A new approach to risk ranking by use of WGS data to measure the extent of linkage between *Salmonella* from food and clinical cases of human salmonellosis in England

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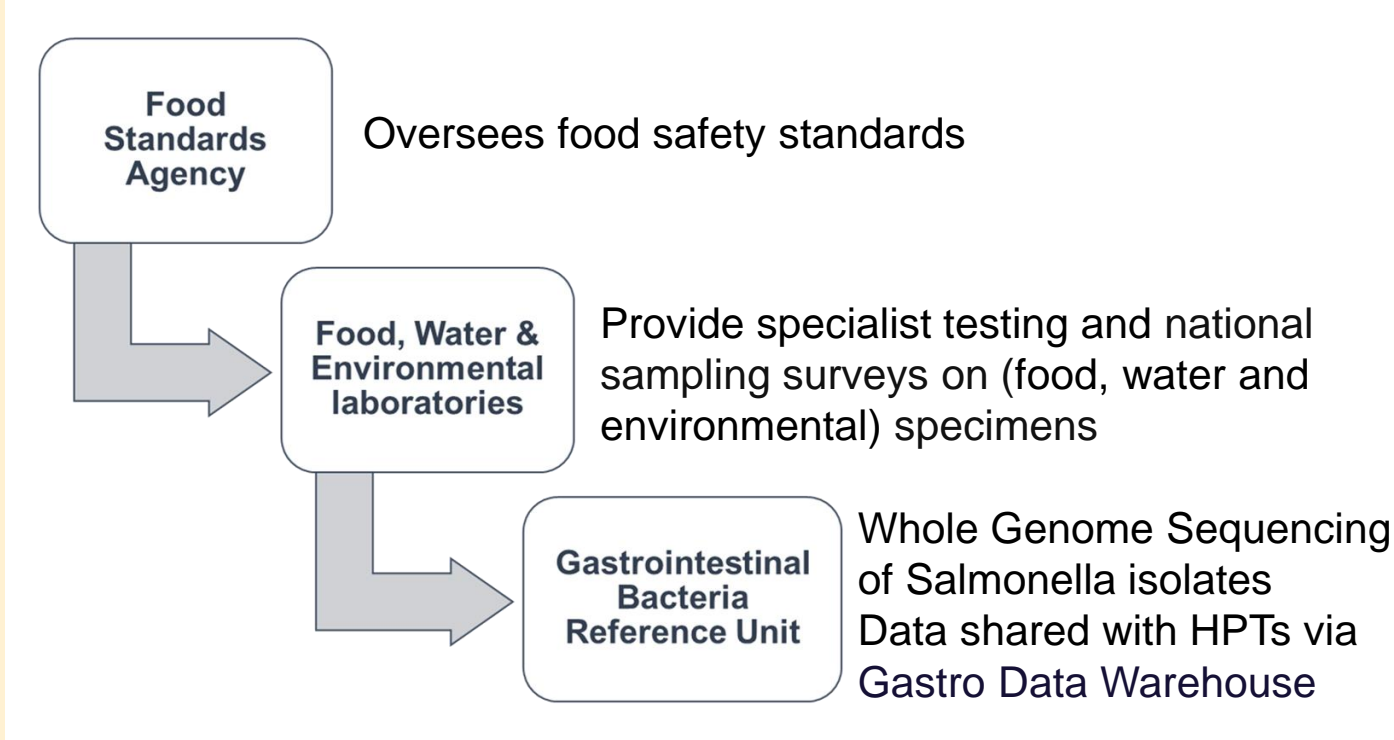
INTRODUCTION

Figure 1. Food safety regulation, testing and surveillance in England. HPT: Health Protection Team

Infections caused by non-typhoidal *Salmonella*, which is a notifiable pathogen, pose a huge societal burden. It is estimated that, in the UK, *Salmonella* cases exceed 31,600 per year (1). Therefore, collaborative and multi-disciplinary efforts, as well as surveillance and outbreak detection are crucial for infection control.

In England, UK Health Security Agency (UKHSA) is responsible for surveillance and outbreak investigations of human salmonellosis (Figure 1).

UKHSA implemented Whole Genome Sequencing (WGS) in 2015 as a method for identification and supporting outbreak investigations.

A detection of an outbreak is characterised using the epidemiological data in combination with single nucleotide polymorphism (SNP) analysis and sample testing (2). Nevertheless, there can be delays between the onset of a foodborne illness and recognition of an outbreak. This study aimed to quantify and rank specific food/pathogen combinations most often linked to human cases as a method to evaluate previous sampling activities, support timely decisions for food testing and outbreak investigations.

METHODS

1. Harmonise the data on food classification and sampling methods (Figure 2).
2. Compare isolates recovered during 2015-2020 in the UK from: 43,614 cases of salmonellosis; 799 from food; 131 from the environment; and 3 from pet food.
3. Establish genetic links between the clinical and non-clinical isolates using:
   - pairwise comparisons of SNPs – matched by ≤5SNP single linkage cluster (SLC)
   - *Salmonella* Hierarchical Clustering (HC) using EnteroBase core genome MLST (cgMLST), at the 5 (HC5) allelic clustering level to determine whether the non-clinical isolates were linked to any human isolates within the UK and globally (3).
4. The SNP and cgMLST analysis was used to risk rank food types and serovars in relation to those linked to the cases of salmonellosis.
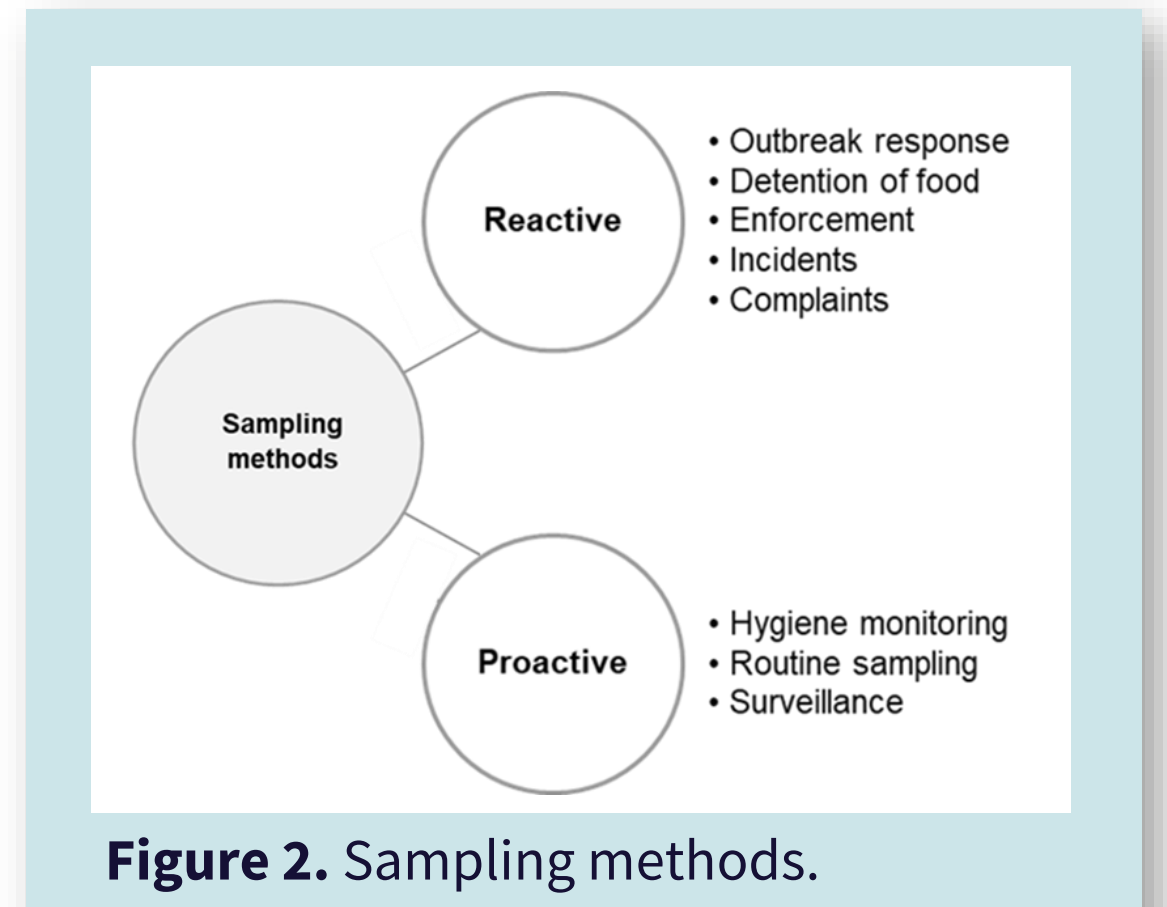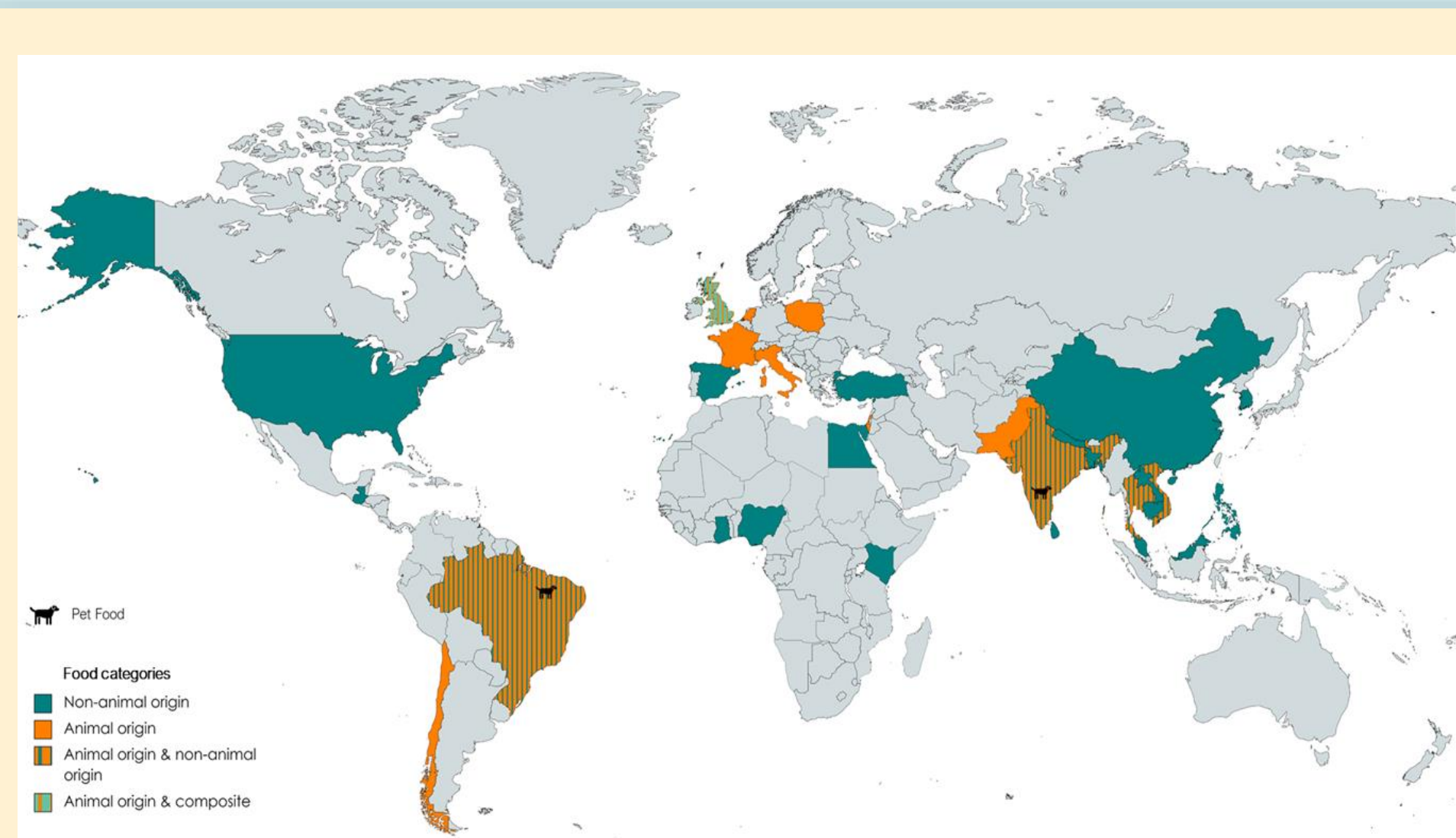

Figure 2. Sampling methods.

RESULTS

Table 1. Risk ranking of Food Products. The table shows food samples which were microbiologically linked to ≥ 5 human cases by SNP typing (5SNP SLC threshold). The numbers of human cases within the UK and globally at the HC5 level.

Human cases 5 SNP	UK – HC5	Global** - HC5	Category	Subcategory	Country	Serovar	EBG	ST	SNP Address	HC5 ID
490 (2012-2020)	906 (2012-2020)	151 (2016-2020)	Animal	Chicken	Netherlands ^^	Enteritidis	4	11	1.1.2.12.590.%	1358
242 (2008-2018)	260 (2014-2018)	25 (2015-2019)	Animal	Boiled Egg	- ^^	Enteritidis	4	11	1.2.3.151.151.783.%	1955
204 (2014-2020)	324 (2012-2020)	184 (2016-2020)	Animal	Egg	N/D ^^	Enteritidis	4	11	1.2.3.18.359.360.%	2037
80 (2014-2017)	76 (2014-2017)	0	Composite	Pease Pudding	- ^	Braenderup	24	22	1.1.1.1.1.%	1403
62 (2015-2020)	83 (2015-2020)	0	Non-animal	Mixed salad	- ^^	Kedougou	291	1543	1.1.1.2.2.4.%	1297
33 (2018)	853 (2005-2020)	454 (2002-2020)	Animal	Pork - Cooked Sliced	UK ^^	Typhimurium	1	34	1.1.1.124.3255.4475.%	302
30 (2015-2020)	32 (2015-2020)	2 (Ireland, 2017; France, 2019)	Animal	Egg	Poland ^^	Enteritidis	4	3748	1.1.2.1148.1901.2268.%	60189
19 (2016)	19 (2016)	1 (Ireland, 2016)	Animal / Composite	Egg - products / Arancini	Italy / UK ^^	Enteritidis	4	11	1.2.3.18.2246.2777.%	53148
13 (2018-2020)	12 (2018-2020)	21 (20x France, 2018-2020; 1x Australia, 2018)	Animal	Chicken	- ^	Typhimurium *	1	34	1.1.1.124.3817.4504.%	97275
11 (2016-2018)	13 (2016-2020)	1 (Ireland, 2017)	Animal	Chicken	- ^	Typhimurium*	1	34	1.1.1.124.2623.3121.%	60283
9 (2016-2019)	11 (2016-2020)	0	Animal	Dried Fishmaws	India ^	Virchow	9	16	1.8.8.9.9.9.%	115050
7 (2012,2014, 2018)	19 (2012-2018)	0	Animal	Pork Sausage	UK ^	Typhimurium	1	34	1.1.1.124.495.500.%	1743
6 (2015 & 2018)	10 (2015-2018)	2 (Ireland, 2017; France, 2019)	Composite	Kebab Meat	UK ^	Infantis	31	32	1.1.36.45.45.45.%	28658

* Typhimurium – monophasic; ** not UK; Reason for sampling: ^ Routine, ^^ Reactive (investigation of incidents or outbreaks)

Figure 3. Map showing where *Salmonella*-contaminated foods originated from.
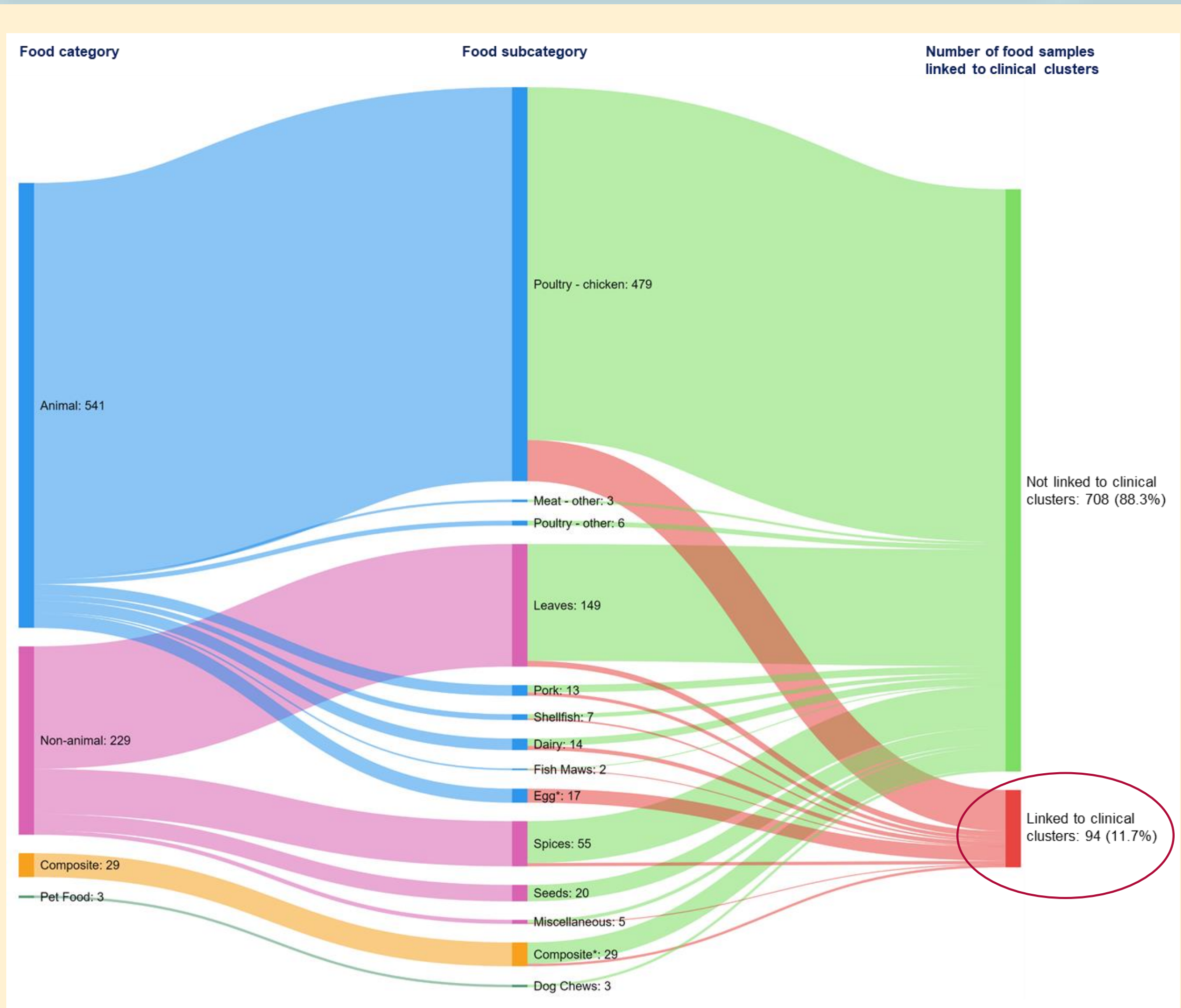

Figure 4. Sankey diagram illustrating the proportion of food samples (per category and subcategory) having potential association with clinical clusters, at the 5 SNP level.

- Approximately 49% of *Salmonella*-contaminated food products (2015-2019) came from Brazil; of these, ~98% were chicken, Figure 3.
- Of the *n* = 803 food isolates, 88% did not have an association with cultures from salmonellosis cases. The remaining 12% were associated with clinical cases, Figure 4.
- The highest proportion of linked cases were attributed to poultry (~42%) and egg products (~40%), Figure 5.
- In total, *n* = 986 clinical cases of salmonellosis were genetically linked to *S.* Enteritidis, predominantly isolated from animal origin foods, Table 1.
- Food products associated with the highest numbers of clinical cases were tested due to reactive sampling, Table 1.
- cgMLST analysis (HC5) showed the global impact of *Salmonella* in the food chain, where the persistence of the same pathogen is noticeable over the years and in multiple countries, Table 1.
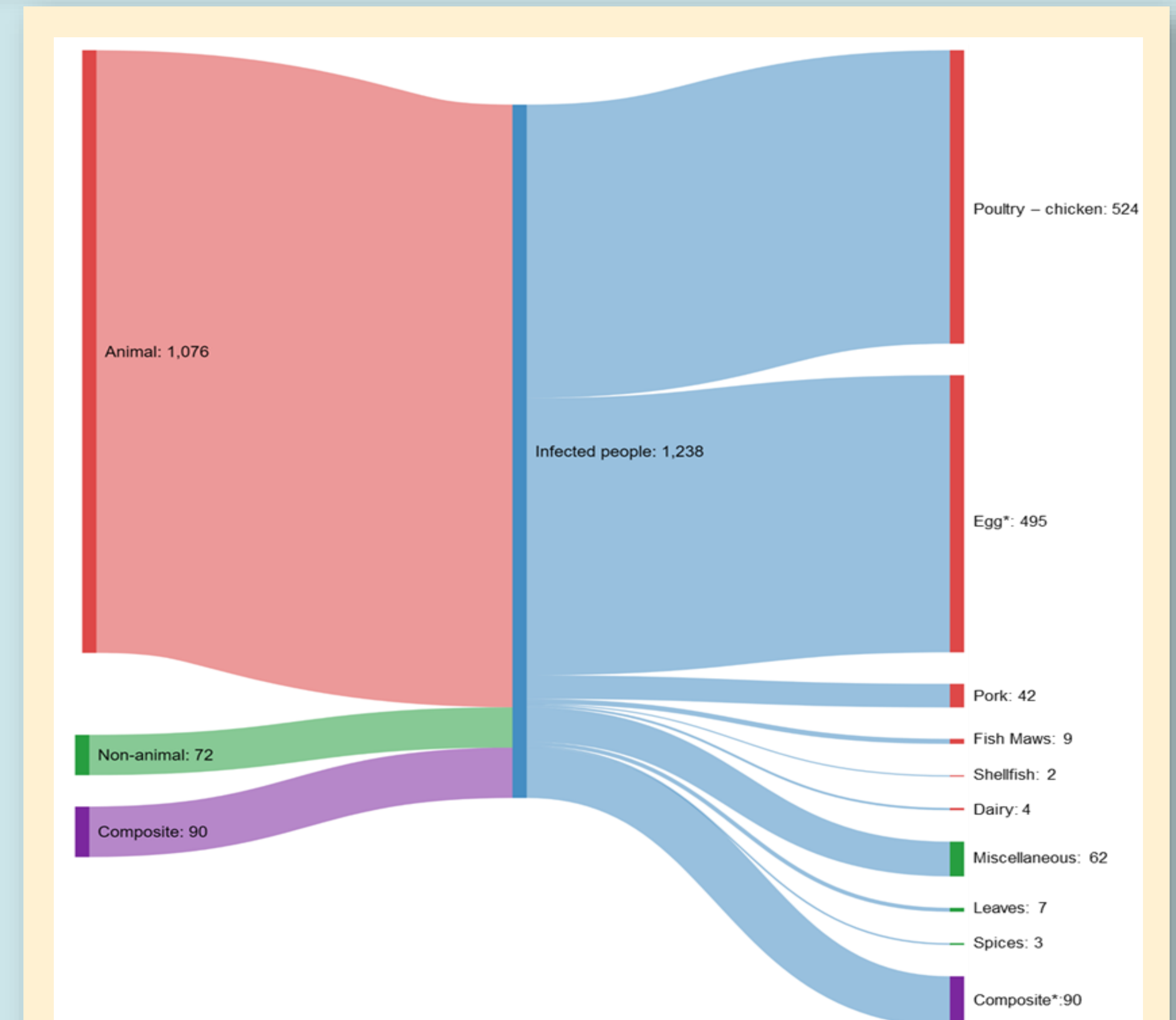

Figure 5. Sankey diagram showing the numbers of human cases genetically linked to the food products (per food category and subcategory), at the 5 SNP level.

DISCUSSION & CONCLUSIONS

- Analysis of WGS data collected over the period of 5 years enabled a previously unavailable understanding of *Salmonella* transmissions within the food chain, by establishing microbiological links of *Salmonella* from food and clinical cases of human salmonellosis.
- Of 43,614 cases of laboratory-confirmed salmonellosis (2015-2020), only 1,238 were genetically linked with the consumption of food products analysed in this study. This may be due to, other than food, sources of infection (such as contact with animals such as reptiles, the environment) or to un-tested food chains.
- A high proportion of food products that were linked to human cases was tested due to the reactive sampling such as in response to an outbreak investigation, detention of food at import, enforcement, incidents or complaints. This study demonstrates that routine monitoring of food plays a crucial role in infection control.
- With global trade and travel, the tracing of contaminated-food sources can be challenging; publicly available data such as on EnteroBase can aid in the understanding of the worldwide impact of *Salmonella* in the food chain.
- Majority of *Salmonella* found in tested food (88%) did not have an association with a clinical case, and a small proportion of *Salmonella* found in food products (11%) were associated with clinical cases – predominantly of animal origin.
- The highest proportion of cases was attributed to poultry and egg products (the majority contaminated with *S.* Enteritidis). Food of non-animal origin was more likely to be associated with sporadic or clusters of <5 cases that had not been previously identified.
- The risk ranking approach (food/pathogen combinations) represents a novel method that can be used for future microbiological risk assessments, prioritisation of genomics development, food sampling, and outbreak investigation.

ACKNOWLEDGEMENTS

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