

Diversity and Trends in the National MLVA Databases and Data Interpretation during Outbreak Investigations



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Session VI
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Objectives

- ❑ **Describe the trends observed in the national MLVA national databases**
- ❑ **Discuss the current MLVA interpretation guidelines in the context of two outbreak investigations**

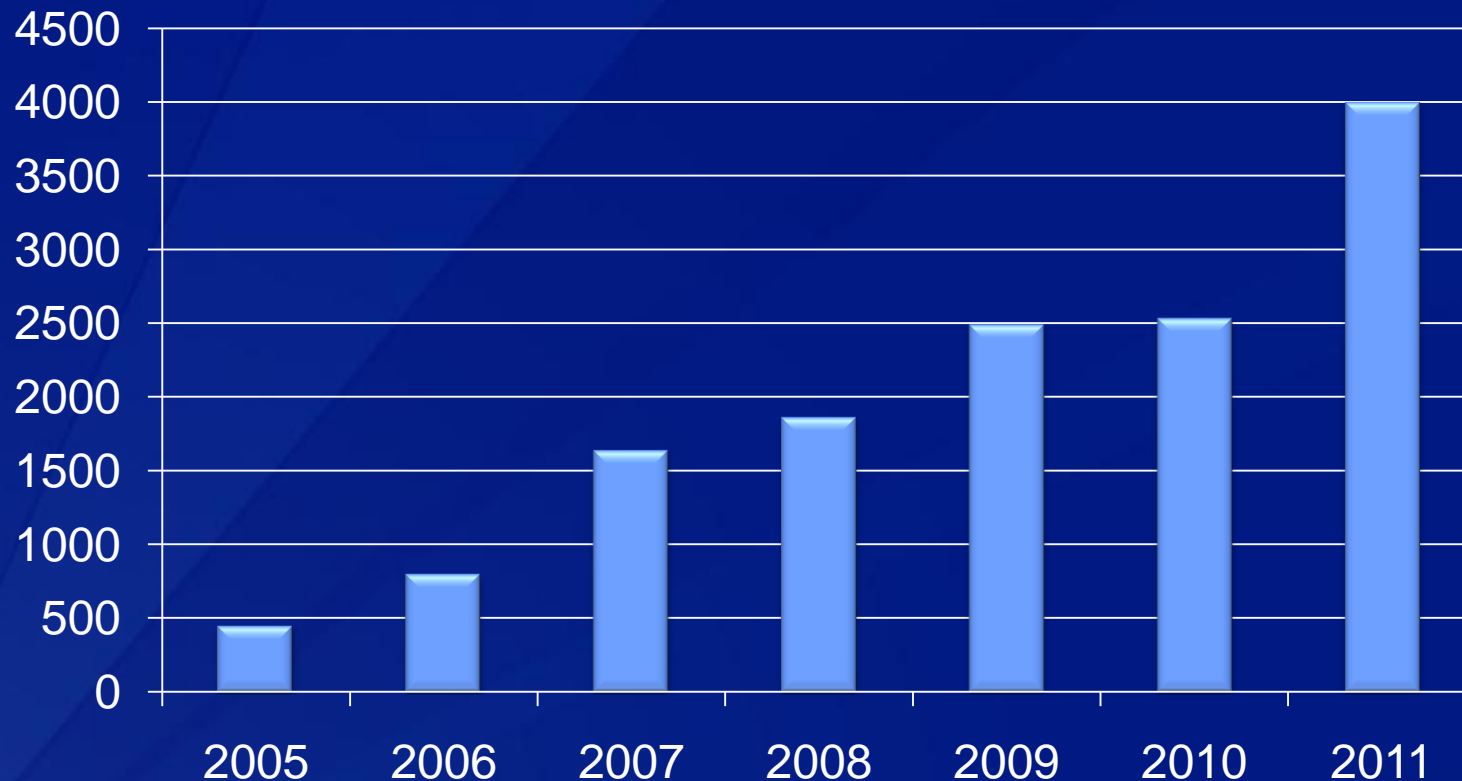
MLVA Overview

❑ Multiple-locus Variable Number Tandem Repeat Analysis (MLVA)

- Targets select VNTRs in the bacterial genome
- Useful in foodborne outbreak surveillance
- Implementation
 - Used as a complementary technique to PFGE
 - Focused on select emerging multi-state clusters prioritized by CDC PulseNet Lab
 - As well as special investigations by CDC, USDA, FDA, and local / state epidemiologists

PulseNet MLVA activity, 2005-2011

MLVA patterns submitted to PulseNet databases¹



¹Numbers apply to fiscal years

E. coli O157:H7, *Salmonella ser.* Enteritidis, and *Salmonella ser.* Typhimurium databases

DIVERSITY AND TRENDS

General Overview of *E. coli* MLVA Database

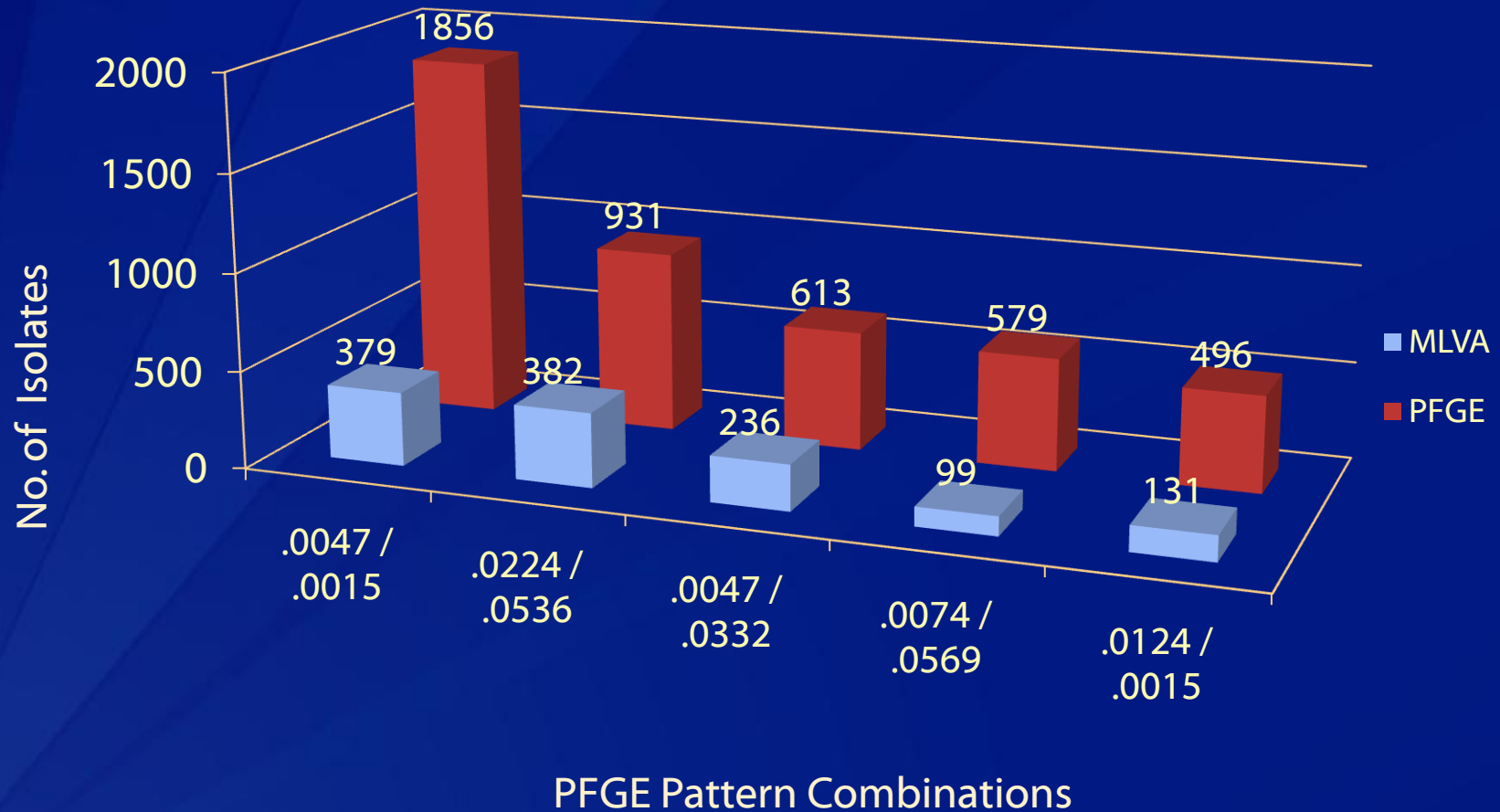
- Total of 5,150 isolates
- USDA isolates account for 6%
- Null alleles detected for VNTR3, VNTR9, VNTR36, and VNTR37

Top 5 MLVA patterns

Rank	VNTR_3	VNTR_34	VNTR_9	VNTR_25	VNTR_17	VNTR_19	VNTR_36	VNTR_37	No. Isolates	Frequency
1	10	7	16	4	4	7	9	7	227	4.37%
2	9	6	17	3	4	8	8	5	115	2.22%
3	12	6	13	3	6	11	-2	6	87	1.68%
4	14	7	14	4	4	7	8	7	80	1.54%
5	8	7	10	4	4	7	10	7	78	1.50%

1. Spinach (0609MLEXH-2); 1007INEXH-1
2. NE Beef (0806OHEXH-1mlc)
3. Cookie dough (0905MAEXH-1); 0809OREXH-1; 1007LACEXH-1
4. Taco Bell (0612MLEXH-2)
5. Schnuck's salad bar (1110MOEXH-2)

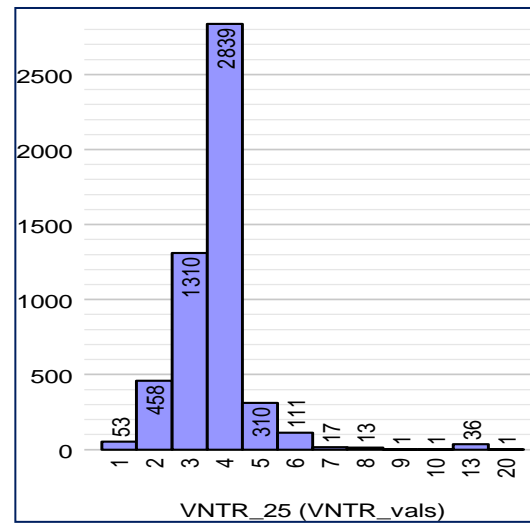
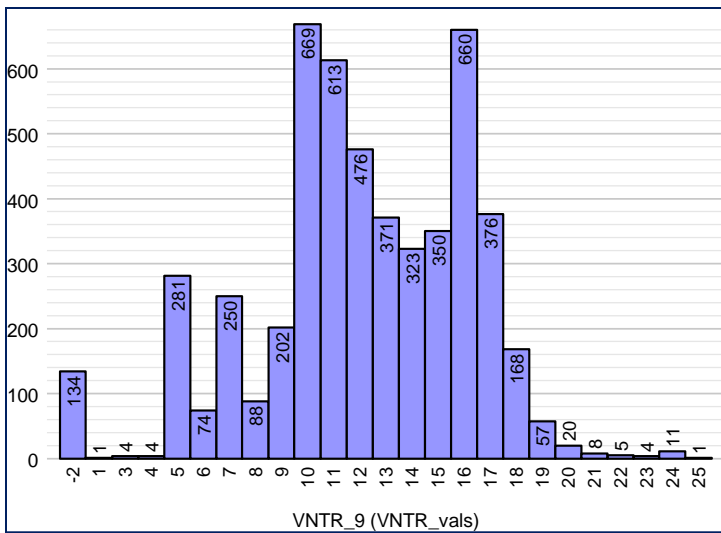
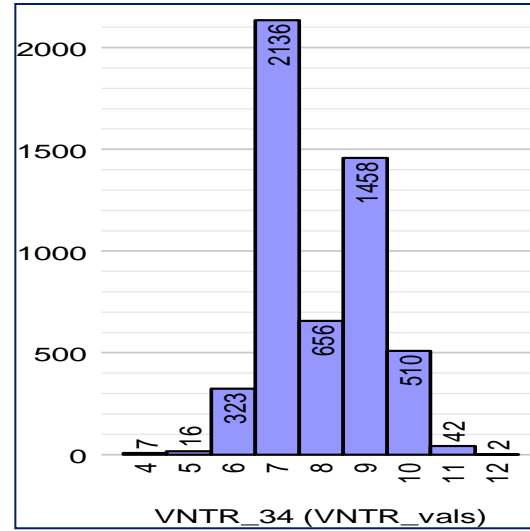
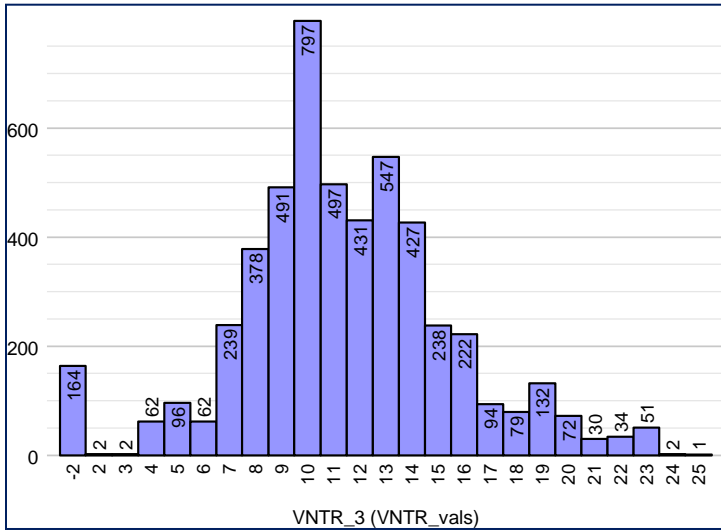
Top 5 *E. coli* O157:H7 PFGE Pattern Combinations in the MLVA database



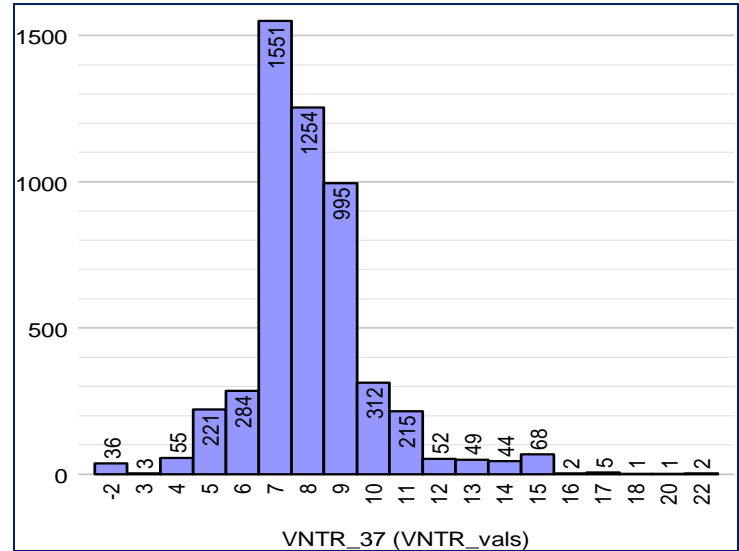
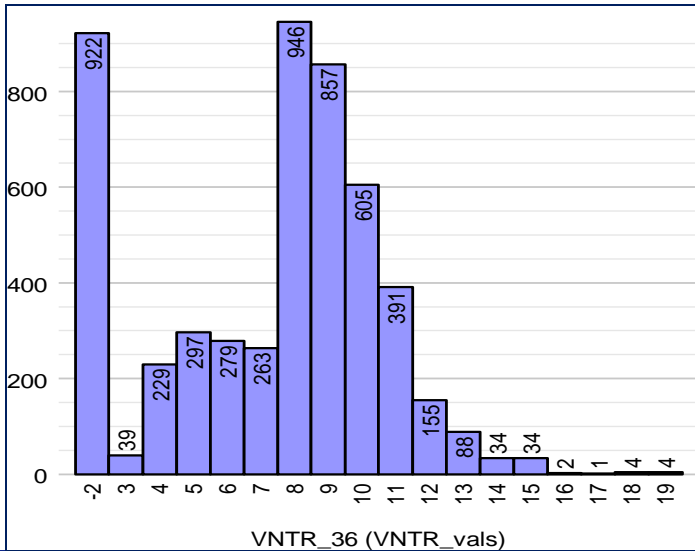
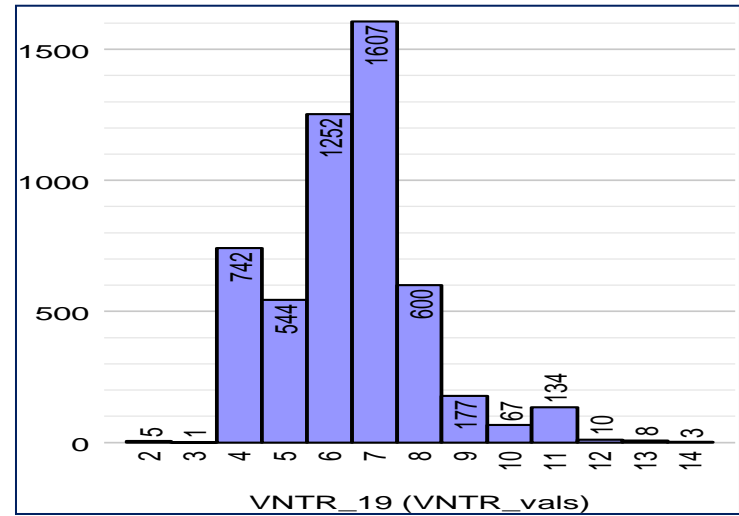
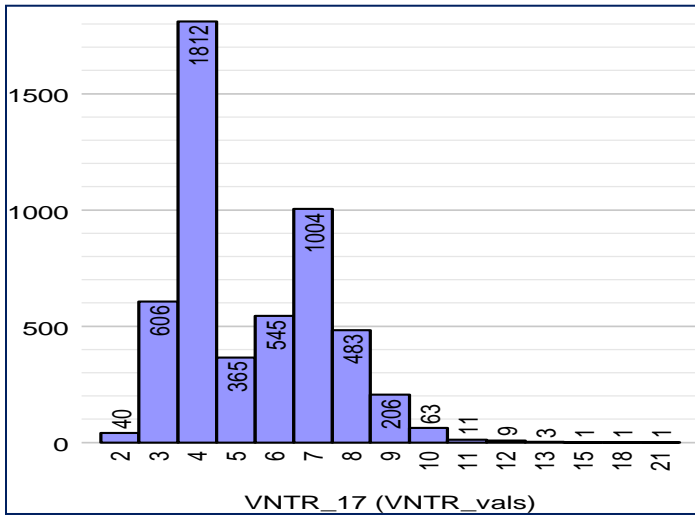
MLVA Diversity Among the Most Common PFGE Pattern Combinations

1. EXHX01.0047/.0015: 159 Patterns; 379 isolates
2. EXHX01.0224/.0536: 104 Patterns; 382 isolates
3. EXHX01.0047/.0332: 56 Patterns; 236 isolates
4. EXHX01.0074/.0569: 42 Patterns; 99 isolates
5. EXHX01.0124/.0015: 40 Patterns; 131 isolates

R1 VNTR Distributions



R2 VNTR Distribution



VNTR36 Null allele

- 922 Total Isolates with VNTR36 null allele (17.9%)
- Most common PFGE patterns associated
 - .0224 / .0536 (377 isolates)
 - .0125 / .0570 (212 isolates)

VNTR3 Null allele

- 164 Total Isolates have a null allele at VNTR3 (3.2%)
- USDA food / environment isolates account for 71 / 164 (43%)
- No dominant PFGE pattern association

VNTR9 Null allele

- 134 Total isolates with VNTR9 null alleles (2.6%)
- USDA food / environment isolates account for 33 / 134 (25%)
- No dominant PFGE pattern association

Minimum Spanning Tree – *E. coli* O157:H7

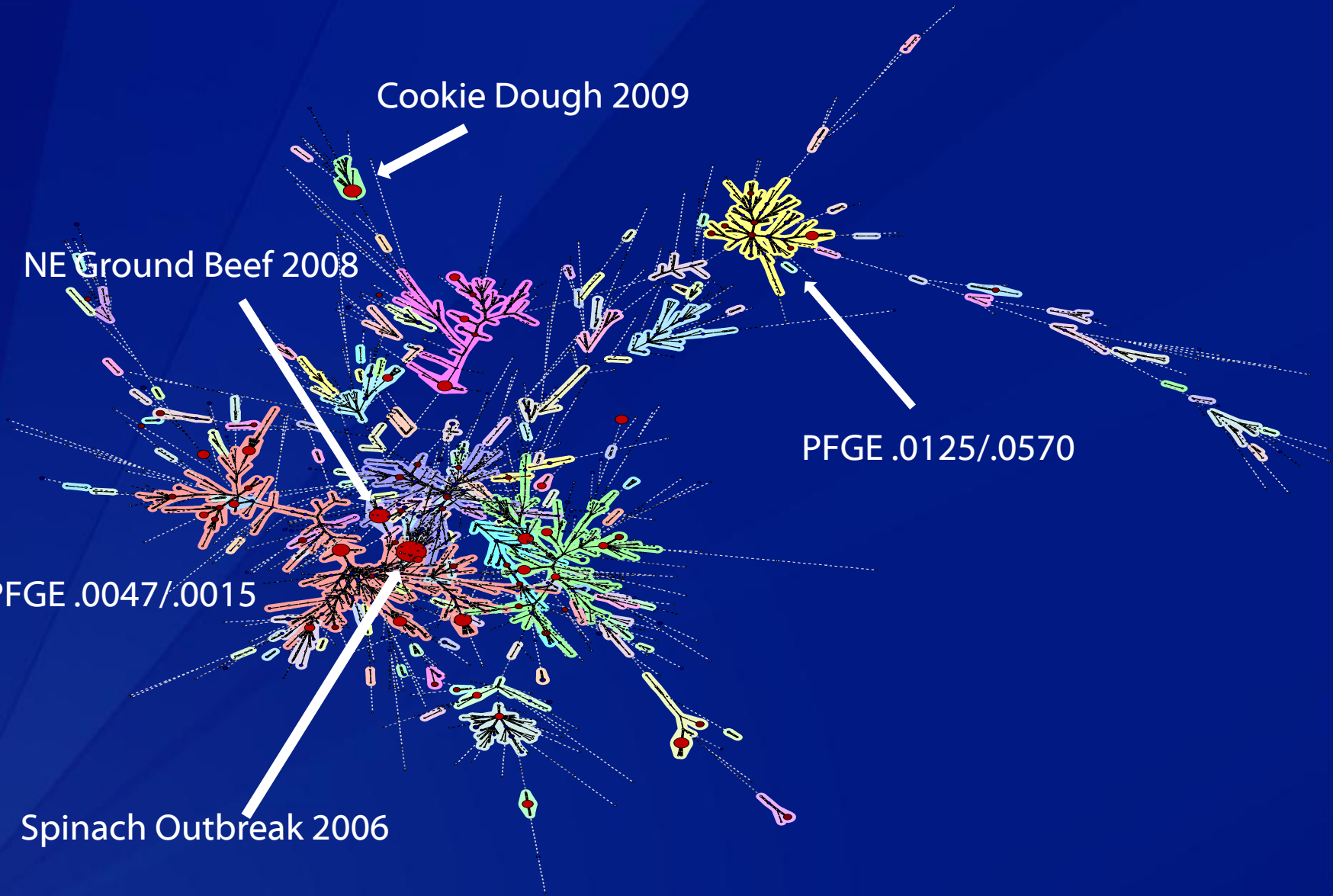
Cookie Dough 2009

NE Ground Beef 2008

PFGE .0125/.0570

PFGE .0047/.0015

Spinach Outbreak 2006



General Overview *Salmonella* serovar Typhimurium MLVA Database

- ❑ **Total of 4,432 isolates**
- ❑ **Null alleles detected for ST5 and STTR10**

MLVA of most common PFGE defined strains

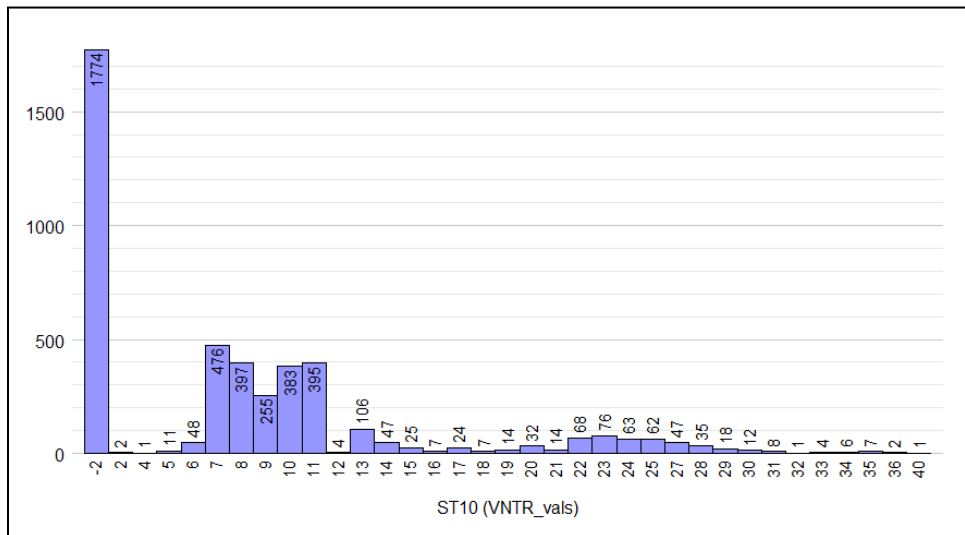
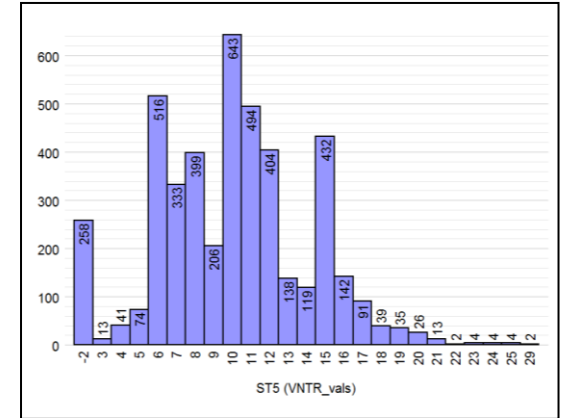
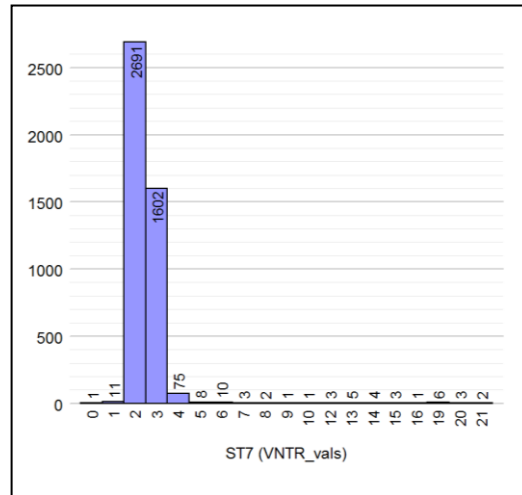
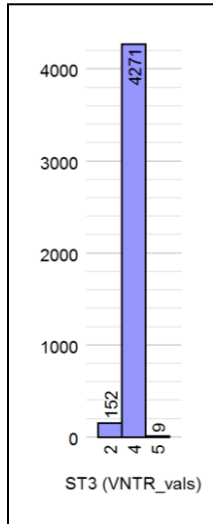
1. JPXX01.0003: 153 profiles among 234 isolates
2. JPXX01.0621: 83 profiles among 375 isolates
 - 0607INJPX-1c (food retailer)
 - 1012INJPX-1 (sprouts)
3. JPXX01.0001: 47 profiles among 98 isolates
4. JPXX01.0146 : 60 profiles among 133 isolates
5. JPXX01.1212: 32 profiles among 84 isolates

Top 5 MLVA patterns

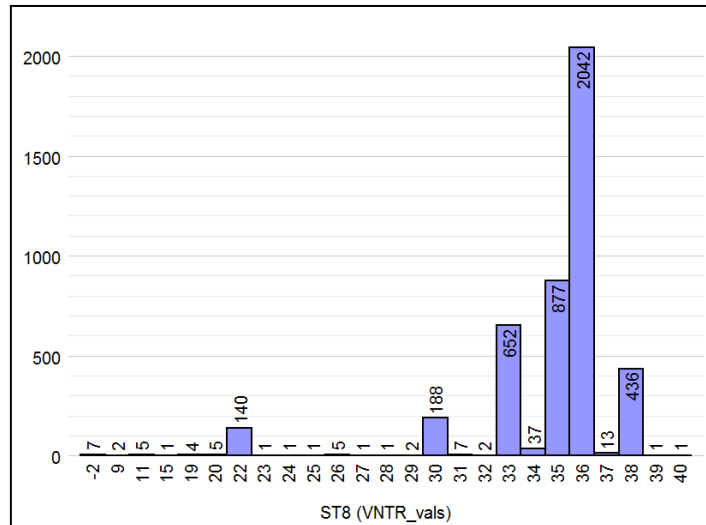
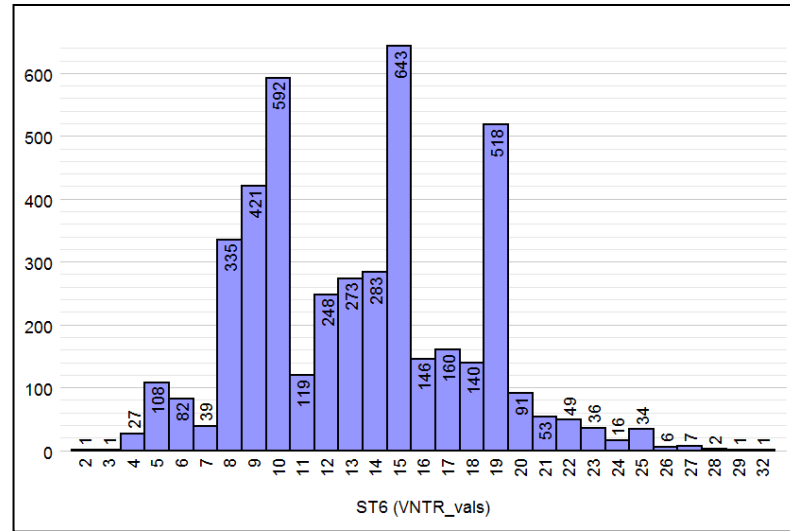
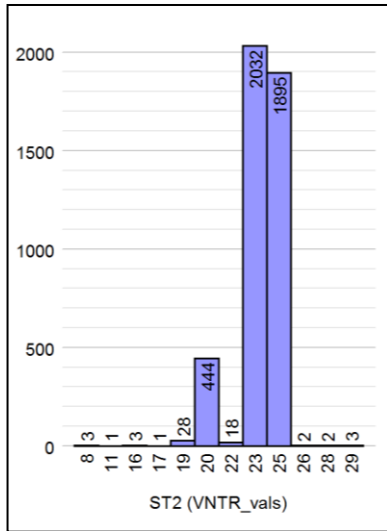
Rank	ST3	ST5	ST7	STTR10	ST2	ST6	ST8	No. Isolates	Frequency
1	4	15	2	-2	20	15	38	342	7.72%
2	4	10	2	7	25	19	36	296	6.68%
3	4	11	2	11	23	9	36	249	5.62%
4	4	6	2	10	25	9	36	116	2.62%
5	4	6	2	8	25	10	36	108	2.44%

1. 0909MAJPX-1
 - almost all have PFGE pattern JPXX01.0177
2. 1010MLJPX-1 (lab-acquired infections)
 - all except two have PFGE pattern JPXX01.0014
3. 0811SDCJPX-1c / 0811MLJPX-1c (peanut butter)
4. 1012INJPX-1
 - almost all have pattern JPXX01.0621
5. 1005MLJPX-1; 1111IAJPX-1
 - Most have PFGE pattern JPXX01.1071

R1 VNTR Distributions



R2 VNTR Distributions

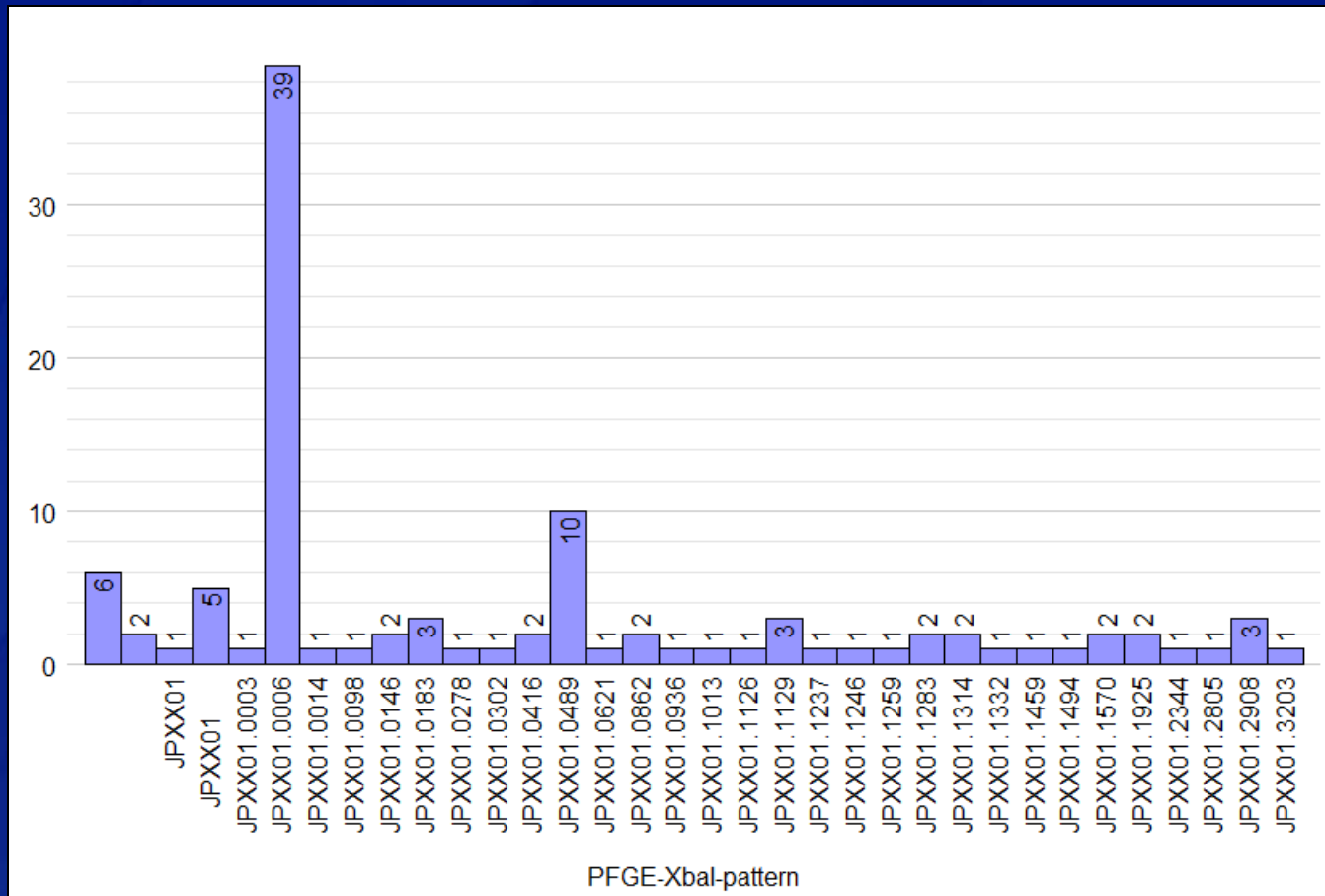


Null alleles

- ST5 - Null allele prevalence $\approx 5.82\%$
 - 258 isolates out of 4432 total
- STTR10 Null allele prevalence $\approx 40.0\%$
 - 1774 isolates out of 4432 total

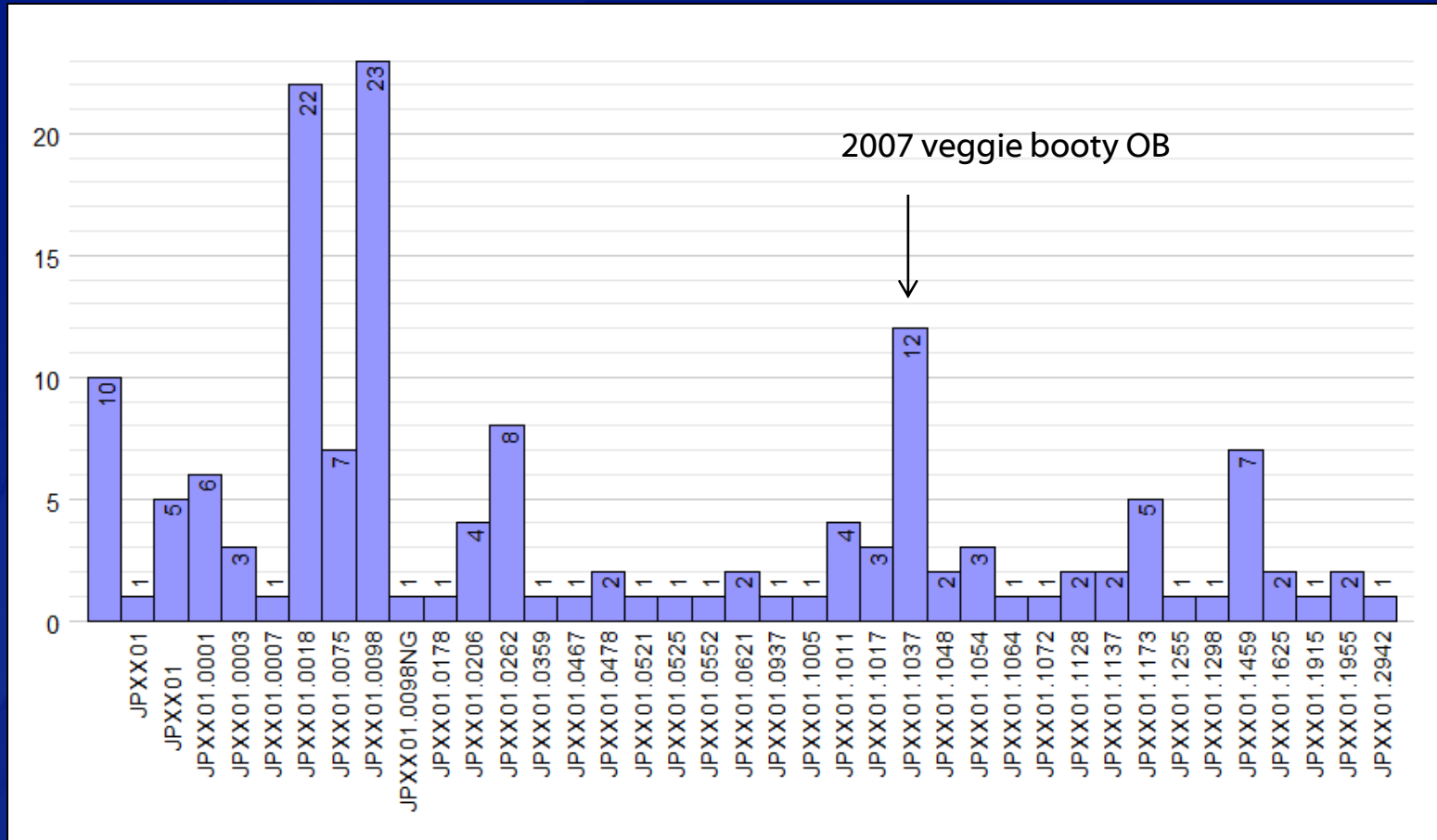
ST5 and STTR10 - both null alleles

- 104/4432 (2.35%)
- Typhimurium only



Only ST5 a null allele

- 153/4432 (3.45%)
- A majority Typhimurium, some l4,5,12:i:-



Pattern
JPXX01.0003/552
complex

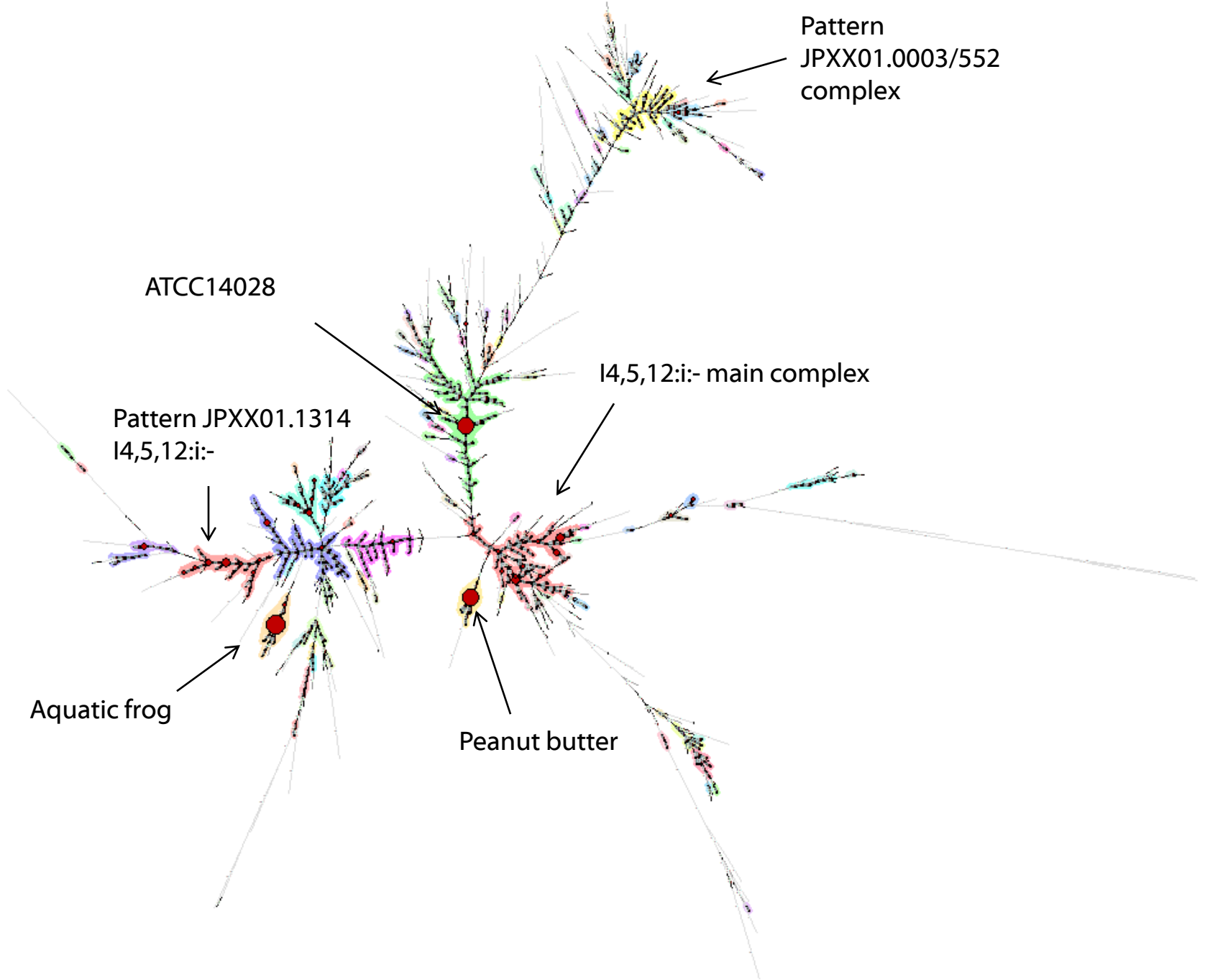
ATCC14028

14,5,12:i:- main complex

Pattern JPXX01.1314
14,5,12:i:-

Aquatic frog

Peanut butter



General Overview Salmonella serovar Enteritidis **MLVA Database**

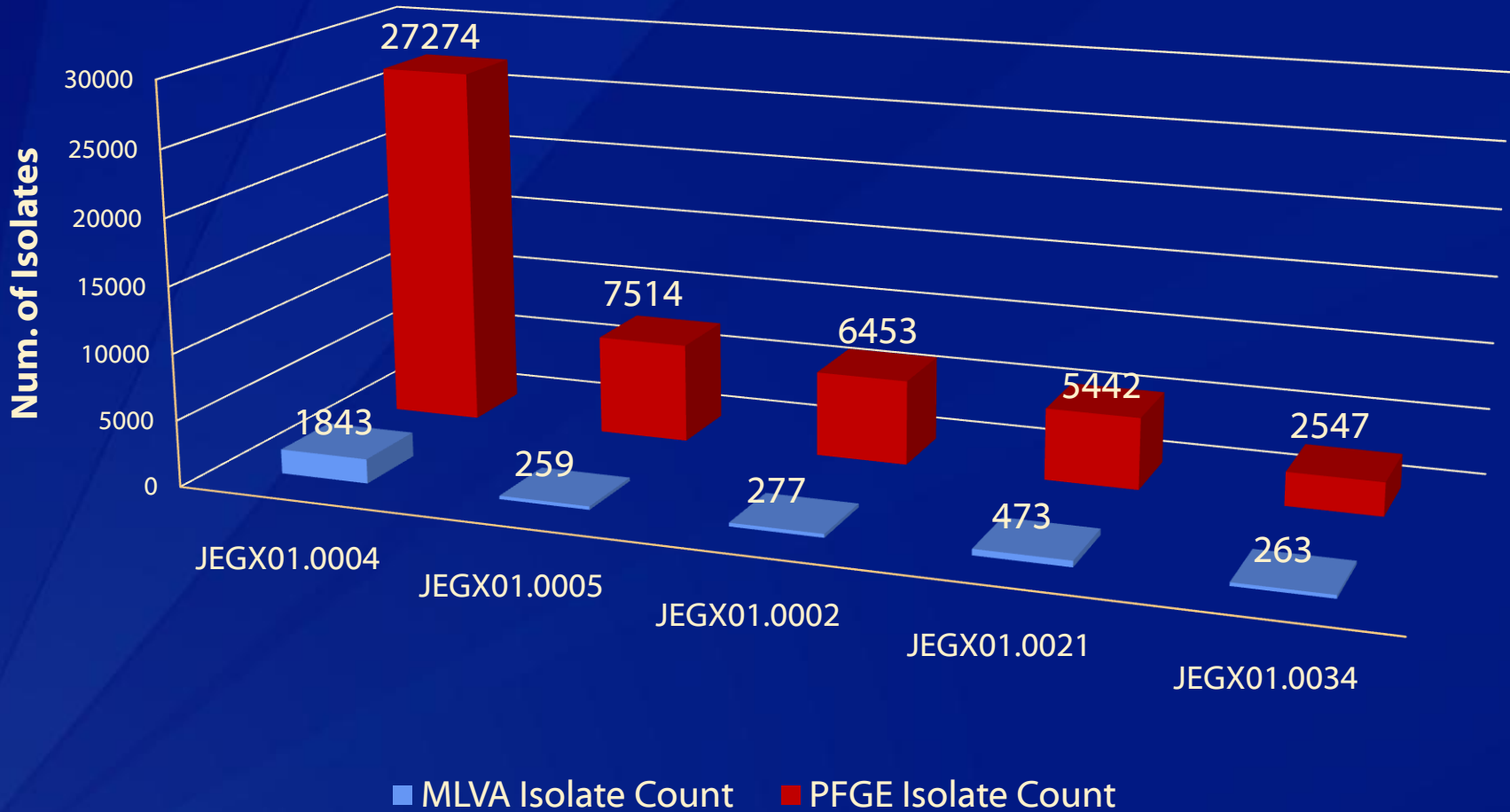
- ❑ **4,138 *S. Enteritidis* isolates**
- ❑ **Null alleles detected for VNTR1, VNTR3, VNTR2, and more rarely VNTR9**

Top 5 MLVA patterns

Rank	VNTR_1	VNTR_2	VNTR_8	VNTR_6	VNTR_9	VNTR_3	VNTR_5	No. Isolates	Frequency
1	6	8	1	10	2	4	10	1304	31.5%
2	6	7	1	10	2	4	9	221	5.34%
3	6	6	2	10	2	4	11	198	4.79%
4	6	8	1	10	2	4	11	183	4.42%
5	6	7	1	10	2	4	10	156	3.77%

1. 2010 Shell Eggs (1006PAJEG-1, 1006SDCJEG-1, 1006TXJEG-1, 1007COJEG-1, 1007IAJEG-1 and 1008MNJEG-1)
2. 2010 Shell Eggs
3. 1004MLJEG-1 (OR, JEGX01.0005)
4. 2010 Shell Eggs
5. 2010 Shell Eggs

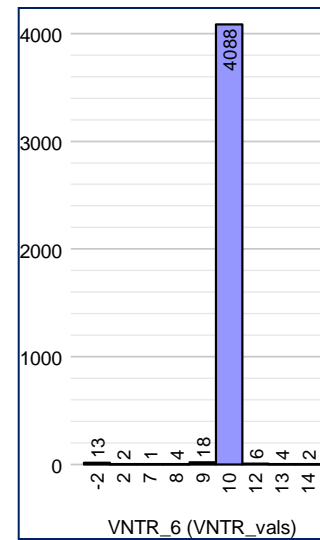
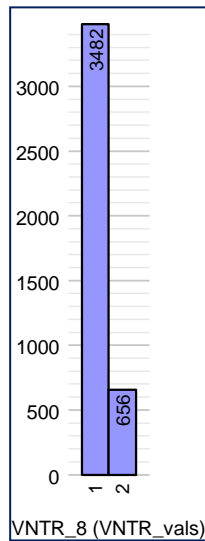
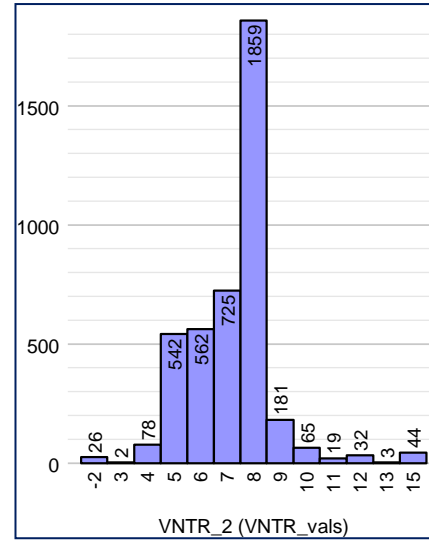
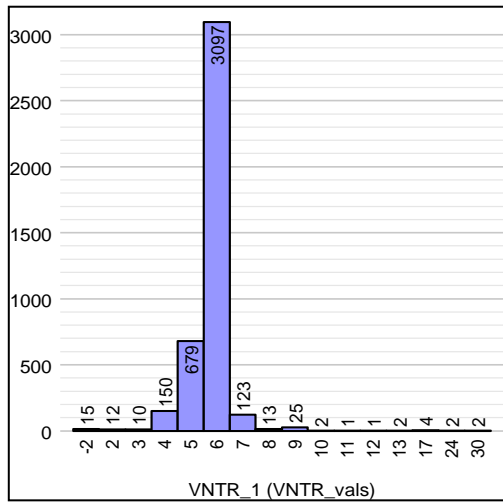
Comparison of Top 5 *Salmonella* serotype Enteritidis PFGE Patterns and their Occurrence in the MLVA and PFGE National Databases



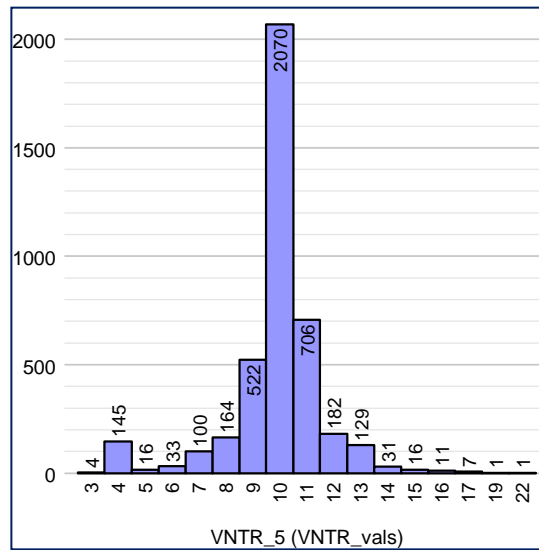
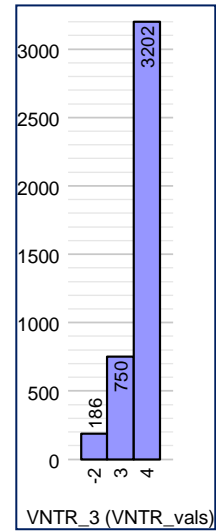
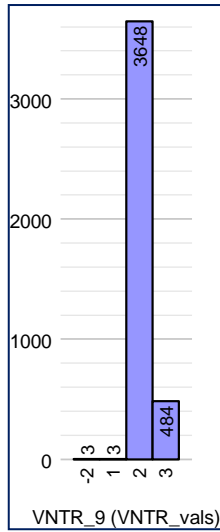
MLVA Diversity Among the Most Common PFGE XbaI Pattern

1. JEGX01.0004: 113 Patterns; 1843 isolates
2. JEGX01.0005: 21 Patterns; 259 isolates
3. JEGX01.0002: 30 Patterns; 277 isolates
4. JEGX01.0021: 28 Patterns; 473 isolates
5. JEGX01.0034: 28 Patterns; 263 isolates

R1 VNTR Distributions



R2 VNTR Distributions



VNTR3 Null allele

- 165 Total Isolates have a null allele at VNTR3 (3.99%)
- 129/165 have Xba patterns JEGX01.0012 and JEGX01.0013 (78%)
 - Almond pattern
- 130/165 have indistinguishable MLVA pattern
- No specific source type

Salmonella serovar Enteritidis MLVA Database

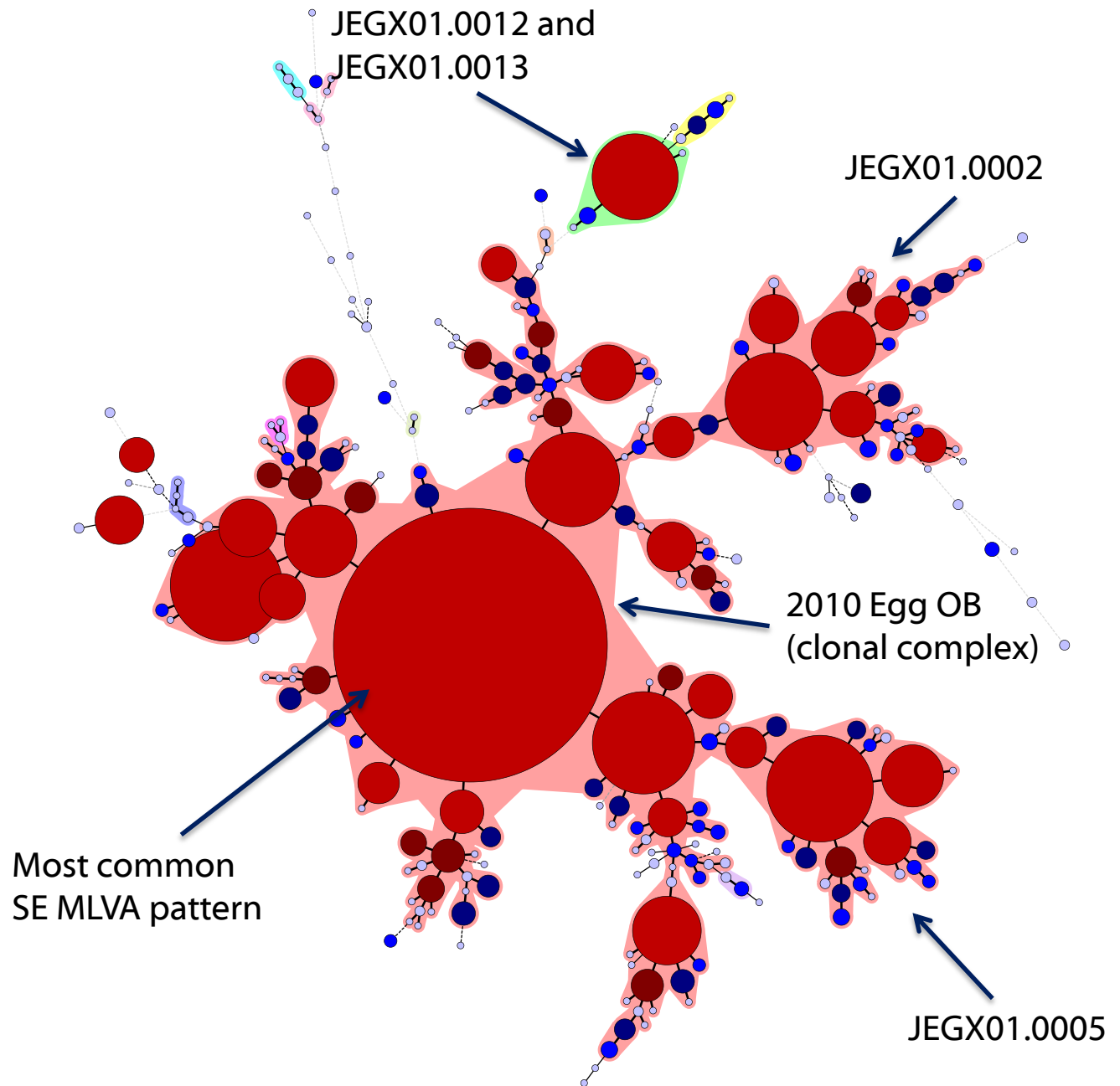
□ *Salmonella* difference fragment (SDF) negative strains

- *sdf* is located on the chromosome¹
- MLVA
 - Loci missing – VNTRs 1, 2, and 3
 - VNTRs 1 and 3 have a much lower diversity
 - VNTR2 highly variable²

1. Agron, P.G., et. al. (2001). Appl Environ Microbiol 67, 4984–4991.

2. Cho S., et. al. 2007. FEMS Microbiol. Lett. 275:16–23

**Minimum
Spanning
Tree for all
Salmonella
serovar
Enteritidis
Isolates in the
SE MLVA
Database**

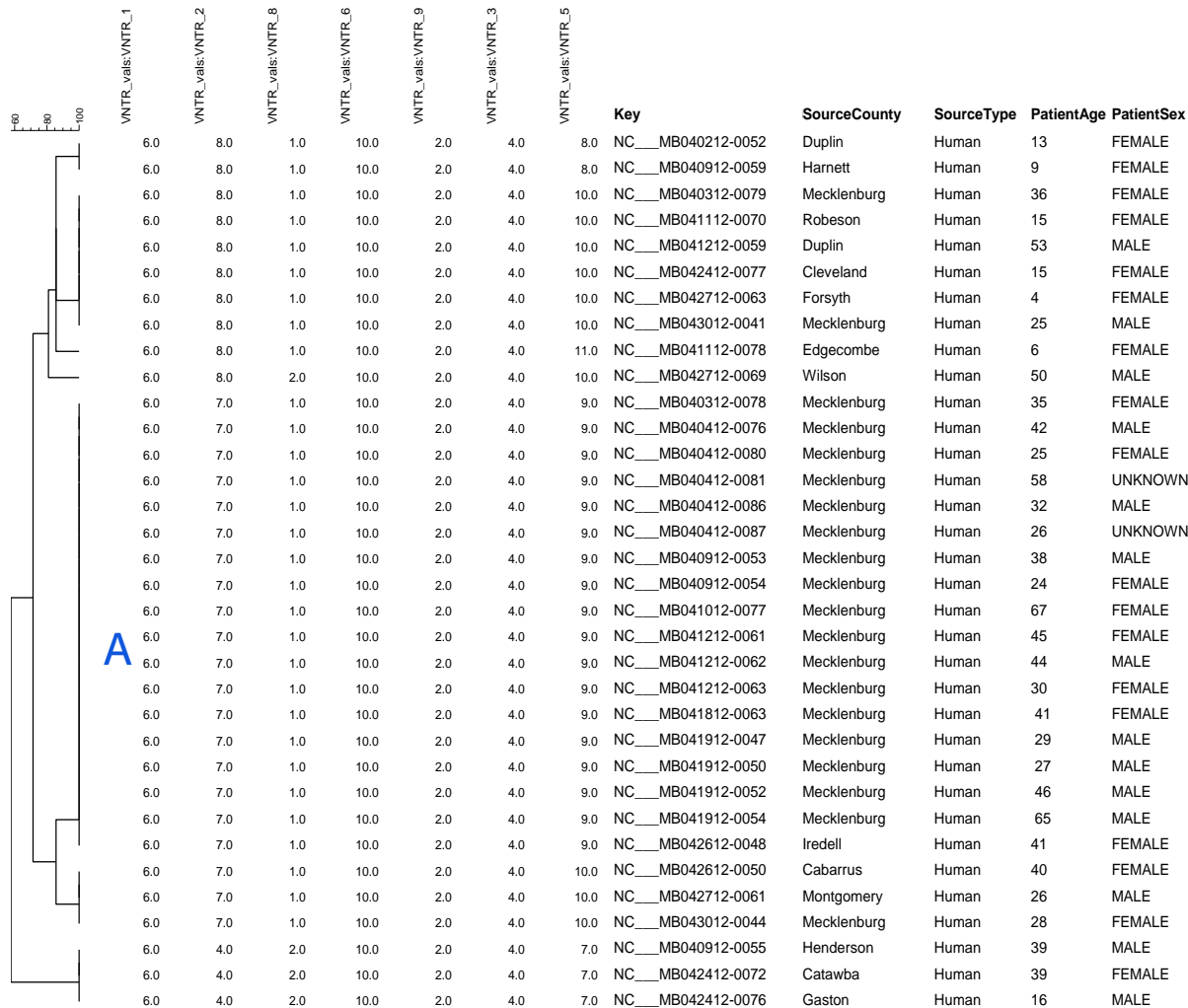


USE OF MLVA IN OUTBREAK INVESTIGATIONS

MLVA in Outbreak Investigations

- ❑ **MLVA has been shown to be a very useful tool in outbreak investigations**
 - Separates background cases from outbreak related cases
 - Instrumental in assessing relatedness of closely related PFGE patterns
- ❑ **Molecular subtyping data alone cannot be used to identify a source**
 - Must have good epidemiological data as well

Salmonella Enteritidis outbreak associated with a NC restaurant - 2012



- First detected April 2012
- PFGE XbaI Pattern: JEGX01.0004
 - Most common *Salmonella* Enteritidis PFGE pattern

E. coli O157:H7 outbreak associated with a romaine lettuce - 2012

	VNTR_3	VNTR_34	VNTR_9	VNTR_25	VNTR_17	VNTR_19	VNTR_36	VNTR_37
AZ___PI11299012	8	7	10	4	4	7	10	7
GA___12C0067922	8	7	10	4	4	7	11	7
CA___M11X04840	8	7	10	4	4	7	9	7
CA___M11X04040	8	8	10	4	4	7	10	7
CA___M11X04713	8	7	10	4	4	7	10	8

- PFGE Pattern combinations:
 - *Xba*I pattern: EXHX01.0047
 - *Bln*I pattern: EXHA26.0015/ EXHA26.1381
- Most common *E. coli* O157:H7 PFGE pattern (EXHX01.0047/.0015)

Summary

- MLVA is a useful technique in foodborne surveillance and outbreak investigations
- Trends in the databases can be used to increase our ability to interpret data during investigations.
- The PulseNet MLVA databases are biased and do not represent true diversity in the population.
- MLVA data interpretation will always have to be coupled with epidemiological information

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Thank you

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