Teagasc
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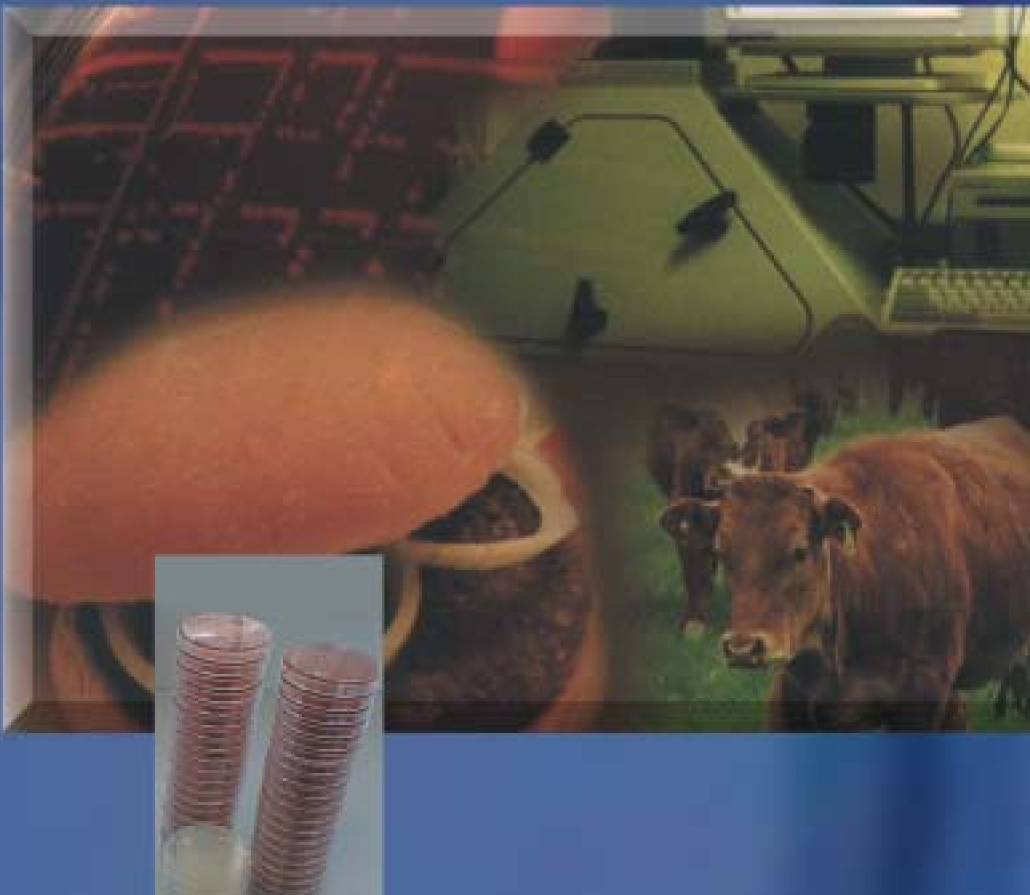
A European Study on Animal, Food and Biomedical Aspects of *E. coli* O157:H7



The National Food Centre

RESEARCH & TRAINING FOR THE FOOD INDUSTRY

RESEARCH REPORT NO 51

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A EUROPEAN STUDY ON ANIMAL FOOD & BIOMEDICAL ASPECTS OF *E.COLI* 0157:H7

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PREFACE

Verocytotoxin producing *Escherichia coli* (VTEC) and, in particular, strains of serogroup O157, have emerged as significant pathogens causing a range of severe and potentially fatal illnesses. The European Union has recognised the threat posed by *E coli* O157:H7 and the need to devise control strategies based on an understanding of VTEC pathogenicity, transmission, survival and growth. It acknowledges the importance of informing farmers, veterinarians, food producers and health authorities so that each of these groups can act appropriately to reduce the overall hazards posed by these organisms. To contribute to the development and dissemination of effective control strategies, the European Commission funded this Concerted Action Project [1].

Thirty one groups from 12 European countries participated together with many international research leaders from the USA and Australasia working on VTEC. This demonstrates the success of the project in promoting collaboration among scientists from veterinary, food and biomedical backgrounds. The result was effective communication of the latest research findings, and the means of their application, to end users working in diverse areas of food safety and public health. The project also served as a forum to make recommendations on the direction of future research.

This report presents an overview of the recommendations emanating from the project and the most recent research findings on VTEC.

[1] 'A European study on animal, food, and biomedical aspects of verocytotoxigenic E. coli including serotype O157:H7, an emerging pathogen' (CT98-3935) within the Agriculture and Agro-industry Framework IV Research Programme (1998 - 2001).

Laboratory diagnostic methods

Routine techniques for VTEC focus almost exclusively on the detection of *E. coli* O157:H7 while the detection of other VTEC organisms is considerably more difficult and is reliant on the use of molecular technology.

There are many problems with the developed techniques for *E. coli* O157:H7. A major challenge is the isolation of *E. coli* O157:H7 from complex sample types (environmental, animal faeces, food and clinical specimens). Often the sample will contain low numbers of VTEC, very high levels of background flora and natural inhibitors of bacterial growth which interfere with isolation and subsequent detection of the pathogen. In many samples, VTEC are present in an injured or stressed condition and unless a recovery step is built into the protocol these cells may not be recovered, giving a false negative result. There is a necessary trade off between the need to incorporate antibiotics and other inhibitory agents into the enrichment broth and agar to enhance selectivity and the potential inhibition of stressed cells by these selective agents.

The use of immunomagnetic beads coated with antibodies against *E. coli* O157 is now regarded as the most sensitive method for the isolation of the pathogen from enriched broth. The bead / *E. coli* O157 complex is plated onto a differential agar. Sorbitol McConkey agar (SMAC) which exploits the lack of sorbitol fementation by *E. coli* O157:H7 is the most commonly used agar. The selectivity of SMAC and other differential agars can be increased by the addition of low concentrations of selective agents including cefixime, tellurite or rhamnose. Recent research indicates that while the addition of these selective agents are necessary to isolate the pathogen from heavily contaminated samples, they may themselves be inhibitory to *E. coli* O157, particularly if the pathogen is present in low numbers or in a stressed condition. It is now recommended that the standard concentration of these selective agents should be halved when examining samples in which cells are stressed.

Techniques to improve the speed of the testing protocols for VTEC, in particular serotype O157, have been developed based on immunological or DNA techniques [polymerase chain reaction (PCR)]. Typing of strains remains a specialised skill which is carried out principally by national reference laboratories and is used as a research tool. The lack of standardisation in cultural techniques, rapid detection methods and typing procedures remains a problem.

Viruleence and clinical aspects of VTEC

Verocytotoxigenic *E. coli* (VTEC) are significant human pathogens and although there are many serotypes of VTEC, *E. coli* O157 are the most important in terms of severity of human illness. In addition to *E. coli* O157, four other serogroups (O111, O26, O103 and O145) have been identified by the World Health Organisation (WHO) as emergent pathogens in Europe. The series of events in *E. coli* O157:H7 infection starts with ingestion of the pathogen. The pathogen survives the acidic environment of the stomach and passes into the large intestine. Colonisation is achieved by the formation of a characteristic lesion on the cells of the large intestine (the attaching and effacing lesion). Thereafter, the VTEC cells multiply and release a toxin called verotoxin into the blood stream. The target cells for the toxin are in the kidneys, brain and large intestine. The spectrum of disease caused by VTEC can range from mild diarrhoea to severe bloody diarrhoea. Complications including haemolytic uraemic syndrome (HUS) and thrombotic thrombocytopaenic purpura (TTP) can occur in some cases . The infectious dose is lower than for other enteric pathogens and has been reported to be fewer than 50 organisms.

The administration of antibiotics in the treatment of VTEC infection remains controversial as bactericidal drugs are believed to increase release of verocytotoxin from VTEC cells into the blood stream. Recent clinical trials using inert substances (i.e. Synsorb-Pk) which preferentially absorb verocytotoxins in the gut indicate that they may reduce the risk of patients developing HUS.

The virulence factors in VTEC include the toxin (verotoxin 1 and verotoxin 2), the production of attaching-effacing lesions and the secretion of plasmid-mediated haemolysins.

The reputation of O157:H7 as the 'hamburger bug' has long been called into question as many other foodstuffs have now been identified as vehicles of infection. While food and water still account for a significant proportion of outbreaks, person-to-person spread was reported to be important, particularly among vulnerable groups such as children under 5 years and the elderly, particularly in settings such as crèches and nursing homes. The number of cases and outbreaks attributable to direct and indirect faecal contact is causing increasing concern in many countries. Contact with animal faeces at 'petting zoos', open farms and from faecally-contaminated mud was reported to be responsible for 14% of outbreak cases in England and Wales. A further study in Scotland suggested that direct and indirect contact with farm animal faeces may be responsible for as many as 50% of sporadic VTEC cases.

Transmission and control measures for VTEC

Farming environment

Faecal contamination is one of the primary contributory factors to the persistence of VTEC in the environment and is a potential vector for infection of animal populations. It contributes to the transmission of VTEC to humans through the contamination of food crops and water sources, and by direct contact. As animals, particularly cattle, provide the basis for several transmission routes to humans, the prevalence and epidemiology of VTEC within the animal population is of considerable interest. Sources of transmission of VTEC to animals may include the soil in pens, water troughs and animal attendants.

Farm management practices have been shown to affect the general carriage and spread of VTEC. Thus care in the provision of non-contaminated feed and water can reduce the prevalence of these organisms. Inappropriate manipulations of diet, which alter the gut flora, may cause undesirable faecal shedding of VTEC, which is also increased by transport stress and antibiotic therapy. Interventions, which may reduce the prevalence and the extent of transmission of VTEC within herds, include reduction in stocking, housing and grouping densities.

VTEC survives on pasture land in faeces and in slurry posing a number of direct and indirect risks. The pathogen may be directly recycling within cattle herds during grass consumption, or within silage or other forage. Effective silage fermentation can reduce the numbers of VTEC in contaminated or slurry treated grass. However, poorly fermented silage may spread VTEC among ruminants fed such silage. Appropriate handling of manure is important to control spread of this pathogen and limit the significant risk of human infection from direct contact with manure or slurry within farming activities, farming families or during transient contact with contaminated farmland. Recognition of such risks may lend greater emphasis to procedures to prevent contact between humans or animals and fresh slurry. It may be necessary to hold slurry for extended periods prior to spreading on farmland or use in the production of food crops, particularly foods that are to be consumed in the raw or minimally-processed state. Alternatively, it may be necessary to develop methods for the destruction of VTEC in manures and slurry for example by the application of heating and/or "sterilising" gases. However, practical and economic methods to eliminate VTEC from manure and slurry are not currently available.

VTEC can survive for days or weeks in water including rivers and lakes and spread widely throughout the environment, contaminating other farms and wildlife, and recreational waters, posing a direct threat to drinking water. Water supplies, including locally abstracted well or river water destined for human or animal consumption, should receive sufficient treatment to inactivate VTEC. VTEC can persist in sediments in contaminated water troughs for up to 8 months, making them an important source of reinfection and transmission. Simple cleaning, based solely on removal of sediments, is not effective. Combined treatments which remove sediments and provide clean drinking water to food animals by, for example, chlorination of the water supply, significantly reduces the risk of VTEC carriage.

Raw meat

VTEC may be present in the gut and faeces of cattle presented for slaughter. During the slaughter process, these and other food pathogens may be transferred to carcasses, workers, factory surfaces and equipment. It is generally accepted that the extent to which dung adheres to hides of cattle influences the levels of microbial contamination on the derived carcasses. To control such risks, many countries have already implemented or are developing "clean cattle policies" which aim to reduce the VTEC contamination of carcasses and derived raw meat products. The entry of faecal material into the abattoir and subsequent cross-contamination can be limited by visual *ante mortem* inspection of the cleanliness of the hides. Strategies for the processing of dirty animals may include: rejection of animals with excessively dirty hides; washing of the animals; hide trimming or clipping; slaughter of dirty animals at the end of the kill period, and reducing the speed of slaughter line.

Hide removal

The hide should be removed in a manner that avoids contact between the hide and the carcass. This can be achieved by a number of measures including the use of hide pulling equipment. All equipment used in the dehiding operation should be sterilised in water at 82°C to prevent cross-contamination from the hide to the carcasses.

Evisceration

During evisceration, VTEC may be transferred to the carcass from the intestines, stomach contents, oral cavity and oesophagus. The risk of faecal contamination of the carcass at evisceration can be reduced by "rodding," a technique used to separate the oesophagus from the trachea and diaphragm. Bagging and tying of the bung can also help prevent contamination of the carcass.

Chilling

Carcasses are normally chilled to a surface temperature of below 7°C within 12 hours. The chilling parameters (air temperature, relative humidity, air speed and carcass spacing) which can achieve a reduction in VTEC numbers have not yet been established.

Decontamination measures

Treatments designed to decontaminate carcasses include washing with cold (10-15°C), warm (15-40°C) or hot (75-85°C) water, spraying the carcasses with acetic or lactic acids or combinations of these procedures. The most effective of these procedures are hot water washing and organic acid sprays. However, it should be noted that while organic acids are widely used for beef carcass decontamination in the USA, they are not permitted under EU regulations.

Steam pasteurisation involves the removal of surface water from the carcass after which steam is applied to reduce pathogen numbers. Damage to the carcass surface is limited by immediate chilling with water. This technique is commonly used in the USA and is now being implemented in Europe.

Steam vacuuming, ionising and non-ionising irradiations, ultrasonic and high pressure have also been proposed as potential decontamination measures but as yet are not widely used.

Ground meat

Ground meat such as minced beef has been associated with a number of outbreaks of VTEC infection. Ground beef is a high-risk product because pathogens on the surface of the meat are mixed into the product during the mincing process. Commonly used preservatives, such as 3% sodium lactate, have no significant effect on the survival of VTEC in such products. The primary control measure therefore remains adequate cooking of the product (70°C for 2 min or meat juices run clear). VTEC will survive during the normal shelf life of ground beef products e.g. chill storage for up to 7 days or frozen storage for several months.

Fermented meat

These products are normally rendered microbiologically safe by a combination of the effects of fermentation i.e. pH reduction, end products of fermentation and other additives. However, VTEC can survive the fermentation process and persist in such products, leading to foodborne illness. This is due to their ability to survive the low pH, high salt, and nitrite in fermented products. Concerns in relation to the survival of VTEC in fermented meats have led the USDA to recommend that processing protocols should achieve a 10^5 cfu/g decline in numbers of *E. coli* O157:H7. Manipulation of the intrinsic factors in the fermentation process will not achieve this target and additional hurdles such as a heat treatment step are therefore necessary in the process. In the absence of a thermal processing step, an extended fermentation or maturation period may prove effective in limiting pathogen numbers.

Dairy products

Many human infections with *E. coli* O157:H7 have been linked to raw milk contaminated with cattle faecal material. Pasteurised milk has also been implicated in human infections but only where inadequate pasteurisation or post process contamination was reported. *E. coli* O157:H7 was observed to grow in both raw and pasteurised milk stored at 15°C and 7°C, while at 5°C *E. coli* O157:H7 failed to grow, but did survive, with a small reduction in numbers of the pathogen after 28 days of storage.

The association of this pathogen with raw milk poses the possibility of VTEC survival in dairy products. In hard cheeses, the potential for survival or growth of the pathogen is significantly lower than in the high moisture soft and semi-soft cheeses. The additional hurdles imposed during the hard cheese manufacturing process, including the low water activity and pH as a result of the curing process, and the competing microflora reduces the survival and growth potential of the pathogen. However, survival of the pathogen has been reported after 60 days of cheese ripening. Therefore the indications are that the additional hurdles imposed during cheese manufacture will be insufficient to prevent the growth or survival of the pathogen in cheese produced from contaminated milk. The risk which VTEC poses in farmhouse cheeses, which are produced from unpasteurised milk in order to retain flavours and aromas which are heat sensitive, is particularly high.

Yoghurt, which has a typical pH of 4.0, is generally produced from pasteurised milk to which lactic acid starter cultures are added. Because of the acid tolerance of *E. coli* O157:H7, the final pH of these products will be insufficient to prevent the survival of VTEC in product which has been produced from improperly pasteurised milk or which has been contaminated post-pasteurisation by poor hygiene practices. This may have particular relevance to small, home or farm-based manufacturers. The addition of fruits or other raw materials, which have been insufficiently washed or disinfected, constitutes an extra risk factor.

E. coli O157:H7 is also able to survive freezing in ice–cream but the organism will not grow in this environment and it may be sub-lethally stressed.

The above data indicate that the most important measures to ensure safety of milk and dairy products are adequate pasteurisation, the avoidance of post-pasteurisation contamination and storage at temperatures of 5°C or below.

Fruit and vegetables

Fruit and vegetables have been implicated in outbreaks of *E. coli* O157:H7 poisoning. Sources of contamination include cattle manure used as a fertilizer, run off water from cattle feed-lots, and irrigation water. The largest outbreak of *E. coli* O157:H7 reported to date has been associated with radish sprouts and a wide range of vegetable & fruit products (apple cider, apple juice, radishes, lettuce and alfalfa sprouts) have been identified as vehicles of infection for VTEC. Minimally-processed vegetables and salads pose particular risks because once contaminated they are not subject to sufficient processing to reduce or eliminate such contamination.

Controlling the quality of fertilisers and irrigation waters is important in the control of VTEC on ready-to-eat vegetables or vegetable products. Irrigation of such foodstuffs by untreated wastewater is prohibited although enforcement of such measures is difficult. Inorganic fertilisers are preferable to faecal-based fertilisers but inorganic fertilisers are inappropriate for use in organic production techniques. In these situations, properly composted manure which has been subject to a heat treatment should be used.

Once harvested, the levels of the pathogen on the surface of the vegetable may be reduced by washing or rinsing; however, water rinsing alone reduces bacterial contamination of vegetables by less than one log cycle. Under poorly controlled conditions, washing may even spread the pathogen to previously uninfected product. Chlorine may be added to the water to enhance the sanitising effect of the washing process. Despite its efficiency on organisms suspended in water, its effect on micro-organisms attached to the surface of vegetables is limited. For this reason and because of general concerns about the impact of chlorine in the environment, its use as a vegetable decontaminant is prohibited in several European countries.

The most effective mechanism to control the growth of VTEC on minimally processed vegetables is refrigeration from the point of harvest to the point of sale although there have been no reports of refrigeration reducing VTEC numbers on vegetables. Care must be taken to ensure appropriate core temperature is achieved rapidly and temperature fluctuations during processing and transportation are kept to a minimum.

Not all products are suitable for refrigeration and some may be damaged by this process. Refrigeration may be combined with modified atmosphere packaging to extend product shelf-life. However, this has not been shown to reduce VTEC numbers on minimally-processed vegetable products.

Fruit juices

Particular care should be exercised during the production of fruit and vegetable juices as low levels of contamination on the raw ingredient may well be passed across to the final product and indeed may be concentrated by the processing procedure. Such products should be subject to a control system such as pasteurisation to ensure the safety of the final product. The addition of food preservatives such as organic acids, potassium sorbate and sodium benzoate have proved effective in controlling VTEC numbers in apple juice products. However the use of such preservatives is subject to legislative control and is not popular with consumers. Recently, novel procedures such as high-pressure treatments have been successfully applied to control VTEC in fruit juices negating the need for the inclusion of preservatives. Irradiation and ultraviolet light have also been shown to be effective in reducing numbers of *E. coli* O157:H7. The more widespread adoption of such novel techniques will improve the safety of juice products.

There is an increasing consumer demand for freshly-squeezed fruit and vegetable juices which are sold from high street juice bars. In general, such juices are unpasteurised and are judged by the consumer to be superior in flavour to "treated" juices. The control of VTEC in such juices relies on the use of high quality produce, extensive cleaning and sanitation of the fruit, the processing equipment and food contact surfaces, coupled with training of personnel in food hygiene.

The complete elimination of VTEC and other food-borne pathogens from minimally-processed fruit and vegetable products is currently impossible and indeed these products may provide suitable conditions for the growth and multiplication of these pathogens. Thus, these products must be regarded as unsafe and be treated accordingly. A certain degree of control may be achieved by the prevention of contamination in the first place by good agricultural practices and by good manufacturing practice and refrigeration.

Food contact surfaces

Food preparation surfaces and utensils with which food comes into contact can be a source of cross-contamination by pathogens including VTEC. *E. coli* O157 has been shown to survive for extended periods on stainless steel at refrigeration temperature and can grow on plastic cutting boards in the presence of meat juices. Recent research indicates that *E. coli* O157:H7 dies off faster on copper than on stainless steel. Effective sanitation of work surfaces and equipment remains a primary control point in reducing the risk of pathogen cross-contamination.

Consumer education

Consumer education on hygienic handling and adequate cooking of food can play a role in reducing the incidence of VTEC infection and indeed other food borne pathogens. The newly set up national food safety agencies in some European countries including Ireland, UK and France have taken a proactive role in this regard and have launched both media campaigns and school awareness programmes on food safety.

VTEC is destroyed by heat, but it is very important that adequate and accurate information is given to consumers about storage and handling of high risk products. For example, advice should emphasise the need to properly defrost frozen products, "cook meat until the juices run clear and no meat remains pink", and prevent recontamination of cooked products. Control of VTEC in retail and domestic environments will require improvements in hygienic practices among food handlers and consumers. Enhanced measures to prevent cross-contamination from high risk products such as "raw" animal products, especially fresh meat and unpasteurised milk, to processed or ready-to-eat foods are essential. At retail level, such improvements should include physical and operational separation of activities involving raw and processed product types. In domestic kitchens, consumers should wash hands, utensils after working with raw foods, have separate chopping boards for raw and cooked foods and prevent contact between domestic animals and any food products or food contact surfaces. Effective control of the increased challenges posed by VTEC mean that there must be a continual process of food safety education among retail and service food handlers, and consumers.

CONCLUSIONS

- A 6 hour enrichment, followed by immunomagnetic separation and then plating onto Sorbitol McConkey agar containing cefixime and tellurite, is regarded as the most sensitive cultural method for the detection of *E. coli* O157:H7 pathogen from food samples.
- The administration of antibiotics in the clinical treatment of VTEC remains controversial as they may increase the release of verotoxins into the blood stream.
- Direct contact with animal faeces at 'petting zoos', open farms and from faecally-contaminated mud is now recognised as an important mode of transmission of VTEC. In addition, person-to-person spread particularly among vulnerable groups such as children under 5 years and the elderly, in settings such as crèches and nursing homes is a very important mode of transmission in outbreak situations.
- VTEC can persist in contaminated water troughs for up to 8 months. Combined treatments which remove sediments and provide clean drinking water to animals, i.e. by chlorination of water, can reduce the spread of VTEC in cattle herds.
- The entry of faecal material into the abattoir and potential for cross-contamination from pathogens including VTEC on to carcasses can be limited by visual *ante motem* inspection of the hide cleanliness.
- Steam pasteurisation can be carried out to reduce pathogen numbers including VTEC on beef carcasses
- Controlling the quality of fertilisers and irrigation waters is important in the control of VTEC on ready-to-eat vegetables and salads.
- *E. coli* O157 has been shown to survive for extended periods on stainless steel at refrigeration temperature and can grow on plastic cutting boards in the presence of meat juices.
- Control of VTEC in retail and domestic environments will require improvements in hygienic practices among food handlers and consumers.

RECOMMENDATIONS TO INDUSTRY

The ability of VTEC to survive in many environments including water, soil, grass, manure, sewage etc. makes it unlikely that complete suppression can be achieved by the application of control measures at a single source. Thus, effective control strategies must consider the multiple points at which VTEC can gain access to the human food chain. The persistence, and the ability of very small numbers of these organisms to establish life-threatening infections with serious long term clinical consequences, particularly among "at risk" sections of the human population, mean that many elements of our food safety strategies will have to be improved.

Measures to control VTEC during food production, processing, distribution, at retail level and during commercial/ domestic preparation do not differ greatly from measures used to control other food pathogens. Therefore the best approach to control VTEC and indeed other pathogens is to implement HACCP principles into the food safety management systems at all stages of food production and distribution. The pathogen does however have an unusual tolerance to low pH and so should be considered a higher risk than other pathogens in products which have a low pH, are minimally-processed or are not cooked before consumption. A hazard analysis or risk assessment on current practices should be carried out for products in these categories. Other high risk products include dairy products produced from unpasteurised milk. The most important measures to ensure safety of milk and dairy products are adequate pasteurisation, the avoidance of post-pasteurisation contamination and storage at temperatures of 5°C or below. The control of VTEC in minimally-processed ready-to-eat vegetables and in fresh unpasteurised fruit/vegetable juices relies on the use of high quality produce, extensive cleaning and sanitation of the produce, the processing equipment and food contact surfaces. The primary control measure for minced meat remains adequate cooking (70°C for 2 min or until meat juices run clear). However, because of the risks posed by cross-contamination it is essential that raw and cooked food should be physically separated at all times during processing, storage, distribution and preparation. Food preparation surfaces and utensils with which food comes into contact can also be a source of cross-contamination by pathogens including VTEC. Training of personnel working in food preparation and food service industries coupled with consumer education on hygienic handling and adequate cooking of food can play a role in reducing the incidence of VTEC infection and indeed other food-borne pathogens.

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