Farmhouse and
Artisan
Cheese & Dairy Producers
European Network

Shiga Toxin-producing *Escherichia coli* in relation to farmhouse and artisanal dairy products with reference to FACEnetwork's position.

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What is FACEnetwork?

Farmhouse and Artisan Cheese and dairy producers' European network (FACEnetwork) is an association which aims to represent and defend the interests of farmhouse and artisan cheese and dairy producers on a national and European level.

FACEnetwork was founded in February 2013 following initial informal meetings which began in 2009.

Members of the network include cheese and dairy producer associations as well as other stakeholders such as technical centres, universities and individuals. Members represent 14 countries across the European Economic Area (EEA): Austria, Bulgaria, Finland, France, Germany, Ireland, Italy, Luxembourg, Netherlands, Norway, Poland, Spain, Sweden and the United Kingdom and includes 11 producer associations which represent 13 countries in total.

The network is continually growing with more countries and organisations expressing interest or becoming involved and we have forged links with organisations in Portugal, Romania and Greece.

The sector that FACEnetwork represents is composed of:

Farmhouse cheese and dairy producers who process milk from their own livestock according to traditional methods.

Artisan cheese and dairy producers who collect milk from local farms and process it according to traditional methods.

The milk can come from cows, sheep or goats while the dairy products may be pasteurised or unpasteurised. Often these are family enterprises manufacturing locally-recognised products. These businesses can contribute considerably to the economic output of rural areas and are important to the local community - especially where other employment opportunities are limited.

The main characteristic of the sector is the fact that the milk is typically processed at or very close to the point of production - a feature which contributes to the high level of hygienic quality necessary for traditional processes. It is important to note that the word "local" refers only to the processing (of milk collected locally) and not to retail. It is true that direct or short supply chains are often preferred by small producers nevertheless many farmhouse and artisanal producers sell all or some of their products through longer circuits via *affineurs*, wholesalers or supermarkets.

The Scope of this Paper

The discussion of methods of analysis and the conclusions reached in this paper relate exclusively to cheese made from raw or pasteurised milk through the addition of lactic starter cultures. Raw drinking milk and other dairy products are excluded.

What is Shiga toxin-producing *Escherichia coli*?

Although *E. coli* is classified as a single species, it is, in fact, a large group of strains that have diverse characteristics. While most *Escherichia coli* strains are harmless commensals, living in the gut and in the environment, a small proportion of strains are capable of causing human illness. Shiga toxin-producing strains of *Escherichia coli* (STEC), have been increasingly identified as a group of emerging major pathogens since the identification of *E. coli* O157 in 1982.

Pathogenic STEC, alternatively known as Verocytotoxin-producing *E. coli* (VTEC), have a low infectious dose, considered to be 10-100 cells. These strains can cause diarrhoea and haemorrhagic colitis and a proportion of patients may develop Haemolytic Uraemic Syndrome (HUS), a severe sequela characterised by acute kidney failure. HUS is estimated to occur in around 10% of *E. coli* O157:H7 infections and the long term health consequences may be particularly severe for children under the age of five years.

Enterohaemorrhagic *E. coli* (EHEC) are defined as a subset of STEC isolated in cases of human disease and include the most pathogenic serotypes (MPS): O157:H7, O26:H11, O103:H2, O145:H28 and O111:H8. While many STEC strains have been identified, not all of them have been associated with human disease.

The pathogenicity of strains was defined by Karmali *et al.* 2003 (*6*) using a scale from A to E where strains in Groups A and B are characterised by multiple virulence factors and an association with severe disease as well as moderate to high incidence and greater frequency of involvement in outbreaks. Strains in Groups C to E are progressively 'lower risk' being associated with less severe clinical outcomes, being involved in fewer outbreaks or they have not been reported as human pathogens.

More recently, the Karmali seropathotype model has been challenged; 85% of STEC infections in the period 2007-2010 could not be fully serotyped (*2*). The model is further complicated by the fact that routine testing has previously been able to detect only *E. coli* O157 so the true incidence of other strains in illness may be under-reported. In 2011, the emergence of an entero-aggregative *E. coli* (EAEC) O104:H4 strain in Germany and France caused around 3900 infections and approximately 50 deaths. At the time, the serotype was exceptionally rare and would have been classified as type C. It was not considered to be a significant pathogen but has since been added to the list of 'most pathogenic strains'.

E. coli is able to share genetic material with other micro-organisms, including genes which encode for virulence factors or confer antibiotic resistance. Horizontal gene transfer in which genes are passed from neighbour to neighbour rather than vertical transfer from parent cells is a particular threat as it allows new pathogenic strains to emerge unpredictably – as demonstrated by the 2011 outbreak. If pathogenicity is hard to define then all STEC may need to be considered <u>potential</u> pathogens.

Like other *E. coli*, STEC are heat-labile and are inactivated by the standard milk pasteurisation process; lower heat-treatments such as milk thermisation or a high-scald temperature for curd may reduce numbers but may not cause complete inactivation.

EEA Incidence Data

Analysis of the data presented in the *EFSA/ECDC Joint Technical Report 2011*(*3*) reveals that, of the 3731 confirmed cases of STEC infection reported in the European Economic Area (EEA) in 2009, over 90% were from countries represented by FACEnetwork - including 236 cases of HUS. Some countries reported more cases (UK: 1339) while others had fewer or no confirmed cases (Spain: 14, Luxembourg: 5, Bulgaria: 0, Poland: 0). In some countries the incidence of HUS as a proportion of the confirmed cases of STEC infection was low (UK: 2%) while others were high (France: 67%) though this may reflect the monitoring system which targets surveillance of HUS in children under the age of fifteen years.

Foods identified as posing a risk of transmission of STEC infection include raw, undercooked or minced meat - especially beef; fresh produce including sprouted seeds; raw milk and dairy products; and unpasteurised fruit- or vegetable-juices.

It is important to note that the EFSA/ECDC data represent all reported incidents and not only those relating to cheese. Confirmed infections arising from the consumption of cheese are believed to be few in number.

Methods of Detection

A number of validated methods have been described for the detection of STEC. The European Union reference method for the detection of *E. coli* O157 is described by EN ISO Standard 16654:2001. As the infectious dose is very low for STEC, an enrichment of the test sample is incubated for 22 hours to enable the detection of low numbers of STEC. The incubation temperature (37°C) is lower than that used for the detection of non-pathogenic strains of *E. coli* (44.5ºC) since the maximum growth temperature of *E. coli* O157:H7 is 42.5ºC. (*10*).

Immunomagnetic separation of the incubated enrichment culture is carried out using magnetic beads that carry antibodies homologous for the O157 antigen. Entrapment of these beads enables *E. coli* O157 to be separated from a competitive microflora, thus improving the selectivity of the test. The beads are then plated onto selective-diagnostic media, incubated for 21 hours. Colonies displaying characteristic reactions for *E. coli* O157 (presumptive positives) are confirmed by a series of biochemical tests. The development of the immunomagnetic bead technique has been specific for *E. coli* O157 and it has limited application for other *E. coli* serogroups.

More recently, quantitative Polymerase Chain Reaction (qPCR) techniques have been developed to detect genes which encode virulence factors such as *stx* (or *vtx*) which encodes the Shiga toxin. Analysis may detect *stx* alone or in combination with adhesion factors such as *eae* (a gene that encodes Intimin which is associated with EHEC), or *aggR* and *aaiC* which are markers for EAEC; or serogroup genes. The presence of *stx* genes in a food may be interpreted as a presumptive presence of STEC; however, confirmation of the presence of STEC requires isolation of the organism itself. It is possible for these molecular techniques to detect genetic material from dead cells though this is arguably less likely when PCR is carried out on an enrichment broth.

There are a number of variants of the *stx* gene, *stx2* being associated with the most severe disease (*1*) however the interpretation of the presence of a single gene or gene combination is not straight-forward. Stressed cells may shed their *stx* gene while some EHEC have been shown to be *eae* negative. Where analysis is based on detection of *stx* genes an *stx*-positive strain without additional virulence factors and which was not associated with human disease would be detected; an *eae*-positive MPS which has lost its *stx* gene would not be.

The EFSA opinion of 2013 states that "there is no marker or combination of markers(s) that defines the potential of a VTEC [STEC] strain to cause human disease" (*2*).

STEC in EU Regulation

An STEC criterion applicable to dairy products is not specified in Regulation (EC) 2073/2005 following the opinion of the Standing Committee on Veterinary Measures relating to Public Health that applying an end-product standard for *E. coli* O157 would be unlikely to deliver meaningful risk reductions, but a criterion is implied in Regulation (EC) 178/2002: *"Food shall not be placed on the market if it is unsafe"*. Where food business operators apply their own criterion, "absence in 25g" is usually specified as a minimum. Some Member States are in favour of setting a criterion for STEC. Farrokh *et al* (2012) (*5*) conducted a thorough review of the significance of STEC in dairy products and concluded that end product testing is not an effective control strategy.

A 'Guidance Document on the application of article 14 of Regulation (EC) № 178/2002 as regards food contaminated with Shiga toxin-producing *Escherichia coli* (STEC)' is currently being prepared by DG Health & Food Safety and is currently in its fifth draft. Previous drafts of the guidance document have based interpretation upon:

- Presence of *stx* genes in the food sample,
- Presence of *stx* genes in an isolated *E. coli* or;
- Presence of *stx* genes in combination with *eae* or *aggR*/aaiC in an isolated *E. coli*.

The fifth draft of the guidance document defines the presence of *stx* genes in an <u>isolated</u> *E. coli* as "presence of STEC", stating that the presence of additional virulence factors would characterise the hazard with a lower level of uncertainty. The guide recommends that the level of uncertainty should not be higher than that of "presence of STEC" giving the example of presumptive presence of STEC (*stx* detected in an enrichment culture) as being at a higher level. It is the responsibility of each Member State to evaluate the interpretation in relation to the risk profile of the food and adopt their own interpretation.

Evaluation of Literature relating to Cheese

We reviewed the literature relating to *stx* presence in cheese. Only a few published studies have investigated this subject.

Each study used slightly different methodology while the types of cheese used also varied slightly. In some studies, this information was not presented while in others, such as the study by Madic *et al*, 'cooked' cheeses (which are produced using a high-scald temperature typically >50°C) were excluded in preference for soft and uncooked hard

cheeses *(7)*. It is likely that the high frequency of *stx* identification in the study reflects careful sample selection in favour of soft and uncooked cheeses. Likewise, the lower percentage of *stx* positive samples in the study of Swiss hard and semi-hard cheeses undertaken by Zweifel *et al*. (*13*) possibly reflects the number of 'cooked' cheeses likely to be present in the sample pool. The table below shows the number of samples in each study, the percentage of total samples in which the *stx* gene was detected and the percentage of total samples in which the presumptive presence of *stx* was confirmed by isolation.

Cheeses in Study (& Reference)	**№ samples**	***stx* positive samples**	***stx* positive *E. coli* isolated**
Raw Milk Cheese (*11*)	1039	13.09%	3.08%
Raw Milk Soft Cheese (*13*)	80	10.00%	6.25%
Raw Milk Semi-Hard & Hard Cheese (*13*)	1422	5.41%	1.69%
Uncooked & Soft Raw Milk Cheese (*7*)	400	29.75%	3.75%
Cheese – type unspecified (*9*)	603	9.95%	1.00%
Raw Milk Cheese (*4*)	180	30.55%	11.66%
Pasteurised Cheese (*4*)	45	8.89%	2.22%
Raw milk Cheese (*12*)	153	11.11%	0.00%

Whether made from raw or pasteurised milk, cheese has a complex microflora. The ability of organisms such as Non-Starter Lactic Acid Bacteria (NSLAB) and other ripening organisms to develop in cheese is well known while starter organisms typically decline in number during maturation. Members of the Enterobacteriaceae such as *Hafnia alvei* may be *stx* positive and are deliberately added to some dairy products as ripening cultures. The study undertaken by Vivegnis, El Lioui, Leclercq, Lambert and Decallone (*12*) demonstrates one of the problems associated with an interpretation based on the presumptive presence of STEC. Five (3.26%) *stx* positive samples were isolated, however these were identified as *Hafnia* in four samples and *Enterobacter* in one. As the isolated samples were found to be species other than *E. coli* they could not be classified as STEC. The isolated strains lacked other virulence factors necessary to cause illness.

While much of the work undertaken on dairy products concentrates on raw milk products, the paper by Fach, Perelle, Dialasser and Grout studied a limited number of pasteurised milk cheeses but identified *stx* in 9% of samples and obtained *stx*-positive isolates in 2% of samples (*4*).

There is limited information on the presence of *stx* genes in cases of asymptomatic carriage of STEC by humans. When evaluating the presence of other enteric pathogens such as *Salmonella*, Pradel *et al* (2000) (*9*) concluded that the healthy carriage state could possibly account for up to 1.5% of the hospitalised children in their study.

Perrin *et al* (2015) (*8*) carried out a quantitative risk assessment of HUS linked to MPS-STEC in raw milk soft cheese and demonstrated risk reduction of up to 98% based on preharvest interventions such as hygienic improvements in the milk supply, vaccination or use of probiotics. A risk reduction of up to 96% was seen by introduction of microbiological criteria alone or in combination with preharvest interventions.

Other possible interventions mentioned in study by Perrin *et al* but not included in the calculation include the detection and isolation of STEC-shedding cows and the use of antimicrobial agents or bacteriophages. Hygienic improvements at farm level may reduce the risk of STEC but complete eradication from the farm environment may not be possible. FACEnetwork is currently working on a guide to good hygienic practice.

Conclusion

Based upon the limited number of studies, there is some consistency as to the high level of STEC presumptive positive detections in cheese. We believe that the evidence of outbreaks relating to cheese is strikingly low in relation to the apparent presence of the *stx* gene so as to cast doubt on the reliability using an interpretation based upon "presumptive presence of STEC". Confirmation of presence of a pathogen is one of the fundamental principles of microbiological analysis and a move to molecular techniques should not be marked by a lower degree of certainty than has been acceptable up to now.

The characterisation of STEC as "presence of *stx* in an isolated *E. coli*" while preferable to "presumptive presence" may still result in a high number of *stx* positive products being recalled in relation to the outbreak data. We urge EU Member States, where possible, to consider an interpretation based upon higher levels of certainty such as "presence of *stx* with either *eae* or *aggR*/*aaiC* in an isolated *E. coli"* or other similar combinations of markers or virulence factors in cheese.

Where Member States base their interpretation upon lower levels of certainty they run the risk of disadvantaging small businesses within their own country while products imported from another Member State or a third country may be produced under a less strict interpretation.

While STEC studies in dairy products have tended to concentrate on raw milk cheeses, further information is required on the wider prevalence of *stx* genes in pasteurised products. We consider that although the presence of *stx* may be considered to be of concern from an epidemiological point of view, there is currently insufficient information to support the withdrawal or recall of cheeses on the basis of a presumptive presence of STEC. Furthermore, we recommend that the prevalence of *stx* genes in pasteurised milk cheeses is studied in greater depth and the presence of *stx* genes in the human population should be better understood before a robust evaluation of the risk to consumers and its minimisation can be made.

Finally, we urge Competent Authorities to engage with technical experts working in the farmhouse and artisanal dairy sector in their own countries as we consider that the exchange of information may contribute to our common objective of protecting consumer health without placing unnecessary burdens upon farmhouse and artisanal enterprises.

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