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Escherichia coli 0157:H7: Implications for HACCP on the farm and in the abattoir

The National Food Centre

RESEARCH & TRAINING FOR THE FOOD INDUSTRY

RESEARCH REPORT NO 6

Contents

ESCHERICHIA COLI O157: H7: IMPLICATIONS FOR HACCP ON THE FARM AND IN THE ABATTOIR

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SUMMARY

Experiments were designed to assess the risks associated with *Escherichia coli* O157:H7 on the farm, through the abattoir and into the butcher shop. Data was also generated for application in model building and the reliability of pathogen models for predicting pathogen growth in different foods was examined. The main results of this work are:

- *E.coli* O157:H7 survived for fourteen weeks in bovine faeces. Bovine faeces, inoculated with *E. coli* O157:H7 and stored in plastic containers at 10°C in a fridge and outside (where temperatures ranged from -4.2 to 19°C) showed a reduction of about 100,000 cfu (colony forming units) per gram over 99 days. Faeces exposed on grass had a similar reduction after 50 days. These differences were attributed to dilution and washing out by rainfall.
- *E. coli* O157:H7 did not survive the ensiling of grass.
- The transfer rate from contaminated hides to the carcass during routine slaughter was considerably reduced by using a simple pre-slaughter washing regime.
- Of 206 meat samples purchased in Dublin butcher shops, 3.6% of salami samples were contaminated with *E. coli* O157:H7, which had the genes encoding for both verotoxins.
- The growth patterns for three strains of pathogenic *E. coli* O157:H7 (wild type and mutant) were similar in Brain Heart Infusion (BHI) broth at 37°C, supporting the use of laboratory pathogen growth models for predicting *E. coli* O157:H7 growth in foods.

INTRODUCTION

Although *E. coli* O157:H7 has been isolated from sheep (Kudva *et al.*, 1997), pigs (Chapman *et al.*, 1997), deer (Chapman and Ackroyd, 1997) and pigeons (Dell'Omo *et al.*, 1998), it is generally accepted that cattle, with a prevalence of up to 36.8% (Chapman *et al.*, 1997) are the primary source of this pathogen. *E. coli* O157:H7 persists in the rumen and colon of these animals and is passed in the faeces (Brown *et al.*, 1997). However, faecal matter often adheres to the hide and is carried with animals into the abattoir, thus acting as a source of *E. coli* O157:H7 contamination.

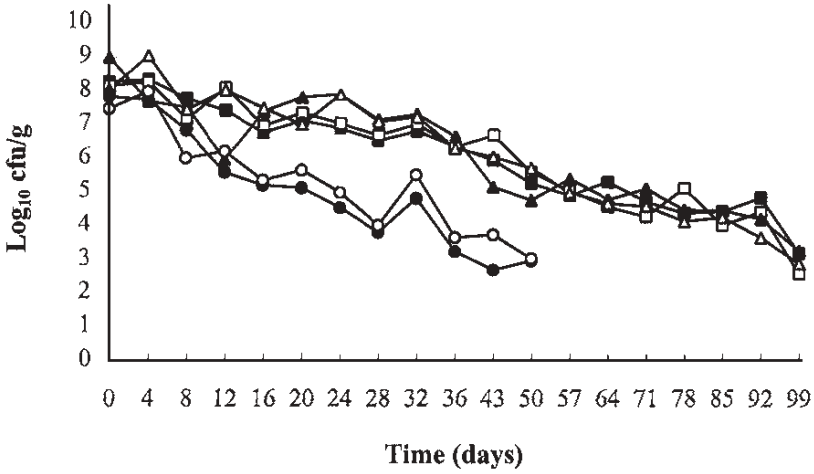
Farm and factory practices which contribute to this contamination process were investigated. Means of reducing the spread of the pathogen on the farm and preventing transfer from dirty hides to beef carcasses in the abattoir were identified for incorporation into Hazard Analysis and Critical Control Point (HACCP) systems. In addition, the growth characteristics of different strains of *E. coli* O157:H7 were examined in the laboratory, providing the necessary data to develop and validate mathematical models which provide decision support when food process modification is contemplated.

The survival characteristics of *Escherichia coli* O157:H7 in the environment

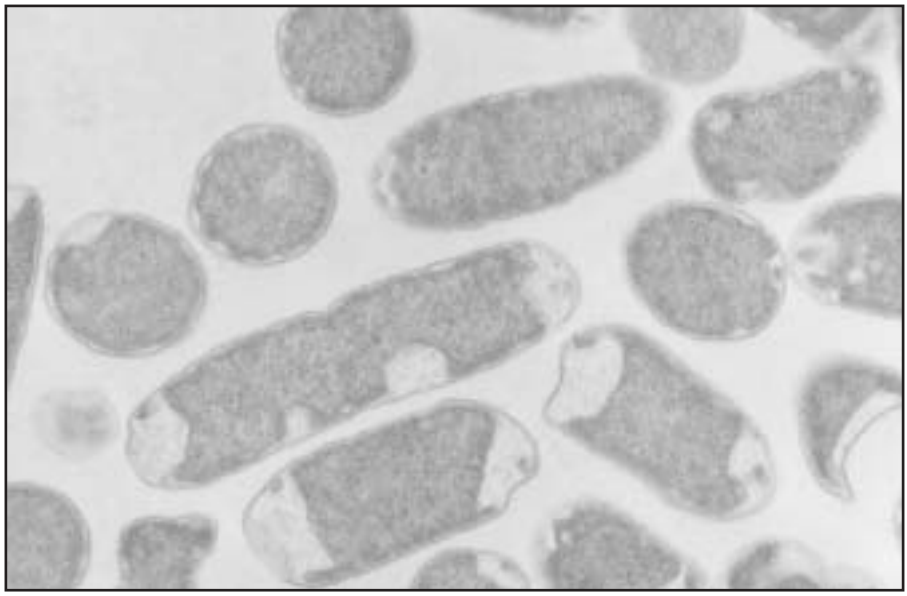
The more widespread *E. coli* O157:H7 is in animal herds, the more likely a contamination event will occur during slaughter. Current farm practices, such as the spreading of manure, might actually facilitate the transmission of this organism. Hancock *et al.* (1994) reported a tentative relationship between such practices and the prevalence of *E. coli* O157:H7 in dairy and beef cattle. However, more recent investigations by the same research team (Hancock *et al.*, 1997) failed to establish any correlation between the prevalence of *E. coli* O157:H7 in cattle and the application of bovine manure to pasture lands.

Whether or not natural fertiliser is an important source of *E. coli* O157:H7 depends on the ability of this organism to survive in the environment. The longer it survives the greater the probability of ingestion by another animal.

The survival characteristics of *E. coli* O157:H7 in bovine faeces were investigated using a non-pathogenic, antibiotic resistant strain. Faecal samples were inoculated with approximately 100,000,000 cfu of the organism per gram and stored in closed plastic containers at 10°C in the laboratory. Concurrently, other samples were left outside in similar containers or exposed on grass. The first treatment showed 100,000 cfu/g decrease over a 99 day period (Fig. 1). A similar result was obtained with the equivalent field samples, despite outdoor temperatures ranging from -4.2°C to 19°C. Exposure on grass for 50 days also resulted in a 100,000 cfu/g decrease. However, using enrichment techniques, the pathogen was still detected in the surrounding soil 99 days after inoculation. These results are consistent with those reported by Maule (1995) and suggest that farming activities which cause contamination of pastures might be an important factor in the spread of *E. coli* O157:H7 among animals.



▲ **Figure 1.** Survival of *E.coli* O157:H7 at 10°C in the laboratory as determined on selective (■) and non-selective (❑) media, outside in field containers as determined on selective (▲) and non-selective (△) media and on the pasture grass as determined on selective (●) an non-selective(❍) media.



Cells of *E. coli* O157:H7

Survival of *Escherichia coli* O157:H7 during the ensiling of grass

The objective of this research was to investigate the survival of *E. coli* O157:H7 during grass fermentations and hence to determine whether or not silage might facilitate the horizontal transfer of this pathogen.

From the same perennial ryegrass, two herbages, one that would readily undergo a lactic acid dominant fermentation (easy to ensile) and one that had a relatively lower sugar content (difficult to ensile) were prepared. Half of each was left untreated while the rest was treated with formic acid (3 ml per kilogram of grass) a procedure currently used on Irish farms. After inoculation to approximately 10,000 cfu/g with a non-pathogenic strain of *E. coli* O157:H7, mini-silos were filled with 6 kg of each treatment and stored at approximately 12°C for 10 days. Samples were removed periodically.

A typical fermentation pattern was observed. As with other coliforms (Seale *et al.*, 1981) *E. coli* O157:H7 disappeared quickly and was not detected after 2 days, regardless of the availability of readily metabolisable sugars or pre-treatment with formic acid.

Emptying the mini-silos to test for *E. coli* O157:H7

Washing the live animal to prevent the transfer of *Escherichia coli* O157:H7 from the contaminated hide to the beef carcass during slaughter

The Pennington report, which investigated the Lanarkshire *E. coli* O157:H7 outbreak, recommended that animals presented for slaughter should be in a clean and appropriate condition (Pennington, 1997). The Clean Livestock Policy (1997) in the UK requires veterinary officers to reject animals that do not comply with this recommendation. In Ireland an amendment of the *Abattoirs Act* 1988 (S.I. No. 425 of 1997) has resulted in similar inspections at

Irish slaughtering facilities. Without doubt, contaminated hides are a major source of *E. coli* O157:H7.

Cleaning the live animal pre-slaughter has therefore become an important consideration. In the United States, Schnell *et al.* (1995) found that dehairing with sodium sulphite solutions did not have a major effect on the total carcass coliform or *E. coli* counts. In view of the low infective dose (Willshaw *et al.*, 1994; Bolton *et al.*, 1996) and the serious illnesses associated with *E. coli* O157:H7 infection, an effective validated method for the complete removal of faecal contamination from live animal hides is needed. Washing the live animal, having shown some potential for decreasing subsequent contamination on carcasses (Childers and Walsh, 1996) was investigated at The National Food Centre.

Inoculated bovine faeces (approximately 100,000,000 *E. coli* O157:H7/g) was spread on the rump area of 30 heifers pre-slaughter. Twenty animals were washed with a power hose (ten for 3 minutes and ten for only 1 minute) while the remainder were left unwashed.

After slaughter the number of *E. coli* O157:H7 on the carcass, tools and butchers' hands were examined. The results demonstrate the removal of *E. coli* O157:H7 from the hide of the live animal and its spread to the butcher's hands, through the abattoir and ultimately onto the carcass (Tables 1, 2 & 3). If done thoroughly, washing the animals pre-slaughter reduced the levels of *E. coli* O157:H7 contamination on the live animal and on the final carcass.

Table 1: The effect of pre-slaughter washing on the levels of *E. coli* O157 :H7 on the live animal

Duration of wash (minutes)	Control	1	3
Number of *E.coli* O157:H7 per cm^2	56.2	51.3	2.2

Table 2: The effect of pre-slaughter washing on the transfer of *E. coli* O157:H7 from the contaminated hides onto the carcass and kill line equipment

Duration of wash (minutes)	Control	1	3
Site swabbed	Number of *E.coli* O157:H7 per ml		
Butcher's hands	3.3	48.9	2.5
Flare	0	0.9	0
Butcher's knife	5.8	2.7	1.5
Chyne saw	6.3	7.1	1.5
Brisket saw	1.4	7.8	0

Table 3: The effect of pre-slaughter washing on the levels of *E. coli* O157 :H7 on the beef carcass

Duration of wash (minutes)	Control	1	3
Site swabbed	Number of *E.coli* O157:H7 per cm^2		
LHS hindquarter	5.0	1.9	0
LHS forequarter	0.6	0.7	1.5
RHS hindquarter	4.3	0.4	1.0
RHS forequarter	3.0	0	0.8

Washing the live animal with a power hose to remove the E. coli O157:H7

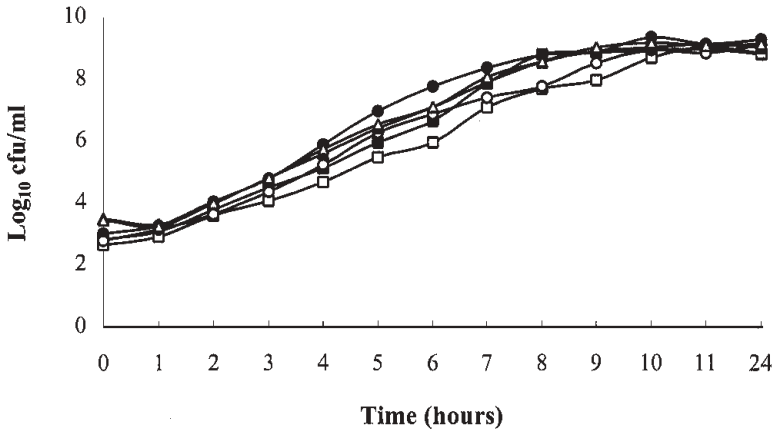
Incidence of *Escherichia coli* O157:H7 contamination on meats purchased from Dublin retail outlets

Meat samples were purchased from Dublin retailers, including 60 beefburgers, 56 salami, 50 minced beef, 23 cooked ham, 11 corned beef and 6 roast beef samples. These were examined using direct plating techniques and enrichment procedures to detect low numbers of this pathogen.

No *E. coli* O157:H7 was detected in the beefburgers, minced beef, ham, corned beef or roast beef. The detection rate in the salami was 3.6% and these *E. coli* O157:H7 had the genes encoding for both verotoxin I and verotoxin II. Even allowing for the small survey size, these figures are alarming given the low infective dose of this pathogen and the lack of further processing of salami before consumption.

Growth curves for *Escherichia coli* O157:H7 in broth: wild type versus mutant

All models currently used to predict the growth of bacteria in food are based on broth cultures. To provide the necessary data to build such a model, the growth patterns of 3 different pathogenic strains of wildtype *E. coli* O157:H7 (*E. coli* O157:H7 ATCC 43895 (control strain), Ent C9490 (the 'Jack in the box' strain) and a strain previously isolated from salami) were studied in Brain Heart Infusion (BHI) broth at pH 7.0 and 37°C. The experiment was then repeated with a mutant of each of these strains, resistant to nalidixic acid and streptomycin sulphate. From an initial inoculum level of around 1,000 cfu/ml, each strain showed a similar pattern of growth, reaching over 100,000,000 cfu/ml after 10 hours at 37°C (Fig. 2)



▲ **Figure 2** The growth pattern of three pathogenic strains of *E. coli* O157:H7 ATCC 43895, wildtype (■) & mutant (□); Ent C9490, wildtype (●) & mutant (○); a salami strain, wildtype (▲) & mutant (△).

CONCLUSIONS

- As the responsibility for food quality is extended back to the primary producer, farm practices such as the spreading of bovine manure and slurry, which may facilitate the spread of *E. coli* O157:H7, need to be re-examined in the light of these results. The use of natural fertiliser, with the obvious financial savings to the farmer must not be condemned outright; however, a risk analysis taking into account environmental, economic and food safety factors needs to be performed. From the food safety perspective, more research is needed. The minimum infective dose needed to establish a carrier state in cattle needs to be investigated as does survival in different types of slurry. Silage does not appear to facilitate the horizontal spread of *E. coli* O157:H7, however further research is ongoing in this area. This study should also assist in the development of HACCP systems for Irish farms
- There is little doubt that removing faecal matter from the hide of an animal before entering the abattoir reduces the potential for subsequent contamination of the carcass. At present this is achieved by clipping or shaving. This work demonstrated that washing with tapwater using a powerhose was effective if done thoroughly. Currently used dehairing techniques should be examined in a similar fashion. Comparison of results would then allow the application of the most effective method for controlling *E. coli* O157:H7 at this stage of the slaughtering process.
- The results of the Dublin area meat survey are alarming since salami does not receive further heat treatment before consumption. However recent research at The National Food Centre has resulted in a pasteurisation step being added to the salami preparation process thus ensuring a 100,000 fold reduction in the levels of any *E. coli* O157:H7 present.

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