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

Final Report

5th March 2009

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by

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1. Summary

The molecular typing by PFGE has now been completed on all *E. coli* O157:H7 isolates from young calves, bovine, veal, prime beef and human cases included in the 2006 PFGE Emergency Response typing project as well as prime beef and veal isolates submitted to ESR up to July 24th 2008. The PulseNet Aotearoa (New Zealand) *E. coli* database contains 723 NZ *E. coli* O157:H7 isolates including 411 human, 189 meat, 118 animal and 4 environmental isolates.

A total of 557 isolates have been analysed by PFGE using both *Xba*I and *Bln*I. When the two PFGE types were combined, 247 *Xba*I:*Bln*I types were observed. Of these types, 117 contained only human isolates (144 isolates), 73 contained only meat isolates (87 isolates), 20 contained only animal isolates (33 isolates) and 2 contained only environmental isolates (2 isolates). The remaining 35 *Xba*I:*Bln*I types contained isolates from more than one source including 19 types containing meat and human isolates (38 meat and 42 human isolates), 7 types containing meat, animal and human isolates (52 meat, 60 animal and 64 human isolates), 5 types containing meat and animal isolates (10 meat and 15 animal isolates) and 4 types containing animal and human isolates (6 animal and 4 human isolates).

The two most prevalent USA *Xba*I types are also amongst the most prevalent *Xba*I types in NZ but the most prevalent NZ *Xba*I type, as well as the fourth and fifth most prevalent NZ *Xba*I types, did not appear in any of the USA postings.

Similarly only two of the five most prevalent USA *Bln*I types have also been seen in NZ. The most prevalent USA type has seldom been seen in NZ but the third most prevalent USA *Bln*I type is the most prevalent *Bln*I type in NZ. The other four most prevalent NZ *Bln*I types, which account for almost a third of the types, did not appear in any of the USA postings.

The vast majority (99.6%) of NZ isolates are distinguished from USA patterns when the *Xba*I and *Bln*I types are combined. Only one NZ *Xba*I:*Bln*I combination has been reported in the PulseNet USA postings. The two NZ isolates with this profile were isolated in 2004; the two USA types, which are uncommon in the PulseNet USA database, were associated with outbreaks (no source identified) in 2007. The incidence of this *Xba*I:*Bln*I combination in other countries that export meat, and other foods, to the US cannot be determined.

2. Introduction

In response to a request from the USA on *E. coli* O157:H7 PFGE types present in New Zealand (NZ), ESR (2006) reported on the genotypes of 242 *E. coli* O157:H7 isolates. These isolates included young calf, bovine, prime beef, veal and related isolates submitted to ESR between January 2002 and April 2006 and human isolates submitted to ESR between January 2003 and May 2006. *Xba*I patterns were obtained for all isolates and a subset of isolates was further typed and *Bln*I patterns obtained.

More recently, 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 have agreed to FSIS implemented changes to its microbiological monitoring programme. These changes will facilitate continued market access to the USA market. The changes involve all *E. coli* O157:H7 isolates being molecular-typed by pulsed field gel electrophoresis (PFGE) and a summary of the PFGE profiles provided to FSIS on a regular basis. NZFSA therefore requires the urgent completion of typing and summarization of the PFGE profiles from the 2006 Emergency Response study as well as subsequent meat isolates.

This aims of this project were:

1. to complete molecular typing by PFGE of all *E. coli* O157:H7 isolates from bovine, young calf, prime beef, veal and human cases typed under the 2006 PFGE typing project,
2. to fully type all subsequent beef and veal isolates to date,
3. to compare NZ *E. coli* O157:H7 PFGE typing information with PulseNet USA postings of *E. coli* O157:H7 types, and
4. to prepare a web-based summary of the *E. coli* O157:H7 PFGE profiles of meat isolates for NZFSA to provide to FSIS

3. Pulsed Field Gel Electrophoresis (PFGE) Analysis

A total of 167 human and 118 meat isolates from the 2006 Emergency Response project were identified as requiring additional analysis to provide complete PFGE information. In addition, 64 additional meat *E. coli* O157:H7 isolates had been received by ESR since that 2006 project and the start of this project. Most (214 of 233, 92%) isolates were able to be resuscitated. The outstanding *Bln*I analysis of 118 meat and 159 human isolates from the Emergency Response project have been completed and 58 new meat isolates have been analysed using both *Xba*I and *Bln*I. The isolates were digested and the bands separated by PFGE using the PulseNet protocol
http://www.cdc.gov/pulsenet/protocols/ecoli_salmonella_shigella_protocols.pdf).

4. BioNumerics Analysis of *Xba*I Patterns

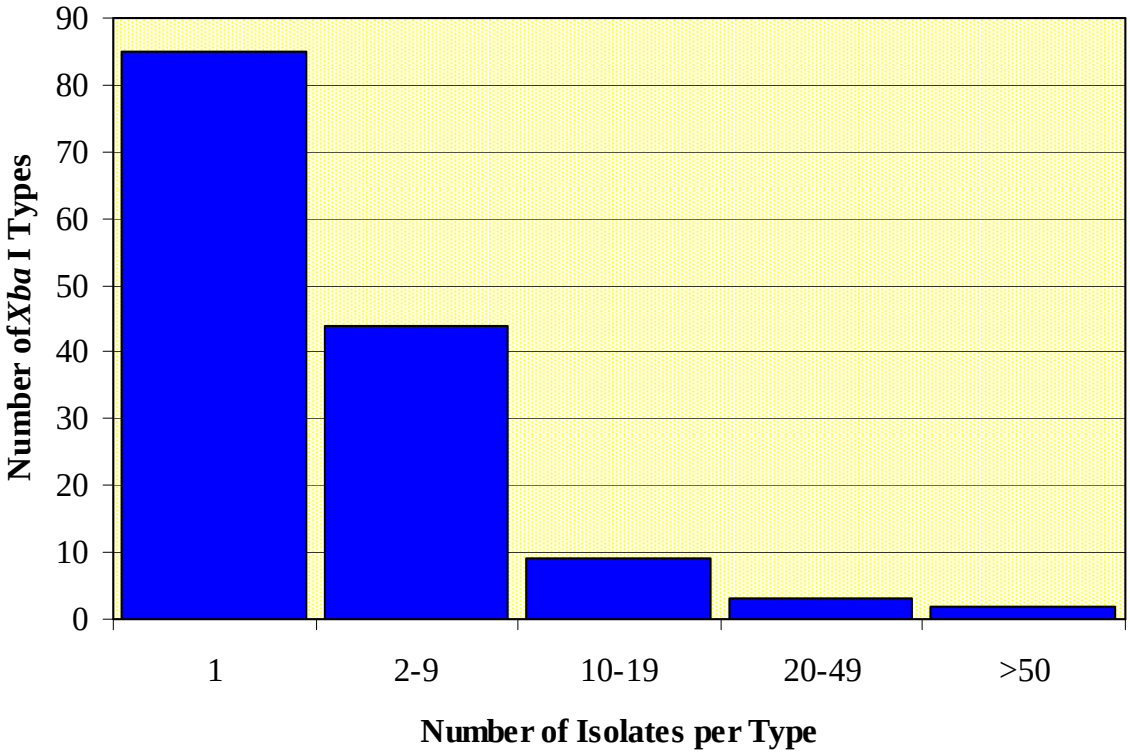
The PulseNet Aotearoa (New Zealand) *E. coli* database contains 723 NZ *E. coli* O157:H7. Of these isolates, 411 (56.8%) were of human origin, 189 (26.1%) were isolated from meat, 118 (16.3%) were of animal origin (hides and faeces), 4 (0.55%) were of environmental origin and 1 (0.14%) of the isolates had no source stated. The majority (163, 86.2%) of meat isolates were veal with the remaining isolates being isolated from prime beef (17, 9.0%), pork (4, 2.1%), sheep (4, 2.1%), and the meat type was not stated for the remaining isolate. All of these isolates have been analysed using *Xba*I, the patterns 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 1.0% 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. While analysis with a single enzyme is sufficient to show that two isolates are different, it is insufficient to demonstrate that the isolates are indistinguishable by PFGE (Gilpin, manuscript in preparation). Analysis with two enzymes is recommended.

Of the 143 *Xba*I types assigned, 66 (46.2%) types contained only human isolates (90 isolates), 28 (19.6%) types contained only meat isolates (37 isolates), 10 (7.0%) types contained only animal isolates (13 isolates), 2 types contained only environmental isolates (2 isolates) and 1 type contained a single isolate for which the source has not been stated. The remaining 36 *Xba*I types contained isolates from more than one source including 18 types containing meat and human isolates (50 meat and 99 human isolates), 9 types containing meat, animal and human isolates (96 meat, 76 animal and 194 human isolates), 4 types containing animal and human isolates (12 animal and 4 human isolates), 3 types containing animal and meat isolates (4 meat and 17 animal isolates), 1 type containing meat, environmental and human isolates (2 meat, 1 environmental and 10 human isolates) and 1 type containing environmental and human isolates (1 environmental and 14 human isolates).

The majority (n=21: 75.0%) of meat-only *Xba*I types contained only veal isolates, with 2 types containing only prime beef, 2 types containing only pork, 2 types containing both veal and prime beef, and 1 type containing only an isolate where the meat type is not stated. Similarly, the majority (n=18: 64.3%) of types containing both human and meat isolates contained only veal isolates, with 5 (17.9%) types containing veal and prime beef isolates, 2 (7.1%) types containing only sheep isolates, 1 type containing only prime beef isolates, 1 type containing only pork isolates, and 1 type containing veal, prime beef and sheep isolates.

The majority (85, 59.4%) of *Xba*I types were represented by only one isolate, while 2 *Xba*I types (Xb0040 and Xb0049) contained over 50 isolates each (Figure 1). The diversity index (Simpson, 1949) for *Xba*I was 91%.

Figure 1: Frequency Distribution of *Xba*I Types



5. BioNumerics Analysis of *Bln*I Patterns

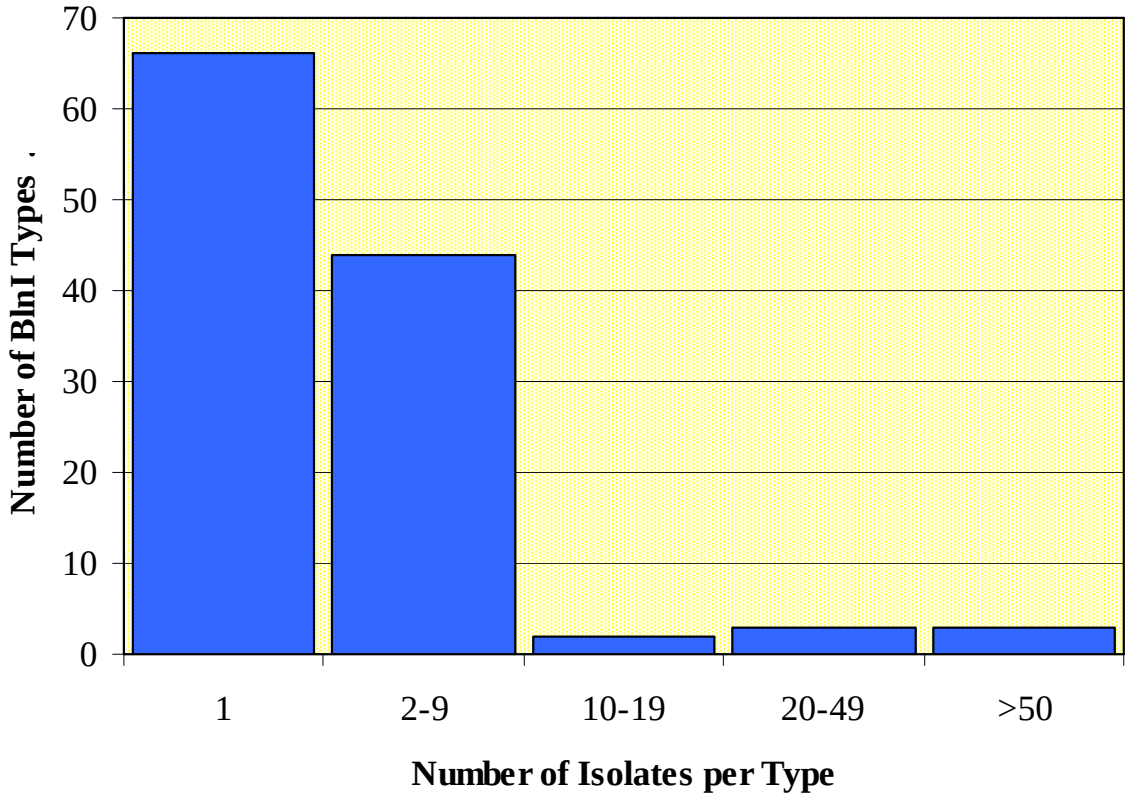
Of the 723 *E. coli* O157:H7 isolates in the PulseNet Aotearoa (New Zealand) *E. coli* database, 557 (77.0%) have been analysed using *Bln*I. The *Bln*I patterns have been loaded into BioNumerics 5.1 and types have been assigned. Cluster analysis was performed using the Dice similarity coefficient and UPGMA dendrogram type with 1.0% optimization and 1.5% position tolerance. Types were primarily assigned through BioNumerics analyses identifying profiles matching with 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. While analysis with a single enzyme is sufficient to show that two isolates are different, it is insufficient to demonstrate that the isolates are indistinguishable by PFGE. Analysis with two enzymes is recommended.

Of the 557 isolates with *Bln*I patterns, 187 (33.6%) were of isolated from meat, 114 (20.5%) were of animal origin, 254 (45.6%) were of human origin, and 2 (0.36%) were of environmental origin. Of the 118 *Bln*I types assigned, 56 (47.5%) types contained only human isolates (83 isolates), 26 (22.0%) types contained only meat isolates (33 isolates), and 2 (1.7%) types contained only animal isolates (3 isolates) and 2 (1.7%) types contained only environmental isolates (2 isolates). The remaining 32 *Bln*I types contained isolates from more than one source including 14 types containing meat and human isolates (28 meat, 44 human isolates), 11 types containing meat, animal and human isolates (115 meat, 100 animal and 124 human isolates), 5 types containing meat and animal types (11 meat and 7 animal isolates), and 2 types containing animal and human isolates (4 animal and 3 human isolates.

The majority (17, 65.4%) of meat only *Bln*I types contained only veal isolates, with 5 (19.2%) types containing only prime beef, 3 types containing only pork isolates and 1 type containing only sheep isolates. Similarly, the majority (16, 64.0%) of *Bln*I types contained both human and meat isolates contained only veal isolates, with 3 (12.0%) types containing only prime beef isolates, 2 (8.0%) types containing sheep only isolates, 2 (8.0%) types containing prime beef and veal isolates, 1 type containing sheep, pork and veal isolates, and 1 type containing veal, prime beef and an isolate for which the type of meat was not stated.

The majority of *Bln*I types (66, 55.9%) were represented by only one isolate while 3 *Bln*I types (Bl0093, Bl0080 and Bl0016) contained over 50 isolates (Figure 2). The diversity index (Simpson, 1949) for *Bln*I analysis of the 557 isolates analysed using *Bln*I was 93%, slightly higher than for *Xba*I.

Figure 2: Frequency Distribution of *Bln*I Types



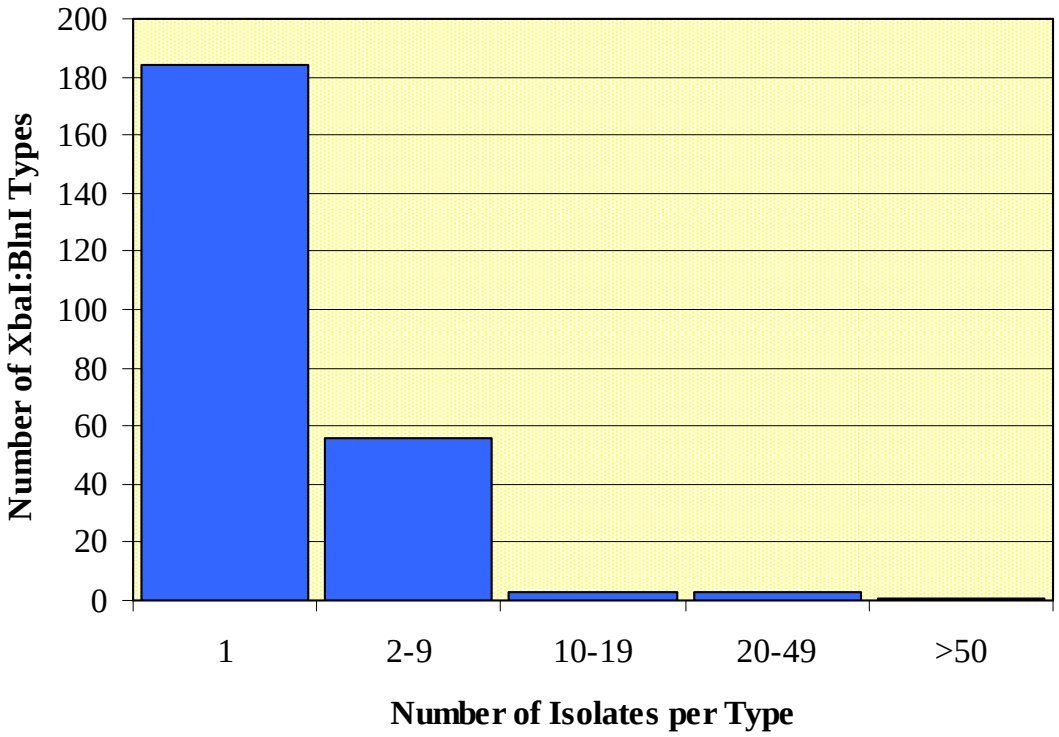
6. Combined *Xba*I:*Bln*I Patterns

When the two PFGE types were combined, 247 *Xba*I:*Bln*I types were observed for the 557 isolates that had been analysed by both enzymes. Of these types, 117 (47.4% of types) contained only human isolates (144 isolates), 73 (29.6%) contained only meat isolates (87 isolates), 20 (8.1%) contained only animal isolates (33 isolates) and 2 (0.8%) contained only environmental isolates (2 isolates). The remaining 35 *Xba*I:*Bln*I types contained isolates from more than one source including 19 types containing meat and human isolates (38 meat and 42 human isolates), 7 types containing meat, animal and human isolates (52 meat, 60 animal and 64 human isolates), 5 types containing meat and animal isolates (10 meat and 15 animal isolates) and 4 types containing animal and human isolates (6 animal and 4 human isolates).

The majority (57, 78.1%) of meat only *Xba*I:*Bln*I types contained only veal isolates, with 9 (12.3%) types containing only prime beef, 4 (5.5%) types containing only pork isolates, 2 (2.7%) types containing only sheep isolates, and 1 (1.4%) type containing an isolate for which no meat type was stated. Similarly, the majority (20, 76.9%) of *Xba*I:*Bln*I types containing both human and meat isolates contained only veal isolates, with 2 (7.7%) types containing veal and prime beef isolates, 2 (7.7%) types containing only prime beef isolates, and 2 (7.7%) type containing only sheep isolates.

The majority of *Xba*I:*Bln*I types (184, 74.5%) were represented by only one isolate while one *Xba*I:*BlnI* type (Xb0040:Bl0093) contained over 50 isolates (26 meat, 27 animal, 16 human isolates) (Figure 3). The *Xba*I:*Bln*I diversity index (Simpson, 1949) for the 557 isolates analysed using both enzymes was 97%.

Figure 3: Frequency Distribution of *Xba*I:*Bln*I Types



7. Comparison with PulseNet USA Postings

Every three months PulseNet USA posts, on their secure website, information on the most prevalent PFGE types and types associated with outbreaks. A word document summarises the new isolates added to the database including the most prevalent *Xba*I types, an Excel worksheet summarizes the outbreak information for the quarter and a bundle of BioNumerics patterns which can be downloaded 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 first quarter of 2008, containing 197 entries, was used to compare 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 comparisons 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 five most common *Xba*I types in the USA, based on the overall frequency figures provided in the 2007 annual summary, were EXHX01.0047 (6.5%), EXHX01.0074 (5.0%), EXHX01.0224 (3.4%), EXHX01.0087 (3.0%), and EXHX01.1343 (2.0%). Of these only two were indistinguishable from NZ *Xba*I types. Xb0049 is indistinguishable from EXHX01.0047, EXHX01.0124 and EXHX01.1486 and is the second most prevalent *Xba*I type in NZ (9.0%) and Xb0168 is indistinguishable from EXHX01.0074 and EXHX01.0697 and is the third most prevalent *Xba*I type in NZ (5.8%). Both had been isolated from meat and human samples. The most prevalent NZ *Xba*I type (Xb0040, 27.5%), as well as the fourth (Xb0138, 5.0%), and fifth (Xb0092, 3.2%) most prevalent NZ *Xba*I types, are distinguishable from all of the USA *Xba*I types we have patterns for. Figure 4 illustrates the USA and NZ *Xba*I types considered indistinguishable. The USA patterns are above the NZ patterns that PulseNet Aotearoa (New Zealand) considers indistinguishable.

The five most common USA *Bln*I types, based on the *E. coli* 2007 Annual Report spreadsheet were EXHA26.0569 (14.5%), EXHA26.0015 (13.0%), EXHA26.0536 (4.1%), EXHA26.0332 (3.3%) and EXHA26.0570 (1.9%). Of these only two are indistinguishable from NZ patterns. Bl0002 (EXHA26.0569) is not very prevalent in NZ with only 2 meat and 2 animal isolates (0.7%) isolates. Conversely Bl0093 (EXHA26.0536) is the most

common (18.3%) *Bln*I type in NZ and has been isolated from meat and human samples. The other four most common *Bln*I types in NZ (Bl0080, 10.6%; Bl0007, 9.3%; Bl0016, 7.9%; and Bl0004, 4.1%) were distinguishable from all USA *Bln*I types we have patterns for. Figure 5 illustrates the USA and NZ isolates with indistinguishable *Bln*I types. The USA patterns are above the NZ patterns that PulseNet Aotearoa (New Zealand) considers indistinguishable.

When analysis was performed using two enzymes, 555 of 557 (99.6%) NZ isolates were different to outbreak and common types found in the USA database. Only one *Xba*I:*Bln*I combination reported in the PulseNet USA postings has also been seen in NZ. Two 2004 isolates, one each from young calf hide and veal carcass, had the *Xba*I:*Bln*I type EXHX01.0248/EXHX01.1294 (Xb0092):EXHA26:0569 (Bl0002) which were associated with outbreaks in the USA during 2007. These *Xba*I:*Bln*I types have prevalences in the USA of 0.41% and 0.20% respectively and the sources of the two outbreaks linked to these types have not been identified. The incidence of this *Xba*I*:Bln*I combination in other countries that export meat, and other foods, to the US cannot be determined.

As demonstrated here the use of a second enzyme remains essential for confirming patterns are indistinguishable. Even then there may be genetic differences which would be identified by additional analysis - for example MLVA (Multiple Loci Variable number tandem repeat Analysis). Isolates with indistinguishable genotypes may still have no epidemiological relationship to one another. The prevalence of PFGE patterns in databases provide useful information, but determining an actual linkage or relationship between isolates with the same genetic profile requires additional investigation.

7.1 Conclusion

The vast majority (99.6%) of NZ isolates are distinguished from USA patterns using a combination of *Xba*I and *Bln*I restriction enzymes. Only one NZ *Xba*I:*Bln*I combination has been reported in the PulseNet USA postings. The two NZ isolates with this profile were isolated in 2004; the two USA types, which are uncommon in the PulseNet USA database, were associated with outbreaks (no source identified) in 2007. The incidence of this *Xba*I*:Bln*I combination in other countries that export meat, and other foods, to the US cannot be determined.

Figure 4: USA and NZ Isolates with Indistinguishable *Xba*I Types

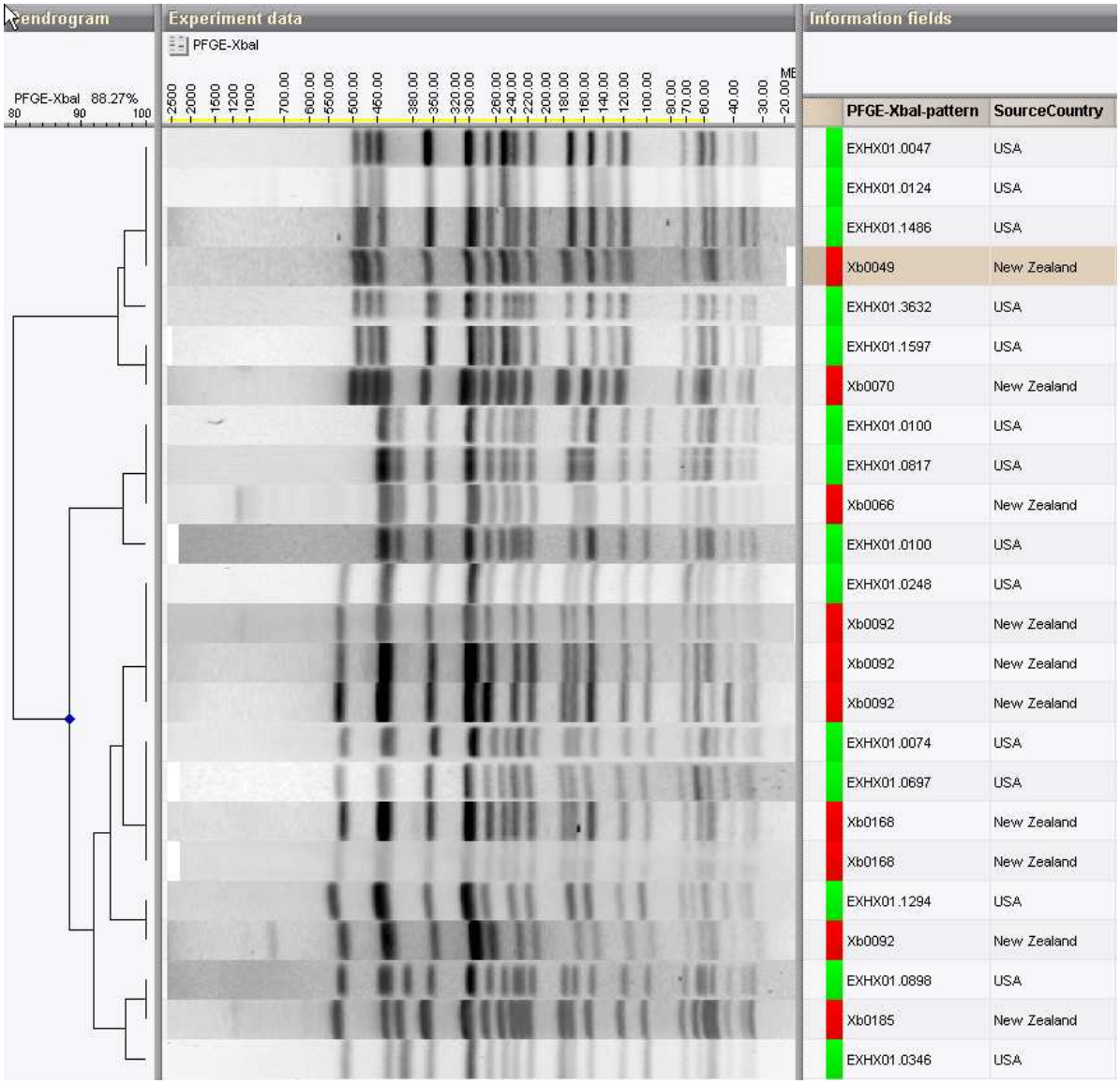
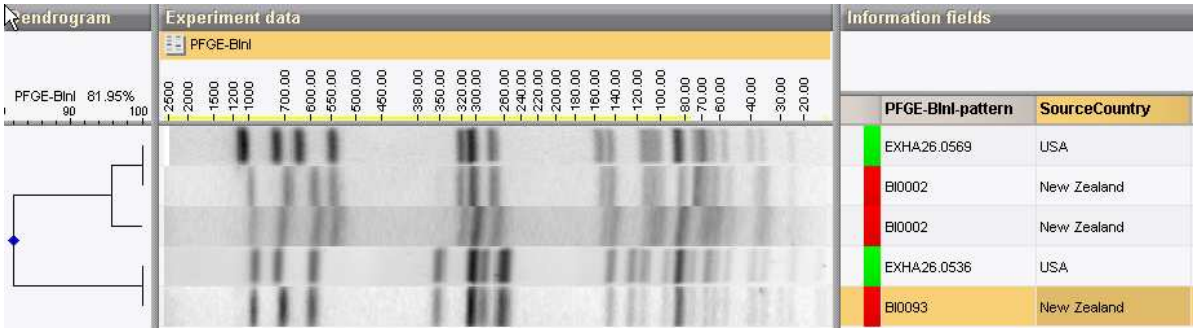


Figure 5: USA and NZ Isolates with Indistinguishable *Bln*I Types



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