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OPINION OF THE

SCIENTIFIC COMMITTEE ON VETERINARY MEASURES RELATING TO
PUBLIC HEALTH

ON

VEROTOXIGENIC *E. COLI* (VTEC)
IN FOODSTUFFS

(adopted on 21-22 January 2003)

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1. EXECUTIVE SUMMARY

VTEC is a group of *E. coli* that produces one or more verocytotoxins (VT). VTEC that have been associated with causing human disease are referred to as human pathogenic VTEC (HP-VTEC) in this report.

The unclear terminology used for describing human pathogenic VTEC (HP-VTEC) confuses the interpretation of scientific findings and reports from monitoring and surveillance programmes, and hampers comparisons. Moreover, the reported incidences of disease caused by HP-VTEC are not comparable within the EU due to variations in the diagnostic methods and procedures, and reporting systems used.

Only a small fraction of all VTEC-types isolated from animals, food or the environment are associated with human illness. However, VTEC O157 is an important cause of bloody diarrhoea and kidney failure (haemolytic uraemic syndrome, HUS). Four principal routes of infection may be identified for VTEC (a) direct contact with infected animals; (b) person to person transmission; (c) foodborne transmission, and (d) transmission through the environment. Foodborne VTEC O157 infections originate from ingestion of foods contaminated by ruminant or human faecal material and where the conditions in the food chain thereafter enable survival. Moreover, the issue of cross-contamination should not be ignored. The infectious dose for VTEC O157 is very low and an infection may result from consumption of contaminated foods in which the bacteria have survived but not necessarily grown. There are currently insufficient analytical tools to identify the burden and main sources of non-O157 VTEC infections in humans.

The following categories of foodstuffs where (VTEC) represents a hazard to public health were identified: raw or undercooked beef and possibly meat from other ruminants; minced and/or fermented beef, and products thereof; raw milk and raw milk products; fresh produce, in particular sprouted seeds, and unpasteurised fruit and vegetable juices; and water. This report should be considered as a risk profile for all these food categories apart from water. Therefore the risk analysis process should continue including developing a risk profile for water for processing, irrigation and drinking and HP-VTEC, with a view to taking appropriate risk management actions.

Microbiological guidelines and corrective actions aimed at reducing faecal contamination along the food chain (e.g., testing for *Enterobacteriaceae*) can contribute to a reduction of associated public health risks including HP-VTEC.

Due to the sporadic occurrence and low prevalence of VTEC O157 found in food commodities representing a risk, applying end product microbiological standards for VTEC O157 is unlikely to deliver meaningful reductions in associated risk for the consumer. However, if there are reasons to suspect a high prevalence of a HP-VTEC, microbiological criteria and appropriate actions might be useful in controlling the risk. Finding HP-VTEC, in particular VTEC O157, in a foodstuff indicates a public health risk necessitating appropriate actions.

2. BACKGROUND

The Community legislation on food hygiene is currently under revision. Proposals for a recast of this hygiene legislation have been submitted to the Council and the European Parliament. In this context the Commission has also started a revision of the microbiological criteria in Community legislation.

The Commission is preparing a comprehensive strategy to set these criteria. This strategy would cover for all foodstuffs the whole production and distribution chain (including retail trade) in line with the proposed new hygiene legislation. Criteria would be set for food products on the market as well as products at different stages of manufacturing processes.

The Scientific Committees have already provided several opinions on the subject of microbiological criteria (SCVPH, 1999, 2000). The opinion on foodborne zoonoses covers the most important foodborne pathogens, including *Salmonella* and verotoxigenic *Escherichia coli* (SCVPH, 2000). These general and comprehensive reports indicate the need for more specific information, in order to put into place appropriate measures to the pathogen considered.

3. TERMS OF REFERENCE

The Scientific Committee on Veterinary Measures relating to Public Health is asked to:

- Identify categories of foodstuffs where Verotoxigenic *E. coli* (VTEC) represents a hazard to public health;
- Evaluate the appropriateness of setting microbiological criteria and,
- Identify where risk profile might be useful.

Considering the common field of interest, the Committee is invited to set up a joint working group including experts from both the Scientific Committee on Veterinary Measures relating to Public Health and from the Scientific Committee on Food.

4. INTERPRETATION OF TERMS OF REFERENCE

The Committee interprets (a) “hazard to public health” as representing a high risk to human health; (b) “appropriateness of setting criteria” as whether the implementation of a microbiological criterion will contribute meaningfully to a reduction of the public health risk posed by the particular pathogen-food commodity combination; and (c) “identifying where a risk profile would be useful” as whether the risk evaluation should be continued, including allocating necessary resources. This may, or may not, proceed to a full risk assessment.

5. INTRODUCTION

Escherichia coli is a species within the Gram-negative family *Enterobacteriaceae*. The species is a normal constituent of the intestinal flora of humans and warm-blooded animals. The pathogenic nature of some serotypes has been known since the early 1900s and virulence factors are today used for identification and characterisation of pathogenic strains. *E. coli* are shed in high numbers in faeces and enter as a contaminant into the environment. Pathogenic *E. coli* can, by different routes of transmission, circulate in the farm environment through foraging from pastures on which manure is spread or where animals graze, and via contaminated water.

The Scientific Veterinary Committee has previously issued a report on Verocytotoxin Producing *Escherichia coli* (VTEC) in 1997 (SVC, 1997). The report concluded that minced beef products such as beef burgers, salami, raw cows' milk and cheese, contaminated pasteurised milk and untreated water were important sources of foodborne VTEC O157 infections. The report included, amongst its recommendations, measures for improved cleanliness of animals when transported; a review of the slaughter processes such as dressing and evisceration with the aim of improving hygiene; the hygienic production of milk and milk products; strict maintenance of the cold chain (processing and distribution); provision of information to food handlers and to consumers with special attention to groups at special risk; and consideration of decontamination procedures before consumption.

The report also identified the need to improve detection methods, including development of rapid methods of detection, and ensure consistency in laboratory methods used in EU Member States.

6. VTEC IN RELATION TO PUBLIC HEALTH

6.1. Nomenclature of VTEC

VTEC is a group of *E. coli* that produces one or more verocytotoxins (VT) also known as Shiga toxins (STX). This group of bacteria has many synonyms. In the USA and to a varying extent in Europe, the notation Shiga-toxin producing *E. coli* (STEC) is used. The term, enterohaemorrhagic *E. coli* (EHEC) was originally used to denote verocytotoxigenic *E. coli* (VTEC) causing haemorrhagic colitis in humans; later EHEC has been used as a synonym for VTEC in the medical domain in some European countries.

VTEC that have been associated with causing human disease are in this report referred to as human pathogenic VTEC (HP-VTEC).

Disease produced by VTEC appears to be associated with a subset of serogroups with the somatic group (O group) O157 as the predominant one. Several other O groups contain verocytotoxin-producing strains that may also cause similar disease in humans, the most common ones being O26, O103, O111, and O145. Most research on VTEC has been done on the serotype O157:H7, which is easily differentiated from other *E. coli* strains by its inability to ferment or slowly fermenting (>24 h) sorbitol, and for which a

rapid and simple test is available. Nevertheless, cases of haemolytic uraemic syndrome (HUS) associated with sorbitol-fermenting HP-VTEC O157:H- have been reported in Germany (Ammon *et al.*, 1999) and caused by HP-VTEC O157 in Scotland (SCIEH, 2002). A schematic representation of the relationship between *E. coli* O157 and VTEC is showed below in Fig 1.

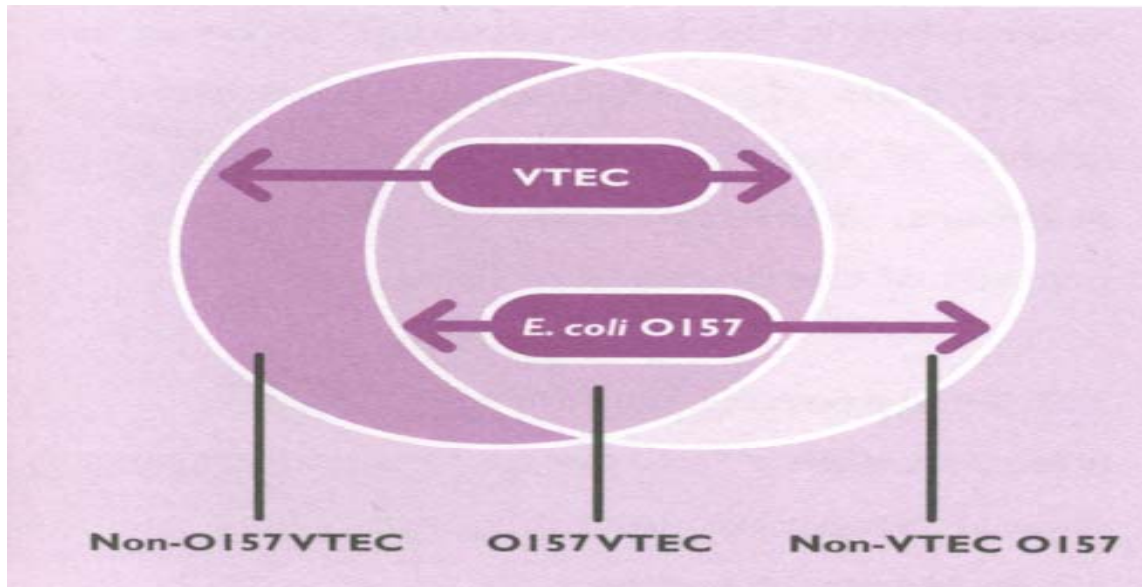


Fig. 1 Schematic representation of the relationship between *E. coli* O157 and VTEC adapted from ACMSF report (1995) on VTEC. London: HMSO. (from "The prevention of *E. coli* O157:H7 infection - a shared responsibility". Food Safety Authority of Ireland (1999).

Other VTEC serotypes are biochemically similar to the commensal *E. coli* strains inhabiting the gastrointestinal tract of humans and warm-blooded animals. Knowledge concerning disease caused by non-O157 HP-VTEC and the sources of these organisms is limited.

VTEC cannot be identified based on their serotype alone. Not all strains belonging to a VTEC-associated serotype produce verocytotoxin, since this ability is encoded by a gene carried on a bacteriophage, which is not always present. Accordingly, any identification of a VTEC needs to be confirmed by the demonstration of production of the verocytotoxin itself or by the presence of the gene (*vtx*) encoding it.

Two main groups of verocytotoxins exist: VT1 and VT2. VT2 may be further subdivided into at least 5 subtypes, VT2, VT2c, VT2d, VT2e and VT2f (Scheutz *et al.*, 2001). The latter two forms have rarely been associated with human disease. VT2e is associated with oedema disease in pigs (Gyles *et al.*, 1988; Weinstein *et al.*, 1988) and VT2f has been found in *E. coli* isolated from pigeons (Schmidt *et al.*, 2000). Strains containing VT1 and the former three VT2-subtypes (VT2, VT2c, and VT2d) are all associated with human disease, but are also found in *E. coli* isolated from animals, often without causing disease in the animals (Blanco *et al.*, 2001, Brown *et al.*, 2001; Schmidt *et al.*, 2001; Wasteson, 2001).

6.2. Disease caused by HP-VTEC

HP-VTEC is an important cause of haemorrhagic colitis in humans in all parts of the world. The infection may simulate non-infectious inflammatory intestinal disease. It is usually self-limiting, but may, in up to 10% of cases, elicit acute renal failure and blood clotting disturbances such as haemolytic uraemic syndrome (HUS) or thrombotic thrombocytopenic purpura (TTP). These complications are especially common in the very young and very old and cause a high case-fatality rate. Sporadic cases as well as outbreaks of disease caused by HP-VTEC commonly occur throughout the world (Mead and Griffin, 1998).

Virulence factors other than the verocytotoxins are implicated in the pathogenesis of VTEC infections in humans. These include intimin (an adhesion molecule) and haemolysin. However, the pathogenesis is not entirely clear and recent studies (Schmidt *et al.*, 1999) indicate that HUS and diarrhoea may be associated with *E. coli* O157:H7 strains that do not produce verocytotoxin. Identification of such virulence factors might be helpful for the detection and identification of non-O157 HP-VTEC from food or live animals.

6.3. Methods for detection of VTEC

The methods for detection of VTEC have recently been thoroughly reviewed (Scheutz *et al.*, 2001; Chapman, 2001 a,b). Direct methods include tests for production of verocytotoxin or presence of verocytotoxin genes (*vtx*) in specimens by a vero cell assay, by ELISA, or by PCR. The tests can also be performed on enrichment cultures or on individual colonies of plate cultures. In addition, *vtx* genes may be detected by colony hybridisation of single bacterial colonies. Wang *et al.*, (2002) suggested a multiplex PCR that should capture all virulence genes such as VT1, VT2, VT2c-f, intimin (*eaeA*), hemolysin (*hlyA*) and genes for serotype O157 (*rfbE*) and flagellum H7 (*fliC*) would be appropriate for HP-VTEC O157 detection.

Indirect methods include detection of sorbitol non-fermenting strains of *E. coli* on sorbitol MacConkey agar and related media, or detection of one of the common VTEC O groups (O26, O103, O111, O145 and O157) by agglutination of suspicious colonies from an agar plate with diagnostic antisera. Recovery of strains belonging to these VTEC O groups may be enhanced by using magnetic beads covered with antibodies directed against these serogroups directly on the specimen or on an enrichment culture. All strains detected by an indirect method should be confirmed by the detection of verocytotoxin production or the presence of *vtx* gene. However, there is no simple method available for the detection of HP-VTEC as such.

Meyer-Broseta *et al.*, (2001) have suggested that the reported prevalences of VTEC O157 from 25 prevalence surveys in primary production were not comparable due to differences in sampling strategies, target populations and laboratory practices. It was also suggested that most of the laboratory techniques employed had a low diagnostic sensitivity, and consequently that

the reported prevalences were underestimates of the true prevalences of VTEC O157.

6.4. Dose-response

The infectious dose for VTEC O157:H7 has been reported to be low. Doyle *et al.*, (1997) suggested that it might be less than 10 organisms ingested. Other studies (FSIS, 1993; Willshaw *et al.*, 1994) have indicated that less than 2 bacteria per 25 gram of foodstuff were sufficient to cause infection. Armstrong *et al.*, (1996) suggested that in a large multi-state VTEC O157:H7 outbreak associated with hamburger patties, the total number of bacteria in each patty prior to heat treatment was less than 700. A consequence of this low infectious dose is that infection may occur without bacterial growth occurring in contaminated food (Anon., 1999). These findings refer to VTEC O157:H7 outbreaks, while the infectious doses for non-O157 VTECs are not well described. It appears that further research is needed to clarify the dose-response curves for VTEC O157 and non-O157 VTEC, and to identify pathogenicity factors that could influence the dose-response.

6.5. Incidence in humans

Among HP-VTEC isolated from human cases, the VTEC serotype O157:H7 is most frequently reported and can be considered as the prototype, but non-O157 VTECs are also associated with human disease (Blanco *et al.*, 2001).

There are indications that, among the zoonotic diarrhoeal diseases, the incidences of VTEC infections are probably the least comparable between countries or even between different regions within the same country. This is because the practices regarding diagnostic methodology and the indications for applying the diagnostic tests vary more between the diagnostic laboratories for VTEC than they do for any other enteric pathogens. However, incidences may be compared between regions with the same diagnostic practices. When this is done, considerable differences in disease burdens are observed. For example in the year 2000 the annual VTEC O157 incidence in Scotland was 39 cases per million inhabitants, while in England and Wales it was 17 (<http://www.show.scot.nhs.uk/scieh/> and <http://www.phls.org.uk/>). There is evidence from recent outbreaks in Scotland that the variations in incidences between regions are related to non-foodborne sources. In the USA (Mead and Griffin, 1998), certain regions also appear to have a high incidence similar to the Scottish situation. Although diagnostic practices vary between countries and regions and make direct comparisons difficult, trends in incidences can still be compared. In Europe, the Community Zoonoses Report for the years 1999-2000 (EC, 2002) indicated a complex picture with strains of the O157 serogroup dominating among VTEC isolates from humans in certain countries (e.g. United Kingdom, Ireland and Sweden). For some countries, a considerable proportion of the reported cases appear to be caused by non-O157 VTEC (see Table 1). Australia also appears to have higher with non-O157 VTEC (CAC/CX/FX/03/5-Add.4, 2002). Moreover, Mead *et al.*, (1999) and Tarr

and Neill (1996) estimated that the fraction of non-O157 infections was between 20-50% of that of VTEC O157:H7 infections in North America.

Table 1: Reported data of human VTEC cases associated with VTEC O157 and non-O157 from the Community Zoonoses Report for the years 1999-2000¹ (EC, 2002)

Country	VTEC cases 1999		VTEC cases 2000	
	O157	Non-O157	O157	Non-O157
Austria	28	2	20	3
Belgium	34	19	-	-
Denmark	11	40	19	41
Finland	17	19	6	12
France	41	8	34	2
Germany	-	-	-	-
Greece	-	-	-	-
Ireland	54	-	45	5
Italy	12	10	-	-
Luxembourg	-	-	-	-
Portugal	-	-	-	-
Spain	15	3	10	1
Sweden	59	-	93	3
The Netherlands	35	2	13	45
Scotland	294	-	197	-
Northern Ireland	42	0	47	-
England and Wales	1084	-	896	-
Norway	10	3	2	5

- = not reported

¹ Numbers reported here may differ from those reported elsewhere and in the literature due to variations in reporting systems.

Table 2: Reported data of haemolytic uraemic syndrome (HUS) cases associated with *Escherichia coli* O157 and non-O157 from the Community Zoonoses Report for the years 1999-2000² (EC, 2002)

Country	HUS cases 1999		HUS cases 2000	
	O157	Non-O157	O157	Non-O157
Austria	2	2	2	3
Belgium	9	0	-	-
Denmark	3	1	1	2
Finland	2	2	0	1
France	41	8	34	2
Germany	-	-	-	-
Greece	-	-	-	-
Ireland	3	-	4	-
Italy	5	8	-	-
Luxembourg	-	-	-	-
Portugal	-	-	-	-
Spain	3	-	-	-
Sweden	1	4	1	3
The Netherlands	-	-	10	2
Scotland	-	-	-	-
Northern Ireland	-	-	-	-
England and Wales	-	-	-	-
Norway	1	0	0	0

- = not reported

A comprehensive collection of references concerning non-O157 VTEC associated with human enterohaemorrhagic disease is available at <http://www.microbionet.com.au/vtactable.htm>.

6.6. Routes of transmission to humans

Four principal routes of infection may be identified for VTEC:

- direct contact with infected animals;
- person to person transmission;
- foodborne (including drinking water) transmission;
- environmental transmission (e.g. swimming in a contaminated lake or swimming pool or contact with environments such as pastures, to which infected animals had access).

² Numbers reported here may differ from those reported elsewhere and in the literature due to variations in reporting systems.

The focus in this report is on the foodborne pathways. The basis for current discussions is the probability of direct or indirect faecal contamination of ruminant or human origin into the food chain, and the survival and possible growth thereafter of VTEC until the point of consumption. However, other transmission pathways should also be noted, if the objective is to control human disease (Mead and Griffin, 1998).

6.7. Important reservoirs of HP-VTEC

According to Duffy *et al.*, (2001), the gastro-intestinal tracts of ruminants, especially cattle, and humans are likely to present the main reservoirs of *E. coli* O157:H7. Other animals apart from cattle from which VTEC O157 has been isolated include sheep, goats, wild deer, pigs and seagulls (Chapman, 2000) as well as pigeons, geese (Anon., 1999) and zebu cattle (Kaddu-Mulindwa, *et al.*, 2001). Other verocytotoxin-producing serotypes (e.g. O26:H11, O103:H2, O111:NM, O113:H21) associated with human bloody diarrhoea and HUS have also been isolated from sheep, calf and cattle faeces (Dorn *et al.*, 1989; Montenegro *et al.*, 1990, Wells *et al.*, 1991).

Different monitoring and surveillance studies have found wide ranging prevalences. The individual prevalence of VTEC O157 in faecal samples may vary widely in different groups of cattle ranging from 0% to 70% (Bonardi *et al.*, 2001). Paiba *et al.*, (2002) found that in UK abattoir surveys the prevalences of VTEC O157:H7 were 4.7% and 1.7% in cattle and sheep, respectively. The highest prevalences were found during the summer, in agreement with the findings of other studies on seasonal variation with more cattle being found positive for VTEC O157 in late summer and early autumn (Chapman *et al.*, 1997; Hancock *et al.*, 1997; De Zutter *et al.*, 1999; Tutene *et al.*, 2002). In a review by Duffy *et al.*, (2001), the reported cattle herd prevalences were above 20% (i.e. herds having one or more animals shedding VTEC O157) while the reported individual prevalences in cattle and sheep ranged between 2 and 8%. Moreover, Duffy *et al.*, (2001) reported prevalences in ground beef and mutton of up to 6%, while in pork and poultry meat the prevalences were up to 1.5% and 4%, respectively. The prevalences of VTEC O157, found in dairy herd, have varied from 0.5% to 36% (Chapman *et al.*, 1997, Hancock *et al.*, 1997) and in beef herds the found prevalence was approximately 25% in Scotland (Synge *et al.*, 2001). In surveys of pigs, the serogroup O157 has been commonly reported (Wray *et al.*, 1993) although none of the isolates from diarrhoeal disease in pigs were shown to produce VT (Woodward *et al.*, 1990).

ICMSF (2002) gave figures for VTEC O157:H7 prevalences based on samples from individual animals, with a prevalence in live cattle ranging from 1.5 to 28% and in beef ranging from 0.1 to 5%.

It has been customary practice to look for VTEC O157 in faecal samples of animals. However, a recent study indicated that the prevalence of VTEC O157 is higher in oral cavity specimens than in faeces of cattle (Keen and Elder, 2002). This observation from a single study needs to be confirmed by findings from other studies, since it would have implications for the

estimation of the animal carriage rate of VTEC O157 and the associated risk assessments of certain meat cuts.

Based on a compilation of data prepared by the Community Reference Laboratory for the Epidemiology of Zoonoses and based on the National Zoonoses Reports for the years 1998 to 2000 (EC, 2002), it appears that the cattle herd prevalence of VTEC O157 ranged between 0 and 13%, the prevalence in individual cattle between 0 and 7%, the beef carcass prevalence between 0 and 1.3%, and the prevalence in minced meat and meat products between 0 and 0.1%.

It appears that the reported prevalences of VTEC O157 at the herd level are higher than at an individual animal level. The prevalence is even lower in carcasses and lowest in meat samples. Therefore, it is important to consider the sampling points in the food chain when recording and comparing the prevalence of VTEC O157.

7. ROLE OF EXTRINSIC AND INTRINSIC FACTORS FOR GROWTH, SURVIVAL AND INHIBITION

7.1. General considerations

In food microbiology much effort has been directed towards defining the conditions capable of limiting microbial growth, because understanding those conditions and applying them in the food chain could extend shelf-life and minimise multiplication of microbes associated with foodborne illness.

For many years laboratory experimentation generated tables of minimum values allowing bacterial growth (e.g., minimum temperatures, a_w and pH). Most studies considered only one factor e.g., the minimum temperature for growth was determined when the pH and a_w were kept near optimal conditions, and taking no account of the type of acid nor the nature of the solute used.

Exploring three controlling factors (temperature, pH, and a_w) simultaneously over a range of conditions relevant to foods and food storage leads to involved experiments and prolonged incubation periods for those conditions where growth is almost inhibited. Semi-automated distribution of the combinations of growth media, and a suite of controlled temperature rooms, enabled growth of a cocktail of ten strains of enteropathogenic strains of *E. coli* to be studied at pH 5.6 to 6.8, NaCl 0-10% (w/v on water), sodium nitrite (0-400 $\mu\text{g/ml}$) and temperature of 10 to 35°C (Gibson and Roberts, 1986).

Since the scientific literature did not provide estimates of growth rates of foodborne pathogenic bacteria across all of the conditions relevant to food production and storage, growth responses were estimated under a range of controlled laboratory conditions being modelled, and the outputs of that model (e.g. growth rate) being compared with independent estimates of

growth rate by other workers taken from the scientific literature (Baranyi and Roberts, 1995; Sutherland *et al.*, 1995).

Models for growth (with pH, a_w and temperature as the controlling factors) often account for the growth response in most, but not all, foods, suggesting that the microbial growth is largely at the surface of the food and is not affected by the food structure, as indicated by Sutherland *et al.*, (1995 and 1997) where the growth rates collected from the literature were compared with the rates generated by the model for the same pH, a_w (or % NaCl) and temperature.

Food products are complex matrices characterised by various parameters (Leistner, 2002) causing difficulties in predicting survival kinetics. At the present time, very few examples of modelling studies describe the effects of combinations of factors on survival of VTEC as well as in dry and semi-dry foods. Growth and survival behaviours of VTEC O157:H7 exposed to a combination of suboptimal conditions such as temperature, pH and NaCl in a simulation medium for red meat (beef gravy) suggest that these conditions can inhibit growth but also induce a prolonged survival (Uyttendaele *et al.*, 2001a). From other studies there are indications that the effect of these factors on limiting growth can be synergistic (Presser *et al.*, 1998).

Increased concern regarding the survival of VTEC in food and in the environment has resulted in the same approach as for growth has been used to model non-thermal death or survival (Whiting, 1993; Shadbolt *et al.*, 1999). In a study of the survival of VTEC O157:H7 (McClure *et al.*, 1999) it appeared that there were difficulties in modelling the survival compared with the growth, as the kinetics of survival is more variable than the kinetics of growth. McClure *et al.*, (1999) and Buchanan *et al.*, (1994) suggested that it would be easier to model the time to reach a particular \log_{10} reduction (e.g. 4 or 6) rather than modelling the survival curve.

7.2. Survival in manure and soil

It appears to be difficult to develop valid models to assess the survival of VTEC O157:H7 in manure and soils. The conditions are not well defined with regard to soil characteristics (i.e. competing microflora in soil and manure, different strains of *E. coli*, different chemical composition of soils, variations in temperature, pH, and a_w). Furthermore, results from laboratory studies may not be valid under field conditions. It appears however, that there is evidence that the VTEC O157 survive in the soil and manure for an extended period, indicating a potential for contamination of fresh produce, surface, drinking or irrigation water. For example, the survival of *E. coli* in two types of soil (loamy clay and silty sands) were studied by Lau and Ingham (2001) who found that *E. coli* survived for 19 weeks and that $\leq 1 \log_{10}$ reduction was observed during the first 3 months. Ogden *et al.*, (2001) studied the survival of VTEC O157 under field and laboratory conditions, and found that the bacteria could survive on land for several months, finding a 3 to 6 \log_{10} reduction in 6 months. Moreover, Bolton *et al.*, (1999) found that a non-toxicogenic strain of *E. coli* O157:H7 inoculated in bovine faeces and stored on grassland, could survive for several months, although an 8 \log_{10} reduction was expected in 6 months. A similar reduction in numbers

after 6 months, was also found by Gagliardi and Karns (2000). Moreover it appeared that the soil type influenced the survival time. Thus, it appears that VTEC O157 has the potential to survive on pastures for several months. There is also a possibility of contamination and survival of VTEC O157 in rivers and lakes downstream from pastures or fields where manure is spread.

7.3. Cooling and freezing

The minimum temperature for growth of VTEC is reported to be 7°C and the highest 44.5°C with an optimum of 37°C (Meng and Doyle, 1998). In milk, growth of VTEC O157:H7 occurred at 7°C (Heuvelink *et al.*, 1998) and it appears that a temperature of ≤5°C is necessary to prevent growth of VTEC O157:H7 (McClure and Hall, 2000).

There is evidence that lower temperatures enhance survival of microorganisms in adverse conditions. Tamplin (2002) found that growth of 9 different strains of VTEC O157:H7, inoculated in retail ground beef and stored at 10°C, was suppressed by the native microflora (>4 log₁₀) compared to the USDA/ARS Pathogen Modelling Program (PMP). In irradiated ground beef, however, the growth rate and the maximum population density were similar to prediction from the PMP.

During frozen storage the number of VTEC O157:H7 declined (Semencheck and Golden, 1998) and that decline may be accelerated in the presence of 6% NaCl, although long periods of >15 months were required for substantial (> 2 log₁₀) reductions (Conner and Hall, 1996).

7.4. pH, pKa and undissociated acid

The growth pH-interval of VTEC O157:H7 ranges between pH 4.4-9.0. Acid adaptation (i.e. following contact with moderate acid environment) induces increased survival, thermotolerance and resistance to lower pH values, such as in apple cider with a pH <4.0 (Miller and Kaspar, 1994). However, depending on their pKa-values, the undissociated acid concentration also has to be considered. There is conflicting evidence on the efficacy of various acids in killing *E. coli*. According to some authors the order of efficiency is acetic acid > lactic acid > citric acid (Davidson, 2002) whereas Buchanan *et al.* (2002) found a different order of inactivating efficiency, i.e. lactic acid > acetic acid > malic acid > citric acid > HCl 0.5% (w/v) at pH 3.0, 37°C. Strain-to-strain variation in acid tolerance and of survivability of normal *E. coli* and of VTEC strains is considerable, but there is no evidence that acquisition of virulence increases acid tolerance or survivability.

7.5. Heat resistance

Heat resistance of VTEC O157:H7 has been studied in beef and is expressed as a D-value, meaning the heat treatment time, in minutes, at a given temperature required to reduce the number of a specific bacterium by one log₁₀ unit, or by 90%. In some regions of the world, regulations specify minimum cooking temperatures for ground beef. Heat treatment at 70°C for 2 min was recommended by the ACMSF (1995) for minced beef and minced

beef products, as well as by the Food Safety Authority of Ireland (Anon., 1999). Such treatment will deliver approximately a 6 log₁₀ reduction of VTEC O157:H7 (Stringer *et al.*, 2000). In the US, the Food Code (FDA, 1999) recommends that ground beef be cooked to an internal temperature of 66°C for 1 min, 68°C for 15 sec, or 70°C for <1 sec. These time-temperature relationships provide a 6.5 log₁₀ or greater reduction for salmonellae, a pathogen of comparable heat resistance to VTEC O157:H7.

As with other food pathogens, the measured thermal resistance of VTEC O157:H7 can be influenced by a range of factors, including the growth conditions and the growth phase of the cells, composition, pH and a_w of the growth medium, growth temperature, holding period before heat treatment, heat shock, the method of heating (e.g. use of open heating system and rate of heating), the medium being heated, including its composition, pH, a_w, choice of humectant, and the recovery conditions (ICMSF, 1980; Stringer *et al.*, 2000).

7.6. Combination with other parameters

The interrelationship between various parameters affecting heat resistance (D-values), such as pH, NaCl, sodium pyrophosphate, is illustrated in Table 3 (Juneja *et al.*, 1999). It was concluded that increasing sodium pyrophosphate concentration decreased heat resistance, whereas increasing NaCl increased heat resistance. There was some discrepancy between the observed values and those predicted by USDA/ARS Pathogen Modelling Program. Nevertheless, predictive models have proved to be useful tools in understanding the survival/growth/death of microorganisms. However, predictions should be validated for each food commodity group against independent data (from the scientific literature), and the actual conditions of (pH, temperature, salinity, etc.) anticipated along the food chain. Microbiological models need to be validated in foodstuffs and predictions should not be relied upon until validation that has been performed.

Table 3: Observed and predicted D-values (in minutes) at 55°C and 60°C of VTEC O157:H7 in beef gravy (Juneja *et al.*, 1999)

Temperature (°C)	pH	NaCl %	Sodium pyrophosphate %	D-value observed	D-value predicted
55	4	0	0	2.8	4.1
55	4	0	0.3	1.9	2.7
55	4	6	0.3	3.5	4.3
60	4	3	0.15	2.1	2.2
60	6	3	0.3	1.8	2.1

7.7. Modified atmospheres

In a four-factor growth model that accounted for the effects of CO₂, NaCl, pH and temperature in tryptone soya broth, Sutherland *et al.*, (1997)

concluded that VTEC O157:H7 survives at CO₂ concentrations of up to 80-100%.

VTEC O157:H7 was unable to grow at 4°C, and was almost totally inhibited at 10°C in ground beef in both high CO₂ /low CO mixture (60%-70% CO₂, 30-40% N₂ and 0.3-0.5% CO) and in high O₂ mixture (70% O₂, 30% CO₂) (Nissen *et al.*, 1999 and 2000).

7.8. Irradiation

In ground beef, a 1 log₁₀ reduction for VTEC O157:H7 has been reported at doses of 0.214 kGy and 0.307 kGy at temperature of 4±1°C and -16±1°C respectively (Clavero *et al.*, 1994). Kamat *et al.*, (2000) found that in ice cream at -72°C, VTEC O157:H19 showed a 1 log₁₀ reduction at 0.2 kGy and concluded that a low irradiation dose (1 kGy) could eliminate the natural number of pathogens present in ice cream without damaging the sensory quality. In general, microbial sensitivity to irradiation is greater at ambient temperatures than at freezing temperatures. Thayer and Boyd (1993) produced a 1 log₁₀ reduction of VTEC O157:H7 with doses ranging from 0.27 kGy at 5°C to 0.42 kGy at -5°C in mechanically deboned chicken meat. Since this organism is found in relatively low numbers, they concluded that an irradiation dose of 1.5 kGy, permitted by the Food and Drug Administration (FDA), would give a 4-5 log₁₀ reduction of VTEC and in practice eliminate it from minced meat.

7.9. Other approaches

Recently, hydrostatic pressure processing (HPP) has been introduced for bactericidal non-thermal food processing. When applied in milk and poultry Patterson and Kilpatrick (1998) showed its efficacy against VTEC O157:H7. Nevertheless they found a need for the combined use of pressure and temperatures. In UHT milk, 400 Mpa/50°C/15 min reduced populations of VTEC O157 by approximately 5 log₁₀ cfu/g. Teo *et al.*, (2001) found that treatment with 615 Mpa for 2 min would reduce the number of VTEC O157:H7 by between 8 and 0.4 log₁₀ cfu/g for grapefruit juice and apple juice respectively.

8. FOOD COMMODITIES OF CONCERN

In view of the known reservoirs, the routes of transmission, the possible effects of various extrinsic factors on bacterium growth/survival/death, and the epidemiological evidence, the following raw materials and derived food commodities have been considered (definitions of products and food commodities in Annex I).

8.1. Meat and meat products

8.1.1. Fresh meat

Based on a compilation of data prepared by the Community Reference Laboratory for the Epidemiology of Zoonoses and based on the National Zoonoses Reports for the years 1998 to 2000 (EC, 2002), the beef carcass prevalences of VTEC O157 were between 0 and 1.3%, and the prevalences in minced meat and meat products between 0 and 0.1%.

Contamination of carcasses generally occurs either directly or indirectly during slaughter (primary processing) or on subsequent handling. Of the main processing steps for slaughtering animals, dehiding, evisceration, trimming, movement to the chill and transport have the highest possibility of transferring VTEC to the carcass or between carcasses.

Duffy *et al.*, (2001) reported VTEC O157:H7 prevalences in ground beef and mutton of up to 6%, while in pork and poultry meat the prevalences were up to 1.5% and 4%, respectively. Chapman *et al.*, (1993a) reported prevalences of VTEC O157 on beef carcasses at abattoir level as high as 8.0% from rectal swab-negative and 30% from rectal swab-positive cattle. Other workers have reported prevalences of VTEC O157 in beef and veal carcasses of less than <1% and no findings in pig carcasses (Daube, 2001). As noted in chapter 6.7, individual prevalences of VTEC O157 in cattle and sheep range from 2% to 8%. Thus, during the slaughter process, it is likely that *E. coli* O157 are frequently present in the animal gut and in the faeces. In US meat packing plants, Ransom *et al.*, (2002) found VTEC O157 prevalences of 3.6% and 0.4% on beef hides and carcasses, respectively.

In slaughter plants it is not possible to fully ensure the absence of human pathogens, including HP-VTEC (ICMSF, 1998). However a number of interventions have been shown to reduce the possibility of contamination of carcasses (Bolton *et al.*, 2001). Prohibiting access to slaughter facilities of visibly soiled animals is one important preventive measure against the dissemination of foodborne pathogens including HP-VTEC (SCVPH, 1997). However, the efficacy of prohibiting entry of animals based on rejection of visibly soiled cattle, to reduce carcass contamination has been questioned (Van Donkersgoed *et al.*, 1997; Jordan *et al.*, 1999b). Knife trimming, water wash and the application of a steam vacuum are additional means to reduce or eliminate visible faecal contamination from carcasses (Castillo *et al.*, 1998a).

Sanitising treatments including hot water sprays (74-80°C), steam pasteurisation, organic acid sprays and other chemicals, e.g. trisodium phosphate (TSP), mixtures of nisin with 50 mM EDTA and acidified sodium chlorite solutions (ASC), have been studied for the reduction or elimination of contamination with VTEC O157:H7 on cattle carcasses (Castillo *et al.*, 2002). By spraying hot water onto different hot carcass surface regions, average reductions of initial counts for VTEC O157:H7 of 3.7 log₁₀/cm² could be obtained (Castillo *et al.*, 1998b). Steam pasteurisation in a chamber

operating above atmospheric pressure reduced VTEC O157:H7 by 3.7-4.4 log₁₀ cycles on surfaces of freshly slaughtered beef (Phebus *et al.*, 1997).

However, spraying of organic acids (acetic, lactic or citric acid) at different concentrations failed to reduce VTEC O157:H7 on beef sirloin pieces (Brackett *et al.*, 1994; Cutter and Siragusa, 1994; Uyttendaele *et al.*, 2001b) unless applied at 55°C (Hardin *et al.*, 1995; Castillo *et al.*, 1998b). Spraying of TSP solutions at 55°C resulted also in a reduction of VTEC O157:H7 on lean beef muscle ranging from 0.8 to 1.2 log₁₀/cm² (Dickson *et al.*, 1994). Furthermore, various mixtures of nisin + lactate, nisin + EDTA and nisin + trisodium-phosphate have also been advocated in inhibiting/reducing faecal contamination of surfaces (Cutter and Siragusa, 1995; Carneiro *et al.*, 1998).

Minced beef and blade-tenderised beef- Undercooking of ground beef or hamburger patties is a common cause of reported VTEC O157:H7 outbreaks. In a previous report concerning VTEC, the Scientific Veterinary Committee identified minced beef products, especially beefburgers, as high-risk foodstuffs (SVC, 1997). A large outbreak in the USA in 1993 (FSIS, 1993) recorded 732 affected people including 4 deaths, and identified undercooked hamburgers distributed from a particular fast food chain as the cause. According to US recommendations, hamburger patties should be cooked to an internal temperature of 68°C (Meng *et al.*, 1994).

Published prevalences on VTEC O157 in ground beef vary from 0% (Lindqvist *et al.*, 1998; Tarr *et al.*, 1999; Willshaw *et al.*, 1993) to 0.7% (Doyle and Schoeni, 1987), 1.3% (Kim and Doyle, 1992) and 2.4% (Sekla *et al.*, 1990). VTEC O157 has also been found in fresh sausage (Chinen *et al.*, 2001).

Duffy *et al.*, (2001) reported that under-cooked ground beef was associated with a number of outbreaks associated with VTEC. Although very few data in relation to illness linked to blade-tenderised steaks are available, the US National Advisory Committee for Microbiological Criteria for Foods concluded on the basis of research data that blade-tenderised beef steaks represent no greater risk than intact beef steaks with regard to VTEC O157:H7, if heated to temperatures above 60°C (AAFHV, 2002).

8.1.2. Fermented meat products (shelf stable raw cured meat products)

Illness due to HP-VTEC has been associated with the consumption of fermented sausages (CDCP, 1994; CDCP, 1995). The main reason for the survival in raw fermented meat products may be attributed to the acid tolerance of VTEC O157:H7 (Benjamin and Datta, 1995). However, in laboratory experiments Tsai and Chou (1996) could completely inhibit growth of VTEC O157:H7 at 37°C in the presence of 200 µg nitrite/ml at pH 5.0.

Shelf-stable raw cured meat products had been assumed to present no health risk to the consumer with regard to foodborne bacterial pathogens at a_w values of <0.91 or at ≤0.95 in combination with a pH-value less than 5.2. (Leistner and Rödel, 1976). However, Glass *et al.*, (1992) demonstrated the

survival of VTEC O157:H7 in fermented meats and recommended that manufacturers should use meat that contains no or very few VTEC O157:H7. Two strategies are possible; either to buy the beef from meat plants where the cattle faeces are tested for VTEC O157 without any positive results, or to use an intensive sampling plan to ensure that the concentration of VTEC O157 is for example below 1 per 500 g (ICMSF, 2002; Pruett *et al.*, 2002). However, it is difficult to ensure that raw meat is completely free of HP-VTEC by microbiological testing alone (Annex IV).

After two outbreaks had been linked directly to the consumption of contaminated fermented meats, VTEC O157:H7 was found on pre-sliced pepperoni from delicatessen counters. Subsequent investigations (Tilden *et al.*, 1996) revealed that the methods used to produce the salami were representative of procedures used industry-wide to produce Italian style salami, and complied with existing regulations and recommended good manufacturing practices (GMP). Moreover, contamination in the plant was not widespread and a limited amount of contaminated meat had been introduced into one or more of the 140 kg batches of slicing salami included in the lot implicated. As a result, the U.S. Department of Agriculture (USDA) Food Safety and Inspection Service (FSIS) developed guidelines (Reed, 1995) for sausage manufacturers to validate processes to ensure a 5 log₁₀ unit (5D) reduction in counts of VTEC O157:H7.

The five options currently specified by the USDA/FSIS are:

- (1) Utilise a heat process equal to 63°C for 4 min
- (2) Include a validated 5D inactivation treatment
- (3) Conduct a 'hold and test' programme for finished product
- (4) Propose other approaches to assure at least a 5D inactivation
- (5) Initiate a HACCP system that includes raw batter testing and a 2D inactivation.

Linking HP-VTEC to fermented meats led to widespread research to determine the extent of inactivation and of survival during the process and subsequent storage (Calicioglu *et al.*, 1997; Faith *et al.*, 1997, 1998 a,b,c; Hinkens *et al.*, 1997; Nissen and Holck, 1998; Riordan *et al.*, 1998).

8.2. Milk and milk products

8.2.1. Unpasteurised milk

VTEC O157:H7 has been identified as a possible contaminant of raw milk (Bryan, 1983). Since VTEC O157:H7 can be present in the intestinal content and faeces of dairy cows (Rice *et al.*, 1999; Heuvelink *et al.*, 1998; Upton and Coia, 1994), faecal contamination of raw cow's milk during its collection on dairy farms is recognised as a major VTEC transmission route (Tauxe *et al.*, 1997). Due to contamination of raw milk and/or improper processing, cow's milk has been implicated in foodborne outbreaks (Chapman *et al.*,

1993b; Keene *et al.*, 1997; Martin *et al.*, 1986; Trevena *et al.*, 1996). Whereas contamination of milk may be uncommon, in a summary of outbreaks of VTEC O157:H7 in the US from 1982 to 1995, Wachsmuth *et al.*, (1997) reported that raw milk was responsible for 5% of the outbreaks.

VTEC O157:H7 has also been isolated from milk filters (Heuvelink *et al.*, 1998). Reported estimates on the prevalence of VTEC O157:H7 in raw cow's milk range from 0 to 10% (Coia *et al.*, 2001; Neaves *et al.*, 1994; Hancock *et al.*, 1998; Heuvelink *et al.*, 1998). However, according to surveys carried out in various countries, for example Scotland (Coia *et al.*, 2001) the Netherlands (Heuvelink *et al.*, 1998) and Sweden (Jonsson *et al.*, 2001), the prevalence of VTEC O157:H7 in raw cow's milk at farm level seems to be extremely low.

8.2.2. Milk products

Unlike other strains of VTEC whose growth is impeded during the cheese making process due to low pH and salt addition (Park *et al.*, 1973), VTEC O157:H7 is relatively acid-tolerant (Reitsma and Henning, 1996) and has been isolated from soft cheese (Quinto and Capeda, 1997) and reported in an outbreak of HUS following consumption of contaminated soft cheese manufactured from unpasteurised milk (Deschenes *et al.*, 1996). Growth of VTEC O157:H7 has been observed in soft Hispanic type of cheese (Kasrazadeh and Genigeorgis, 1995) and cottage cheese (Arocha *et al.*, 1992). Coia *et al.*, (2001) found no VTEC O157:H7 in 739 raw-milk cheeses over a 2-year period, with pH values and a_w values ranging between 4.0 and 8.5 (mean of 6.0) and 0.75 - 0.99 (mean of 0.95), respectively. However, Casenave *et al.*, (1993) reported from France an outbreak of HUS associated with consumption of raw milk cheeses where the VTEC serogroup O119 was implicated.

The problems are the sporadic breakthroughs of faecal contamination that might carry HP-VTEC into the milk and subsequently in the milk products or cross-contamination of milk and milk products after pasteurisation. Point prevalence estimates from baseline studies are not very helpful in measuring the incidence of such breakthroughs or cross-contamination.

Consumption of ice cream or cream manufactured from unpasteurised milk can present a comparable risk to milk products. However, no reports of VTEC infections related to ice cream were located.

8.3. Fresh produce (fruits and vegetables)

8.3.1. Reported cases

Sprouted seeds, cantaloupes, carrots, apples, and leaf lettuces have been contaminated with VTEC O157:H7 and involved in outbreaks (Solomon *et al.*, 2002; Beuchat, 1996; De Rover, 1998). With cutters and slicers the contamination can be transmitted to unprotected surface areas facilitating bacterial growth. Various types of cutting have been shown to result in a six to seven-fold increase in microbial numbers (Garg *et al.*, 1990) and salad bar

vegetables have occasionally been implicated in VTEC outbreaks (Barnett *et al.*, 1995; Beuchat, 1996). Survival or growth of VTEC O157:H7 on shredded lettuce was not affected by packaging under modified atmospheres (Abdul-Raouf *et al.*, 1993).

Historically, fruits have been considered a low risk food with regard to VTEC infections. However, recently several reported foodborne illness outbreaks were attributed to the consumption of fruits. Cantaloupe has been associated with VTEC O157 infections (Anon., 1993b; Del Rosario and Beuchat, 1995) and two outbreaks of illness were associated with VTEC O157:H7 in unpasteurised apple juices³ in the Western United States and British Columbia, Canada (McCarthy, 1996, CDC, 1996).

Numerous international outbreaks involving VTEC O157:H7 associated with sprouted seeds have been reported (NACMCF, 1999a). The largest outbreak involved VTEC O157:H7 in contaminated radish sprouts, with over 6,000 infected people in Japan (Ministry of Health and Welfare of Japan, 1997; Michino *et al.*, 1999). Several other Japanese outbreaks have been associated with sprouted radish seeds (Ministry of Health and Welfare of Japan, 1997).

Microbiological analyses have shown that alfalfa seeds routinely contain high levels of microorganisms, including coliforms and faecal coliforms (NACMCF, 1999a; Taormina *et al.*, 1999b). The source of pathogens can be the seeds, contaminated water or workers. VTEC O157:H7 will multiply during sprout germination (Andrews *et al.*, 1982; Brown and Oscroft, 1989; NACMCF, 1999b) since conditions under which seeds are sprouted (growing time, temperature, moisture and nutrients) are ideal for bacterial proliferation (Feng, 1997; Taormina and Beuchat, 1999a). An increase of 100 to 1000 fold (Prokopowich and Blank, 1991; Feng, 1997) can occur and bacterial numbers may exceed 10^7 per gram without affecting the appearance of the product (Taormina and Beuchat, 1999b).

No effective decontamination process has been identified that will substantially reduce or eliminate pathogens in sprouts (NACMCF, 1999b). Nevertheless, by washing seeds with chlorinated water one can achieve some degree of reduction (Beuchat *et al.*, 2001).

8.3.2. Pre- and post- harvest conditions affecting the risk of VTEC O157 along the fresh produce food pathway

Fruits and vegetables can become contaminated with VTEC O157 whilst growing in fields, or during harvest, handling, washing/cleaning, processing, distribution, retail, preparation, and final use (Beuchat, 1996; De Rover, 1998). Contamination may be due to the use of improperly treated manure as fertiliser, exposure to faecally contaminated irrigation or washing water or contacts with animals, birds, or insects pre and post harvest. The extent and

³ The terms cider and apple juice can cause confusion as the term 'cider' in the USA signifies unfermented apple juice, while in Europe 'cider' is understood as fermented apple juice, which therefore contains alcohol.

the impact of this kind of contamination on consumer health are unclear, since limited data are available.

Irrigation - A number of recent VTEC O157:H7 outbreaks in the USA have been linked to irrigation with contaminated water (CDC, 1999b). The transfer of foodborne pathogenic microorganisms from irrigation water to fruits and vegetables will depend on the irrigation technique used (e.g. sprinklers) and on the nature of the produce e.g. carrots or lettuce (NACMCF, 1999b). It may be noted that VTEC O157 will survive for prolonged periods in fresh water, especially at low temperatures (Maule, 2000; McDowell and Sheridan, 2001; Wang and Doyle, 1998; Warburton *et al.*, 1998).

Fertilising - Sewage, manure, slurry, sludge and compost of human and animal origin are commonly used as organic fertilisers for fruit and vegetable production. Several epidemiological investigations have identified manure as the source of contamination of VTEC outbreaks (Tauxe *et al.*, 1997; Nguyen-the and Carlin, 2000; Hancock *et al.*, 1998; Randall *et al.*, 1999; Lung *et al.*, 2001). The microbiological process during composting or aeration is not well understood and Tauxe *et al.*, (1997) recommended that the adequacy of existing methods and regulations for composting needed to be reviewed, since the composting of solid manure or aeration of liquid manure may not ensure elimination of VTEC O157:H7.

Post-harvest treatment of fruits and vegetables includes handling, storage, transportation and cleaning. During this process, conditions may arise which lead to cross-contamination of the produce from other agricultural materials, from the workers, or from the environment (e.g. animals and water).

It is not possible to rely solely on disinfection to control contamination by pathogens and Beuchat (1999) concluded that prevention of contamination at all points of the food chain is preferred over the application of decontamination. In the European Chilled Food Federation (ECFF) review (2000), the potential implications and appropriate controls for VTEC in fruits and vegetables are discussed. The report concludes that there is a general lack of guidance available on the control of VTEC at the primary production level, and guidance on effective control measures for VTEC.

8.4. Herbs and spices

Herbs and spices could present a hazard since the production practices are similar to those used for vegetables, and fertiliser and irrigation water and the storage environment can also be a source of contamination. Untreated whole and ground spices have been reported to harbour coliform bacteria (Pafumi, 1986), but *E. coli* was less frequently isolated (Baxter and Holzapfel, 1982). Continuous vigilance appears to be justified, as there has recently been a large outbreak associated with parsley and cilantro in the USA, although, this outbreak was associated with *Shigella* and ETEC (CDC, 1999a).

To date, no published data are available on the occurrence of VTEC in spices or herbs, but the absence of reports of isolation of these pathogens could be due to the effects on microorganisms of the ionising technologies usually applied to these products.

8.5. Cross-contamination

In order to study the risk of cross-contamination at slaughter, Bonardi *et al.*, (2001) isolated this organism from 7.3% (stool-negative cattle) to 35.3% (stool-positive) of the carcasses. Most of the contaminated carcasses from stool-negative animals were adjacent on the production line to those of stool-positive cattle. Sub-typing of the VTEC O157 isolates confirmed that cross-contamination during slaughter might occur by direct or by indirect (e.g., contaminated equipment) contact. Cross-contamination occurred more frequently in highly mechanised slaughterhouses with a high slaughter capacity (Bonardi *et al.*, 2001). Elder *et al.*, (2000) found a correlation between VTEC O157 prevalences in faeces, hides and carcasses of cattle in a study Midwestern USA.

In a Dutch survey, Heuvelink *et al.* (2001) found that around 45% of 33 EU-approved cattle and calf slaughterhouses inspected had structural deficiencies likely to lead to structural cross-contamination of carcasses, by direct carcass-carcass contact or by indirect contact through floors, walls or steps. In 39% of the slaughterhouses, cleaning and disinfection procedures were inadequate, causing an increased risk for indirect cross-contamination. Nevertheless, in this study no VTEC O157 was isolated from any of the pooled carcass samples (33x10) tested.

During further storage, transport and processing (de-boning, cutting, mincing), cross-contamination may occur directly due to poor hygiene and infected food handlers, or indirectly through equipment and utensils. Cross-contamination can also occur by insufficient separation between cooked products or ready-to-eat foods and raw material. For example, cross-contamination between raw beef and processed meats coupled with inadequate refrigeration was thought to be the cause of a Scottish VTEC outbreak (Pennington Group Report, 1997).

Filtration of collected raw milk at the farm can cause cross-contamination via the filter itself. Cross-contamination may also occur at further processing stages due to an inadequate cleaning and disinfection procedure at the pasteurisation station. VTEC O157:H7 has been associated with a large outbreak (more than 100 persons affected) due to the consumption of pasteurised milk. During inspection of the processing plant the same phage-type was isolated from pipes to the bottling machine as well as from rubber seals from the same machine. Findings thus suggest post-process contamination (Upton and Coia, 1994). Although fermented dairy products manufactured from pasteurised milk are commonly considered to be intrinsically safe, various fermented products have been implicated in VTEC outbreaks due to post-processing contamination (Dineen *et al.*, 1998; Morgan *et al.*, 1993). Massa *et al.*, (1997) only observed small reductions (0.8-2 log₁₀ units) in VTEC O157:H7 numbers during processing and storage

of yoghurt. Similar rates of inactivation have been observed for cheddar cheese (Reitsma and Henning, 1996) and in other dairy products inoculated with VTEC O157:H7, e.g. sour cream and buttermilk (Dineen *et al.*, 1998). Hence, after recontamination of yoghurt, sour cream and buttermilk, VTEC O157 bacteria were able to survive for 12, 28 and 35 days respectively. Survival times in Colby, Feta and Romana cheeses were 27, 27 and 30 days, respectively (Hudson *et al.*, 1997).

Bulk mayonnaise was found to be the vehicle for transmission of VTEC O157:H7 in an outbreak in the United States in 1993 (Anon., 1993a). The primary source for VTEC O157:H7 appeared to be cross-contamination of the mayonnaise from fresh beef.

8.6. Food commodities representing a health risk

It appears that risk factors for human exposure to HP-VTEC are linked to either direct or indirect exposure to ruminants and ingestion of food commodities contaminated by faecal contents from ruminants or humans. This exposure could be minuscule given that the infectious dose could be as low as 10 bacteria (Doyle *et al.*, 1997). The identified foodborne pathways for exposure were raw or undercooked beef, unpasteurised milk and products thereof, fresh fruits and vegetables or products thereof that have been contaminated by manure or exposed to contaminated irrigation or processing water (Tables 4 and 5) and contaminated drinking water. Moreover, further exposure could result from cross-contamination at the primary and secondary production stages by faecal contents from wild or domestic ruminants or humans, or cross-contamination from raw meat products. It appears that sprouts might be a particular risk vegetable since the bacteria might multiply during sprouting.

The commodities of most concern are considered to be raw or undercooked beef, especially minced meat, meat products and fermented meat products. Other ruminant meats (e.g., mutton, venison, and goat meat) should also be considered. Other commodities of concern include raw milk and products thereof (such as raw milk cheese) as well as contaminated surface water, fresh produce in particular sprouted seeds, and unpasteurised fruit or vegetable juices. In addition, foodstuffs that were cross-contaminated with HP-VTEC after the last processing step (e.g. heat treatment) and where the remaining parts of the food chain enable growth and/or survival of the bacteria will represent a hazard for the consumer.

The occurrence of contamination with HP-VTEC appears to be sporadic in many foodstuffs. A point prevalence measure, the usual outcome of a baseline monitoring study, may in this case not reflect the risks associated with the monitored foodstuff. Instead, the true risk of consumer exposures to HP-VTEC is the result of cumulative exposures of HP-VTEC coupled with conditions enabling multiplication and survival along the food chain thereafter.

Table 4: Example of foodborne and waterborne outbreaks caused by VTEC O157 (Willshaw *et al.*, 2000; Duffy *et al.*, 2001). References are given for additional reported outbreaks.

Year	Country	Location	Total no. of cases	HUS no. of cases	Fatalities no. of cases	Transmission
1982	USA	Community ⁴	26			Hamburgers
1985	England	Nursing home	49		1	Handling raw vegetables*
1985	Canada	Nursing home	73	12	17	Sandwiches* person to person
1989	USA	Community	243	2	4	Water*
1990	Japan	Kindergarten	319		2	Water*
1991	England	Fast food restaurant	23	3		Beefburgers*
1992	Swaziland		20000			Contaminated potable water
1993	England	Dairy farm	7	3		Raw milk
1993	Wales	Community	17	1		Butcher meat
1993	USA	Fast food restaurant	732	55	4	Beefburgers
1994	Scotland	Community	100	9	1	Pasteurised milk
1994	USA	Community	19	5	0	Dried fermented salami (Doyle <i>et al.</i> , 1997)
1996	Japan	Schools, Community	5727	>100	3	White radish sprouted seeds*
1996	Scotland	Community, Nursing home	501	27	21	Cooked meats, gravy
1996	USA, Canada	Community	66	12	1	Unpasteurised apple juice
1996	Germany	Community		28		Sausages (mortadella, teewurst) (Ammon <i>et al.</i> , 1999). Note Sorbitol-fermenting VTEC O157:H
1996	USA	Community	61			Mesclun lettuce from one grower (Hilborn <i>et al.</i> , 1999)
1999	USA	Restaurant	72			Shredded iceberg lettuce
2000	Canada		2000			Potable water
2002	Sweden	Community	28	3		Cold smoked sausage of beef origin (Hjertkvist <i>et al.</i> , 2002)

* suspected transmission

⁴ The term community in regard to outbreaks refers to a widely disseminated epidemic throughout a village, neighbourhood, city or region.

Table 5: Examples of foodborne outbreaks caused by VTEC non-O157 from Willshaw *et al.*, (2000). References are given for additional reported outbreaks.

Year	Country	Location	Total no. of cases	HUS no. of cases	Fatalities no. of cases	Transmission	Serotypes
1984	Japan	Elementary school	100			Unknown	O145:H-
1986	Japan	Orphanage	22		1	Unknown	O111:H-
1991	Japan	Elementary school	234			Unknown	O111:H-
1991	Japan	Elementary school	89			Unknown	O?:H19
1992	Italy	Community		9		Unknown	O111:H-
1992	France	Community		10		Person to person	O111
1994	USA	Community	19			Pasteurised milk	O104:H21
1995	Australia	Community	>200	22	1	Mettwurst	O111:H-
1995	Spain		13			Drinking water	O111:H-
1999	USA	Teenage Campers	56				O111:H8 (CDC, 2000)
2001	USA	Community	18			Pasteurised milk later contaminated	O104:H21 (Feng <i>et al.</i> , 2001)

9. EVALUATE THE APPROPRIATENESS OF SETTING MICROBIOLOGICAL CRITERIA

9.1. General considerations

Whether the implementation of a microbiological criterion might contribute meaningfully to the reduction of public health risks, should be judged for each pathogen food commodity combination.

It might be difficult to show a uniform reduction in associated public health risks following the introduction of a criterion since the initial prevalence in a food lot or process will vary. For example, production and processing practices, heterogeneity of pathogen distributions, and regional or seasonal variations might affect the prevalence of contamination and consequently the efficacy of a criterion. The risk reduction afforded by the implementation of microbiological criteria is correlated with the prevalence of contaminated food items, the number of samples taken and testing negative, and the efficiency of the analytical method used. If the diagnostic sensitivity of methods is less than 100%, the risk reduction afforded by microbiological criteria will be reduced accordingly. On the other hand if the diagnostic specificity is less than 100%, there is a risk of false positives and a need for confirmatory testing procedures (see Annexes II and III).

End product testing using microbiological criteria may have limited usefulness for food safety for a number of reasons, including low prevalence of the pathogen, or low diagnostic sensitivity of the testing procedure applied. While the finding of a pathogen in a foodstuff may indicate a problem for public health, necessitating appropriate risk management action; the failure on the other hand to detect a pathogen in a food product does not necessarily mean that the pathogen is absent from that food product, process or food lot.

Nevertheless, microbiological testing can be used in monitoring programmes along the food chain, for documentation purposes, HACCP, as an indicator of adherence to Good Hygienic Practices (GHP), on-the spot checks, monitoring the suitability of raw materials or food ingredients, and the hygienic status of the processing environment, all of which play an important role in maintaining food safety. Even if the application of a microbiological criterion does not result in a marked change in average prevalence of the pathogen, its implementation might facilitate official surveillance and inspection, and imposition of corrective action in the case of any unfavourable findings. Moreover, the use of a criterion can yield very useful results when collated and analysed on a national or regional scale, i.e., baseline prevalence studies that can be helpful in assessing risks associated with a particular pathogen. The use of equivalent methodologies is crucial for yielding comparable results.

If there are situations where a high prevalence is suspected e.g., indicated by trace back investigations, microbiological criteria may be useful in reducing the risk (Annex III). However, even the taking of a large number of samples cannot guarantee the absence of a pathogen, merely that the presence (prevalence or concentration) is less than a certain limit with for example 95% confidence (Annex IV).

9.2. Food safety concepts

A short outline of current food safety concepts will be presented before discussing the usefulness of microbiological criteria. In considering food safety issues, the answers to the following questions are helpful:

1. What is the microbiological concern associated with the food (hazard)?
2. What is the frequency of its occurrence and consequences?
3. Are there appropriate control options available?
4. How can such control options be implemented and what is the expected efficacy?
5. How may control measure(s) be put into operation?

The setting of microbiological criteria is one risk management option available for managers to control a hazard. Microbiological criteria and taking of appropriate corrective action of food found to be contaminated can contribute to better food safety. However, it appears that in many cases they

are not sufficient as a solitary control option, and there is a need for an integrated control strategy i.e., process controls of hazard analysis critical control points (HACCP) is one example. The process of managing a food safety problem is described in the Codex Committee for Food Hygiene (CCFH) document on risk management (CX/FH, 2001). Microbiological criteria should thus be implemented in the context of a risk analysis to clarify the benefits to public health and the cost effectiveness of the criteria.

Hazard analysis critical control points (HACCP) - The recognition that food safety cannot be assured by end-product testing alone led to the development of the concepts of HACCP, to supplement Good Agricultural Practice (GAP), Good Hygiene Practice (GHP), and Good Manufacturing Practice (GMP). The HACCP system was conceived by the Pillsbury Company, together with the National Aeronautics and Space Administration (NASA) and the U.S. Army Laboratories at Natick, who developed this system to ensure the safety of astronauts' food. In the thirty years since then, the HACCP system has become the generally accepted method for food safety assurance. The recent growing worldwide concern about food safety by public health authorities, consumers and other concerned parties and the continuous reports of foodborne outbreaks have given a further impetus to the application of the HACCP system. The HACCP system achieves process control by identifying hazards and critical control points in the process and establishing critical limits at these control points for the identified hazards (i.e., microbiological criteria), establishing systems for monitoring the critical control points and indicating suitable corrective actions if the critical limits are exceeded, and establishing suitable verification and documentation procedures (WHO, 1998).

Risk profile – Elaboration of a risk profile is the initial step in the risk management of a food safety problem. The risk profile should provide as much information as possible to the risk managers to guide further actions and it should be carried out in collaboration between risk assessors and risk managers. The outcome of the risk profile should guide the risk managers either to develop a control strategy or to commission a formal risk assessment.

Risk assessment - The purpose of the risk assessment is to enable the risk managers to make informed decisions on management options to be taken. The risk profile will assist the managers in defining specific questions that should be addressed. The outcome of a risk assessment is a risk estimate i.e., the likelihood and severity of adverse effects that occur in given population with associated uncertainties (CAC/GL-30, 1999).

Appropriate Level of Protection (ALOP) is a quantification of the disease burden within a country linked to the implementation of food safety systems. ALOP is derived from a risk assessment and is expressed as e.g., the likelihood to suffer a food related illness from a food serving, or the number of cases per 100,000 consumer years. The setting of an ALOP is a risk management decision.

Food Safety Objectives (FSO) may be an important element in guidance on options to be taken for the future safety of foods. The concept is still evolving and no definition has yet been agreed upon. The proposed definition in the Codex document (CX/FH, 2001) is as follows: “the maximum frequency and/or concentration of a microbiological hazard in a food at the time of consumption that provides the appropriate level of health protection (ALOP)”. The FSO does not guide an operator on the control options, such as microbiological criteria, to be taken.

9.3. Microbiological testing and criteria

9.3.1. Microbiological testing

Many different types of microbiological testing can be used to assure the safety of foods. Comparisons are easier if sampling procedures and microbiological methods are equivalent.

Microbiological testing in monitoring and surveillance can be used for:

- identifying trends in human illness caused by foodborne pathogens e.g., sentinel studies,
- establishing baseline prevalences in primary production and in later stages of the food chain, i.e., testing foods in distribution or at retail,
- estimating the load of bacterial pathogens in foods reaching the consumer (e.g., when assessing exposures of a pathogen),
- measuring compliance with good hygienic practices, and
- measuring the effect of intervention measures such as control programs.

When sampling procedures and microbiological methods are standardised, monitoring allows inferences to be made about the safety of food derived from more than one batch (lot) of food, as occurs with animals from different farms at a slaughterhouse, or with large consignments of food at a port-of-entry.

With similarly standardised sampling procedures and microbiological methods, monitoring can establish the baseline prevalences of bacterial pathogens in foods e.g. as in the US baseline studies in meat and poultry, and assist risk analyses.

Investigational sampling – is both intensive and focussed. It is mainly used by the food industry to investigate foods when a process is suspected of failure, or when foods have been stored accidentally under inappropriate conditions. The results of investigational sampling are therefore not comparable to the results from baseline studies.

9.3.2. Microbiological criteria

The Codex document on Principles for the establishment of Microbiological Criteria (CAC, 1997a) used the following definition for **microbiological criteria** – “*a microbiological criterion for foodstuffs defines the acceptability of a product or food lot based on the absence or presence, or number of microorganisms including parasites and/or quantity of their toxins/metabolites, per unit(s) of mass, volume, area or lot*”.

The Scientific Committee on Veterinary Measure relating to Public Health also gave a definition for a microbiological criterion in its opinion of 1999 (SCVPH, 1999). The definition differs from the Codex definition in that the word “*process*,” is included before the word “*product*”, extending the use of microbiological criteria to the whole food chain.

The SCVPH already stressed in a previous Opinion that the mere existence of microbiological criteria does not protect consumer health. The use of Good Hygienic Practice (GHP) and Hazard Analysis Critical Control Point (HACCP) systems will be more important in ensuring that pathogens are eliminated, or minimised to the extent that they cannot cause illness (SCVPH Opinion, 1999).

The intention of microbiological criteria is to ensure the health of the consumer by providing safe, wholesome food products, and to meet the requirements of fair practices in trade. Thus, the introduction and implementation of a criterion should not be an *ad-hoc* measure, but rather the outcome of a deliberate process. Hence, a “*microbiological criterion should be established and applied only where there is a definitive need for it and where it can be shown to be effective and practical*” (EC, 1997).

A microbiological criterion should include (CAC, 1997b) a statement of the micro-organisms of concern (e.g., *Salmonella* or VTEC *O157*); the analytical methods for their detection and/or quantification; a plan defining the number of field samples to be taken and the size of the analytical unit; the microbial limits acceptable at that particular point in the food chain; and the number of analytical units samples that should conform to these limits. Moreover, the criterion should state the foodstuff to which criterion applies, the point(s) in the food chain where the criterion applies, and any actions foreseen if the criterion is not met.

When applying criteria for assessing products, it is essential that only appropriate tests are applied to those foods and at those points in the food chain that offer maximum consumer benefits in terms of food safety (CAC, 1997b).

It appears that there is a consensus that microbiological criteria should not be applied arbitrarily, but rather as the outcome of a deliberate process to achieve the optimal food safety.

Microbiological criteria can be applied differently, as:

- Microbiological standards,
- Microbiological guidelines, or
- Specifications.

Microbiological standards – are mandatory criteria based on legal requirements, where failure to comply results appropriate actions e.g., reprocessing, rejection or destruction of the food.

Microbiological guidelines – may be established during production and processing, or on the end-products, and should be based on best practices. Manufacturers and food inspectors use guidelines for the verification of safe and hygienic production, and corrective actions in the process are taken when the guidelines are exceeded. Such guidelines should be established to detect deviations from the food process representing a danger for human health or hygiene failures.

Specifications - microbiological criteria used for contractual purposes by food businesses must not be confused with legal requirements of official control purposes (EC, 1997). Specifications are not discussed in this document.

9.3.3. Considerations of sampling and laboratory techniques

Having decided upon the need for a microbiological criterion for a particular food, aspects of the sampling and microbiological techniques are considered.

If the prevalence of a pathogen in the food lot or the diagnostic sensitivity of the procedure applied is low, and/or its distribution in the food is heterogeneous, the probability of detecting the pathogen will be low (WHO, 1988; ICMSF, 2002).

A lot - A lot (batch) is a quantity of food or food units produced and handled under uniform conditions. Which implies that the pathogens are homogeneously distributed within a lot, as occurs with liquid foods. However, regarding levels of microbial contamination and distribution this rarely occurs with most of the solid foods. This heterogeneity is magnified when a lot is not well defined, as occurs with animals at a slaughterhouse or a large consignment of food. If a consignment is, in fact, made up of several different lots, the stringency of a given sampling plan and its ability to discriminate between acceptable and non-acceptable production may be reduced. Consequently, a poorly defined lot will reduce the efficacy of microbiological criteria.

Stage of processing - other considerations might include stage of processing and where in the food chain the samples are taken. The risk reduction from the application of the criteria will be correlated with the prevalence of pathogens in the foodstuff at the particular sampling point. However, application of microbiological criteria might be of limited relevance to public health if the foodstuff undergoes, for example, heat treatment *after* sampling but *before* consumption.

Pooling of samples – enables reduction of laboratory effort while maintaining the stringency of sampling plans where a single positive results in rejection of the consignment. Considerable cost reductions of analyses can be achieved by pooling analytical units. Alternatively, pooling allows examination of large numbers of analytical units, increasing the stringency of examination, without increasing laboratory effort. This approach is suitable for dried foods and foods of high moisture content including eggs, poultry meat, meat and meat products (Silliker and Gabis, 1973; Gabis and Silliker, 1974). However, Christensen and Gardener (2000) noted that the advantages of pooling were greatest when the prevalence was low (<5%), but this advantage will decrease as the prevalence increases. If the samples from a food lot are pooled into one, it is not possible to assess the ‘within lot’ prevalence of the pathogen, only the qualitative question of absence or presence of the pathogen. Moreover, the sensitivity of pooled sampling may also be influenced by the detection limits of the analytical procedure and possible dilution effects due the pooling procedure. The effect of pooling of samples will depend on factors such as true prevalence of the pathogen, pool size, amount of specimen to be tested, number of pooled tests and the comparative performance of pooled and individual tests. Therefore, the appropriateness of pooling procedures should be judged on a case-by-case basis for each pathogen commodity combination having regard to all these factors.

Test characteristics - if the tests used have a perfect diagnostic sensitivity and specificity (100%) the measured apparent prevalence will equal the true prevalence. See Annexes II and III for more detailed discussions of predictive values and test characteristics and the calculated risk reductions by application of microbiological criteria.

If the prevalence of a pathogen in a batch is low, or the diagnostic sensitivity of the procedure applied is low, and/or its distribution in the food is heterogeneous, the consequent probability of detecting the pathogen is also low (WHO, 1998; ICMSF, 2002).

9.4. Appropriateness of setting criteria for VTEC O157

The hazard for the consumer originates from the direct or indirect faecal contamination of ruminant or human origin into the food chain, and the survival or possible growth thereafter of the agent until the point of consumption. This faecal contamination may or may not contain HP-VTEC. Finding HP-VTEC, in particular VTEC O157, in a foodstuff indicates a public health risk necessitating appropriate actions. However, due to the sporadic occurrence and low prevalence of VTEC O157 found in food commodities representing a risk, applying end product microbiological standards is unlikely to deliver meaningful reductions in associated risk for the consumer.

Where prior evidence (e.g., trace back investigations or results of on-the-spot inspections), suggests faecal contamination of the food or the category of food, or the high prevalence of a HP-VTEC, the application of microbiological criteria and implementation of appropriate actions for the

foods found to be contaminated can deliver meaningful reductions in risk to the consumer.

Moreover, microbiological guidelines and corrective actions aimed at reducing faecal contamination along the food chain e.g., testing for *Enterobacteriaceae* (ICMSF, 1988; Mossel, 1967), can contribute to a reduction of public health risks including HP-VTEC.

10. VTEC AND USEFULNESS OF A RISK PROFILE

10.1. Previous risk assessments

Two quantitative risk assessments (QRA) for *Escherichia coli* O157 have been published for ground beef hamburgers. One Canadian model estimated the probability of illness from a single meal for adults to be 5 per 100,000 meals (Cassin *et al.*, 1998). For the very young, the probabilities of haemolytic uraemic syndrome (HUS) and death were estimated to be 3.7 and 0.19 per million meals consumed, respectively.

The exposure model used by Nauta *et al.*, (2001) predicted that approximately 0.3% of ground beef patties are contaminated with VTEC O157. Of these contaminated patties, over 60% were contaminated with one cfu only and only 7% contained more than 10 cfus. In the Dutch study, the probability of a single VTEC O157 bacteria resulting in illness was estimated to be approximately 0.5%, and around 1,300 cases of gastroenteritis associated with VTEC O157 in steak tartare were predicted per year. Compared with the estimated total number of VTEC O157 cases (2000 cases) based on epidemiological data, it appears that a large fraction is associated with “steak tartare” consumption, however there were large uncertainties regarding the estimates. Nauta *et al.*, (2001) found the effects of growth of VTEC O157 during retail and domestic storage to be negligible, and the effects on consumer behaviour by advocating the consumption of well-cooked steak tartare to be questionable. Hence, interventions at the farm level or at slaughter were considered more suitable to reduce the VTEC O157 associated risks compared with interventions at the consumer level.

In a US draft baseline risk assessment study, the prevalences of VTEC O157:H7 in grinder loads (for raw beef patties) were estimated to be between 40% and 88% in the low prevalence season (winter) and between 61% and 94% in the high prevalence season (summer). The prevalence of VTEC O157:H7 contaminated (cooked) servings was estimated to be between 2.8 and 14.3 per 100,000 in winter and between 6.7 and 30.3 per 100,000 in summer. Among the contaminated servings, 95% had less than 10 VTEC O157:H7. Approximately, 1.7 and 0.6 per million ground beef servings consumed during summer and winter, respectively, were predicted to result in illness. Although children under 5 years of age consume fewer (7%) and smaller servings (44g compared with 90g), the risk of this age group was predicted to be 2.5 times higher than for the rest of the population (FSIS, 2002).

An overview of the assessments conducted during the last years appears in Table 6 as reported by Codex Alimentarius.

Table 6: Risk assessments for VTEC O157:H7 as reported by Codex Alimentarius Commission (CAC/CX/FH/ 03/5-Add.4 table 3, 2002)

Country	Topic	Reference
Australia	ground beef	Lammerding, 1999
Australia	STEC in ground beef	Lammerding, 1999
Canada	ground beef hamburgers	Cassin, 1998
Canada	Seeds/beans and sprouted seeds	Personal communication Health Canada
Canada	Unpasteurised fruit juice/ciders	Personal communication Health Canada
Canada	Pre-harvest husbandry practices	Jordan, 1999 a,b
N. Ireland	Beef/beef products	http://www.science.ulst.ac.uk/food/E_coli_risk_Assess.html
The Netherlands	Raw fermented products	http://www.research.teagasc.ie/vteceurope/S+Gprog/hoornstrasg.html
The Netherlands	Steak tartare	RIVM report 257851003/2001*
USA	Ground beef	http://www.fsis.usda.gov/_oppde/rdad/frpubs/00-023Nreport.pdf
USA	Tenderized vs non tenderized beef steak	Personal communication USDA

*Nauta *et al.*, 2001

11. COMMODITIES WHERE A RISK PROFILE MIGHT BE USEFUL

In the terms of reference to this opinion the Committee was asked to identify where a risk profile might be useful in respect of HP-VTEC. This report can be considered a risk profile for:

- raw or undercooked beef and possibly other ruminant meats (sheep, goat and deer),
- minced and/or fermented beef, and products thereof,
- raw milk and raw milk products,
- fresh produce, in particular sprouted seeds, and
- unpasteurised fruit and vegetable juices.

For all these foodstuffs the risk analysis process should continue with a view to taking appropriate risk management actions. In the further risk

management activities, one should take advantage of the risk assessments already done for HP-VTEC.

It is suggested that a risk profile with regard to HP-VTEC should be developed for water intended for drinking, processing or irrigation because of HP-VTEC outbreaks associated with water from different sources.

12. CONCLUSIONS

12.1. General conclusions

- Human pathogenic verotoxigenic *E. coli* (HP-VTEC) are responsible for human illness ranging from diarrhoea to HUS and TTP, sometimes with a lethal outcome. Most of the reported cases were associated with HP-VTEC O157.
- The terminology used to describe human pathogenic VTEC (HP-VTEC), VTEC, STEC, EHEC, *Escherichia coli* O157:H7, or *E. coli* O157; is confusing and creates difficulties in the interpretation of reports.
- Only a small fraction of all VTEC-types isolated from animals, food or the environment are associated with human illness.
- Foodborne VTEC O157 infections originate from the direct or indirect faecal contamination, of ruminant or human origin, of the food chain, and the survival of the agent until the point of consumption. The infectious dose for VTEC O157 is very low and infection may result from eating contaminated food where the bacteria have survived but not necessarily grown.
- There is currently insufficient information to identify the burden and main sources of non-O157 VTEC infections in humans.
- The reported incidences of disease caused by HP-VTEC are not comparable within the EU due to variations in the diagnostic methods and procedures, and the reporting systems used.
- Outbreaks strains of VTEC O157 are unusually acid-tolerant.
- No routine method to detect all HP-VTEC types is available apart for O157.
- Water contaminated with VTEC O157 and O111 has been associated with several HP-VTEC outbreaks.

12.2. Categories of foodstuffs where verotoxigenic *E. coli* (VTEC) represents a hazard to public health

The following categories have been identified:

- raw or undercooked beef and possibly meat from other ruminants,

- minced and/or fermented beef and products thereof,
- raw milk and raw milk products,
- fresh produce, in particular sprouted seeds, and
- unpasteurised fruit and vegetable juices.

12.3. Appropriateness of setting microbiological criteria

- The risk reduction afforded by microbiological criteria is correlated with the prevalence of defected items in the food lot, the sampling plan and the diagnostic sensitivity of the testing procedure.
- Failure to detect a pathogen in a food product does not ensure that the pathogen is absent from that food product.
- Finding HP-VTEC, in particular VTEC O157, in a foodstuff indicates a public health risk necessitating appropriate actions.
- Due to the sporadic occurrence and low prevalence of VTEC O157 found in food commodities representing a risk, applying end product microbiological standards for VTEC O157 is unlikely to deliver meaningful reductions in associated risk for the consumer. However if there are reasons to suspect a high prevalence, microbiological criteria and appropriate actions may be useful in controlling the risk.
- Microbiological guidelines aimed at reducing the faecal contamination along the food chain, e.g. testing for *Enterobacteriaceae*, can contribute to a reduction of public health risks including HP-VTEC.

12.4. Identify where risk profile might be useful

- This report can be considered a risk profile for: raw, undercooked or undercooked beef and possibly meat from other ruminants, minced and/or fermented beef, and products thereof, raw milk and raw milk products, fresh produce, in particular sprouted seeds, and unpasteurised fruit and vegetable juices.
- A risk profile would be useful for water intended for drinking or processing or irrigation with regard to HP-VTEC.

13. RECOMMENDATIONS

- A common terminology, case definition, diagnostic and reporting procedures would help to clarify the epidemiological situation within the EU regarding HP-VTEC.
- Haemolytic uraemic syndrome (HUS) should be made compulsorily notifiable throughout the EU.

- A risk profile should be developed for water intended for drinking or processing or irrigation with regard to HP-VTEC.
- A microbiological standard should be implemented only when a meaningful reduction of the public health risk of that particular pathogen food commodity combination can be anticipated.
- It is currently not appropriate to set microbiological standards for HP-VTEC in general and for VTEC O157 in particular. However, if a high prevalence is suspected in the food, microbiological criteria and appropriate actions will reduce the public health risk.
- The potential of microbiological guidelines e.g. testing for *Enterobacteriaceae* to reduce the faecal contamination along the food chain and thereby reduce the public health risks concerning HP-VTEC should be explored.
- Methods to detect non-O157 HP-VTEC should be developed and applied in the food chain.

14. GLOSSARY

ALARA – As Low As Reasonably Achievable

ALOP – Acceptable Level Of Protection

CCFH – Codex Committee for Food Hygiene

CFU – Colony Forming Unit

EHEC – Enterohaemorrhagic *Escherichia coli*

FSO – Food Safety Objective

HP-VTEC – Human Pathogenic Verotoxigenic *Escherichia coli*

HUS – Haemolytic Uraemic Syndrome

STEC – Shigatoxin producing *Escherichia coli* (synonym with VTEC)

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16. ANNEXES

16.1. Annex I: Product definitions of food commodities as used in the text

Fresh meat - Minced meat and meat preparations - Meat products

Meat: all parts of domestic bovine animals (including the species bubalus bubalis and bison bison) swine, sheep, goats and solipeds that are suitable for human consumption (Directive 64/433/EEC);

Farmed game Meat: all parts of wild land mammals and wild birds including the species referred to in Article 2 (1) of Directive 90/539/EEC and ratites (rattae)-bred, reared and slaughtered in captivity which are fit for human consumption;

Minced meat: meat which has been minced into fragments or passed through a spiral-screw mincer (Directive 94/65/EEC);

Meat preparations: meat within the meaning of Article 2 of Directives 64/433/EEC (fresh meat), 711/118/EEC (poultry meat) and 92/45/EEC (game) and satisfying the requirements of Articles 3, 6 and 8 of Directive 91/495/EEC (rabbits and farmed game) which has had foodstuffs, seasonings or additives added to it or which has undergone a treatment insufficient to modify the internal cellular structure of the meat and thus to cause insufficient to modify the internal cellular structure of the meat and thus cause the characteristics of fresh meat to disappear (Directive 94/65/EEC);

Meat products: products prepared from or with meat which have undergone treatment such that the cut surface shows that the product no longer has the characteristics of fresh meat (Directive 77/99/EEC). Its related treatments are chemical or physical process such as heating, smoking, salting, marinating, curing or drying, intended to lengthen the preservation of meat or animal products whether or not associated with other foodstuffs, or a combination of these various processes (Directive 77/99/EEC);

Prepared meat meals: wrapped meat products corresponding to culinary preparations, cooked or pre-cooked and preserved by cold (Directive 77/99/EEC);

Milk and milk-based products (Directive 92/46/EEC)

Raw milk: milk produced by secretion of the mammary glands of one or more cows, ewes, goats, buffaloes, which has not been heated beyond 40°C or undergone any treatment that has an equivalent effect

Milk for the manufacture of milk-based products: either raw milk for processing or liquid or frozen milk obtained from raw milk, whether or not it has undergone an authorised physical treatment, such as heat treatment or thermisation, or is modified in its composition, provided that these modifications are restricted to the addition and/or removal of natural milk constituents

Heat-treated drinking milk: either drinking milk intended for sale to the final consumer and to institutions, obtained by heat treatment and presented in the forms defined in Annex C, Chapter I.A.4 (a), (b), (c) and (d) or milk treated by pasteurisation for sale in bulk at the request of the individual consumer.

Milk-based products: milk products, namely products exclusively derived from milk, it being accepted that substances necessary for their manufacture may be added, provided that these substances are not used to replace in part or in whole any milk constituent, and composite milk products, namely products of which no part replaces or is intended to replace any milk constituent and of which milk or a milk product is an essential part either in terms of quantity or for characterisation of the product.

Heat treatment: any treatment involving heating that causes, immediately after it has been applied, a negative reaction on the phosphate test

Thermisation: the heating of raw milk for at least 15 seconds at a temperature between 57°C and 68°C such that after treatment the milk shows a positive reaction to the phosphatase test.

Fresh produce (Fruits and vegetables)

Fruit: The fleshy edible part of a perennial plant associated with the development of a flower.

Vegetable: The fresh edible portion of an herbaceous plant consumed either raw or cooked. The edible part may be a root, tuber, stem, bud, bulb, petiole or leafstalk, leaf and a mature or immature fruit.

Sprouts⁵: Germinated (sprouted) seeds of alfalfa, mung beans and other seeds that are usually eaten raw.

ICMSF-definitions (1998)

Cream: the fat-rich part of milk that is separated by skimming or by other techniques.

Cultured or fermented milks: milk products intended for consumption after fermentation by lactic acid bacteria.

Cheese: the product of coagulation of casein coagulation in the milk, followed by separation and removal of the whey from the curd. Apart from certain fresh cheese, curd is then textured, salted, formed, pressed and finally ripened. Cheese varieties include fresh, soft, semisoft, hard as well as blended cheeses.

Ice cream and ice milk: formulated milk products intended for consumption in the frozen or partially frozen state.

⁵ In this report the term sprouted seeds are used to avoid confusion.

16.2. Annex II Test characteristics

If the tests used have a perfect diagnostic sensitivity and specificity (100%) the measured apparent prevalence will equal the true prevalence: that is $A+B$ will equal $A+C$ and $(B=C=0\%)$.

The definition of sensitivity and specificity is as follows:

Sensitivity = # test positive (a) / # diseased or contaminated (a+c)

Specificity = # test negative (d) / # healthy or not contaminated (b+d)

	Contaminated	Not contaminated	
Test +	A	B	A+B
Test -	C	D	C+D
	A+C	B+D	N

If as normal the tests are imperfect (sensitivity and specificity < 100%) must be calculated the true prevalence ($A+C$) the apparent prevalence ($A+B$) and the diagnostic sensitivity and specificity are known according to the formula:

True prevalence = $(\text{Apparent prevalence} + \text{specificity} - 1) / (\text{Sensitivity} + \text{Specificity} - 1)$

Important parameters for the interpretation of results are the predictive values for positive (PVPT) and negative test results (PVNT) according the formulae:

$PVPT = \# \text{ contaminated (A)} / \# \text{ test positive (A+B)}$

Or in terms of prevalence, sensitivity and specificity

$PVPT = [\text{prevalence} * \text{sensitivity}] / [(\text{prevalence} * \text{sensitivity}) + (1 - \text{prevalence}) * (1 - \text{specificity})]$

$PVNT = \# \text{ not contaminated (D)} / \text{test negative (C+D)}$

$PVNT = [(1 - \text{prevalence}) * \text{specificity}] / [(1 - \text{prevalence}) * \text{specificity} + \text{prevalence} * (1 - \text{sensitivity})]$

It should be noted that the predictive values vary with the prevalence in particular if the prevalence is low and specificity is less than 100% there will be a large fraction of false positive samples.

For example if the prevalence is 10% and the sensitivity and specificity is 90% the predictive values are

$PVPT = (0.1 * 0.9) / [(0.1 * 0.9) + (1 - 0.1)(1 - 0.9)] = 0.09 / 0.18 = 50\%$ and

$PVNT = (1 - 0.1) * 0.9 / [(1 - 0.1) * 0.9 + 0.1 * (1 - 0.9)] = 0.81 / 0.82 = 99\%$.

If the prevalence is only 1% the predictive values will be:

$$PVPT = (0.01*0.9)/[(0.01*0.9)+(1-0.01)(1-0.9)]=0.009/0.108= 8\% \text{ and}$$

$$PVNT = (1-0.01)*0.9/[1-0.01)*0.9 + 0.01*(1-0.9) = 0.89/0.892 = 99.9\%.$$

Thus, if the prevalence of a pathogen is very low, and the specificity cannot be assumed to be equal to 100% there is a tangible risk of false positive results when applying the tests. Thus, a positive test results would in this case not mean the presence of a pathogen and there is a need for confirmatory tests before the presence of a pathogen is concluded. Another approach would be the three class sampling plans that are applied for dealing with the similar problem of false positives in the context of microbiological criteria.

A particular effect of a sensitivity that is less than 100% is that the efficient sample diminishes. For example if taking 60 samples from a food lot and one positive sample rejects the lot, the probability of at least one sample testing positive for prevalence of 5% is:

$$1-(1-\text{prevalence})^{\#\text{samples}} = 1-(1-0.05)^{60} = 0.954 \text{ or } 95\%$$

if however, the sensitivity is 50% this probability will decrease:

$$1-(1-\text{prevalence} * \text{sensitivity})^{\#\text{samples}} = 1-(1-0.05*0.5)^{60} = 0.781 \text{ or } 78\%$$

this might be compensated by increasing the sample size for example to 120:

$$1-(1-\text{prevalence} * \text{sensitivity})^{\#\text{samples}} = 1-(1-0.05*0.5)^{150} = 0.952 \text{ or } 95\%.$$

If the sensitivity is less than 100% it is possible to compensate for this by increasing the number of samples taken, whereas problems regarding specificity should be addressed by using confirmatory tests or e.g., three class sampling plans.

16.3. Annex III: Correlation of risk reduction linked to microbiological criteria with prevalence of contaminated foodstuffs

Based on the assumption of perfect tests i.e., diagnostic sensitivity=specificity=100% and the binomial formulae $1-(1-\text{prevalence defectives})^{\#\text{sample size}}$, (Vose, 1996) one can estimate the risk reduction afforded by the microbiological criteria as illustrated in Tables 7-9. This risk reduction is the fraction of food lots that will be rejected given a prevalence of defectives, number of samples taken and the number of positive samples accepted (in these tables zero).

Table 7: The effect of prevalence contaminated food items and sample size on the risk reduction (%) for different 2-class sampling plans ^{a)}.

Sampling plan	n=1 c=0	n=5 c=0	n=10 c=0
Prevalence of defective items	Risk reduction on fraction of rejected food lots in %		
0.1%	0.1	0.5	0.9

1%	1	4.9	9.6
5%	5	23	40
10%	10	41	65
20%	20	68	89

a) The risk reduction is described by the formula $1-(1-\text{prevalence defectives})^{\wedge\text{sample size}}$. Here n denotes sample size and c the number of sampled food items that can be defective and the whole lot still accepted. The number of food items in the lot is assumed to be large (>5000) thus the binomial distribution can be applied. Note that these numbers presume random sampling from the whole food lot.

It appears that the risk reduction is somewhat limited when using a few samples such as 5 or 10 without accepting the finding of defective food items and the prevalence is low. For example by taking 5 samples and assuming a 5% the prevalence the risk reduction is around 23%, that is 23% of the contaminated food lots will be rejected (Table 7).

Table 8: The effect of prevalence contaminated food items and sample size on the risk reduction (%) for different 2-class sampling plans using a higher number of samples ^{a)}.

Sampling plan	n=15 c=0	n=30 c=0	n=60 c=0	n=100 c=0
Prevalence of defective items	Risk reduction or fraction of rejected food lots in %			
0.1%	1.5	3	5.8	9.5
0.5%	7.2	14	26	29
1%	14	26	45	63
2%	26	45	70	87
5%	54	79	95	99

a) The probability of acceptance is described by the formula $1-(1-\text{prevalence defectives})^{\wedge\text{sample size}}$. Here n denotes sample size and c the number of sampled food items that can be defective and the whole lot still accepted. The number of food items in the lot is assumed to be large (>5000) thus the binomial distribution can be applied. Note that these numbers presume random sampling from the whole food lot.

The risk reduction produced by the microbiological criteria is limited if the prevalence of contaminated food items is low. However, this can be compensated for some extent by increasing the number of samples to be taken (Tables 7 and 8).

For example, if a food lot of 10000 items has 1% prevalence of contaminated items where 10 samples analysed and no positive samples found (n=10, c=0), the risk reduction is 9.6%, consequently 90% of the food lots will be accepted (Table 7). If the number of samples is higher (n=100, c=0) the risk reduction will be 63%, consequently 27% of the lots will be accepted (Table 8).

However, whether the risk reduction is meaningful can be questioned if the prevalence approaches 0.1% (Table 8). For prevalences between 0.5% and 20% the question whether the risk reduction is meaningful or not, ought to be answered on a case-by-case basis. Cost-effectiveness and comparison with other risk management

options available would be important parameters to consider when addressing the question of meaningful risk reduction.

One complication is that Tables 7 and 8 assume perfect tests with diagnostic sensitivity and specificity equal to 100%. However, the effect of lesser sensitivity will be a diminished the risk reduction afforded by microbiological criteria. Table 9 gives an example of the effect of criteria of the sensitivity is 50%, that is one will detect 50% of the truly contaminated food items; while the specificity is 100%, that is all truly negative food items will test negative. In this case the formulae will be $1 - (1 - \text{prevalence defectives} * \text{sensitivity})^{\text{sample size}}$ (Gardener and Greiner, 1999). In this case the risk reduction will be reduced. For example for food lots with 1% prevalence and applying a microbiological criteria of 60 samples, without accepting defectives will give a risk reduction of 45% and 26%, for diagnostic sensitivities of 100% and 50%, respectively.

Table 9: The effect of prevalence contaminated food items and sample size and diagnostic sensitivity here assumed to be 50% and specificity is 100% on the risk reduction (%) for different 2-class sampling plans ^{a)}.

Sampling plan	n=15 c=0	n=30 c=0	n=60 c=0	n=100 c=0
Prevalence of defective items	Risk reduction or fraction of rejected food lots in %			
0.1%	0.7	1.5	3.0	4.9
0.5%	3.7	7.2	14	22
1%	7.2	14	26	39
2%	14	26	45	63
5%	32	53	78	92

^{a)}The risk reduction is described by the formula $1 - (1 - \text{prevalence defectives} * \text{sensitivity})^{\text{sample size}}$. Here n denotes sample size and c the number of sampled food items that can be defective and the whole lot still accepted, here c=0. The number of food items in the lot is assumed to be large (>5000) thus the binomial distribution can be applied. Note that these numbers presume random sampling from the whole food lot.

It can be concluded that the risk reduction afforded by microbiological criteria is reduced if the diagnostic sensitivity is less than 100%. Moreover, the risk reduction afforded by microbiological criteria is correlated with the prevalence of contaminated food items, the number of samples and the diagnostic sensitivity of the testing procedure.

16.4. Annex IV: Sampling plans and their performance.

Cases and sampling plan performance assuming a standard deviation of 0.8; lots having the calculated mean concentrations or greater will be rejected with at least 95% probability.

	Cases, sampling plans and calculation of their performance		
Type of hazard	Conditions reduce hazard	Conditions cause no change in hazard	Conditions may increase hazard
Indirect	case 4 (3-class, $n=5$, $c=3$) e.g. $m=1000/g$, $M=10000/g$ Mean conc.=5128/g	Case 5 (3-class, $n=5$, $c=2$) e.g. $m=1000/g$, $M=10000/g$ Mean conc.=3311/g	case 6 (3-class, $n=5$, $c=1$) e.g. $m=1000/g$, $M=10000/g$ Mean conc.=1819/g
III. Moderate	case 7 (3-class, $n=5$, $c=2$) e.g. $m=1000/g$, $M=10000/g$ Mean conc.=3311/g	Case 8 (3-class, $n=5$, $c=1$) e.g. $m=1000/g$, $M=10000/g$ Mean conc.=1819/g	case 9 (3-class, $n=10$, $c=1$) e.g. $m=1000/g$, $M=10000/g$ Mean conc.=575/g
II. Serious	case 10 (2-class, $n=5$, $c=0$) e.g. $m=0/25g$ Mean conc.=3.2/100g (1 cfu/32g)	Case 11 (2-class, $n=10$, $c=0$) e.g. $m=0/25g$ Mean conc.=1.2/100g (1 cfu/83g)	case 12 (2-class, $n=20$, $c=0$) e.g. $m=0/25g$ Mean conc.=5.4/1000g (1 cfu/186g)
I. Severe	case 13 (2-class, $n=15$, $c=0$) e.g. $m=0/25g$ Mean conc.=7.4/1000g (1 cfu/135g)	Case 14 (2-class, $n=30$, $c=0$) e.g. $m=0/25g$ Mean conc.=3.6/1000g (1 cfu/278g)	case 15 (2-class, $n=60$, $c=0$) e.g. $m=0/25g$ Mean conc.=1.9/1000g (1 cfu/526g)

Table 8.5 from ICMSF (2002) Microbiological Testing in Food Safety Management, Kluwers Academic.

17. ACKNOWLEDGEMENTS

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