

# Surveillance of Shiga toxigenic *Escherichia coli* in Australia

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## Abstract

All Australian States and Territories have low rates ( $\leq 0.32$  cases per 100,000 population) of notification for Shiga toxin-producing *Escherichia coli* (STEC), except for South Australia where the rates are ten-fold higher at 2.58 cases per 100,000 population. To explore possible reasons for the variation in rates we surveyed public health reference laboratories to determine the methods used and number of specimens tested for these organisms. Only five of eight jurisdictions routinely conducted testing for STEC, and polymerase chain based tests were most common. Culture was also common and in one jurisdiction that tests specimens with culture, approximately 1.2 per cent of specimens were positive. The notification rates for different jurisdictions reflected the number of specimens tested, with jurisdiction testing  $\leq 500$  specimens having rates  $\leq 0.32$  cases per 100,000 population. The use of culture as a test method may also influence notification rates. Public health agencies must consider the number of specimens tested in interpreting surveillance data. *Commun Dis Intell* 2005;29:366–369.

Keywords: diagnoses, *Escherichia coli*, Shiga toxin, surveillance

## Introduction

Shiga toxin-producing *Escherichia coli* (STEC) was first reported as a significant foodborne pathogen in the United States of America (USA) where it caused outbreaks of gastroenteritis associated with the consumption of undercooked beef mince in 1982.<sup>1</sup> In Australia, there have been eight reported outbreaks of STEC. The largest outbreak with 23 cases of haemolytic uraemic syndrome occurred in South Australia in 1995, as a result of *E. coli* O111:H contaminated mettwurst.<sup>2</sup> However, most STEC infections in Australia are sporadic with between 43–60 cases notified to health departments each year.<sup>3,4</sup> In 2001, the majority of jurisdictions reported low rates of STEC notification (0–0.4 STEC cases/100,000 population).<sup>5</sup> The exception was South Australia with a notification rate of 1.7 STEC cases per 100,000 population. South Australia's notification rate increased to this rate of notification following the introduction of testing of all bloody stools with a polymerase chain reaction test (PCR) in 1997, as a response to the large STEC outbreak in 1995.<sup>2</sup>

Reports have shown that different surveillance<sup>6</sup> and diagnostic methods<sup>7</sup> are critical factors in determining the number of STEC infections identified in the community. This report describes surveillance and diagnostic practices in Australian reference laboratories that may influence notification of STEC in Australia.

## The survey

We surveyed State and Territory reference laboratories in September 2003 with a semi-structured questionnaire on screening and diagnostic practices for STEC. OzFoodNet epidemiologists conducted face-to-face interviews with staff of reference laboratories for each Australian State and Territory. In Australia, most STEC screening and diagnosis is carried out in each jurisdictional reference laboratory. This information was collated and crude proportions calculated using Microsoft Excel.

## Results

Reference laboratories in three jurisdictions, Tasmania, the Australian Capital Territory and the Northern Territory reported that they do not carry out any testing for STEC. If STEC testing is requested these laboratories refer samples to other interstate reference laboratories.

Reference laboratories in Queensland, Western Australia, New South Wales and South Australia have standing requests for other laboratories in the jurisdiction to send stool samples for testing. The screening criteria for each of these reference laboratories to determine if a sample was tested for STEC were: if the test was requested by the physician; if the patient had clinical evidence of

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recent bloody diarrhoea; and/or if the stool sample had evidence of macroscopic blood. Western Australian laboratories also tested stool samples if there was evidence of blood microscopically. The number of samples tested for STEC during 2002, ranged from 141 samples in Queensland to 1,665 samples in South Australia (Table 1). Most of the 123 specimens tested in Victoria were presumptive STEC isolates sent from other laboratories.

Diagnostic methods varied between reference laboratories and are described in Table 2. The Queensland laboratory reported using a combination of PCR, chromogenic agar, sorbitol MacConkey agar, the Premier EHEC ELISA (Meridian Diagnostics, Inc) and a chromatography method using Duopath (Merck). The New South Wales laboratory used chromogenic agar and some regional laboratories also used either PCR, chromogenic agar, sorbitol MacConkey agar and washed sheep blood agar plates. Victoria used a number of methods including culture, immunological assays to detect toxin and PCR to test mainly

presumptive STEC isolates. The South Australian laboratory used PCR only and the Western Australian laboratory used sorbitol MacConkey agar only.

South Australia was the only jurisdiction that did not routinely attempt to culture for STEC isolates if a stool sample was positive (i.e. by PCR). Presumptive STEC isolates were confirmed by PCR methods in all jurisdictions. Toxin activity was also carried in tissue culture by Victoria and Western Australia. Flagella and phage typing was only available in the Victorian reference laboratory. Pulse field gel electrophoresis was carried out for cluster investigations by Queensland, Victorian and South Australian laboratories but no common method was used.

In Western Australia, approximately 1.2 per cent of the tested samples/specimens were positive for STEC. For other jurisdictions the percentage of tested samples/specimens that met jurisdictional screening criteria and were positive varied from 2.3 per cent to 6.9 per cent (Table 1).

**Table 1. Sample testing rates for STEC in Australian State Reference Laboratories**

Jurisdiction	Number of laboratories referring samples	Samples tested (2002)			Notification rate 2002 (per 100,000 population)
		Number of specimens tested	Number of specimens positive	Percentage of specimens positive	
Queensland	20	141	5	3.5	0.14
New South Wales	13	145	10	6.9	0.16
Victoria	12	123*	5	4.1	0.11
South Australia	4	1,665	39	2.3	2.58
Western Australia	Not available	500†	6	1.2	0.32

\* Most of these specimens were presumptive isolates.

† Approximate number only.

**Table 2. Laboratory methods commonly used (80–100% of the time) by Jurisdiction Reference Laboratories to identify STEC in faeces**

Laboratory method used	State				
	NSW	Qld	SA	WA	Vic
ELISA detection of toxin in stool		✓			
Pre-enrichment (PES) of stool			✓		✓
Culture on Sorbitol MacConkey agar		✓		✓	✓
PCR for <i>stx1</i> & <i>stx2</i> of stool					✓
PCR for <i>stx1</i> & <i>stx2</i> of PES		✓	✓		✓
Immunological toxin detection		✓			✓
Toxin detection in isolate (Duopath)		✓			
Chromogenic agar culture	✓	✓			

## Discussion

There is a wide range of diagnostic practices used for testing for STEC among jurisdictional reference laboratories in Australia. These practices lead to vastly different notification rates between individual jurisdictions, ranging from 0.14 to 2.58 cases per 100,000 population.

Although many jurisdictions had the same screening criteria for testing samples and request that other laboratories send bloody stools for STEC screening, there is a large range in the number of specimens tested, from 123 in Victoria to 1,665 specimens tested in South Australia. The difference in the numbers of specimens tested could be due to a number of reasons, including whether the reference laboratory conducts primary diagnosis (South Australia, Western Australia), which would allow easier access to specimens for testing.

The percentage of tested samples/specimens that were positive for STEC in Australia varied from 1.2 per cent to 6.9 per cent, which may be due to the origin of specimens, whether they are presumptive isolates, and the diagnostic method used. The types of diagnostic tests used ranged from culture only in Western Australia and in New South Wales to a range of methods including culture, immunological methods to detect toxin and PCR methods in Queensland and Victoria. It has been reported that culture methods for detection of STEC are less sensitive than other methods such as PCR or the Premier EHEC ELISA.<sup>7,8</sup> This may explain part of the reason why in Western Australia, where specimens were tested by culture, there was a low proportion of specimens testing positive for STEC. However, Western Australia did have a higher notification rate ( $\leq 0.32$  cases per 100,000 population) than Queensland, Victoria and New South Wales, which is likely due Western Australia testing a much larger number of stool specimens than these other jurisdictions. South Australia had the highest notification rate of 2.58 cases per 100,000 population, which could be due to the testing of all bloody stools with a PCR based method. South Australia and the Hunter Valley region of New South Wales obtained similar proportions of samples positive for STEC when using similar PCR-based methods.<sup>9</sup>

The differences in notification rates between jurisdictions could also be in part due to real differences in prevalence. This has been observed in the USA, with northern states having a higher prevalence of STEC O157:H7 than southern states, which may be associated with large rural populations in northern states and contact with farm animals.<sup>7</sup> There could also be other differences that may affect notification rates including variability in health care systems, access to

medical care, farm animal husbandry practices and susceptibility of the population (younger and older age groups are at higher risk of STEC infection).

With the exception of South Australia, most rates of STEC notification in Australian jurisdictions are lower than those reported internationally, including the USA<sup>10</sup> and Wales.<sup>11</sup>

This survey of reference laboratories and STEC cases notified in Australia indicate that the number of stool samples tested and the sensitivity of the diagnostic test may explain much of the variability in notification rates between jurisdictions.

Since this survey, some jurisdictions have made changes to the surveillance of STEC. In the first half of 2004 and January 2005 respectively, Western Australia and Victoria started using PCR to diagnose STEC in stool samples. Victoria also increased the number of stools tested. New South Wales is proposing similar changes. These changes are likely to increase notification rates and should also give a better understanding of the prevalence of STEC in Australia.

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## References

1. Riley LW, Remis RS, Helgerson SD, McGee HB, Wells JG, Davis BR, *et al.* Hemorrhagic colitis associated with a rare *Escherichia coli* serotype. *N Engl J Med* 1983;308:681–685.
2. Community outbreak of haemolytic uremic syndrome attributable to *Escherichia coli* O111:NM—South Australia, 1995. *MMWR Morb Mortal Wkly Rep* 1995;44:550–551, 557–558.
3. Australian Government Department of Health and Ageing. Communicable Diseases Network Australia—National Notifiable Diseases Surveillance System. Notifications of STEC/VTEC, Australia 1996–2002. Australian Government Department of Health and Ageing. Canberra; 2003.
4. OzFoodNet Working Group. Foodborne disease investigation across Australia: annual report of the OzFoodNet network, 2003. *Commun Dis Intell* 2004;28:359–389.

5. OzFoodNet Working Group. Enhancing foodborne disease surveillance across Australia in 2001: the OzFoodNet Working Group. *Commun Dis Intell* 2002;26:375–406.
6. Gavin PJ, Thomson Jr RB. Diagnosis of enterohemorrhagic *Escherichia coli* infection by detection of Shiga toxins. *Clin Micro Newsletter* 2004;26:49–54.
7. Fey PD, Wickert RS, Rupp ME, Safranek TJ, Hinrichs SH. Prevalence of non-O157:H7 Shiga toxin-producing *Escherichia coli* in diarrheal stool samples from Nebraska. *Emerg Infect Dis* 2000;6:530–533.
8. Mackenzie AM, Lebel P, Orrbine E, Rowe PC, Hyde L, Chan F, *et al*. Sensitivities and specificities of premier *E. coli* O157 and premier EHEC enzyme immunoassays for diagnosis of infection with verotoxin (Shiga-like toxin)-producing *Escherichia coli*. The SYNSORB Pk Study investigators. *J Clin Microbiol* 1998;36:1608–1611.
9. Doyle R, Watson K, Unicomb LE, Lancer JA, Wise R, Ratcliff R, *et al*. Laboratory surveillance of Shiga toxin producing *Escherichia coli* in South Australia and the Hunter Health Area, New South Wales, Australia. *Commun Dis Intell* 2004;28:390–391.
10. Preliminary FoodNet data on the incidence of foodborne illnesses—selected sites, United States, 2000. *MMWR Morb Mortal Wkly Rep* 2001;50:241–246.
11. Chalmers RM, Parry SM, Salmon RL, Smith RM, Willshaw GA, Cheasty T. The surveillance of vero cytotoxin-producing *Escherichia coli* O157 in Wales, 1990 to 1998. *Emerg Infect Dis* 1999;5:566–569.