DIAGNOSTIC AND PUBLIC HEALTH MANAGEMENT PRACTICES OF FOODBORNE BACTERIAL DISEASES

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by

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1 SUMMARY

There is variability in the methods used by clinical laboratories in New Zealand to isolate and identify the pathogens (*Campylobacter* spp., *Listeria monocytogenes*, *Salmonella* spp., *Yersinia enterocolitica* or *Y. pseudotuberculosis*, and verocytotoxigenic *E. coli* (VTEC)/shigatoxin-producing *E. coli* (STEC)) being investigated in this study, but the methods do not appear to have changed significantly over the last five years. The testing protocols adopted in a laboratory are at the discretion of laboratory management; cost, time, effectiveness and staff capability are all factors that affect decision-making on methods. The data collected during this survey of laboratories carried out between February and June 2010 provides a benchmark to investigate future questions regarding the influence of laboratory practice on disease notifications. The laboratory data has also exposed some areas where testing might be improved or standardised, though the impact of any these activities on notification rates must be carefully managed.

The practices reported by Public Health Unit (PHU) staff during the survey also varied. The practices around de-notifying cases and investigating non-O157 VTEC/STEC cases are likely to have some influence over regional notification rates. There is no standard approach for investigating non-O157 VTEC/STEC cases at the PHU or laboratory levels. The Ministry of Health notification guidelines include all verocytotoxin-producing *E. coli*, not just O157 (Ministry of Health 1998, 2007, 2009). The PHU survey data has also revealed that salmonellosis and campylobacteriosis cases are less likely to be investigated than other diseases. The main impact of this is a reduction in the amount of information that might be available for attribution and intervention studies.

The evaluation of laboratory methods and PHU practices in District Health Boards (DHBs) with high VTEC/STEC notification rates and DHBs with low VTEC/STEC notification rates did not reveal any differences between the activities in these DHBs that could account for the disparate notification rates. However differences in the criteria used by laboratories to determine if samples are tested for VTEC/STEC could account for low incidence rates in some DHBs.

2 INTRODUCTION

Evidence from outbreak investigations and epidemiological studies of human enteric diseases is increasingly used as a source of data for risk assessments and source attribution. However, the application is often restricted by the strength of the evidence presented and its interpretation (Adlam *et al*., 2010). A range of reports have described variation in the present system of public health investigation and the management of identified cases of human enteric diseases (e.g. Lake & Sexton, 2009; Lake *et al.*, 2005; Pirie, 2005). Furthermore, geographical variations in disease incidence have long been described in New Zealand but the aetiologies of these have not been identified.[1]

It is possible that these variations and other contributory factors are a result of laboratories using different diagnostic protocols for analysing clinical samples (e.g. faecal samples) from

[1] The geographical variation of notified diseases can be viewed in the Annual Surveillance Summaries produced by ESR for the NZFSA available at http://www.nzfsa.govt.nz/science/foodborne-disease-reports/FW10040_FBI_report_May_2010.pdf.

human cases, or from Public Health Units (PHUs) taking different approaches to investigating notifiable diseases and responding to information they receive from laboratories and the national Enteric Reference Laboratory (ERL) at ESR.

It is also important to identify whether changes in the protocols applied by individual diagnostic laboratories and PHUs could influence local and national disease notification rates over time.

The objectives of this study were:

1. To determine individual human laboratory practices in diagnosing human campylobacteriosis, listeriosis, salmonellosis, yersiniosis, and infection by verocytotoxigenic *E. coli* (VTEC)/shigatoxin-producing *E. coli* (STEC);[2]
2. To determine individual PHU practices in response to laboratory notifications/ERL reporting of these diseases;
3. Using VTEC/STEC infection as an example, evaluate the influences of laboratory and PHU practices on District Health Board (DHB) notification data.

2.1 Disease rates over time

The incidence and prevalence of campylobacteriosis, salmonellosis, yersiniosis, listeriosis and VTEC/STEC infection are reported annually for New Zealand.[1] These data are collated from EpiSurv, the New Zealand notifiable disease data base.[3]

Table 1 summarises the number of cases and case rates for the years 2005 to 2009 for the five notifiable diseases being investigated in this study. The rate of campylobacteriosis decreased over this period (although recent data indicates rates are increasing since 2008), and the rate of VTEC/STEC infection increased.

[2] The acronym VTEC was derived from the pathological effect of the toxin on Vero cells in tissue culture and the acronym STEC was derived from the toxins being similar to those produced by *Shigella dysenteriae* (Chart, 2000). The acronyms are now used synonymously.
[3] EpiSurv is managed by ESR on behalf of the Ministry of Health.

Table 1: Number of cases and case rates for campylobacteriosis, listeriosis, salmonellosis, VTEC/STEC infection and yersiniosis (New Zealand, 2005-2009)[1]

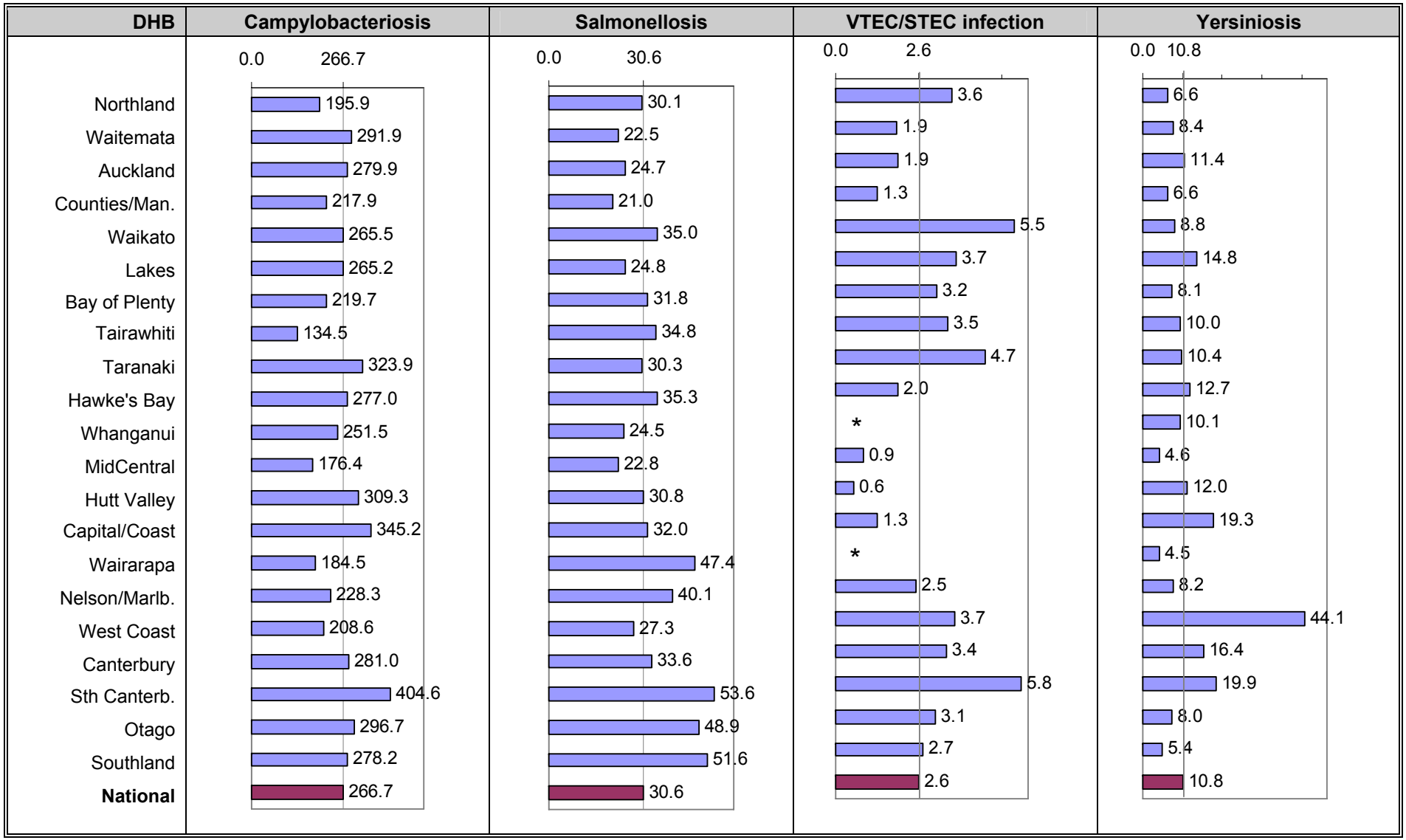
Year	Campylobac-teriosis		Listeriosis		Salmonellosis		VTEC/STEC infection		Yersiniosis	
	No. cases	Rate[2]	No. cases	Rate	No. cases	Rate	No. cases	Rate	No. cases	Rate
2005	13,836	334.8	20	0.5	1,382	33.4	92	2.2	383	9.3
2006	15,873	379.4	19	0.5	1,335	31.9	87	2.1	453	10.8
2007	12,778	302.2	26	0.6	1,275	30.1	100	2.4	502	11.9
2008	6,694	156.8	27	0.6	1,345	31.5	124	2.9	508	11.9
2009	7,176	166.3	28	0.6	1,129	26.2	143	3.3	431	10.0

[1] *Source: EpiSurv data as at February 2010.*
[2] *Rate: Annual rate of notified cases per 100,000 population.*

2.2 Geographical variation in disease rates

The five-year annualised rates for campylobacteriosis, salmonellosis, VTEC/STEC infection and yersiniosis are summarised in Figure 1 for each DHB. For listeriosis, 11 of the 21 DHBs had less than five cases reported over the five year period 2005 to 2009, therefore DHB rates have not been calculated.

Figure 1. Five year annualised rates (cases per 100,000 population) by DHB for four enteric diseases (New Zealand, 2005-2009)



** Rate not calculated as total cases for the period 2005-2009 was less than five.*

2.3 Changes to the notification process

Campylobacteriosis, listeriosis, salmonellosis and yersiniosis are all on the Ministry of Health list of notifiable diseases (Ministry of Health, 2009). Single cases of disease caused by verocytotoxic *E. coli* (VTEC/STEC infection) are included in the list of notifiable diseases as a footnote to the category "acute gastroenteritis" and are reported on a specific case report form.

Prior to December 2007 only clinicians were required to report notifiable diseases to the local Medical Officer of Health. From 21 December 2007 both laboratories and clinicians have been required to report notifiable diseases under the Health Act 1956.

In December 2007 the Ministry of Health published laboratory notification guidelines to support the changes to the health legislation (Ministry of Health, 2007). Laboratories are required to notify their local Medical Officer of Health of the isolation and identification from humans of any *Campylobacter* species, any *Salmonella* species, *Listeria monocytogenes*, and isolation of *Yersinia enterocolitica* or *Yersinia pseudotuberculosis*. Laboratories must also notify the Medical Officer of Health at a PHU of the isolation of presumptive VTEC/STEC, as indicated by the detection of Shiga toxin from faeces or Stx1 and/or Stx2 genes, or the isolation of sorbitol-negative *E. coli*, *E. coli* O157 or enterohemolysin producing *E. coli* (Ministry of Health, 1998, 2007).

Prior to December 2007, clinicians and laboratories notified PHUs primarily by telephone or fax. From December 2008 the laboratories have been able to report disease notifications electronically via EpiSurv using electronic messaging standards (based on HL7 messages) developed for this purpose. All ESR laboratory results are now reported using electronic messaging and a number of local laboratories are also using this method.

3 METHOD

3.1 Laboratory practices

Objective one was investigated using five surveys, one for each of *Campylobacter* spp., *Listeria* spp., *Salmonella* spp., STEC/VTEC and *Yersinia* spp. The *Yersinia* survey was developed by ESR prior to this project for another ESR project, piloted with one clinical laboratory and ESR laboratory staff, and implemented using the web-based survey supplier SurveyGizmo (http://www.surveygizmo.com). All other laboratory surveys were designed by ESR staff with input from NZFSA, based on a modification of the *Yersinia* survey, and administered via the internet using SurveyGizmo. The questions for the laboratory surveys are included in Appendix 1.

All clinical laboratories who had referred isolates to ESR during the extended *Yersinia* biotyping project in 2008/2009 (Pirie and Williman, 2008) and all medical microbiology laboratories identified from the International Accreditation New Zealand (IANZ) website were invited to participate in the surveys (a total of 36 laboratories; Appendix 2).

The laboratories completed the *Yersinia* survey in February 2010 as part of the ESR *Yersinia* Capability Fund project (to be reported 2010/11). The results from this survey were made available for incorporation into this study.

The laboratories had approximately three weeks to complete each of the other four surveys during April – June 2010. Non-respondents were followed up by e-mail and telephone.

In 2006 ESR surveyed 35 community and hospital laboratories on their methods as part of a larger study on Acute Gastrointestinal Illness (AGI) (King *et al*., 2007). The results of the AGI survey are compared with these 2010 results where similar questions were asked.

The results and discussion are presented in Section 4. Detailed results from the survey are available in appendices 5-9.

3.2 Public Health Unit practices

Objective two was investigated using four surveys, one for each of campylobacteriosis, listeriosis, salmonellosis and STEC/VTEC infection. The listeriosis survey focussed on *L. monocytogenes* because laboratories are only required to notify PHUs of infections of this *Listeria* species (Ministry of Health, 2007).

The surveys were designed by ESR staff and administered via the internet using the web-based survey supplier SurveyGizmo (http://www.surveygizmo.com). The questions for the PHU surveys are included in Appendix 3.

A list of New Zealand's PHUs is presented in Appendix 4. There are 12 regional PHUs that cover the 20 DHBs in New Zealand. There are 20 PHU offices.

All 20 PHU offices were contacted by telephone or e-mail. Each PHU office was invited to participate in the survey and asked to name a key contact. It was common for PHUs that had

more than one office to nominate one contact if the offices shared the same policies for investigating gastrointestinal illness.

The laboratories had approximately three weeks to complete each of the four surveys during April – June 2010. Non-respondents were followed up by e-mail and telephone.

Yersiniosis was investigated in an earlier survey of the Medical Officers of Health (Pirie *et al.*, 2008). This survey was sent to each public health office with a Medical Officer of Health (17 participants). The results of this survey are presented where similar questions were asked.

The results and discussion are presented in Section 5. Additional results from the survey are available in Appendix 10.

3.3 The influence of laboratory and PHU practices on DHB VTEC/STEC infection notification data

The possible influences that laboratory and PHU practices have on DHB notification rates for VTEC/STEC infection were examined by combining the results of the laboratory and PHU surveys with VTEC/STEC notification data from EpiSurv for the period 2005 to 2009.

The practices of laboratories and PHUs providing services to the three DHBs with the highest notification rates for VTEC/STEC infection were compared with the practices of laboratories and PHUs providing services to the three DHBs with the lowest notification rates for VTEC/STEC infection.

The results and discussion are presented in Section 6.

4 LABORATORY SURVEY: RESULTS AND DISCUSSION

This section provides a high-level summary of the results from the five surveys with some explanation, followed by a discussion. The full results from each survey are available in the appendices:

- Appendix 5: *Campylobacter* survey;
- Appendix 6: *Listeria* survey;
- Appendix 7: *Salmonella* survey;
- Appendix 8: VTEC/STEC survey;
- Appendix 9: *Yersinia* survey.

4.1 Participating laboratories

The response rates for the five laboratory surveys were as follows:

- 32/36 (89%) for the *Campylobacter* survey;
- 29/36 (81%) for the *Listeria* survey;
- 34/36 (94%) for the *Salmonella* survey;
- 33/36 (92%) for the VTEC/STEC survey;
- 36/36 (100%) for the *Yersinia* survey.

All of the laboratories participated in at least three surveys.

These laboratories provided services across all New Zealand's DHBs (Table 2). Please note that individual laboratories may provide services for more than DHB.

Table 2: Number of laboratories providing testing services for each District Health Board (DHB), by pathogen[1]

DHB	No. laboratories providing testing services for:				
	Campylobacter spp.	*Listeria* spp.	*Salmonella* spp.	VTEC/STEC	*Yersinia* spp.
Northland	4	4	4	4	4
Waitemata	3	3	2	2	3
Auckland	3	3	2	2	3
Counties Manukau	3	3	2	2	3
Waikato	3	1	4	4	5
Lakes	1	1	2	1	1
Bay of Plenty	2	2	2	2	3
Tairawhiti	1	1	1	1	1
Taranaki	2	1	2	2	2
Hawke's Bay	2	2	2	2	2
Whanganui	1	1	1	1	1
MidCentral	1	1	1	1	1
Hutt Valley	2	2	2	2	2
Capital and Coast	2	2	2	2	2
Wairarapa	1	1	2	1	1
Nelson Marlborough	1	1	1	2	1
West Coast	1	1	1	2	1
Canterbury	3	3	2	2	3
South Canterbury	2	2	2	2	2
Otago	2	2	2	2	2
Southland	1	1	1	1	1

[1] *Number of laboratories is based on those that responded to each survey.*

4.2 Testing criteria

All of the laboratories tested for *Campylobacter* spp. and *Salmonella* spp. as part of a standard faecal screen. Almost all of the laboratories (35/36) also tested for *Yersinia* spp. as part of a standard faecal screen.

Criteria were applied by 22/33 laboratories to decide if a faecal sample should be tested for VTEC/STEC. The most common criteria listed by these laboratories were the presence of blood in the specimen, indicative clinical details (e.g. history of bloody diarrhoea or haemolytic-uremic syndrome) and the age of the patient.

Faecal samples are not usually tested for *Listeria* spp.; laboratories usually test other sample types (e.g. blood) for this pathogen. Eight laboratories either did not test faecal samples for *Listeria* spp. or referred such samples to other laboratories for testing. Criteria were applied by 21/29 laboratories to decide if a faecal sample should be tested for *Listeria* spp. and the majority of these laboratories would only test a faecal sample for *Listeria* spp. upon request.

4.3 Isolation methods

The majority of laboratories were using isolation methods that have been in place prior to 2006:

- 25/32 for isolation of *Campylobacter* spp. (2 did not know);
- 11/19 for isolation of *Listeria* spp. (3 did not know);[4]
- 30/34 for isolation of *Salmonella* spp. (2 did not know);
- 23/33 for isolation of VTEC/STEC (3 did not know);
- 27/36 for isolation of *Yersinia* spp. (6 did not know).

4.3.1 *Campylobacter* spp.

There were five laboratories that began using their reported isolation methods from 2006 onwards. The methods used by four of these laboratories were the same as the laboratories that began using their isolation methods prior to 2006. The fifth laboratory used an enzyme immunoassay. This assay would give faster confirmation of *Campylobacter* spp. than the more conventional methods.

4.3.2 *Listeria* spp.

There were five laboratories that began using their reported isolation methods from 2006 onwards. All five laboratories directly inoculated either Oxford *Listeria* agar or PALCAM agar.[5] One laboratory additionally inoculated two blood agar plates, one of which was kept at 4°C for five days. *Listeria* enrichment broth cultures were also set up by 2/5 laboratories, one of whom subcultured to PALCAM and Aztreonam agars and the other subcultured to PALCAM agar and fraser broth. Other than the low temperature blood plate (*Listeria* spp. are able to grow at 4°C), these methods were similar to the laboratories that began using their methods prior to 2006.

4.3.3 *Salmonella* spp.

There were two laboratories that began using their reported isolation methods from 2006 onwards. Only one of these two laboratories reported a method that differed to methods reported by all of the other laboratories. This laboratory directly inoculated the same agars (xylose-lysine-deoxycholate, hektoen) and the same broth (selenite) as the other laboratories, but the broth was also subcultured to a chromogenic Salmonella agar.

4.3.4 VTEC/STEC

There were seven laboratories that began using their reported isolation methods from 2006 onwards. The methods used by six of these laboratories were the same as the laboratories that began using their isolation methods prior to 2006. The seventh laboratory referred all samples to ESR for testing.

[4] 29 laboratories submitted responses to the *Listeria* survey. The results presented exclude 10 laboratories that did not test faecal samples for *Listeria*, referred such samples to other laboratories, or provided methods for blood or swab samples.

[5] PALCAM, polymyxin acriflavine lithium-chloride ceftazidime aesculin mannitol.

No laboratories used molecular testing (PCR) to detect VTEC/STEC. Two laboratories use immunoassay toxin kits which would confirm toxin production of any VTEC/STEC strain, one is trialling a kit and one is considering adopting the use of a kit for isolates from children.

4.3.5 *Yersinia* spp.

There were three laboratories that began using their reported isolation methods from 2006 onwards. The methods used by these three laboratories are very similar to those laboratories that began using their isolation methods prior to 2006.

In this study 27/36 laboratories reported an incubation temperature of 28°C compared with 7/29 laboratories reporting an incubation temperature of 25-28°C in the AGI study). Enrichment broths were incubated at 37°C in all cases. *Yersinia* spp. are able to grow at lower temperatures and these conditions help to inhibit the growth of other organisms on agar plates, particularly after subculture from enrichment broths.

4.4 Identification methods

The numbers of laboratories that were using identification methods that have been in place prior to 2006 were:

- 22/32 for isolation of *Campylobacter* spp. (2 did not know);
- 15/26 for isolation of *Listeria* spp. (5 did not know);[6]
- 19/34 for isolation of *Salmonella* spp. (2 did not know);
- 15/33 for isolation of VTEC/STEC (4 did not know, 2 did not answer);
- 21/36 for isolation of *Yersinia* spp. (8 did not know).

4.4.1 *Campylobacter* spp.

There were eight laboratories that began using their reported identification methods from 2006 onwards. All of the methods used by seven of these laboratories were also used by laboratories that began using their identification methods prior to 2006. The eighth laboratory did not report any identification methods as this laboratory used an enzyme immunoassay test directly on the faecal sample (see Section 4.3.1).

4.4.2 *Listeria* spp.

There were six laboratories that began using their reported identification methods from 2006 onwards. All of the methods used by these six laboratories were also used by laboratories that began using their identification methods prior to 2006.

4.4.3 *Salmonella* spp.

There were 13 laboratories that began using their reported identification methods from 2006 onwards. The methods used by 12 of these laboratories were also used by laboratories that began using their identification methods prior to 2006. The thirteenth laboratory reported

[6] 29 laboratories submitted responses to the *Listeria* survey. The results presented exclude three laboratories that did not provide information on identification methods.

that they screened isolates using a lactose test and used an API10S biochemical panel for identification (method in place since 2006).

4.4.4 VTEC/STEC

There were 12 laboratories that began using their reported identification methods from 2006 onwards. One of these 12 laboratories forwarded suspect isolates to ESR so did not report any identification methods. All of the methods used by the remaining 11 laboratories were also reported by laboratories that began using their identification methods prior to 2006, except for a citrate screening test that was used by one laboratory (method in place since 2007).

It is possible that there has been an increase in the use of latex agglutination and biochemical tests; 8/11 laboratories that began using their identification methods from 2006 used latex tests and 10/11 laboratories in the same group used biochemical tests. However, the survey question did not request the laboratories to report what was in place prior to this time period.

4.4.5 *Yersinia* spp.

There were seven laboratories that began using their reported identification methods from 2006 onwards. All of the methods used by these laboratories were also used by laboratories that began using their identification methods prior to 2006.

4.5 Referral to the ERL

All of the laboratories referred *Salmonella* isolates to the ERL, 32/33 laboratories referred VTEC/STEC isolates and 28/29 laboratories referred *Listeria* isolates. *Yersinia* isolates were referred to the ERL by 23/36 laboratories; the remaining 13 laboratories had criteria for referring isolates (when requested by ESR, for confirmation of unclear results, for confirmation of *Y. pestis*, or for invasive isolates from blood). The Ministry of Health guidelines on Direct Laboratory Notification of Communicable Diseases (Ministry of Health, 2007) do not require laboratories to refer *Campylobacter* isolates to the ERL. Serotyping and PFGE is undertaken in some campylobacteriosis outbreak situations at the request or approval of the Ministry of Health. A list of tests available from ESR Infectious Disease Laboratories can be viewed at http://www.esr.cri.nz/SiteCollectionDocuments/ESR/PDF/CommunicableDisease/ListoftestsAugust09.pdf.

4.6 Notification guidelines

All of the laboratories reported that they followed the Ministry of Health guidelines on Direct Laboratory Notification of Communicable Diseases (Ministry of Health, 2007) except for one laboratory in the VTEC/STEC survey and two laboratories in the *Salmonella*, *Listeria* and *Campylobacter* surveys, which all reported 'unknown'.

4.7 Reporting to Public Health Surveillance Units

Most of the laboratories had facilities to report results to PHUs by direct electronic transfer from the laboratory management systems, and some laboratories used more than one method (e.g. phone, fax):

- *Campylobacter* spp.: 23/32 reported results by electronic transfer and 7 laboratories reported results using more than one method.
- *Listeria* spp.: 20/29 reported results by electronic transfer and 5 laboratories reported results using more than one method.
- *Salmonella* spp.: 23/34 reported results by electronic transfer and 8 laboratories reported results using more than one method.
- VTEC/STEC: 21/33 reported results by electronic transfer and 16 laboratories reported results using more than one method.

This question was not asked in the *Yersinia* survey but it is likely that the results would have been similar to the other surveys.

4.8 Discussion

From the available results there does not appear to be any significant change in the isolation and identification methods used by laboratories since 2006.

4.8.1 *Campylobacter* spp.

All methods currently being used are suitable for the isolation of *Campylobacter jejuni* and *Campylobacter coli.* However, other *Campylobacter* species (e.g. *C. upsaliensis* and *C. concisus*) are increasingly being recognised as important causes of human gastrointestinal disease and there is evidence that some of these methods might inhibit non-*jejuni/coli Campylobacter* species (Acke *et al.*, 2009; Byrne *et al.*, 2001). Non-*jejuni/coli* species can be recovered by using a filtration technique (López *et al.*, 1998) but no laboratories reported using this method.

Additionally, laboratories that incubate isolates at 42°C may have a higher isolation rate of *C. jejuni* and *C. coli* than those incubating at 35-37°C. These species are thermotolerant and the higher temperature can inhibit other competing microflora. However, the higher temperature may also inhibit other species of *Campylobacter*.

Eight laboratories speciate isolates based on the Hippurate test. This is a standard test to confirm a *Campylobacter* isolate as *C. jejuni*. However, the ERL has observed an increase in the number of Hippurate-negative strains of *C. jejuni* being isolated, particularly in the Auckland area. The ERL can identify several Hippurate-negative *Campylobacter* species by PCR: *C. coli*, *C. fetus*, *C. lari*, *C. hyointestinalis* and *C. upsaliensis* as well as Hippurate-positive and Hippurate-negative strains of *C. jejuni*. Laboratories are not required to routinely send *Campylobacter* isolates to the ERL.

4.8.2 *Listeria* spp.

Specimens from sterile sites (e.g. blood, cerebral spinal fluid, amniotic fluid) are more commonly used than faecal specimens for the isolation of *Listeria* spp. The methods used in New Zealand for the isolation of *Listeria* spp. from faeces follow methods recommended by the US Department of Agriculture and The Netherlands Government Food Inspection Service (Bille, 2007). The methods used are recognised as being robust by these agencies.

The laboratories refer all *Listeria* isolates to the ERL for further subtyping and the ERL enters the results into EpiSurv. Consequently, all laboratory-confirmed listeriosis cases are captured accurately.

4.8.3 *Salmonella* spp.

All of the methods used by the laboratories would identify *Salmonella* spp. and all of the laboratories can distinguish by one or more methods the serotypes *S*. Typhi and *S*. Paratyphi A. All laboratories refer *Salmonella* isolates to the ERL for confirmation of *Salmonella* spp. and serotype identification. The ERL also does additional testing to identify *S*. Paratyphi B, *S.* Paratyphi B var Java and *S.* Paratyphi C.

The WHO Global Foodbourne Infections Network (formerly WHO GLOBAL Salm-Surv) has recently published a Laboratory Protocol for the biochemical identification of salmonellae (Mikoleit, 2010). The algorithm in this document would be a valuable addition to the current algorithm in the Ministry of Health guidelines for notification of infectious diseases (Ministry of Health, 2007).

4.8.4 VTEC/STEC

All of the methods used by the laboratories would identify *E. coli* O157. Laboratories that only do biochemical testing refer isolates to their central laboratories for confirmation using latex agglutination, and one laboratory refers the isolate directly to ESR for confirmation.

Only two laboratories test for non-O157 VTEC/STEC using an immunoassay kit, which detects production of Shiga toxin 1 and 2. If the isolate does not react with O157 latex the isolate is referred to ERL for further serotyping.

One laboratory refers sorbitol-negative, O157 latex-negative isolates as well as a mixed sweep from the primary plate to ESR to test for verocytotoxin production. It should be noted that the majority of non-O157 isolates are sorbitol positive. The ERL has isolated toxin-producing strains from these mixed sweeps which have been of clinical significance.

Most of the laboratories reported that they applied criteria to decide if a faecal sample should be tested for VTEC/STEC. The criteria were fairly similar but not consistent across the laboratories. The selection of samples for VTEC/STEC testing might influence detection rates.

One laboratory is considering testing all samples from children (age not specified) for Shiga toxin 1 and Shiga toxin 2 using an immunoassay kit. Another laboratory is to begin routinely analysing all faecal samples for VTEC/STEC on the recommendation of a recent report (Gould *et al.*, 2009).

4.8.5 *Yersinia* spp.

More laboratories are incubating CIN agar plates at the recommended temperature range of 25-30°C, though it appears that this has not lead to any noticeable change in the yersiniosis notification rate. One laboratory was using MacConkey agar to isolate *Yersinia* spp., the others used CIN agar which helps recognition of *Yersinia* colonies and is more inhibitive for other organisms. The biochemical testing in use can identify *Yersinia* spp.

Preliminary results from the *Yersinia* Capability Fund Project indicate that *Y. pseudotuberculosis* is inhibited by pre-enrichment selenite broth. The study results also indicate that an incubation time of 48 hours is required to detect *Y. psuedotuberculosis* on CIN agar, whether or not pre-enrichment was used. This could explain the apparently low incidence of *Y. pseudotuberculosis* in New Zealand. The full results of this study and recommendations on laboratory methodology will be reported in spring 2010.

Laboratories are required to notify PHUs of the isolation of *Y. enterocolitica* and *Y. pseudotuberculosis*; 30/36 laboratories reported they could identify *Y. enterocolitica* and 21/36 laboratories reported they could identify *Y. pseudotuberculosis*. The laboratories that could not identify these species sent *Yersinia* isolates to a laboratory that did have this capability or to the ERL. *Y. enterocolitica* isolates received by ERL are biotyped to identify potential pathogenic strains. The ERL identifies other *Yersinia* spp. using phenotypic methods.

4.8.6 Overall comment

The isolation methods used in New Zealand laboratories are largely comparable to conventional methods used elsewhere (O'Brien *et al.*, 2010) and the available information indicates that there has been very little change in methods in the last five years.

With sufficient resources, the isolation of *Campylobacter* spp., *Yersinia* spp. and VTEC/STEC might be improved.

The *Campylobacter* isolation methods favour *C. jejuni* and *C. coli* and the few laboratories that speciate *Campylobacter* isolates focus on identifying *C. jejuni*. Laboratories are only required to notify isolation of *Campylobacter* spp. and are not required to send isolates to the ERL. While the ERL is able to identify other *Campylobacter* species, any isolates sent to the ERL by laboratories are the output of these biased isolation methods. However, any modification of the methods used by laboratories needs to be managed carefully, as this may impact campylobacteriosis notification rates.

Seven laboratories use an enrichment broth for isolation of *Yersinia* spp., of which two reported using a *Yersinia*-specific broth. These two laboratories service the West Coast and Canterbury DHBs; the West Coast DHB had the highest annualised yersiniosis notification rate in New Zealand (44.1/100,000, see Figure 1) and Canterbury DHB the fourth highest (16.4/100,000, Figure 1). *Yersinia* spp. can be out-competed by other microflora present in samples and, while would be expected that a sample from a yersiniosis case would yield a high concentration of *Yersinia* spp., an additional enrichment step with a *Yersinia*-specific broth alongside direct plating might increase detection. However, the preliminary results

from the *Yersinia* Capability Fund Project indicate that selenite broth enrichment may inhibit some *Yersinia* spp.

There appeared to be widespread use of criteria for deciding whether a sample should be tested for VTEC/STEC and the responses indicated that these criteria were similar, but not the same, across laboratories. Encouraging standardisation of the criteria would improve consistency between the laboratories that apply this protocol, but there were some laboratories which indicated that VTEC/STEC testing is a part of a standard faecal screen so inter-laboratory differences will not be resolved. If criteria are to remain in place, there may be some benefit in evaluating the effectiveness of these criteria by, for example, enhancing VTEC/STEC testing and determining the proportion of positive samples that would not have been tested under a reference set of criteria.

It was also evident from laboratory respondents' comments that there was an increased awareness of the clinical importance of non-O157 VTEC/STEC, but laboratories did not have consistent protocols and procedures in place for these organisms. A recent MMWR report (Gould *et al.*, 2009) recommends that:

- All faecal samples should be tested routinely for VTEC/STEC;
- Immunoassay testing to detect Shiga toxin production is followed by confirmatory serology for O157;
- Toxin-producing strains which are O157-negative should be referred for further serotyping.

Currently there are no national testing protocols for the isolation of non-O157 VTEC/STEC in New Zealand. The Ministry of Health guidelines (Ministry of Health, 2007) could be extended to include additional criteria for testing isolates and the existing flowchart could be amended to encourage notification of presumptive O157 or non-O157 *E. coli*.

5 PHU SURVEY: RESULTS AND DISCUSSION

This section summarises results from the four PHU surveys and, where relevant, the separate yersiniosis survey (Pirie *et al.*, 2008). Additional results are available in Appendix 10.

5.1 Participating PHUs

All 17 Medical Officers of Health responded to the yersiniosis survey.

Ten of the 12 regional PHUs nominated one key contact to respond to the other four surveys. The responses from each of these ten PHUs represent all of the offices within each PHU (where there is more than one office) and all of the DHBs they provide services to (where they cover more than one DHB). The remaining two PHUs provided separate responses for each DHB they provided services to. This brought the total respondents to 15 (Table 3).

Table 3: DHBs covered by each PHU/PHU office responding to the four surveys

Participating PHU/PHU office	DHBs covered by PHU/PHU office
Northland Primary and Community Health Services	Northland
Auckland Regional Public Health Services	Waitemata, Auckland, Counties Manukau
Waikato Public Health Unit	Waikato
Toi Te Ora Public Health Unit	Bay of Plenty, Lakes
Tairawhiti Public Health Unit	Gisborne/Tairawhiti
Taranaki Public Health Unit	Taranaki
Hawke's Bay Public Health Unit	Hawke's Bay, Chatham Islands
MidCentral Public Health Unit: Palmerston North office	MidCentral
MidCentral Public Health Unit: Wanganui office	Whanganui
Hutt Valley Regional Public Health	Capital and Coast, Wairarapa, Hutt Valley
Nelson Public Health Unit	Nelson Marlborough
Community and Public Health: Greymouth office	West Coast
Community and Public Health: Christchurch office	Canterbury
Community and Public Health: Timaru office	South Canterbury
Public Health South	Otago, Southland

The response rates for the four PHU surveys were as follows:

- 15/15 (100%) for the campylobacteriosis survey;
- 14/15 (93%) for the listeriosis survey;
- 15/15 (100%) for the salmonellosis survey;
- 15/15 (100%) for the VTEC/STEC infection survey.

The listeriosis survey results do not include information of PHU activities in the MidCentral DHB.

5.2 Notifications

5.2.1 Sources and methods of notification

PHUs receive notifications from the ERL, General Practitioners (GPs), hospital clinicians and local laboratories. These sources use varied methods to communicate notifications of campylobacteriosis, *L. monocytogenes* infection, salmonellosis and VTEC/STEC infection to the PHUs:

- ERL: Most of the PHUs received notifications by electronic notification. Notifications of VTEC/STEC infection were also commonly received by a posted hard copy. Telephoned notifications were rarely received from the ERL.
- GPs: The PHUs mostly received notifications from GPs by phone calls and faxed hard copies. The use of electronic notification was rare.
- Hospital clinicians: The PHUs most commonly received telephoned notifications from hospital clinicians. Electronic notifications and posted hard copies were rarely received.
- Local laboratories: The PHUs most often received notifications from local laboratories by faxed hard copies; the other communication methods were used, but less often.

See Appendix 10 for detailed results.

The PHUs reported other sources from which they receive notifications:

- Other PHUs (usually as a transferred case in EpiSurv);
- Patients (self-reported notifications);
- Infection control nurses.

At the time of the yersiniosis survey in 2007, 8/17 PHUs received notifications directly from laboratories.

5.2.2 Laboratory notification of isolated pathogens to PHUs

All respondents reported that laboratories notified them of all isolations of *Campylobacter* spp., *Listeria* spp., *Salmonella* spp. and VTEC/STEC, without consideration of the clinical illness of patients submitting the samples for analysis.

5.2.3 Notifications entered into EpiSurv

All respondents reported that they entered all notified cases of campylobacteriosis, *L. monocytogenes* infection, salmonellosis and VTEC/STEC infection into EpiSurv, with one exception. One respondent reported that patients identified with *L. monocytogenes* infection were recorded in EpiSurv only if they had a clinically compatible illness.

In the yersiniosis survey in 2007, 15/17 respondents reported entering all yersiniosis notifications into EpiSurv. Of the remaining two PHUs, one only entered cases that met the case definition, and the other only entered *Y. enterocolitica* and *Y. pseudotuberculosis*.

The PHUs were also specifically asked if the species of *Campylobacter* was identified in campylobacter notifications and whether the PHU enters the *Campylobacter* species into EpiSurv. Eight respondents did not receive *Campylobacter* species information with the

notification and one respondent did not know whether species information was received. Of the six respondents who did receive *Campylobacter* species information, one always received species data and five sometimes received notifications with species. One respondent always entered the information into EpiSurv, four sometimes entered this data and one never entered species data into EpiSurv.

The participants in the yersiniosis survey were also asked about the *Yersinia* species information they received. One PHU always received *Yersinia* species information, 10/17 sometimes received species information and 4/17 never received species information. Two PHUs did not know or did not answer the question. Species information was received from local laboratories, GPs and the ERL. Four PHUs entered all *Yersinia* species results into EpiSurv. The policy for the remainder of PHUs varied; some only entered the "unusual species i.e. the non-*Y. enterocolitica* species".

5.3 Investigation and follow up of notified cases

5.3.1 Investigation of cases

Respondents to the survey were asked to indicate whether PHU staff investigated cases. The responses to this question are summarised in Table 4. All cases of O157 VTEC/STEC infection or typhoidal salmonellosis were reported as being investigated. All cases with *L. monocytogenes* infection were also investigated, though one respondent was unsure of their policy as they had not encountered any listeriosis cases in recent years. There were varied PHU investigation practices for investigating cases of campylobacteriosis, non-typhoidal salmonellosis and non-O157 VTEC/STEC infection. PHUs that only investigated some cases of campylobacteriosis focussed on investigating cases in high risk occupations such as food handlers or health care workers. PHUs that only investigated some cases of non-typhoidal salmonellosis focussed on patients in high risk groups, e.g. children aged less than five years. PHUs that only investigated some cases of non-O157 VTEC/STEC infection often did so because they had initiated an investigation before the laboratory typing was completed. Further details are in Appendix 10.

Table 4: Summary of case investigation practices[1]

Disease notification		Cases investigated (No. of PHU respondents)			
		All cases	Some cases[2]	No cases	Unsure
Campylobacteriosis		8	5	2	0
L. monocytogenes infection	Perinatal	13	0	0	1
	Non perinatal	13	0	0	1
Salmonellosis	Typhoidal	15	0	0	0
	Non-typhoidal	13	2	0	0
VTEC/STEC infection	O157	15	0	0	0
	Non-O157	9	4	2	0

[1] *The interpretation of "investigation" may have varied by respondent. See Section 5.5.2.*
[2] *The reported criteria for which cases are investigated are listed in Appendix 10.*

In the yersiniosis survey, 16/17 PHUs would investigate all yersiniosis cases that are notified; the remaining PHU would only investigate a geographic cluster of cases or when two or more cases are potentially linked to a common source.

5.3.2 Investigation of asymptomatic cases

The PHUs were asked if they investigated asymptomatic cases. A summary of their responses is shown in Table 5. Almost all respondents investigated asymptomatic cases of VTEC/STEC infection; one respondent reported that asymptomatic cases were investigated if laboratory results reported markers of pathogenicity. A range of responses were recorded for the other pathogens and the PHUs listed criteria for deciding which cases would be investigated (see Appendix 10).

Table 5. Summary of asymptomatic cases investigated by respondents and pathogen

Disease notification	Cases investigated (No. of PHU respondents)			
	All cases	Some cases[1]	No cases	Unsure
Campylobacteriosis	6	4	5	0
L. monocytogenes infection	9	2	0	3
Salmonellosis	10	5	0	0
VTEC/STEC infection	13	1	0	1

[1] *The criteria used by respondents in their decision to investigate selected asymptomatic cases are listed in Appendix 10.*

5.3.3 Percentage of notifications that are investigated

The PHUs were asked to estimate the percentage of notifications that were investigated.

Twelve of the 14 respondents reported that their PHU investigated *Campylobacter* notifications. Of these 12 respondents, seven investigated all *Campylobacter* notifications with the remaining five reporting an estimate of 99%, 90%, 70-80%, 34% and 2 % of *Campylobacter* notifications investigated.

All (14) respondents reported investigating all *L. monocytogenes* notifications

Fourteen of the respondents reported investigating all *Salmonella* notifications with the only exceptions being cases that could not be contacted. One respondent reported investigating 19% of notified *Salmonella* cases in 2009.

All respondents reported investigating all VTEC/STEC notifications with some exceptions where cases could not be contacted and cases were denotified.

5.3.4 Commencing investigation of notified cases

All respondents who investigated notified cases reported beginning their investigation when the notification was received, with the following exceptions:

- One respondent indicated that investigation of notified cases of campylobacteriosis began after the GP notification was received.
- Two respondents indicated that investigation of VTEC/STEC cases started after confirmation of VTEC/STEC infection was received (one of these did not record receipt of VTEC/STEC notifications from their local laboratory but received notifications through other sources).

5.3.5 Method(s) used to investigate cases

The methods of investigation that were reported most frequently by respondents were "phone call to case" and "visit to case". A number of respondents also indicated that their PHUs used a posted questionnaire (Table 6). The staff most often involved in investigations were Health Protection Officers (HPOs) (Table 7).

Table 6. Method of investigation reported by respondents for different pathogens

Cases investigated by[1]	Campylobac-teriosis	*L. mono-cytogenes* infection	Salmonellosis	VTEC/STEC infection
Phone call to case	12	11	14	13
Visit to case	7	12	13	14
Questionnaire posted out	8	1	5	0
Letter to case	1	0	0	0
Case report form faxed to notifier	0	1	0	0

[1] *Respondents could select more than one method.*

Table 7. Staff involved in investigation of disease cases by pathogen

Cases investigated by[1]	Campylobac-teriosis	*L. mono-cytogenes* infection	Salmonellosis	VTEC/STEC infection
Health Protection Officer (HPO)	12	14	13	12
Environmental Health Officer	3	0	6	0
Medical Officer of Health	0	4	1	3
Designated officer (non-HPO)/technical officer	0	1	1	1
Communicable Disease Nurse	0	0	0	1

[1] *Respondents could select more than one type of worker.*

In the yersiniosis survey, 12/17 PHUs reported that HPOs undertake investigations of yersiniosis cases. Environmental Health Officers are involved in five PHUs and EpiSurv Coordinators were responsible for investigation in two PHUs. Fourteen PHUs make a phone call to the case as part of the investigation. Six PHUs post out questionnaires and two administer the questionnaires in person.

5.3.6 Follow-up of postal questionnaires

Of the respondents who reported that their PHU used postal questionnaires to investigate cases, only some followed up questionnaires that were not returned:

- Campylobacteriosis: Two respondents reported that their PHUs followed up postal questionnaires that were not returned, and five respondents reported that their PHU did not follow up non-returned postal questionnaires.

- *L. monocytogenes* infection: The one PHU using a postal questionnaire followed up non-returned questionnaires.
- Salmonellosis: Three respondents reported that their PHUs followed up postal questionnaires that were not returned, and two respondents reported that their PHU did not follow up non-returned postal questionnaires.

The PHUs using postal questionnaires to investigate cases were asked to estimate a response rate. The estimated response rates ranged between 10% and 93%, where estimates were provided:

- Campylobacteriosis: Where non-returned postal questionnaires were followed up (2 PHUs), the estimates were 10% and 93%. Where postal questionnaires were not followed up (5 PHUs), the estimates were around 50% (2 PHUs), 60-70% (2 PHUs) and 80%.
- Salmonellosis: Where non-returned postal questionnaires were followed up (3 PHUs), the estimates were 60%, 80% and 90%. Both PHUs reporting that postal questionnaires were not followed up estimated 60%.

Participants in the yersiniosis survey estimated questionnaire return rates of between 57% and 95%.

5.3.7 Use of a specific questionnaire for investigations

For each disease, approximately half of respondents reported that their PHUs used a specific questionnaire for the investigation of cases (Table 8).[7]

Table 8. Use of specific questionnaire for investigation of cases by PHU staff

Questionnaire used	Campylobacteriosis	*L. monocytogenes* infection	Salmonellosis	VTEC/STEC infection
Specific questionnaire – developed by PHU	8	8	8	7
Specific questionnaire – ESR/EpiSurv case report form	2	6	2	4
No specific questionnaire	5	0	5	4

5.3.8 Criteria used in decision to investigate a potential outbreak

Respondents generally indicated that two or more linked cases would be followed up and that investigators would look for common factors amongst all cases investigated. Some respondents referred to the ESR Outbreak Response Manual guidance for when an outbreak should be investigated (Thornley & Baker, 2002):

- Severity of the disease and potential for spread (e.g. If there are hospitalisations or deaths);

[7] The questionnaire (or data collection form) may not necessarily be the same one posted out to patients as part of the investigation methods (Table 6); this distinction was not made in the survey.

- Estimated number of people affected;
- Characteristics of population affected by the outbreak (e.g. vulnerability to serious illness);
- Evidence as to whether illnesses attributable to the aetiologic agent are continuing, and the direction of any trend;
- Rarity of the situation (e.g. an unusual or previously unrecognised pathogen or mode of transmission), the investigation of which may help develop our understanding of the disease;
- Public health importance of the outbreak relative to other competing public health issues and activities;
- Availability of suitable personnel and financial resources;
- Timeliness of notification of illness - investigation weeks after the event is less likely to obtain reliable information;
- Public concern or media interest.

One respondent reported their decision to carry out an analytical epidemiological investigation of a possible salmonellosis outbreak depends on the level of public concern and whether the outbreak is ongoing, the source has been identified, a statistically significant result is likely, a law suit is likely and there are available staff and resources.

5.3.9 Additional comments

Further comments on the follow-up and investigation of notifications can be found in Appendix 10.

5.4 Recording of investigation details in EpiSurv

5.4.1 Recording of negative laboratory test results from ESR in EpiSurv

PHUs were asked how they recorded negative laboratory test results (i.e. no pathogen isolated) in EpiSurv.

Cases with *L. monocytogenes* infection: Eight respondents reported changing the case status to "not a case". Of the remaining respondents (6), two respondents commented that they had never received a negative *L. monocytogenes* report, one reported that "isolation" would be set to "no" under laboratory criteria, one does not record the results in EpiSurv, one was unsure what happened only received positive test results and one respondent did not answer the question.

Salmonellosis cases: Three respondents commented that they only received positive test results. Eight respondents reported changing the case status to "not a case" with one of these respondents also updating the laboratory criteria section. Four respondents reported that a discussion would be held to determine if the status should be changed taking into account other factors e.g. epidemiologic link to confirmed case and the changes made in EpiSurv (e.g. updating of status and laboratory criteria) would be based on that decision.

Cases with VTEC/STEC infection: Twelve responses were recorded for this question. Eleven respondents indicated that they would change the case status to "not a case". One respondent reported also updating the laboratory criteria section and comments section. One

respondent also mentioned assessing the clinical presentation of the case and other evidence before deciding to change the status to "not a case". One respondent reported that negative results were not recorded in EpiSurv.

This question was not asked in the campylobacteriosis survey.

5.4.2 Circumstances for de-notification of cases

PHUs were asked under what circumstances they would consider de-notification of cases. De-notification is when the case status in EpiSurv is changed to "not a case".

The reasons for de-notifying cases varied by disease. Most de-notifications for VTEC/STEC infection were related to negative laboratory results (PHUs can be notified of potential VTEC/STEC infection before confirmation results are available, see Section 2.3). The main reason for de-notifying cases of campylobacteriosis and *L. monocytogenes* infection was the case not meeting the case definition. Not meeting the clinical criteria was the main reason for salmonellosis de-notifications (Table 9).[8]

Table 9. Reasons for de-notifying cases of disease in EpiSurv

Reason for de-notifying case	Campylobac-teriosis	*L. mono-cytogenes* infection	Salmonellosis	VTEC/STEC infection
Case does not meet case definition	6	8	4	3
Negative laboratory results	5	5	5	9
Case does not meet clinical criteria	3	0	6	2
Other [1]	2	1	0	1

[1] *Reasons given were: Never had to deal with this situation (campylobacteriosis), case not tested (L. monocytogenes infection), ESR reports as non-O157 VTEC.*

In the yersiniosis survey, one PHU reported that they would de-notify a case of yersiniosis if they had recorded the notification without knowing the species of *Yersinia*, but later received results that indicated the case was not *Y. enterocolitica* or *Y. pseudotuberculosis*. They would update the EpiSurv case record to "not a case". Eight PHUs reported that they did not consider de-notifying cases of yersiniosis. Six PHUs would consider de-notification if the case did not meet clinical criteria or the laboratory results were negative and three PHUs would consider de-notification if the case did not meet the case definition.

5.4.3 Details of de-notified cases recorded in EpiSurv

The sections on the EpiSurv case report form most frequently updated with details of the reason for de-notifying a case were the comments field and the laboratory confirmation fields (Table 10).

[8] Technically, the case definition includes both laboratory results and clinical criteria (Ministry of Health, 1998).

Table 10. Where details of de-notification is recorded in EpiSurv

Action	Campylobac-teriosis	*L. mono-cytogenes* infection	Salmonellosis	VTEC/STEC infection
Record details in the comments section	11	12	10	9
Select "No" for laboratory confirmation	10	12	12	13
Select 'No' for fits clinical description	6	9	8	4
Other [1]	1	1	0	1

[1] *Reasons given were: No details recorded (campylobacteriosis, VTEC/STEC infection), no testing carried out recorded (L. monocytogenes infection).*

5.5 Discussion

There was over two years between the yersiniosis survey and the four PHU surveys conducted for this study. PHU practices around the management of yersiniosis cases may have changed since the yersiniosis survey so caution is advised when comparing results.

5.5.1 Notification

The responses from the PHUs indicate that approximately half of them now receive electronic notifications from local laboratories.

Test results including speciation are updated directly in EpiSurv by ESR for samples tested by ESR for *Salmonella*, *Yersinia* and VTEC/STEC,

Listeria test results are entered into EpiSurv by PHU staff.

Campylobacter species information is rarely recorded in EpiSurv, largely because laboratories do not routinely speciate isolates. This is expected since laboratories are only required to notify PHUs of isolation of *Campylobacter* spp. from a human case (they do not need to identify an isolate to species level), and are not required to send *Campylobacter* isolates to the ERL for speciation/typing.

In most cases the PHU commenced an investigation upon receipt of the notification. Regarding notifications of VTEC/STEC infection, one particular issue reported by survey participants was the delay in starting an investigation due to waiting for confirmation that the isolate was a toxigenic strain. This was exemplified in one PHU's response where the local laboratory did not notify the PHU of STEC/VTEC cases and the PHU could not commence investigation until confirmation was received from ESR. More guidance around when to start an investigation may be required. This could include guidance on triggers for the investigation of cases and their contacts based on presumptive laboratory results for VTEC/STEC, and information on the likelihood that a positive presumptive laboratory result will be confirmed as an O157 or non-O157 VTEC/STEC case. The flow charts in the Ministry of Health guidance for laboratories (Ministry of Health, 2007) could also be

amended to ensure that laboratories notify PHUs of presumptive VTEC/STEC isolates in the first instance, and not wait until isolate has been confirmed by ERL.

5.5.2 Investigation

All respondents reported that their PHUs investigated all cases of *L. monocytogenes* infection, VTEC/STEC infection and typhoidal salmonellosis.[9] All but one respondent in the yersiniosis survey reported that their PHUs investigated all cases of yersiniosis. The annual number of notified cases with these diseases is small compared to non-typhoidal salmonellosis and campylobacteriosis (Table 1).

The PHU activities towards investigating non-typhoidal salmonellosis and campylobacteriosis cases differed to the other diseases. Some PHUs did not investigate all cases of non-typhoidal salmonellosis, and two respondents reported that their PHUs did not investigate campylobacteriosis cases and five reported only investigating some cases. Additionally, while most investigations were conducted by an HPO personally contacting a case (phone call and visit), several PHUs also used postal questionnaires for investigating salmonellosis and campylobacteriosis cases. Not all PHUs using postal questionnaires for these diseases followed up non-returns. The estimated return rates of questionnaires were mostly between 50 and 90%. The relatively high number of campylobacteriosis and salmonellosis cases may be one factor that limits the capacity of a PHU to investigate all cases of these diseases.

It should be noted that the term "investigate" was not defined so was interpreted by some respondents as a passive process that included provision of advice only, and by others as an active investigation of sources and risk factors. However, the results described above, where rarer diseases are always investigated compared with non-typhoidal salmonellosis and campylobacteriosis, is some evidence that the respondents took a reasonably consistent approach to the survey questions. Despite these reservations about respondents' understanding of the term investigation, it is clear that PHUs have adopted disparate policies around the extent to which individual case notifications are actively managed. The reasons for this policy variation were not explored in the survey, but local priority-setting connected with the availability of resources are likely to be important.

This variability may be addressed by providing evidence-based guidance for PHUs on best practice for investigating and managing cases of commonly-notified enteric infectious diseases, such as campylobacteriosis and non-typhoidal salmonellosis. The guidance would need to consider the objectives of case investigation and management, the cost-effectiveness of different strategies (from national to local) for addressing these objectives, and the resources required in each of the PHUs for the recommended level of investigation.

While variation in PHU case investigation would not strongly impact notification rates, it does affect the quality of surveillance data. Data items such as some demographics (ethnicity, occupation) and risk exposures (environmental exposures, travel history, food histories) cannot be consistently collected in the absence of an interview with the case. This

[9] The respondent for one PHU was unsure of the approach taken to investigating listeriosis cases. This may be due to it being a rarer disease and some PHUs have not had any cases reported in recent years. Ministry of Health guidance (Ministry of Health, 1998) clearly recommends case investigation.

directly affects the extent to which surveillance data can be used to identify common sources of disease.

Not all cases of non-O157 were investigated; two PHUs did not investigate any cases. In the laboratory survey, only three laboratories reported that they tested for non-O157 VTEC/STEC. As discussed earlier (Section 4.8.6), it appears that some guidance around the testing and investigation of non-O157 VTEC/STEC would be valuable.

There was less focus on investigating asymptomatic cases for all diseases except VTEC/STEC infection. A number of respondents reported that they investigated asymptomatic infections of campylobacteriosis. This may be because the case definition for confirmed campylobacteriosis is worded as "a case that is laboratory confirmed" (Ministry of Health, 1998) and therefore does not specifically exclude those without clinical illness.

Approximately half of the respondents reported using a specific questionnaire for a particular disease. It may be helpful to review these questionnaires as part of a future project and make a standard questionnaire available to other PHUs from a centralised source.

5.5.3 Updating EpiSurv with results

For pathogens confirmed and subtyped by ESR (*Listeria* spp., VTEC/STEC, *Salmonella* spp., *Yersinia* spp.) results are automatically entered in EpiSurv. Interestingly, a number of respondents reported that they received *Campylobacter* results from ESR although ESR does not routinely test and report *Campylobacter* test results. When the ERL result differs from that reported by local clinical laboratories, the relevant PHU is responsible for deciding if, and what, changes should be made to the notification in EpiSurv. Details of the discussion and decision-making in the PHU, and the outcome, should be recorded in the comments section of the relevant case record as a minimum.

De-notification of cases directly affects notification rates. PHUs reported that they de-notified cases based on the case no longer meeting the case definition, a negative laboratory result or the absence of clinical criteria. More importantly, the approach to recording de-notifications was inconsistent between PHUs (Table 10). Some guidance on the decision-making and recording of de-notifications might address these inconsistencies and ensure that notification rates are only based on probable and confirmed cases meeting the case definition.

6 THE INFLUENCE OF LABORATORY AND PHU PRACTICES ON DHB VTEC/STEC INFECTION NOTIFICATION DATA: RESULTS AND DISCUSSION

6.1 VTEC/STEC notification rates

The highest VTEC/STEC notification rates in New Zealand for the period from 2005-2009 were reported from South Canterbury (16 cases, 5.8 cases per 100,000), Waikato (98, 5.5 cases per 100,000), and Taranaki DHBs (25 cases, 4.7 cases per 100,000). The lowest VTEC/STEC notification rates in New Zealand for the period from 2005-2009 were reported from the DHBs Hutt Valley (4 cases, 0.6 cases per 100,000 population) and MidCentral (7 cases, 0.9 cases per 100,000). In addition the Wanganui and Wairarapa DHBs reported insufficient VTEC/STEC cases (2 each) during the period to calculate rates (Table 11).

Table 11. VTEC/STEC notifications and hospitalisations as reported in EpiSurv (2005-2009)

DHB	Notifications of VTEC/STEC infection (2005-2009)		Hospitalised cases with VTEC/STEC infection (2005-2009)	
	No.[1]	Rate[2]	No.[1]	%[3]
Northland	28	3.6	9	32.1
Waitemata	49	1.9	21	55.3
Auckland	42	1.9	17	54.8
Counties Manukau	30	1.3	13	59.1
Waikato	98	5.5	18	19.8
Lakes	19	3.7	11	61.1
Bay of Plenty	32	3.2	12	37.5
Tairawhiti	8	3.5	2	50.0
Taranaki	25	4.7	10	41.7
Hawke's Bay	15	2.0	2	13.3
Whanganui	2	n/c	0	n/a
MidCentral	7	0.9	1	14.3
Hutt Valley	4	0.6	0	n/a
Capital and Coast	18	1.3	6	33.3
Wairarapa	2	n/c	0	n/a
Nelson Marlborough	17	2.5	3	17.6
West Coast	6	3.7	4	66.7
Canterbury	84	3.4	10	12.0
South Canterbury	16	5.8	3	18.8
Otago	29	3.1	6	20.7
Southland	15	2.7	2	13.3
Total	**546**	**2.6**	**150**	**29.9**

[1] *Source: EpiSurv notification data.*

[2] *Five-year annualised rate per 100,000 population; n/c, not calculated (number of notifications is <5). Shading indicates DHBs with the highest (darker) and lowest (lighter) rates.*

[3] *Cases with an unknown hospitalisation status have been excluded from the denominator; n/a, not applicable (no hospitalised cases).*

A total of 15 cases were reported in the four DHBs with the lowest notification rates, of which one was hospitalised (13 cases were reported as not hospitalised and the hospitalisation status of one case was unknown). There were at least two hospitalised cases reported for every other DHB in New Zealand.

6.2 VTEC/STEC laboratory services

Each of the three DHBs with the highest VTEC/STEC notification rates was supported by more than one laboratory that provided VTEC/STEC culture and identification services (Table 2):

- South Canterbury DHB is supported by two laboratories.
- Waikato DHB is supported by four laboratories.
- Taranaki DHB is supported by two laboratories.

Five of these eight laboratories routinely tested faecal samples for O157 VTEC/STEC. The remaining three laboratories applied criteria to decide whether to do this test (one served South Canterbury DHB and two served Waikato DHB).

Three of the four DHBs with the lowest VTEC/STEC notification rates were supported by one laboratory that provided VTEC/STEC culture and identification services. Hutt Valley DHB was supported by two laboratories. All five laboratories did not test faecal samples for O157 VTEC/STEC as part of a standard screen, but based this testing on criteria (e.g. age or clinical symptoms of patient, the presence of blood in the sample, by request).

Only one of the 13 laboratories supporting all seven DHBs reported that they tested for non-O157 VTEC/STEC. This laboratory reported that this was a project to test methods, and the project had started in 2010.

6.2.1 Isolation and identification methods

The eight laboratories providing services to the DHBs with the highest VTEC/STEC notification rates have been using the same VTEC/STEC isolation method since before 2006.

Three of the five laboratories providing services to the DHBs with the lowest VTEC/STEC notification rates also have isolation methods that have been in place since before 2006; the other two laboratories began using their methods in 2009 (one of these did not report an isolation method).

All 12 laboratories that reported an isolation method directly inoculated faecal samples on to either CTSMAC or SMAC. Ten laboratories reported an incubation temperature for the plates; all incubated between 35 and 37°C. All but three laboratories incubated the plates for up to 24 hours. The other three laboratories, all providing services to the DHBs with the highest STEC/VTEC notification rates, incubated the plates up to 48 hours. One of the laboratories providing services to a DHB with one of the lowest STEC/VTEC notification rates indicated that they re-incubated the agar plate if the result was negative after 24 hours (i.e. presumptive STEC/VTEC colonies were not visible).

An identification method was reported by 11/13 laboratories; one did not report a method and one has been sending all suspect isolates elsewhere for identification since 2009 (both of these laboratories service DHBs with the lowest VTEC/STEC notification rates). Nine of the laboratories have been using the same method since before 2006; the remaining two have had the methods in place since 2007.

Suspect VTEC/STEC colonies were tested using an O157 latex test and a biochemical identification test by 8/11 laboratories. This includes the three laboratories with reported identification methods that service the DHBs with the lowest VTEC/STEC notification rates. Some of the laboratories analysing samples from DHBs with the highest VTEC/STEC notification rates did additional tests, such as oxidase, urea or indole.

All of the laboratories reported forwarding O157 VTEC/STEC isolates to the ERL for confirmatory testing (one laboratory does this via another).

6.2.2 Notification methods

All of the laboratories providing services to six of the seven DHBs included in this analysis notified these DHBs by electronic transfer (most also sent hard copies by phone or fax). Electronic transfer was not used by any of the laboratories providing services to a DHB with one of the highest VTEC/STEC notification rates; these laboratories communicated notifications by post or fax.

6.3 VTEC/STEC PHU services

The three DHBs with the highest VTEC/STEC notification rates and two of the DHBs with the lowest VTEC/STEC notification rates were each supported by one PHU. The Hutt Valley and Wairarapa DHBs are both under the remit of one PHU, which also has responsibility for the Capital and Coast DHB.

6.3.1 Notifications

All six PHUs were notified of all positive isolations of VTEC/STEC.

Based on reported methods by which PHUs receive notifications, all six PHUs received notifications of VTEC/STEC infection from hospital clinicians, local laboratories and the ERL. Four of the PHUs also received notifications from GPs.

6.3.2 Investigation

All six PHUs:

- Began an investigation when a notification was received;
- Investigated all cases of O157 VTEC/STEC infection;
- Investigated all asymptomatic cases of VTEC/STEC infection.

Four of the PHUs also reported that they investigated all cases of non-O157 VTEC/STEC infection (though none of the laboratories tested for this group of pathogens as part of surveillance; see Section 6.2). One laboratory supporting a DHB with high notification rates

did not investigate these cases. One laboratory supporting a DHB with low notification rates investigated some of these cases; they commented that a positive laboratory result will initiate the investigation, which will continue until the laboratory confirms that the isolated organism is not toxigenic.

6.3.3 Reporting

All six PHUs entered all VTEC/STEC notifications into EpiSurv.

Five of the PHUs would consider de-notification of a VTEC/STEC case in EpiSurv if the laboratory advised them that the isolate was not VTEC/STEC. One PHU would consider de-notification if the case was asymptomatic and did not meet the case definition. All six of the PHUs would provide a reason in the EpiSurv case report form for de-notifying the case.

6.4 Discussion

While several laboratories supporting DHBs with the highest notification rates tested faecal samples for VTEC/STEC as part of a standard faecal screen, there appeared to be some confusion over this question (see Section 5.5.2), so this should not be considered a reason for higher notification rates without further investigation. While some of the PHUs indicated that they investigated non-O157 VTEC/STEC none of the laboratories providing services to these PHUs reported testing for this group of pathogens as part of regular surveillance.

The available information on isolation and identification methods indicated that the methods have remained largely unchanged since 2006-2007. The survey did not ask what methods were in place prior to any changes, so it is not possible to ascertain if any changes were important. Methods may be partially modified over time as new or cheaper reagents are developed and this survey did not seek this level of detail. Despite this, the available information indicated that the methods were fairly similar between those laboratories providing services to the DHBs with the highest VTEC/STEC notification rates and those laboratories providing services to the DHBs with the lowest VTEC/STEC notification rates, with two notable exceptions:

- The laboratories directly inoculated faecal samples on to either CTSMAC or SMAC agar and incubated these agar plates between 35-37°C. All but four laboratories incubated the agar plates for up to 24 hours; these four laboratories, three of which provided services to the DHBs with the highest STEC/VTEC notification rates, incubated the agar plates up to 48 hours. While CTSMAC is more inhibitory towards other faecal flora, SMAC is still a suitable isolation agar. Manufacturers of these agars recommend incubation at 35-37°C for 18-24 hours. Non-sorbitol fermenting colonies (an indicator of VTEC/STEC) appear clear and colourless on these agars and they can be small and difficult to see after 18-24 hours incubation. It is possible that the four laboratories that incubate for a longer period are more successfully isolating VTEC/STEC, however this would need to be investigated further.
- Some of the laboratories analysing samples from DHBs with the highest VTEC/STEC notification rates did additional tests, such as oxidase, urea or indole. These tests can be used as part of a screening panel to either identify or eliminate the presence of *E. coli*. This reduces the number of more expensive identification tests required but would not contribute to a higher isolation rate.

All laboratories sent isolates to the ERL for confirmation and the ERL electronically reports their results to EpiSurv. ESR surveillance and laboratory staff actively monitor the correct recording of cases in EpiSurv. Therefore it is very unlikely that cases are either not entered into EpiSurv or entered incorrectly into EpiSurv.

The practices for investigation and de-notification did not appear to differ markedly between the six PHUs and it is unlikely these could impact on the numbers of cases reported in EpiSurv.

Other regional features of the six DHBs studied, such as agricultural activities, may have more influence on the VTEC/STEC notification rates than the laboratory services or PHU activities.

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APPENDIX 1: LABORATORY SURVEYS

All of the surveys followed a similar template. This is presented below, where *this pathogen* is used in place of the specific pathogen that was the subject of the questionnaire. There were additional or modified questions for each of the surveys and these are listed below. Questions 10-14 were not asked in the *Yersinia* survey, Question 10 was not asked in the *Listeria* survey, and Questions 7 and 10 were not asked in the *Campylobacter* survey.

QUESTIONNAIRE:

1. Is testing for *this pathogen* part of a standard faecal screen at your laboratory?

   If no, please specify the criteria used for testing.
2. Please specify the collection and transportation methods for specimens tested for *this pathogen* at your laboratory.
3. Please describe the isolation process (including use of selective media, enrichment steps, incubation time and temperature) for *this pathogen* in your laboratory.
4. When did your laboratory begin using this isolation method(s) for *this pathogen*?
5. Please describe the identification processes (including tests and/or kits used, incubation times and temperatures) for *this pathogen* in your laboratory.
6. When did your laboratory begin using this identification process for *this pathogen*?
7. Are all isolates *of this pathogen* sent from your laboratory to ESR's Enteric Reference Laboratory?

   If no, please specify the criteria used for referral of isolates to ESR.
8. Which DHB area(s) does your laboratory provide *this pathogen's* testing services for?
9. Do you have any other comments regarding *this pathogen* testing?
10. Did your laboratory take part in the 2005 Acute Gastrointestinal Illness (AGI) Study?
11. Do you follow the Ministry of Health Guidelines Direct Laboratory Notification of Communicable Diseases December 2007?

    (http://www.surv.esr.cri.nz/LabSurv/Documents/dln-national-guidelines-dec07.pdf)
12. How are reports for *this pathogen* sent to Public Health Surveillance Units? (tick all that apply; *options: Electronic transfer from Laboratory Management System, Email, Hard copy report by fax, Hard copy report by post, Phone call, Other (please specify)*)
13. Would you like to receive a copy of the summarised data from this survey?

Please confirm details for your laboratory and the person completing this form so that we can contact you if any clarification is required.

Additional questions for the *Campylobacter* survey:

- Do you speciate isolates?
- Alternative to Question 12: What are your notification procedures for *Campylobacter* to Public Health Surveillance Units?

Additional questions for *Listeria* survey:

- Are methodology and identification procedures the same for blood, CSF amniotic fluid, etc?

  If no, please describe the methods used.

- Alternative to Question 7: Do you forward isolates to ESR Kenepuru Science Centre?
- Do you speciate isolates?
- Alternative to Question 12: What are your notification procedures for *Listeria* to Public Health Units/Medical Officers of Health?

Additional questions for *Salmonella* survey:

- If you have not covered this in the Identification section (question 4), please specify the serology performed in your laboratory.
- Alternative to Question 12: What are your notification procedures for *Salmonella* to Public Health Surveillance Units?

Additional questions for VTEC/STEC survey:

- Do you perform any molecular testing for O157 VTEC/STEC in your laboratory?
- Does your laboratory test for non-O157 VTEC/STEC?

  Please describe the isolation process (including use of selective media, enrichment steps, incubation time and temperature) for non-O157 VTEC/STEC in your laboratory.

  When did your laboratory begin using this isolation process for non-O157 VTEC/STEC?

  Please describe the identification processes (including tests and/or kits used, incubation times and temperatures) for non-O157 VTEC/STEC in your laboratory.

  When did your laboratory begin using this identification process for non-O157 VTEC/STEC?

Additional questions for *Yersinia* survey:

- Please indicate which species of *Yersinia* can be identified in your laboratory.
- Is your laboratory able to identify biotypes of *Y. enterocolitica*?
- Alternative to Question 7: Under what circumstances are *Yersinia* isolates sent from your laboratory to ESR's Enteric Reference Laboratory?

APPENDIX 2: LABORATORIES RECEIVING THE SURVEYS

Laboratory Name	Location
Aotea Pathology	Wellington
Canterbury Health Laboratories	Christchurch
Capital and Coast Microbiology Laboratory	Wellington
Dargaville Hospital Laboratory	Dargaville
Diagnostic Medlab	Auckland
Grey Hospital Laboratory	Greymouth
Hawke's Bay District Health Board	Hastings
Health Waikato Laboratory	Hamilton
Hutt Valley District Health Board	Lower Hutt
Kaitaia Hospital Path Lab.	Kaitaia.
LabCare Pathology	New Plymouth
Laboratory Services Rotorua	Rotorua
Labplus	Auckland
Labtests	Auckland
Medlab Central	Palmerston North
Medlab South Blenheim	Blenheim
Medlab South Ltd	Christchurch
Medlab Timaru	Timaru
Medlab Wairarapa	Masterton
Middlemore Hospital	Auckland
Northland Pathology	Whangarei
Pathlab Bay of Plenty	Tauranga
Pathlab Waikato Ltd	Hamilton
Southern Community Laboratories	Christchurch
Southern Community Laboratories	Dunedin
Southern Community Laboratories	Oamaru
Southern Community Laboratories Hawke's Bay	Hastings
Southern Community Laboratories Southland Hospital	Invercargill
Taranaki Medlab	New Plymouth
Taumarunui Hospital Laboratory	Taumarunui
Te Kuiti Hospital	Te Kuiti
Thames Hospital Laboratory	Thames
TLab	Gisborne
Waitemata District Health Board/North Shore Hospital	Auckland
Whakatane Hospital Laboratory	Whakatane
Whangarei Hospital Laboratory,Microbiology.	Whangarei

APPENDIX 3: PHU SURVEYS

The purpose of the PHU questionnaires was to identify, for each pathogen, practices used by PHUs in the following situations:

- On receipt of a disease notification;
- In the follow up and investigation of symptomatic and asymptomatic cases;
- In outbreak situations;
- In determining case status for notified cases.

All of the PHU questionnaires followed a similar template. This is presented below, where <pathogen> is used in place of the specific pathogen that was the subject of the questionnaire. There were additional pathogen-specific questions for each of the questionnaires and these are listed below. Question 22 was not asked in the campylobacteriosis survey.

QUESTIONNAIRE:

Response to Notifications

1. In your PHU, are <pathogen> notifications received from any of the following sources? Please also indicate the method(s) of notification.

	Electronic notifications	Hard copy report by fax	Hard copy report by post	Phone call	Other (specify)
General Practice	( )	( )	( )	( )	( )_____
Hospital clinician	( )	( )	( )	( )	( )_____
Local laboratory	( )	( )	( )	( )	( )_____
ESR Enteric Reference Laboratory	( )	( )	( )	( )	( )_____

2. Does your PHU receive notifications from any sources not listed in question 1?

( ) Yes
( ) No
( ) Unknown

3. Please provide details of the source and method of notification.

4. In regard to laboratory notifications, which isolations of <pathogen> are notified to your PHU?

( ) All positive isolations
( ) Only positive isolations where case is known to have clinical illness
( ) Other (please specify)

5. Are all <pathogen> notifications received by your PHU (from sources identified in Q1 and Q2 above) entered into EpiSurv?

( ) Yes
( ) No
( ) Don't know

6. If no, please give details of types of <pathogen> notifications not entered.

7. Do you have any other comments regarding notification of <pathogen>?

8. When does your investigation of notified <pathogen> cases begin?

( ) When initial notification received
( ) After confirmation by ESR
( ) Other (please specify)
( ) Not applicable (no investigation of cases)

Follow up and investigation of <pathogen> cases

9. Does your PHU investigate <pathogen> cases?

( ) Yes, all cases
( ) Yes, some cases
( ) No
( ) Don't know

10. Which cases are investigated?

11. Does your PHU investigate asymptomatic <pathogen> cases?

( ) Yes, all cases
( ) Yes, some cases
( ) No
( ) Don't know

12. Which asymptomatic <pathogen> cases are investigated?

13. What method(s) are used to investigate <pathogen> cases in your PHU? (tick all that apply)

( ) Visit to case
( ) Phone call to case
( ) Questionnaire posted out
( ) Other (please specify)

14. Who investigates the <pathogen> cases in your PHU area? (tick all that apply)

( ) Medical Officers of Health
( ) Health Protection Officers
( ) Environmental Health Officers
( ) Other (please specify)

15. Does your PHU use a specific questionnaire for <pathogen> investigations?

( ) Yes
( ) No
( ) Don't know

16. If yes, where does the questionnaire used by your PHU originate from?

( ) Unknown origin
( ) Acquired from
( ) Developed by PHU

17. Is the postal <pathogen> questionnaire followed up if not returned?

( ) Yes
( ) No
( ) Don't know

18. What is the approximate response rate for the <pathogen> questionnaire?

19. What is the approximate percentage of <pathogen> notifications that are investigated (all methods of investigation) in your PHU?

20. Please outline the criteria used by your PHU in your decision to investigate a potential <pathogen> outbreak.

21. Do you have any other comments regarding follow up and investigation of <pathogen> notifications?

Recording/Updating the Case Status in EpiSurv

22. How are negative <pathogen> (i.e. no <pathogen>isolated) laboratory test results from ESR recorded by your PHU in EpiSurv?

23. Under what circumstances would your PHU consider de-notification (i.e. changing the case status in EpiSurv to 'not a case') for <pathogen> cases ?

24. What details regarding the reason for denotifying a <pathogen> case does your PHU record in the EpiSurv case report form (tick all that apply)?

( ) Select 'No' for lab confirmation
( ) Select 'No' for fits clinical description
( ) Record details in the Comments section
( ) Do not provide any details
( ) Other (please specify)

25. Do you have any other comments regarding case status for <pathogen> cases?

PHU Details

26. Which DHB area(s) does your <pathogen> survey response cover?

Please confirm details for your PHU and the person completing this form so that we can contact you if any clarification is required.

PHU
City where office is located
Name of person completing this form
Job Title/Position
Email Address
Phone Number

The following questions for specific pathogens were included with the relevant questionnaire.

Additional questions for the *Campylobacter* survey:

Does the laboratory notification include the species of Campylobacter identified?

( ) Yes, always
( ) Yes, sometimes
( ) No
( ) Don't know

Does your PHU enter the species for Campylobacter cases into EpiSurv?

( ) Yes, always
( ) Yes, sometimes
( ) No
( ) Don't know

Additional questions for the *Listeria* survey:

Does your PHU investigate perinatal *L. monocytogenes* cases?

Which perinatal cases are investigated?

Does your PHU investigate non-perinatal *L. monocytogenes* cases?

Which non-perinatal cases are investigated?

Additional questions for the *Salmonella* survey:

Does your PHU investigate typhoidal *Salmonella* cases?

Which typhoidal cases are investigated?

Does your PHU investigate non-typhoidal *Salmonella* cases?

Which non-typhoidal cases are investigated?

Do you request stool samples from asymptomatic contacts of a *Salmonella* case?

( ) Yes
( ) No
( ) Unknown

Additional questions for the VTEC/STEC survey:

Does your PHU investigate O157 VTEC cases?

Which O157 VTEC cases are investigated?

Does your PHU investigate non O157 VTEC cases?

Which non O157 VTEC cases are investigated?

APPENDIX 4: PUBLIC HEALTH UNITS RECEIVING THE SURVEY

PHU	Office location(s)	DHBs covered
Northland Primary and Community Health Services	Whangarei	Northland
Auckland Regional Public Health Services	Takapuna, Auckland, Manukau	Waitemata, Auckland, Counties Manukau
Waikato Public Health Unit	Hamilton	Waikato
Toi Te Ora Public Health Unit	Whakatane, Tauranga, Rotorua	Bay of Plenty, Lakes
Tairawhiti Public Health Unit	Gisborne	Gisborne/Tairawhiti
Taranaki Public Health Unit	New Plymouth	Taranaki
Hawke's Bay Public Health Unit	Napier	Hawke's Bay, Chatham Islands
MidCentral Public Health Unit	Palmerston North, Wanganui	MidCentral, Whanganui
Regional Public Health	Lower Hutt	Capital and Coast, Wairarapa, Hutt
Nelson Marlborough Public Health Unit	Nelson, Blenheim	Nelson Marlborough
Community and Public Health	Christchurch, Timaru, Greymouth	West Coast, Canterbury, South Canterbury
Public Health South	Dunedin	Otago, Southland

APPENDIX 5: CAMPYLOBACTER LABORATORY SURVEY RESULTS

Results are presented as reported by the laboratories.

Aims of survey

- To identify practices used by New Zealand laboratories for the confirmation/identification of *Campylobacter* spp. including the criteria for testing, isolation and identification methods.
- To ascertain notification procedures to Public Health Surveillance Units for *Campylobacter*-positive isolates.

Participating laboratories

Thirty-two laboratories submitted responses to this survey (89% response rate). These laboratories provided services across all New Zealand's DHBs (Table 12). Five laboratories provided services for more than one DHB.

Table 12: Number of laboratories providing *Campylobacter* testing services for each District Health Board (DHB)

DHB	No. laboratories
Northland	4
Waitemata	3
Auckland	3
Counties Manukau	3
Waikato	3
Lakes	1
Bay of Plenty	2
Tairawhiti	1
Taranaki	2
Hawke's Bay	2
Whanganui	1
MidCentral	1
Hutt Valley	2
Capital and Coast	2
Wairarapa	1
Nelson Marlborough	1
West Coast	1
Canterbury	3
South Canterbury	2
Otago	2
Southland	1

Testing criteria

All 32 (100%) laboratories tested for *Campylobacter* spp. as part of a standard faecal screen.

In the AGI study, 33/35 (94%) laboratories reported that they tested for *Campylobacter* spp. routinely as part of an enteric screen and two (6%) listed their own criteria (testing depended on the clinical history, symptoms and condition of the specimen).

Isolation and identification methods

Seventy-eight percent of the laboratories used an isolation method that was in place prior to 2006 and 69% used an identification method that was in place prior to 2006 (Table 13).

Table 13: When the laboratories began using the *Campylobacter* isolation and identification methods reported in the survey

Time period	No. laboratories providing a response	
	Isolation method	Identification method
PriorTo2006	25	22
2006	1	2
2007	0	1
2008	0	1
2009	3	4
2010	1	0
Unknown	2	2
Total laboratories	**32**	**32**

Agar plates were used by 31/32 of the laboratories for isolating *Campylobacter* spp. The agars reported were:

- Charcoal cefoperazone deoxycholate agar (CCDA) (16 laboratories);[10]
- Campylobacter agar (no further detail) (6 laboratories);
- Campylobacter blood free agar (no further detail) (5 laboratories);
- Modified CCDA (mCCDA) (1 laboratory);
- CAT (cefoperazone, amphotericin B, Teicoplanin) agar (2 laboratories);
- Prestons blood free agar (1 laboratory).

The remaining laboratory used an enzyme immunoassay test, "*Campylobacter* Specific Antigen EIA."

Incubation conditions for agar plates were reported by 31 laboratories. Three of these laboratories incubated agar plates at 35-37°C and the remainder incubated agar plates at 42°C. Twenty-seven laboratories reported that the agar plates were incubated under a CO_2 (5% or 10%) or microaerobic atmosphere (the remaining 4 laboratories did not report atmospheric conditions). The most common incubation period was 48 hours/2 days (reported

[10] CCDA is marketed by Fort Richard Laboratories as "Campylobacter Isolation Agar Blood Free". Laboratories that reported using blood free campylobacter agar sourced from Fort Richard Laboratories were included in the CCDA group.

25 of the 29 laboratories reporting an incubation period). Four laboratories incubated agar plates for 72 hours.

The agars used by the laboratories surveyed in the AGI study were the same as those reported in this study, with three exceptions; three laboratories in the AGI study reported using Skirrows agar, and one laboratory reported using campylobacter isolation media with blood. The incubation conditions were similar to those reported in this study (28/31 laboratories incubated at 42°C, and 12 laboratories reported microaerobic or CO_2 conditions even though this information was not specifically requested).

Identification methods were reported by 31/32 laboratories. These laboratories used a number of screening tests to identify *Campylobacter* isolates and species, which are summarised in Table 14. No further tests were carried out.

Table 14: *Campylobacter* spp. identification methods reported by laboratories

Screening tests	No. laboratories reporting method
- Gram stain	28
- Oxidase	25
- Colony morphology/appearance	13
- Growth under microaerophilic conditions	8
- Motility/microscopy	8
- Hippurate hydrolysis	7
- Sensitivity to nalidixic acid and cephalothin	3
- Sensitivity to erythromycin and ciprofloxacin	3
- DNA sequencing	1

The AGI study did not specifically ask for identification methods so the results cannot be compared.

Speciation of *Campylobacter* isolates was undertaken in 8/32 (25%) of the laboratories. One of these laboratories commented that only *C. jejuni* was identified at species level. It should be noted that speciation of this group is known to be highly challenging and only limited accuracy is expected with the above testing.

Notification guidelines

The Ministry of Health guidelines on Direct Laboratory Notification of Communicable Diseases (Ministry of Health, 2007) were followed by 30/32 laboratories (94%). The remaining two laboratories did not know if they followed these guidelines.

Reporting to Public Health Surveillance Units

Twenty-three (72%) of the laboratories reported *Campylobacter* results to Public Health Surveillance Units by electronic transfer from their laboratory management system (Table 15). Results were also commonly communicated by fax. Seven laboratories used more than one method. One laboratory used an "other method"; via the hospital internal mail system.

Table 15: Methods used by laboratories to communicate *Campylobacter* results to the Public Health Service Units

Method	No. labs
Electronic transfer from Laboratory Management System	23
Hard copy report by fax	9
Hard copy report by post	6
Phone call	3
Email	0
Other method	1

Additional comments

One laboratory commented that they had observed an increased number of hippurate-negative isolates since November 2008. A positive result for hippurate is one identification method used to confirm a *Campylobacter* isolate as *C. jejuni*. PCR testing by ESR confirmed some of these hippurate-negative isolates as *C. jejuni*.

One laboratory is currently trialling Bolton Broth for the enrichment of *Campylobacter* spp.

APPENDIX 6: LISTERIA LABORATORY SURVEY RESULTS

Results are presented as reported by the laboratories.

Aims of survey

- To identify practices used by New Zealand laboratories for the confirmation/identification of *Listeria* spp. including the criteria for testing, isolation and identification methods.
- To ascertain notification procedures to Public Health Surveillance Units for *Listeria*-positive isolates.

Participating laboratories

Twenty-nine laboratories submitted responses to this survey (81% response rate). These laboratories provided services across all New Zealand's DHBs (Table 16). Five laboratories provided services for more than one DHB.

Table 16: Number of laboratories providing *Listeria* spp. testing services for each District Health Board (DHB)

DHB	No. laboratories
Northland	4
Waitemata	3
Auckland	3
Counties Manukau	3
Waikato	1
Lakes	1
Bay of Plenty	2
Tairawhiti	1
Taranaki	1
Hawke's Bay	2
Whanganui	1
MidCentral	1
Hutt Valley	2
Capital and Coast	2
Wairarapa	1
Nelson Marlborough	1
West Coast	1
Canterbury	3
South Canterbury	2
Otago	2
Southland	1

Testing criteria

Only one laboratory tested for *Listeria* spp. as part of a standard faecal screen. However, further information from this laboratory suggested that criteria were applied (a faecal sample from a maternity patient).

Four laboratories did not test faecal samples for *Listeria* spp. Two of these laboratories also reported that they only tested blood cultures.

Where requested to test a faecal sample for *Listeria* spp., four laboratories referred these samples to other laboratories for testing. One of these laboratories also reported that they tested other types of samples for *Listeria* spp.

Of the remaining 20 laboratories:

- 13 only tested a faecal sample for *Listeria* spp. if requested;
- 4 would test a faecal sample if the clinical details were indicative of listeriosis (e.g. pregnant woman);
- 3 would test a faecal sample if the clinical details indicated a possibility of listeriosis or if the test was requested.

Five of these laboratories reported that blood samples were the preferred specimens for *Listeria* spp. testing.

Eight laboratories also reported other types of samples they tested for *Listeria* spp.:

- Urogenital swabs (tested on request) (reported by 1 laboratory);
- Stillborn specimens (reported by 2 laboratories);
- Rectal swabs of pregnant women with abdominal pain (reported by 1 laboratory);
- Samples from a new born baby screen (e.g. body swabs, faecal sample, gastric aspirate) (reported by 1 laboratory).

In the AGI study, 1/33 (3%) laboratories reported that they test for *Listeria* spp. routinely as part of an enteric screen, 22 (67%) tested by request, eight (24%) did not test for *Listeria* spp. and five (15%) listed their own criteria (all based on clinical details, e.g. pregnancy, symptoms).[11]

Isolation and identification methods

There were four laboratories that did not test faecal samples for *Listeria* spp., and four laboratories that referred faecal samples to another laboratory if this test is requested. Two of the remaining 21 laboratories provided isolation methods for blood or swabs rather than faeces. The responses of these 10 laboratories have been excluded from the summary of isolation methods (leaving 19 laboratories).

Twenty-six laboratories reported identification methods. Identification methods reported by the eight laboratories that did not test faecal samples for *Listeria* spp. or referred such tests to another laboratory were included where this information was provided.

[11] Some laboratories ticked 'request' and also gave their own criteria, hence the sum of these responses exceeds the 33 laboratories submitting answers to this question.

Fifty-eight percent of the laboratories used an isolation method or an identification method that was in place prior to 2006 (Table 17).

Table 17: When the laboratories began using the *Listeria* isolation and identification methods reported in the survey

Time period	No. laboratories providing a response	
	Isolation method	Identification method
PriorTo2006	11	15
2006	0	1
2007	1	2
2008	0	1
2009	2	2
2010	2	0
Unknown	3	5
Total laboratories	**19**	**26**

Of the 19 laboratories from which isolation methods were analysed, 13 laboratories directly inoculated faecal samples on to one type of agar:

- Oxford listeria agar (3 laboratories);
- PALCAM agar (4 laboratories);
- Aztreonam agar (2 laboratories) or Aztreonam agar with sheep blood (1 laboratory);
- Blood agar (2 laboratories);
- Blood/chocolate agar (1 laboratory).

Three laboratories directly inoculated faecal samples on to more than one type of agar:

- Oxford listeria agar + blood agar;
- Oxford listeria agar + blood agar + Colistin Naladixic Acid (CNA) agar;
- Aztreonam agar + blood agar.

Three laboratories did not use any agars.

All of the agar plates were incubated between 35 and 37°C for up to 48 hours (four laboratories reported they examined the plates at 24 hours). Three of the laboratories reported that the incubation was carried out under CO_2 (5%). One laboratory incubated additional blood agar plates at 4°C for five days.

Five laboratories that used agars also directly inoculated faecal samples into broth:

- Listeria enrichment broth (4 laboratories; 28-33°C, overnight-48h), subcultured to PALCAM agar and Fraser broth (2 laboratories; 35-37°C, 48h), or to PALCAM and Aztreonam agars (1 laboratory; 37°C, 24h);
- Nutrient broth (1 laboratory; refrigeration temperature, 1 week), subcultured to blood agar (36°C, 48h, 5% CO_2).

The two laboratories that used Fraser broth would both subculture from this broth to PALCAM agar if the Fraser broth turned dark brown/black.

The three laboratories that did not use agars directly inoculated faecal samples into broth:

- Tryptic soy broth (27°C, 48h), subcultured to blood agar (temperature unspecified, 24h, CO_2 incubator);
- Listeria enrichment broth (27°C, 2 days), subcultured to blood agar (37°C, 24h);
- Meat broth (4°C, for an unspecified number of weeks), subcultured weekly (media and conditions not specified).

The laboratories surveyed in the AGI study reported using Oxford, PALCAM, Aztreonam and CNA agars. Five laboratories reported an enrichment step (two specified *Listeria* enrichment broth). One laboratory reported incubation under CO_2.

The laboratories used various steps to identify *Listeria* spp., which usually included one or more screening tests followed by identification by a biochemical panel (Table 18).

Table 18: *Listeria* spp. identification methods reported by laboratories

Method	No. laboratories reporting method
Screening tests	**23**[1]
- Gram stain	21
- Catalase	16
- Motility	11
- CAMP test/reverse CAMP test[2]	6
- Aesculin	2
- Onithine	1
- Streptococcus latex test	1
Biochemical test	**22**[1]
- API Coryne	10
- BBL crystal identification system	7
- API20E	3
- RapID CB+ panel (Remel)	3
- Vitek II (bioMérieux)	1
- Rapid ID 32 Strep	1

[1] *Number of laboratories reporting at least one of the methods in the category.*
[2] *Christie Atkins Munch Peterson (CAMP) test.*

The AGI study did not specifically ask for identification methods so the results cannot be compared.

The methodology and identification procedures for blood, CSF amniotic fluid, etc. were the same as for faecal samples in 18/21 (86%) laboratories. This excludes the eight laboratories that did not test faecal samples for *Listeria* spp. or referred such tests to another laboratory.

The remaining three laboratories reported differences in the isolation method:

- Instead of Oxford agar, "sterile sites are cultured into enriched media and would have enrichment as well";
- The samples are cultured on Columbia 5% sheep blood agar and CNA if appropriate;
- Blood agar, CAN and PALCAM (35°C, 48h), no broths.

Listeria isolates were speciated in 15/29 (52%) of the laboratories. Excluding the eight laboratories that did not test faecal samples for *Listeria* spp. or referred such tests to another laboratory, 11/21 (52%) laboratories speciated *Listeria* isolates.

Referral to ESR

All but one laboratory (28/29, 97%) reported that they sent *Listeria* isolates to the ESR Kenepuru Science Centre.

Notification guidelines

The Ministry of Health guidelines on Direct Laboratory Notification of Communicable Diseases (Ministry of Health, 2007) are followed by 27/29 laboratories (93%). The remaining two laboratories did not know if they followed these guidelines.

Reporting to Public Health Units/Medical Officers of Health

Twenty (70%) of the laboratories reported *Listeria* results to Public Health Units/Medical Officers of Health by electronic transfer from their laboratory management system (Table 19). Results were also commonly communicated by fax. Five laboratories used more than one method. One laboratory used an "other method"; via the hospital internal mail system.

Table 19: Methods used by laboratories to communicate *Listeria* results to Public Health Units/Medical Officers of Health

Method	No. labs
Electronic transfer from Laboratory Management System	20
Hard copy report by fax	7
Phone call	2
Hard copy report by post	4
Email	0
Other method	1

Additional comments

Four laboratories commented that it is rare for them to receive a request to test a faecal sample for *Listeria* spp. One of these laboratories commented that any isolates they had found were isolated during routine culture of clinical specimens.

One laboratory commented that it is often very difficult to speciate *Listeria* and that they may have to report an isolate as *Listeria* spp. until ESR provides the identification results.

APPENDIX 7: SALMONELLA LABORATORY SURVEY RESULTS

Results are presented as reported by the laboratories.

Aims of survey

- To identify practices used by New Zealand laboratories for the confirmation/identification of *Salmonella* spp. including the criteria for testing, isolation and identification methods.
- To ascertain notification procedures to Public Health Surveillance Units for *Salmonella*-positive isolates.

Participating laboratories

Thirty-four laboratories submitted responses to this survey (94% response rate). These laboratories provided services across all New Zealand's DHBs (Table 20). Three laboratories provided services for more than one DHB.

Table 20: Number of laboratories providing *Salmonella* spp. testing services for each District Health Board (DHB)

DHB	No. laboratories
Northland	4
Waitemata	2
Auckland	2
Counties Manukau	2
Waikato	4
Lakes	2
Bay of Plenty	2
Tairawhiti	1
Taranaki	2
Hawke's Bay	2
Whanganui	1
MidCentral	1
Hutt Valley	2
Capital and Coast	2
Wairarapa	2
Nelson Marlborough	1
West Coast	1
Canterbury	2
South Canterbury	2
Otago	2
Southland	1

Testing criteria

All 34 laboratories (100%) reported that testing for *Salmonella* spp. is part of a standard faecal screen.

In the AGI study, 32/34 (94%) laboratories reported that they tested for *Salmonella* spp. routinely as part of an enteric screen and 2 laboratories (6%) listed their own criteria. In these laboratories, testing for *Salmonella* spp. depended on the clinical history, symptoms and condition of the specimen.

Isolation and identification methods

Eighty-eight percent of the laboratories have been using an isolation method that was in place prior to 2006, and 56% are using an identification method that was in place prior to 2006 (Table 21).

Table 21: When the laboratories began using the *Salmonella* isolation and identification methods reported in the survey

Time period	No. laboratories providing a response	
	Isolation method	Identification method
PriorTo2006	30	19
2006	0	3
2007	0	1
2008	0	5
2009	2	4
Unknown	2	2
Total laboratories	**34**	**34**

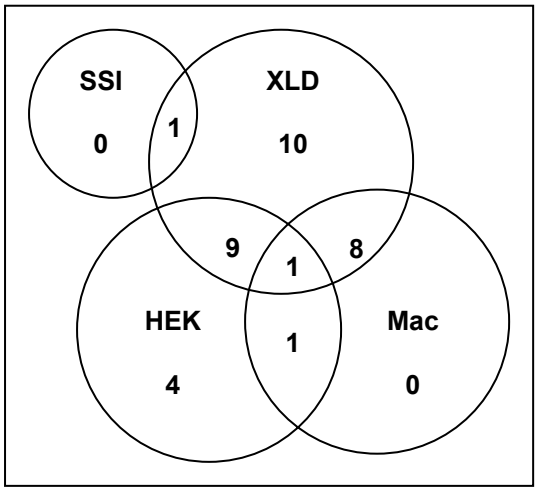
All 34 laboratories directly inoculated various agar plates to isolate *Salmonella* spp. (Figure 2). Xylose lysine deoxycholate (XLD) agar was used by 29/34 (85%) laboratories, often in combination with other agar types. All agar plates were incubated at 35-37ºC (reported by 31 laboratories) under aerobic conditions (reported by 16 laboratories) either overnight (reported by 7 laboratories), for 18-24 hours (reported by 19 laboratories) or for 24-48 hours (reported by 5 laboratories). One laboratory incubated the XLD and MacConkey agar plates at 37ºC for 24 hours followed by room temperature incubation for 24 hours.

Thirty-three laboratories also directly inoculated clinical samples into a broth; 32 inoculated selenite broth or selenite F broth and one inoculated a "gram negative broth". The broths were incubated at 35-37ºC (reported by 29 laboratories) under aerobic conditions (reported by 13 laboratories) overnight up to 24 hours (reported by 29 laboratories), or for 24-48 hours (reported by 2 laboratories). The broths were then subcultured to either XLD agar (19 laboratories), XLD and Hektoen agars (6 laboratories), XLD and MacConkey agars (2 laboratories), MacConkey agar (1 laboratory), XLD/SSI (1 laboratory) or chromogenic Salmonella agar. (1 laboratory), and incubated under similar conditions.

The laboratories surveyed in the AGI study all reported using XLD, MacConkey and Hektoen agars under similar incubation conditions. Four laboratories also used Brilliant Green Agar or Bismuth Sulphite agar, but this practice appears to have stopped. Thirty-two

laboratories reported using an enrichment but the broth was not requested (selenite or selenite F broth had been reported by some laboratories).

Figure 2: The number of laboratories directly inoculating faecal samples to the agars xylose lysine deoxycholate (XLD), statens serum institut (SSI), MacConkey (MacC) or hektoen (HEK) to isolate *Salmonella* spp.



The laboratories used various steps to identify *Salmonella* spp., which usually included one or more of:

- Subculturing positive colonies to additional agars (sometimes to purify colonies);
- Screening tests;
- Detecting the O, H and Vi sera using agglutination tests;
- Screening positive colonies with one or more biochemical tests to identify whether a colony is salmonella.

The methods are summarised in Table 22.

The type of tests and order which they were applied varied between laboratories, though the screening tests were usually applied prior to more expensive biochemical tests.

Table 22: *Salmonella* identification methods reported by laboratories

Method	No. laboratories reporting method
Subculturing to additional broths/agars/reagents	**20**[1]
- Blood agar	10
- Chromogenic agar (e.g. Orientation agar, ChromSalm agar)	9
- MacConkey agar	7
- Acetate agar	4
- Purity plate (agar not specified)	2
- Mueller Hinton agar (via saline broth)	1
Screening tests	**25**[1]
- Urea slope/urea broth	17
- Triple sugar iron (TSI) slope	8

Method	No. laboratories reporting method
- OUMI (ONPG, Urea, Motility, Indol) medium	8
- Indole	4
- Oxidase	5
- Pyrrolidonyl peptidase (PYR)	4
- Citrate	1
- Lysine iron agar (LIA) slope	1
- Lactose	1
- Motility	1
Serology[2]	**33[1]**
- Polyvalent O antisera	22
- Polyvalent H antisera	14
- Polyvalent Vi antisera	15
- Wellcolex latex colour kit	8
- Oxoid latex colour kit	3
Biochemical test[3]	**34[1]**
- API20E	13
- BBL crystal identification system	11
- Microgen (A/B)	8
- RapID One (Remel)	6
- Vitek II (bioMérieux)	3
- Microbact 12A/12B	2
- API10S	1

1. Number of laboratories reporting at least one of the methods in the category.

2. Four laboratories reported that serotyping was carried out at another laboratory. The methods used by these laboratories have been included in the count. Only one of the 34 laboratories did not report undertaking serology.

3. Many laboratories used one test, but would use a second type of test if needed.

The AGI study did not specifically ask for identification methods so the results cannot be compared.

Referral to the ERL

All 34 laboratories reported that they sent *Salmonella* isolates to the ERL; one laboratory sent their isolates to ESR via a hospital microbiology laboratory.

Notification guidelines

Thirty-two laboratories (97%) follow the Ministry of Health guidelines on Direct Laboratory Notification of Communicable Diseases (Ministry of Health, 2007). The remaining two laboratories did not know if they followed these guidelines.

Reporting to Public Health Surveillance Units

Twenty-three (68%) of the laboratories reported *Salmonella* results to Public Health Surveillance Units by electronic transfer from their laboratory management system (Table

23). Results were also commonly communicated by fax and phone. Eight laboratories used more than one method. One laboratory used an "other method"; a hard copy via the internal hospital mail.

Table 23: Methods used by laboratories to communicate *Salmonella* results to the Public Health Surveillance Units

Method	No. labs
Electronic transfer from Laboratory Management System	23
Hard copy report by fax	10
Phone call	6
Hard copy report by post	4
Email	1
Other method	1

Additional comments

One laboratory reported that they are currently trialling three chromogenic media that are formulated to detect *S.* Typhi, *S.* Paratyphi, and lactose fermenting salmonellae. This laboratory is also trialling a media that will help differentiate *Salmonella* spp. from *Citrobacter spp*.

APPENDIX 8: VTEC/STEC LABORATORY SURVEY RESULTS

Results are presented as reported by the laboratories.

Aims of survey

- To identify practices used by New Zealand laboratories for the confirmation/identification of VTEC/STEC including the criteria for testing, isolation and identification methods;
- To ascertain notification procedures to Public Health Surveillance Units for VTEC/STEC laboratory identified cases.

Participating laboratories

Thirty-three laboratories submitted responses to this survey (92% response rate). These laboratories provided services across all New Zealand's DHBs (Table 24). Three laboratories provided services for more than one DHB.

Table 24: Number of laboratories providing VTEC/STEC testing services for each District Health Board (DHB)

DHB	No. laboratories
Northland	4
Waitemata	2
Auckland	2
Counties Manukau	2
Waikato	4
Lakes	1
Bay of Plenty	2
Tairawhiti	1
Taranaki	2
Hawke's Bay	2
Whanganui	1
MidCentral	1
Hutt Valley	2
Capital and Coast	2
Wairarapa	1
Nelson Marlborough	2
West Coast	2
Canterbury	2
South Canterbury	2
Otago	2
Southland	1

Testing criteria

Seventeen laboratories (52%) reported that testing for VTEC/STEC is part of a standard faecal screen. However of these 17 laboratories, seven listed criteria that were used to decide if they would test for VTEC/STEC. This indicated that there was some misunderstanding over the interpretation of "standard faecal screen". One laboratory reported that criteria were applied up until the US Centers for Disease Control and Prevention guidelines in 2009,[12] after which all clinical samples that qualify for culture were tested.

Fifteen laboratories reported that VTEC/STEC were not routinely tested as part of a standard faecal screen and provided criteria for testing. These criteria, plus the criteria listed by the seven laboratories who responded "Yes" to Q1 are summarised in Table 25.

Table 25: Criteria used by laboratories to decide if a human clinical sample should be tested for VTEC/STEC

Criteria	No. laboratories reporting this criteria
Bloody diarrhoea/specimen	17
Relevant clinical details e.g. HUS, history of bloody diarrhoea	13
Red blood cells present in wet film	10
Age of the patient	15
- All cases <13 years old	*4*
- All cases <12 years old	*7*
- All cases <10 years old	*1*
- All cases 6 years old or younger	*4*
- All cases <6 years old	*1*
On request	9
Liquid/fluid sample	3
While cells present in stool	2
All specimens during a period of enhanced surveillance	1

In the AGI study, 7/34 (21%) laboratories reported that they tested for STEC routinely as part of an enteric screen, 13 (38%) only tested if specifically requested by a referring doctor and 26 (76%) listed their own criteria.[13] The criteria listed by these laboratories are similar to those reported in Table 25.

Isolation and identification methods

Seventy percent of the laboratories have been using the same isolation method that was in place prior to 2006 and of these, 15 laboratories (45%) are using an identification method that was also in place prior to 2006 (Table 26). One laboratory had two of the three identification methods in place prior to 2006 but has added the third (a latex test) in the last 2-3 years.

[12] Gould L.H., *et al*. (2009) Recommendations for Diagnosis of Shiga Toxin-Producing *Escherichia coli* Infections by Clinical Laboratories. *Morbidity and Mortality Weekly Report* (Recommendations and Reports) 58(RR12):1-14. Available at http://www.cdc.gov/mmwr/preview/mmwrhtml/rr5812a1.htm

[13] Some laboratories ticked 'request' and also gave their own criteria, hence the sum of these responses exceeds the 34 laboratories submitting answers to this question.

Table 26: When the laboratories began using the VTEC/STEC isolation and identification methods reported in the survey

Time period	No. laboratories providing a response	
	Isolation method	Identification method
PriorTo2006	23	15
2006	0	1
2007	1	4
2008	2	3
2009	3	4
Unknown	3	5[2]
Not applicable[1]	1	1
Total laboratories	**33**	**33**

1. One laboratory only tests for VTEC/STEC when specifically requested and sends the sample to ESR for isolation and identification. This has been in place since 2009.

2. One laboratory did not provide a response to this question so has been included in the "unknown" count.

To isolate VTEC/STEC, ten (30%) laboratories directly inoculated clinical samples to Sorbitol MacConkey (SMAC) agar. The remaining 22 (67%) laboratories directly inoculated clinical samples to a variation of SMAC that assists with the specific isolation of *E. coli* O157:H7 by the addition of a Cefixime-Tellurite supplement (CT-SMAC). VTEC/STEC will appear as colourless colonies on these agars as they do not ferment sorbitol. One laboratory also inoculates MacConkey agar and another also inoculates MacConkey broth for use in identification tests.

The agar plates were incubated at 35-37ºC either overnight (reported by seven laboratories), for 18-24 hours (reported by 13 laboratories) or for 48 hours (reported by eight laboratories, three of which reported checking the plates at 24 hours). Twelve laboratories reported that this was under aerobic conditions. Two laboratories that reported an incubation time of 24 hours reported that they would re-incubate the plate for a further 24 hours if the result was negative (no visible non-sorbitol fermenting bacteria) and one laboratory discarded negative plates after 24 hours.

The laboratories surveyed in the AGI study all reported using SMAC (34% laboratories) or CT-SMAC (66% laboratories). Incubation of the plates was also between 35ºC and 37ºC.

The laboratories used various steps to identify VTEC/STEC, which usually included one or more of:

- Subculturing positive colonies to additional broths, agars (sometimes to purify colonies) or single-reagent tests;
- Screening positive colonies using an immuno-latex agglutination test and/or an immunoassay test which identifies whether a colony belongs to the *E. coli* O157 serogroup;
- Screening positive colonies with a biochemical test which identifies whether the colony is *E. coli*.

The methods are summarised in Table 27.

The type of tests and order which they were applied varied between laboratories, e.g. some laboratories applied the latex test prior to the biochemical kit and others the reverse. One laboratory did not carry out any identification methods but sent all positive colonies to ESR where they were isolated from clinical samples from patients who had relevant clinical details (e.g. bloody diarrhoea, history of HUS).

Table 27: VTEC/STEC identification methods reported by laboratories

Method	No. laboratories reporting method
Subculturing to additional broths/agars/reagents	**17**[1]
- Blood agar	8
- Chromogenic agar (e.g. Orientation agar)	7
- Urea slope/rapid urea/urea broth	6
- Indole	5
- MacConkey agar	4
- Purity plate (agar not specified)	4
- Oxidase	3
- Sorbitol sugar	2
- CTSMAC agar	1
- UTI agar	1
- Citrate	1
Immunoassay test	**2**[1]
- Premier Enterohemorrhagic *E. coli* Enzyme-Immunoassay (EHEC EIA) (Meridian Bioscience)	1
-ImmunoCard STAT! *E coli* O157 : H7 (Meridian Bioscience)	1
Immuno-latex test	**25**[1]
- *E. coli* O157 Latex Test Kit (Oxoid)	10
- Prolex Blue *E. coli* O157 Latex Test Reagent Kit (Prolab)	6
- *E. coli* O157:H7 Latex Test (no brand identified)	3
- RIM *E. coli* O157:H7 Latex Test (Remel)	2
- Wellcolex *E. coli* O157:H7 Rapid latex agglutination test (Remel)	2
- O157 antisera, H7 agglutination, *E. coli* O157 H antisera (Remel)	1
Biochemical test	**26**[1]
- BBL crystal identification system	5
- Microbact 12A/12E	2
- Microgen (12A)	4
- API20E	5
- API10S	1
- RapID One (Remel)	5
- Vitek II (bioMérieux)	3

[1] *Number of laboratories reporting at least one of the methods in the category.*

None of the laboratories carried out any molecular testing of VTEC/STEC isolates.

The AGI study did not specifically ask for identification methods so the results cannot be compared.

Referral to the ERL

Thirty-two laboratories reported that they sent O157 VTEC/STEC isolates to the ERL. The remaining laboratory sent their isolates to a hospital microbiology laboratory.

Testing for non-O157 VTEC/STEC

Three laboratories tested for non-O157 VTEC/STEC. Their methods are summarised in Table 28. Another laboratory commented that if non-O157 VTEC/STEC is requested the stool is sent to a reference laboratory.

Table 28: The isolation and identification methods used by three laboratories that test for non-O157 VTEC/STEC

Isolation process		Identification process	
Method(s)	**Used since[1]**	**Method(s)**	**Used since[1]**
SMAC agar (37ºC).	Prior to 2006	Oxidase. Remel Rapid GN (4h, 37ºC), API20E (overnight 37ºC). Sorbitol and Prolex O157 Latex agglutination negative *E. coli* isolates are sent to ESR to test for verocytotoxin production. A second nutrient agar slope of mixed gram negative bacteria taken from a sweep of the original SMAC plate is sent to ESR to test for verocytotoxin to detect any non-*E. coli* O157 verocytotoxin producers.	*(Not reported but likely to be prior to 2006)*
CTSMAC and MacConkey broth.	Prior to 2006	EHEC EIA. Any reactive samples are referred to ESR (original sample as well as reactive MacConkey broth)	Prior to 2006
(Not reported, but likely to be CTSMAC, 37ºC, 24-48h)	2010	Novitec Verotoxin 1&2 Elisa Kit.	2010

[1] *When the laboratory began using this method.*

Notification guidelines

Thirty-two of these laboratories (97%) follow the Ministry of Health guidelines on Direct Laboratory Notification of Communicable Diseases (Ministry of Health, 2007). The remaining laboratory did not know if they followed these guidelines.

Reporting to Public Health Surveillance Units

Twenty-one (64%) of the laboratories reported VTEC/STEC results to Public Health Service Units by electronic transfer from their laboratory management system (Table 29). Results were also commonly communicated by fax and phone. Sixteen laboratories used more than one method. Of the four laboratories using an "other method", the results of two laboratories are communicated via a hospital, the results from one laboratory are communicated as a hard copy through hospital internal mail, and the results from the remaining laboratory are

transferred automatically via the laboratory management system but the mode (i.e. fax or electronic) was unknown.

Table 29: Methods used by laboratories to communicate VTEC/STEC results to the Public Health Service Units

Method	No. labs
Electronic transfer from Laboratory Management System	21
Hard copy report by fax	11
Phone call	11
Hard copy report by post	7
Email	0
Other method	4

Additional comments

Four laboratories commented on the isolation of non-O157 VTEC/STEC. The comments indicated a growing awareness of the clinical significance of non-O157 VTEC/STEC and the lack of a standard, "user friendly" method to detect these bacteria.

APPENDIX 9: YERSINIA LABORATORY SURVEY RESULTS

Results are presented as reported by the laboratories.

Aims of survey

- To identify practices used by New Zealand laboratories for the confirmation/identification of *Yersinia* spp. including the type of test used, isolation and identification methods
- To identify species and biotypes of *Yersinia* recognized across the country
- To provide information that can be used to guide diagnosis, interpretation and use of *Yersinia* biotyping results and examine the validity of *Yersinia* notifications
- To make recommendations on testing methods for *Yersinia* spp.

Participating laboratories

Thirty-six submitted responses to this survey (100% response rate). The 36 laboratories provided services across all New Zealand's DHBs (Table 30). Five laboratories provided services for more than one DHB.

Table 30: Number of laboratories providing *Yersinia* testing services for each District Health Board (DHB).

DHB	No. laboratories
Northland	4
Waitemata	3
Auckland	3
Counties Manukau	3
Waikato	5
Lakes	1
Bay of Plenty	3
Tairawhiti	1
Taranaki	2
Hawke's Bay	2
Whanganui	1
MidCentral	1
Hutt Valley	2
Capital and Coast	2
Wairarapa	1
Nelson Marlborough	1
West Coast	1
Canterbury	3
South Canterbury	2
Otago	2
Southland	1

Testing criteria

Yersinia testing is part of a standard faecal screen for 35 of the 36 laboratories surveyed. The one laboratory that does not routinely test for *Yersinia* spp. performs a *Yersinia* test if specifically requested.

In the AGI study, 32/34 (94%) laboratories reported that they test for *Yersinia* spp. routinely as part of an enteric screen and 2 laboratories (6%) listed their own criteria. In these laboratories, testing for *Yersinia* depended on the clinical history, symptoms and condition of the specimen.

Isolation and identification methods

Seventy-five percent of the laboratories have been using an isolation method that was in place prior to 2006, and 58% are using an identification method that was in place prior to 2006 (Table 31).

Table 31: When the laboratories began using the *Yersinia* isolation and identification methods reported in the survey

Time period	No. laboratories providing a response	
	Isolation method	Identification method
PriorTo2006	27	21
2006	1	1
2008	1	4
2009	1	2
Unknown	6	8
Total laboratories	**36**	**36**

Seven respondents reported using an enrichment step for *Yersinia* spp.:

- Selenite broth, incubated at 35-37ºC for 18-24 hours (reported by 5 laboratories);
- *Yersinia* selective enrichment (YSE) broth incubated at 28ºC for 18 hours (reported by 2 laboratories).

To isolate *Yersinia* spp., 35/36 (97%) laboratories either directly inoculated clinical samples or inoculated the enrichment broth on cefsulodin irgasan novobiocin (CIN) agar. One laboratory directly inoculated clinical samples on MacConkey agar.

Twenty-seven laboratories incubated the CIN agar plates at 28ºC; the incubation times were:

- 18-24 hours (11 laboratories);
- 24 hours, followed by 24 hours at room temperature (2 laboratories);
- 48 hours (the 1 laboratory);
- 48 hours, with a check of the plates at 24 hours (13 laboratories).

Eight laboratories incubated the CIN plates at 37ºC for either 18-24 hours (7 laboratories) or 48 hours, with a check of the plates at 24 hours (1 laboratory). The laboratory that used MacConkey agar incubated these plates for 24 hours at 37°C.

All of the 34 laboratories reporting a *Yersinia* isolation method in the AGI study used CIN agar. Eight laboratories also reported using enrichment (selenite, selenite F or ossmer broth). Seven laboratories incubated the CIN agar at 25-28°C, 13 laboratories at 30°C, 8 laboratories between 35-37°C and one laboratory at room temperature and 37°C.

All laboratories reported using an identification process for *Yersinia* spp. All of the laboratories used one or more biochemical tests (Table 32).

Table 32: *Yersinia* biochemical identification methods reported.

Method[1]	No. laboratories reporting method
- API20E	13
- API10S	1
- BBL crystal identification system	9
- Microgen (A/B)	5
- RapID One (Remel)	5
- Vitek II (bioMérieux)	3
- Microbact 12A	2
- Microbact 12E	1

[1] *Many laboratories used more than one method.*

The AGI study did not specifically ask for identification methods so the results cannot be compared.

Yersinia species

Most of the laboratories were able to identify *Y. enterocolitica* (30/36, 83%), followed by *Y. pseudotuberculosis* (21/36 laboratories, 58%) (Table 33).

Table 33: Number of laboratories able to identify each *Yersinia* spp.

Yersinia spp.	No. laboratories
Y. enterocolitica	30
Y. pseudotuberculosis	21
Y. frederiksenii	18
Y. kristensenii	18
Y. intermedia	16
Y. pestis	9
Y. ruckeri	6
Y. aldovae	5
Y. rohdei	4
Y. bercovieri	4
Y. mollaretii	4
Y. aleksiciae	1

None of the laboratories were able to identify biotypes of *Y. enterocolitica*, but refer to ESR as requested.

Referral to the ERL

Twenty-three laboratories (64%) referred all *Yersinia* isolates to ESR and one laboratory referred all *Yersinia* isolates that were not *Y. enterocolitica*. The other laboratories reported criteria for referring *Yersinia* isolates to ESR:

- When requested by ESR (5 laboratories);
- For confirmation of unclear results (4 laboratories);
- For confirmation of *Y. pestis* (2 laboratories);
- Invasive isolates from blood (2 laboratories).

APPENDIX 10: ADDITIONAL PHU SURVEY RESULTS

Notifications

A summary of the notification sources and methods of notification is presented in Table 34.

Table 34: Number of respondents receiving notification from each source and method of notification for reporting by disease

Source	Communication method	Campylobacteriosis (n=15)	*L. monocytogenes* infection (n=14)	Salmonellosis (n=15)	VTEC/STEC infection (n=15)
ESR	*Total responses*[1]	*8*	*10*	*15*	*15*
	Hard copy report by fax	5	4	2	4
	Electronic notifications	7	10	14	14
	Hard copy report by post	4	5	6	10
	Phone call			1	2
General Practice	*Total responses*[1]	*15*	*12*	*15*	*12*
	Hard copy report by fax	12	9	15	11
	Electronic notifications	1	1		1
	Hard copy report by post	5	2	5	4
	Phone call	12	10	11	8
Hospital clinician	*Total responses*[1]	*15*	*14*	*15*	*12*
	Hard copy report by fax	6	7	8	5
	Electronic notifications	1	1	1	1
	Hard copy report by post	1	2	1	4
	Phone call	11	10	11	9
Local laboratory	*Total responses*[1]	*14*	*13*	*15*	*14*
	Hard copy report by fax	10	10	11	12
	Electronic notifications	5	6	7	5
	Hard copy report by post	4	6	6	7
	Phone call	3	4	5	4
Other	Total responses[1]	5	0	2	0

1. Some respondents reported more than one method for each source.

Additional comments were received regarding notification:

<u>Campylobacteriosis</u>
Four respondents made the following comments

- We only check if the case is in a high risk occupation. If the case is, then we would give advice. We would investigate an outbreak of campylobacter.

- The species is entered if we have that information.
- Usually only *Campylobacter jejuni* notified.
- We used to sometimes get species notified but we don't now.

L. monocytogenes infection
Two respondents noted that they have not had any notifications for some years.

Salmonellosis
One respondent noted that they "do not receive clinical notifications in the absence of confirmatory laboratory test results".

VTEC/STEC infection
Four respondents provided comments regarding issues with notification of cases with VTEC/STEC infection:

- We had a recent case notified to us from the lab saying a VTEC had been isolated. However subsequent ESR confirmatory analysis indicated that it was not a VTEC. This is an administrative/quality issue that we have discussed with the lab in terms of how they initially report the result.
- Diagnosis of VTEC/STEC in rural areas is confounded where GPs or hospital clinician have limited exposure to these illnesses making clinical assessment difficult. Following this the collection of biological samples for confirmation may not be taken or if done may not include screening fro VTEC/STEC.
- Mostly notifications come through E-notifications. Our local lab can identify *E. coli* O157:H7 by latex testing and will notify us only when they are sure of their results, but they cannot complete toxin testing.
- Please continue to notify any probable VTEC case as labs are currently doing.

Criteria for selective investigation of cases

For each pathogen, the PHUs were asked whether they investigated all cases or some cases infected with the pathogen, or whether they did not investigate cases infected with the pathogen. "Don't know" was also an option they respondents could select. The results are summarised in Table 4.

Some respondents indicated that only some notified cases of campylobacteriosis, non-typhoidal salmonellosis or non-O157 VTEC/STEC infection were investigated, and listed criteria used to decide if these cases should be investigated.

Campylobacteriosis cases: Five respondents investigated "some" campylobacteriosis cases. They reported the following criteria:

- Only high risk occupations.
- High risk groups – food handlers, early childhood centres, health care workers, water supply workers, etc.
- High risk (food handlers and under 5 year olds) are investigated by TLAs, non high risk are sent questionnaires by PHS.
- High risk categories as follows: Those who are
  - providing health care or support to the sick or elderly or
  - working or helping out in an Early Childhood Centre or playgroup or

  - handling or preparing food in a cafe/restaurant, food outlet etc or
  - a child that has this infection and goes to a playgroup or early childhood centre.
- An EHO will investigate those in a high risk group.

Salmonellosis cases: Two respondents reported investigating "some" non-typhoidal salmonellosis cases in the following situations:

- Children aged less than 5 years.
- Patient in a high risk group for transmission or outbreak associated case.

Cases of VTEC/STEC infection: Four respondents investigated "some" cases with VTEC/STEC infection. They reported the following criteria:

- Where the final typing is received after investigations have begun.
- We investigate all cases on initial notification. In some instances the case has turned out to be a non O157 VTEC.
- If clinically severe or if lab results report markers of pathogenicity.
- Investigation is initiated as soon as a lab result is confirmed as VTEC. The investigation continues until the lab confirms that the organism is not toxigenic.

Criteria for selective investigation of asymptomatic cases

For each pathogen, the PHUs were asked whether they investigated all asymptomatic cases or some asymptomatic cases infected with the pathogen, or whether they did not investigate asymptomatic cases infected with the pathogen. "Don't know" was also an option they respondents could select. The results are summarised in Table 5.

While several PHUs investigated all asymptomatic cases of campylobacteriosis, *L. monocytogenes* infection, salmonellosis or VTEC/STEC infection, some PHUs only investigated some of these cases and listed criteria used to decide if these cases should be investigated.

Campylobacteriosis cases: Four respondents investigated "some" asymptomatic cases. They reported the following criteria:

- Only cases with high risk occupations.
- The cases that are considered high risk i.e. those who are
  - Providing health care or support to the sick or elderly or
  - Working or helping out in an Early Childhood Centre or Playgroup or
  - Handling or preparing food in a cafe/restaurant, food outlet etc or
  - A child that has this infection and goes to a playgroup or Early childhood centre.
- Where it is not known that the case is asymptomatic until after investigation has commenced.
- To establish whether the case has symptoms or is linked to further symptomatic cases.

Cases with *L. monocytogenes* infection: Two respondents investigated "some" asymptomatic cases. They reported the following criteria:

- Cases may be related to a cluster.

- Where there may be an association with a case or we are ruling out a clinical symptoms.

Salmonellosis cases: Five respondents investigated "some" asymptomatic cases. They reported the following criteria:

- If associated with a cluster or other risk factors.
- We investigate all *Salmonella* cases & if on investigation we find that a case is asymptomatic we wouldn't investigate further but some investigation has occurred. If we were told at the time of notification they were asymptomatic we wouldn't investigate.
- All typhoidal *Salmonella* cases, regardless of symptoms; non-typhoidal *Salmonella* cases aged less than 5 years, regardless of symptoms. Regarding non-numbered question below: stool samples are requested from asymptomatic contacts of typhoidal *Salmonella* cases. If non-typhoidal *Salmonella*, stool samples are requested from contacts who are food handlers.
- Those where we receive the notification and only discover upon investigation that they were asymptomatic or are related to an outbreak.
- If case in high risk group.

Additionally, seven respondents reported requesting stool samples from asymptomatic contacts of a salmonellosis case.

Cases with VTEC/STEC infection: One respondent reported only investigating asymptomatic cases if laboratory results reported markers of pathogenicity.

Comments regarding follow up and investigation of notifications

Campylobacteriosis notifications:

- We normally only do postal questionnaires however from November last year to March this year we were taking part in a study where cases were phoned.
- All notified cases are sent a questionnaire to complete unless they are in a high risk occupation, are very young, have been notified previously or are part of family outbreak - these cases will be followed up by phone (sent a questionnaire if phone contact not made).
- We used to have issues with the turn around times when EHO's were investigating cases but we have taken all notified diseases cases back for investigation by the PHU.
- The majority of cases are sent a letter.
- We only follow up asymptomatic cases to the point when we identify that the case is asymptomatic case and there are no other associated cases we also assess the chances of an incidental finding associated with other hospital tests or conditions.
- Animal section of CRF needs to be reconsidered as not all animals are unwell when carrying *Campylobacter*.
- High risk cases are investigated the same day.

Notifications of *L. monocytogenes* infection:

- With respect to asymptomatic cases we do not know that is the case until investigation has commenced.
- Would always sample any suspect food remaining under refrigeration and send to ESR.

- Notifications are rare (2 respondents).

Salmonellosis notifications:

- Typing helpful. Only ask for stool sample in an asymptomatic contact who is a food handler of a case.
- Would be useful to have clear guidance around clearance criteria for high risk household contacts of non typhoid *Salmonella* cases.
- *Salmonella* Typhi - Close contacts faecal specs requested if no other source identified for the case.
- MOHs have clinical oversight where necessary and sign off on all *Salmonella* investigations undertaken.
- A questionnaire will be sent to *Salmonella* cases if all other attempts to contact the case fail.
- Time taken to be notified of a confirmed case of typhoid fever/paratyphoid fever from ESR may mean that a case is originally investigated as *Salmonella*, i.e. quite different questionnaires & approach.
- We use a questionnaire for interviews, but do not post this out.

Notifications of VTEC/STEC infection:
Three respondents reported issues with regard to timeliness of confirmation of results and decisions regarding when to begin an investigation:

- We have issues with some lab notifications & identifying from the results if they are in fact a case i.e. an interpretation issue. Some lab notifications end up being entered on EpiSurv as not a case following interpretation of the lab results. There can also be a delay in receiving notifications to follow up on i.e. specimen taken 23 Feb, received ESR 2 March, e-notification 8 March.
- There is one important part of VTEC notification that is not covered in the questions above. Knowing that full confirmation of VTEC by ESR takes some time to come through, we commence investigation of cases identified by the local laboratory to be both sorbitol-negative and O157 PCR-positive, in advance of ESR confirmation. This misses the minority that have VTEC but not O157, but these will ultimately be picked up by ESR. How is this addressed elsewhere in NZ?
- Would be good to have clarity around when an investigation should start i.e. undertake investigation from the initial lab notification or on receipt of a confirmatory result from the ESR reference lab.

Four respondents provided further details of their PHU protocols for investigation and follow up of cases:

- Cases and contacts and appropriate clearances/exclusions are followed up as per PHU protocol.
- Clearance specimens are required from all cases but this can sometime prove problematic with non-compliance. The same applies to clearances for contacts.
- All cases are investigated given this disease is rare and infrequent notification in our region.
  We have interpreted "investigation" as incorporating a simple check of commonalities between info collected to complete CRFs, through to completing an environmental

investigation if thought necessary to identify a potential source. A questionnaire for VTEC would be developed if there is a cluster of cases identified.

- We have stated we investigate non-symptomatic cases as we need to follow-up to see if they meet the case definition and they may be in a high risk group and or have high risk contacts.