with the stx_1 subtype, while stx_2 includes both the stx_2 and stx_{2c} subtypes.

[1] STEC is most commonly used in North America and other countries outside Europe, while VTEC has been more commonly used in Europe.

Laboratory methods for detection of STEC in food, animal feeding stuffs, and animal faeces

The methods used for detection of STEC may vary between laboratories, particularly regarding verification and characterization of virulence factors, but there may also be variations in isolation techniques. In this report the most common methods assumed to be in use are briefly discussed. For further details, the report refers to the laboratories at the National Veterinary Institute and Norwegian School of Veterinary Science.

Detection of *E. coli* O157

The method for detection of *E. coli* O157 in foods and animal feeding stuffs is based on the method recommended by Nordic Committee on Food Analysis (NMKL 164) (65). The method is qualitative, and includes a selective enrichment for both 6-8 hours and 18-24 hours, followed by immunomagnetic separation (IMS) using magnetic beads coated with antibodies against surface antigen O157, and plating of the separated culture onto selective solid media. Suspected *E. coli* O157 isolates are usually confirmed by O157 agglutination tests and further investigated by PCR for the presence of shiga toxin genes (stx_1 and stx_2) and the intimin gene (*eae*).

The method used for detection of *E. coli* O157 in faeces from animals is a modified method of NMKL 164 (Personal communication; Torkjel Bruheim, National Veterinary Institute, Trondheim).

At the National Veterinary Institute, the IMS method used for detection of *E. coli* O157, and also *E. coli* O103, in foods and faecal samples has been further modified by inclusion of an ELISA step (91), in which ELISA positive samples are further plated onto selective agar for confirmation and characterization of isolates.

Detection of other serogroups

There is no internationally standardised method for detection of other serogroups of STEC, such as O26, O103, O111, and O145, in food, feeding stuffs, and faeces from animals. However, methods similar to NMKL 164, using IMS with magnetic beads coated with antibodies against *E. coli* surface antigens O26, O103, O111 and O145, respectively, are available and may be used for the detection of these *E. coli*. Both IMS and IMS-ELISA were used for detection of *E. coli* O103 during the 2006 outbreak. Further, as for *E. coli* O157, suspected isolates are verified and investigated by PCR for the presence of stx_1, stx_2 and *eae*.

Indicator bacteria

The presence of *E. coli* or related bacteria might be indicative of contamination with STEC, and their level can represent a measure of the probability of a pathogen being present. In situations where the possibility of detecting a possible pathogen directly is low, or too expensive, indicator organisms are often used. In particular, use of indicator organisms may be most typically appropriate during routine monitoring. During outbreak situations or where epidemiological understanding of the situation is sparse, analyses for the actual pathogen is usually more appropriate. At present, the two most relevant indicator organisms for STEC are *E. coli* and *Enterobacteriaceae*. *Enterobacteriaceae* are currently used as indicators of faecal or general contamination in foods in EU.

The method used in Norway for enumeration of *E. coli* as an indication of faecal contamination in food is method NMKL 125 (63), and NMKL 144 (64) is used for *Enterobacteriaceae*.

A specific advantage of using indicator organisms is that they are almost always present, and thus may be used as a running quality assurance system in a Hazard Analysis of Critical Control Point (HACCP) system. Direct detection of various STEC in foods has several disadvantages, compared to using indicator organisms:

1. The method is qualitative (+/-) and does not give any information about the level of contamination.
2. The sensitivity of the method is low, as STEC and other potential pathogens may be unevenly distributed throughout a product and occurs in a small part only.
3. Serogroup characteristics are used for detection of STEC, and as there are many serogroups of STEC, detection is complicated by choice of serogroup to be included in the analysis.

Sampling

It is often poorly understood that establishing a laboratory system in which detection of specific pathogens could serve as a tool for identification of “contaminated” foods, and thus prevent such products reaching the market, would be a monumental task. Bacteria are typically unevenly distributed in foods, and extensive sampling of each lot would be necessary to obtain a realistic picture. Sampling for pathogens must be extremely focused and based upon epidemiological information.

The meat products discussed in this report all come from animals where various STECs are commonly found in the intestinal contents. These bacteria may be transferred to the meats, and this contamination is typically erratic, and often linked to mishaps, accidents or poor slaughter hygiene.

As documented later in this report, the meat industry should be able to produce raw materials for dry-cured sausages with such a low level of STEC that specific analyses for them should be unnecessary and irrelevant.

The limited importance of detection of pathogens is well illustrated by parts of the Norwegian *Salmonella* programme: *Salmonellae* are sporadically detected in lymph nodes of slaughtered animals and whilst the programme identifies approximately 1/1000 sampled carcasses as positive, it has been estimated that approximately 3000 (1200-6000) slaughter pigs with *Salmonella* in lymph nodes enter the market each year. Thus the direct public health relevance of this part of the programme is marginal (75).

Before starting a specific sampling scheme for STEC, a thorough risk assessment should be conducted, including all aspects of sampling, as well as method sensitivity and specificity.

Hazard identification

Hazard identification is implicit in the title of this report and in the terms of reference, and further comment is unnecessary.

Background

Disease caused by STEC (EHEC) was identified for the first time in 1982, when strains of a previously uncommon serotype of STEC, O157:H7, were implicated in two outbreaks of haemorrhagic diarrhoea in the USA. Since then, outbreaks of STEC O157:H7 infections have occurred, and continue to occur, throughout the world, and are especially reported from industrialised countries. Human cases and outbreaks due to STEC strains belonging to serotypes other than O157:H7, including O26:H11, O111:H8, O103:H2, and O145:H21, are being increasingly reported, and presently comprise more than 150 different serotypes (41). Detailed information regarding STEC/EHEC: their pathogenity, virulence factors, toxins, mechanisms of intestinal adhesion etc. can be found in a number of review articles (8,9,19). The incidence of human STEC infections is low compared to the most common foodborne bacterial pathogens, such as *Campylobacter* and *Salmonella*. However, STEC may be associated with more severe illness, such as bloody diarrhoea and HUS, which makes it a pathogen of high public health significance. Data on outbreaks that include clinical, epidemiological and microbiological information, indicate that illness results from very low infective doses of *E. coli* O157 - <100 cells (85). HUS usually occurs in children <5 years of age and the elderly (66), and may result in death. Sequelae from HUS may include chronic kidney disease, hypertension, and CNS disorders. Diarrhoea caused by STEC is usually self-limiting. Antimicrobial therapy is controversial and usually contra-indicated, as such treatment may increase the risk of patients developing HUS, due to an increased release of toxins (24,103).

STEC are mainly regarded as emerging zoonotic pathogens in developed countries, and have alarmed public health authorities worldwide and raised debate on the microbiological safety of foodstuffs. Foods of animal origin, and food exposed to animal manure, including vegetables irrigated with contaminated water, are considered as major sources of STEC transmission to humans.

Common food vehicles identified in outbreaks and traced sporadic cases include meat products such as hamburgers, ground meat and cured/fermented sausages made of raw meat, as well as unpasteurised milk and products from unpasteurised milk. However, an increasing

number of outbreaks have also been associated with consumption of raw or minimally processed foods.

Norway - STEC infections in humans

The first known case of human STEC infection (caused by *E. coli* O157:H7) in Norway was detected in 1992. However, STEC infection in humans did not become a mandatorily notifiable disease to the Norwegian notification system for infectious diseases (MSIS) until 1995.

From 1994 to 2005 a total of 125 cases was notified to MSIS; of these, 61 (48%) were domestically acquired cases, 54 (44%) were imported cases, while for 10 cases (8%) the place of acquisition was unknown. *E. coli* O157:H7 accounted for 58% of the reported cases (72/125). However, in the counties served by the regional laboratories in Trondheim and Tromsø, where PCR methods for identifying specific pathogenicity factors have been used since the late 1990s, the proportion of O157:H7 is only about 25% (Table 1). This corresponds with data from Denmark and other continental European countries and may represent more realistic numbers (Enter-net annual report 2004

www.hpa.org.uk/hpa/inter/enter-net/Enter-net%20annual%20report%202004.pdf). Non-O157 cases comprise a number of different serogroups, in addition to several isolates that have been untypable with the sera used (Table 1). STEC O26 and O103 have been the most common non-O157 STEC reported in Norway (Figure 1 and Table 1).

The *stx*-profile is known for 100 of the 125 strains from cases with STEC infection notified in this period in Norway. Of nine strains isolated from HUS patients, only one had both stx_1 and stx_2, whereas eight had stx_2 alone. Of the STEC strains from patients with other symptoms (mainly gastroenteritis), 51% (46 strains) possessed both a stx_1 and, stx_2 22% (20 strains) had stx_1 alone, and 27% (25 strains) had stx_2 alone (Table 2).

The number of notified cases of human STEC infections is highest in the county of Sør-Trøndelag. This concerns non-O157 cases in particular, and to a certain extent also O157 cases. This “skewing” of notified serotypes and groups may be mainly because most medical microbiological laboratories in Norway only had methods for detection of STEC O157:H7, whereas the regional laboratory for Sør-Trøndelag (Trondheim), as previously mentioned, had implemented methods for identifying pathogenicity factors. Another possible reason for the geographical differences in notified STEC infections may be different indications used for

testing human samples for STEC. The national recommendations, as implemented from 1996, recommends testing for STEC in patients with bloody diarrhoea and HUS (45). However, at least one laboratory (the regional laboratory for Sør-Trøndelag in Trondheim) began testing samples from all children <2 years with diarrhoea from 2001.

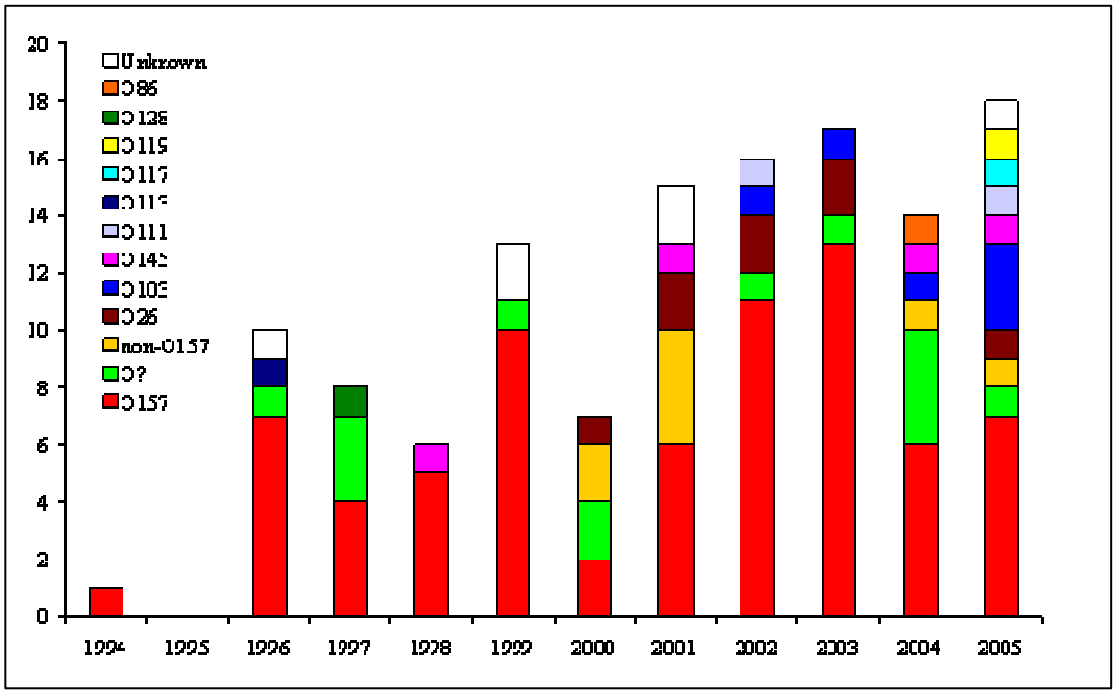

Figure 1. Verified STEC-infections in Norway 1994-2205.

Table 1. Human STEC infections by O-serogroup, county of residence and place of infection (Norway vs. abroad); MSIS 1994-2005.

County	O157	O26	O103	O145	O111	O113	O117	O119	O128	O86	Non-O157	Unknown	Total
Østfold	2												2
Akershus	11											1	12
Oslo	3				1							1	5
Hedmark	3												3
Oppland	7												7
Buskerud	2		1										3
Vestfold	2												2
Aust-Agder	2												2
Vest-Agder	6										1	1	8
Rogaland	7		2				1			1			11
Hordaland	6												6
Sogn og Fjordane											1		1
Møre og Romsdal	1												1
Sør-Trøndelag	9	4	2	2	1	1		1	1		18		39
Nord-Trøndelag	2	3									1		6
Nordland	5												5
Troms	1	1	1	2							1	3	9
Finnmark	3												3
Total	**72**	**8**	**6**	**4**	**2**	**1**	**1**	**1**	**1**	**1**	**22**	**6**	**125**
Infected in Norway	30	4	5	3		1		1		1	12	4	61
Infected abroad	38	4		1	2		1				6	2	54
Unknown	4		1						1		4		10

Diarrhoea-associated haemolytic uraemic syndrome (HUS D+)

Diarrhoea-associated HUS (HUS D+) can be seen at any age group, but is primarily affecting infants and children.. At least 80% of childhood HUS is attributable to infection with STEC (104). Among laboratory-verified cases, serogroup O157 is the most common, although other serogroups have also been implicated (5) and may be under-diagnosed. The peak incidence of HUS is in children <5 years of age. HUS is reported to be the most common cause of acute renal failure in children today (4,27).

As many as 2-10% of cases diagnosed with STEC O157 progress to HUS D+, while the proportion of children who develop HUS D+ after infection with other STEC is unknown and may vary considerably with strain (12,32,51). Reports from many countries indicate that 50 to 80% of sporadic cases of HUS D+ are caused by non-O157 STEC infection (28).

Approximately 85% of children recover from HUS if given supportive care. The case fatality rate, during the acute phase is high (3-5%), and older children and adults have poorer prognoses. STEC-associated HUS D+ is mainly seen in young children and in the elderly with sub-optimal immune responses, however it may occur at any age (101).

During the period 2001 to 2005, nine cases of HUS D+ caused by STEC infection were reported to MSIS. Eight of the nine cases were children 0-9 years of age. Three of the *E. coli* isolates were O157, two were O103, while the rest of the isolates belonged to different serogroups. All isolates were stx_2 positive, and one was also stx_1 positive (Table 2).

The actual incidence of HUS in the Norwegian population and the population “at risk” is unknown, as only cases from whom STEC have been isolated are currently reported to the MSIS register. The aetiological agent is often not found in patients, and we may therefore assume that the incidence is underestimated.

Table 2. Distribution of *stx* by serogroup and clinic in human patients in the period 1994-2005, data from MSIS and Reference laboratory of enteric pathogens, Norwegian Institute of Public Health

	***E. coli* O**	***stx*$_1$**	***stx*$_2$**	***stx*$_{1+2}$**	**Unknown**	**Total**
HUS	O157		3			3
	O26		1			1
	O103		2			2
	O145		1			1
	O111			1		1
	O86		1			1
Total HUS			*8*	*1*		*9*
Other	O157		17	36	16	69
Symptoms	non-O157	7	6	8	1	22
	O26	4	1		2	7
	O103	4				4
	O145	2		1		3
	O111	1				1
	O113			1		1
	O117	1				1
	O119	1				1
	O128		1			1
	Unknown				6	6
Total other		*20*	*25*	*46*	*25*	*116*
Total		**20**	**33**	**47**	**25**	**125**

Norwegian outbreaks

Before 2006, only two small outbreaks of STEC infection were registered in Norway, both caused by *E. coli* O157. The first was a small outbreak in Kristiansand (33), with four laboratory-confirmed cases, and was notified to MSIS as a result of contact tracing. The source of infection was believed to be contaminated kebabs made from Norwegian beef. In another outbreak, in 1999, also with four cases, salad was implicated as the possible source of infection based on epidemiological investigation followed by inspection of the production plant, but no definitive source was identified (44).

The 2006 outbreak

In the outbreak in 2006, 17 persons were diagnosed as infected with *E. coli* O103 (later typed as serotype O103:H25). All isolates were *eae* positive. Only two of the ten patient isolates were *stx* positive, indicating loss of genes encoding *stx*. Ten of the patients, all children, developed HUS and one died. Multilocus variable-number tandem-repeats analysis (MLVA) of the patient isolates showed that all had identical MLVA profiles (49). Identical and closely related profiles (single-locus variants) were also detected in *E. coli* O103 isolates from several lot-numbers of the incriminated dry-cured sausage products. As with the majority of the patient isolates, all sausage isolates were invariably *stx* negative, but *eae*-positive. For more information about the outbreak, refer to www.fhi.no/ecoli or www.ecoliutvalget.no.

Because of the severity of the illness, it is very unlikely that there have been other undetected STEC outbreaks of similar or greater magnitude in Norway.

An increasing incidence?

During the first half of the 1990s only a few cases of STEC infection in humans were notified to MSIS and the notifications tended to occur rather sporadically. This may be due to a relatively low prevalence and incidence of STEC infection, but other factors may also have contributed, including:

- Lack of knowledge among medical practitioners about the illness - and thereby limited testing of patient samples;
- Low sensitivity of the diagnostic methods in use;
- Insufficient routines among medical practitioners or laboratories for notification of cases to MSIS.

For these reasons it is difficult to assess the actual incidence of human STEC infections during this period. Similarly, estimating the magnitude of under-diagnosed and under-reported human STEC infection/disease is problematic.

From the end of the 1990s, the quality of available data has probably improved, due to a variety of reasons including:

- Increased awareness/vigilance among medical practitioners and veterinarians regarding STEC infections,
- More stringent criteria for testing in the medical microbiological laboratories regarding analysis of human, faecal specimens for STEC (45).

- An increase in the use of novel techniques, e.g. PCR for the $stx_{1/2}$ genes, as well as improved techniques for serotyping of bacteria which are non-O157:H7 *E. coli*.

Given that the patterns of under-diagnosis and under-reporting to MSIS have probably been relatively stable, then the annual incidence of STEC cases in humans in Norway over the same time period has probably also been relatively stable. However, the degree of under-reporting, and thereby the true incidence, remains uncertain. This may concern non-O157 cases in particular.

Compared to Sweden, where there has been a known epidemic clone of *E. coli* O157 for many years, the Norwegian incidence of notified cases has been markedly lower during the same time period. The notification systems in the two countries have so many similarities that it can be assumed that the observed difference in incidence of *E. coli* O157 is real. However, as the actual incidence of other STEC infections is very uncertain, comparison of incidence data between the two countries is difficult.

Norway - EPEC infections in humans

Notification of typical EPEC infections to the Norwegian notification system for infectious diseases (MSIS) is also mandatory. Only strains belonging to the typical EPEC serogroups have historically been notified, and indications for diagnosis have been diarrhoea in hospitalised children less than two years. From 1994 to 2005, between ten to 60 cases were notified annually.

However, as with STEC infections, incidence varies between counties, possibly due to diagnostic and reporting differences. As under-reporting of the illness is probable, the true incidence of this infection in Norway is unknown.

However, subsequent to the 2006 *E. coli* outbreak, several requests to the national reference laboratory have been made regarding atypical EPEC, and more O serogroups have been reported (Table 3).

Table 3. EPEC (typical and atypical) and STEC infections notified to MSIS, 2001-2006, by O-serogroup

	2006		2001-2005	
O-group	STEC	EPEC	STEC	EPEC
O157	7		43	
O26	6	2	7	2
O103	26	3	6	
O145	3	5	3	3
O111		2	2	
O117	1		1	
O119	1		1	
O86			1	
O128	1	1		3
O121	1	2		
O104	1			
O146	1			
O2	1			
O55		8		
O127		4		2
O125		2		
non-O157	2		13	
Unknown		8	3	59
Total	51	37	80	69

E. coli in domestic animals and meats

The following section describes the occurrence and transportation of possibly pathogenic varieties of *E. coli* through the meat chain, from live animals at the farm through slaughter, and into the market, either directly to consumers, or in final processing into products such as dry-cured sausages. For each step, possible intervention measures are also discussed.

STEC/EPEC in the domestic animal reservoir

STEC have been isolated from several different domestic and wild animal species worldwide, including cattle, sheep, goats, deer, pigs, horses, cats, dogs, chickens, wild birds, pigeons and rats (10,99). However, the prevalence and distribution of STEC are not well described for all these species, and domestic ruminants have been considered to be the principal reservoirs of STEC with relevance for human infections. Cattle have been the suspected domestic ruminant

source in most human cases, and small ruminants have not been the subjects of as many studies as cattle.

More than 400 STEC serotypes have been isolated from ruminants, but some serotypes are isolated more often than others, and associations between serotypes, and the *stx* variants present with particular animal hosts have been described. From cattle, serotypes O20:H19, O22:H8, O26:H11, O45:H8, O91:H21, O113:H4/H21 and O116:H21 with associated *stx* variants stx_1, stx_2 and/or stx_{2c} are reported as some of the most common serotypes, while stx_{1c}– and/or stx_{2d}– positive *E. coli* of serotypes O5:NM, O91:H14, O128:H2 and O146:H8/H21 are among the most common isolates from sheep, but there is regional variation in the most frequently reported serotypes on a global basis (10,17).

The most commonly isolated STEC serotypes from Norwegian cattle and sheep are O113:H4/H21, O91:H21, O22:H8 (90) and O5:H-, O6:H10, O91:[H14], O128:[H2] and O174:[H8] (90,91), respectively (Table 4). These typical sheep and cattle STEC isolates usually do not carry *eae* and are regarded as less pathogenic to humans. The significance that these sheep and cattle *eae*-negative STEC isolates may have, with respect to less severe human infections, is unknown. Some *eae*-positive STEC isolates have also been associated with diarrhoea in young calves up to four-months old, lambs and goat kids. These isolates are mainly stx_1 positive, but the primary cause of diarrhoea is considered to be *eae*. Also, *eae*-positive, *stx* negative *E. coli* (atypical EPEC) of specific serotypes have been associated with diarrhoea in young calves and lambs (31,37,97,98) and in recent years there has been an increasing awareness of healthy ruminants being a reservoir of EPEC. More serotypes, belonging to both typical and atypical EPEC, are continuously being described.

In pigs, STEC is a well-known cause of oedema disease. The majority of these oedema disease isolates belong to serogroups O138, O139 and O141 (99). Oedema disease STECs are not considered pathogenic to humans and are not further described in this report.

Comparison of prevalence results obtained in different studies is complicated by the use of different detection methods. In general prevalences of STEC reported from studies around the world vary extensively, from <40 to 60% herd prevalences, and from 20 to 100% animal prevalences (reviewed in (10,17)). In general, the occurrence of STEC in ruminants is high (probably mostly of the typical sheep and cattle serotypes), reaching perhaps as high as 100%. The occurrence of STEC has been reported to be higher in sheep than in cattle. This is consistent with results from studies in Norway showing animal prevalences of about 65% in cattle and between 80 and 100% in sheep (46,88-91) (Table 4). The same studies reported a

herd prevalence of about 50% and 100% in sheep, and 100% in cattle. No differences in STEC prevalence between regions were detected in these studies.

Serogroups O26, O103, O111, O145 and O157

Prevalence studies on STEC O157:H7 in ruminants have been performed throughout the world. Occurrence in sheep has generally been considered to be lower than that in cattle, but fewer studies have been conducted in sheep. Reported prevalences vary extensively between countries, partly due to variation in detection methods and study design, but also due to regional and geographical variations. Herd prevalences reported are from less than O.5% to more than 90%, with animal prevalence from less than 0.5% to more than 20% (reviewed in (10,17)). Pigs are not considered to be major source of STEC associated with human cases. However, STEC O157 prevalences of between 0.2 and 2% have been reported. In contrast to these reports, a few countries in South-America and Asia have reported a relatively high frequency of STEC O157 in pigs (99).

Studies performed in Norway from 1995 to 1999 reported cattle herd prevalences of STEC O157 of approximately 0.5-1%, and animal prevalences of approximately 0.2-0.3% (39,92). One study on imported beef cattle found a higher herd prevalence of 7.1%, and an animal prevalence of 4.6% (93). Only one study has focused on detecting herd prevalence of STEC O157 in sheep. The study did not detect any STEC O157 (39). STEC O157 was, however, detected in two out of 1976 (0.1%) pigs from 832 herds (herd prevalence = 0.24%). A follow-up study revealed another STEC O157 positive pig from one of these herds (39). The STEC O157 isolates from these studies all carried stx_2 and *eae*, and some isolates also carried stx_1. The results from these studies are summarised in Table 4.

There are less data on the other well known human pathogenic serotypes, O26:H11, O111:H8, O103:H2 and O145:H21, in the animal reservoir. The limited data available indicate geographical variations for these serotypes similar to those for O157:H7, and to some extent this reflects the occurrence of human cases in the same area.

In a small Norwegian study conducted in 2000, 1.6% of the animals in one flock of sheep were positive for STEC O103 (89) (Table 4). The isolates were not H-typed, but carried stx_1 and *eae*. Two isolates were later retested as *stx* negative and it was assumed that genetic loss had occurred. In addition *stx* negative isolates were detected from 62 of the total 96 samples tested (the isolates were not tested for *eae*). Two studies in cattle have attempted to detect

serogroups O26, O103, O111, and O145. One of these studies reported the detection of *eae*-negative STEC O103 in 3.2% of the herds, and none of the other STEC were detected. However, the studies did detect *stx* negative *E. coli* of different serogroups as follows: O26 from 6.5 and 20%, O145 from 2.6 and 10.9%, and O111 from 1.5% of the herds. Of these, only a few of the O26 and O103 isolates were *eae*-positive (35).

International studies also report *stx* and *eae*-negative *E. coli* isolates of these serogroups (O26, O103, O111, O145 and O157), indicating that these are relatively common in the microbial flora of animals. Also, atypical EPEC isolates of these serogroups from ruminants have been reported (3,11,69,71,98). However, since most studies, both national and international, have focused on detecting and characterizing STEC, the data and knowledge on ruminant EPEC is sparse. Strain variation in pathogenicity factors and mobile genetic elements is an important part of the explanation of the wide spectrum of virulence seen within the STEC and EPEC groups, and is a key aspect to consider in understanding their ecology (71). The relationship and ratio between *stx* and *eae*-negative *E. coli*, *stx* negative and *eae*-positive *E. coli* (atypical EPEC), *stx* positive and *eae*-negative *E. coli* (STEC), and *stx* and *eae*-positive *E. coli* (STEC) of a serotype, is unknown and the risk that this reservoir represents as a source for generating new human pathogenic STEC variants, and for human health, needs further investigation.

Table 4. Results from Norwegian studies documenting the occurrence of various *E. coli* serotypes in domestic animals.

Animal species	*E. coli* serotype	*stx* variant	*eae*	Positives/tested (%)	Method	Comments	Reference
Cattle		stx_1 stx_2		57/197 herds (29) 137/1970 animals (7)	PCR on IMS material	Dairy cattle in 3 southern regions	(92)
Cattle	O157:H7	stx_1 stx_2	*eae*	2/197 herds (1) 6/1970 animals (0.3)	IMS	Dairy cattle in 3 southern regions	(92)
Cattle (imported)	O157:H7	stx_2	*eae*	23/504 animals from 99 farms (4.6)	IMS	Imported beef cattle, 1991-1995	(93)
Cattle	O157:H7	stx_2	*eae*	3/848 herds (0.35) 3/1541 animals (0.19)	IMS	Southwest part of Norway	(39)
Sheep	O157:H7			0/605 flocks 0/665 animals	IMS	Southwest part of Norway	(39)
Pigs	O157:H7	stx_2	*eae*	2/832 herds (0.24) 2/1976 animals (0.1)	IMS	Southwest part of Norway	(39)
Cattle	O157:H7	stx_2	*eae*		AIMS	Fluctuation study, one farm	(100)
Sheep	O157:H7	stx_2	*eae*		AIMS	Fluctuation study, one farm	(100)
Sheep	O103:H?	stx_1	*eae*	2/124 animals (1.6)	AIMS-ELISA	One flock studied	(91)
Cattle	O103:H?	*stx*	neg	5/155 herds (3.2) (STEC O26, O111, O145 not detected)	IMS	Pooled samples from beef cattle	(35)
Sheep		*stx*		61/124 flocks (49)	PCR on faeces with primers covering most *stx* variants	Samples from all over Norway	(88)
Sheep	O5:H-, O6:H10, O91:[H14], O128:[H2]	stx_{1c} stx_{2d}	neg		Hybridization method with *stx*-targeted probes	Isolated (Urdahl *et al.* 2001)	(89)
Sheep		*stx*		7/7 flocks (100) 113/129 animals (87.6)	PCR on faeces with primers covering most *stx* variants	Farms from one valley	(90)
Sheep	O5:H-, O6:H10, O91:[H14], O128:[H2], O174:[H8]	stx_{1c} stx_{2d}	neg		Hybridization method with *stx*-targeted probes		(90)
Cattle		*stx*		4/4 herds (100) 51/79 animals (64.6)	PCR on faeces with primers covering most *stx* variants	Farms from one valley	(90)
Cattle	O113:H4/H21, O91:H21, O22:H8	stx_2 stx_1 stx_{2d}	neg		Hybridization method with *stx*-targeted probes	Mainly stx_2	(90)
Cattle		*stx*		50/50 herds (100) 415/680 animals (61)	PCR on faeces with primers covering most *stx* variants	Dairy cattle around Oslo	(46)
Cattle	O157:H7			0/50 herds	AIMS	Dairy cattle around Oslo	(46)

Possible pre-harvest (farm-level) interventions

Intervention strategies at farm-level are difficult to establish and need to be based on fundamental epidemiological knowledge of the occurrence and on-farm ecology of the bacteria.

On-farm ecology

Even though some serotypes of STEC and EPEC have been associated with diarrhoea in young animals, and diarrhoea caused by STEC has been shown experimentally in newborn ruminants, STEC O157 is not regarded as a common cause of diarrhoea in animals, and ruminants are regarded as asymptomatic shedders of STEC and EPEC. However, young animals, between 2 and 4 months, and up to two years of age, tend to shed more STEC and EPEC of all serotypes, including the human case associated O26:H11, O111:H8, O103:H2, O145:H21 and O157:H7, than younger and older individuals. About two months shedding of STEC O157 is regarded as typical in ruminants. In recent years animals described as “high shedders” or “super shedders” of STEC O157 have attracted attention, with “super shedders” being defined as animals that shed more than 10^4 CFU/g faeces (up to 10^{6-7} CFU/g faeces). Whether some animals may remain as “super shedders” for a prolonged time period is a theory under current discussion. Such animals would constitute a higher risk of transferring bacteria to other animals, to the environment and to carcass during slaughter (52,53,67,68,73). There is also seasonal variation in shedding patterns of STEC and EPEC in ruminants, with a peak during summer months and in early autumn.

Various risk factors for occurrence of STEC in ruminants have been discussed but due to considerable differences in management practices around the world it is difficult to draw conclusions. Norwegian data indicate that loose-housing dairy barns and high animal density may be risk factors for the occurrence of STEC in ruminants (94). High animal density increases faecal-oral contact and thereby may increase the rate of transmission between animals and may prolong the farm infection period.

Farm-level interventions

Since STEC of certain serotypes are widespread in the ruminant reservoir, and are probably established as part of the normal intestinal flora in these animals, complete elimination of STEC is impossible. However, any reduction of STEC in the ruminant reservoir will reduce

the level of contamination of the human food chain, and consequently reduce the potential number of human infections.

To date no specific management strategies have been demonstrated to be successful at decreasing the occurrence of STEC or EPEC in the ruminant reservoir. However, as transmission of *E. coli* between animals occurs through the faecal-oral route, interruption of this route is one possible approach. It is difficult to assess the significance of transmission between ruminants through grooming and social activities, but management practices facilitating a high degree of faecal-oral contact might nevertheless be considered as possible critical points for interventions. STEC O157:H7 can survive in farm environment for months, depending on temperature, water activity etc. Good general hygiene practices are therefore important management interventions for decreasing on-farm transmission.

Contaminated food and water may be important routes, both for introducing new STEC and EPEC strains to flocks/herds, and for transmission between animals within a flock/herd. Therefore, hygienic principles should be applied to ruminant feed and water, and strategies implemented to prevent or minimise faecal contamination of feed and water troughs.

There are many reports in the literature on the influence of different feeding regimes and dietary factors on the survival and shedding of *E. coli* in general, and STEC O157 in particular (17), but conclusions from these reports are inconclusive or even conflicting. It has also been suggested that withholding feed before slaughter, in order to reduce gut fill, could reduce carcass contamination, but as withholding feed modifies gastrointestinal flora, the result may actually be an increase in shedding. Other possible interventions to influence survival and shedding which have been discussed in the literature include the use of probiotics, antigen-specific bacteriophages, and vaccination (16,47)). Further studies are needed on the use of these methods to control STEC and EPEC at farm-level.

STEC in the abattoir and in meats

Since STEC (and/or EPEC) occur among the normal microbial flora of the gastrointestinal tract of animals, STEC can be transferred to carcass meat during dressing, and have been found on carcasses, and in processed meat such as minced or ground beef. STEC can survive freezing, and frozen products such as beef burgers may represent a hazard to the consumer if inadequately cooked. As previously described, meat from ruminants is an important source of STEC infection for humans, while meat from pigs is regarded as less important, and poultry meat as probably not at all (29). Food handlers may contaminate meat and meat products during processing.

From 1998 until summer 2004, there was a national programme for detecting STEC O157 in cattle, sheep, and goat carcasses. This programme detected a carcass prevalence of 0.06% for cattle and 0.03% for sheep. None of the 510 goat carcasses tested were positive (35). This programme demonstrated that it is not that uncommon to find specific *E. coli* on randomly selected carcasses, a reflection of the transportation of pathogens from the intestines of slaughter animals to carcasses.

There are several points in the food chain, from farm to table, at which control measures can be implemented to prevent or minimise the spread of pathogens from mammalian slaughter animals, via meat and meat products, to man (Table 4). It is possible to reduce or limit the spread if strict hygienic procedures are adhered to during dressing. During the operations following dressing, (i.e. chilling, cutting and deboning), further spread of STEC may occur (13). However, during processing, it may be possible to prevent growth of STEC by protective cultures, as shown for strains of *E. coli* O157 (14), although there may be variations between *E. coli* serotypes and strains. Packaging under a modified atmosphere might also limit the growth (61). Finally, it should be emphasized that the treatment of beef carcasses with hot water, steam, or organic acids at the end of the slaughter lines, as used in the USA, has been shown to be an efficient tool for significantly reducing contamination with STEC (78,96).

Transportation, slaughter, and dressing and slaughter hygiene

The traditional slaughter lines for pigs and ruminants are open processes, with many opportunities for contamination of carcasses with STEC. Proper management of slaughter lines uses HACCP and Good Hygienic Practice (GHP) systems, focused on limiting the spread. A proper hazard analysis is the basis for the identification of Critical Control Points

(CCPs) on a processing line, for the specification of critical limits to be used when monitoring the process, for corrective actions when the process is not properly controlled, and finally for verification of the effectiveness of the HACCP plan. With the exception of decontamination, for the CCPs identified for slaughtering practices, only partial control can be achieved and the ability to eliminate risk is limited (83) (Table 5). However, the slaughter process of pigs includes some process steps where the number of STEC may be reduced, such as scalding and singeing/flaming.

Table 5. Hygienic aspects and preventive actions with respect to bacterial hazards during slaughter and dressing procedures. Based on published articles (7,13)

Process step	**Hygienic aspect**	**Preventive actions**
Transportation	Due to increased animal stress, higher numbers of STEC might be shed in faeces during transport. Contamination from other animals and herds.	Cleaning & disinfection of vehicles.
Lairage	Cross contamination between animals.	Cleaning & disinfection.
Clipping of sheep and lambs	Contamination of animals with faeces during transport.	Clipping of sheep and lambs in the lairage before slaughtering
Stunning	Contamination from tools.	Cleaning & decontamination of tools.
Bleeding (killing)	Contamination from tools.	Cleaning & decontamination of tools.
Rodding of ruminants	Contamination of the carcass via the oesophagus.	Sealing of the oesophagus.
Scalding (only pigs)	Reduction of bacterial levels.	Time/temperature.
Dehairing (only pigs)	Contamination from machines.	Cleaning & disinfection.
Singeing/flaming (only pigs)	Reduction of bacterial levels.	Time/temperature.
Polishing (only pigs)	Contamination from machines.	Cleaning & disinfection.
Skinning/dehiding (only ruminants)	Contamination between animals and from the animals themselves.	Cleaning & decontamination of tools(two-knife method). Skilled personnel.
Evisceration	Contamination from intestines, tongue, pharynx, tonsils and tools.	Enclosure of rectum. Cleaning & decontamination of tools (two-knife method). Skilled personnel.
Carcass splitting (not lambs/sheep)	Contamination via splitter saw.	Line-speed; water temperature.
Post-mortem inspection	Cross-contamination.	Cleaning & decontamination of tools (two-knife method).
Final trimming	Cross-contamination.	Cleaning & decontamination of tools (two-knife method).
Grading	Cross-contamination.	Cleaning & decontamination of tools (two-knife method).
Decontamination with hot water, steam or organic acids (USA)	Significant reduction of bacteria.	Time/temperature (water/steam); Concentration (organic acids) etc.
Chilling	Reduced growth of bacteria.	Time/temperature.
Cutting and deboning	Possible growth of bacteria. Cross-contamination.	Time/temperature.

Transportation to slaughter

Stress effects on animals may affect shedding and spread of STEC. Stress can predispose latently-infected animals to shed high numbers of STEC by increasing peristaltic activity. Contamination of the environment (trucks, equipment etc.) and use of the same transport and other equipment by different herds favours spread of STEC among slaughter animals, and subsequently in the lairage and on the slaughter line. It is legally permissible to keep animals in the lairage for up to 72 hours, but ideally all animals should be slaughtered on the day of arrival to reduce the risk of spreading STEC. During the transport of sheep and lambs the wool is often contaminated with faeces.

Handling of unclean animals for slaughter

It is the farmers' responsibility to take adequate measures to ensure the cleanliness of animals intended for slaughter.

Skinning/dehiding

Unclean animals have implications for the skinning process. Adjustments may be made, depending on how dirty the lot is. Adjustments may include: rejection of dirty lots, washing of animals, hide trimming or clipping, and slaughter of dirty animals at the end of the day. Other adjustments may be slowing the slaughter line down and/or adding extra people at certain stations, and compensation for extra time or yield loss.

Removal of hides should be carried out in a manner that avoids contact between the outside of the skin and the carcass. In order to avoid transferring STEC, hands and equipment that touch the outside of the skin should not come in contact with carcass meat.

At positions such as skinning/dehiding and evisceration in particular, it is important that the operators are skilled and experienced. During seasonal slaughtering, such as lamb slaughtering, staffing might be problematic due to a lack of skilled personnel and therefore relevant and adequate training plans and training programmes for operators in the abattoir are essential.

Evisceration

During evisceration there is a particular risk that STEC may be spread to the carcass meat from the intestines, stomach content, oral cavity and oesophagus. The critical operations are circumcising of rectum, and removal of the intestinal tract and the pluck set. Improved slaughtering methods, including enclosure of the anus into a plastic bag after rectum-

loosening, are important in this context (58). The importance of this procedure has been shown during dressing of lamb, as illustrated in Figure 2 (Nesbakken et al., in prep.). The oesophagus should be sealed so that the ruminal contents do not leak from the oesophagus at any stage. A technique termed ”rodding” may be used to free the oesophagus from the trachea and diaphragm and includes closure of the oesophagus by a rubber ring or plastic clip close to the diaphragm.

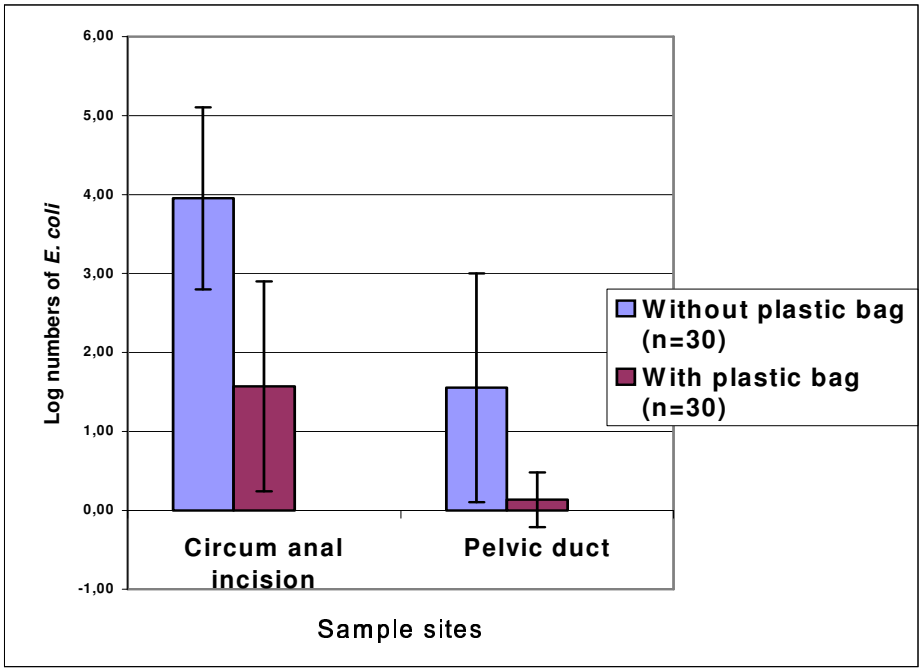

Figure 2. The average numbers and standard deviations of *E.coli* per 100 cm^2 sample sites on lamb carcasses (Nesbakken et al., in prep).

The significance of proper evisceration and possible recontamination were demonstrated in a study from UK, in which *E. coli* O157 were isolated from 7 (30%) of 23 carcasses of rectal-swab positive cattle and from 2 (8%) of 25 carcasses of rectal swab-negative cattle (20).

Table 6. Information about the slaughter lines in three abattoirs (results presented in Figure 3) (Nesbakken et al., in prep.)

Abattoir, type of slaughter line	**Rodding**	**Position of carcass during preparation before mechanical dehiding**	**Position of carcass during mechanical dehiding**	**Position of carcass during circum-anal incision and removal of rectum. Use of plastic bag during this procedure?**	**Slaughter rate per hour**
A - Hamjern ("new")	After bleeding	Hanging by three or all four legs.	Hanging by all four legs.	Hanging by hind legs. No bagging	300-330
B – Nordøy** (Principles used also in New Zealand)	No rodding	Hanging by three or all four legs.	Hanging by forelegs	Hanging by all four legs. No bagging*	270
C – Traditional bench slaughter with some "modern" adjustments	In connection with mechanical dehiding	Lying on bench	Hanging by forelegs	Hanging by hind legs. Bagging	170

* Rectum is cut and a few centimetre of rectum is left in the pelvic duct. Later on the circum anal incision is performed and the rest of rectum is removed

** The slaughter line most often used in Norway

In a study performed in three Norwegian abattoirs (Table 6), slaughter hygiene was evaluated. The numbers of *E. coli* from four different sampling sites on lamb carcasses from the three different abattoirs are presented in Figure 3 (Nesbakken et al., in prep.). The slaughter line in abattoir B represents the prototype most often used in Norwegian abattoirs in 2006/2007. The sampling sites in Figure 3 represent the following procedures:

- Circum-anal incision and pelvic duct: Removal of rectum,
- Chest outside: Removal of hide,
- Neck: Rodding.

Although the numbers of carcasses were limited, based on relevant 100 cm^2 sampling sites (circum-anal incision and pelvic duct) it could be concluded that the use of the plastic bag technique during circum-anal incision and removal of rectum results in a 1 – 2 log reduction of *E.coli* (Figures 2 and 3). The effect of rodding is not possible to interpret from the results, but as the fluid from rumen also contains *E. coli,* rodding at an early stage of the slaughter line is clearly important.

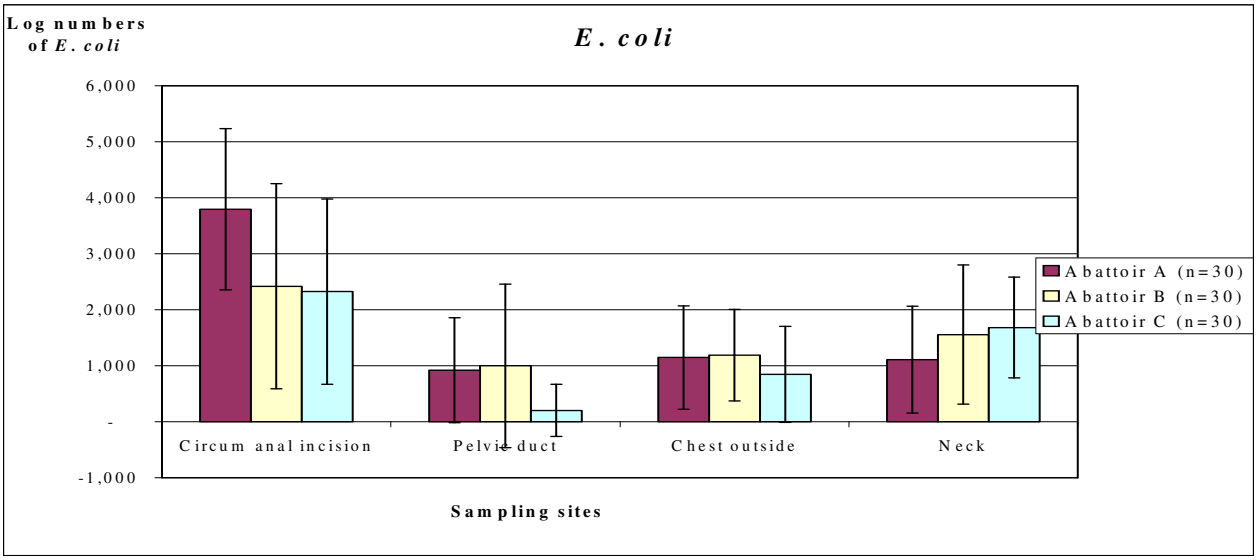

Figure 3. The average numbers and standard deviations of *E.coli* per 100 cm^2 sample sites on lamb carcasses at three different abattoirs in 1999 (Nesbakken et al., in prep).

Additional comments; Slaughtering of pigs

To reduce the likelihood of carcass contamination with STEC and other intestinal bacteria, it is essential, in the case of pigs, to withdraw their feed for up to 12 hours before slaughter in order to empty the stomach (7).

Scalding of pigs should be carried out at a water temperature of at least 60°C. Singeing or flaming effectively reduces STEC at the carcass surface. Cleaning and disinfection of polishing equipment, including the lashes, preferably by a cleaning-in-place (CIP) system, is particularly important. If this is not done properly, STEC might grow overnight on the polishing equipment and may spread to the carcasses processed during the next working day.

Splitting the carcass

Contact between the splitter or saw and the rectal incision is possible, and may result in spreading of STEC during the splitting procedure. Therefore the splitting machinery should always be disinfected, following splitting of the carcass, and before re-use (13).

Meat inspection, final trimming and grading

During post-mortem meat inspection, final trimming, and grading palpations and incisions may result in cross contamination with STEC.

Decontamination

Whilst various techniques for reducing carcass contamination are used in North America, these are presently not accepted in the EU. However, the use of some of these techniques might have a positive effect in reducing the incidence of human food-borne illness in Europe also. According to EU Regulation (EC) No 852/2004 (25), article 3, point 2 and annex II, chapter VII, point 3 and 5, only the use of potable water, water or steam are allowed in this context. If HACCP and GHP have been established, and function in an effective way, decontamination of carcasses can be useful in reducing accidental or unnoticed contamination, especially with matter of faecal origin that may contain pathogens (78). The decontamination methods at the end of the beef slaughter lines, such as automatic steam, hot water (>70°C), or organic acid treatment of whole carcasses in chambers, as used in the USA, seem to significantly reduce the numbers of *E. coli* on beef carcasses (78). A reduction of bacterial counts by 1 – 3 logs, depending on the initial bacterial counts and the decontamination process chosen, has been reported (78). Today, such approaches are probably the most efficient tools against STEC in the meat chain, and in the USA, these measures, together with other interventions, have resulted in a decrease in the frequency of *E. coli* O157:H7 in ground beef (38,87) and consequently a significant decline in the number of human cases with this infection (18,38). In conclusion, the decline in the incidence of STEC O157 infections observed in recent years suggest that coordinated efforts by regulators and industry, have been effective in reducing contamination and illness related to ground beef (57).

The steam-vacuum method used in Norwegian abattoirs during the lamb-slaughtering season (autumn 2006), is a procedure by which visible contamination on carcasses is removed by hand-operated equipment using steam and vacuum. Use of steam-vacuum has been tested during slaughtering of beef and lamb (80), where comparison of numbers of aerobic microorganisms on surfaces not steam-vacuumed and surfaces steam-vacuumed, demonstrated that steam-vacuum processes were associated with decreases in aerobic microrganisms of 1.11–1.49 logs on sheep carcasses, and 1.32–1.76 logs on beef carcasses. In a study on pig carcasses (82), the numbers of aerobic microorganisms were decreased by 0.75–1.15 logs on steam-vacuumed surfaces.

A study at the Norwegian School of Veterinary Science (Hassan et al., in prep.) investigated the use of the steam-vacuum process and its effect on the levels of *E. coli* on sheep carcasses. Out of 39 carcasses on which *E. coli* was detected before steam-vacuum, a reduction in numbers was observed in 37, no effect in one, and an increase in one carcass. The overall effect detected is described in Figure 4, with deletion of the one extreme data point.

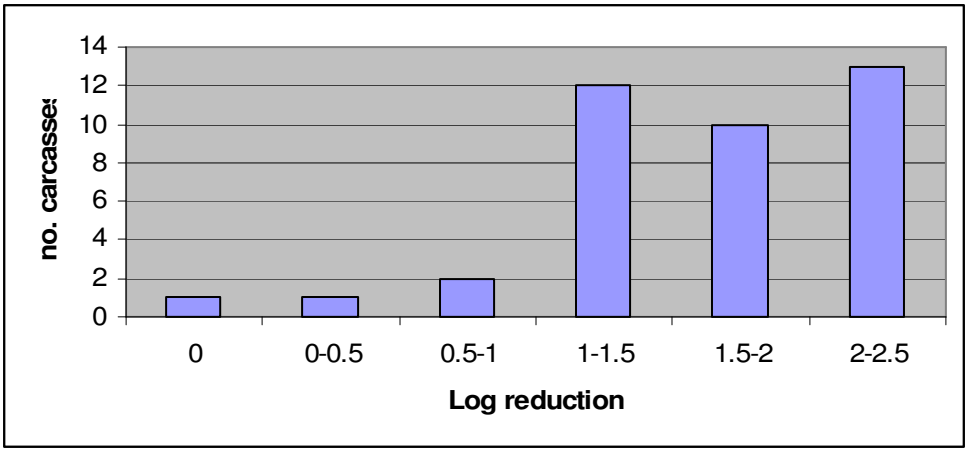

Figure 4. Effect on steam-vacuum on the level of *E. coli* on sheep carcasses, expressed as log reduction (Hassan et al., in prep.).

Vosough et al. (96) predicted that the occurrence of STEC-contaminated quarters of dairy beef can be decreased from approximately one-third to one-sixth by implementing any one of six decontamination methods. Reduction of STEC population from the surface of beef quarters and corresponding elimination probabilities of all CFU counts (%) from carcass quarters are shown in Table 7.

Table 7. Reduction of STEC population from the surface of beef quarters and corresponding elimination probabilities of all CFU counts from carcass quarters (96)

Intervention	Reduction (log CFU/cm^2)		Reference	Estimated elimination probability, Pd (%)
	Mean	S.E.		
Hot-water wash[b]	0.75	0.49	(70)	34.69
Lactic acid	2.70	0.49	(70)	68.75
Steam-vacuum	3.11	0.49	(70)	76.01
Trimming	3.10	0.49	(70)	75.83
Hide-wash with ethanol	5.00	0.20	(54)	83.33
Steam-pasteurisation	3.53	0.49	(70)	83.17
Irradiation	6.00	0.49[a]	(55)	99.48

[a] Assuming the same standard error as the other interventions;

[b] In this case the temperature was 35°C, but in the USA the temperature is usually >70°C

Based on this report, taking into account the EU regulations (25), and with considerations of the practical Norwegian context, use of hot water (>70°C), steam-pasteurisation and steam-vacuum are probably the most appropriate interventions

Simulating the effect of improved slaughter hygiene and decontamination

The following comments focus on the quantitative aspects of the risk assessment, where simulated numbers are given for expected effects of established processes in the abattoir. The work by Nesbakken et al. (in prep.) and preliminary results from Hassan et al. (in prep.) suggest that implementation of optimal slaughter hygiene, combined with the use of the steam-vacuum method, can be expected to have a significant effect on the levels of *E. coli* on meat surfaces.

A preliminary stochastic simulation model was developed, based upon the available information. Table 8 presents the input variables in the model. The model was run using

standard Monte Carlo simulation techniques in the Excel Add-In @RISK, as described by Vose (95).

Table 8. Input variables in the stochastic simulation model, showing the assumed effect of intervention during slaughter in the reduction of the number of *E. coli* on carcasses

Variable	Lower limit	Expected value	Upper limit	Function in model using log units
Improved slaughter hygiene, reduction of *E. coli*	90%	95%	99%	RiskPert (-2,-1.3, -1)
Decontamination, reduction of *E. coli*	50%	99%	99.6%	RiskPert (-2.4, -2, -0.3)
High contamination: *E. coli*/ 100 cm^2	1000	3000	5000	RiskPert (3, 3.5, 3.7)
Medium contamination: *E. coli*/ 100 cm^2	100	300	1000	RiskPert (2, 2.5,3)

The model was run in 10000 iterations using Latin Hypercube sampling. Based upon this model, an expected effect of 3.15 (90% interval 2.4-3.8 log) for a combination of slaughter hygiene improvement and decontamination with vacuum-steam was indicated, while the effect of decontamination alone was 1.78 (1.1-2.3) log units. Figure 5 shows the results from this simulation.

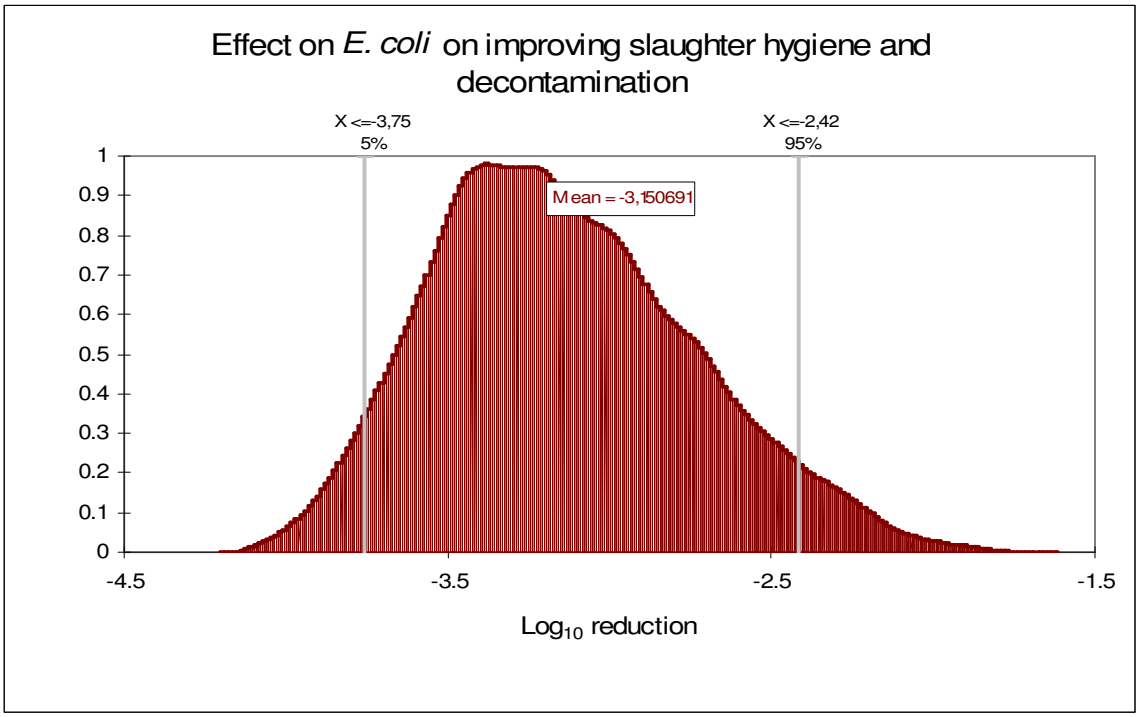

Figure 5.

By combining the results on expected counts on carcasses as reported by Nesbakken, a simulation was established using two different scenarios (Table 8):

- A high contamination scenario with expected value 3000 *E. coli*/ 100 cm^2, (range 1000-5000/ 100 cm^2)
- A moderate contamination scenario with expected value 300 *E. coli*/ 100 cm^2 (range 100-1000 / 100 cm^2)

The simulations based upon these scenarios showed that with the higher contamination, a level of <10 *E. coli*/ 100 cm^2 can be obtained; at the moderate contamination a much lower level can be achieved (**Figure 6**).

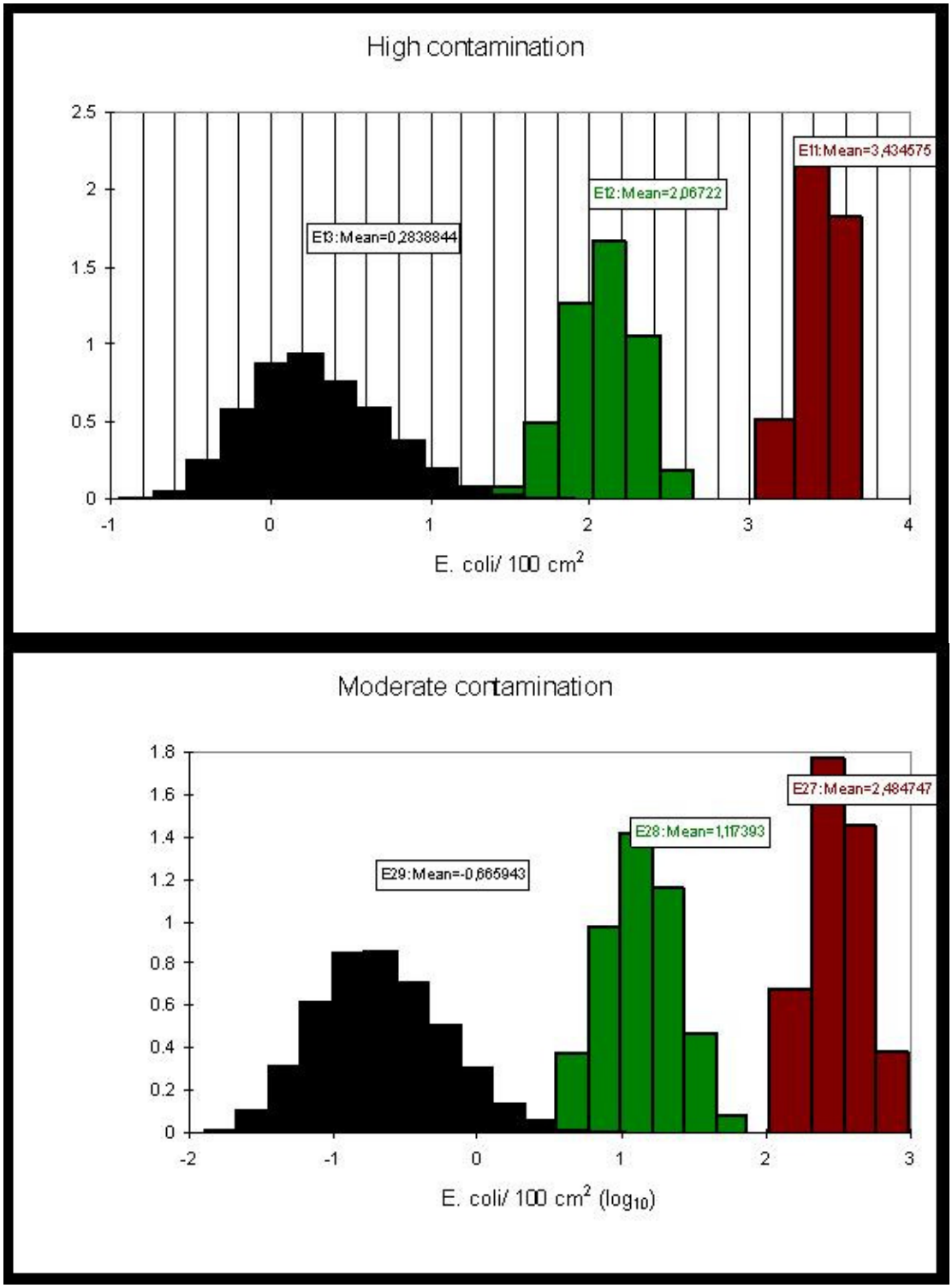

Figure 6. Results from a Monte Carlo simulation model showing the predicted levels of *E. coli*/ 100 cm^2 on a highly contaminated carcass (upper) and a moderately contaminated surface. Red columns indicate levels without intervention, green with implementation of proper slaughter hygiene, and black with a combination of slaughter hygiene and decontamination.

Provided that these procedures are properly implemented, a significant reduction in the transportation, of not only generic *E. coli,* but also potentially pathogenic STEC/ EPEC, into the market in general and specifically into the production of dry-cured sausages, should be expected.

It is not possible to relate the level of *E. coli* directly with the probability of extensive growth of *E. coli* in dry-cured sausages. Also, as indicated by studies at The Norwegian Food Research Institute, and commented upon in elsewhere in this report, it is understood that, assuming growth, the starting level of *E. coli* is not decisive. However, it must be assumed that the probability of extensive growth of *E. coli* in dry-cured sausages will also be significantly reduced if the full hygienic effect of proper slaughter hygiene and decontamination is utilised. With the described intervention at slaughter level, the effect will also be relayed into all segments of the market, and not only that for dry-cured sausages.

During autumn 2006, decontamination procedures were used extensively during sheep slaughter, but no extra measures were used during cattle slaughter. Cattle are generally slaughtered in a more hygienic way than sheep, and the plastic bag technique in particular is more commonly used. This is not because slaughter of cattle is technically easier than that of sheep, but because it is less dominated by seasonal slaughter and inexperienced staff. Nevertheless, if special measures are implemented for sheep, consideration should also be given to using the same approach for slaughtering of cattle and pigs. Inclusion of a decontamination step at the end of the slaughter-line for all three major meat animals might result in a significant reduction in the number of faecal bacteria reaching consumers through contamination of meat and meat products.

Chilling

Subsequent chilling of meat products is required to prevent multiplication of STEC. For effective chilling, carcasses must be appropriately spaced to allow adequate circulation of cold air. In this context, the shortest time and lowest temperature possible, which does not interfere with the aging of the meat, is important.

Deboning and cutting

Growth or spread of STEC may occur during deboning/cutting from handling, and the environment (conveyer belts, cutting tables, tools etc.). Therefore, cold temperatures are recommended in this department are recommended (meat temperatures of below 7ºC and air

temperatures of below 12ºC) and the duration of stay should be prolonged no longer than is necessary.

Production of dry-cured sausages

Dry-cured sausages are products in which chopped or ground meat are mixed with salt and curing agents, and subjected to a fermentation process, followed by a period of ripening/drying. Cured sausages are stable products and may be stored for months or years. Table 9 shows the processes involved in the production of dry-cured sausages in greater detail.

The production of dry-cured sausages traditionally starts by mixing ingredients, and often adding some kind of starter culture, typically consisting of lactobacilli and sometimes micrococci to speed up fermentation and obtain the desired lowering of pH (range 4.6-5.2). Sausages are hung in a climate chamber, with temperature and humidity regulated in order to support the desired growth rate of the starter culture; a temperature ranging between 20-27°C and a relative humidity (RH) of 90-94% for 2 days are typical conditions. During this phase the sausages may be cold smoked one or more times. After the required pH drop is achieved, the temperature and humidity in the climate chamber are gradually reduced to achieve flavour and colour development, and drying of the product. Alternatively, the sausages are moved to a drying chamber with temperatures normally in the range 14-16°C and humidity about 85% RH. This maturation phase typically lasts for between 14 and 28 days. During maturation, the sausages may be cold smoked one or more times.

Dry-cured sausages are found in a wide range of varieties throughout the world. The industrial production of dry-cured sausages in Norway is similar to the industrial production in other countries using standard technological processes, including starter culture. A large number of small-scale producers are also in the market, using a wide variety of techniques and processes. In the following section, standard industrial processing is considered, followed by some comments specific to small-scale production.

The annual production of dry-cured sausages in Norway is approximately 5000 metric tonnes, and about 20% is from small-scale production.

Table 9. Description of the typical industrial production process for dry-cured sausages, with added comments of relevance for possible growth/ survival of STEC

Process step	**Time/ Temperature**	**Possible intervention**	**Comments related to STEC**
Delivery of meat and lard to sausage producing plant	Fresh: 0-96hrs/ 2-4^{o}C Frozen: N.A./ -18^{0}C	Temperature control. Inspection of vehicle and driver. Establish limits for *E. coli* in meat and lard	Under normal circumstances STEC/EHEC will not be able to grow
Frozen storage	N.A./-18	None	Stable
Thawing	72-96 hrs/ between -2 and -7^{o}C Surface temperature not defined.	Temperature/ time 4 - 7°C	At 4-7°C in raw meat, growth will be slow (lag time ~ 100 hrs, G-time >10 hrs)
Mincing and addition of lard, spices, starter culture, sugar.	8 hrs/ between -4 and -6°C	Wash/disinfection between batches	Heat or radiation treatment of spices
Stuffing	1 hr/ -2°C	Wash/disinfection between batches	No growth of STEC
Temperating	6-12 hrs/ -2 ->16-25°C	Time/ Temperature	Time/ Temperature may support growth of STEC
Fermenting	72-96 hrs/ 16-25°C	A rapid lowering of pH	STEC will grow at temperatures used, especially if pH development is delayed
Maturation and drying	1-4 weeks/12*-14°C	Avoid case hardening	Drying may result in a dry outer edge inhibiting drying process, giving an increased a_w
Slicing	8 hrs/14-18°C	Wash and disinfection between batches	Recontamination may occur.
Storing	Months/4°C or room temperature	Store at room temperature	STEC more stable at 4 °C than at room temperature.

*Maturation and drying at 12-14°C are recommended and used by most industrial producers in Norway. STEC is less stable at temperatures >4°C than <4°C.

Some relevant characteristics of the process

Figure 7 describes how pH, a_w and lactic acid bacteria develop throughout dry cured sausage production. Two scenarios are shown, with storage in vacuum for 5.5 months at 4 and 20ºC, respectively. Starter culture is added at the normal quantity of approximately 10^6 CFU/g. In the first 9 days, pH falls approximately one unit, in 21 days water activity reaches 0.9, and growth of lactic acid bacteria peaks at around 10^8-$5x10^8$ CFU/g.

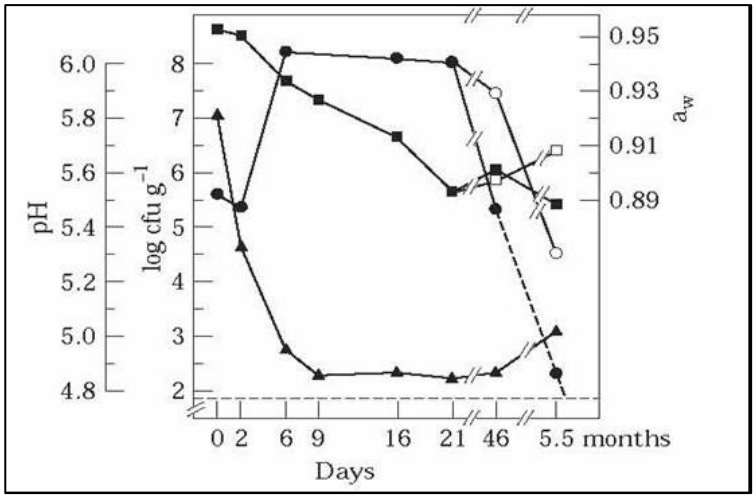

Figure 7. Development of pH, water activity and microbial flora in processing of dry-cured sausages ▲= pH ; ■=$a_w$stored at 20°C; □=a_w stored at 4°C; ●= log CFU/g at 20°C; ○=log CFU at 4°C (62).

The production steps and possible influence on STEC

In the following section, key elements of the process steps are discussed briefly, with focus on factors linked to contamination, survival and growth of *E. coli* O157:H7. It is assumed that other STEC also may have similar characteristics, but large serotype and strain variations may occur.

Raw materials; meat

The meat ingredients are typically frozen at the onset of production. Some producers use fresh meats at cold room temperatures together with frozen meat in 10–20 kg blocks. The fresh meat at + 4 °C is used to bring the mincing temperature of the meat fraction up to (-4°C) – (-6°C). Dependent on the temperatures of the defrosted / frozen blocks the proportion of fresh meat normally corresponds to 0-30%. The lard is always frozen.

Thawing

Freezing and thawing can kill, inactivate or damage *E. coli*. In a study performed by Doyle et al. (23), numbers of non-pathogenic *E. coli* were reduced 10-fold at -25.5ºC over 38 weeks,

but little or no change in population numbers was noted for *E. coli* O157:H7 in ground beef at -20ºC over a 9 month period. Thawing of frozen blocks of meat prior to production may be conducted at temperatures that support the onset of growth of STEC, or provide conditions for a lag phase.

Mincing and addition of other ingredients

Lard is frozen when added to the meat mixture, and the energy provided by the mechanical treatment results in the final batter temperature being between -4 and -6°C.

Spices are important potential sources of STEC contamination in the process of dry-cured sausages. Industrial dry-cured sausage producers have defined limits and demands on the bacteriological quality of the spices/herbs used in production. However the limits set for bacterial quality (including absence of *Salmonella*) do not represent any guarantee of absence of STEC.

Different treatments of spice/herb ingredient may give diverse results regarding bacterial survival of STEC from these ingredients.

- **Heat treatment** typically results in a 4-6 log reduction in the number of vegetative bacteria present.
- **Irradiation** (gamma-irradiation) is considered the most reliable and efficient method. However, as the use of irradiated spices must be declared, most industries have stopped using irradiated spices and introduced heat treatment instead. However, some of the larger producers have recently returned to the use of irradiated spices.
- **Gas treatment (ethylene oxide)** is not allowed in Europe.

For further information about spices, refer to the risk assessment currently in progress by the Panel on Biological Hazards, Norwegian Scientific Committee for Food Safety.

Other additional ingredients include: nitrite-salt, dextrose (or other refined sugars), ascorbic acid and starter culture. Provided appropriate handling is maintained, it is improbable that STEC contamination will occur from these ingredients. .

Fermentation

It has been documented that *E. coli* O157:H7 may multiply during the fermentation step (26,62). Some strains of STEC are more acid tolerant than others, which may be an important consideration in assessing the risk for STEC being relayed through to consumers. Ideally, the

starter culture should rapidly lower the pH of the batter to below 5.2. Any delay in this process may increase the risk of STEC growth.

Maturation, drying and storing

During maturation at 12-14°C the number of STEC reduces with time (43,60,62), at a rate which is significantly affected by both pH and temperature (72). In general, STEC reduction is faster at lower pH and higher temperatures. From a meta-analysis of the results of 12 individual studies Ross and Shadbolt (74) estimated that drying and storing at room temperature (20°C) of dry-fermented sausages reduces the number of *E. coli* O157:H7 by approximately 1 – 2 log per month. This has been supported by results published by Nissen and Holck (62).

Small-scale production of dry-cured sausages

Small-scale and organic small-scale production of dry-cured sausages follows a variety of different processes that may be poorly controlled. The producers may use meat from pigs, sheep, goats, horses, deer, reindeer and moose, either alone or in mixtures, while fat, when used, is usually pork lard as in industrial production. Whilst most producers use starter cultures, some producers rely on fermentation from endogenous lactic acid bacteria or use back-slopping (addition of previously fermented products). Fermentation may take place in kitchens at 18-25°C over periods from 2 to 7 days, while drying and maturation often take place in unheated buildings where the outdoor climate may strongly influence the process. When spontaneous fermentation is used, fermentation at refrigerated temperatures for up to 6 weeks may be employed. The spices used may be local variants of herbs, or industrial, heat-treated spices.

No data are available that can be used to evaluate the risk of STEC occurrence, growth or reduction from such products.

Possible interventions in the production of dry-cured sausages

In the current Norwegian production and processing of dry-cured sausages, there is no individual step which could be described as a CCP. However, some factors will contribute to either lowering the probability of growth/ survival, or of causing a die-off of STEC over time.

Raw materials

All production processes should use raw materials of the best possible microbiological quality, including spices that have undergone appropriate treatment. If decontamination is used, the level of *E. coli* / STEC will be minimal. This may be difficult for small-scale producers where meat may be of different origins.

Starter culture and fermentation temperature

It is possible to limit the probability of growth of *E. coli* by using an efficient starter culture to obtain a rapid lowering of pH. While this step may be used in an industrial setting with controlled production, this may not be feasible for small-scale producers.

Maturation and drying

Drying may result in a dry outer edge inhibiting the drying process, resulting in an increased water activity in the inner part of the sausage. Maturation and drying conditions of 12-14°C and < 85% RH are used (48) by most industrial producers in Norway.

Storage

It has been documented (62) that a one-month storage at room temperature of a produced dry-cured sausage may cause a 1 log reduction of the *E. coli*. This may be the simplest intervention for small-scale production, where other production factors are not fully controlled. STEC is less stable at temperatures >4°C than <4°C

Final heat-treatment

STEC, like other gram-negative bacteria, are readily destroyed by heat. However, heat resistance is strongly determined by strain, physiological state, and the matrix in which the bacteria are found. A low pH reduction may increase sensitivity to heat, while a reduction of water activity (a_w), or a high fat content, can increase heat resistance. Precondition of organisms, such as previous exposure to stress conditions, may also affect heat resistance.

According to Stringer et al. (2000) (81), heat treatment at 70ºC for 2 min results in a 6 log reduction of STEC O157:H7. However, this treatment will alter the product taste, as the border between fat and meat becomes blurred due to melted fat.

Health Canada, http://www.hcsc.gc.ca/fnan/legislation/guideline_fermented_sausages-direc) investigated which physical treatments would be necessary to obtain a 5 log reduction of O157:H7 (Table 10). As demonstrated with the last two treatments proposed, shorter heating times are possible at lower pH.

Table 10. Processes validated as achieving a 5 log or greater reduction of *E. coli* O157:H7 (Health Canada, 2006, http://www.hcsc.gc.ca/fnan/legislation/guideline_fermented_sausages-direc) in fermented dry-sausages.

Fermentation chamber temperature in °C	**pH at the end of fermentation process**	**Casing diameter**	**Subsequent process (dry, hold or heat)**
21	5.0	55 mm	Heat 1 hr at 43°C and 6 hrs at 52°C
32	4.6	55 mm	Hold at 32°C for 6 days
32	4.6	55 mm	Heat 1 hr at 43°C and 6 hrs at 52°C
32	4.6	56-105 mm	Heat 1 hr at 38°C, 1 hr at °43C, 1 hr at 48°C then 7 hrs at 52°C.
32	5.0	56-105 mm	Heat 1 hr at 38°C, 1 hr at 43°C, 1 hr at 48°C then 7 hrs at 52°C.
36	5.0	56-105 mm	Heat 53°C internal temperature for 1 hr and dry at 20°C and 65% RH to a moisture protein ratio of 1.6:1
43	4.6	55 and more	Hold at 43°C for 4 days
43	4.6	56-105 mm	Hold at 43°C for 4 days
43	5.0	56-105 mm	Hold at 43°C for 7 days

A five log reduction of STEC can be archived by any of the suggested heat treatments, provided correct pH and correct diameter of sausage.

Figure 8. Visual changes in fermented dry-sausages after heat treatment of final products (36). The sausages were vacuum packed and heat-treated in a water bath for the temperatures and times indicated. The effects of heat treatment on visual characteristics can probably be ignored.

Options for interventions

In the following sections, possible interventions in the meat chain for producing dry-cured sausages are briefly summarised. Particular focus is given to processes that may be controlled, and which have a predictable effect in a population. Technological measures during slaughter and processing of meats may be used as reliable measures to reduce the number of STEC reaching Norwegian consumers. Some of these have already been implemented during sheep slaughter 2006, and it is possible that the main routes of STEC contamination have already been controlled.

Outbreaks or a "normal" situation

As STEC infections are currently a focus of attention, it can be expected that the reported incidence of human STEC infections will increase. During an outbreak situation all options should be available for sampling and intervention procedures, while in a "normal" or non-outbreak situation, sufficient time should be available for proper planning and a full risk assessment, before expensive sampling or other intervention programmes are started. While

pre-harvest interventions may be efficient in some situations, they may also be misleading or give a false feeling of consumer safety if not properly designed or without proper aims.

Pre-harvest interventions

As described in previous sections in the report, there are no farm level interventions that have been documented to reduce the level of STEC in the domestic animal population. The main responsibilities of the farmer are associated with providing the best possible general hygiene during animal production, and sending animals to slaughter as clean as possible.

Monitoring and surveillance programmes in the pre-harvest stage

It may be tempting to start a surveillance programme as a tool for identifying herds or flocks with potentially pathogenic STEC, in order to avoid animals harbouring these bacteria reaching the consumer, or contaminating meats for further processing. However, such a programme is likely to provide only a false sense of security, as it is not possible to ensure that any animal slaughtered does not harbour potentially pathogenic STEC. There is currently a specific lack of information about the real occurrence of STEC and EPEC in Norway, including not only the outbreak variety of STEC O103, but but also other potentially pathogenic serogroups. There is an obvious requirement for baseline studies, investigating not only STEC O103, but also other potentially pathogenic STECs in the animal chain. Only information from a properly designed and extensive baseline study on STEC may indicate the utility of establishing a risk-based monitoring or surveillance programme in the pre-harvest stage. Isolates from baseline studies on various domestic animals would also provide a better basis for comparison with human isolates.

Interventions during the slaughter process

It is well-documented that improved slaughter hygiene, with full implementation of rodding in ruminants, plastic bags during evisceration in all mammalian species, and improvement of slaughter lines, will reduce the number of *E. coli* or STEC in meats. Furthermore, decontamination using steam-vacuum, or other, techniques may further reduce the microbial load. The benefit would be most evident in sheep, but decontamination could also be used for cattle and pigs. With proper hygiene and thermal decontamination, a level of up to 3 log units reduction of the *E. coli* /STEC is within reach for sheep, probably less for cattle and pigs where slaughter hygiene is already superior.

Monitoring and surveillance programmes in the meat industry

With proper implementation of full hygiene measures, the bacterial load on carcasses should be reduced to a level where specific analysis for STEC or other pathogens becomes irrelevant and does not contribute to consumer protection. Monitoring of the levels of indicator bacteria, such as generic *E. coli* or *Enterobacteriaceae,* should be selected as a method to assess and document the quality of the slaughter process.

Interventions during production and storage of dry-cured sausages

Production of dry-cured sausages with STEC absent or at the lowest possible level, should be based upon using meats with the lowest possible level of *E. coli,* and other raw materials (spices) without any contamination from faecal bacteria. It has been well documented that by modifying the processes during fermentation it is possible to reduce the probability of STEC growth, whilst prolongation of sausage storage will reduce any residual STEC populations. To optimise STEC reduction, a combination of production modification and heat-treatment of the final product can be implemented which may reduce the number of *E. coli*/ STEC by a factor of 5 log units.

Whilst these measures may be more readily applicable in the industrial setting, small-scale producers face a special challenge. If fermentation and temperatures are not full controlled, the safest procedure is probably to extend the storage or maturation time for dry-cured sausages, with an expected 1 log unit reduction occurring per month of storage.

Answers to the questions in the terms of reference

The following sections attempts to provide brief answers to the questions raised in the Terms of Reference. Some quantitative estimates are given here, but for more detailed information the text in the relevant sections of the report should be consulted.

Qualitative aspects to be addressed in the risk assessment

Q1: Have there been any changes in the distribution of STEC and EPEC in the domestic animal reservoirs (e.g. cattle, sheep, and pigs) during recent years?

There is no indication that there have been any significant changes in the distribution of STEC and EPEC in the domestic animal reservoir in recent years. However, a lack of comparative data, especially regarding other *E. coli* O-groups than O157, means that there is considerably uncertainty on this issue. There have not been any domestic studies regarding

EPEC, and the available domestic data is from a few studies on STEC. There are more data from cattle than from sheep (and goats), and only one study has been conducted in pigs. The virulence factors of EPEC and STEC are encoded on mobile genetic elements that enable new variants to develop through horizontal gene transfer and/or mutations.

Q2: Have there been any changes in the epidemiological pattern of STEC infections in people in Norway during recent years?

The incidence of reported human STEC infections in Norway has been low, and relatively stable, over the last ten years before the outbreak in 2006, with between 10 and 20 cases notified annually. Improved diagnostics may lead to an increase in reporting, and in recent years more STEC-infections of serotypes other than O157:H7 have been reported. It is probably that there has been a degree of underreporting of all serotypes, especially those other than O157:H7. Increased awareness, changes in diagnostic methods, and new legislation concerning mandatory notification of diarrhoea associated HUS, may result in a larger number of cases being reported in the future. This trend has already been observed following the 2006 outbreak. It is difficult to assess the extent to which there is, or has been, transmission of STEC from the various animal sources, thereby resulting in variations in the pattern of human disease.

Q3: Identify the groups at risk for STEC infections.

The incidence of STEC infection varies by age group, with the highest incidence of reported cases occurring in children. While children and elderly are more susceptible to more severe illness, such as HUS, people of all ages can suffer from STEC infection. Additionally, if older children or adults develop HUS, then their prognosis for recovery is poorer.

Q4: Describe the variations in occurrence of virulence factors in the different STEC-serotypes (and atypical EPEC) isolated from animals, foods and humans and the relevance for pathogenicity in humans:

a. Animals

Typical cattle STEC serotypes (O113:H4/H21, O91:H21, O22:H8) are associated with *stx* variants stx_1, stx_2 and/or stx_{2c}, while typical sheep STEC serotypes (O5:H-, O6:H10, O91:[H14], O128:[H2] and O174:[H8]) are associated with stx_{1c} and/or stx_{2d}. These typical

sheep and cattle STEC isolates do not usually carry *eae*. There is a lack of data on *eae*-positive, *stx* negative *E. coli* (atypical EPEC) of these serotypes. STEC of specific serotypes carrying stx_{2e} cause oedema disease in pigs.

Based on international studies, the *stx* variants of the well-known human pathogenic serotypes, O26:H11, O111:H8, O103:H2, O145:H21 and O157:H7, occurring in the animal reservoir are regarded as being stx_1, stx_2 and/or stx_{2c}. However, subtyping of *stx* has not generally been performed on Norwegian animal isolates, nor has subtyping of *eae*. All the domestic STEC O157 cattle isolates investigated have carried stx_2 and *eae*, and some isolates have also carried stx_1.

International and domestic studies report both *stx* and *eae*-negative *E. coli* as well as *stx* negative, *eae*-positive *E. coli* of these O-groups, indicating that these are relatively common in the microbial flora of animals. However, the relationship and ratio between *stx* and *eae*-negative *E. coli*, *stx* negative, *eae*-positive *E. coli* (EPEC), *stx* positive, *eae*-negative *E. coli* (STEC), and *stx* and *eae*-positive *E. coli* (STEC) of any serotype are unknown, as is the influence of free bacteriophages.

b. Foods

The variation in occurrence of virulence factors of STEC in food should reflect the variation present in the animal and environmental reservoir, and also perhaps the variations observed in human infections. Any food chain in which STEC is present may result in the production of food with STEC contamination. Specific properties, such as pH or a_W tolerance, of some serotypes or strains of a specific serotype may result in increased survival in specific food products. There is no information about the level of STEC in Norwegian foods, and thus the variation in occurrence of virulence factors of STEC in Norwegian foods is also unknown.

c. Humans

Of the STEC-strains isolated from human patients (excluding HUS-patients) in Norway during 1995-2005, 51% (46 strains) possessed both stx_1 and stx_2, 22% (20 strains) had stx_1 alone and 27% (25 strains) had stx_2 alone (Table 2). Of nine strains isolated from HUS patients, eight had stx_2 alone, while only one had both stx_1 and stx_2. Subtyping of *stx* has not been performed on these isolates. For 25 strains, information about *stx*-profile is not available, but all the strains were *eae*-positive.

The most important virulence characteristic of a human pathogenic STEC-strain is the ability to produce and release Stxs, but not all Stx-producing bacteria cause HUS. There is evidence for an association between-variant and severity of disease. Strains of *E. coli* harbouring only stx_2 have been significantly more frequently associated with the development of HUS than those only harbouring stx_1, or harbouring both stx_1 and stx_2. In addition stx_{2c} has been associated with HUS, whereas stx_{2d} may be considered as a "low-pathogenicity Stx-producing *E. coli*". stx_{2e} and stx_{2f} can be considered as non-pathogenic for humans. Most human pathogenic STEC strains carry eae, which mediates the attachment of the bacteria to host cells. Human pathogenic STEC also possess a variety of other virulence factors, but these are almost never sought when identifying STEC. There are some reports suggesting a possible association between HUS and Stx negative *E. coli,* and also a few reports regarding eae-negative STEC. These cases might be as a result of virulence factors, present at the time of disease, being subsequently genetically lost, and are therefore absent during laboratory examination. In sporadic cases these strains will probably not be identified as human pathogenic *E. coli*. When *eae*-positive, they may be reported as atypical EPEC, but otherwise they might not be recognized.

The pathogenic potential of STEC / EPEC, according to the presence or non-presence of virulence factors, may be classified as in Table 11. This table is based upon present information, but is not suitable for direct use by risk managers. In order to differentiate between pathogenic and probably non-pathogenic strains of STEC, subtyping of *stx* is also necessary, but is not normally performed. Thus, with the diagnostic methods presently in use, it is not possible to differentiate with certainty between pathogenic and non-pathogenic subgroups of STEC.

Table 11. Pathogenic potential of STEC /EPEC classified by two of the known virulence factors (*eae* and *stx*), referring to the clinical situation with detected bacteria from human patients. The table cannot be directly used to evaluate isolates from animals or foods.

Serogroup	***stx*[1]**	***eae* +**	***eae* -**
O157, O26, O103, O145, O111	*stx* +	Highly pathogenic	May be pathogenic
	stx -	May be highly pathogenic [2]	Probably apathogenic
Other O-groups	*stx* +	May be highly pathogenic	May be pathogenic[3]
	stx -	Pathogenic [2, 4]	Considered apathogenic

[1]. Some *stx* subtypes (stx_{2e}, stx_{2f}) are less associated with disease in humans than others, however subtyping is rarely done.
[2] Loss of *stx* genes may have occurred during cultivation
[3] Some O-groups in this category have frequently been diagnosed from patients with diarrhoea (and in some rare cases HUS) in some countries (for example O91, O146, O128, O113).
[4] Pathogenic if classified as typical EPEC. Another group, the "atypical EPEC" can cause mild diarrhoea

Q5: Are current laboratory techniques (including indicator organisms) sufficient for providing reliable results regarding STEC and their pathogenicity factors (e.g. *stx*$_1$/*stx*$_2$ genes, *eae* gene)?

No practical methods are presently available for large-scale use as part of a monitoring/ surveillance programme in the food chain. Detection of STEC and assessing their pathogenicity is currently a qualitative method, and does not enable direct quantification of the level of STEC in products that may be contaminated. If STEC sampling is used routinely, it may be detected on a random basis, but it should be expected that most contaminated lots would not be detected.

Industrial chain control and HACCP systems require quantitative analyses that enable continuous monitoring of the hygienic level in the production. At present, the most relevant methods for this type of chain control are quantification of indicator bacteria such as generic *E. coli* or *Enterobacteriaceae*.

Modern laboratory techniques are a prerequisite for epidemiological surveillance. The available techniques are sufficient for outbreak investigation or epidemiological tracing. One limitation of the methods is linked to that the potential for STEC to shed their *stx* genes, and thus present as atypical EPEC or STEC with "lost *stx*-genes". However, in epidemiological tracing, this situation can be addressed by using specific typing, or employing MLVA or other molecular methods.

Quantitative aspects to be addressed in the risk assessment

Q1: What magnitudes of risks are associated with consumption of dry-cured sausages with the current production process?

The annual production of dry-cured sausages in Norway is approximately 5000 metric tonnes and about 20% is from small-scale production. There is no CCP in the current production of dry-cured sausages in Norway. With the exception of the outbreak of STEC O103 infection during 2006, there is no information about human disease (STEC or EPEC, or any other infectious agent) associated with the consumption of dry-cured sausages produced in Norway. The low incidence of outbreaks of human STEC infections in Norway may imply that the risk is small; however, as the sources of sporadic human STEC infections are generally not identified, dry-cured sausages can not be excluded in these cases.

Due to the focus on slaughter hygiene following the 2006 outbreak, the risk of the recurrence of further similar incidents may already have been reduced. A direct estimate of the risk is not possible from our knowledge of the current disease pattern and available data.

Q2: Describe, and if possible quantify, the effects of interventions in the meat production line on the level of STEC on carcasses, or in the processing of meat by a: pre-harvest intervention or b: at slaughter:

a. Pre-harvest intervention

No specific management strategies have so far been demonstrated to be successful in decreasing the occurrence of STEC or EPEC in the ruminant reservoir. However, any reduction of STEC in the ruminant reservoir will reduce the level of contamination in the human food chain, and thus consequently have a beneficial effect on the potential number of human infections. At present, the only appropriate advice is to ensure good general hygiene practices, and to attempt to limit the number of faecal bacteria transmitted between animals and herds/ flocks.

b. At slaughter

i- General slaughter hygiene

The use of the plastic bag technique during circum-anal incision and removal of the rectum results in at least a 1 log reduction of *E. coli*, based on relevant 100 cm^2 sampling sites. Due to the fact that fluid from the rumen may also contain *E. coli*, rodding at an early stage of the slaughter line is important.

Because of faecal contamination of the wool during transport, clipping of sheep and lambs should be performed after reception in lairage, and not at farm-level. To limit shedding of STEC in faeces, the animals should not be stressed during transport and handling.

In addition, at the slaughter line positions of skinning/dehiding and evisceration, it is important that the operators are skilled and experienced. During seasonal slaughtering, such as lamb slaughtering, staffing of the slaughter line might be difficult due to lack of skilled personnel. Accordingly, relevant training programmes for operators in the abattoir are important.

Implementation of proper hygiene during slaughter will have the most significant effect in sheep slaughter, where an estimated 90-99% reduction (1-2 log units) of contamination may be achievable. The effect in cattle or pigs may be less obvious, but is still of relevant magnitude.

ii- Decontamination procedures

Decontamination methods at the end of the beef slaughter lines (treatment of whole carcasses by steam, hot water or organic acids) as used in USA, apparently have significant effects on the numbers of *E. coli* on beef carcasses. Reductions in bacterial counts by 1 – 3 log, depending on the initial bacterial counts and the decontamination process employed, have been described. The use of steam-vacuum, which has been used in Norway on lamb carcasses, seems to reduce the numbers of *E. coli* by an average of 1–2 logs. This result supports those data available from the producers of this system, who have documented an effect of a 1.1 – 1.5 log reduction, based on a comparison of numbers of aerobe microorganisms on surfaces of sheep carcasses which have, or have not, been steam-vacuumed. Norwegian experiments suggest a reduction in the magnitude of 99% (2 log units) on the levels of *E. coli* on sheep carcasses.

A combination of proper slaughter hygiene and decontamination may be an efficient measure for reducing consumer exposure, not only to STEC, but also to other enteric bacteria such as *Salmonella* and *Yersinia enterocolitica.* In the absence of definitive documentation on methods for controlling STEC at the pre-harvest stage, The general population effect on interventions during the slaughter process indicates this stage as the primary intervention point.

The meat industry has already exerted considerable efforts in improving the slaughter hygiene in many slaughterhouses. Thus, in these slaughterhouses, the next step of any importance would be introduction of appropriate decontamination procedures.

Q3: Describe critical control points, and if possible quantify, the effect of different interventions during the production of dry-cured sausages regarding: a: Raw material quality (meats, sugar, spices, etc.), and b: Production parameters (temperatures, recipes, maturation times, etc.).

With the current production processes of Norwegian dry-cured sausages, it is not possible to identify CCPs where the potential presence of STEC may be controlled or eliminated. However, a strict use of starter culture and an increased fermentation temperature has the potential to reduce the probability of STEC growth significantly. Provided that there is an acceptable standard of hygiene in the raw materials, these two measures will represent a substantial improvement. No data are available to estimate the quantitative effects of these steps. Before implementing such changes into production of dry-cured sausages, possible effects on other pathogens should also be taken into consideration.

A longer storage, or maturation period, of the final product will reduce the level of STEC reaching the consumer. An estimated 90% of *E. coli* STEC dies off per month of storage at, or above, 12°C (Table 10). This may be the only practical option for small-scale producers, in which the production processes are not fully controlled.

It is, however, possible to establish a production system where a 5 log reduction of the STEC level is attained, by using a combination of temperature/starter culture and a final heat treatment step (Table 10).

Q4: Describe and quantify the risks associated with consumption of dry-cured sausages?

Quantifying the direct risks associated with consumption of dry-cured sausages is not possible with the data currently available. A description of the possible risk reductions achieved by various interventions is provided under Q2 and Q3. Given that the interventions described in Q2 and Q3 are implemented, the presence of STEC in dry-cured sausages should be extremely rare. The main risk linked to dry-cured sausages will then be caused by recontamination during slicing and packaging, or by the consumer. Due to STEC reduction occurring more rapidly at higher temperatures, room temperature is preferable during storage at the retail level.

Main data gaps

Data gaps have been described and discussed in the previous chapters of this report. However, a few are further emphasized below:

- The true public health burden of pathogenic *E. coli* in Norway is unknown. Hopefully, more information will become available in the ensuing years due to the increased focus on STEC following the 2006 outbreak. Furthermore, the sources of human STEC infections, including sporadic cases, are frequently not identified. STEC infections generally are animal origin, and as more cases are reported, it should be possible to undertake studies to identify the dominant routes of transmission.
- The occurrence of various *E. coli* serotypes, and the composition of their virulence factors, present in the animal reservoir (ruminants and pigs) is unknown. Properly planned baseline studies may provide more information and also supply a better basis for comparison with human isolates
- The interpretation of results from laboratory studies, including those concerned with pathogenicity factors and relationships between *E. coli* of the same serotype with various pathogenicity factors present, remains an obstacle to our understanding of STEC epidemiology.

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