

PFGE ANALYSIS OF MEAT ISOLATES OF *E. COLI* O157:H7 IN NEW ZEALAND (2011)

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By Dr Stephen On, Food Programme Leader

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(2011)**

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Dr Stephen On
Food Programme Leader

Brent Gilpin
Report Author

Angela Cornelius
Peer Reviewer

Institute of Environmental Science & Research Limited
Christchurch Science Centre
Location address: 27 Creyke Road, Ilam, Christchurch
Postal address: P O Box 29 181, Christchurch, New Zealand
Website: www.esr.cri.nz

A CROWN RESEARCH
INSTITUTE



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(2011)**

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A. Summary

Following on from the PFGE report of isolates to the end of December 2010 ([*PFGE Typing of Meat Isolates of E. coli O157:H7 in New Zealand*](#); ESR, March 2011), this report describes the results of PFGE analysis of an additional 47 *E. coli* O157:H7 isolates from meat received by ESR during the period 1 January 2011 to 31 December 2011. All of the isolates have been analysed by PFGE using both *Xba*I and *Bln*I. When the two PFGE types were combined 30 *Xba*I:*Bln*I types were observed.

Of the 30 *Xba*I:*Bln*I types 25 were new patterns not previously been seen in the New Zealand database. The remaining five patterns were indistinguishable using two enzymes from patterns previously analysed.

An unusual feature of these isolates was the absence of the PFGE pattern Xb0040 which has historically been frequently observed among bovine isolates. Whether this is evidence of a change in genotypes in the population, or simply an anomaly of sampling, will only be revealed once analysis of future years' isolates is completed.

All of the 47 New Zealand bovine isolates were distinguishable using two enzymes from the 2011 USA isolates reported by PulseNet USA as part of recognised outbreaks.

The genotyping performed was used to respond to two queries. The first a New Zealand based query as to whether isolates from the same premise were of the same genotype. They were all different genotypes which may indicate different contamination events. The second request from the USA was part of a supplier implication investigation. Comparison of the gel images, enabled us to conclude that we had NOT seen the implicated *Xba*I:*Bln*I pattern in New Zealand.

B. 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 (now incorporated into the Ministry for Primary Industries, MPI) 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 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 December 2010 ([*PFGE Typing of Meat Isolates of E. coli O157:H7 in New Zealand*](#); ESR, March 2011), this report describes the results of PFGE analysis of an additional 47 *E. coli* O157:H7 isolates from meat received by ESR during the period 1 January 2011 to 31 December 2011.

C. PFGE Analysis of Meat Isolates

The PulseNet Aotearoa (New Zealand) *E. coli* Database contains 1501 New Zealand *E. coli* O157:H7 isolates including 928 human isolates and over 573 bovine 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 806 have also been analysed using *Bln*I. All meat isolates since 2006 have been analysed with two enzymes.

Of these isolates, 47 were meat *E. coli* O157:H7 isolates received by ESR during the period 1 January 2011 to 31 December 2011. Comparisons were made with 491 previously analysed bovine isolates, and 268 human isolates with two enzyme data.

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. Assignment of strains to a common type does not imply strain identity.

A total of 19 *Xba*I types and 18 *Bln*I types were observed for the 63 meat isolates of *E. coli* O157:H7. When the two PFGE types were combined, 30 *Xba*I:*Bln*I types were observed among these isolates. Twenty-one of these patterns were seen only once, six of the patterns were seen twice, one pattern three times, one pattern 5 times and one pattern 6 times.

Twenty-five of these 30 types had new patterns not previously been seen in the New Zealand database. The remaining five patterns were indistinguishable using two enzymes from six bovine and seven human isolates previously analysed (Table 1). The human isolates were recovered over a range of years prior to 2011.

A summary of all PFGE patterns observed among New Zealand bovine and human isolates (1993-2011) compared with those found among 2011 meat isolates is contained in Appendix 1.

Table 1. Comparison of 2011 meat isolates with patterns previously observed in the database

PFGE Pattern	2011 Meat Isolates	Previous Isolates	
		Bovine	Human
Xb0358:BI0093	6	1	
Xb0365:BI0093	5		
Xb0367:BI0093	3		
Xb0019:BI0093	2	3	1
Xb0202:BI0150	2	1	
Xb0049:BI0091	2		6
Xb0202:BI0188	2		
Xb0352:BI0007	2		
Xb0358:BI0179	2		
Xb0331:BI0007	1	1	
Xb0049:BI0183	1		
Xb0049:BI0187	1		
Xb0351:BI0007	1		
Xb0353:BI0093	1		
Xb0357:BI0016	1		
Xb0357:BI0034	1		
Xb0357:BI0080	1		
Xb0357:BI0093	1		
Xb0358:BI0016	1		
Xb0358:BI0180	1		
Xb0359:BI0093	1		
Xb0361:BI0035	1		
Xb0362:BI0093	1		
Xb0363:BI0093	1		
Xb0365:BI0185	1		
Xb0366:BI0182	1		
Xb0367:BI0181	1		
Xb0368:BI0184	1		
Xb0369:BI0093	1		
Xb0370:BI0186	1		
Total	47	6	7

A notable feature of the 2011 bovine isolates is the absence of any with the pattern Xb0040. This genotype remains common among human cases (Table 2).

Table 2. Frequency of the PFGE pattern Xb0040 among human and bovine isolates

Year	<i>Bovine Isolates</i>			<i>Human Isolates</i>			Totals
	Isolates	Xb0040	%	Isolates	Xb0040	%	
2003	24	19	79%	78	24	31%	102
2004	69	9	13%	76	27	36%	145
2005	59	27	46%	84	26	31%	143
2006	135	50	37%	75	26	35%	210
2007	19	8	42%	91	21	23%	110
2008	88	37	42%	76	14	18%	164
2009	73	25	34%	137	59	43%	210
2010	57	15	26%	115	44	38%	172
2011	47	0	0%	135	59	44%	182
Totals	571	190	33%	867	300	35%	1438

Comparison with 2011 New Zealand human isolates

During 2011, 135 *E. coli* O157:H7 isolates from human cases were analysed by PFGE using the *XbaI* enzyme. On the basis of a single enzyme comparison, only five genotypes (22 isolates) were indistinguishable from the 2011 meat bovine patterns. Only five of these human isolates were analysed using *BlnI* (All pattern Xb0049), and these five were all of a different *BlnI* pattern to the meat isolates. Without a complete set of *BlnI* genotypes, it is not possible to comment on whether there is any overlap in patterns of these human isolates with the 2011 meat isolates. However, if any do, it is less than 17 of the 135 human isolates.

Table 3. Comparison of *Xba*I patterns of 2011 meat isolates with 2011 human isolates

PFGE- <i>Xba</i> I-pattern	Bovine	Human
Xb0358	10	
Xb0365	6	
Xb0049	4	16
Xb0202	4	3
Xb0357	4	
Xb0367	4	
Xb0019	2	1
Xb0352	2	
Xb0351	1	1
Xb0353	1	1
Xb0331	1	
Xb0359	1	
Xb0361	1	
Xb0362	1	
Xb0363	1	
Xb0366	1	
Xb0368	1	
Xb0369	1	
Xb0370	1	
Xb0040		41
Xb0168		11
Xb0014		5
Xb0040a		4
Xb0040g		4
Xb0048		4
Xb0105		3
Xb0138		3
Xb0039		2
Xb0040d		2
Xb0040e		2
Xb0040z		2
Xb0200		2
Xb0263		2
Xb0015		1
Xb0040b		1
Xb0040c		1
Xb0040f		1
Xb0040j		1
Xb0061		1
Xb0092		1

PFGE- <i>Xba</i> I-pattern	Bovine	Human
Xb0124		1
Xb0156		1
Xb0179		1
Xb0254		1
Xb0296		1
Xb0305		1
Xb0332		1
Xb0335		1
Xb0336		1
Xb0338		1
Xb0343		1
Xb0345		1
Xb0354		1
Xb0355		1
Xb0356		1
Xb0371		1
Xb0372		1
Xb0373		1
Xb0374		1
Grand Total	47	135

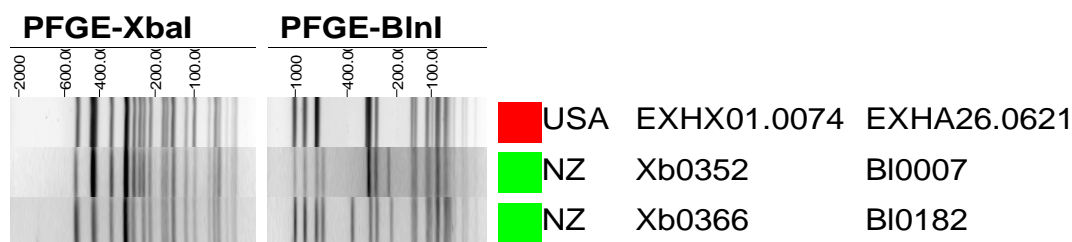
D. Comparison with PulseNet USA Postings

PulseNet USA posts on their secure website information on the PFGE types associated with outbreaks in the USA. A bundle of BioNumerics patterns for 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. A total of 40 *E. coli* O157 isolates from USA outbreaks from 2011 were downloaded and compared with the 47 meat isolates.

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 become 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 laboratories. 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.

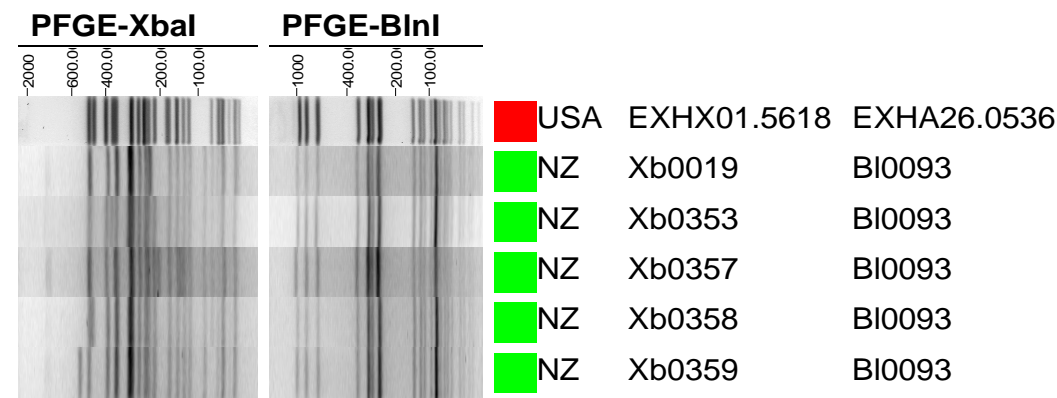
All of the 47 bovine isolates were distinguishable from the 2011 USA isolates using two enzymes.

Two of the USA patterns were similar with one enzyme as shown in Figures 1 and 2. However addition of second enzyme data confirms that the isolates are distinguishable. This does however reinforce the importance of comparing isolates with more than one enzyme.



The USA isolate is from an outbreak linked to lettuce

Figure 1. Isolates with similar *Xba*I patterns (New Zealand isolates green, USA isolate red)



The USA isolates is from a ground beef outbreak

Figure 2. Isolates with similar *BlnI* patterns (New Zealand isolates green, USA isolate red)

E. Queries during 2011

Two queries have been responded to using this genotyping data.

Query 1: In May 2011, Nicola Dermer (MPI) asked as to whether four isolates from the same premises/lab, were of the same genotype. Comparison of PFGE patterns identified that they were all distinguishable from each other (Figure 3). There was also some variability in the STEC PCR and H7 results.



Figure 3. Comparison of four New Zealand isolates

Query 2: In January 2012, Philip Bronstein, Ph.D. Staff Officer, Microbiologist, USDA/FSIS/OPHS/MD/MIB, requested comparison of PFGE patterns as part of a multi-source *E.coli* O157:H7 supplier implication investigation. Request was made to Jason Frost (MFAT), and communicated via Roger Cook (MPI).

Comparison of the gel image, enabled us to conclude that we have NOT seen the implicated *XbaI:BlnI* pattern in New Zealand. As shown in Figure 4, there are several bands differences between the implicated pattern and the closest New Zealand patterns, for both enzymes.



Figure 4. Comparison New Zealand patterns with USA isolate M-04873

F. Conclusions

A total of 47 *E. coli* O157:H7 isolates from meat received by ESR during the period 1 January 2011 to 31 December 2011 were analysed by PFGE using both *Xba*I and *Bln*I. When the two PFGE types were combined 30 *Xba*I:*Bln*I types were observed.

Twenty-five of these patterns had not previously been seen in the New Zealand database. The remaining five patterns were indistinguishable using two enzymes from patterns previously analysed. PFGE pattern Xb0040 has historically been frequently observed among bovine isolates. It was not observed among the 2011 isolates. Whether this is evidence of a change in genotypes in the population, or simply an anomaly of sampling, will only be revealed once analysis of future years' isolates is completed.

All of the 47 New Zealand bovine isolates were distinguishable using two enzymes from the 2011 USA isolates reported by PulseNet USA as part of recognised outbreaks.

Appendix 1: PFGE patterns observed among New Zealand bovine and human isolates (1993-2011) compared with those found among 2011 meat isolates

Only isolates with two enzyme data are presented.

PFGE Pattern	2011 Meat Isolates	Previous Isolates	
		Bovine	Human
Xb0358:BI0093	6	1	
Xb0365:BI0093	5		
Xb0367:BI0093	3		
Xb0019:BI0093	2	3	1
Xb0202:BI0150	2	1	
Xb0049:BI0091	2		6
Xb0202:BI0188	2		
Xb0352:BI0007	2		
Xb0358:BI0179	2		
Xb0331:BI0007	1	1	
Xb0049:BI0183	1		
Xb0049:BI0187	1		
Xb0351:BI0007	1		
Xb0353:BI0093	1		
Xb0357:BI0016	1		
Xb0357:BI0034	1		
Xb0357:BI0080	1		
Xb0357:BI0093	1		
Xb0358:BI0016	1		
Xb0358:BI0180	1		
Xb0359:BI0093	1		
Xb0361:BI0035	1		
Xb0362:BI0093	1		
Xb0363:BI0093	1		
Xb0365:BI0185	1		
Xb0366:BI0182	1		
Xb0367:BI0181	1		
Xb0368:BI0184	1		
Xb0369:BI0093	1		
Xb0370:BI0186	1		
Xb0040:BI0093		52	10
Xb0168:BI0007		29	10
Xb0040a:BI0080		25	2
Xb0092:BI0007		20	2
Xb0040a:BI0093		14	
Xb0040i:BI0093		12	2
Xb0164:BI0139		12	

PFGE Pattern	2011 Meat Isolates	Previous Isolates	
		Bovine	Human
Xb0138:BI0093		11	2
Xb0165:BI0139		10	
Xb0095:BI0041		8	
Xb0040g:BI0093		7	4
Xb0090:BI0007		7	1
Xb0137:BI0132		7	
Xb0156:BI0093		6	4
Xb0138:BI0080		6	2
Xb0014:BI0036		6	
Xb0158:BI0031		5	
Xb0197:BI0099		5	
Xb0049:BI0004		4	16
Xb0040:BI0044		4	
Xb0040d:BI0036		4	
Xb0092:BI0068		4	
Xb0138:BI0023		4	
Xb0049:BI0108		3	6
Xb0040:BI0036		3	1
Xb0040:BI0132		3	1
Xb0049:BI0001		3	1
Xb0139:BI0112		3	1
Xb0040:BI0085		3	
Xb0040:BI0088		3	
Xb0138:BI0143		3	
Xb0144:BI0132		3	
Xb0154:BI0093		3	
Xb0040:BI0016		2	23
Xb0019:BI0016		2	2
Xb0040:BI0080		2	2
Xb0164:BI0116		2	2
Xb0021:BI0093		2	1
Xb0040j:BI0093		2	1
Xb0061:BI0027		2	1
Xb0091:BI0057		2	1
Xb0009:BI0129		2	
Xb0012:BI0093		2	
Xb0018:BI0080		2	

PFGE Pattern	2011 Meat Isolates	Previous Isolates	
		Bovine	Human
Xb0040a:BI0031		2	
Xb0040a:BI0085		2	
Xb0040g:BI0132		2	
Xb0040p:BI0154		2	
Xb0048:BI0050		2	
Xb0092:BI0002		2	
Xb0094:BI0057		2	
Xb0110:BI0093		2	
Xb0123:BI0033		2	
Xb0138:BI0076		2	
Xb0158:BI0158		2	
Xb0163:BI0080		2	
Xb0163:BI0143		2	
Xb0179:BI0093		2	
Xb0207:BI0040		2	
Xb0239:BI0070		2	
Xb0241:BI0040		2	
Xb0265:BI0007		2	
Xb0040p:BI0016		1	3
Xb0138:BI0016		1	3
Xb0168:BI0068		1	3
Xb0049:BI0050		1	2
Xb0138:BI0017		1	2
Xb0163:BI0076		1	2
Xb0164:BI0035		1	2
Xb0012:BI0080		1	1
Xb0048:BI0004		1	1
Xb0049:BI0084		1	1
Xb0112:BI0094		1	1
Xb0138:BI0107		1	1
Xb0004:BI0054		1	
Xb0010:BI0130		1	
Xb0012:BI0034		1	
Xb0012:BI0112		1	
Xb0013:BI0110		1	
Xb0015:BI0171		1	
Xb0021:BI0076		1	
Xb0028:BI0133		1	
Xb0033:BI0016		1	
Xb0033:BI0080		1	
Xb0033:BI0093		1	
Xb0033:BI0177		1	
Xb0039:BI0178		1	

PFGE Pattern	2011 Meat Isolates	Previous Isolates	
		Bovine	Human
Xb0040:BI0035		1	
Xb0040:BI0107		1	
Xb0040:BI0139		1	
Xb0040:BI0159		1	
Xb0040:BI0172		1	
Xb0040:BI0175		1	
Xb0040b:BI0036		1	
Xb0040c:BI0022		1	
Xb0040c:BI0093		1	
Xb0040c:BI0154		1	
Xb0040d:BI0128		1	
Xb0040e:BI0152		1	
Xb0040g:BI0034		1	
Xb0040i:BI0077		1	
Xb0040i:BI0080		1	
Xb0040j:BI0080		1	
Xb0040k:BI0080		1	
Xb0040l:BI0093		1	
Xb0040l:BI0139		1	
Xb0040l:BI0170		1	
Xb0040m:BI0093		1	
Xb0040o:BI0093		1	
Xb0040p:BI0080		1	
Xb0040p:BI0133		1	
Xb0044:BI0081		1	
Xb0044:BI0084		1	
Xb0048:BI0108		1	
Xb0048:BI0117		1	
Xb0048:BI0176		1	
Xb0049:BI0016		1	
Xb0049:BI0037		1	
Xb0049:BI0113		1	
Xb0049:BI0117		1	
Xb0049:BI0118		1	
Xb0049:BI0144		1	
Xb0050:BI0002		1	
Xb0050:BI0007		1	
Xb0051:BI0030		1	
Xb0054:BI0115		1	
Xb0057:BI0072		1	
Xb0066:BI0169		1	
Xb0067:BI0056		1	
Xb0070:BI0119		1	

PFGE Pattern	2011 Meat Isolates	Previous Isolates	
		Bovine	Human
Xb0094:B10067		1	
Xb00xx:B10050		1	
Xb0110:B10043		1	
Xb0119:B10035		1	
Xb0123:B10002		1	
Xb0123:B10082		1	
Xb0125:B10016		1	
Xb0126:B10139		1	
Xb0127:B10035		1	
Xb0133:B10033		1	
Xb0138:B10088		1	
Xb0138:B10132		1	
Xb0138:B10151		1	
Xb0154:B10080		1	
Xb0158:B10076		1	
Xb0158:B10077		1	
Xb0158:B10114		1	
Xb0163:B10093		1	
Xb0164:B10088		1	
Xb0168:B10041		1	
Xb0168:B10097		1	
Xb0168:B10100		1	
Xb0168:B10174		1	
Xb0171:B10109		1	
Xb0189:B10166		1	
Xb0197:B10139		1	
Xb0199:NOCUT		1	
Xb0202:B10165		1	
Xb0203:B10007		1	
Xb0204:B10007		1	
Xb0206:B10111		1	
Xb0209:B10007		1	
Xb0210:B10041		1	
Xb0214:B10121		1	
Xb0216:B10028		1	
Xb0221:B10093		1	
Xb0222:B10093		1	
Xb0224:B10077		1	
Xb0225:B10132		1	
Xb0226:B10017		1	
Xb0226:B10080		1	
Xb0227:B10022		1	
Xb0228:B10126		1	

PFGE Pattern	2011 Meat Isolates	Previous Isolates	
		Bovine	Human
Xb0230:B10093		1	
Xb0232:B10133		1	
Xb0233:B10080		1	
Xb0233:B10114		1	
Xb0238:B10007		1	
Xb0240:B10057		1	
Xb0242:B10153		1	
Xb0243:B10070		1	
Xb0261:B10007		1	
Xb0262:B10156		1	
Xb0263:B10068		1	
Xb0264:B10007		1	
Xb0266:B10157		1	
Xb0267:B10007		1	
Xb0268:B10071		1	
Xb0269:B10093		1	
Xb0285:B10007		1	
Xb0286:B10007		1	
Xb0294:B10036		1	
Xb0295:B10093		1	
Xb0298:B10001		1	
Xb0299:B10081		1	
Xb0329:B10033		1	
Xb0347:B10080		1	
Xb0040:B10022			4
Xb0012:B10095			3
Xb0070:B10110			3
Xb0155:B10046			3
Xb0194:B10101			3
Xb0012:B10083			2
Xb0020:B10022			2
Xb0040:B10094			2
Xb0040d:B10080			2
Xb0040g:B10080			2
Xb0040i:B10016			2
Xb0070:B10004			2
Xb0138:B10022			2
Xb0138:B10035			2
Xb0012:B10016			1
Xb0012:B10076			1
Xb0016:B10016			1
Xb0018:B10024			1
Xb0019:B10094			1

PFGE Pattern	2011 Meat Isolates	Previous Isolates	
		Bovine	Human
Xb0021:B10016			1
Xb0021:B10022			1
Xb0021:B10035			1
Xb0021:B10089			1
Xb0021:B10158			1
Xb0024:B10014			1
Xb0026:B10076			1
Xb0027:B10078			1
Xb0029:B10093			1
Xb0029:B10094			1
Xb0030:B10019			1
Xb0031:B10016			1
Xb0035:B10039			1
Xb0035:B10061			1
Xb0036:B10139			1
Xb0037:B10022			1
Xb0040:B10074			1
Xb0040:B10087			1
Xb0040:B10158			1
Xb0040a:B10016			1
Xb0040b:B10093			1
Xb0040g:B10016			1
Xb0040g:B10029			1
Xb0040g:B10082			1
Xb0040g:B10094			1
Xb0040i:B10022			1
Xb0040l:B10080			1
Xb0040r:B10004			1
Xb0040r:B10084			1
Xb0041:B10019			1
Xb0044:B10050			1
Xb0044:B10086			1
Xb0044:B10103			1
Xb0048:B10032			1
Xb0048:B10042			1
Xb0048:B10081			1
Xb0049:B10018			1
Xb0049:B10020			1
Xb0049:B10026			1
Xb0049:B10032			1
Xb0049:B10081			1
Xb0049:B10082			1
Xb0049:B10096			1

PFGE Pattern	2011 Meat Isolates	Previous Isolates	
		Bovine	Human
Xb0049:B10098			1
Xb0049:B10106			1
Xb0049:B10108a			1
Xb0049:B10176			1
Xb0049:B10177			1
Xb0053:B10061			1
Xb0054:B10048			1
Xb0054:B10051			1
Xb0054:B10061			1
Xb0054:B10137			1
Xb0057:B10103			1
Xb0058:B10007			1
Xb0059:B10092			1
Xb0061:B10084			1
Xb0062:B10068			1
Xb0062:B10070			1
Xb0063:B10105			1
Xb0070:B10021			1
Xb0070:B10038			1
Xb0078:B10013			1
Xb0082:B10104			1
Xb0085:B10010			1
Xb0086:B10034			1
Xb0093:B10007			1
Xb0096:B10007			1
Xb0096:B10069			1
Xb0107:B10080			1
Xb0120:B10023			1
Xb0121:B10055			1
Xb0124:B10094			1
Xb0136:B10049			1
Xb0138:B10015			1
Xb0138:B10025			1
Xb0138:B10043			1
Xb0138:B10044			1
Xb0138:B10045			1
Xb0138:B10047			1
Xb0138:B10053			1
Xb0138:B10087			1
Xb0138:B10102			1
Xb0138:B10114			1
Xb0158:B10016			1
Xb0172:B10012			1

PFGE Pattern	2011 Meat Isolates	Previous Isolates	
		Bovine	Human
Xb0179:BI0079			1
Xb0181:BI0070			1
Xb0183:BI0007			1
Xb0185:BI0071			1
Xb0186:BI0011			1
Xb0191:BI0061			1
Xb0191:BI0073			1
Xb0191:BI0158			1
Xb0192:BI0061			1
Xb0195:BI0038			1
Xb0200:BI0075			1
Xb0200:BI0084			1
Xb0200:BI0090			1
Xb0201:BI0016			1
Xb0229:BI0017			1
Xb0231:BI0022			1
Grand Total	47	491	268