

Whole Genome Sequencing of *Salmonella* Enteritidis

Dave Boxrud

Molecular Epidemiology Supervisor

Minnesota Department of Health Laboratory

Outline

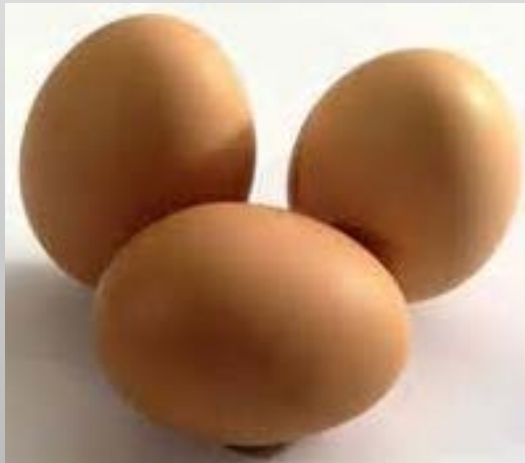
- Background
- Project goals
- Retrospective project
- Prospective project

Salmonella Enteritidis (SE)

- Second most common serotype in US
- Associated with poultry and eggs
- Very clonal
 - 4 PFGE types comprise 76% of database
- Many PHLs do not perform PFGE
- MDH performs PFGE with 2 enzymes (MLVA available)
- MDH identified many SE outbreaks in the past

SE Outbreaks

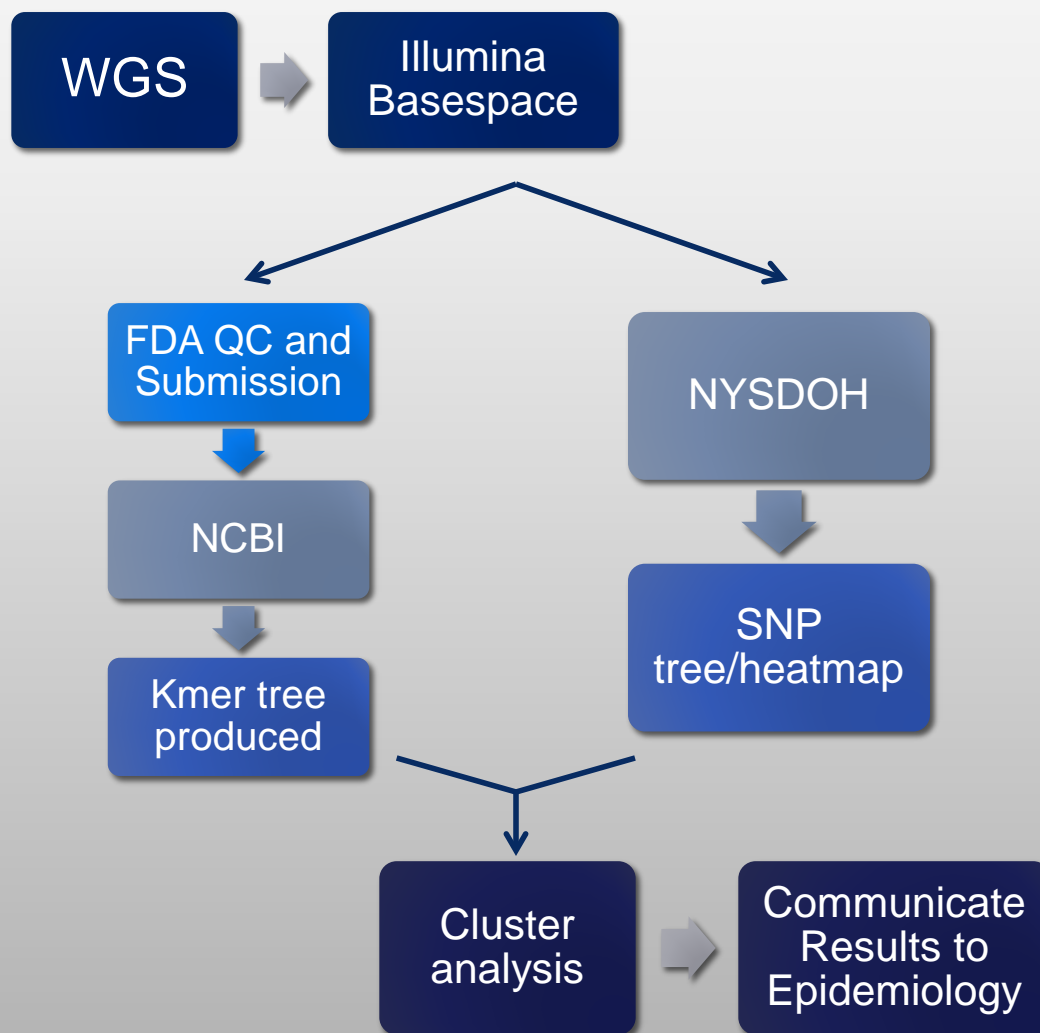
- Schwanns ice cream (1994)-224,00 cases*
- Shell eggs (2010)-54,000 cases**
- Common patterns slows outbreak identification



*blahblah

**blah blah

MDH WGS Data Flow



Criteria for Evaluation of Subtyping Methods (ESGEM)*

- Stability
- Typeability
- Discriminatory power
- Epidemiological concordance
- Reproducibility

*van Belkum et al. Clinical Microbiology and Infectious Disease, 2007.

SE Study Questions

Retrospective

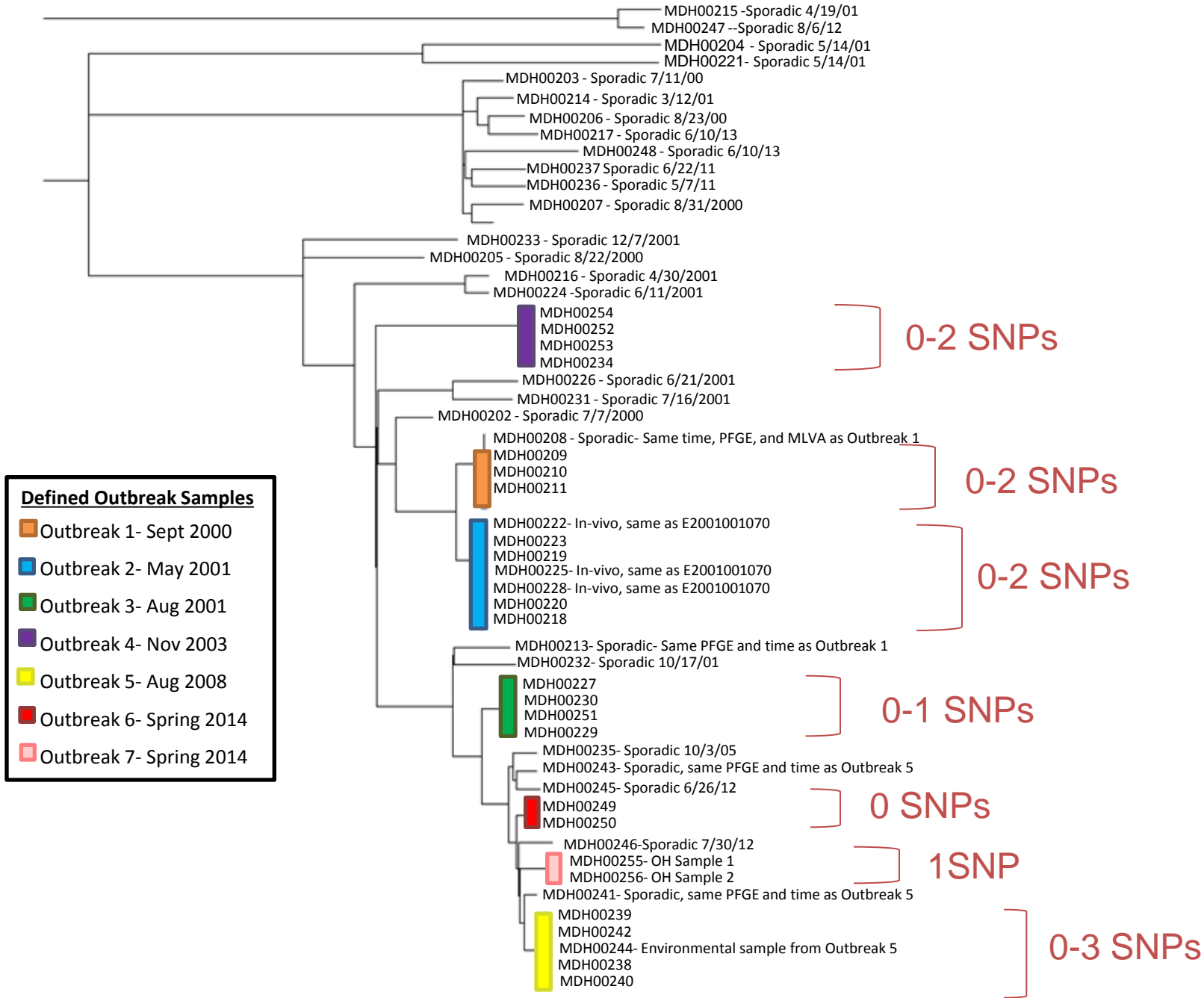
- Are WGS types similar within an outbreak?
- Are non-outbreak types different than outbreak?
- Are non-outbreak types different from each other?
- Are types consistent within person over time?

Prospective

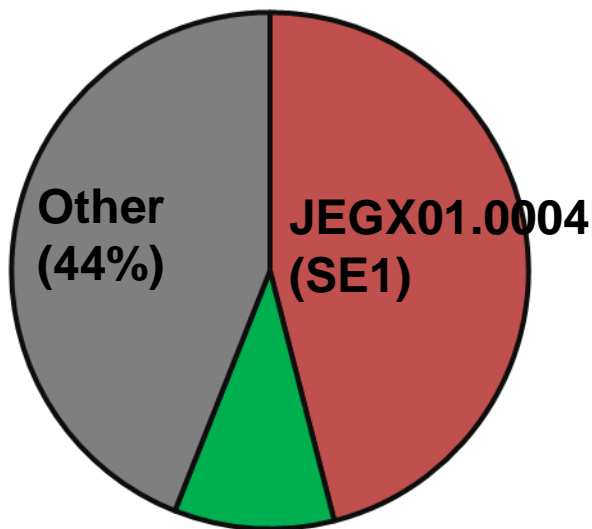
- Can WGS and epi be used to identify outbreaks?
- Is WGS better at outbreak ID than current methods?

Retrospective Samples (n=55)

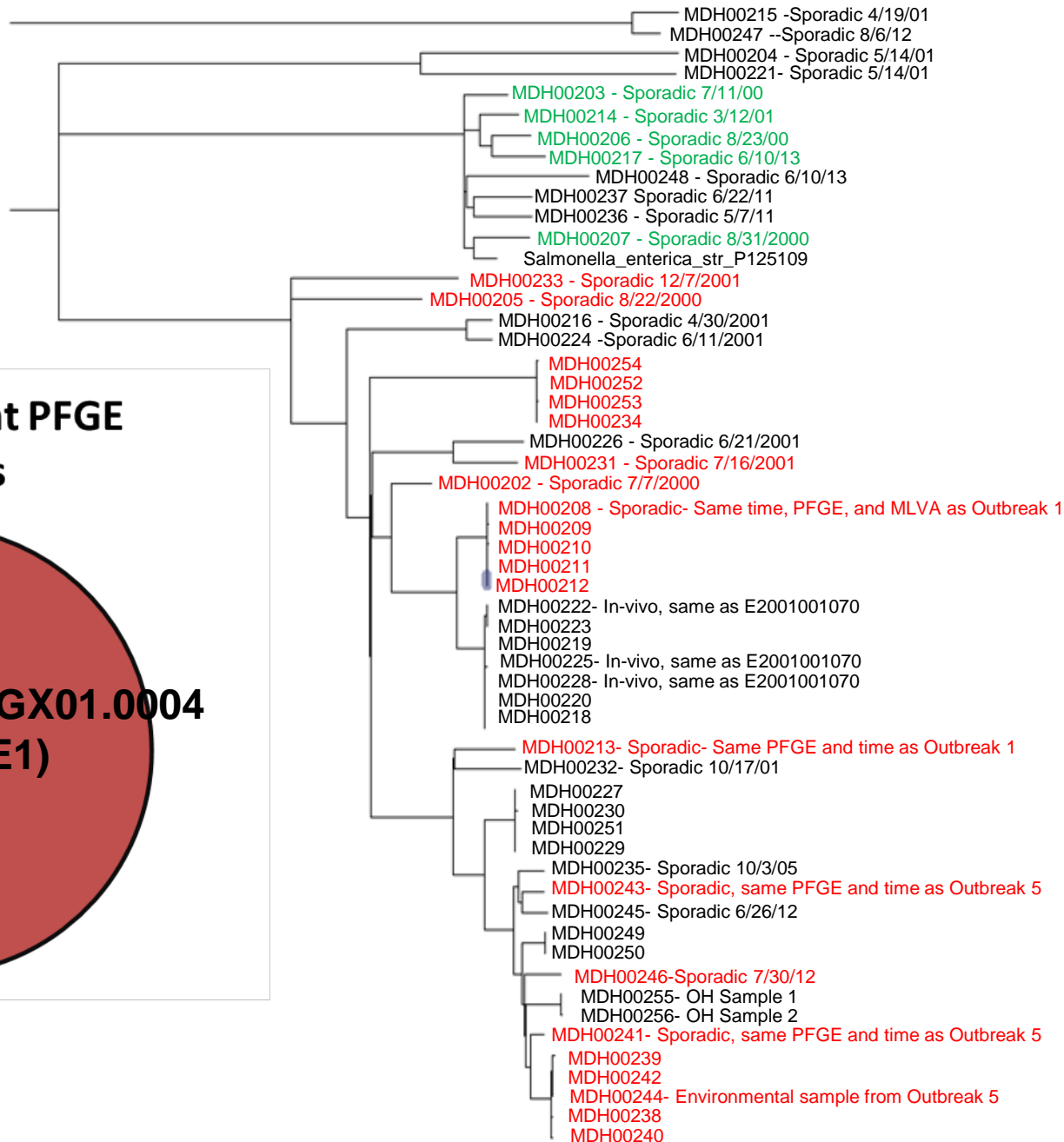
- Well characterized isolates (PFGE and MLVA), exposure information (epi)
- 7 separate outbreaks (n=25)
- 22 sporadic isolates
- *In vivo* (n=4)
- 4 suspect isolates (same subtype/time as OB, no known OB exposure)



Most Prevalent PFGE Patterns



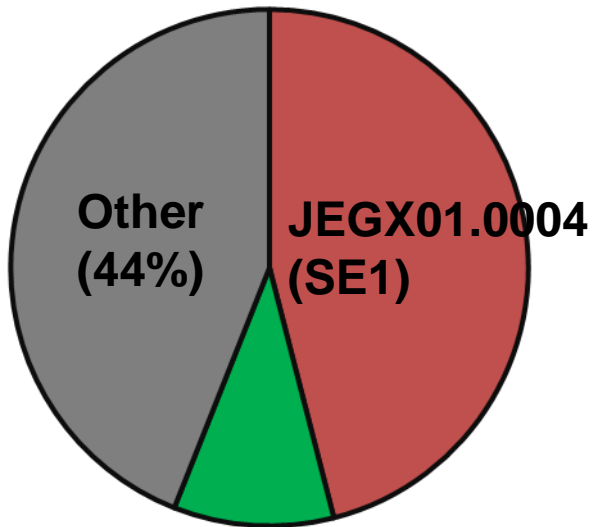
JEGX01.0002
(SE11)



0.05

Common PFGE Patterns (sporadic)

Most Prevalent PFGE Patterns



**JEGX01.0002
(SE11)**

Average SNP Differences Within PFGE Type

- Pattern 4 – 138 SNPs
- Pattern 2 – 58 SNPs

Retrospective Study Conclusions

- Few SNP differences within outbreak
- Sporadic isolates look different than outbreaks
- WGS stable
- All isolates type able by WGS
- High amount of diversity

Prospective Study

- April 1-December 31st, 2014
- All isolates have PFGE and WGS performed in real-time (WGS goal TAT <1 week)
- Each cluster (identified by any method) will be investigated as possible outbreak
- FB epi interviews all cases with trawling questionnaire
- N=159, 21 unique primary PFGE patterns

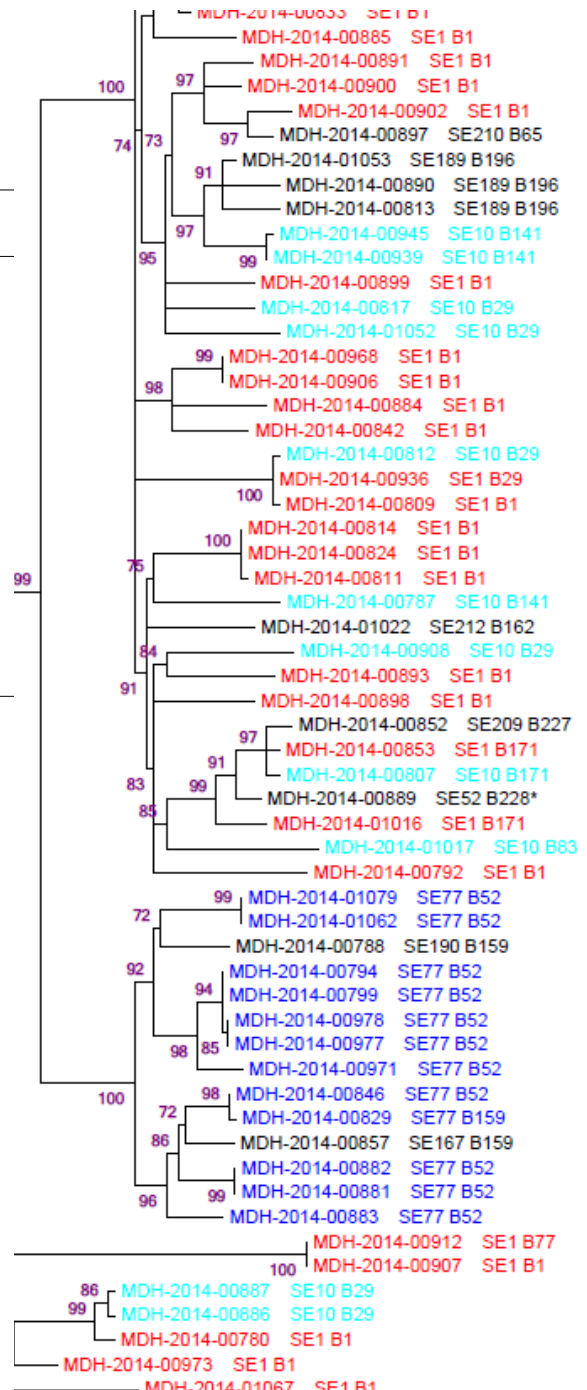
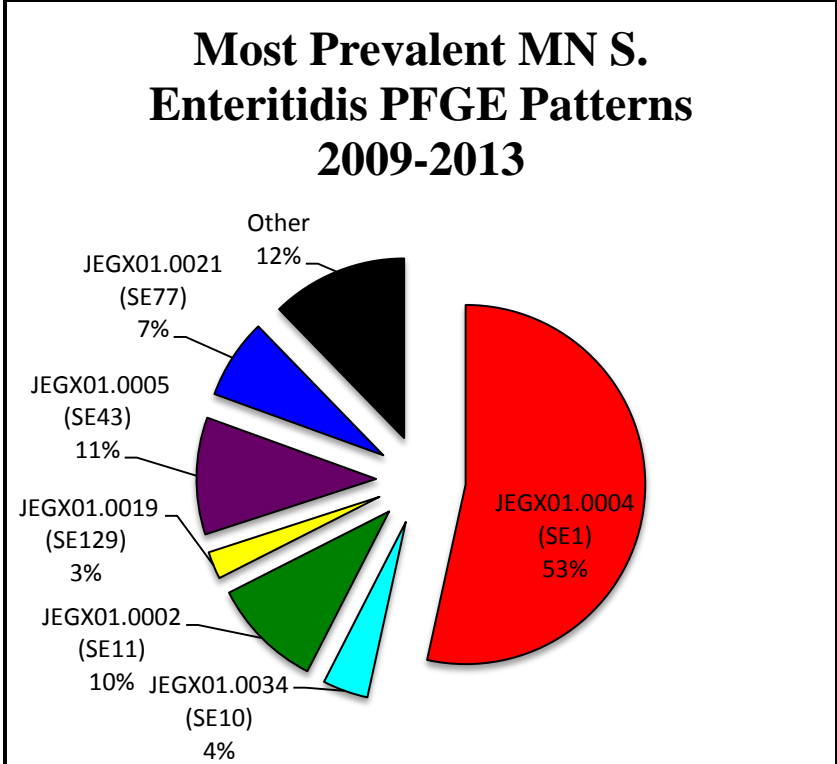
Cluster Definition

- Prospective study, any isolates:
 - Indistinguishable PFGE (Xba1 and Bln1) type within month
 - <10 SNPs difference within month
 - Originally <20 SNPs difference

Interviewing Cases: Minnesota Basic Philosophy

- Interview all cases in surveillance
- Interview ASAP
- Collect details on specific exposures
 - Dates
 - Restaurant, grocery store names
 - Brand names
 - Open-ended food histories
- Dynamic investigation approach

SE Prospective Study Results- By PFGE pattern (April- December 2014)



SNP Differences in Sporadic Isolates

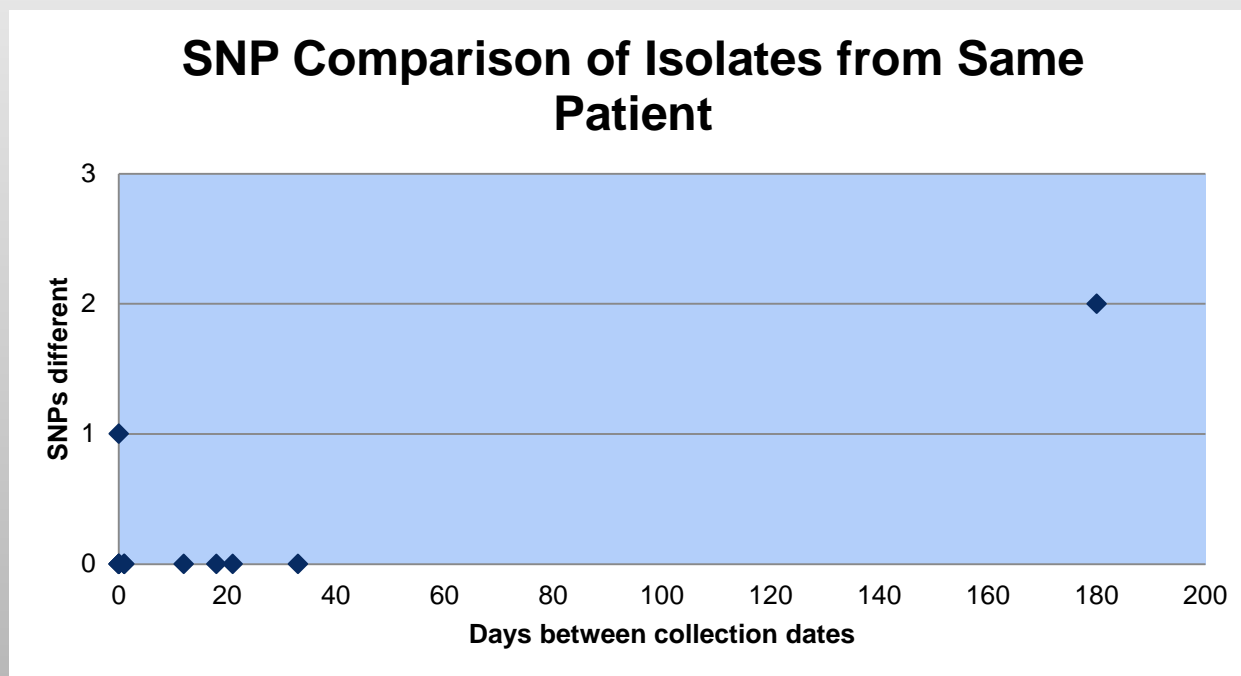
MN PFGE Pattern	CDC PFGE pattern	N	Average SNP differences with PFGE pattern
SE1	4	56	93
SE43	5	41	56
SE11	3	16	82
SE77	21	13	32
SE10	34	13	77

Prospective Results-cluster analysis

	PFGE	WGS
# of clusters	12	25
Mean # in cluster (range)	9.2 (2-44)	3 (2-8)

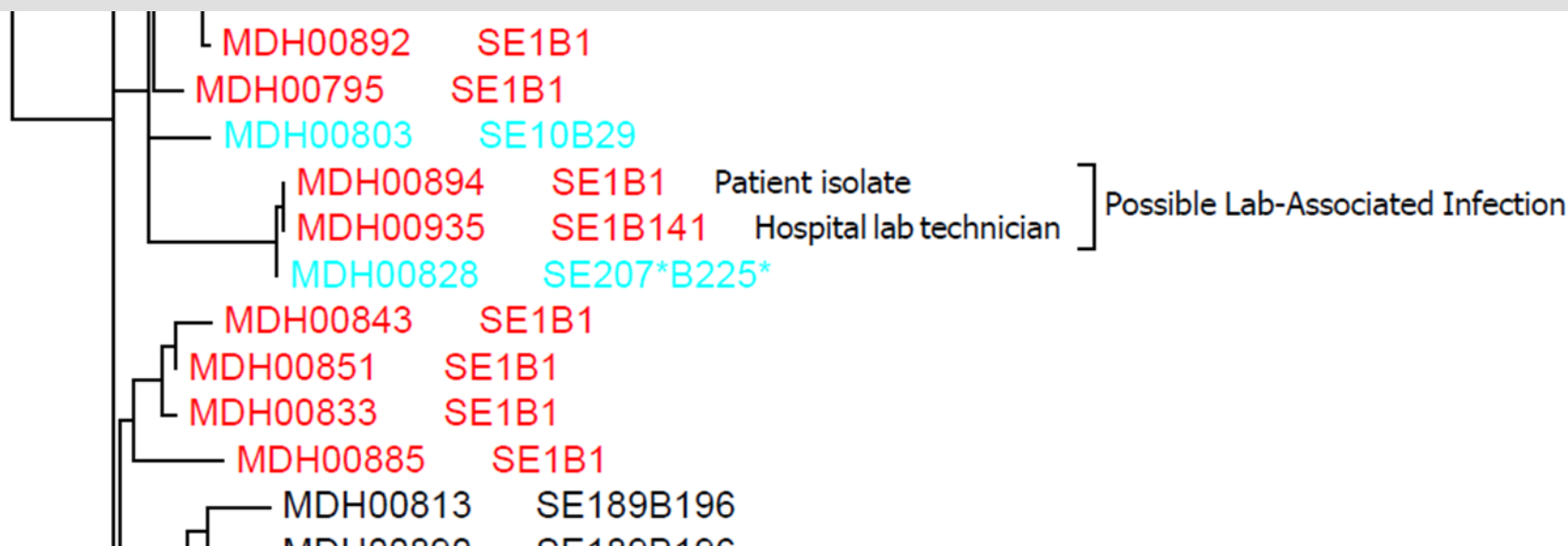
Occurrences of Multiple Isolates Collected from Same Patient

- N=7
- Time between collection dates ranges from 0 days (same collection date) to 6 months later.



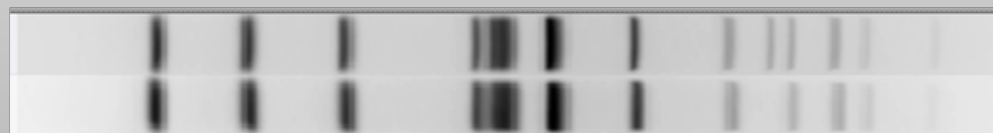
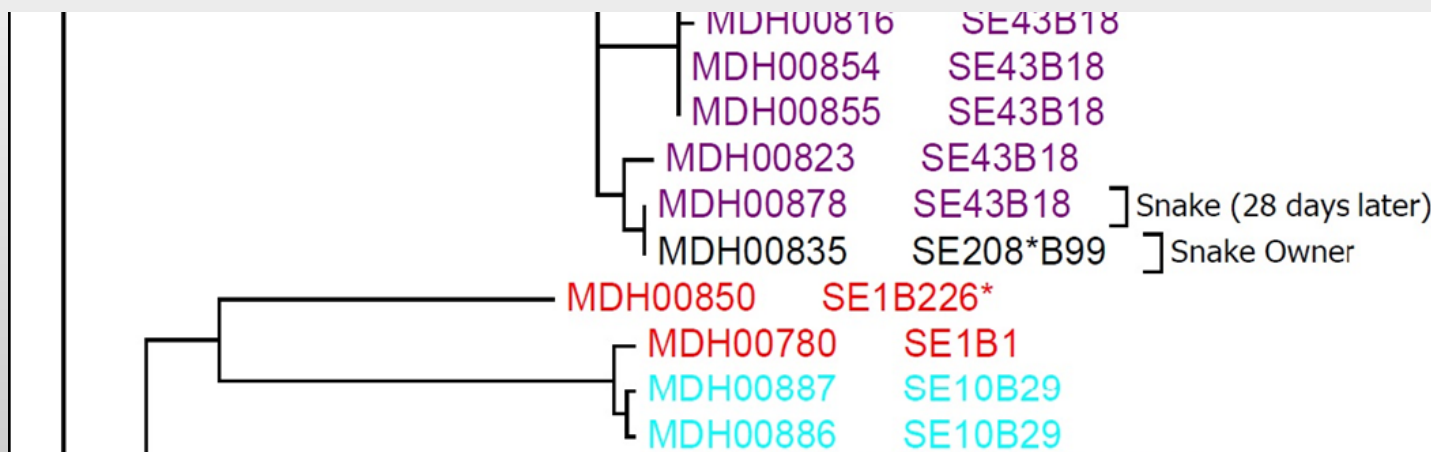
Possible Lab Associated Infection

- Patient 894-Collected late July
- Lab worker 935-Collected late August
- 1 SNP different
- Additional possibly lab-associated Shigella infections in same time-period- possible lab contamination/safety issues

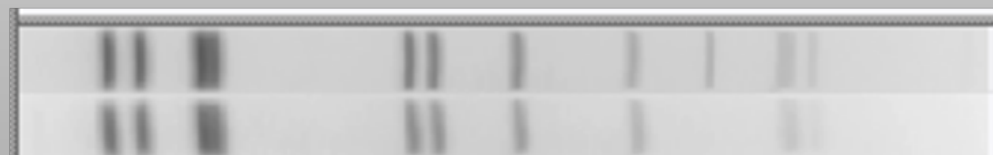


Pet Associated Infection

- Different PFGE patterns between Snake and Snake Owner
- 0 SNPs between Snake and Owner, 7 SNPs between those and other not linked cluster isolate



Xba1



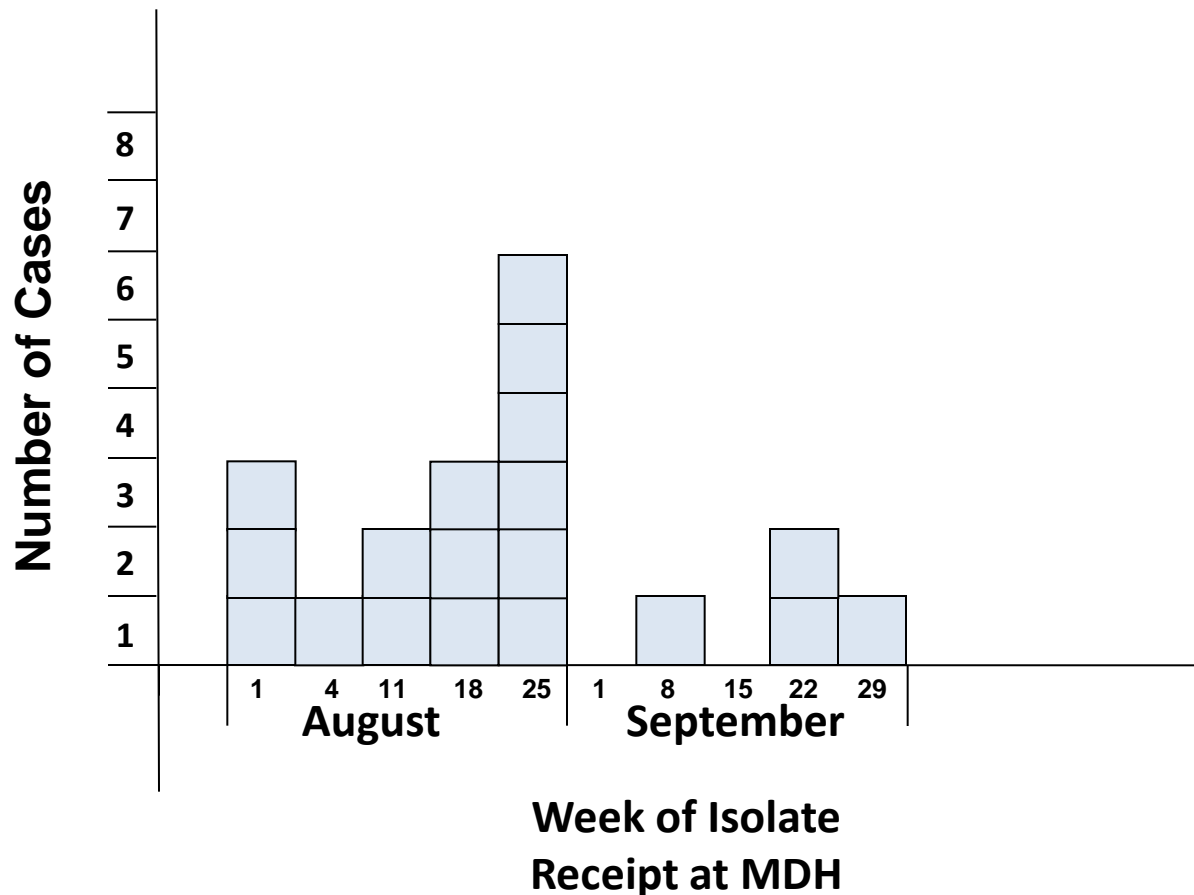
Bln1

Frozen Chicken Product

- 8 isolates, 0 SNPs different
- Collection dates: 8/17/14 through 9/27/14

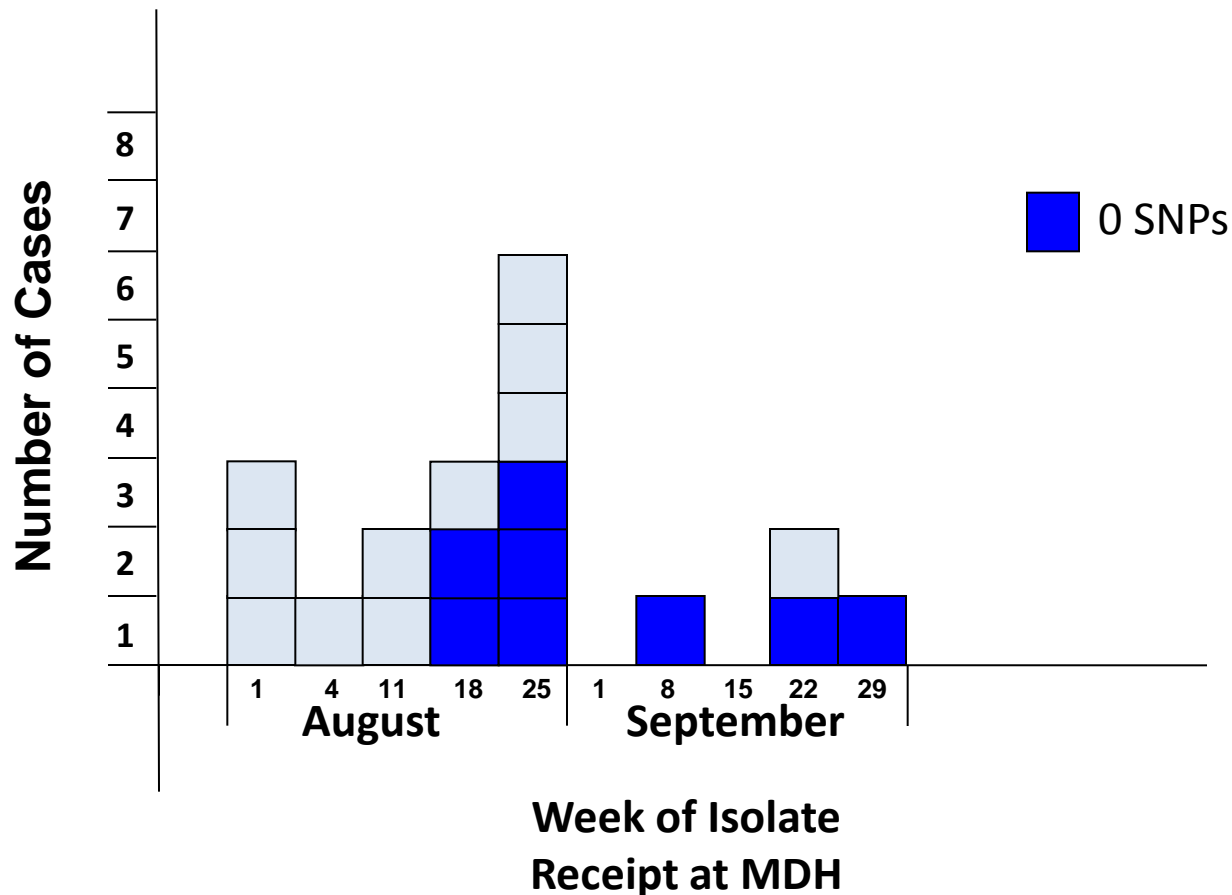


All *Salmonella* Enteritidis SE1B1* Isolates Received August-September 2014 (n=19)



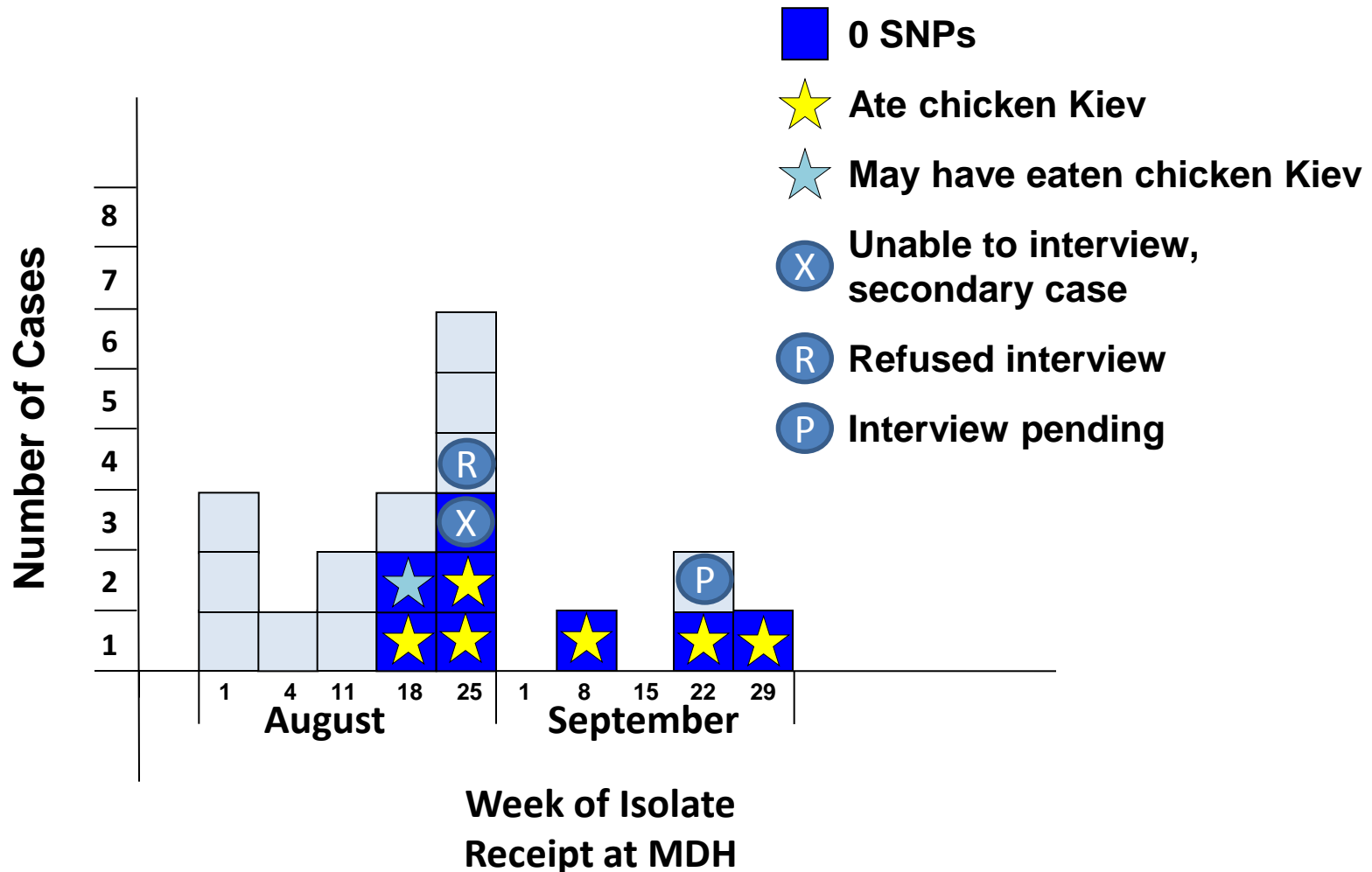
*JEGX01.0004 (SE1)

All *Salmonella* Enteritidis SE1B1* Isolates Received August-September 2014 (n=19)



*JEGX01.0004 (SE1)

All *Salmonella* Enteritidis SE1B1* Isolates Received August-September 2014 (n=19)



*JEGX01.0004 (SE1)



News Release

FOR IMMEDIATE RELEASE: October 23, 2014

Salmonella cases linked to raw, frozen chicken entrees

Consumers urged to cook raw chicken products thoroughly

ST. PAUL, Minn. – State health and agriculture officials said today that six recent cases of salmonellosis in Minnesota have been linked to raw, frozen, breaded and pre-browned, stuffed chicken entrees. The implicated product is Antioch Farms brand A La Kiev raw stuffed chicken breast with a U.S. Department of Agriculture stamped code of P-1358. This product is sold at many different grocery store chains.

Investigators from the Minnesota Department of Health (MDH) and the Minnesota Department of Agriculture (MDA) determined that six cases of *Salmonella* infection from August and September 2014 were due to the same strain of *Salmonella* Enteritidis. One person was hospitalized for their illness.

“Our DNA fingerprinting found that the individuals were sickened by the same strain of *Salmonella*,” said Dr. Carlota Medus, epidemiologist for the Foodborne Diseases Unit at MDH. “The Minnesota Department of Agriculture collected samples of the same type of product from grocery stores and the outbreak strain of *Salmonella* was found in packages of this product.”

MDH-2014-00905

- MDH01000 E2014018451-Chicken Kiev, Case home A
- MDH00998 E2014018447-Chicken Kiev, Case home A
- MDH00975 E20014017243-Case
- MDH00967 E2014015983- Case
- MDH00930 E2014014440-Case
- MDH00933 E2014014680-Case
- MDH00938 E2014014860-Case
- MDH00941 E2014015146-Case
- MDH00943 E2014015240-Case
- MDH00972 E2014016878-Case
- MDH00979 E2014018280-Same person as E2014016878 (MDH00972)
- MDH00980 E2014018133-Chicken Kiev, retail sample
- MDH00981 E2014018134-Chicken Kiev, retail sample
- MDH00982 E2014018135-Chicken Kiev, retail sample
- MDH00983 E2014018136-Chicken Kiev, retail sample
- MDH00984 E2014018137-Chicken Kiev, retail sample
- MDH00985 E2014018138-Chicken Kiev, retail sample
- MDH00988 E2014018435-Chicken Kiev, retail sample
- MDH00996 E2014018443-Chicken Kiev, Case home A
- MDH00997 E2014018445-Chicken Kiev, Case home A
- MDH01002 E2014018455-Chicken Kiev, Case home B
- MDH00999 E2014018449-Chicken Kiev, Case home A

0 SNPs different

2 SNPs different

MDH00884

Conclusions

- WGS satisfies criteria for a good typing method
 - Stability
 - Typeability
 - Discriminatory power
 - Epidemiological concordance
 - Reproducibility
- WGS has challenges
 - Communication of results
 - No pattern designation
- Bioinformatics is challenging

Conclusions

- Need better understanding of WGS and outbreaks-epi critical-may be different criteria for different species

Acknowledgements

- FDA
- New York DOH
 - Bill Wolfgang, Pascal Lapierre, Mike Palumbo, Bioinformatics Core
- Minnesota Lab
 - Angie Jones, Vicki Lappi, Enterics lab, PFGE lab
- Minnesota epi
- Minnesota Dept Agriculture
- CDC, NCBI