PFGE Analysis of Meat Isolates of *E. coli* O157:H7 in New Zealand

30th October 2009

Prepared as part of a New Zealand Food Safety Authority
contract for scientific services

by

Angela Cornelius

PFGE Analysis of Meat Isolates of
E. coli O157:H7 in New Zealand

30th October 2009

Stephen On
Food Safety Programme Leader

Angela Cornelius
Report Author

CONTENTS

Figures

Tables

1.   Summary

Following on from the PFGE report of isolates to the end of 2008 (*PFGE Typing of Meat Isolates of E. coli O157:H7 in New Zealand*; ESR, March 2009), this report describes the results of PFGE analysis of an additional 55 *E. coli* O157:H7 isolates from meat received by ESR to 1 October 2009, and includes nine isolates associated with the AgResearch/AsureQuality "Investigating *E. coli* O157:H7 False Positives" project.

All of the isolates have been analysed by PFGE using both *Xba*I and *Bln*I. When the two PFGE types were combined 33 *Xba*I:*Bln*I types were observed.

The nine isolates from the "Investigating *E. coli* O157:H7 False Positives" project had 9 *Xba*I types, 6 *Bln*I types and 9 *Xba*I:*Bln*I types. All of these isolates were distinguishable from USA PFGE types.

The majority (92.3%) of PFGE patterns of NZ *E. coli* O157:H7 meat isolates currently available for 2009 were distinguishable from USA patterns. Only one NZ 2009 *Xba*I:*Bln*I combination has been reported in the PulseNet USA postings. The USA outbreak was in 2005 and had no source reported. The incidence of this *Xba*I:*Bln*I combination in other countries that export meat, and other foods, to the USA cannot be determined from the available data.

2.   Introduction

In response to initiatives by the United States of America (USA) to further control *E. coli* O157:H7 in the USA beef supply, NZFSA and industry agreed in January 2008 to molecular-type by pulsed field gel electrophoresis (PFGE) all *E. coli* O157:H7 isolates detected under the New Zealand (NZ) *E. coli* O157:H7 monitoring programme (O157 MP), and a to provide a summary of the PFGE profiles to FSIS on a regular basis.

Following on from the PFGE report of isolates to the end of 2008 (*PFGE Typing of Meat Isolates of E. coli O157:H7 in New Zealand*; ESR, March 2009), this report describes the results of PFGE analysis of an additional 55 *E. coli* O157:H7 isolates from meat received by ESR to 1 October 2009, and includes nine isolates associated with the AgResearch/AsureQuality "Investigating *E. coli* O157:H7 False Positives" project.

3.   Methods and Results

The PulseNet Aotearoa (New Zealand) *E. coli* Database contains 893 New Zealand *E. coli* O157:H7 isolates including 462 human, 311 meat, 116 animal and 4 environmental isolates. PFGE analysis was carried out using the PulseNet protocol http://www.pulsenetinternational.org/protocols/protocols.asp. All isolates have been analysed using *Xba*I and 662 have also been analysed using *Bln*I. Of these isolates, 55 were meat *E coli* O157:H7 isolates received by ESR in 2009 for which the PFGE analysis had been completed by the 1st October 2009. All of the 2009 isolates have been analysed using *Xba*I and *Bln*I.

The *Xba*I and *Bln*I patterns were loaded into BioNumerics 5.1 (Applied Maths, Kortrijk, Belgium) and types assigned. Similarities between patterns were calculated using the Dice coefficient with band matching parameters of 0.5% optimization and 1.5% position tolerance. Interstrain relationships were assessed by cluster analysis using the Unweighted Pair-Group with Mathematical Average (UPGMA) method. Types were primarily assigned based on BioNumerics marking the isolates as 100% similarity but these results were modified, as necessary, following visual inspection of the patterns. Assignation of strains to a common type does not imply strain identity.

A total of 24 *Xba*I types and 23 *Bln*I types were observed for the 55 meat isolates of *E. coli* O157:H7. When the two PFGE types were combined, 33 *Xba*I:*Bln*I types were observed among these isolates.

3.1.   BioNumerics Analysis of Isolates from "Investigating *E. coli* O157:H7 False Positives" Project

The results for the nine isolates from the "Investigating *E. coli* O157:H7 False Positives" project are summarised in Table 1 and Figure 1.

Table 1:   Summary of PFGE Results for Isolates from "Investigating *E. coli* O157:H7 False Positives" Project

CSC__ERL Lab No	Submitting Lab No	*Xba*I Type	*Bln*I Type
CSC__ERL09-2285	1049370-13	Xb0179	Bl0093
CSC__ERL09-2286	1049384-13	Xb0014	Bl0036
CSC__ERL09-2287	1048971-13	Xb0040	Bl0093
CSC__ERL09-2350	1051232-13	Xb0158	Bl0031
CSC__ERL09-2378	1051691-13	Xb0127	Bl0035
CSC__ERL09-2419	1054039-13	Xb0110	Bl0043
CSC__ERL09-2420	1054051-13	Xb0092	Bl0007
CSC__ERL09-2557	1056398-13	Xb0264	Bl0007
CSC__ERL09-2558	1055927-13	Xb0265	Bl0007

Figure 1:   *Xba*I and *Bln*I PFGE Images of Isolates from "Investigating *E. coli* O157:H7 False Positives" Project
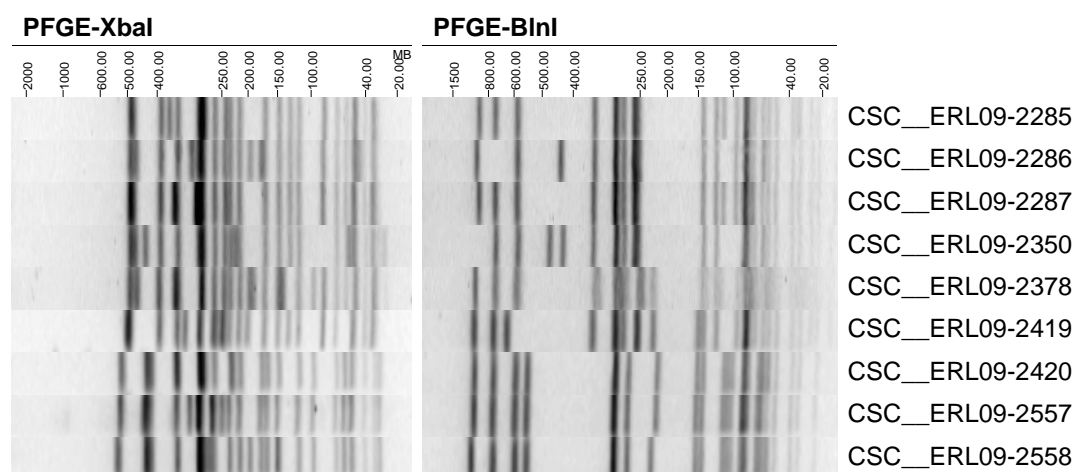
3.2.   Frequency and Distribution of PFGE Types Observed in Isolates from "Investigating *E. coli* O157:H7 False Positives" Project

The PulseNet Aotearoa (New Zealand) *E. coli* database was interrogated for isolates with the same PFGE type as these nine isolates. The results are summarised Tables 2-4.

Care must be taken when interpreting this data as matching PFGE types is evidence for, not proof of, a relationship between isolates.

Table 2:   Sources of Isolates with *Xba*I Types Observed in Isolates from "Investigating *E. coli* O157:H7 False Positives" Project

*Xba*I Type	Number of Isolates	Sources
Xb0179	6	Human, meat
Xb0014	3	Meat
Xb0040	325*	Human, meat, animal
Xb0158	22	Human, meat
Xb0127	2	Human, meat
Xb0110	3	Human, meat
Xb0092	30	Human. meat, animal
Xb0264	1	Meat (new type)
Xb0265	2	Meat (new type)

* most common *Xba*I type in NZ

Table 3:   Sources of Isolates with *Bln*I Types Observed in Isolates from "Investigating *E. coli* O157:H7 False Positives" Project

*Bln*I Type	Number of Isolates	Sources
Bl0093	129**	Human, meat, animal
Bl0036	11	Human, meat
Bl0031	6	Meat, animal
Bl0035	10	Human, meat
Bl0043	2	Human, meat
Bl0007	69	Human, meat, animal

** most common *Bln*I type in NZ

Table 4:   Sources of Isolates with *Xba*I:*Bln*I Types Observed in Isolates from "Investigating *E. coli* O157:H7 False Positives" Project

*Xba*I:*Bln*I Type	Number of Isolates	Sources
Xb0179:Bl0093	2	Meat
Xb0014:Bl0036	3	Meat
Xb0040:Bl0093	91***	Human, meat, animal
Xb0158:Bl0031	3	Meat
Xb0127:Bl0035	1	Meat (other isolate not typed with *Bln*I)
Xb0110:Bl0043	1	Meat (other isolates not typed with *Bln*I)
Xb0092:Bl0007	19	Human. meat, animal
Xb0264:Bl0007	1	Meat (new type)
Xb0265:Bl0007	2	Meat

*** most common *Xba*I:*Bln*I type in NZ

3.3   Comparison with PulseNet USA Postings

Every three months, PulseNet USA posts on their secure website information on the PFGE types associated with outbreaks in the USA. Until the first quarter of 2008, the website also included a summary of the most prevalent *Xba*I types. A bundle of BioNumerics patterns for the post prominent types and those associated with outbreaks can be downloaded from the website and loaded into a local database. This information is available for viewing and downloading by all members of PulseNet International. The postings from 2003 to the end of September 2009 were thus compared with the PulseNet Aotearoa (New Zealand) *E. coli* database.

PulseNet USA pattern designations use a 10 digit code. EXH stands for *E. coli* O157:H7, X01 stands for *Xba*I, A26 stands for *Bln*I, while the final four digits are a sequential pattern number assigned in order of first submission to the database. The comparison of very similar patterns relies on a degree of individual subjectivity which change as databases get larger and different people are involved. While very small differences in patterns may be reflective of differences in genotype, they may also in some cases reflect methodological differences that can arise, particularly when isolates are analysed over many years from many labs. Outbreak investigations usually have defined time periods for comparisons (in USA, the default is a 60 day window) which makes this less of an issue.
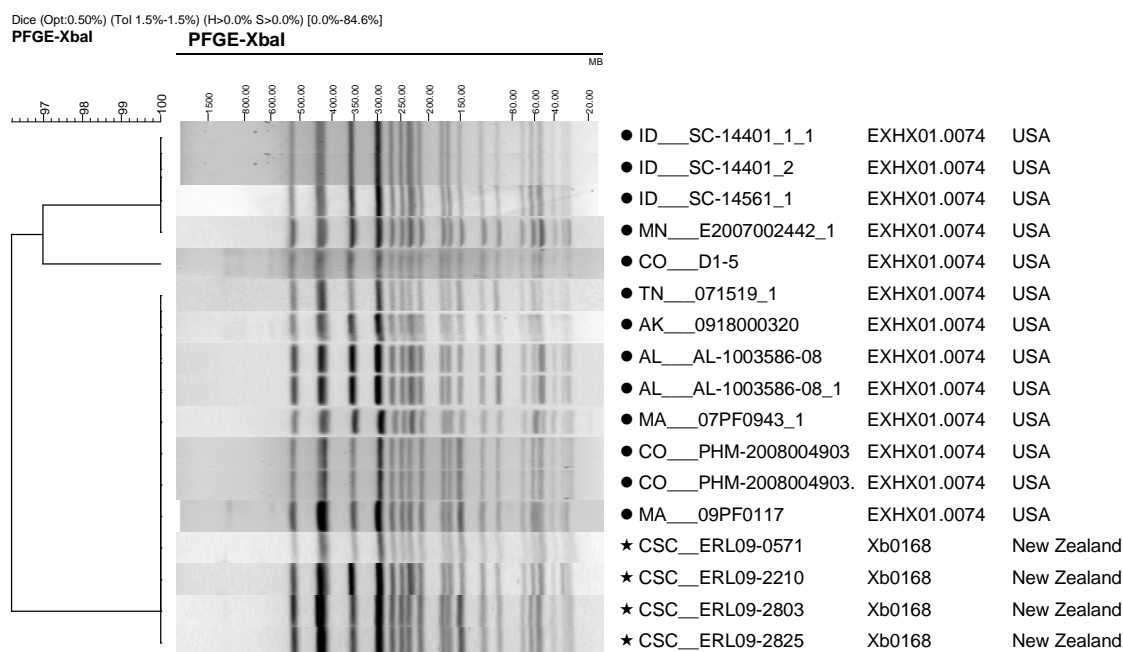
In the comparison below we have, in several instances, identified more than one USA pattern that we report as indistinguishable from a single NZ pattern. This does not mean that they are the same, simply that we can not reliably distinguish them from one another using that single enzyme. That is one reason that two enzymes are highly recommended for all comparisons. The approach we have taken builds from the assumption that all isolates are indistinguishable, and that typing allows us to distinguish them. We have therefore a very high confidence that all isolates with different PFGE types, are in fact different due to the underlying genotype, not due to methodological issues. Isolates which remain indistinguishable can then be further interrogated using both epidemiological and laboratory approaches to evaluate the nature of these indistinguishable isolates.

The 55 NZ meat *E. coli* O157:H7 isolates currently available for 2009 were compared with all of the PulseNet USA postings available at the 1st October 2009.

All of the isolates from the "Investigating *E. coli* O157:H7 False Positives" project were distinguishable from patterns reported in the PulseNet USA postings.

Only one USA outbreak with both *Xba*I and *Bln*I types reported had a PFGE type that was indistinguishable from NZ meat *E. coli* O157:H7 isolates from 2009 (Figures 2 and 3). The outbreak (0506ml-3c), which has no source recorded, occurred in 2005 and had the *Xba*I:*Bln*I type EXHX01.0074 (Xb0168):EXHA26.0585 (Bl0007). Four (7.3%) of the NZ meat *E. coli* O157:H7 isolates currently available for 2009 had this PFGE type and they are not related geographically or temporally. The incidence of this *Xba*I:*Bln*I combination in other countries that export meat, and other foods, to the USA cannot be determined from the data available.

Figure 2: USA and NZ Isolates with the *Xba*I Pattern EXHX01.0074/Xb0168

Dice (Opt:0.50%) (Tol 1.5%-1.5%) (H>0.0% S>0.0%) [0.0%-84.6%]

PFGE-XbaI   PFGE-XbaI

ID___SC-14401_1_1	EXHX01.0074	USA
ID___SC-14401_2	EXHX01.0074	USA
ID___SC-14561_1	EXHX01.0074	USA
MN___E2007002442_1	EXHX01.0074	USA
CO___D1-5	EXHX01.0074	USA
TN___071519_1	EXHX01.0074	USA
AK___0918000320	EXHX01.0074	USA
AL___AL-1003586-08	EXHX01.0074	USA
AL___AL-1003586-08_1	EXHX01.0074	USA
MA___07PF0943_1	EXHX01.0074	USA
CO___PHM-2008004903	EXHX01.0074	USA
CO___PHM-2008004903.	EXHX01.0074	USA
MA___09PF0117	EXHX01.0074	USA
CSC__ERL09-0571	Xb0168	New Zealand
CSC__ERL09-2210	Xb0168	New Zealand
CSC__ERL09-2803	Xb0168	New Zealand
CSC__ERL09-2825	Xb0168	New Zealand

Figure 3: USA and NZ Isolates with the *Bln*I Pattern EXHA26.0585/Bl0007

Dice (Opt:0.50%) (Tol 1.5%-1.5%) (H>0.0% S>0.0%) [0.0%-79.5%] [99.8%-100.0%]

PFGE-BlnI   PFGE-BlnI

CSC__ERL09-0571	New Zealand
CSC__ERL09-2210	New Zealand
CSC__ERL09-2803	New Zealand
CSC__ERL09-2825	New Zealand
WA___13292	USA
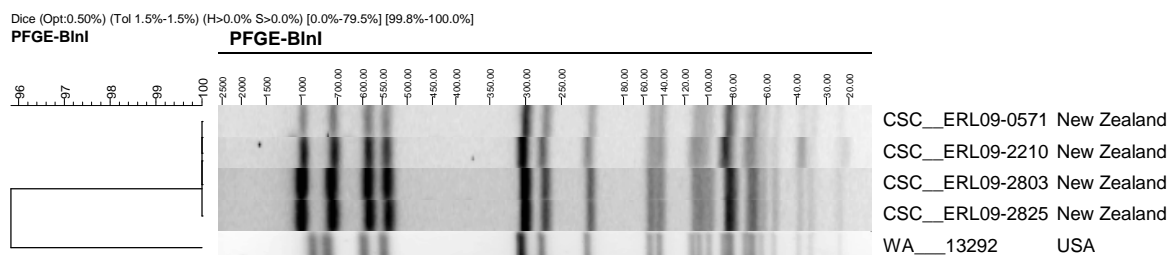
4.   Conclusions

The nine meat *E. coli* O157:H7 isolates currently available for 2009 had 9 *Xba*I, 6 *Bln*I and 9 *Xba*I:*Bln*I types, all of which were distinguishable from types posted on PulseNet USA.

The majority (92.7%) of the currently available 2009 *E. coli* O157:H7 isolates from meat are distinguished from USA patterns using a combination of *Xba*I and *Bln*I restriction enzymes. Only one 2009 *Xba*I:*Bln*I combination has been reported in the PulseNet USA postings, for a 2005 outbreak in which the source has not been reported.