

Unravelling the Mysteries of Yersiniosis

Progress update: November 2021

The purpose of this report is to provide an update of the results to date for the Health and Research Council (HRC) study entitled “Unravelling the mysteries of yersiniosis”.

Information about the project can be found on the [ESR website](#).

If further information is required, then please contact us on: yersiniahrc@esr.cri.nz.

Highlights

- A case-control study has begun focusing on cases of yersiniosis notified to Canterbury, South Canterbury, and the West Coast District Health Boards. Risk-factor questionnaires have been completed for 56 notified cases (89% of notified cases) who have consented or pre-consented to use the data for the study.
- Control individuals are being recruited from a New Zealand Health Survey Cohort database and interviewing has commenced. Access to the cohort was delayed until November 2021. In the interim, 29 individuals were recruited as preliminary controls which allowed for piloting the control interviews and to provide some interim data.
- *Yersinia enterocolitica* (YE), the predominate bacterial species associated with yersiniosis has been detected in source samples tested to date. Clinically relevant YE types were confirmed in 15.2% (15 out of 99) of samples. These have been predominately from raw pork available at retail in Christchurch. Source sampling began in July and is ongoing.
- Whole genome sequencing (WGS) analysis has identified genetic clusters of isolates obtained from clinical cases from the study region and Auckland (from a separate surveillance activity) as well as isolates from raw pork product purchased in Christchurch.
- An interview schedule has been developed to interview Māori health experts, Māori health providers and focus groups with lay Māori participants, to begin exploring why Māori are amongst the lowest notified for yersiniosis in New Zealand.

Case-control study to identify risk-factors associated with yersiniosis in New Zealand

Interviews of notified yersiniosis cases and controls are on going

- The study is currently focusing on cases of yersiniosis notified to Canterbury, South Canterbury, and the West Coast District Health Boards. A risk-factor questionnaire is administered by telephone by Community and Public Health (CPH) to reported yersiniosis cases soon after notification. Data used for the study are from individuals consenting to share their risk-factor responses for research purposes. Risk-factor questionnaires captured by the study began on 25th June 2021.
- From 25th June until the 30th November 2021 (inclusive), 63 notified cases in the study region were available from EpiSurv¹. Those notified cases consisted of 52% female and 48% male with age ranges from under one years old to over 90 years old (median age is 41 years, mean age is 41.5 years)
 - Telephone interviews were undertaken by CPH with 59 notified cases, of which 54 have consented to participate in the study and two are at pre-consent stage (representing 89% of notified cases). Three cases declined to participate or were not able to be recontacted for consent. Three cases were not able to be interviewed by CPH.
- Ten (18%) of the consented cases were hospitalised due to yersiniosis.
- As a part of the public health surveillance processes, isolates from **all** notified cases of yersiniosis are referred to ESR's Enteric Reference Laboratory for confirmation. Of the 63 notified cases, 59 clinical isolates have been analysed to date. Eighty-eight percent of isolates (n = 52) were *Y. enterocolitica* (YE) and the remaining seven case isolates (12%) were identified as *Y. pseudotuberculosis*. Isolates from four of the cases are yet to be analysed.
- Bioserotyping for YE indicated that YE Biotype 1A predominated amongst the isolates from all notified cases with 42% (25 out of 59), followed by YE bioserotype 2/3, O:9 with 39% (23 out of 59) and YE bioserotype 2/3, O:5, 27 was identified for 7% (4 out of 59). To date, YE bioserotype 4, O:3 has not been observed amongst the notified cases.
- The study region was in Level 4 and 3 COVID-19 restrictions between 17th August and 9th September 2021. The number of notified yersiniosis cases in the study area between these periods remained constant when compared to past surveillance data. This may suggest that risk-exposures associated with yersiniosis were not affected by these restrictions.
- Control individuals (i.e., persons that have not been diagnosed with yersiniosis, or had yersiniosis like symptoms, or enteric infections in the 14 days prior to the questionnaire) are being interviewed from a New Zealand Health Survey Cohort (NZHSC). Access to the cohort was delayed until November 2021. In the interim, 29 individuals were recruited as preliminary controls which allowed for piloting the control interviews and to provide some interim data. These individuals represent those residing in Canterbury who either work at ESR, Christchurch or a contact recruited through an interviewed ESR staff member control participant.
- Case and control data is currently not adequate to undertake robust analyses for study purposes. Case and control interviews will continue to strengthen the dataset and analyses in the coming months.

¹ ESR undertakes the surveillance of notifiable diseases in New Zealand, including yersiniosis, on behalf of the Ministry of Health. EpiSurv is New Zealand's national notifiable disease database.

Yersinia enterocolitica has been detected during source sampling

- Testing of source (food and environmental) samples began in July 2021 and is focused on YE as it is the *Yersinia* species most identified amongst the clinical cases in New Zealand.
- To date, ninety-nine different samples have been tested for YE (Table 1). Most samples (n = 88) were purchased from various retailers across Christchurch. Eleven food and environmental samples were collected from consenting cases. YE was not confirmed in any of the samples obtained from cases.
- Clinically relevant YE bioserotypes (2/3, O:9; 2/3, O:5, 27 and/or 4, O:3) were confirmed in 15.2% (15 out of 99) of samples tested. The predominant sample type tested was raw NZ pork, which included mince and other products such as diced and stir fry cuts of fresh pork (n = 35). Of these samples, 13 samples were confirmed to contain pathogenic YE. The remaining confirmed YE positive samples included a sample each of raw pork sausages and raw lamb mince.
- Five samples of pork mince were found to contain more than one YE clinically relevant bioserotype.
- YE Biotype 1A is considered non-pathogenic internationally but is notifiable in New Zealand. YE Biotype 1A has been confirmed in 25% (25 out of 99) of samples tested (not shown in Table 1). Most of these samples (n = 18) were of raw pork or other pork products. The remaining confirmed YE Biotype 1A were from other raw meat types (chicken [n= 1], beef [n = 1] and lamb [n = 3]) and a non-pork raw sausage sample (n = 1). Fifteen other samples that are presumptive positive for YE Biotype 1A are still being processed and confirmed.
- Sampling and testing will be ongoing throughout the study guided by the case risk factor information.

Table 1: Number of samples tested and confirmed with pathogenic *Y. enterocolitica*.

Sample type	Positive	Negative	Total
Meat and product - Raw			
Raw pork mince	12	13	25
Raw pork - other	1	9	10
Raw lamb	1	8	9
Raw beef	0	8	8
Raw chicken	0	5	5
Raw pork sausages	1	1	2
Raw (non-pork or flavoured) sausages	0	3	3
Pet food	0	2	2
Meat - Processed, Ready-to-eat			
Bacon	0	8	8
Ham	0	3	3
Ready-to-eat pork	0	1	1
Ready-to-eat sausages	0	6	6
Other food products			
Baby spinach	0	2	2
Blueberries (frozen)	0	8	8
Raspberries (frozen)	0	3	3
Environmental			
Faeces pig	0	1	1
Faeces sheep	0	2	2
Water	0	1	1
Grand Total	15 (15.2%)	84	99

Whole genome sequencing of YE has identified genetic clusters between cases and source isolates

- Whole genome sequencing (WGS) analysis provides the most accurate method to confirm the identification of a bacterial isolate and is considered the most discriminatory method to determine the genetic relationship between isolates of interest.
- This study is performing WGS on all clinical isolates from notified cases within the study region. As of 30th November 2021, WGS has been completed on 56 isolates from notified cases.
- For source sampling, a single isolate per bioserotype per sample was selected for WGS.
- Multilocus sequence type (ST) 12, 14 and 18 (using the McNally 7-gene scheme) correlates with bioserotypes 2/3, O:9; 2/3, O:5, 27 and 4, O:3, respectively. WGS analysis is performed using single-nucleotide polymorphism (SNP) analysis, where genomes of isolates identified as ST12, ST14 and ST18 are mapped and compared to selected NZ 'reference' genomes of the same ST. Genomic SNP clusters are assigned to isolates that share 0-5 SNP differences between them (Table 2).

Table 2: Genetic clusters for *Y. enterocolitica* isolates identified as ST12 and ST14 for clinical isolates obtained from the HRC study region.

ST (Bioserotype) Cluster/Singleton ^a	Number of clinical isolates ^b		Number of source isolates ^c
	HRC study regions	Other surveillance	
ST12 (2/3 O:9)			
ST12_2016_C_06	8	14	3
ST12_2017_C_01	1	1	1
ST12_2020_C_07	1	1	0
ST12_2021_C_02	3	1	0
ST12_2021_C_04	1	2	1
ST12_2021_C_05	2	3	3
Singleton	7	7	0
ST14 (2/3 O:5, 27)			
ST14_2017_C_01	2	N/A	1
Singleton	2	N/A	0

^aGenomic SNP clusters are assigned to isolates that share 0-5 SNP differences between them. The ST12 clusters contain clinical isolates from the HRC study region and the Auckland region. A singleton is an isolate that is not genetically related to any other isolate of the same bioserotype within the ESR database.

^b Collected as a part of the HRC study, and a separate surveillance activity conducted in the Auckland region. The surveillance activity focused on bioserotype 2/3, O:9 as it was predominating bioserotype amongst clinical cases. No Auckland region clinical isolates identified as 2/3, O:5, 27 were whole genome sequenced and not available (N/A) for cluster analysis.

^cOnly source isolates obtained as a part of the HRC study.

- Genetic clusters of YE isolates identified as ST12 and YE ST14 obtained from clinical cases from the study region as well as isolates from raw pork product purchased in Christchurch has been observed. This signals that isolates within each cluster share a close genetic relationship and may indicate a common source of infection.
- Genetic clustering of YE obtained from pork samples from different retailers potentially suggest a common source of YE.
- YE Biotype 1A from clinical cases are genetically distinct from each other using SNP analysis. This indicates that these cases are not genetically linked.

Public health investigation into an increase in yersiniosis cases in Auckland

- During June/July 2021, an increase in YE bioserotype 2/3 O:9 was reported in the Auckland region (including Auckland, Waitemata and Counties Manukau DHBs) and an epidemiological investigation was conducted by the Auckland Regional Public Health Service (ARPHS). This included administering an extended case questionnaire alongside prospective WGS of case isolates. The Ministry of Health funded WGS of Auckland case isolates to support the public health investigation.
- Epidemiological data collected by ARPHS from case interviews identified an association of YE biotype 2/3 serotype O:9 infection with consumption of pork products.
- Genetic clustering of YE isolates was observed from cases in the Auckland region with Canterbury cases and also pork isolates obtained in from Canterbury based businesses (Table 2). These results are indicative of a dispersed common foodborne source affecting multiple regions of New Zealand (there was no evidence to indicate Canterbury cases had travelled to the Auckland region or vice versa during this time period).
- The ARPHS epidemiological study and the WGS of cases from the Auckland region ceased on 17th August 2021.

Eating raw pork associated with illness

- One of the consenting cases interviewed reported eating raw pork mince in a speciality dish known as Mett (Figure 1). A household member of the case prepared the dish, and reportedly was also ill, although only briefly. The interviewed case reported symptoms lasting for a fortnight and required antibiotics.
- The YE isolate from the case was sequenced and found to cluster with other cases identified in Canterbury and Auckland region (cluster ST12_2016_C_06 outlined Table 2), notified at approximately the same time. This cluster also contained three YE isolates from raw pork mince purchased in Christchurch.



Figure 1: Mett is a preparation of minced raw pork that is popular in Germany, Poland and in Belgium; a similar preparation is made from beef. It is normally served with salt and black pepper, and sometimes with garlic, caraway or chopped onion, and eaten raw, usually on a bread roll <https://en.wikipedia.org/wiki/Mett> (image: <https://commons.wikimedia.org/wiki/File:Mettbroetchen.jpg>).

Study to determine why Māori have the lowest notified rates of yersiniosis (and other enteric diseases)

As a part of the first year of the research, we have established the Māori advisory group, gained ethics approval, and co-developed the interview and focus group questions and study design with the Māori advisory group. We are now in the process of setting up interviews and the focus groups for data collection.

Other developments

Burden of disease of Yersiniosis in New Zealand

Information about the symptoms and severity of disease including complications that those diagnosed with yersiniosis are experiencing is being collected through interviews. Among the initial cases, diarrhoeal illness has been experienced for more than four weeks by some cases, and cases have had up to 14 days away from work or school.

Using genomics to learn more about *Yersinia*

We are exploring the genomes of all the *Yersinia* that are being collected in the study. Some of the areas we are exploring include:

- Establishing the population structure for *Yersinia* and which types are circulating in New Zealand.
- Finding out if YE Biotype 1A found in New Zealand clinical cases are pathogenic and ways to detect them better in foods.
- Antimicrobial resistance genes that *Yersinia* may harbour in New Zealand.

Acknowledgements

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