Canadian Food Inspection Agency | Agence canadienne d'inspection des aliments

Canadian Food Inspection Agency

Application of WGS technologies in a food microbiology lab—is it possible to have too much information?

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Our vision:
To excel as a science-based regulator, trusted and respected by Canadians and the international community.

Our mission:
Dedicated to safeguarding food, animals and plants, which enhances the health and well-being of Canada's people, environment and economy.

Canada

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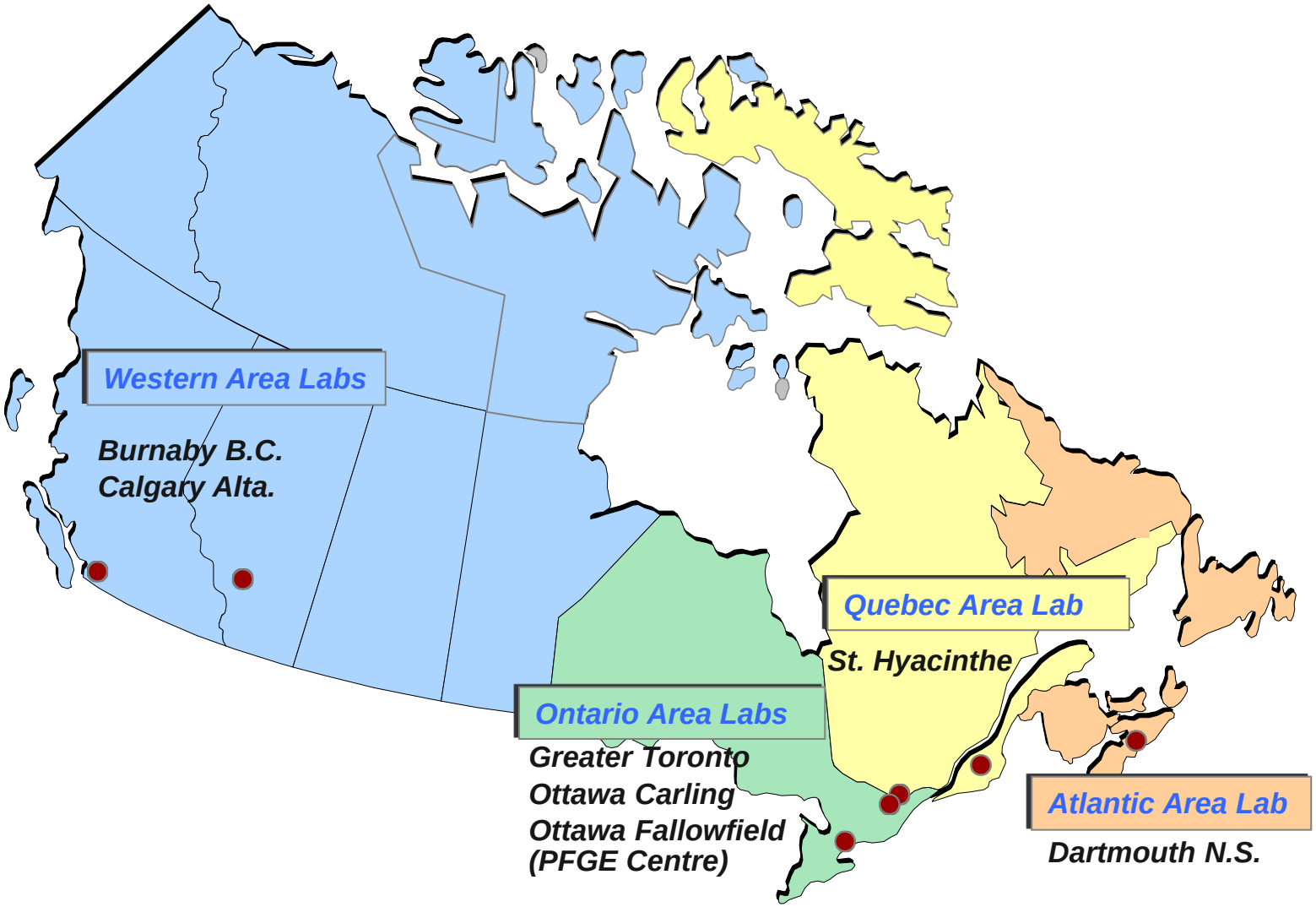
Overview

- Canadian Food Inspection Agency
- WGS at the CFIA
  - Foodborne pathogen sequencing
  - Sequencing stories
  - Interpretation of WGS data

The Canadian Food Inspection Agency

- Canada's largest science-based regulatory agency
- responsible for the delivery of all federally-mandated programs for food inspection, plant and animal health, and consumer protection as it relates to food
- mandated to safeguard Canada's food supply and the plants and animals upon which safe and high-quality food depends
- *Key objective to protect Canadians from preventable health risks, by verifying compliance of the food industry with applicable standards through comprehensive inspection and testing activities (safety, nutrition, authenticity & composition)*

Food Microbiology Laboratories



Food Microbiology Testing

Routine Monitoring:

Enforcement of Standards established by Health Canada, International Organizations

- Food Programs:
  - Meats, Fish, Dairy & Egg products, honey
  - Fresh & Processed Fruits and Vegetables
  - Manufactured Foods (bread, infant formula, spices…)
  - Food processing environment samples
- Indicator organisms: Generic *E. coli*, coliforms, yeast, mold…
- Food-borne pathogens (bacterial, viral, parasitic)
- Safety parameters – Salt content, pH, Aw

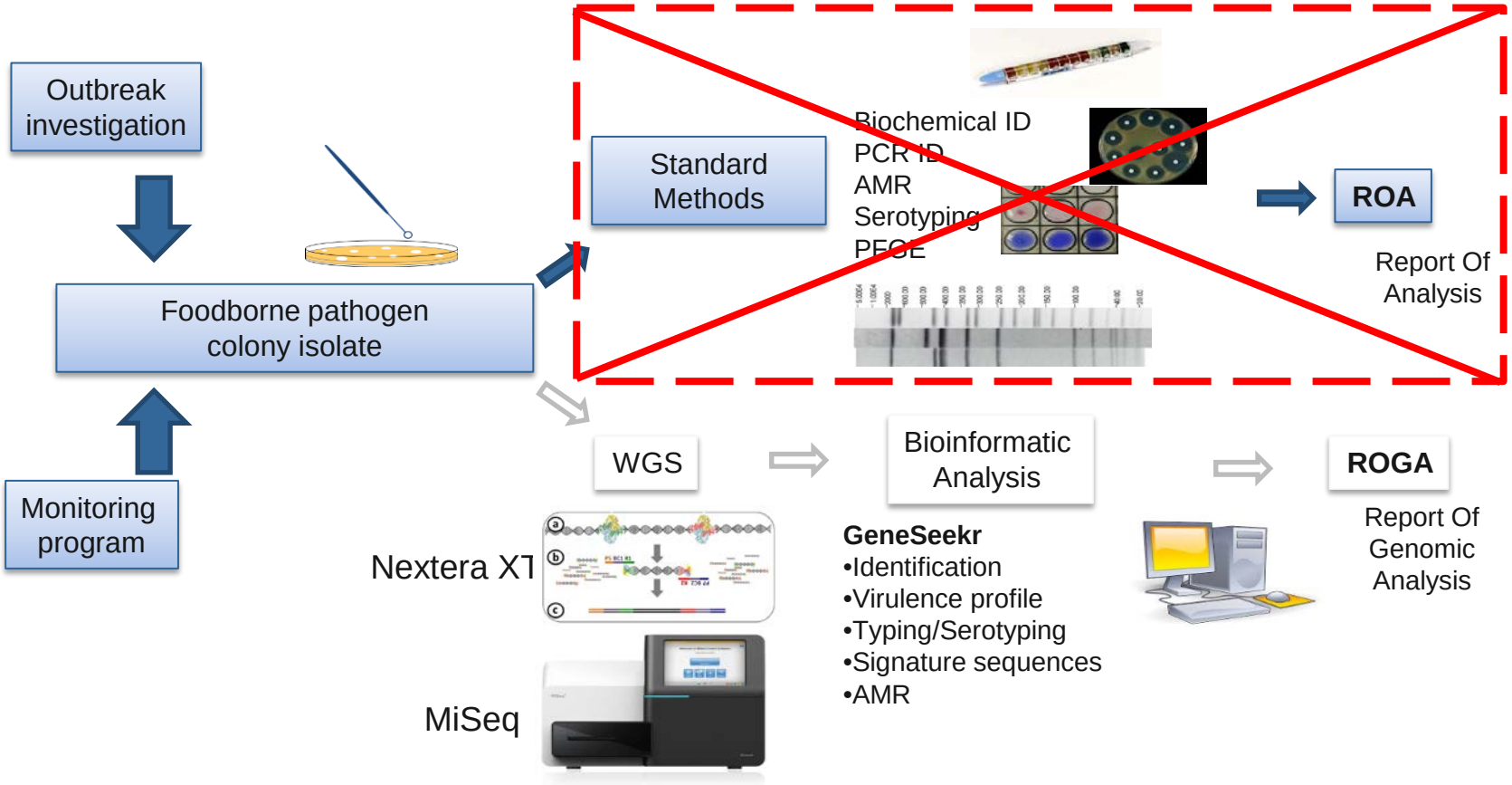
Targeted surveys

Consumer complaints

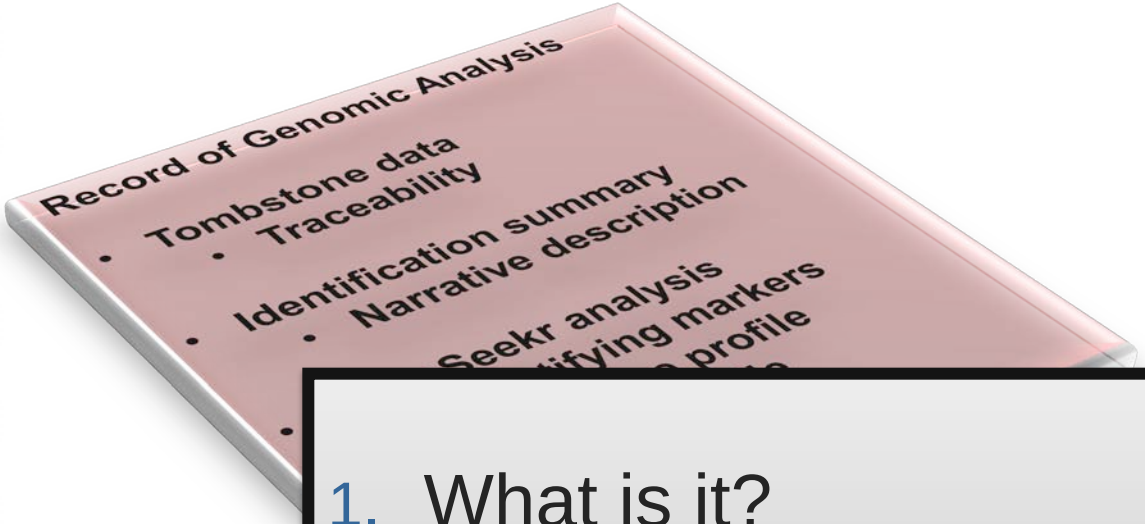
Outbreak investigation

- Source attribution
- Scope of contamination
- Hazard mitigation
- PFGE (PulseNet)

Integration of WGS in regulatory food microbiology



Record of Genomic Analysis (ROGA)



1. What is it?
2. Is it dangerous?
3. Have we seen it before?

Ex. 1: *Salmonella*

- **Marker identification**
  - ***nvA***

(Achtman)

analysis
DT104 ACSSuT

Ex. 2: Shiga-t

- **Marker identification**
  - ***eae*, *stx1*, *stx2*, *wzx*, *uidA***
- **Serotyping**
  - **O-Typer**
  - **CGE**
- **Shigatoxin subtyping**
  - **e.g., *E. coli* O26:H11 *stx2$_c$***
- **MLST**

genes

- **Marker identification**
  - ***hlyA, inlJ, IGS***
- **MLST**

Ex. 4: *V. parahaemoliticus* .

- **Marker identification**
  - ***GroEL, R72h, trh, tdh***
- **MLST**

Have we seen this before?

resolution

```
5' -----CAGTTTCCCGGGGGAAACC----- 3'
3' -----GTCAAAGGGCCCCTTTGG----- 5
```

PFGE <200 bp/indel

MLST ~3000 bp

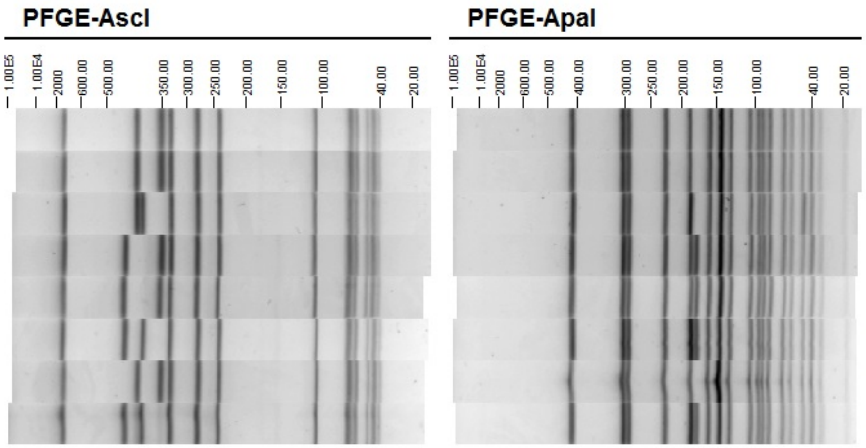
rMLST ~20 000 bp

cgMLST ~200 000 bp

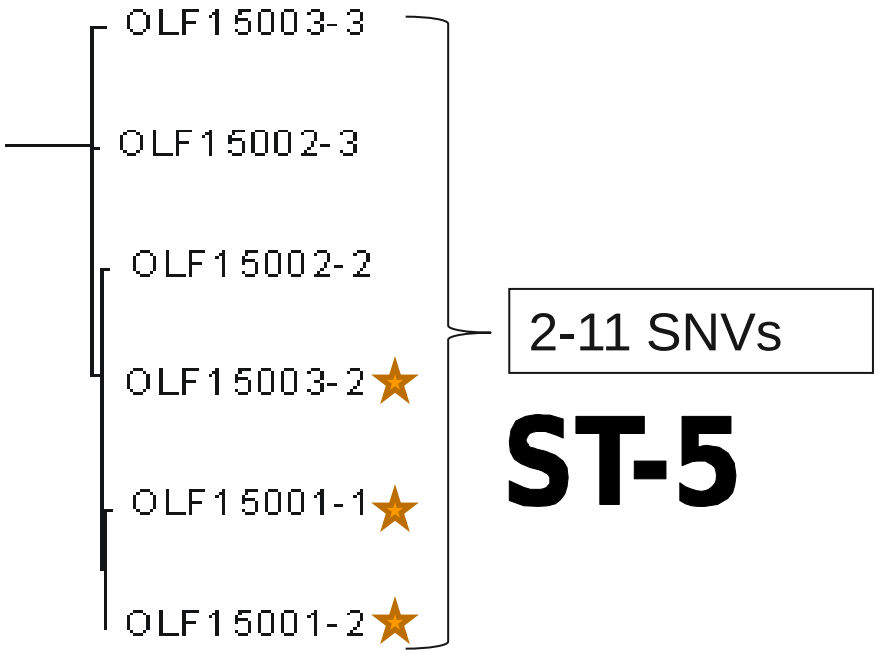
wgMLST
SNV analysis ~2-5 Mbp

http://www.phdcomics.com/comics.php?f=1156

PFGE: distinct patterns for related strains



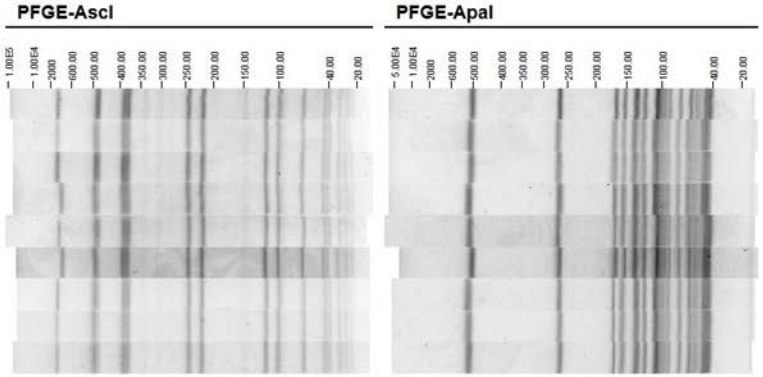
OLF15001-1	LMACI.0028	LMAAI.1184	RTE Fish ★
OLF15001-2	LMACI.0028	LMAAI.1184	RTE Fish ★
OLF15002-1	LMACI.0037	LMAAI.1184	Environmental
OLF15002-2	LMACI.0213	LMAAI.0489	Environmental
OLF15002-3	LMACI.0213	LMAAI.0489	Environmental
OLF15003-1	LMACI.0337	LMAAI.0489	Environmental
OLF15003-2	LMACI.0028	LMAAI.1184	Environmental ★
OLF15003-3	LMACI.0337	LMAAI.0489	Environmental



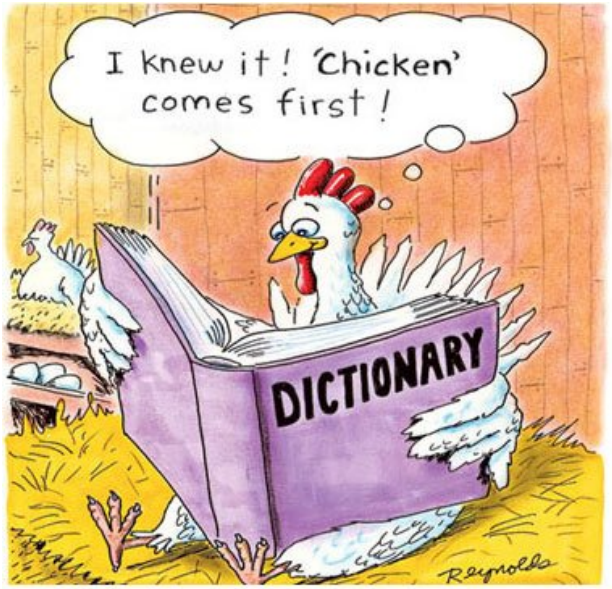
- 3 PFGE Patterns
- Different genome sizes
- Closely linked isolates

PFGE: same pattern for unrelated strains

Which came first—the chicken or the egg?



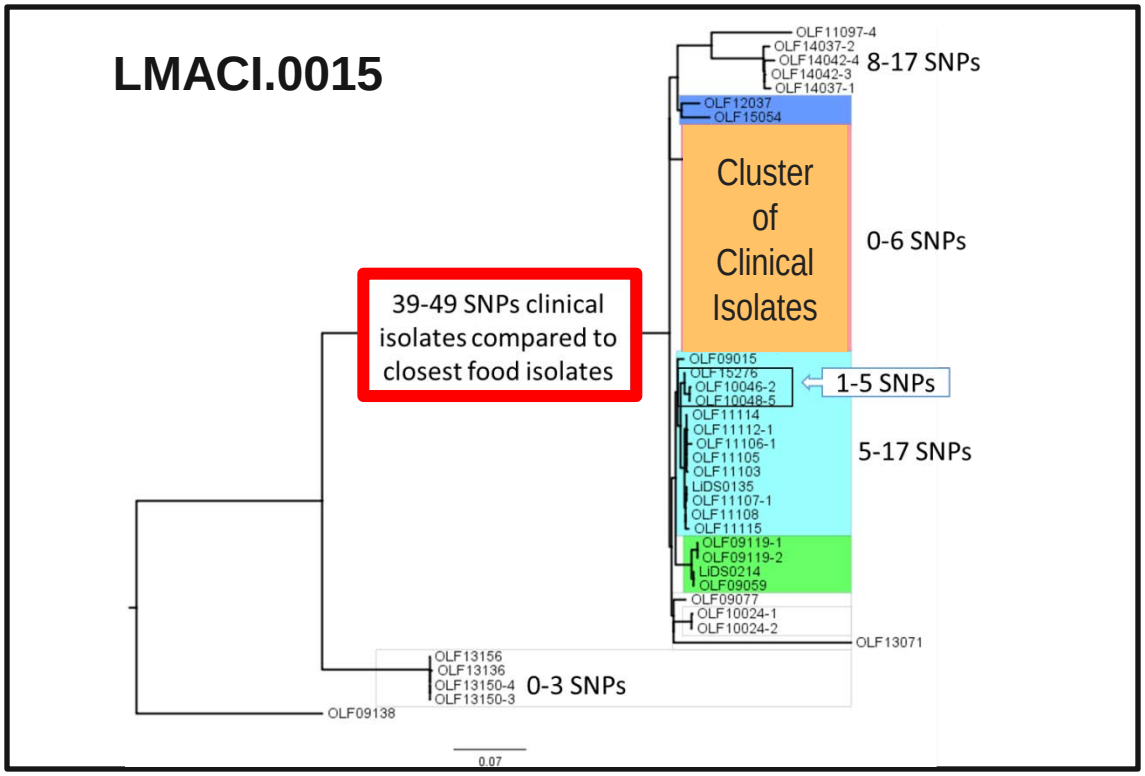
OLF15032	LMACI.0316	LMAAI.0005	Liquid Egg
OLF15034-1	LMACI.0316	LMAAI.0005	RTE Meat
OLF15035-1	LMACI.0316	LMAAI.0005	RTE Meat
OLF15036-1	LMACI.0316	LMAAI.0005	RTE Meat
OLF15037-1	LMACI.0316	LMAAI.0005	RTE Meat
OLF15038-1	LMACI.0316	LMAAI.0005	RTE Meat
OLF15040	LMACI.0316	LMAAI.0005	RTE Meat
OLF15041-1	LMACI.0316	LMAAI.0005	RTE Meat
OLF15042-1	LMACI.0316	LMAAI.0005	RTE Meat



- Isolates were not closely linked: 274 SNVs

Outcome: No need to investigate link between liquid egg/RTE chicken

Exclusion of unrelated food isolates from an *L. monocytogenes* cluster

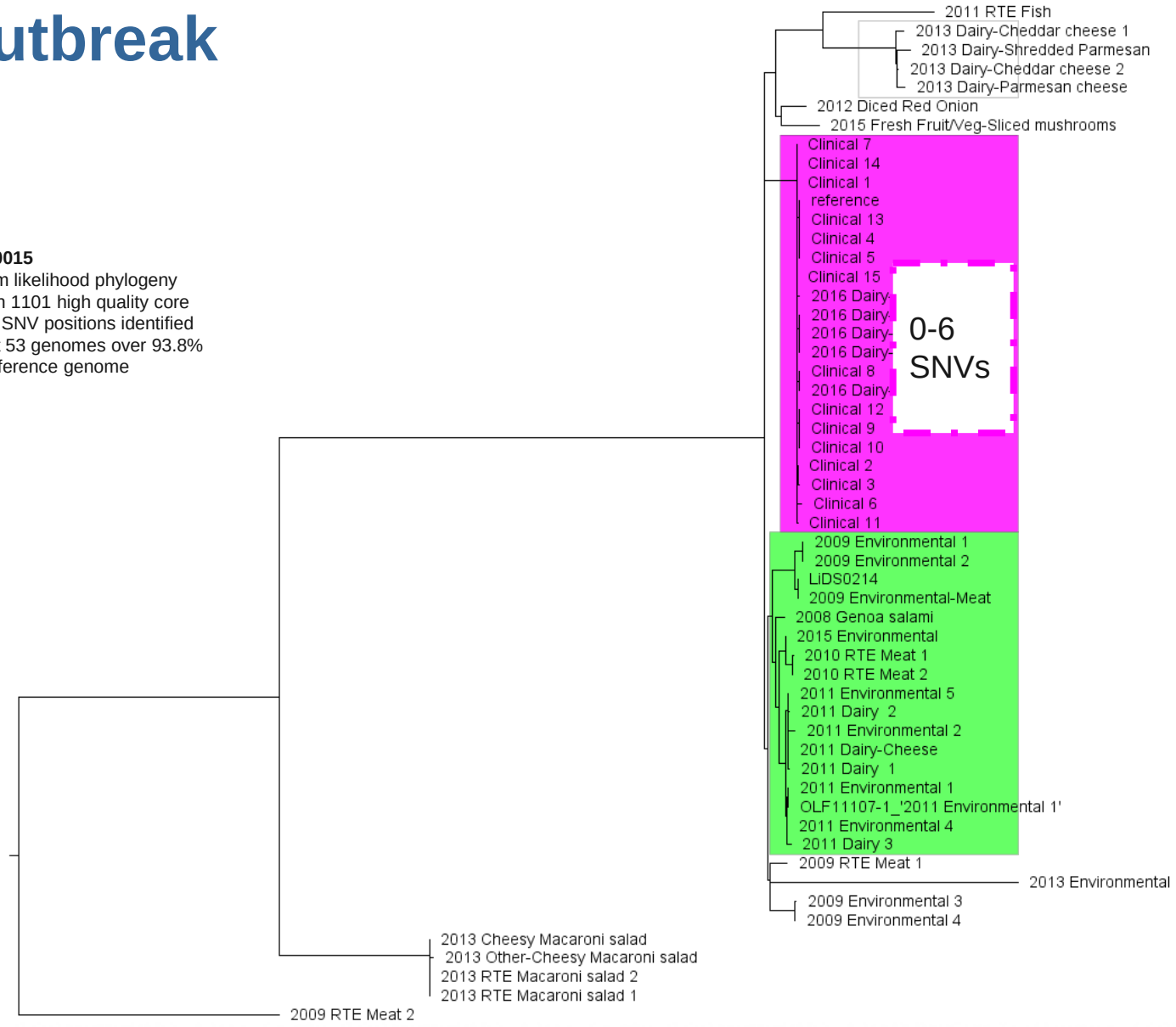


Outcome:

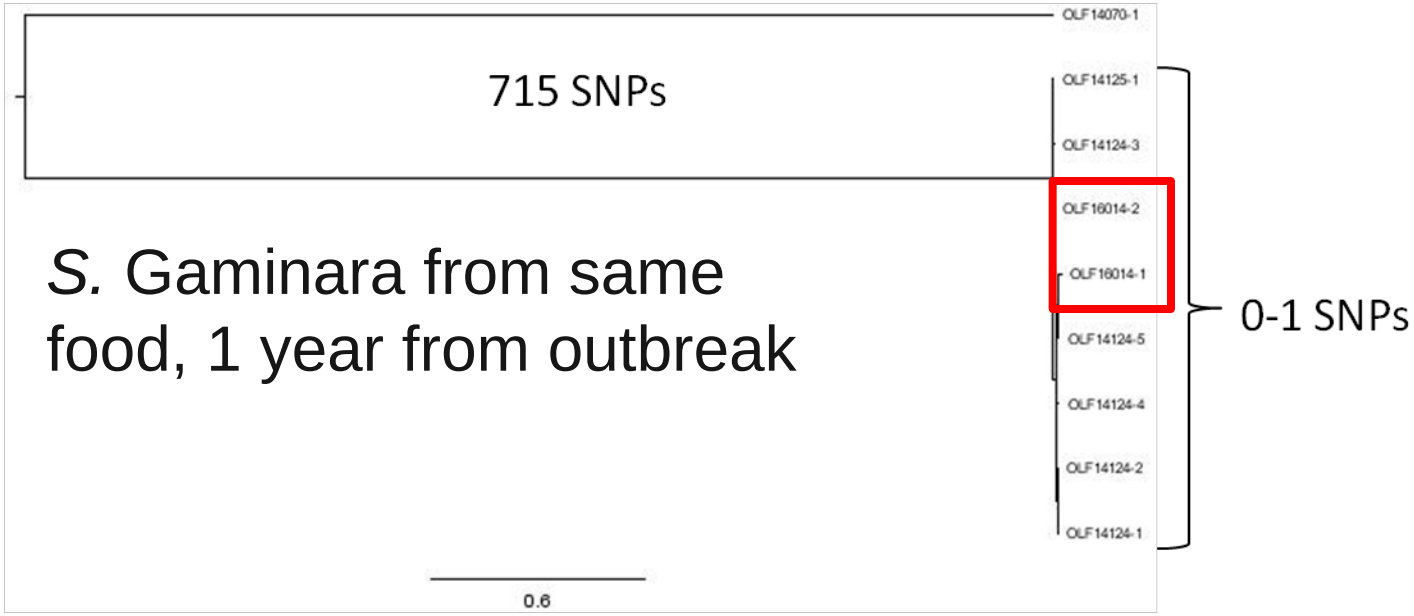
•Food isolates with the same PFGE pattern were not associated with the clinical cases and could be excluded from investigation

Confirmation of source of *L. monocytogenes* outbreak

LMACI.0015
Maximum likelihood phylogeny based on 1101 high quality core genome SNV positions identified amongst 53 genomes over 93.8% of the reference genome



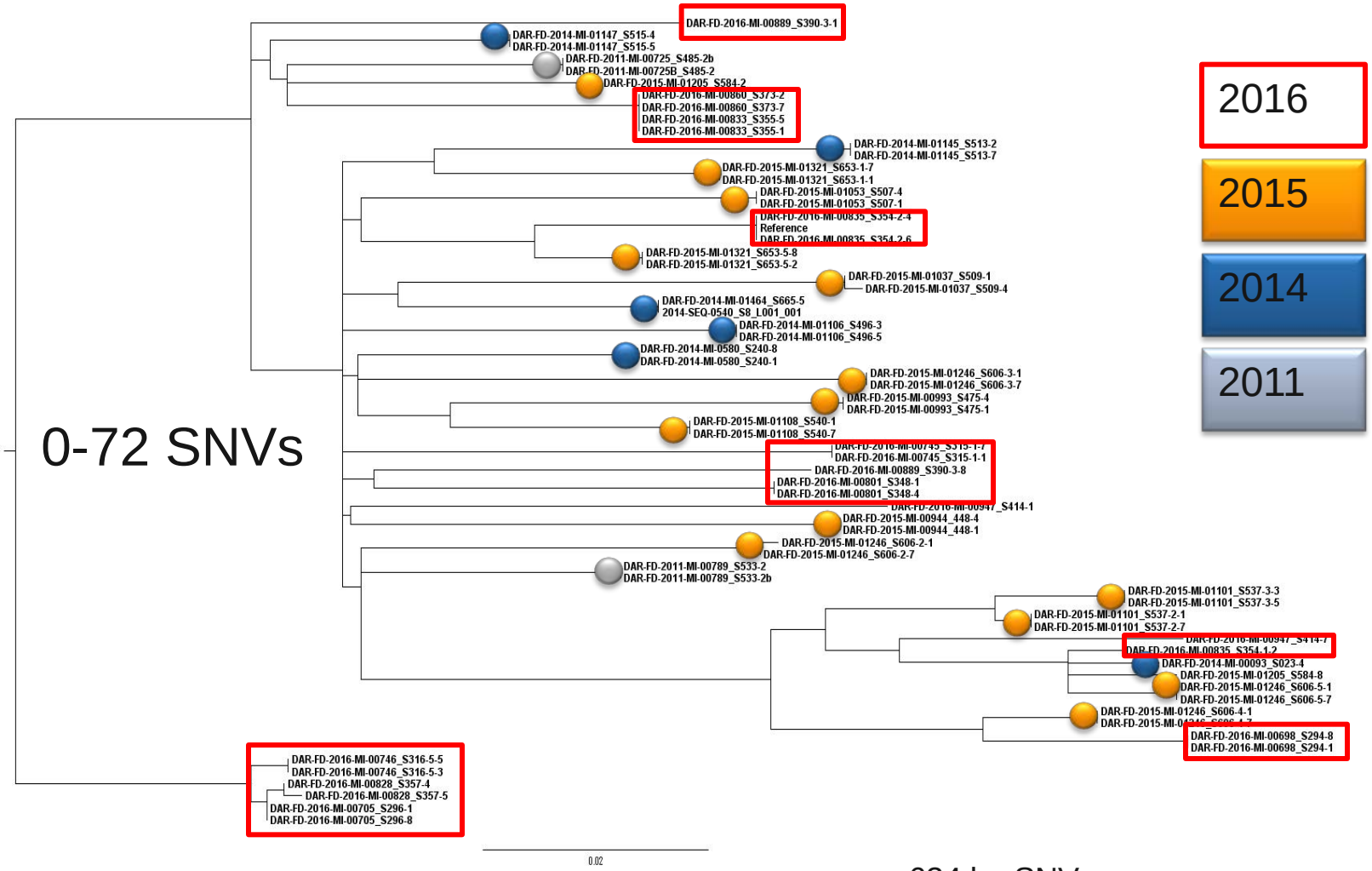
Evidence of Persistent contamination



S. Gaminara from same food, 1 year from outbreak

Outcome: Close match is evidence that contamination is from same source

One Source of contamination? Many?



S. Typhimurium

634 hq SNV
67 genomes
92.3% reference

The WGS advantage:

- Distinguish new isolate from isolates in the laboratory
- Identify persistent contamination*
- Confirm linkage (or not) to clinical strains*

*Interpretation of SNV data?

It's complicated…

Changes in *E. coli* inoculated onto lettuce

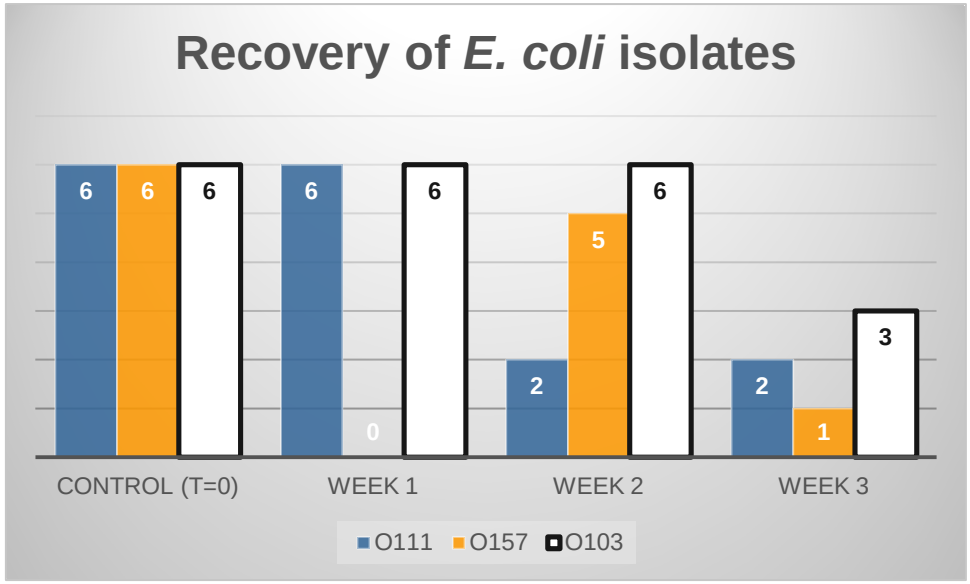
O103
O111
O157

inoculate →

0-3 weeks

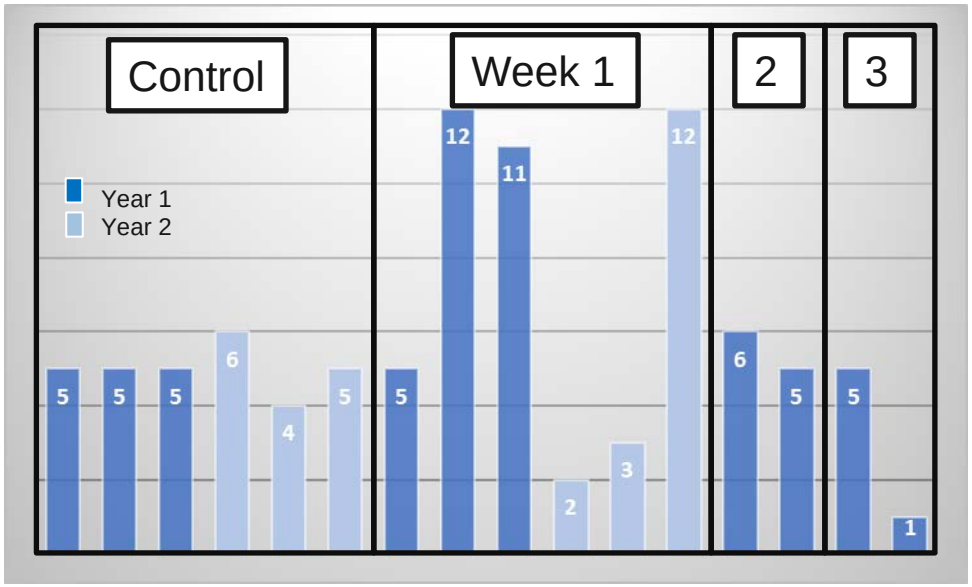
recover →

O103 → 21 isolates
O111 → 16 isolates
O157 → 12 isolates



Changes in *E. coli* inoculated onto lettuce

O157 → 12 isolates → 0 SNV
O103 → 21 isolates → 1 SNV
O111 → 16 isolates → 93 SNVs



Conclusions

- WGS is one source of information that can be useful for a food safety investigation
- WGS often provides evidence that foods can be excluded from an investigation
- High-resolution WGS-based typing data should not be interpreted in the absence of epidemiological information

Acknowledgements

OLC R&D Section team

Research Scientists:
Burton Blais, Section Head
Calvin Lau
**Dominic Lambert*

Research Biologist:
Martine Gauthier

Bioinformaticians:
Adam Koziol
Andrew Low
Forest Dussault

Research Technicians:
Mylène Deschênes
Paul Manninger

Students:
Austin Markell
Ashley Cooper
Jackson Eyres
Michael Knowles
Tanis McMahon
Susan Van Zanten

CFIA collaborators:
Ray Allain
Johanna Murphy
Mohamed Elmufti
Annie Locas

PHAC
Ed Taboada
John Nash
Catherine Yoshida
Peter Kruczkiewicz
Gary Van Domselaar

AAFC
Pascal Delaquis