Advanced technology (including WGS) to perform microbial food safety risk assessments and hazard identification

Norval Strachan

School of Biological Sciences

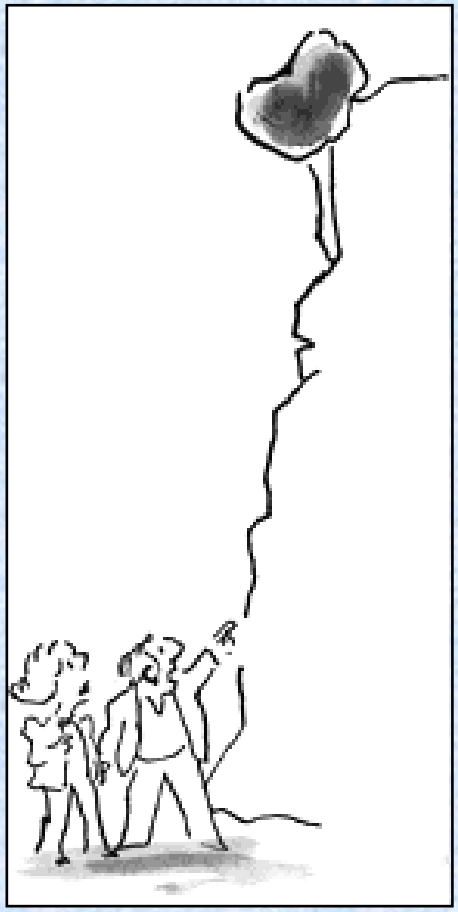
University of Aberdeen

And

Chief Scientific Adviser

Food Standards Scotland

Risk assessment



www.sciencecartoonsplus.com

Risk assessment

- **Hazard** - A biological agent in a food with the potential to cause an adverse health effect
- **Risk** - A function of the probability of an adverse health effect and the severity of that effect, consequential to a hazard(s) in food
- **Risk assessment** - The systematic, scientific evaluation of known or potential risks

www.barfblog.com

CODEX, 2014

Risk assessment

Statement of Purpose of Risk Assessment

1. Hazard Identification
2. Exposure Assessment
3. Hazard Characterisation
4. Risk Characterization

www.barfblog.com

Hazard Identification

- Generally qualitative process to identify hazards
- Problem – risk assessment frequently ignores heterogeneity between organisms of a species Look at two examples employing whole genome sequencing

Hazard Identification example 1

Contents lists available at ScienceDirect

International Journal of Food Microbiology

journal homepage: www.elsevier.com/locate/ijfoodmicro

First step in using molecular data for microbial food safety risk assessment; hazard identification of *Escherichia coli* O157:H7 by coupling genomic data with in vitro adherence to human epithelial cells

Annemarie Pielaat [a,*], Martin P. Boer [b], Lucas M. Wijnands [a], Angela H.A.M. van Hoek [a], El Bouw [a], Gary C. Barker [c], Peter F.M. Teunis [a,d], Henk J.M. Aarts [a], Eelco Franz [a]

Studied 38 strains of *E. coli* O157 and how they attached to human epithelial cells (Caco-2)

***In vitro* gastrointestinal tract system**

Hazard Identification WGS example 1

- Attachment assay results
- Challenge linking genome data (5.5 Mbases) with adherence assays
- Isolates sequenced and 27,980 SNP's obtained

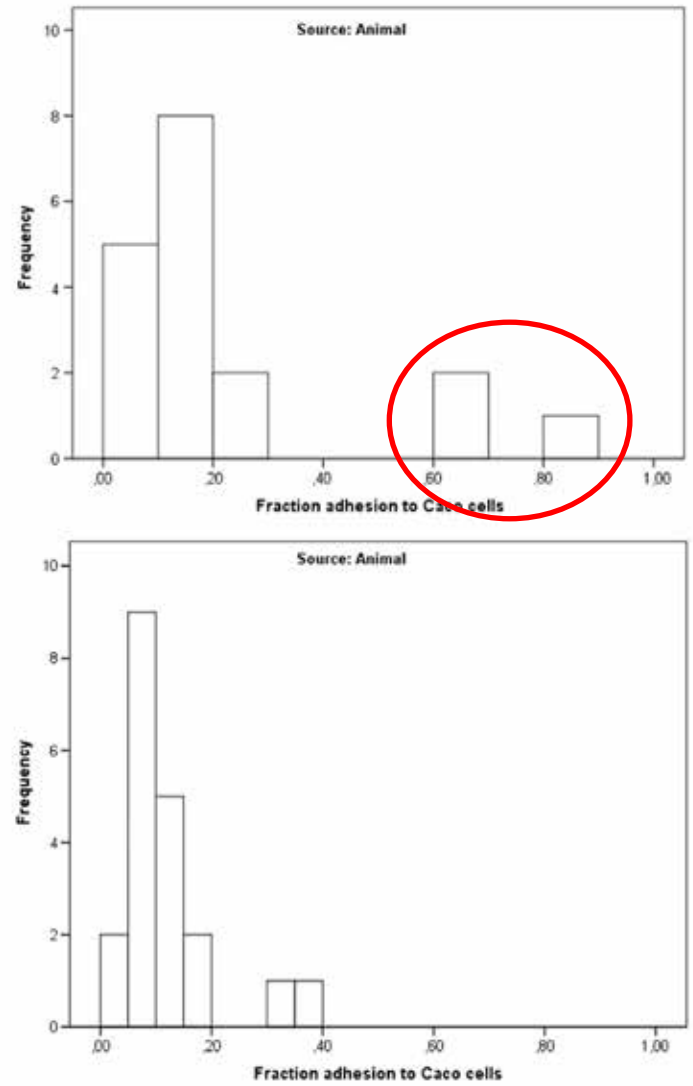
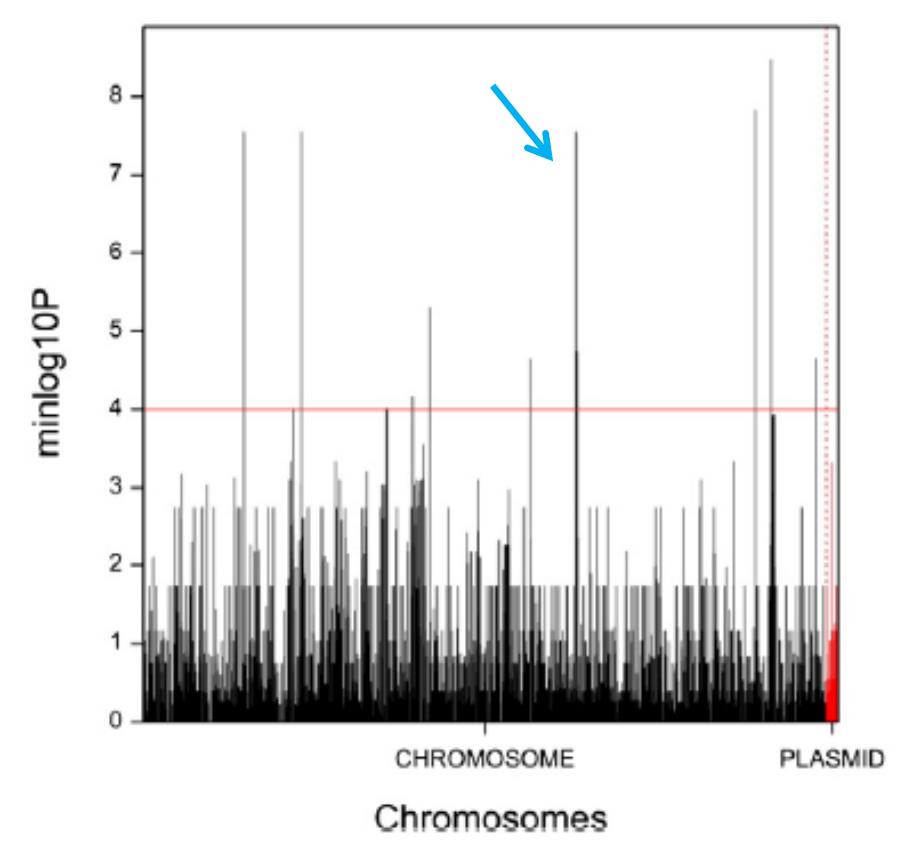


Fig. 1. Frequency distributions of the fractional adhesion for the different STEC strains to Caco-2 cells (separated for human and animal strains).

Hazard Identification example 1

- Initially 17 SNPs then after correction for sample structure 1 SNP stood out as a potential biomarker
- Further work needs done on additional strains



Hazard Identification Example 2

PNAS

Support vector machine applied to predict the zoonotic potential of *E. coli* O157 cattle isolates

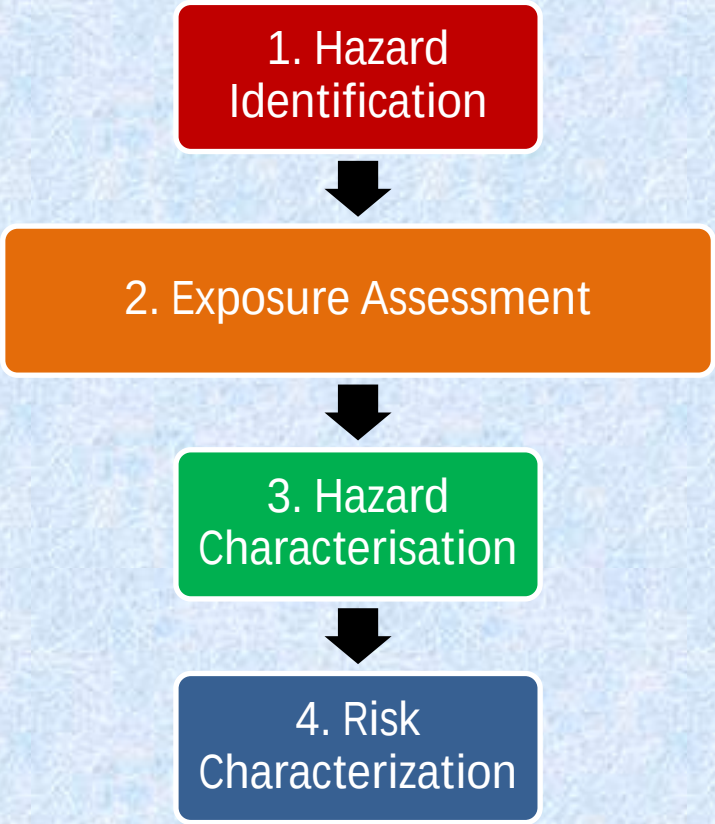
Nadejda Lupolova[a], Timothy J. Dallman[b], Louise Matthews[c], James L. Bono[d], and David L. Gally[a,1]

[a]Division of Immunity and Infection, The Roslin Institute and The Royal (Dick) School of Veterinary Studies, The University of Edinburgh, Midlothian EH25 9RG, United Kingdom; [b]Public Health England, National Infection Service, London NW9 5EQ, United Kingdom; [c]Institute of Biodiversity, Animal Health and Comparative Medicine, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow G12 8QQ, United Kingdom; and [d]US Meat Animal Research Center, Agricultural Research Service, United States Department of Agriculture, Clay Center, NE 68933

Edited by Roy Curtiss III, University of Florida, Gainesville, FL, and approved August 2, 2016 (received for review May 2, 2016)

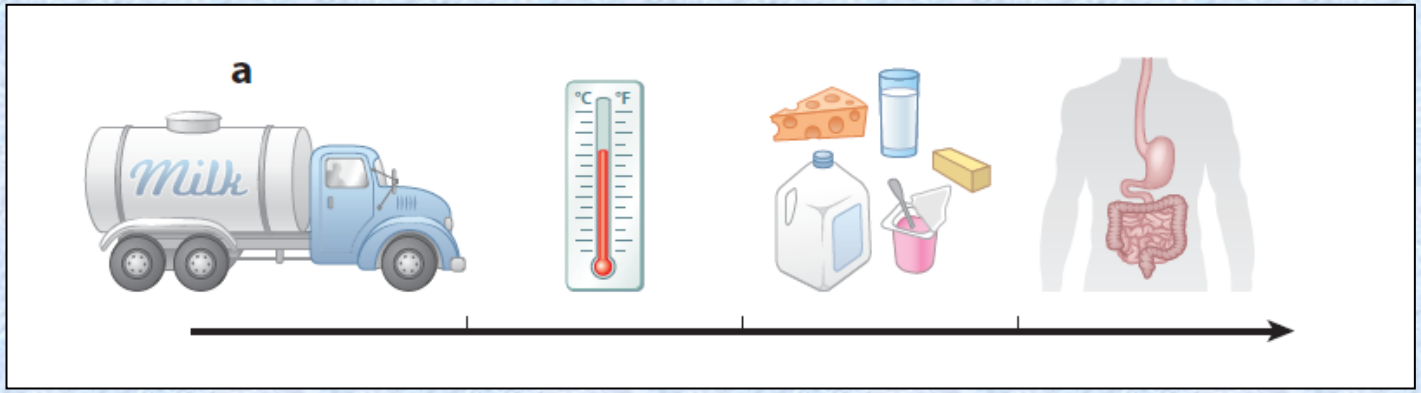
"We demonstrate that only a small subset of bovine strains is likely to cause human disease, even within previously defined pathogenic lineages"

Exposure Assessment Example



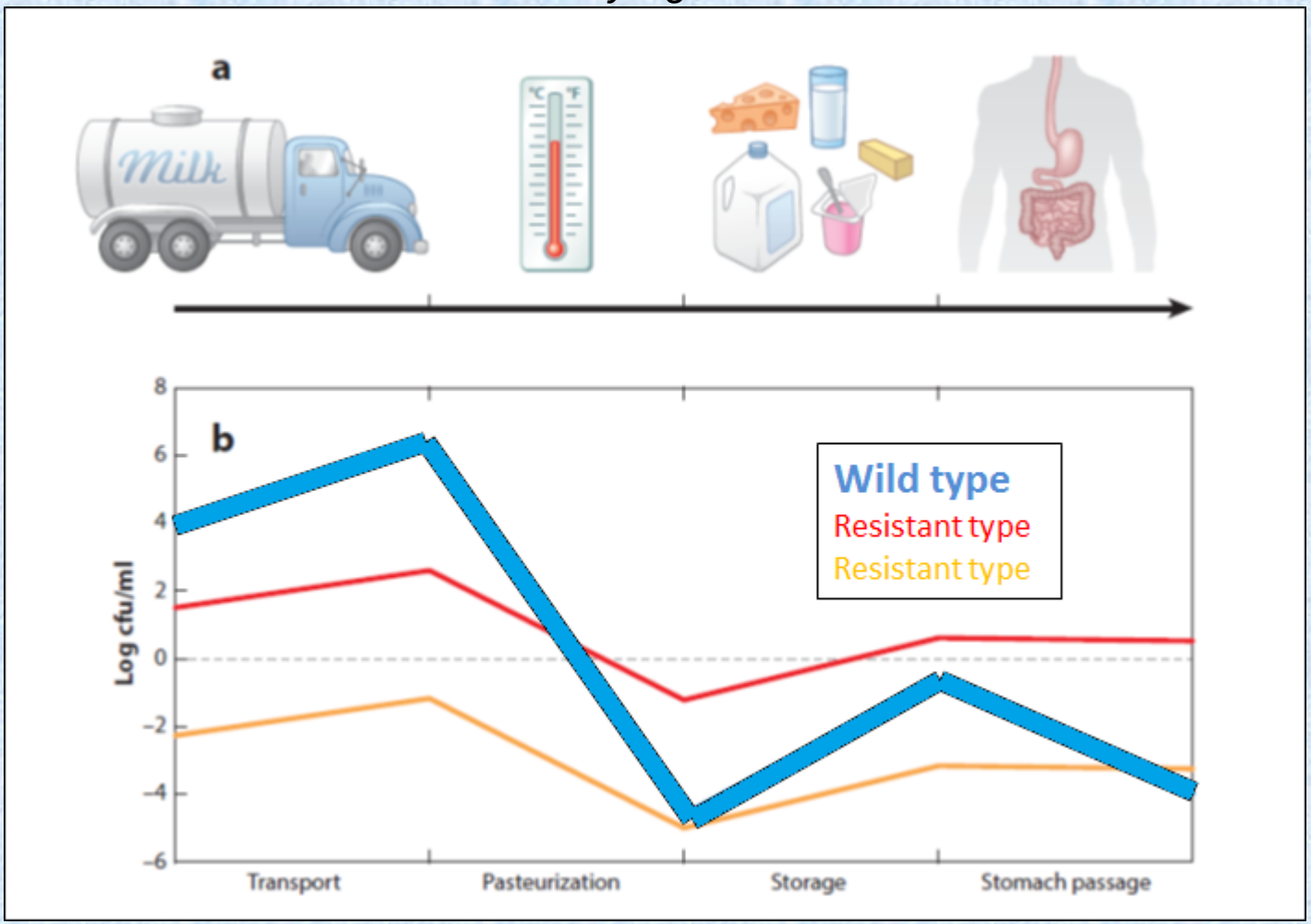
Exposure Assessment Example

Listeria monocytogenes in milk



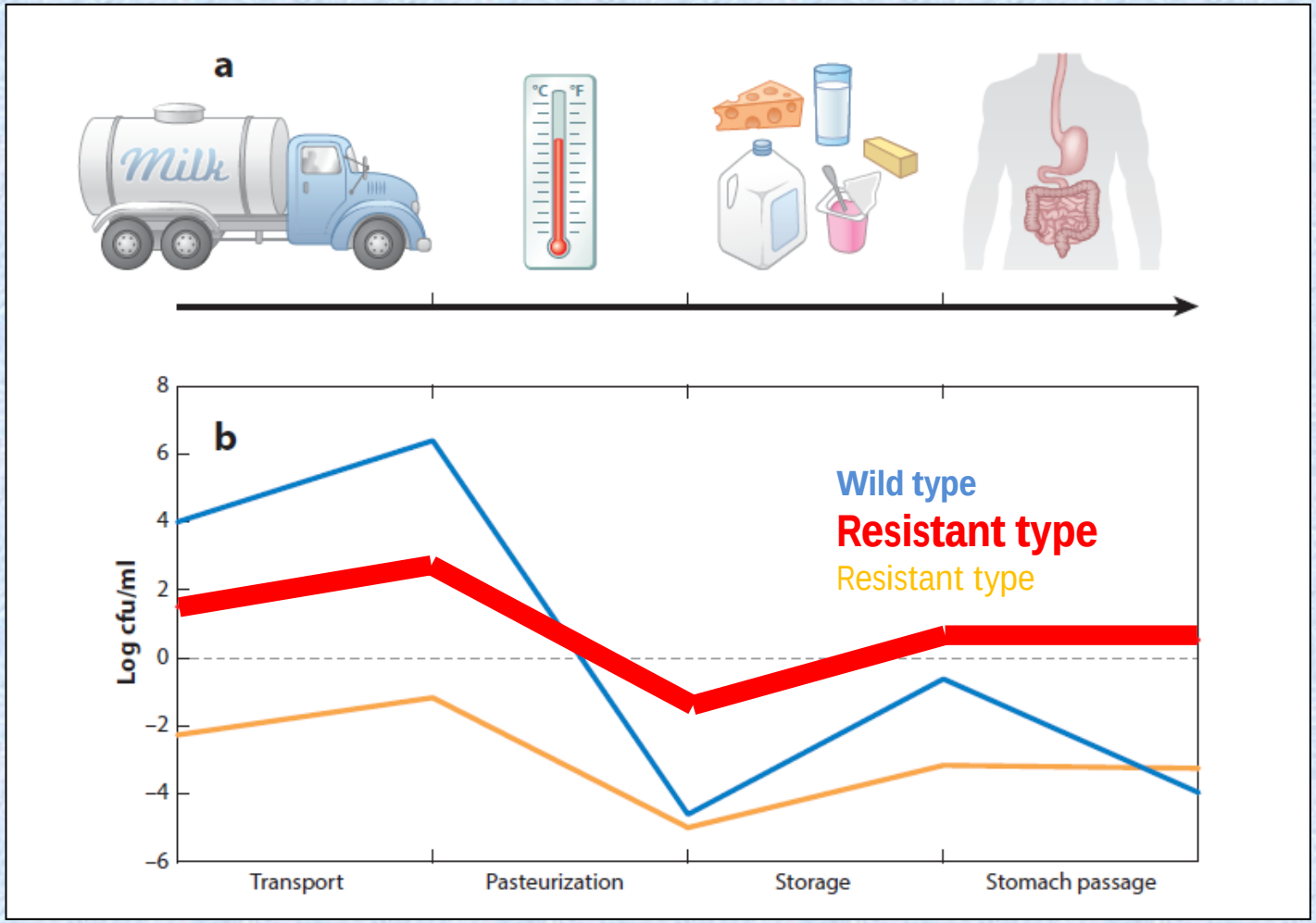
Exposure Assessment Example

Listeria monocytogenes in milk

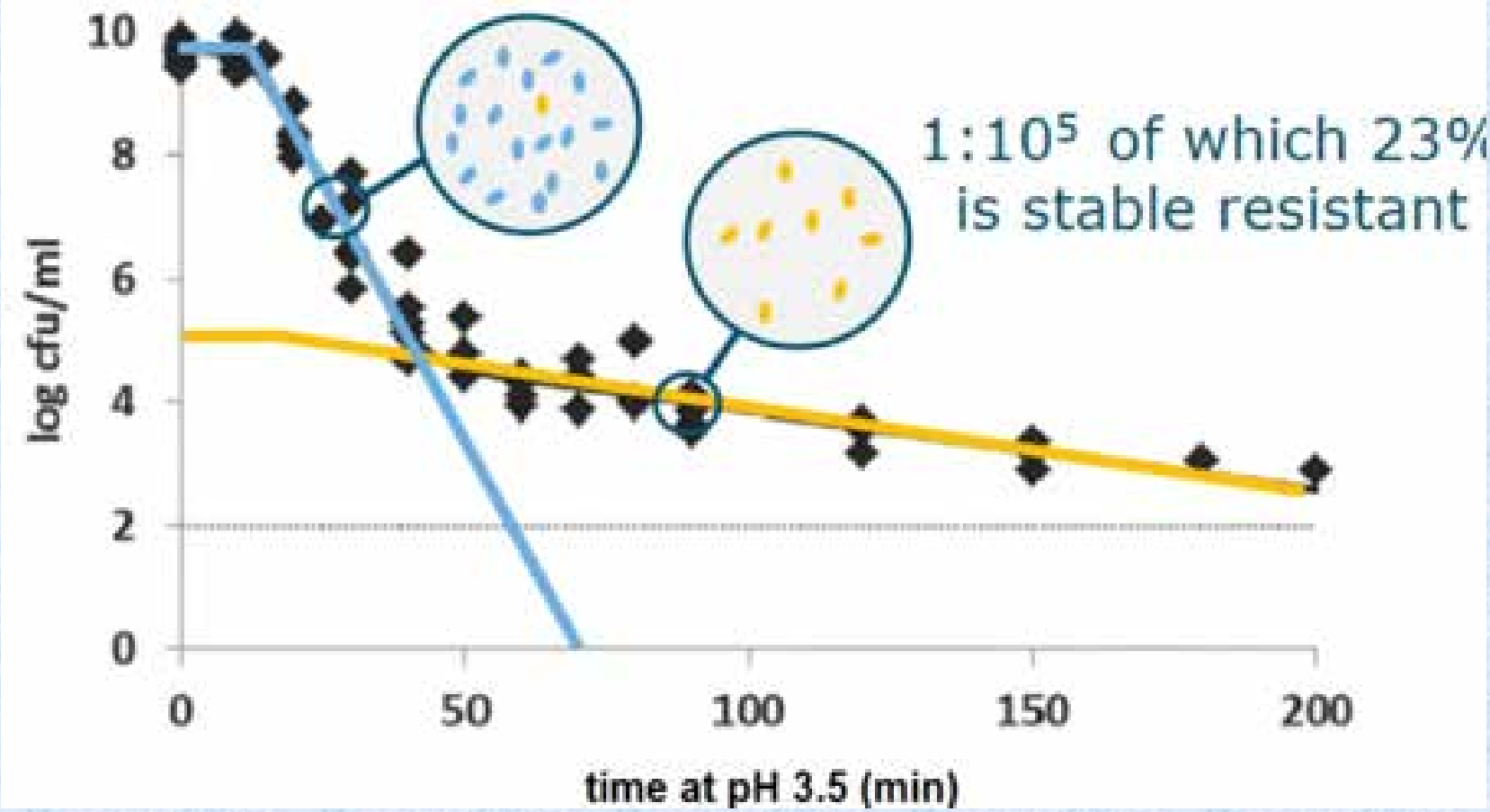


Exposure Assessment Example

Listeria monocytogenes in milk

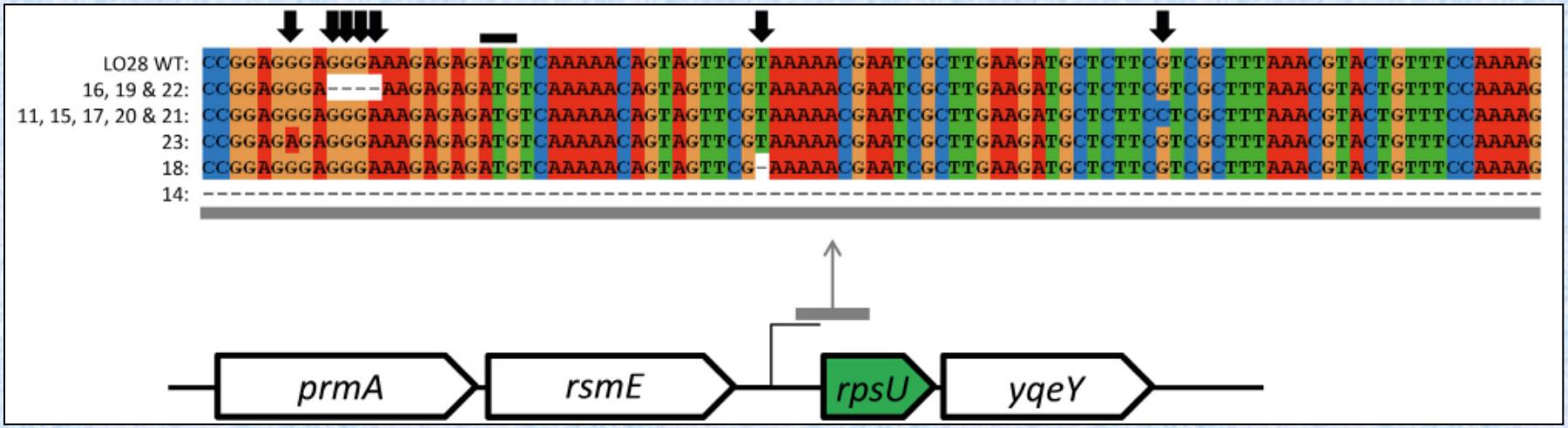


Exposure Assessment Example



Metselaar et al. , IJFM, 2013

Exposure Assessment Example



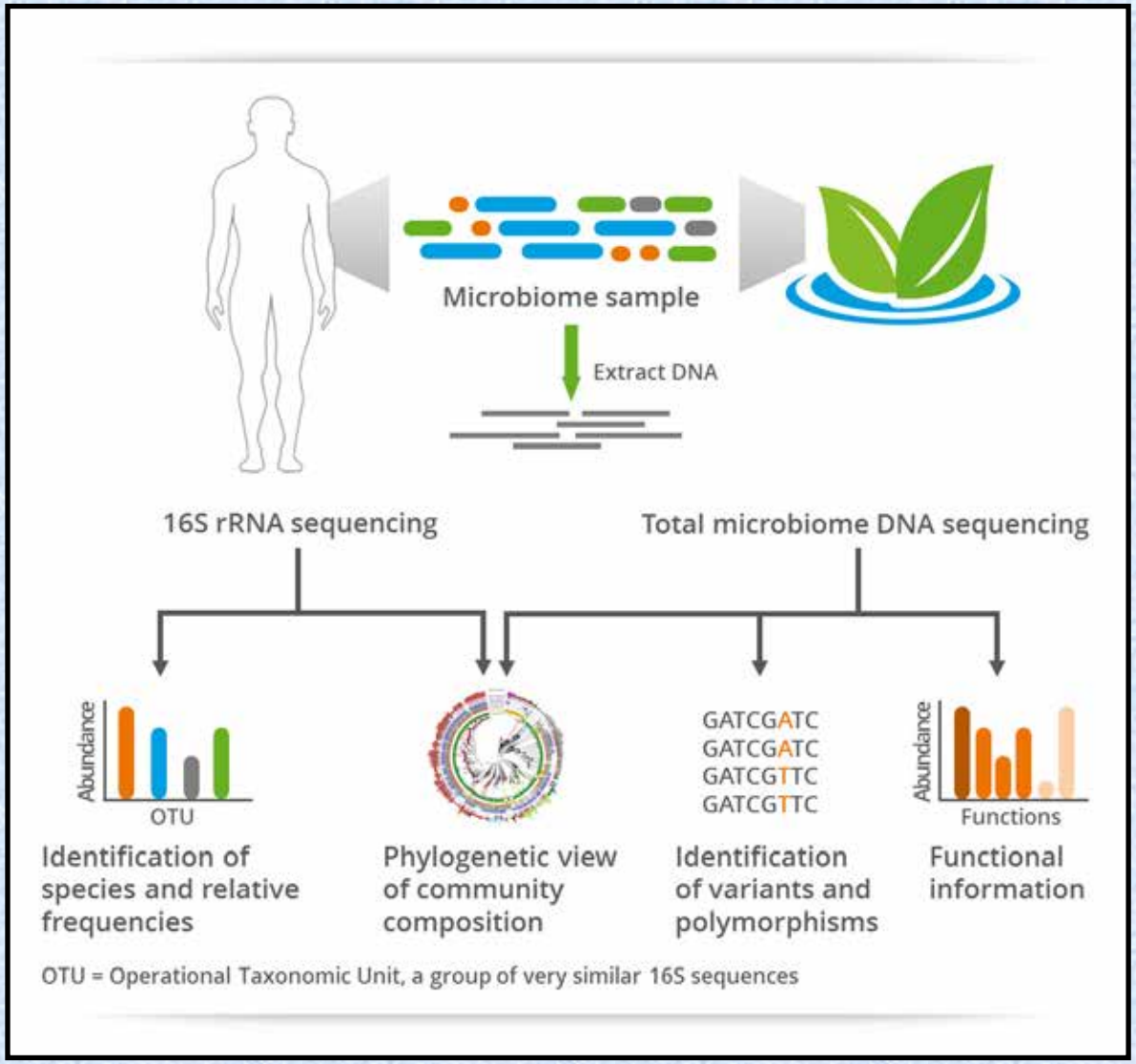
- Whole genome sequencing detected mutations associated with the *rspU* gene.
- Resistant listeria need to be considered in exposure assessment.
- Isolates from human listeriosis cases should be scanned for these mutants by WGS to determine their significance

Exposure Assessment Example Metagenomics

- Most microbiology studies bacteria on their own and away from the food. Not the real world!
- Culturing bacteria leads to biases.
- Metagenomics allows culture independent analysis of microbial populations.

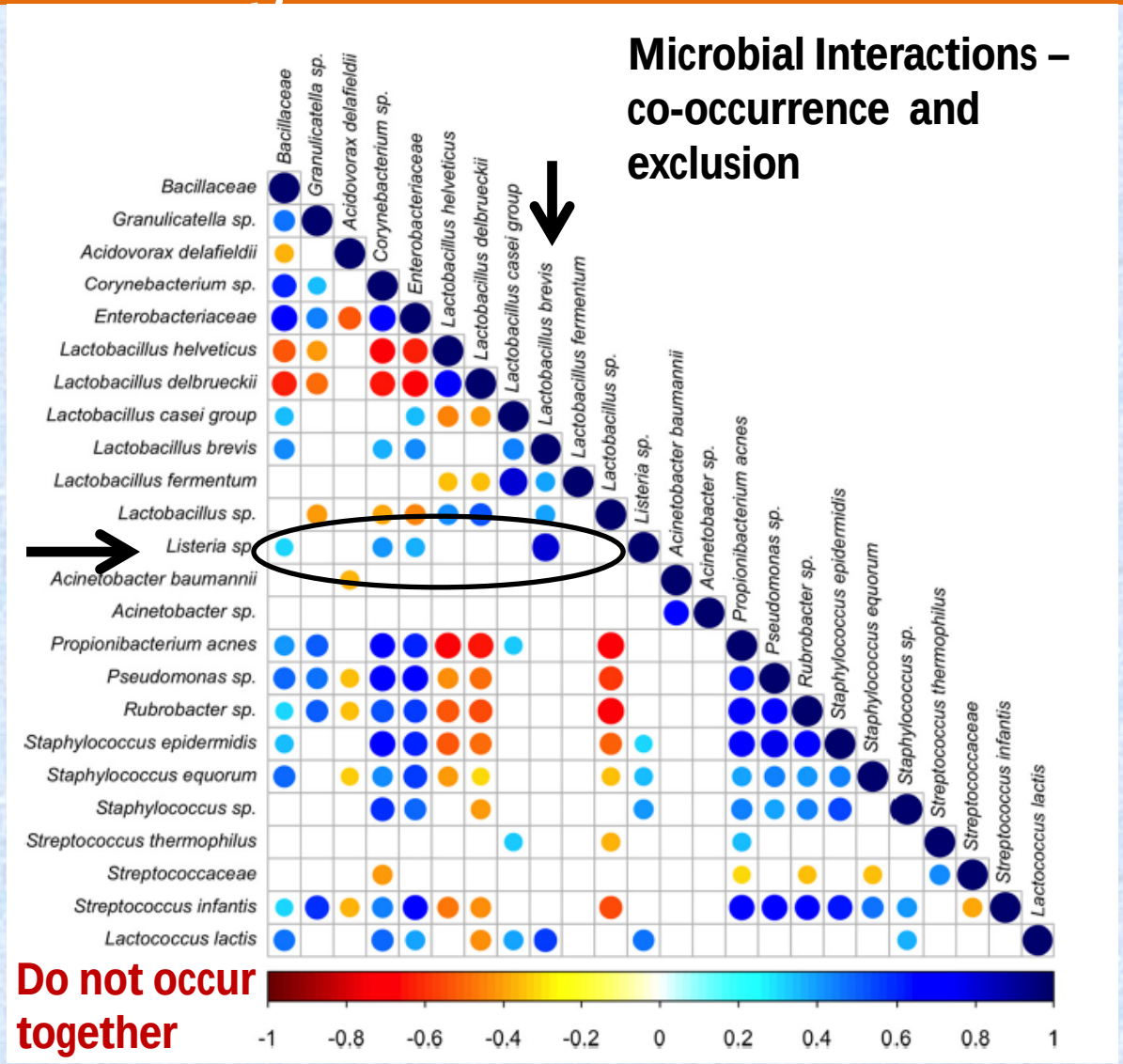
www.thehealthsite.com

Exposure Assessment Example Metagenomics



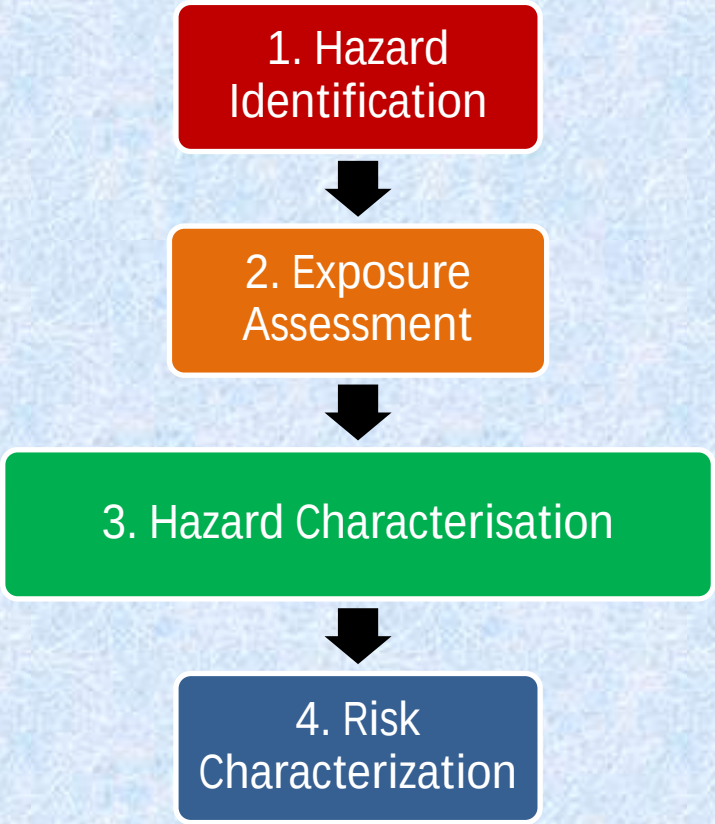
https://www.gatc-biotech.com/en/expertise/targeted-sequencing/16s-rrna-analysis.html

Exposure Assessment Example Metagenomics – Italian Cheese



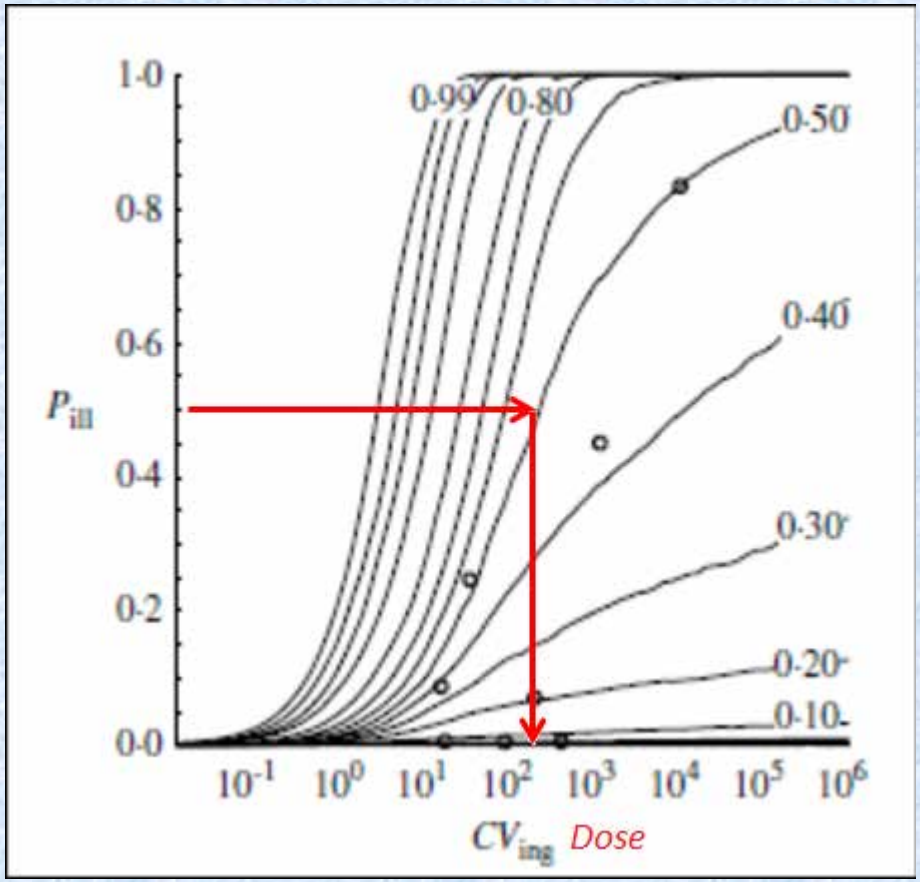
Alessandria et al., AEM, 2016

Hazard Characterization



Hazard Characterization

- Dose Response
  - Ingestion of pathogen
  - Colonisation
  - Production of toxins etc
- Severity of disease (Toxin type)



Teunis et al., Epi and Inf, 2008

Hazard Characterization Example - WGS

- Shigatoxins two main types 1 & 2
- Sub-types: stx1a to stx1f and stx2a to stx2g
- Potency in mouse: stx2d > stx2a >stx 2c > stx1 (Fuller, Inf & Immun 2011)
- STEC toxin typing method developed from the next generation sequencing reads

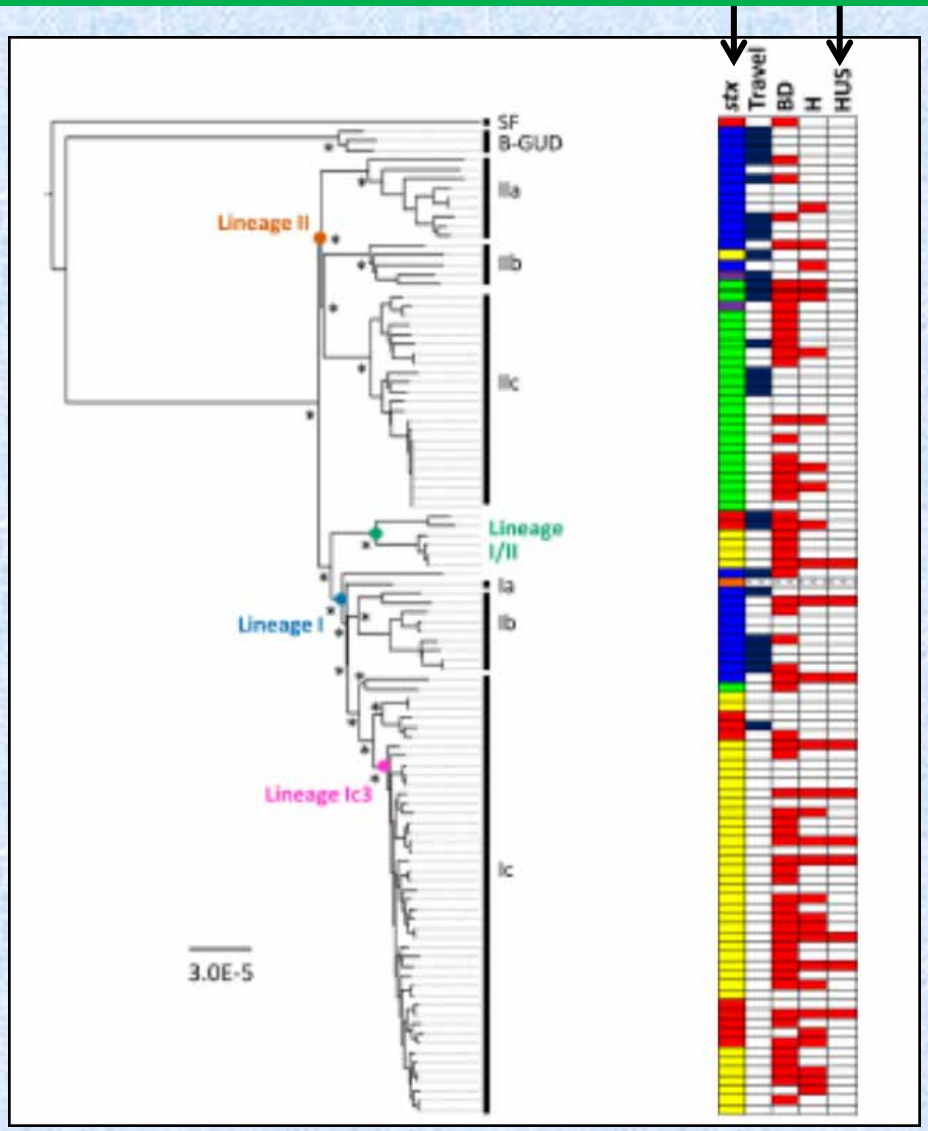
PeerJ

Insight into Shiga toxin genes encoded by *Escherichia coli* O157 from whole genome sequencing

Philip M. Ashton[1], Neil Perry[1], Richard Ellis[2], Liljana Petrovska[2], John Wain[3], Kathie A. Grant[1], Claire Jenkins[1] and Tim J. Dallman[1]

Hazard Characterization Example - WGS

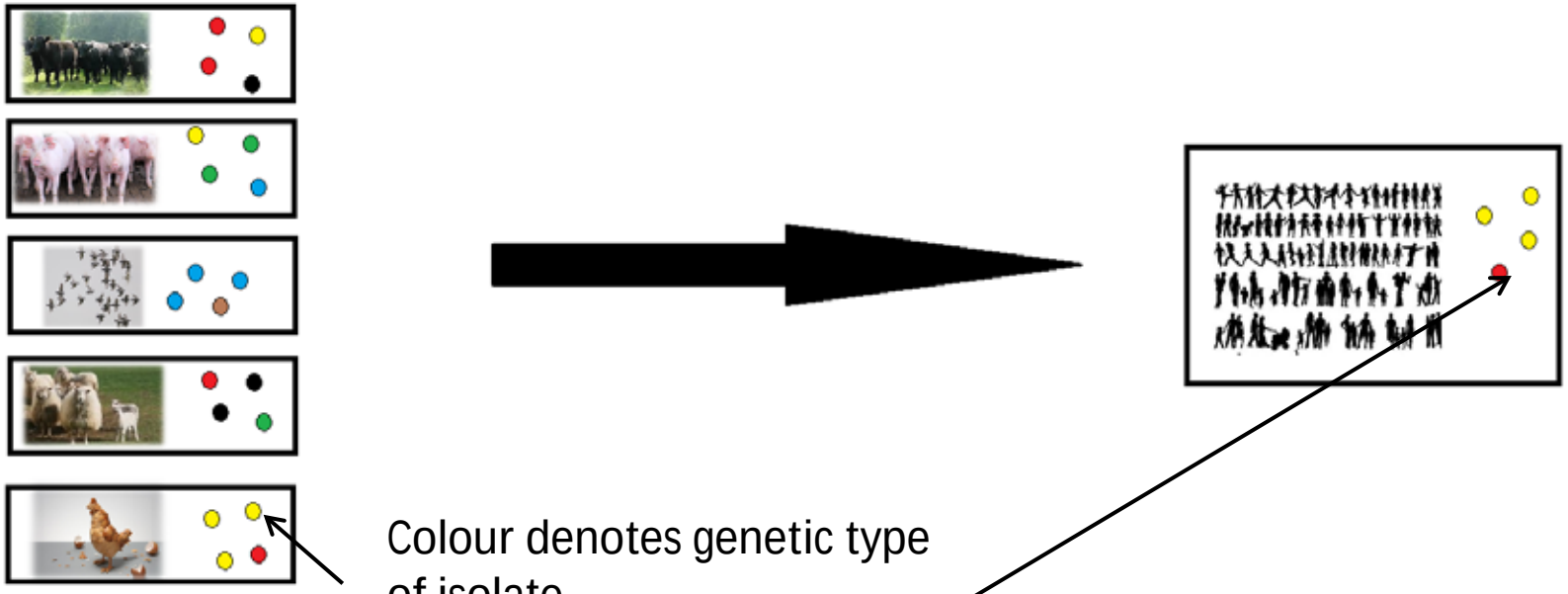
- Sequencing applied to 105 Scottish clinical isolates (Holmes, JClinMic 2015).
- All HUS cases involved stx2 of which 8/10 involved stx2a (red and yellow)



What are the sources of human infection?

Risk Assessment

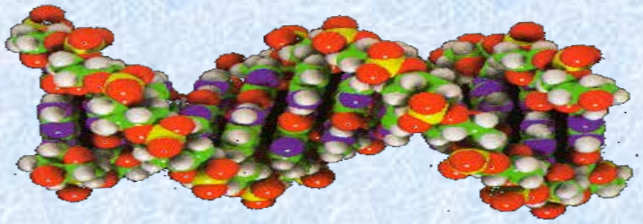
Source Attribution using Microbial Sub-typing



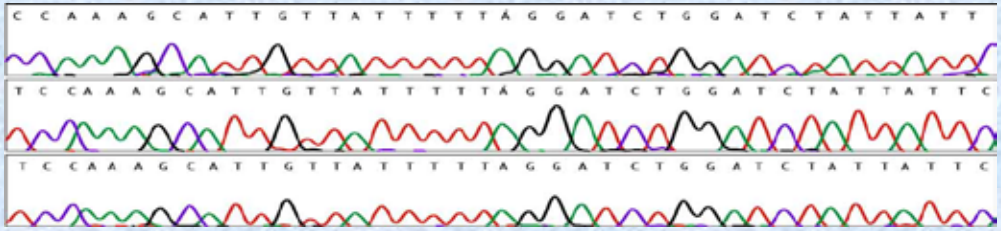
Campylobacter Source Attribution by MLST

MLST method

Isolate DNA



Sequence

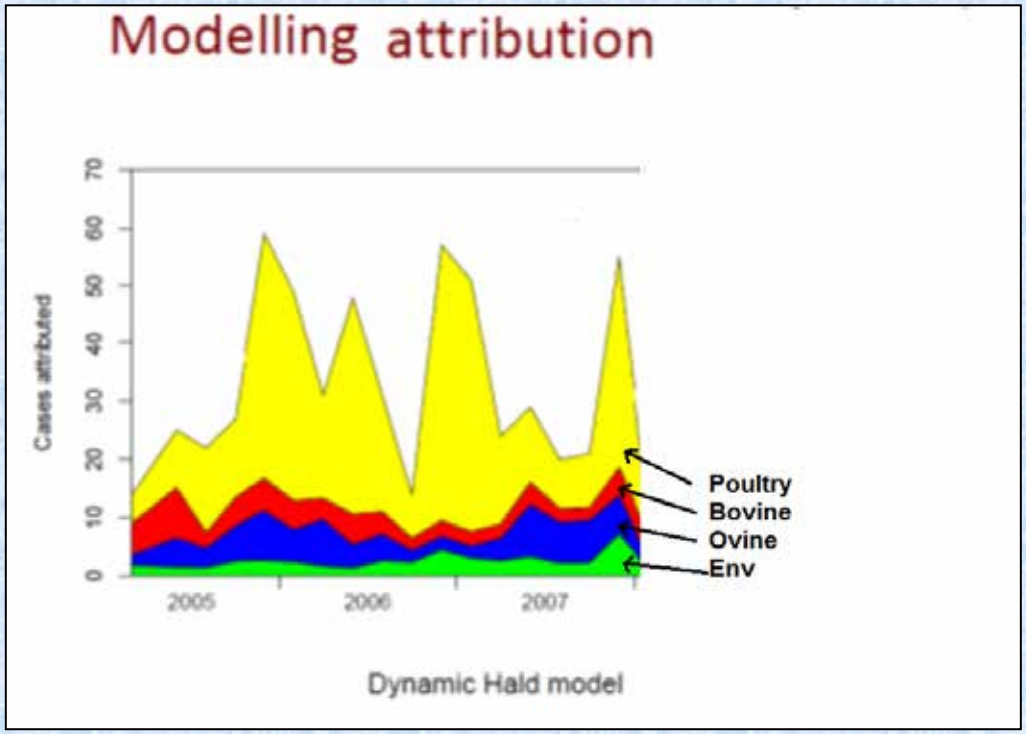


*aspA***9** *glnA***2** *gltA***4** *glyA***62** *pgm***4** *tkt***5** *uncA***6**

ST-257

Source Attribution In New Zealand

Manawatu Region in North Island shows predominance of poultry isolates in human population



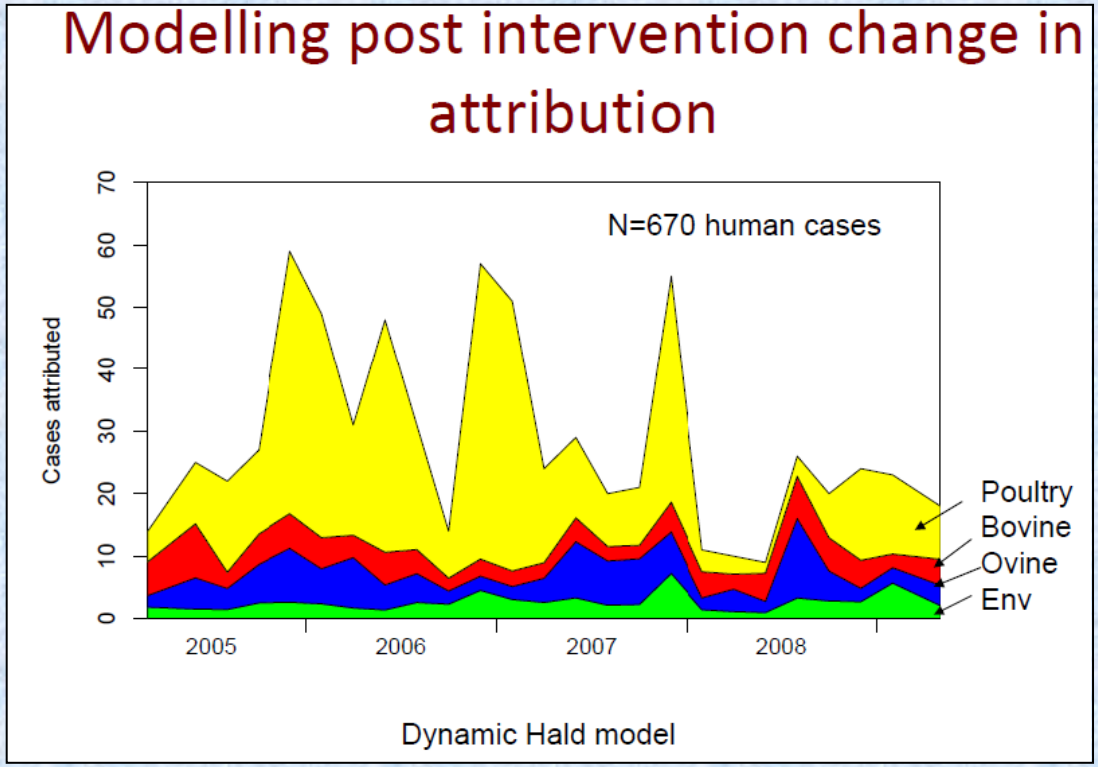
French et al www.foodsafety.govt.nz

Risk Management

New Zealand poultry interventions included

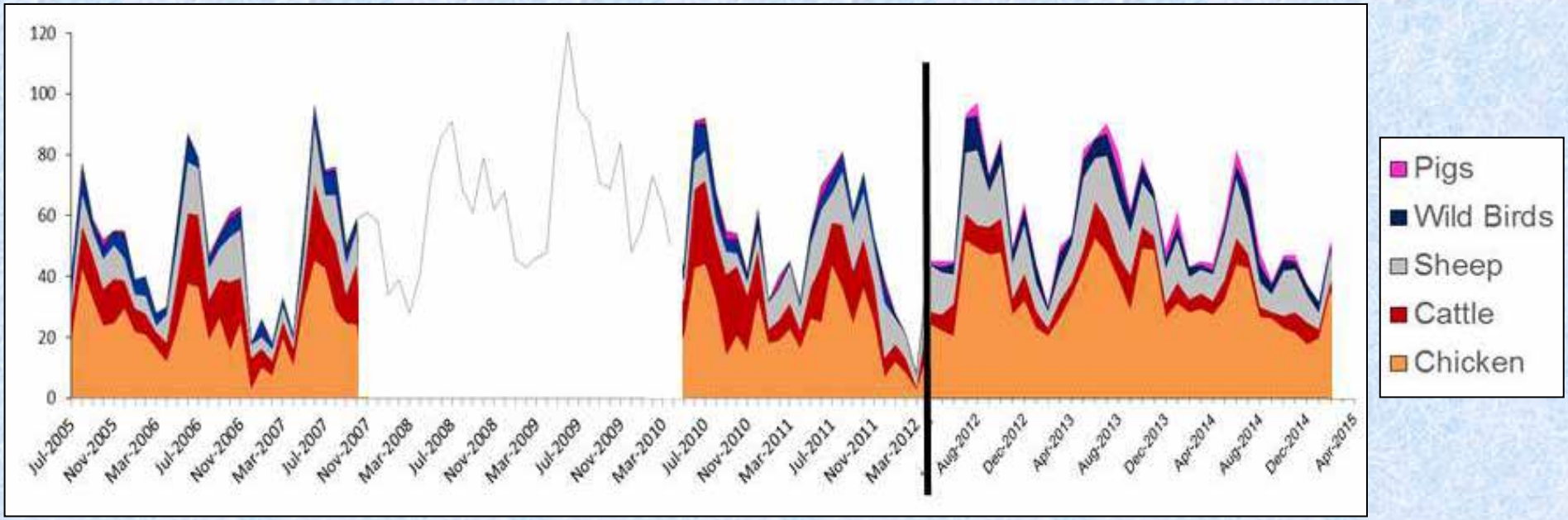
- Improve procedures for catching birds and cleaning crates
- Monitoring and enumerating Campylobacter levels on birds after immersion chiller (April 2007)
- Improving immersion chilling (2006-2008)
- Mandatory targets for Campylobacter levels on poultry after primary processing (April 2008)
- Voluntary use of leak-proof packaging
- Enhanced consumer education (1998)
- Enhanced surveillance and source attribution

Source Attribution Monitoring Risk Management



74% reduction in *Campylobacter* cases associated with poultry in 2008 (Sears et al., EID 2011)

Source Attribution of Campylobacter in NE Scotland



Retail chicken remains the main source of Campylobacter in NE Scotland

Food Standards Agency food.gov.uk

Food Standards Scotland For safe food and healthy eating

iCamps3 Report
www.foodstandards.gov.uk

UNIVERSITY OF ABERDEEN

Source Attribution of Listeria monocytogenes in Europe

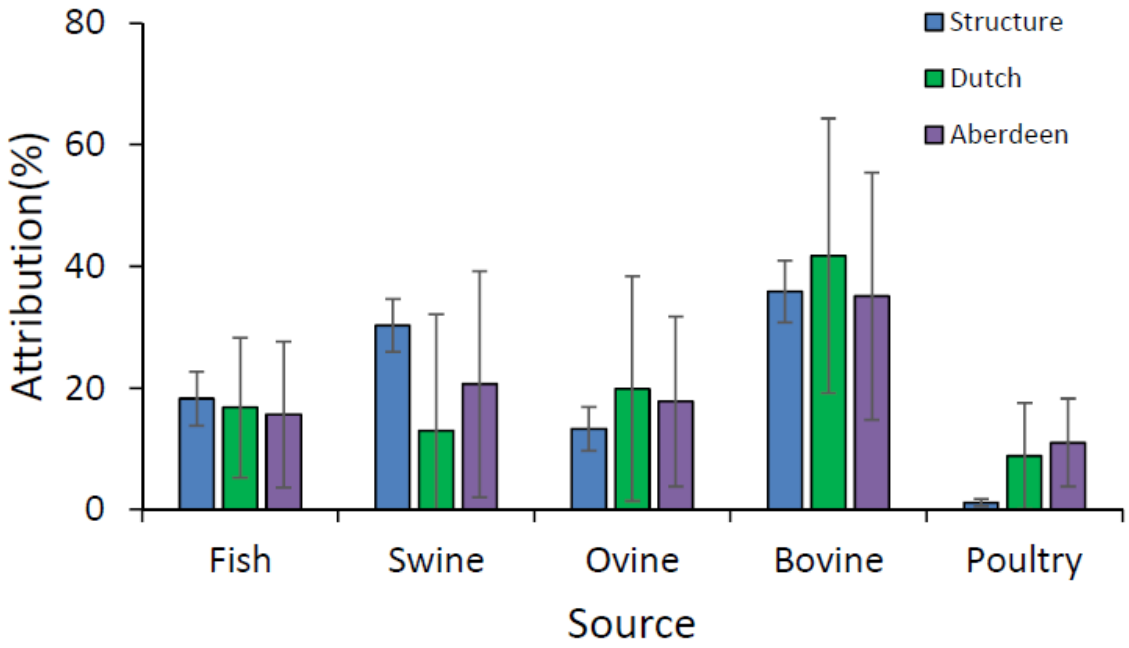


Figure 7.7.: Source attribution of human cases by 1748 locus cgMLST data utilising STRUCTURE, Dutch and Aberdeen models (error bars denote 95% confidence intervals)

Nielsen et al., 2017, EFSA report

Take Home Messages

- Next generation sequencing examples for the hazard identification, exposure assessment and hazard characterisation steps of RA exist
- This work is in its infancy but the opportunities are huge
- Vital that the genetic data are linked to the appropriate phenotypic data
- WGS has direct application to source attribution and risk management