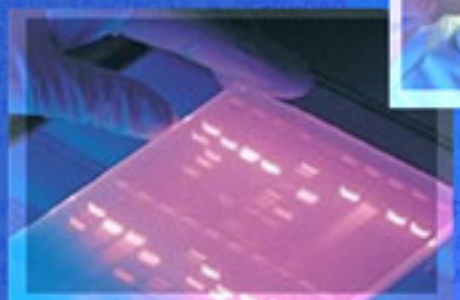
Division of Enteric, Foodborne and Waterborne Diseases

Report of the 2001

Canadian Laboratory Survey

National Studies on Acute Gastrointestinal Illness

NSAGI



Health Canada

Report of the 2001 Canadian Laboratory Study

National Studies on Acute Gastrointestinal Illness

Division of Enteric, Foodborne and Waterborne Diseases

PPHB, CIPDC, Health Canada

Report prepared by James Flint

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EXECUTIVE SUMMARY

Passive surveillance systems are particularly sensitive to the limitations associated with case loss (the failure to capture all community cases for a given disease). Quantifying this loss and identifying factors that influence the likelihood of counting cases are important steps towards enhancing the interpretation of past and current surveillance data and developing strategies to improve the surveillance system itself. The NSAGI Canadian Laboratory Study is one in a series of studies examining public health reporting within the Canadian enteric disease surveillance system.

The NSAGI Canadian Laboratory Survey was administered to 470 microbiology laboratories across Canada; 408 (87%) responded. This study identified a number of inter-laboratory and inter-provincial/territorial variations in criteria for testing stool specimens. A small number (3%) of specimens were rejected because no transport media was used, the stool was fully formed or the container was damaged or contaminated. Routine testing for *Salmonella*, *Shigella*, *Campylobacter, E. coli and* Y*ersinia* was common with 100%, 99%, 97% 96% and 95% respectively of laboratories routinely testing for these bacteria. Other pathogens, such as *Plesiomonas* and *Vibrio,* were routinely tested by fewer laboratories (54% and 38% respectively). Differences in laboratory policies regarding (a) the testing of repeat specimens, (b) testing specimens from inpatients who have been hospitalised over a certain length of time, (c) testing fully formed stool specimens and (d) testing stool received without transport media were noted. When comparing the effect of these policies on the likelihood of identifying enteric pathogens, there were few statistically significant relationships.

Overall, participating laboratories tested (culture and/or molecular methods, including toxin detection) 459 982 stool specimens for enteric bacterial pathogens (excluding *C. difficile*) in the year 2000. In comparison, 392 023 stool specimen were examined for enteric parasites, 177 696 for *C. difficile* and 14 051 for enteric viruses. Of the laboratories testing for viruses, 74% indicated never testing for astrovirus and 69% never testing for small round structured viruses (SRSV), caliciviruses, Norwalk or Norwalk-like viruses. On average, 5.0%, 7.6%, 15.3% and 18.9% of stool specimens tested for bacteria, parasites, *C. difficle* and viruses respectively, were positive. The overall proportion of *tests* positive for a bacterial, parasitic or viral pathogen was 8.8% (the sum of all positive isolations divided by the sum of all specimens tested for bacteria, parasites and viruses). Assuming that the total number of cases submitting stool is equal to the total number of stools submitted for bacterial testing, the overall proportion of *cases* for which a pathogen was identified was 29.4% (sum of all positive isolations divided by number of stools submitted for bacterial testing).

The pathogen yield from stool found in this study is in line with other international studies. American and European studies examining stool from inpatients admitted with AGI have documented positivity rates for enteric bacteria ranging from 1.2% - 6.1% (Bauer *et al*, 2001; Fan *et al*, 1993; Rohner *et al*, 1997; Zaidi *et al*, 1999). Community based studies in the Netherlands and UK found higher bacterial positivity rates of 16% and 19.5% respectively (Matty de Wit *et al*, 2001, Wheeler *et al*, 1999). While enhanced stool sampling and testing in the UK resulted in 47.8% of stools testing positive for bacterial pathogens (Tompkins *et al*, 1999).

Future research should concentrate on improving pathogen yield to maximize treatment efficacy and surveillance value.

Table Of Contents

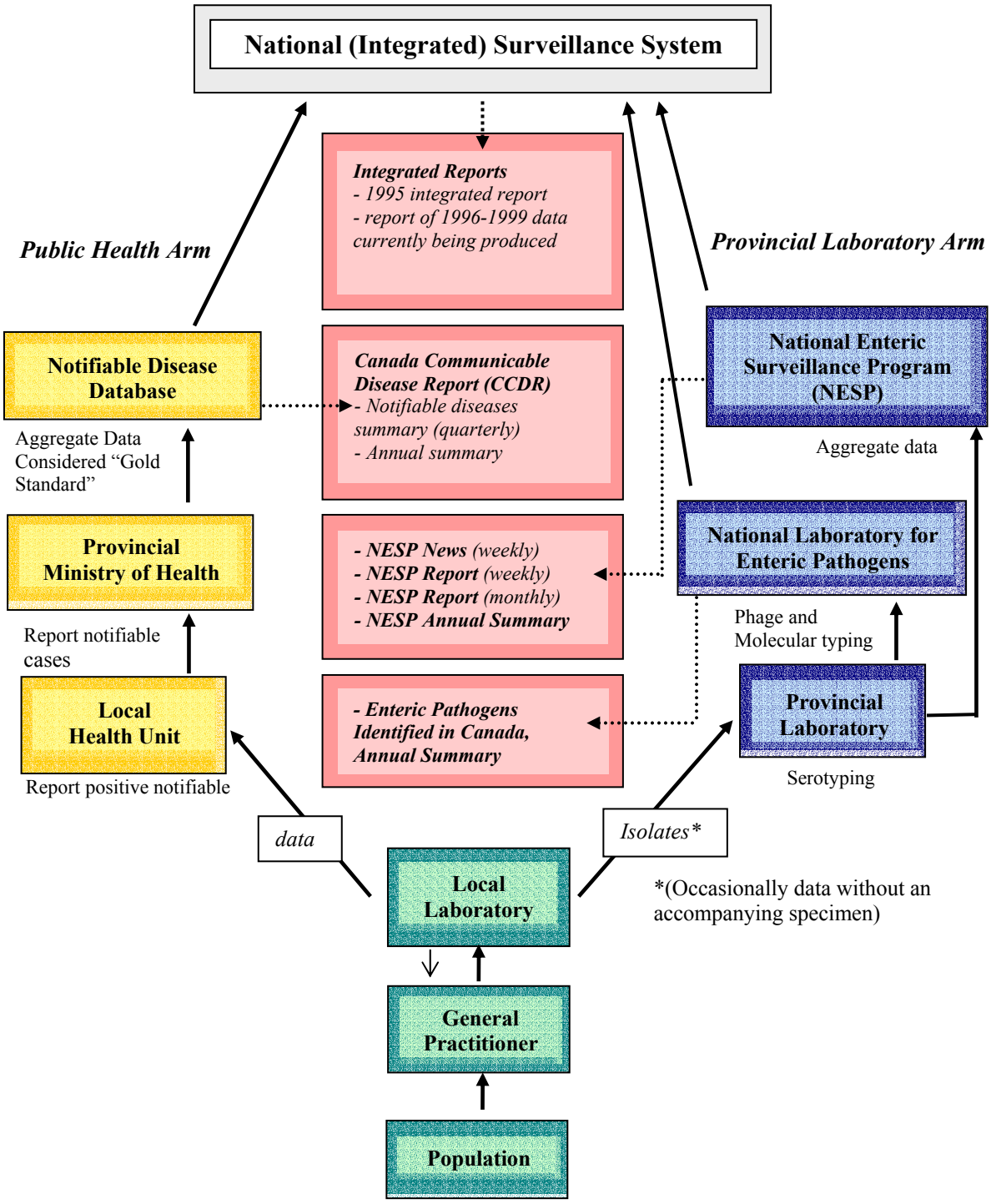
1. INTRODUCTION

Accurate estimates of enteric disease prevalence and burden are central to the development of sound public health policies. There are, however, limitations associated with passive surveillance systems that compromise the accuracy and quality of the data used to make policy decisions. The under-reporting of disease magnitude and the variation in factors that affect the likelihood of a case entering and being retained in the surveillance system are important limitations. Health Canada's National Studies on Acute Gastrointestinal Illness (NSAGI) aims to address these limitations by gathering data to understand the variables influencing the proportion of cases captured and quantifying the proportion of cases lost at various points in the surveillance system. This report summarizes the findings of the 2001 Canadian Laboratory Survey; a study focusing on the laboratory interface of Canada's national enteric disease surveillance system.

1.1 Where are Cases 'Lost'?

The Canadian national enteric disease surveillance system has two arms, the public health arm and the provincial laboratory arm (Figure 1). For a case to reach the national level, a number of critical steps must be taken. The patient with an acute gastrointestinal illness (AGI) must consult a physician, the physician must request a stool specimen and the patient must comply in providing a specimen. In turn, the specimen must be transported to the laboratory and test positive for an enteric pathogen that is reportable within the province. From here, the system diverges. For the case to be captured by the public health arm, the laboratory must report the positive isolation to a local health authority directly, or via a physician, who will then report the case to the province and the province to the national level. The strength of the Public Health arm lies in the additional epidemiological information collected by local health authorities. The provincial laboratory arm on the other hand, requires the front line laboratory to forward the isolate (or in some cases the data without the isolate) to the provincial laboratory. Following additional testing on the isolate, the provincial laboratory then reports the result to the national enteric surveillance program. Its strength rests in the additional microbiological or molecular characterization of the pathogen implicated in the infection. The front line laboratory receiving the stool specimens is pivotal in deciding if a case is included or excluded in either arm of the national surveillance system.

Figure 1. Canada's national enteric surveillance systems: flow of information and end reports produced



1.2 Case Loss at the Laboratory Interface

As case definitions for nationally reportable diseases require a laboratory confirmed diagnosis, a patient will not be retained in the system unless a positive identification is made. This is generally true for provincial surveillance systems as well, although, in some instances symptom based diagnoses may be reportable. The following are reasons why a stool specimen submitted from a patient with symptoms of AGI may result in a negative finding;

- *the symptoms of AGI may have resulted from a non-infectious cause,*
- *the pathogens may have died during specimen transport (excessive time delays during transport or inappropriate transport conditions will impact on pathogen survival),*
- *the stool specimens may arrive in a condition unsuitable for testing at the laboratory (i.e. the container may be damaged or the specimen contaminated),*
- *the number of pathogens in the stool may be below the sensitivity threshold of the methods used in the laboratory,*
- *the laboratory may not test the stool specimen because of established protocol (i.e. if the stool came from an inpatient* hospitalised *for more than 3 days etc.),*
- *the laboratory may not look for the pathogen responsible for the illness,*
- *there may be human or mechanical error during laboratory testing or interpretation of results, and*
- *in the case of co-infection, testing may end following identification of the first pathogens resulting in a negative finding secondary pathogen(s).*

Even when an enteric pathogen is found in a stool specimen, there may be situations where the health units are not notified of the finding or the provincial laboratory is not forwarded the isolate. This will also result in case loss.

Many of the testing and reporting policies that influence the likelihood of a case being captured in the public health or provincial laboratory arms are developed by each individual laboratory. Variations in these policies between laboratories or over time will introduce reporting biases into the surveillance system.

1.3 The NSAGI Laboratory Survey Objectives

The NSAGI Laboratory Survey is one of four studies making up the first component of the NSAGI project. The primary aims of the 2001 Canadian Laboratory Study are as follows:

a) *Quantify the proportion of stool specimens that are positive for an enteric pathogen, and*

b) *Examine inter-laboratory variations in key factors influencing whether an etiological agent is identified as it passes through the laboratory interface and understand how such variations may affect the interpretation of surveillance data.*

2. METHODS

2.1 Survey Development

A draft version of the NSAGI Laboratory Survey was developed after reviewing similar surveys conducted in the United States and previous studies conducted by Health Canada. Following internal evaluation, the draft survey was sent to all provincial laboratory directors and other national and international experts for comment. After pre-testing, the survey was piloted in a single province. Following a review of the survey instrument, the survey was administered to all other provinces and territories.

The survey was translated into French by designated Health Canada translators and reviewed by bilingual microbiology experts at the Quebec Provincial Laboratory, the Laboratory for Foodborne Zoonoses and the Division of Enteric, Foodborne and Waterborne Diseases. Copies of the English or French surveys are available on request.

2.2 Provincial Collaboration

In addition to providing input into the survey content and design, the provincial laboratory directors were invited to co-sign a cover letter accompanying the survey.

2.3 Sampling Frame

The names and contact details for all Canadian laboratories licensed to do microbiological testing on stool specimens were requested from provincial licensing bodies in 2000. Following the compilation of a complete national database, each laboratory was contacted by telephone to identify an appropriate survey respondent. This initial telephone conversation also served as a pre-study introduction. Provincial laboratories were not requested to complete the questionnaire as the focus was on primary isolation laboratories.

2.4 Data Management and Analysis

Two separate databases were managed. One contained contact information and response dates. The other contained data from the surveys. No personal or laboratory identifiers were recorded in the second database to ensure confidentiality.

Databases were stored within a secure building and on a password protected network. Back-up disks were kept in a locked filing cabinet.

Data from the surveys were entered into EpiData v. 2.0 (The EpiData Association Odense Denmark 2001). Analysis and data presentation were performed using S-PLUS 2000 (Mathsoft Inc), SAS (SAS Institute Inc) and Microsoft Excel 97 (Microsoft Corporation).

3. RESULTS

3.1 Survey Response Rate

A total of 536 laboratories licensed to perform microbiological tests on stool specimens were identified. Following telephone contact, 66 laboratory representatives indicated that, although licensed to perform microbiology on stool specimens, their laboratory did not receive any stool specimens in the year 2000. Of the 470 remaining laboratories, 87% (n=408) responded to the survey. The response rate by province/territory ranged from 76%-100%.

3.2 General Information about Responding Laboratories

Of the 408 responding laboratories, the majority (87.3%, n=356) were hospital-based laboratories while 9.8% (n=40) were private, 2.7% (n=11) were 'other' and 0.2% (n=1) did not respond to this question. 'Other' laboratories included clinics or health care centres (n=6), regional laboratories (n=2), a long-term care facility (n=1), a mental care centre (n=1) and a referral centre (n=1).

When asked to describe the population served by their laboratories, 77% of all laboratories indicated primary care patients, 58% indicated patients consulting private physicians, 22% indicted tertiary care patients and 6% indicated 'other'. Other populations served included emergency patients, health service personnel, intermediate and long term care residents, people living on reserves and people living in institutions.

3.3 Referral and Rejection of Stool Specimens

3.3.1 Referral of stool specimens

The percentage of laboratories indicating that they received stool specimens and performed on-site testing on all or a percentage of the specimens is summarized in Table 1. When comparing testing capabilities, hospital laboratories more frequently performed on-site testing (culture and/or molecular methods, including toxin detection) of enteric parasites and viruses than private laboratories or clinics.

Table 1. Laboratories involved in on-site testing of all or a portion of stool specimens

	All Laboratories (n=408)		Hospital Laboratories (n=365)		Private Laboratories (n=40)		Other Laboratories (n=11)	
	%	number	%	number	%	number	%	number
Bacteria	67	274	66	241	75	30	27	3
Parasites	31	126	30	107	18	18	9	1
Viruses	10	42	11	39	2.5	1	18	2

Of the laboratories doing on-site testing, 85%, 49% and 38% of laboratories indicated testing *all* of stool specimens for bacteria, parasites and viruses respectively (Table 2). For bacteria, the 15% of laboratories that did not do on-site testing on all specimens referred on average 12% (range 1%-60%) of specimens. The 51% not testing all specimens for parasites, referred on average 6.4% (range 1%–90%) of specimens and the 62% not testing all specimens for viruses referred on average 51% (range 1%–99%) of specimens.

Table 2. The number and percentage of laboratories that do onsite testing and test 100% of specimens received by their laboratory.

	Bacteria		**Parasites**		**Viruses**	
	%	number	%	number	%	number
All	**85%**	**233**	**49%**	**62**	**38%**	**16**
Hospital	86%	206/241	46%	49/107	36%	14/39
Private	87%	26/30	67%	12/18	0%	0/1
Other	33%	1/3	100%	1/1	100%	2/2

When laboratories referred stool specimens to a second laboratory, 59%, 55% and 46% respectively indicated always receiving positive results for bacteria, parasites and viruses reported back to them (Table 3). Less than 10% of laboratories indicated never having positive isolations reported to them when referring specimens.

Table 3. Summary of the frequency with which a laboratory referring specimens receives reports on positive isolations.

	Always (100%)	Routinely (80-99%)	Sometimes (20-79%)	Rarely (1-19%)	Never	Don't know
Bacteria	59%	6%	7%	15%	6%	8%
Parasites	55%	9%	6%	17%	3%	10%
Viruses	48%	5%	5%	15%	6%	22%

The majority of laboratories indicated that when a specimen was referred to another laboratory, they were not responsible for reporting positive findings to a local health unit/authority (Table 4). In some cases, the primary laboratory or both laboratories were identified are being responsible for reporting.

Table 4. Laboratory responsible for reporting positive isolations of 'reportable pathogens' when a specimen referral has been made.

	Primary Lab* Reports (%)	Secondary Lab‡ reports (%)	Primary & Secondary Lab report (%)	Don't know (%)
Bacteria	8	68	15	8
Parasites	14	60	16	6
Viruses	18	54	15	9

* primary lab is the laboratory that first receives the stool specimen.
‡ secondary lab is the laboratory isolating the pathogen.

Table 5 summarizes the percentage of laboratories that always, routinely, sometimes, rarely and never receive positive results from secondary laboratories when they (a) refer 100% of stool specimens and (b) indicate that they are responsible for reporting positive identification to the local health authority.

Table 5. Reporting of positive results to primary laboratory when primary laboratory refers all specimens and reports positive findings to health authority

	Bacteria (n=31)		**Parasites (n=84)**		**Viruses (n=117)**	
	percentage	number	percentage	number	percentage	number
Always (100%)	64.5%	20	66.7%	56	45.3%	53
Routinely (80-99%)	6.5%	2	4.8%	4	6.8%	8
Sometimes (20-79%)	6.5%	2	4.8%	4	6.8%	8
Rarely (1-19%)	19.4%	6	16.7%	14	14.5%	17
Never (0%)	0.0%	0	0.0%	0	1.7%	2
Don't know	3.2%	1	6.0%	5	17.1%	20
Missing	0.0%	0	1.2%	1	7.7%	9

3.3.2 Referral of bacterial isolates to provincial laboratories

Overall, 94% (n=258) of laboratories across all provinces indicated referring isolates to the provincial public health laboratory. *Salmonella. E. coli and Shigella* were the most commonly referred pathogens with 84%, 79% and 70% of laboratories sending *some* portion of their non-outbreak isolates. The *frequency* with which each laboratory sends isolates is summarized in Table 6.

The percentage and number of laboratories sending *Campylobacter*, *E. coli*, *Salmonella* and *Shigella* isolates to their provincial laboratory, broken down by hospital versus private laboratory, is shown in Table 7.

Table 6. Percentage and number of laboratories sending the following enteric isolates to their provincial laboratory (n-outbreak = non outbreak related cases).

		Always 100%		*Routinely* 80-99%		*Sometimes* 20-79%		*Rarely* 1-19%		*Never* 0%		*Don't know*		*Missing*		*NA**	
		%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n
Aeromonas	n-outbreak	9.9%	27	0.4%	1	0.4%	1	6.9%	19	30.3%	83	4.4%	12	34.3%	94	13.5%	37
	outbreak	10.2%	28	0.0%	0	0.0%	0	0.7%	2	10.6%	29	7.3%	20	55.8%	153	15.3%	42
Campylobacter	n-outbreak	15.0%	41	0.4%	1	0.7%	2	22.3%	61	28.8%	79	4.7%	13	25.2%	69	2.9%	8
	outbreak	16.4%	45	0.4%	1	0.0%	0	4.7%	13	12.0%	33	8.8%	24	51.8%	142	5.8%	16
Clostridium	n-outbreak	0.7%	2	0.0%	0	0.0%	0	0.4%	1	9.9%	27	4.0%	11	38.3%	105	46.7%	128
	outbreak	2.6%	7	0.0%	0	0.0%	0	0.0%	0	4.0%	11	4.4%	12	43.1%	118	46.0%	126
E. coli O157	n-outbreak	60.6%	166	0.7%	2	0.4%	1	11.7%	32	3.6%	10	4.0%	11	11.3%	31	7.7%	21
	outbreak	38.3%	105	0.7%	2	0.4%	1	2.6%	7	2.2%	6	7.7%	21	39.1%	107	9.1%	25
E. coli (other)	n-outbreak	2.2%	6	0.0%	0	0.0%	0	0.7%	2	5.1%	14	2.6%	7	7.7%	21	81.8%	224
	outbreak	1.5%	4	0.0%	0	0.0%	0	0.4%	1	1.8%	5	3.3%	9	11.3%	31	81.8%	224
Plesiomonas	n-outbreak	10.6%	29	0.0%	0	0.0%	0	3.6%	10	29.2%	80	4.4%	12	25.9%	71	26.3%	72
	outbreak	10.9%	30	0.0%	0	0.0%	0	0.4%	1	9.5%	26	7.7%	21	45.6%	125	25.9%	71
Salmonella	n-outbreak	63.9%	175	3.3%	9	2.2%	6	13.5%	37	1.8%	5	5.5%	15	8.8%	24	1.1%	3
	outbreak	38.7%	106	0.7%	2	0.4%	1	3.3%	9	2.9%	8	7.3%	20	44.2%	121	2.6%	7
Shigella	n-outbreak	56.2%	154	1.5%	4	1.5%	4	9.9%	27	9.9%	27	5.1%	14	14.2%	39	1.8%	5
	outbreak	36.1%	99	1.5%	4	0.7%	2	0.4%	1	5.1%	14	8.0%	22	45.6%	125	2.6%	7
Vibrio	n-outbreak	24.8%	68	0.4%	1	0.4%	1	5.1%	14	9.1%	25	4.7%	13	19.7%	54	35.8%	98
	outbreak	16.1%	44	0.4%	1	0.0%	0	0.4%	1	4.7%	13	6.9%	19	34.3%	94	37.2%	102
Yersinia	n-outbreak	38.3%	105	0.0%	0	0.0%	0	8.0%	22	21.5%	59	5.1%	14	24.1%	66	2.9%	8
	outbreak	26.6%	73	0.0%	0	0.0%	0	0.4%	1	8.8%	24	8.8%	24	51.8%	142	3.6%	10

**Not applicable, the laboratory does not test for this pathogen*

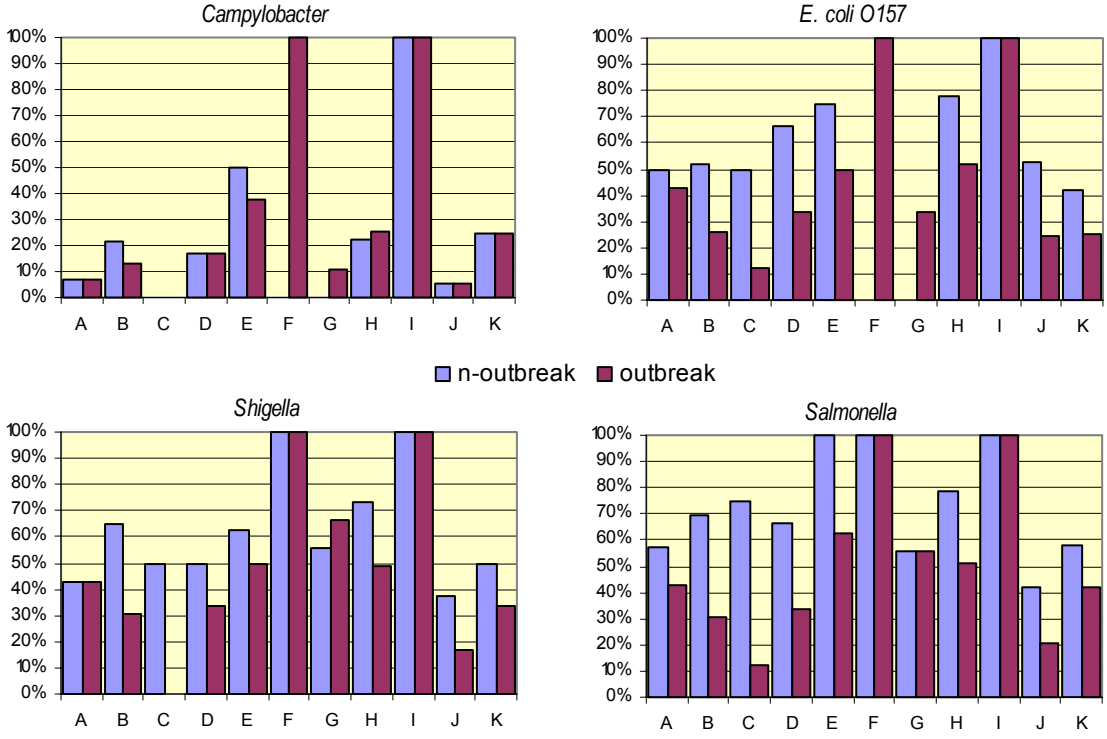
Table 7. Percentage and number of hospital based laboratories (H) and private laboratories (P) sending the following isolates to their provincial laboratory

			Always 100%		*Routinely* 80-99%		*Sometimes* 20-79%		*Rarely* 1-19%		*Never* 0%		*Don't know*		*Missing*		*NA**	
			%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n
Campylobacter	H	n-outbreak	16.2%	39	0.4%	1	0.8%	2	20.3%	49	29.0%	70	5.4%	13	25.3%	61	2.5%	6
		outbreak	18.3%	44	0.4%	1	0.0%	0	3.7%	9	12.9%	31	10.0%	24	49.4%	119	5.4%	13
	P	n-outbreak	6.1%	2	0.0%	0	0.0%	0	36.4%	12	27.3%	9	0.0%	0	24.2%	8	6.1%	2
		outbreak	3.0%	1	0.0%	0	0.0%	0	12.1%	4	6.1%	2	0.0%	0	69.7%	23	9.1%	3
E. coli O157	H	n-outbreak	60.6%	146	0.8%	2	0.4%	1	11.6%	28	3.7%	9	4.6%	11	10.8%	26	7.5%	18
		outbreak	40.2%	97	0.8%	2	0.4%	1	2.5%	6	2.5%	6	8.7%	21	36.1%	87	8.7%	21
	P	n-outbreak	60.6%	20	0.0%	0	0.0%	0	12.1%	4	3.0%	1	0.0%	0	15.2%	5	9.1%	3
		outbreak	24.2%	8	0.0%	0	0.0%	0	3.0%	1	0.0%	0	0.0%	0	60.6%	20	12.1%	4
E. coli (other)	H	n-outbreak	2.1%	5	0.0%	0	0.0%	0	0.8%	2	5.0%	12	2.9%	7	7.9%	19	81.3%	196
		outbreak	1.7%	4	0.0%	0	0.0%	0	0.4%	1	2.1%	5	3.7%	9	10.4%	25	81.7%	197
	P	n-outbreak	3.0%	1	0.0%	0	0.0%	0	0.0%	0	6.1%	2	0.0%	0	6.1%	2	84.8%	28
		outbreak	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	18.2%	6	81.8%	27
Salmonella	H	n-outbreak	63.9%	154	3.7%	9	2.1%	5	12.9%	31	2.1%	5	6.2%	15	8.3%	20	0.8%	2
		outbreak	41.1%	99	0.8%	2	0.4%	1	3.3%	8	3.3%	8	8.3%	20	40.7%	98	2.1%	5
	P	n-outbreak	63.6%	21	0.0%	0	3.0%	1	18.2%	6	0.0%	0	0.0%	0	12.1%	4	3.0%	1
		outbreak	21.2%	7	0.0%	0	0.0%	0	3.0%	1	0.0%	0	0.0%	0	69.7%	23	6.1%	2
Shigella	H	n-outbreak	56.4%	136	1.7%	4	1.2%	3	8.3%	20	10.4%	25	5.8%	14	14.9%	36	1.2%	3
		outbreak	38.6%	93	1.7%	4	0.8%	2	0.0%	0	5.4%	13	9.1%	22	42.7%	103	1.7%	4
	P	n-outbreak	54.5%	18	0.0%	0	3.0%	1	21.2%	7	6.1%	2	0.0%	0	9.1%	3	6.1%	2
		outbreak	18.2%	6	0.0%	0	0.0%	0	3.0%	1	3.0%	1	0.0%	0	66.7%	22	9.1%	3

**Not applicable, the laboratory does not test for this pathogen*

The frequency of sending *all Campylobacter*, *E. coli* O157, *Shigella* and *Salmonella* isolates to the provincial laboratory, by province, is shown in Figure 2.

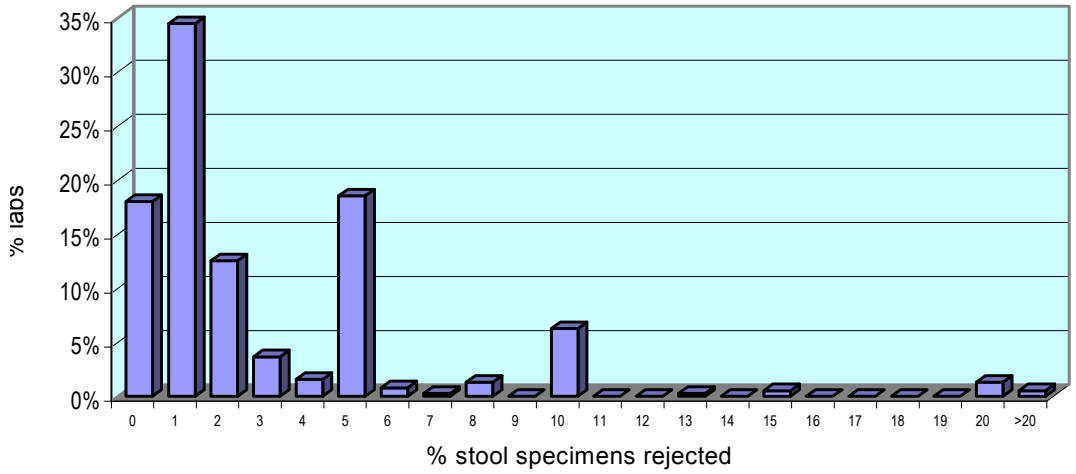
Figure 2. Laboratories sending all isolates (non-outbreak and outbreak) to the provinces; comparison between provinces. (Provinces represented by A – K).



3.3.3 Bacteria – rejection of stool specimens

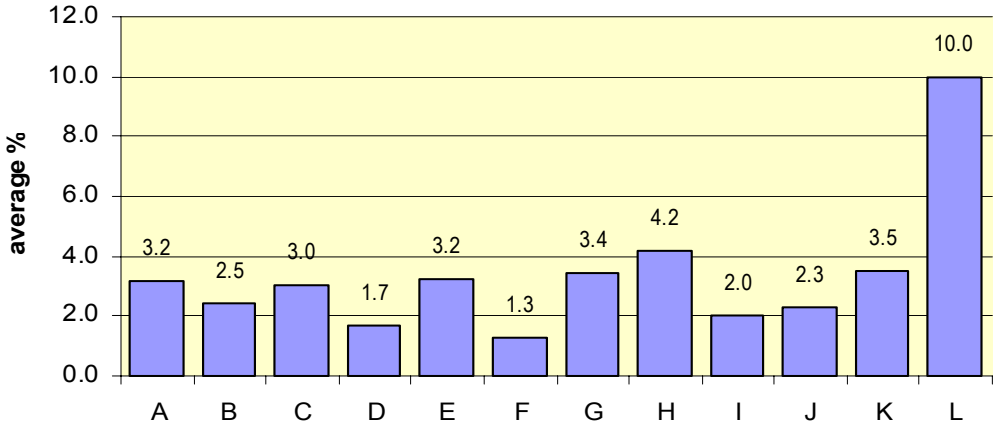
Stool specimens that arrive at a laboratory may be rejected without culturing or referral for several reasons (e.g., not enough stool was provided, the stool collection container arrived damaged, there was an excessive time delay between collection and receipt at laboratory, the specimen arrived without transport media). On average, approximately 3.1% (range 0% – 50%) of all stool specimens that arrived at a laboratory were rejected without testing or referral. The average proportion of stools rejected only differed slightly depending on whether the laboratory tested 100% of stool specimens received or referred 100% of stool specimens (3.4% and 3.0% respectively). Figure 3 shows an approximate percentage of stool specimens that are received by Canadian laboratories each month and are rejected without culturing or referral.

Figure 3. Percentage of stool specimens rejected when they arrive at the laboratory



The variation in the average percentage of stool specimens rejected by province is shown in Figure 4.

Figure 4. Average percentage of stool specimens rejected when they arrive at the laboratory, comparison by province (A-L represents provinces/territories).



The laboratories were asked how often stool specimens were received with (a) transport media, (b) without transport media but on ice or refrigerated and (c) without transport media, ice or refrigeration. The results from outpatients and inpatients are summarized in Figure 5 and the data for outpatients alone is summarized in Figure 6.

Figure 5. Percentage of laboratories that receive stool specimens with transport media, without transport media, on ice/refrigerated and without transport media, ice or refrigeration from outpatients and inpatients (n=274).

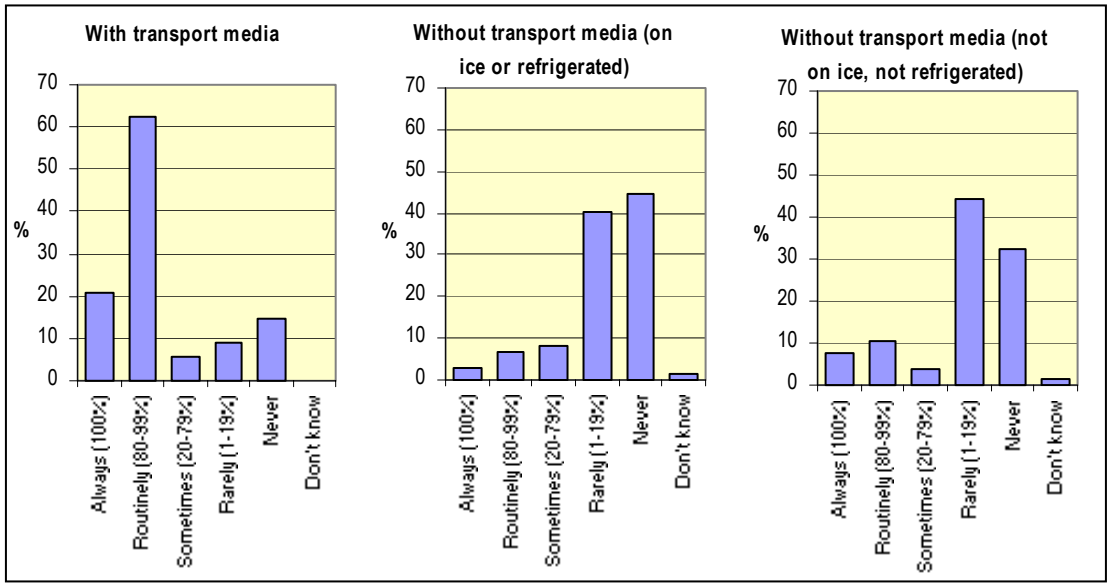
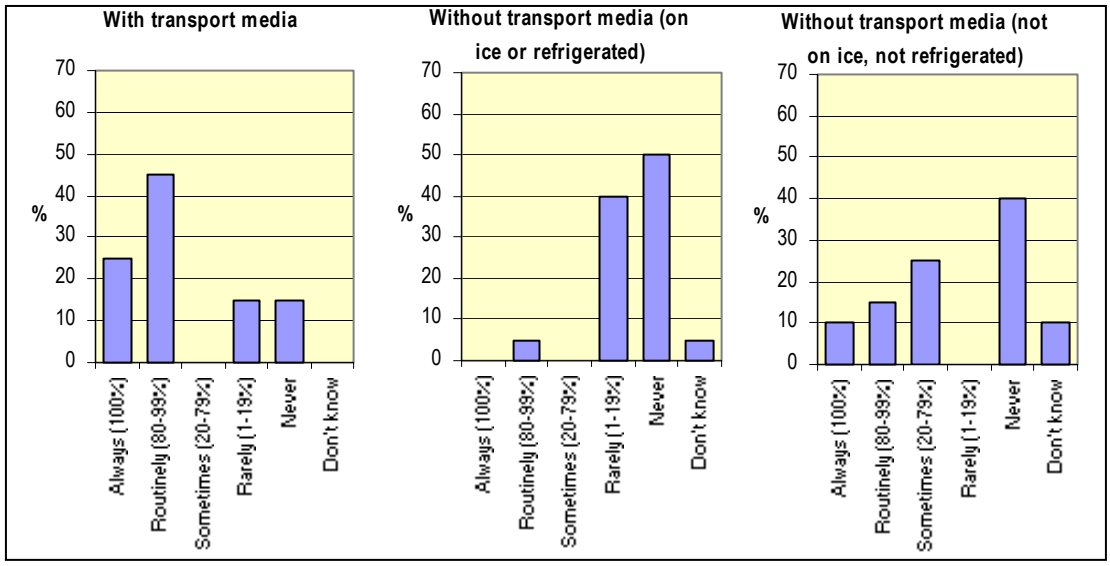


Figure 6. Percentage of laboratories that receive stool specimens with transport media, without transport media, on ice/refrigerated and without transport media, ice or refrigeration from outpatients only (n=20).



When a laboratory receives a stool specimen from an outpatient without transport media, usual routine tests are performed by 40% of laboratories. Thirty percent of laboratories performed routine tests only if certain conditions were met (e.g. if the specimen is received within a certain time period following collection) and 5% of laboratories rejected the specimen. One quarter of the laboratories indicated never having received a stool specimen from outpatients without transport media.

Laboratories were also asked how they handle stool specimens that are fully formed (i.e. no indication of diarrhoea), the results are shown in Table 8.

Table 8. Percentage of laboratories abiding by the following protocols when they receive stool that is fully formed (A-K represents provinces/territories)

	National	A	B	C	D	E	F	G	H	I	J	K
Reject the specimen without any testing	**8%**	0%	2%	0%	0%	25%	0%	22%	12%	0%	5%	8%
Reject the specimen, except when testing for a specific pathogen has been requested	**4%**	0%	5%	0%	0%	0%	0%	11%	3%	0%	8%	0%
Test the specimen as usual	**64%**	64%	45%	50%	83%	63%	100%	22%	63%	100%	75%	83%
Test the specimen, except when testing for a specific pathogen has been requested	**23%**	36%	48%	50%	17%	13%	0%	44%	20%	0%	10%	8%
Don't know	**1%**	0%	0%	0%	0%	0%	0%	0%	2%	0%	1%	0%
Missing	**1%**	0%	2%	0%	0%	0%	0%	0%	0%	0%	1%	0%

When asked about testing multiple specimens from a single individual, 74% of laboratories indicated having some kind of criteria in place for outpatients and 66% for inpatients. With the exception of one province/territory, the majority of laboratories within a province/territory did have a limit on the number of specimens tested by a single inpatient or outpatient (Table 9).

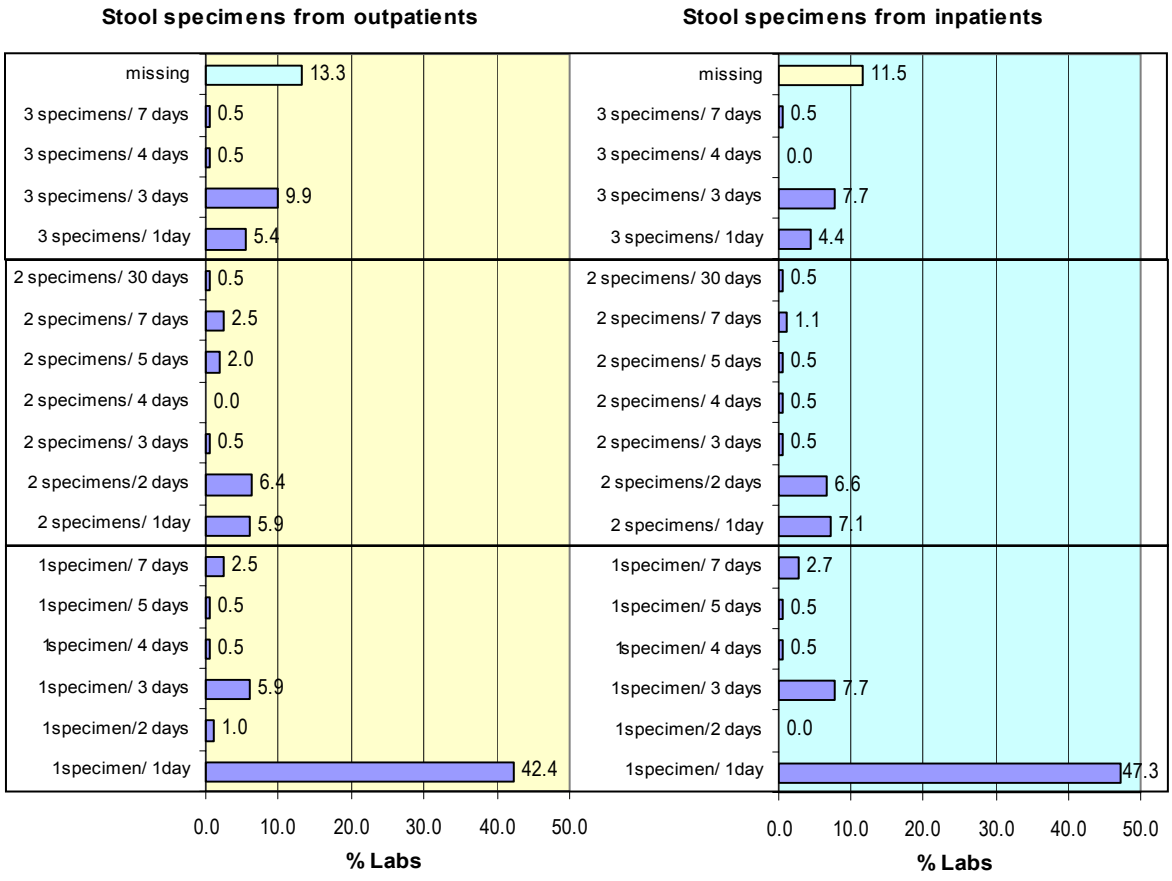
Table 9. Percentage of laboratories limiting testing on repeat specimens (A-K represents provinces/territories)

		National	A	B	C	D	E	F	G	H	I	J	K
Outpatients	Yes	**74%**	93%	77%	63%	67%	38%	100%	89%	65%	100%	82%	83%
	No	**24%**	0%	21%	25%	33%	63%	0%	11%	32%	0%	18%	17%
	N/A†	**2%**	7%	0%	13%	0%	0%	0%	0%	3%	0%	0%	0%
	Don't know	**0%**	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
	Missing	**0%**	0%	2%	0%	0%	0%	0%	0%	0%	0%	0%	0%
Inpatients	Yes	**66%**	79%	65%	38%	50%	25%	100%	89%	65%	100%	69%	83%
	No	**25%**	0%	19%	25%	50%	75%	0%	11%	24%	0%	29%	17%
	N/A‡	**7%**	14%	14%	38%	0%	0%	0%	0%	10%	0%	0%	0%
	Don't know	**0%**	0%	0%	0%	0%	0%	0%	0%	1%	0%	0%	0%
	Missing	**1%**	7%	2%	0%	0%	0%	0%	0%	0%	0%	1%	0%

NA = not applicable because laboratory does not receive stool specimens from †outpatients or ‡inpatients

When asked about specific policies, 42% and 47% of laboratories indicated only accepting one specimen per day from outpatients and inpatients respectively. Policy variations are shown in Figure 7.

Figure 7. Criteria for rejecting a stool specimen based on the number of submissions from a single inpatient or outpatient over a set time period (percentage of laboratories indicating having a policy in place).



Approximately one third of laboratories have criteria in place for rejecting a stool specimen submitted from an inpatient based on the length of the patients' hospitalisation (Table 10). With the exception of two provinces/territories, the majority of laboratories did not have a policy in place limiting testing based on length of hospitalisation.

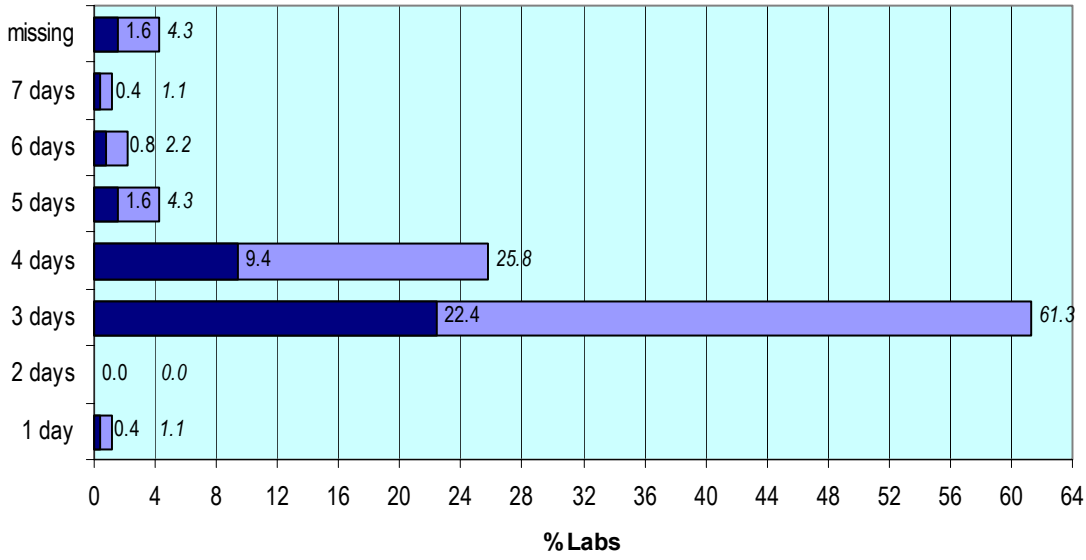
Table 10. Percentage of laboratories limiting testing based on length of hospitalisation (A-K represents provinces/territories).

	National	A	B	C	D	E	F	G	H	I	J	K
Yes	**34%**	29%	23%	13%	17%	13%	0%	56%	43%	0%	31%	58%
No	**58%**	57%	60%	50%	83%	88%	100%	44%	47%	100%	68%	42%
N/A	**7%**	14%	14%	38%	0%	0%	0%	0%	10%	0%	0%	0%
Don't know	**1%**	0%	0%	0%	0%	0%	0%	0%	1%	0%	1%	0%
Missing	**0%**	0%	2%	0%	0%	0%	0%	0%	0%	0%	0%	0%

NA = not applicable because laboratory does not receive stool specimens from inpatients

Of those laboratories having a policy in place for rejecting stool based on length of hospitalisation (n=93), 61% indicated rejecting stool specimens after 3 days of hospitalisation. Of all laboratories receiving stool from inpatients (n=254), 22% had a policy in place that saw specimens rejected after 3 days of hospitalisation (Figure 8).

Figure 8. Percentage of (a) all laboratories receiving stool from inpatients (dark bars) and (b) laboratories with a rejection policy based on hospitalisation in place (light bars), that rejected stool specimens if patient has been hospitalised for 1, 2…7 days.

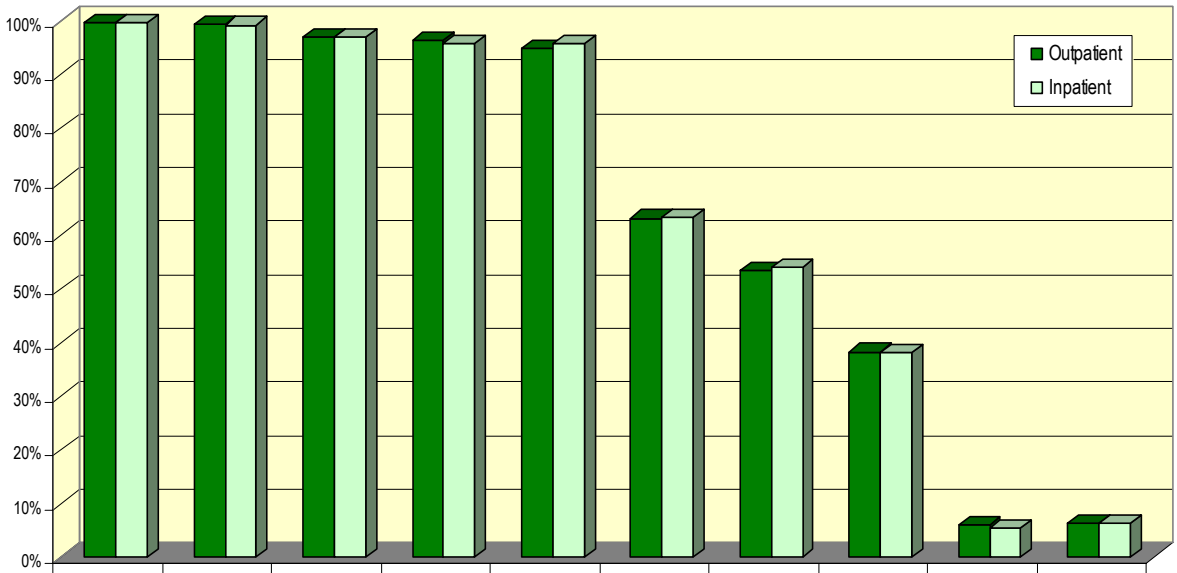


3.4 Testing Stool Specimens

3.4.1 Bacterial pathogens tested

The bacterial pathogens included on routine tests varied from laboratory to laboratory and province to province. The majority of laboratories indicated testing for the same set of pathogens regardless of whether the stool specimen came from an inpatient or outpatient. *Salmonella, Shigella, Campylobacter, E. coli* and *Yersinia* were the pathogens most commonly included in a routine stool test (Figure 9).

Figure 9. Percentage of laboratories testing for the following enteric pathogens in a routine stool test received from outpatients and inpatients.



	Salmonella	Shigella	Campylobacter	E. coli	Yersinia	Aeromonas	Plesiomonas	Vibrio	Clostridium	Other
Outpatient	100%	99%	97%	96%	95%	63%	54%	38%	6%	6%
Inpatient	100%	99%	97%	96%	96%	63%	54%	38%	6%	6%

While only 6% of laboratories indicated culturing stool for *Clostridium spp*, 60.6% of laboratories did tests (using culture and/or non-culture methods) for *Clostridium difficile* (65% of hospitals labs, 27% of private laboratories and 67% of other laboratories).

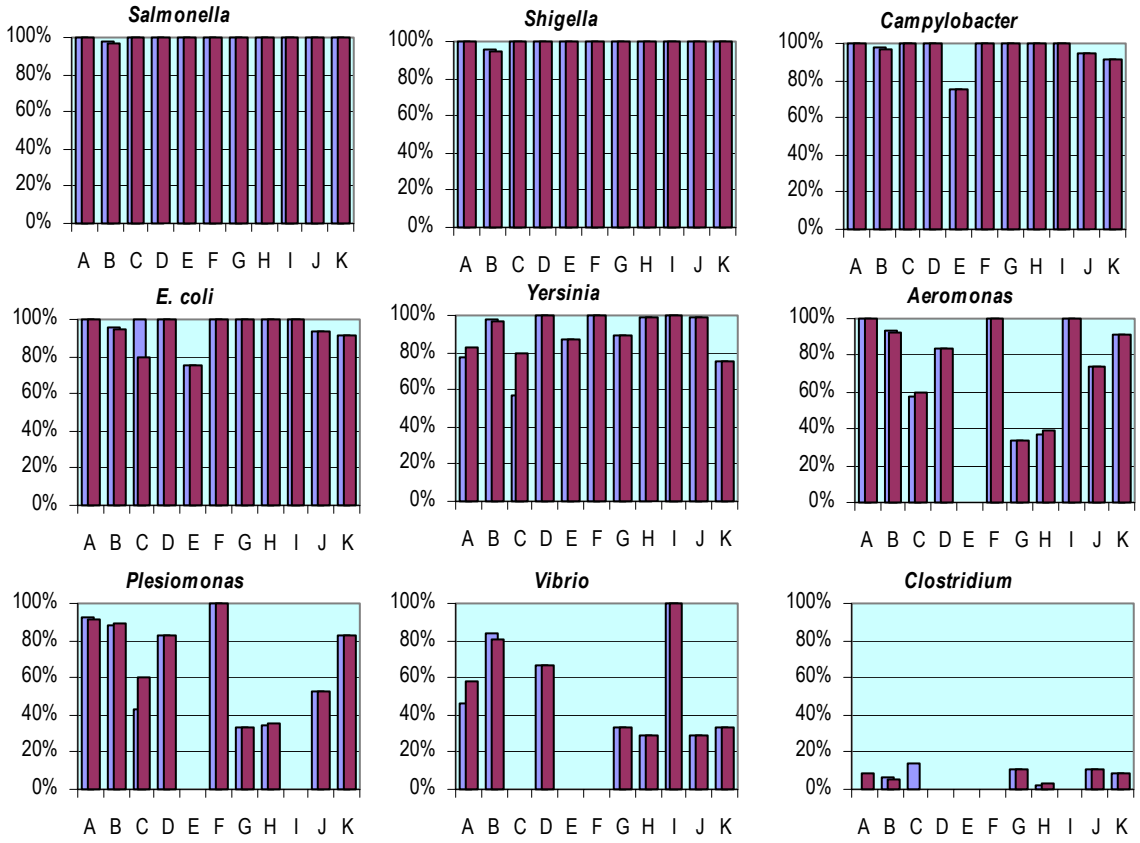
A summary of other pathogens routinely tested is shown in table 11.

Table 11. Other enteric pathogens routinely tested when stool specimens are received from outpatients and inpatients.

Outpatient	**Percentage of labs**	**Number of labs**
Staphylococcus aureus	3.0%	8
Edwardseilla spp.	4.1%	11
Pseudomonas spp.	1.1%	3
Bacillus cereus	0.4%	1
Streptococcus spp.	0.7%	2
Vancomycin Resistance Enterococcus	0.4%	1
Inpatient		
Staphylococcus aureus	3.1%	8
Edwardseilla spp.	4.2%	11
Pseudomonas spp.	1.2%	3
Bacillus cereus	0.4%	1
Streptococcus spp.	0.8%	2
Vancomycin Resistance Enterococcus	0.8%	2

An inter-provincial comparison of enteric bacteria routinely tested is shown in Figure 10.

Figure 10. Laboratories routinely testing (culture and/or molecular methods, including toxin detection) outpatient and inpatient stool specimens for the following enteric pathogens within each province/territory (provinces/territories are represented by capital letters A–K, light bars behind = inpatients, dark bars in front = outpatients).



Laboratories were also asked about pathogens they tested for if specifically requested by the physician (Table 12).

Table 12. Percentage of laboratories that do not routinely test for the following enteric pathogens unless specifically requested to by a physician.

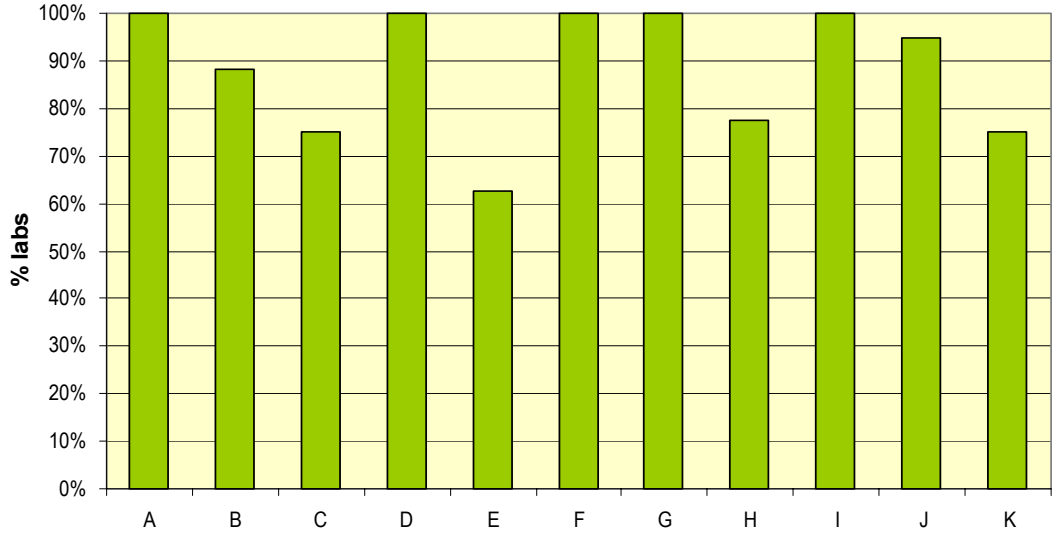
	Outpatient		Inpatient	
	%	number	%	number
Aeromonas	20%	55	20%	51
Campylobacter	0.4%	1	0.4%	1
Clostridium	14%	39	17%	44
E.coli	5%	14	6%	14
Plesiomonas	20%	54	20%	50
Salmonella	0%	0	0%	0
Shigella	0%	0	0%	0
Vibrio	26%	69	26%	65
Yersinia	3%	7	3%	7
No additional testing	52%	140	48%	121

Only 10% of laboratories indicated using non-culture methods for primary detection of enteric bacterial pathogens and this was predominantly (66% of the time) for primary detection of *C. difficile* toxin. *E. coli* was the only other pathogen indicated as being identified primarily by non-culture methods.

3.4.2 Antimicrobial resistance testing on bacterial pathogens

Across the country, 236 laboratories (86%) are involved in testing one or more enteric pathogens for resistance to antibiotics. The inter-provincial variation in the percentage of laboratories performing resistance testing is summarized in Figure 11.

Figure 11. Percentage of laboratories in each province involved in sensitivity testing on enteric pathogens (A – K represents provinces).



ALL Labs	100%	88%	75%	100%	63%	100%	100%	78%	100%	95%	75%
Hospital Labs	100%	89%	80%	100%	63%	100%	100%	75%	100%	92%	67%
Private Labs	100%	100%	67%	0%	0%	0%	0%	93%	0%	1%	8%
Other Labs	100%	0%	0%	0%	0%	0%	0%	0%	0%	1%	0%

The pathogens most commonly tested for antimicrobial resistance include *Shigella spp*, *Salmonella typhi* and *Salmonella paratyphi*. The frequency of resistance testing by pathogen is summarized in Table 13.

Table 13. Percentage and frequency of laboratories testing enteric pathogens for antimicrobial sensitivity (percentage and number given for all laboratories, hospital laboratories (H) and private laboratories (P).

		Always (100%)			Routinely (80-99%)			Sometimes (20-79%)			Rarely (1-19%)			Never (0%)			NA			DK			Missing		
		ALL	H	P	ALL	H	P	ALL	H	P	ALL	H	P	ALL	H	P	ALL	H	P	ALL	H	P	ALL	H	P
Aeromonas	%	**40%**	40%	40%	**1%**	2%	0%	**0%**	0%	0%	**6%**	7%	0%	**16%**	13%	40%	**11%**	12%	7%	**1%**	1%	3%	**24%**	25%	10%
	n	**110**	97	12	**4**	4	0	**0**	1	0	**16**	16	0	**43**	31	12	**30**	28	2	**4**	3	1	**66**	61	3
Campylobacter	%	**8%**	7%	17%	**1%**	1%	0%	**0%**	0%	0%	**4%**	4%	3%	**61%**	61%	63%	**3%**	3%	0%	**1%**	1%	0%	**22%**	24%	13%
	n	**23**	17	6	**2**	2	0	**0**	0	0	**11**	10	1	**166**	146	19	**9**	7	0	**2**	2	0	**61**	57	4
Clostridium	%	**0%**	0%	0%	**0%**	0%	0%	**0%**	0%	0%	**0%**	0%	0%	**18%**	20%	7%	**42%**	38%	73%	**1%**	1%	0%	**39%**	41%	20%
	n	**0**	0	0	**0**	0	0	**0**	0	0	**1**	0	0	**49**	47	2	**116**	92	22	**2**	2	0	**106**	100	6
E. coli O157	%	**29%**	29%	70%	**1%**	1%	3%	**1%**	0%	3%	**4%**	4%	0%	**36%**	35%	50%	**8%**	7%	10%	**1%**	1%	0%	**20%**	22%	10%
	n	**30**	70	8	**3**	3	1	**2**	1	1	**10**	10	0	**99**	84	15	**22**	18	3	**2**	2	0	**56**	53	3
E. coli	%	**1%**	1%	2%	**0%**	0%	3%	**0%**	0%	3%	**0%**	0%	0%	**7%**	7%	3%	**76%**	75%	83%	**1%**	1%	0%	**15%**	16%	7%
	n	**2**	2	0	**1**	0	1	**1**	0	1	**1**	1	0	**18**	17	1	**209**	181	25	**2**	2	0	**40**	38	2
Plesiomonas	%	**36%**	36%	86%	**3%**	3%	0%	**0%**	0%	0%	**4%**	4%	0%	**15%**	12%	40%	**24%**	24%	20%	**1%**	2%	0%	**17%**	19%	3%
	n	**98**	86	11	**7**	7	0	**1**	1	0	**10**	10	0	**41**	29	12	**67**	59	6	**4**	4	0	**46**	45	1
S. typhi	%	**68%**	65%	157%	**2%**	2%	0%	**1%**	1%	0%	**4%**	5%	0%	**3%**	4%	0%	**1%**	0%	0%	**2%**	2%	3%	**19%**	20%	7%
	n	**186**	157	27	**5**	5	0	**3**	3	0	**12**	12	0	**9**	9	0	**2**	1	0	**6**	5	1	**51**	49	2
S. paratyphi	%	**65%**	62%	149%	**1%**	2%	0%	**1%**	2%	0%	**5%**	5%	3%	**5%**	6%	3%	**1%**	0%	0%	**2%**	2%	0%	**19%**	21%	7%
	n	**177**	149	26	**4**	4	0	**4**	4	0	**14**	13	1	**15**	14	1	**2**	1	0	**5**	5	0	**53**	51	2
S. typhimurium	%	**53%**	54%	129%	**1%**	2%	3%	**3%**	2%	3%	**11%**	10%	20%	**11%**	10%	23%	**1%**	0%	0%	**2%**	2%	0%	**19%**	20%	10%
	n	**144**	129	13	**4**	4	1	**7**	6	1	**29**	23	6	**30**	23	7	**2**	1	0	**6**	6	0	**52**	49	3
Salmonella other	%	**53%**	54%	131%	**1%**	2%	3%	**3%**	3%	3%	**12%**	10%	23%	**12%**	10%	23%	**1%**	0%	0%	**1%**	2%	0%	**17%**	18%	10%
	n	**145**	131	12	**4**	4	1	**8**	7	1	**32**	25	7	**32**	25	7	**2**	1	0	**4**	4	0	**47**	44	3
Shigella	%	**72%**	70%	169%	**2%**	2%	0%	**0%**	0%	0%	**3%**	4%	0%	**3%**	2%	7%	**1%**	1%	0%	**1%**	1%	0%	**17%**	19%	7%
	n	**197**	169	26	**6**	6	0	**1**	1	0	**9**	9	0	**8**	6	2	**4**	3	0	**2**	2	0	**47**	45	2
Vibrio	%	**30%**	30%	72%	**1%**	1%	3%	**1%**	0%	3%	**3%**	3%	3%	**9%**	9%	17%	**36%**	35%	43%	**1%**	2%	0%	**18%**	20%	3%
	n	**82**	72	9	**3**	3	1	**2**	1	1	**8**	7	1	**26**	21	5	**99**	84	13	**4**	4	0	**50**	49	1
Yersinina	%	**55%**	56%	135%	**2%**	2%	0%	**0%**	0%	0%	**4%**	4%	0%	**16%**	14%	40%	**3%**	2%	10%	**1%**	2%	0%	**18%**	20%	3%
	n	**151**	135	14	**5**	5	0	**0**	1	0	**10**	10	0	**45**	33	12	**9**	5	3	**4**	4	0	**50**	49	1

Of the laboratories involved in resistance testing, 41% (n=135) used the Kirby-Bauer (disk diffusion) method, 37% (n=102) use the Vitek system, 23% (n=64) the MicroScan system, 5% (n=14) E-test, 5% (n=13) agar dilution, 1% (n=2) broth dilution and 1% (n=2) indicated using BioMerieux ATB.

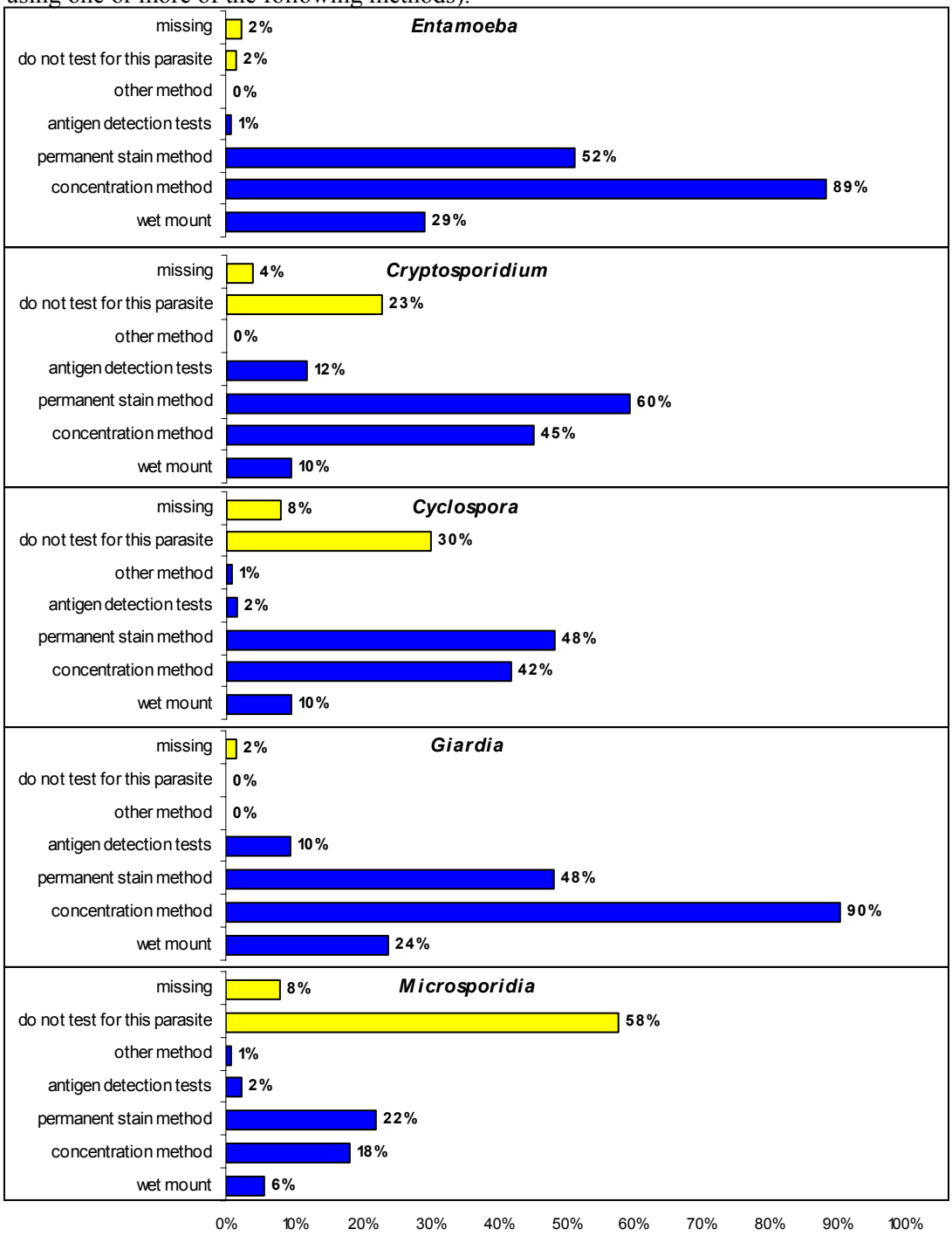
Data were recorded quantitatively by 23% of laboratories and qualitatively by 82% of laboratories. A total of 73% of laboratories stored their resistance data on a laboratory computer system.

When asked about the reason(s) for conducting resistance testing, 35% of laboratories indicated testing was done because a physician or infectious disease specialist requested, or might request, the information, 66% because it was part of routine laboratory practice and 7% because their laboratory was participating in an antimicrobial resistance research or surveillance program. Six percent recorded 'other' reasons for doing sensitivity testing, including compliance with NCCLS and QMPLS recommendations.

3.4.3 Methods for testing bacteria, parasites and viruses

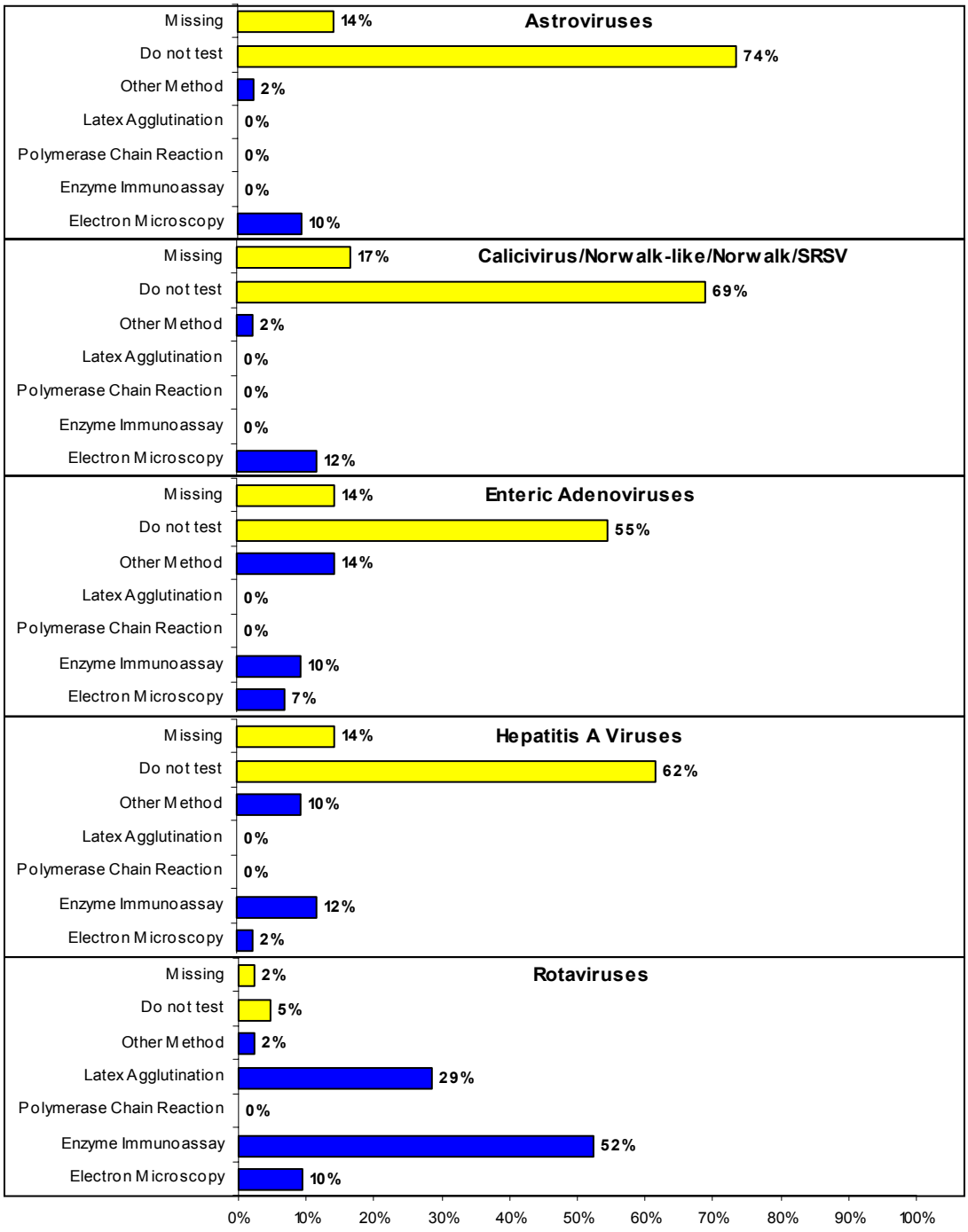
The most common method used for identifying parasites was the concentration method (e.g. formalin-ethyl acetate), followed by permanent stain and wet mount (Figure 12). *Giardia* was the parasite tested in the most number of laboratories (n = 126) followed by *Enatmoeaba* (n = 124), *Cryptosporidium* (n = 97), *Cyclospora* (n = 88) and *Microsporidium* (n = 53).

Figure 12. Methods used for parasite testing (percentage of laboratories testing for parasites using one or more of the following methods).



For viral testing, EIA was most commonly used, followed by EM and latex agglutination (Figure 13). 'Other methods' included diarlex, tissue culture and dry spot latex. Rotavirus was clearly the most commonly tested virus with 40 laboratories testing for it, this was followed by Enteric Adenoviruses (n = 19), Hepatitis A virus (n = 16), Calicivirus/Norwalk-like/Norwalk/SRSV (n = 13) and finally Astroviruses (n = 11)

Figure 13. Methods used for viral testing (percentage of laboratories testing for viruses using one or more of the following methods).



3.4.4 Number of stool specimens tested and the percentage positive

The number of stool specimens tested (culture and/or molecular methods, including toxin detection) for bacterial, parasitic and viral pathogens in the year 2000 are shown in Table 14. Two provinces/territories did not test any stool specimens for *C. difficile*, Parasites or Viruses.

Table 14. Total number of stool specimens tested in the year 2000 (provinces/territory indicated by 1-11)

Total # stool specimens tested				
	Bacteria (excl. C. difficile)	**C. difficile**	**Parasites**	**Viruses**
National	**459 982**	**177 696**	**392 023**	**14 051**
1	177 554	42 287	186 393	4438
2	108 899	65 729	68 072	3787
3	62 001	27 206	51 117	860
4	50 823	21 349	39 806	2400
5	18 683	5647	14 817	179
6	14 864	7374	9994	1606
7	10 223	2587	10 476	115
8	9413	4791	5494	249
9	6138	726	5854	417
10	728			
11	656			

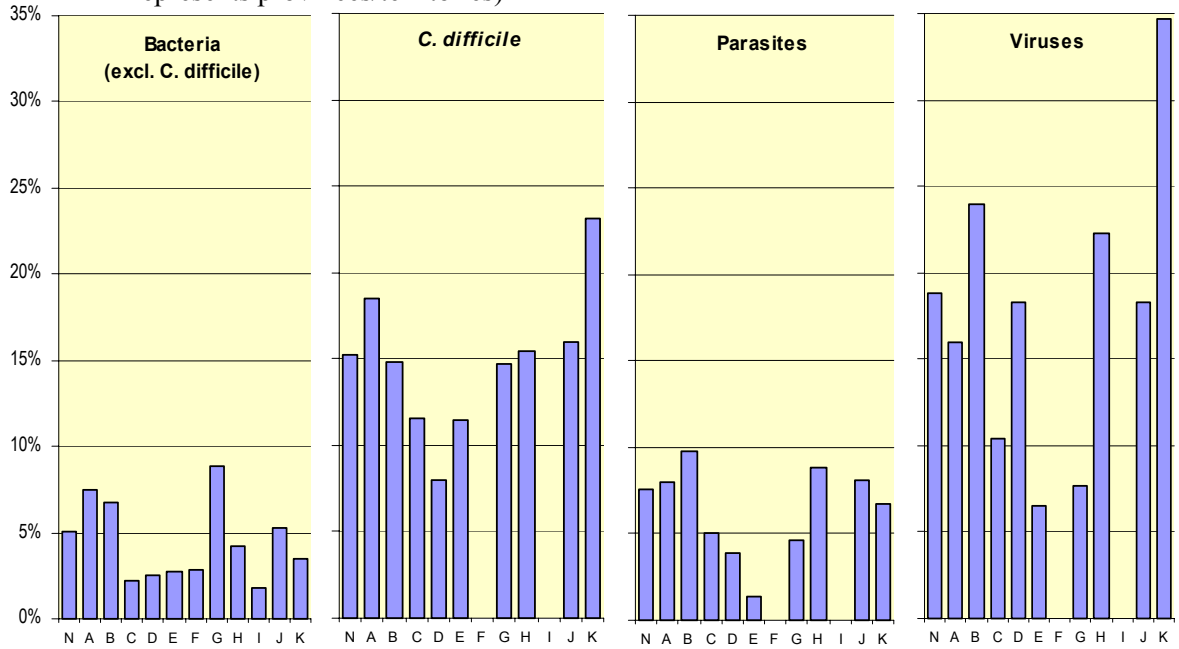
Stool specimens tested for enteric bacteria were the least likely to yield a positive isolation. On average, 5.0% of all stool specimens were positive when tested for an enteric bacterial pathogen (excluding *C. difficile*). When testing for *C. difficle*, 15.3% of stool specimens were positive. For parasites, 7.6% of specimens were positive and for viruses, 18.9% were positive. Isolation rates by province are shown in Table 15.

Table 15. Average, minimum and maximum percentage of stools having a pathogen positively identified, comparison across provinces/territory (A-K represents provinces/territory)

Percent Positive (range)												
	Bacteria (excl. *C. difficile*)			*C. difficile*			Parasites			Viruses		
	Av.	Min	Max	Av.	Min	Max	Av.	Min	Max	Av.	Min	Max
National	**5.0%**	0.0%	45.7%	**15.3%**	1.7%	49.4%	**7.6%**	0.0%	28.2%	**18.9%**	0.0%	64.3%
A	**7.5%**	3.0%	25.0%	**18.5%**	11.2%	31.5%	**8.0%**	2.6%	15.2%	**16.0%**	10.3%	21.8%
B	**6.8%**	0.1%	33.4%	**14.8%**	1.7%	28.2%	**9.7%**	1.1%	27.2%	**24.0%**	3.1%	64.3%
C	**2.2%**	0.0%	4.9%	**11.6%**	5.7%	18.9%	**5.1%**	1.7%	11.8%	**10.4%**	10.4%	10.4%
D	**2.6%**	1.7%	3.4%	**8.0%**	5.1%	11.4%	**3.9%**	0.8%	10.0%	**18.2%**	7.4%	38.2%
E	**2.7%**	1.1%	6.3%	**11.5%**	9.4%	13.5%	**1.4%**	0.8%	2.5%	**6.5%**	6.4%	6.7%
F	**2.9%**	2.9%	2.9%	**-**	-	-	**-**	-	-	**-**	-	-
G	**8.9%**	0.5%	45.7%	**14.8%**	5.7%	29.4%	**4.6%**	1.6%	8.2%	**7.6%**	7.6%	7.6%
H	**4.3%**	0.3%	11.1%	**15.5%**	5.6%	32.8%	**8.8%**	2.6%	13.6%	**22.3%**	6.7%	41.4%
I	**1.8%**	1.8%	1.8%	**-**	-	-	**-**	-	-	**-**	-	-
J	**5.3%**	0.4%	19.8%	**16.0%**	2.8%	47.7%	**8.1%**	0.0%	28.2%	**18.3%**	0.0%	49.4%
K	**3.5%**	0.5%	8.6%	**23.1%**	6.4%	49.4%	**6.7%**	2.4%	12.5%	**34.6%**	34.6%	34.6%

Figure 14 highlights the inter-provincial/territorial variation in the percentage of stool specimens positive for enteric pathogens.

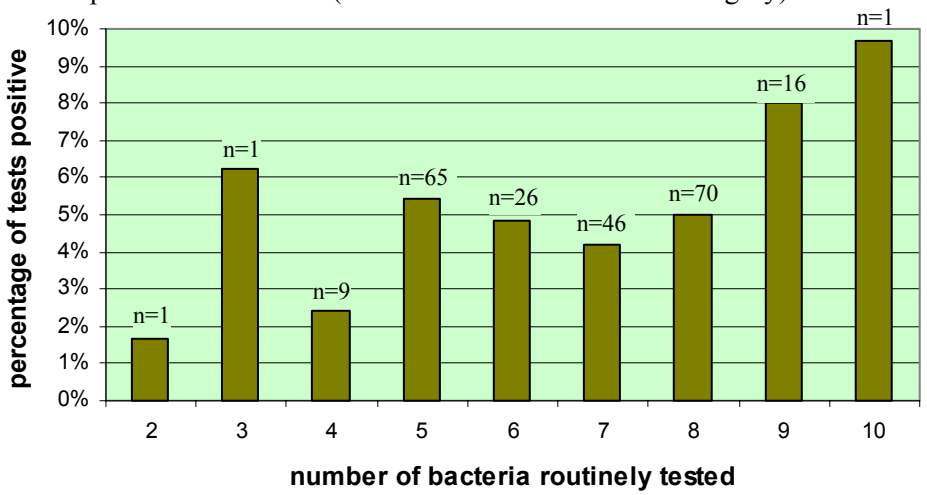
Figure 14. Comparison of positive stools tested (percent positive) by province (N = National, A – K represents provinces/territories)



3.4.5 Impact of variables on percentage of stools positive

When comparing the total number of pathogens included on a routine test with the portion of stool specimens positive for bacterial pathogens an increasing trend was observed (Figure 15). This correlation was not, however, statistically significant (p=0.1851). Laboratories testing for ≤4 bacteria on a routine stool test isolated pathogens from 2.7% of stool specimens, while laboratories testing for ≥5 bacteria isolated bacteria had a significantly higher yield of 5.1% (p=0.090).

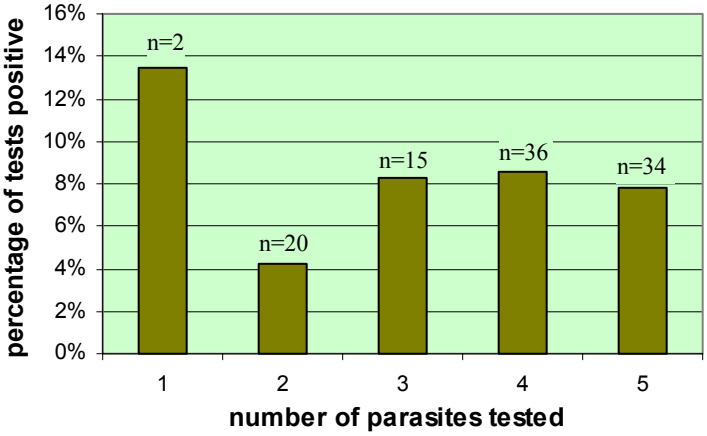
Figure 15. Number of bacteria routinely tested compared to the overall percentage of stool specimens positive for bacteria (n=number of laboratories in category).



There was no statistically significant correlation between the total number of stool specimens tested for bacteria by an individual laboratory and the proportion of positive tests recorded by the same laboratory (p=0.9206).

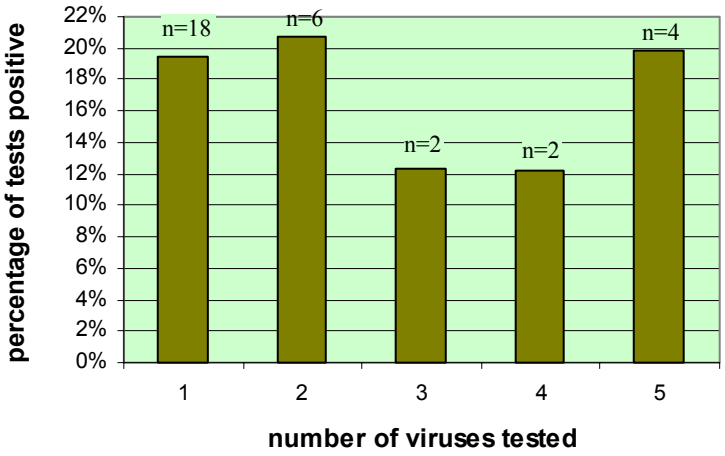
The number of parasites tested for and the relative percentage of positive tests is shown in Figure 16. When comparing laboratories testing one or two parasites with those testing three to five, there was a statistically significance increase in the mean proportion of specimens yielding a positive result (5.1% vs. 8.2%, p=0.0273).

Figure 16. Number of parasites routinely tested compared to the overall percentage of stool specimens positive for bacteria (n=number of laboratories in category).



Likewise, there was no statistically significant correlation (p=0.7578) between the number of viruses tested for in a laboratory and the percentage of positive tests (Figure 17). The low numbers of labs testing viruses makes comparison tenuous.

Figure 17. Number of parasites routinely tested compared to the overall percentage of stool specimens positive for bacteria (n=number of laboratories in category).



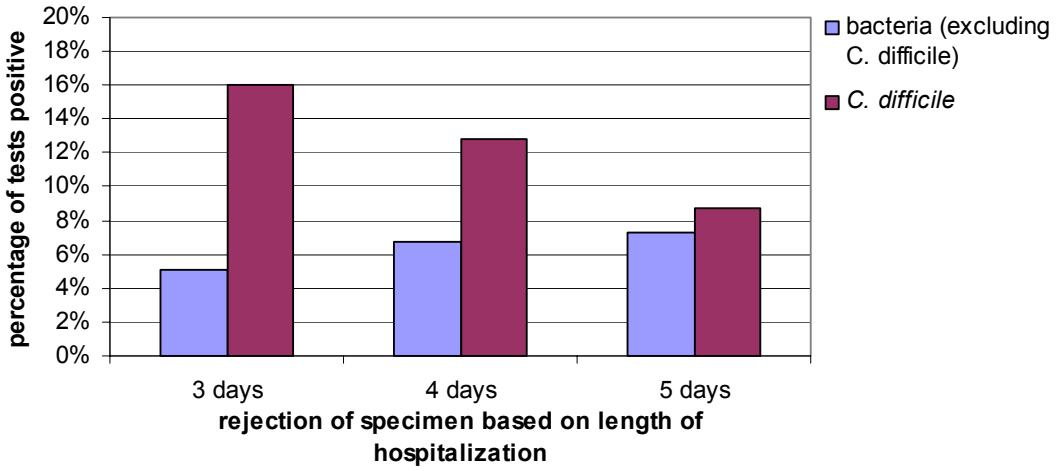
The positivity rate for bacteria according to various testing criteria are compared in Table 16. No statistically significant differences were noted.

Table 16. Comparison between laboratory's testing policy and percent of tests positive for bacteria

Testing Criteria	Percent positive (bacteria)	P-value
Rejecting specimen if stool is fully formed	4.6%	0.2754
Testing specimen if stool is fully formed	5.9%	
No limit on the number of specimens tested from a single outpatient	4.4%	0.4473
Limit on the number of specimens tested from a single outpatient	4.9%	
Only test one stool from a single outpatient per day	5.2%	0.7237
Only test one stool from a single outpatient per 3 or more days	4.5%	
No limit on the number of specimens tested from a single inpatient	4.5%	0.247
Limit on the number of specimens tested from a single inpatient	5.3%	
Only test one stool from a single inpatient per day	4.8%	0.1649
Only test one stool from a single inpatient per 3 or more days	6.3%	

Figure 18 compares the percentage of stool specimens testing positive for bacteria excluding *C. difficile* and *C difficile* with the rejection criteria based on 3, 4, or 5 days of hospitalisation.

Figure 18. Rejection of stool based on length of inpatient hospitalisation compared to percentage of positive tests for bacteria

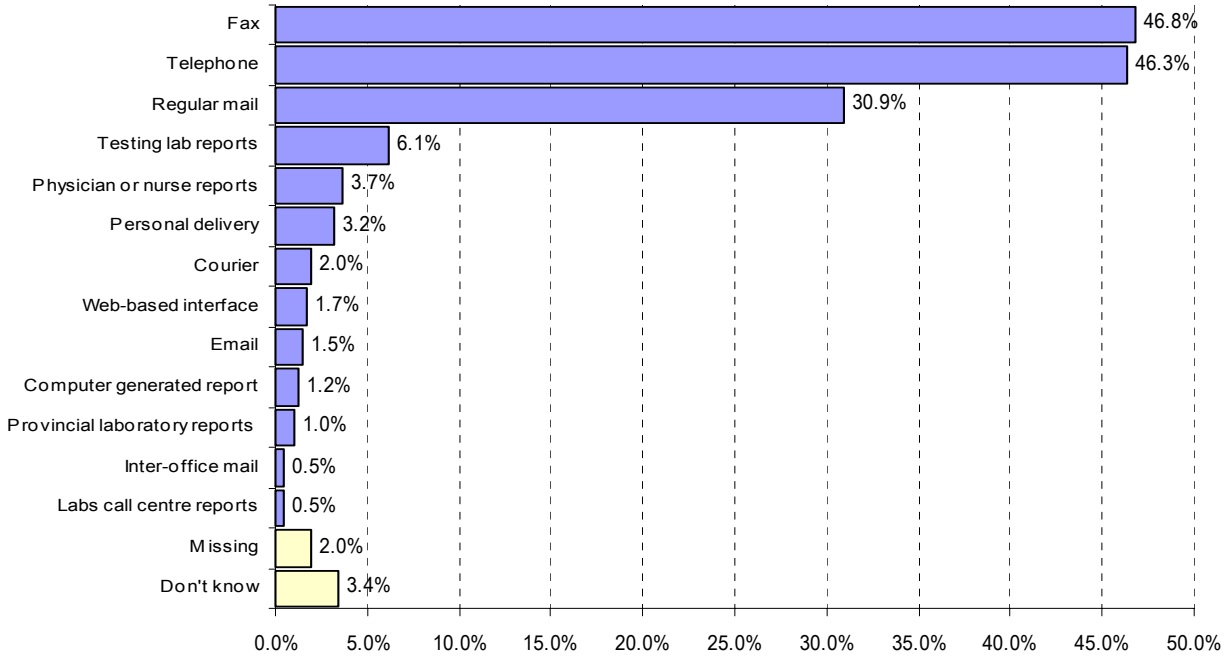


When comparing the percentage of test positive with the type of laboratory (hospital vs. private), no statistically significant differences were noted.

3.5 Recording and Transfer of Information

Regarding the potential for multiple submissions from a single patient being recorded as multiple cases, 35% of laboratories indicated having a mechanism in place to prevent such an event from occurring, 51% said they had no mechanism in place, 9% did not know and 5% didn't answer the question. The actual method of reporting information to the local or regional medical officer of health or local health unit/authority is summarized in Figure 19.

Figure 19. Percentage of laboratories indicating how they reported information to the local or regional medical officer of health.



When a laboratory identifies a reportable pathogen, 51% report to the health unit/authority within which their laboratory is located, 16% report to the health unit within which the patient resides and 4% indicated reporting to the appropriate health authority based on the physicians address. The variation in this reporting practice across the country is summarized in Table 17.

Table 17. Selection of health region/authority to report positive identifications based on patients, physicians or laboratory address (N = national, A – K represents provinces/territories).

	only patient's address	only physician's address	only laboratory address	patient's or physicians address	patients or laboratory's address	physician's or laboratory's address	patient's, physician's or laboratory's address	missing	don't know	not applicable
N	15.9%	4.2%	51.2%	0.2%	5.1%	0.2%	0.5%	4.2%	7.6%	10.8%
A	25.0%	12.5%	33.3%	0.0%	4.2%	0.0%	0.0%	4.2%	8.3%	12.5%
B	12.9%	2.2%	60.2%	0.0%	2.2%	0.0%	0.0%	6.5%	7.5%	21.5%
C	7.7%	6.2%	24.6%	0.0%	0.0%	1.5%	0.0%	12.3%	23.1%	24.6%
D	66.7%	0.0%	33.3%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
E	0.0%	0.0%	75.0%	12.5%	0.0%	0.0%	0.0%	0.0%	0.0%	12.5%
F	50.0%	0.0%	50.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
G	23.1%	23.1%	38.5%	0.0%	0.0%	0.0%	0.0%	0.0%	7.7%	7.7%
H	22.1%	1.1%	51.6%	0.0%	18.9%	0.0%	0.0%	0.0%	4.2%	2.1%
I	0.0%	0.0%	100.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
J	14.9%	2.3%	72.4%	0.0%	0.0%	0.0%	1.1%	3.4%	3.4%	2.3%
K	0.0%	15.4%	61.5%	0.0%	0.0%	0.0%	7.7%	0.0%	7.7%	7.7%
L	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	100.0%

When asked about the reporting of positive results (i.e. notification without an accompanying specimen) to provincial laboratories, 16% of laboratories indicated doing so (Table 18). The pathogens for which this practice most commonly occurred were *Campylobacter spp*, *Yersinia spp* and *E. coli* O157.

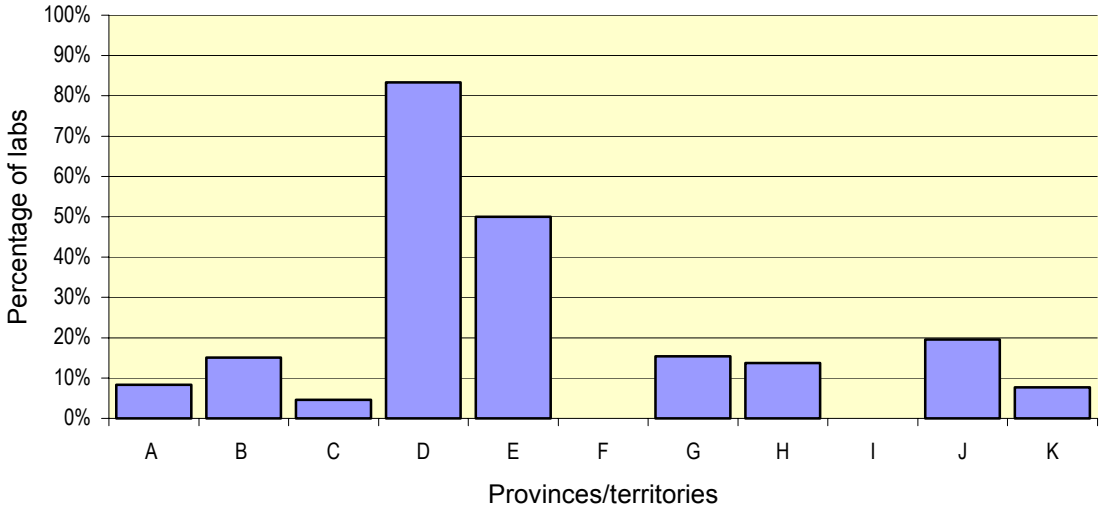
Table 18. Percentage of laboratories that report positive results without an accompanying specimen, and the pathogens for which this practice occurs.

		Percentage of laboratories	Number of laboratories
ALL	Yes	16%	61
	No	68%	268
	Don't know	12%	46
	Missing	5%	18
Hospital	Yes	17%	58
	No	67%	231
	Don't know	11%	37
	Missing	5%	17
Private	Yes	8%	3
	No	78%	31
	Don't know	13%	5
	Missing	3%	1

Pathogens	
Campylobacter spp	44%
Yersinia spp	28%
E. coli	21%
Shigella spp	20%
Salmonella spp	20%
Plesiomonas spp	20%
Aeromonas spp	18%
Vibrio spp	15%
Giardia	13%
Edwardsiella	3%
Rotavirus	2%
Ova and Parasites	2%

The variation by province/territory in the practice of sending information to the provincial laboratory without an accompanying specimen is shown in Figure 20.

Figure 20. Percentage of laboratories reporting positive results without isolates to their provincial public health laboratory, comparison between provinces/territories (A-K represents provinces/territories)



The frequency of reporting positive findings isolated in their laboratory to a local/regional medical officer of health varied greatly depending on the pathogen identified. *C. perfingens* was the least reported bacteria with only 9% of laboratories *always* reporting while Salmonella was the most commonly reported with 92% of laboratories always reporting (Table 19).

Table 19. Frequency with which enteric pathogens, once confirmed, are reported to the local/regional medical officer of health or the local/regional health unit/authority (the number of provinces each pathogen is reportable and the pathogens nationally reportable are also indicated).

Pathogen	# *prov/terr reportable*[a]	*Nationally reportable*	always (100%)	routinely (80-99%)	sometimes (20-79%)	rarely (1-19%)	never (0%)	don't know	missing
Aeromonas	*2*		33%	3%	0%	4%	33%	3%	24%
Campylobacter	*13*	✓	85%	4%	0%	1%	2%	1%	5%
C. perfringens	*2*		9%	0%	0%	1%	19%	4%	67%
C. botulinum	*13*	✓	21%	0%	0%	0%	5%	3%	71%
E. coli O157	*7/1*[†]		91%	2%	0%	2%	0%	1%	4%
E. coli other	*2/1/1/1*[‡]		32%	0%	0%	3%	12%	3%	50%
Plesiomonas	*0*		33%	3%	0%	3%	33%	2%	25%
Salmonella	*13*	✓	92%	2%	0%	1%	1%	1%	3%
Shigella	*13*	✓	91%	2%	0%	2%	1%	1%	3%
Vibrio	*1/13**	✓	58%	2%	0%	3%	8%	2%	27%
Yersinia	*8*		86%	4%	1%	1%	2%	1%	5%
Entamoeba	*0*		71%	2%	7%	0%	6%	2%	13%
Cryptosporidium	*9*	✓	60%	1%	0%	0%	15%	4%	20%
Cyclospora	*7*	✓	54%	1%	0%	1%	14%	1%	28%
Giardia	*13*	✓	88%	2%	0%	0%	2%	2%	8%
Microsporidia	*0*		37%	2%	0%	4%	21%	0%	37%
Astroviruses	*0*		20%	0%	0%	0%	20%	10%	50%
Enteric Adenoviruses	*0*		30%	7%	0%	0%	22%	7%	33%
Rotaviruses	*2*		23%	9%	0%	0%	23%	5%	41%
Norwalk-like/ Norwalk/ Calicivirus/ SRSV	*1*		35%	4%	0%	0%	35%	2%	23%

[a]The number of provinces and territories requiring pathogen to be reportable as of January 29, 2001
[†]Verotoxigenic *E. coli* reportable in 7 prov/terr., Verotoxigenic *E. coli* (including HUS) reportable in 1 prov/terr.
[‡]Pathogenic *E. coli* reportable in 2 prov/terr., enteritis *E. coli* reportable in 1 prov/terr., enteropathogenic *E. coli* reportable in 1 prov/terr., enterotoxogenic *E. coli* reportable in 1 prov/terr.
**Vibrio parahemolyticus* reportable in 1 prov/terr., cholera reportable in 13 prov/terr.

Table 20 highlights inter-provincial/territorial comparisons in the percentage of laboratories that report positive identification with varying frequencies to their local health unit/authority.

Table 20. Frequency with which confirmed enteric pathogens are reported to the local/regional medical officer of health or the local health unit/authority; comparison by province (pathogens included in this table are reportable in all provinces and territories).

Campylobacter

Prov.	always (100%)	Routinely (80-99%)	Sometimes (20-79%)	rarely (1-19%)	never (0%)	don't know	missing
A	93%	0%	0%	0%	0%	0%	7%
B	86%	5%	2%	0%	2%	0%	5%
C	63%	0%	0%	0%	13%	25%	0%
D	67%	33%	0%	0%	0%	0%	0%
E	71%	14%	0%	0%	0%	0%	14%
F	100%	0%	0%	0%	0%	0%	0%
G	89%	11%	0%	0%	0%	0%	0%
H	87%	3%	0%	2%	2%	0%	5%
I	91%	1%	0%	0%	1%	1%	5%
J	50%	17%	0%	8%	8%	8%	8%
K	-	-	-	-	-	-	-

Salmonella

Prov.	always (100%)	Routinely (80-99%)	sometimes (20-79%)	rarely (1-19%)	never (0%)	don't know	missing
A	100%	0%	0%	0%	0%	0%	0%
B	88%	5%	0%	2%	0%	0%	5%
C	75%	0%	0%	0%	13%	13%	0%
D	83%	17%	0%	0%	0%	0%	0%
E	88%	0%	0%	0%	0%	0%	13%
F	100%	0%	0%	0%	0%	0%	0%
G	100%	0%	0%	0%	0%	0%	0%
H	97%	0%	0%	2%	1%	0%	0%
I	94%	0%	0%	0%	0%	1%	5%
J	50%	17%	0%	8%	8%	8%	8%
K	-	-	-	-	-	-	-

Shigella

Prov.	always (100%)	routinely (80-99%)	Sometimes (20-79%)	rarely (1-19%)	never (0%)	don't know	missing
A	100%	0%	0%	0%	0%	0%	0%
B	88%	5%	0%	2%	0%	0%	5%
C	75%	0%	0%	0%	13%	13%	0%
D	83%	17%	0%	0%	0%	0%	0%
E	75%	0%	0%	0%	0%	0%	25%
F	100%	0%	0%	0%	0%	0%	0%
G	100%	0%	0%	0%	0%	0%	0%
H	97%	0%	0%	3%	0%	0%	0%
I	94%	0%	0%	0%	0%	1%	5%
J	50%	17%	0%	8%	8%	8%	8%
K	-	-	-	-	-	-	-

Table 19 continued.

Giardia

Prov.	always (100%)	routinely (80-99%)	sometimes (20-79%)	rarely (1-19%)	never (0%)	don't know	missing
A	100%	0%	0%	0%	0%	0%	0%
B	88%	6%	0%	0%	0%	0%	6%
C	40%	0%	0%	0%	20%	20%	20%
D	83%	17%	0%	0%	0%	0%	0%
E	100%	0%	0%	0%	0%	0%	0%
F	-	-	-	-	-	-	-
G	100%	0%	0%	0%	0%	0%	0%
H	88%	0%	0%	0%	0%	0%	12%
I	88%	0%	0%	0%	2%	2%	9%
J	100%	0%	0%	0%	0%	0%	0%
K	-	-	-	-	-	-	-

4. DISCUSSION

A good understanding of how surveillance data are generated makes the interpretation of the data more accurate and meaningful. Disease magnitude and the change in magnitude are key outcomes of Canada's enteric disease surveillance systems. Both, however, are limited by under-reporting and variation in factors that influence the likelihood of a case being captured or lost within the system.

4.1 Under-reporting figures

It is known from international studies that only a fraction of community AGI cases are reported into national surveillance databases. The UK estimated that 1 out of every 136 community AGI cases were counted in their national database (Wheeler *et al*, 1999) while the US estimated that 1 in 38 *Salmonella* infections were captured at the national level (Angulo, 2001).

In this study, it was found that once a patient with an AGI visits a physician and submits a stool specimen, most specimens are tested. Only 3% of stools specimens in the year 2000 (or the equivalent of approximately 1300 specimens) were rejected because they arrived at laboratories in a condition unfit for testing. Once tested, however, only a fraction of the specimens were positive for an enteric pathogen. Of the stool specimens that were examined for (a) bacteria or bacterial toxins (excluding *C. difficile)*, (b) *C. difficile*, (c) parasites and (d) viruses in the year 2000, 5.0%, 15.3%, 7.6% and 18.9%, respectively, were positive. The overall proportion of *tests* positive for a bacterial, parasitic or viral pathogen was 8.8% (the sum of all positive isolations divided by the sum of all specimens tested for bacteria, parasites and viruses). Assuming that the total number of cases submitting stool is equal to the total number of stools submitted for bacterial testing, the overall proportion of *cases* diagnosed with a laboratory confirmed infection was 29.4% (sum of all positive isolations divided by number of stools submitted for bacterial testing).

The bacterial positivity rates found in this study are similar to published international studies. Zaidi *et al* (1999) conducted a 5-year retrospective study in the United States that tested stool from patients hospitalised with AGI. Of the specimens tested from patients in ambulatory settings, 3.4% (439/12 985) were positive for *Salmonella*, *Shigella*, *Campylobacter*, *Yersinia* or *E. coli* O157:H7. Of the remaining 14 125 specimens taken from all other inpatients, only 1.2% were positive. Another US study, by Fan *et al* (1993), found that 2.6% (29/1097) of stool specimens from inpatients were positive for *Salmonella*, *Shigella* or *Campylobacter*. A study by Bauer *et al* (2001) prospectively collected and tested stool from patients in four European health care centers and found 1.3% of specimens were positive for enteropathogenic bacteria (*Salmonella*, *Shigella*, *Campylobacter*, *Yersinia*, *E. coli* and *Vibrio*). In Switzerland, Rohner and colleagues (1997) identified the *Salmonella*, *Shigella* or *Campylobacter* in 6.1% (856/12253) of stool specimens submitted by hospitalised patients and *C. difficile* in 10.2% (379/3723). An earlier Canadian study, which surveyed 238 hospitals across the country to determine the incidence of *C. difficile*, reported average test positivity rates of 17.2%, 15.3% and 13.2% for hospitals with <300, 300-500 and >500 beds (Alfa *et al*, 1998).

The studies mentioned above were largely hospital based and 'normal' stool collection and testing protocols were followed. In a recent Dutch study, however, stool was requested from *all* patients presenting with AGI to physicians participating in a sentinel network and comprehensive testing procedures were followed. In this case, bacterial pathogens were detected in 16% of specimens, parasites in 8% and viruses in 15% (Matty de Wit *et al*, 2001). In the English IID study, two GP sentinel networks were established. The first group of physicians requested specimens after the usual manner and tested them at local laboratories. Of the 1262 cases submitting stool, 19.5%, 1.6% and 2.7% were positive for bacteria, parasites and viruses respectively (Wheeler *et al*, 1999). The second group of physicians requested stool from all patients presenting with AGI and these specimens were tested for an extensive range of pathogens. In this case, bacterial pathogens were detected in 47.8% of specimens, viruses in 22.0% and parasites in 2.3% (Tompkins *et al*, 1999).

In an earlier Canadian study by Gyorkos *et al* (1987), the isolation rate for parasites was 16.4%, more than twice that found in this study. Comparisons with this study are limited as Gyorko *et al* focused on provincial laboratories rather than front line laboratories. The decline is likely a combination of a true decrease in the incidence of AGI resulting from parasitic infections and changes in testing protocols. According to Canadian Notifiable Disease Data from 1987 to 1999, Amoebiasis and Giardiasis rates (per 100 000 people) dropped from 7.1 to 4.5 and 34.4 to 17.2 respectively (CIDPC, 2001).

4.2 Under-reporting reasons

A test may be negative because the stool specimen (a) contained no pathogens, (b) contained pathogens that were looked for but not identified or (c) contained pathogens that were not looked for. This study found that 91.2% of all tests (or approximately 70% of all cases submitting stool) were negative. It cannot be determined from this study what proportion of these specimens came from patients who became ill from a cause other than infectious. Further research is required to better understand the proportion of AGI that is truly infectious in Canada and thus amenable to preventative interventions.

The second reason for not isolating a pathogen (i.e. pathogen not found) is largely a function of the methodology used. The higher isolation rates for viruses and *C. difficile* are likely reflections of improved sensitivity of the diagnostic methods and the characteristics of the patients usually infected with these enteric pathogens. The sensitivity of the diagnostic method is key in recognizing enteric pathogens of importance. Astroviruses, for example, appeared to be rare causes of AGI, found in less than 1% of children with diarrhea, in the United States when electron microscopy was the method of choice for identification. With the development and use of monoclonal antibodies and enzyme immunoassays, it is now recognized as an important cause of pediatric AGI with prevalence rates of up to 9% in children hospitalised with diarrhea in the United States and other western countries (Glass *et al*, 1996, Mustafa *et al*, 2000) and up to 20% in developing countries (Gaggero *et al*, 1998). Even with culture methods, the addition of an enrichment procedure can notably improve isolation rates. In the UK IID study, 31.5% of *Salmonella*, 4.5% of *Campylobacter*, 78.5% of *Aeromonas* and 64.9% of *Yersinia* isolates were only identified after enrichment procedures (Tompkins *et al*, 1999). If more sensitive methods become feasible for use in front line laboratories, coordination or monitoring of the implementation of new methodologies will be important from a surveillance perspective.

The final reason a test may turn out negative (i.e. pathogen not looked for) is potentially the most significant reason. Pathogens thought to be less common (or less important from a public health perspective) or pathogens that are more difficult (or expensive) to identify are less commonly tested making true comparisons with more commonly tested pathogens impossible. Although *Salmonella*, *Shigella*, *Campylobacter* and *E. coli* are tested routinely on a consistent basis across Canada, there are notable variations in the consistency of routine testing for *Yersinia*, *Aeromonas*, *Plesiomonas* and *Vibrio*. Differences in the frequency of testing parasites and viruses are even more pronounced. Whereas nearly 460 000 stool specimens were tested for bacteria, only 14 051 were tested for viruses. Of the laboratories testing viruses (which only make up 10% of the laboratories overall), 74% indicated never testing for astrovirus and 69% indicated never testing for small round structured viruses (SRSV), calicivirues, Norwalk or Norwalk-like viruses. A number of international studies have highlighted the importance of viral pathogens as etiologic agents of AGI. In the UK, SRSV were the most commonly identified enteric pathogen causing AGI at the community level and the third most common cause at the physician level (Handysides, 1999). In hospitalised children admitted to Australian and French hospitals, 71.5% and 72.2%, respectively of stools specimens submitted were positive for rotavirus (Kirkwood and Bishop, 2001; Bon *et al*, 1999). The potential extent of viral under-reporting in Canada is highlighted by UK figures where only 1 in every 1562 cases of SRSV and one in every 35 cases of rotavirus were reported nationally (Wheeler *et al*, 1999).

Finding a negative laboratory result is not the only reason why a case may be excluded from the national surveillance system. Once a pathogen is identified, it must be reported to the next level in the surveillance chain. When considering the Public Health arm of the surveillance system, not all positive identifications were reported to the local health authority. The frequency of *always reporting* ranged from 9% of laboratories for *C. perfringens* (reportable in 2 provinces) to 92% for *Salmonella* (reportable in all provinces and territories). When considering the Provincial Laboratory arm of the surveillance system, the percentage of isolates sent from the local to the provincial laboratory is key to understanding under-reporting. Overall, 94% of laboratories indicated sending isolates to the provincial laboratory; however, by pathogen this varied considerable. For example, over 60% of laboratories indicated always forwarding *Salmonella* and *E. coli* O157 specimens, whereas only 15% always sent *Campylobacter* isolates, 2% always sent *E. coli* (other than O157) and less than 1% always sent *Clostridium* isolates. In addition, hospital laboratories were nearly three times more likely than private laboratories to send all *Campylobacter* specimens to the provincial laboratory. Only 16% of laboratories indicated sending reports of positive isolation without an accompanying isolate to the provincial laboratory; twice as many hospital laboratories as private laboratories indicated doing this.

4.3 Variations

True trends in disease magnitude can be obscured due to variations in factors that influence the likelihood of identifying a pathogen; this study examined some of these inter-laboratory variations. Trends were noted between positivity rates and differences in testing policies, however, for the most part these trends were not statistically significant. The overall low yield, the low number of laboratories in some of the variable categories and the inter-relatedness of variables may explain why many 'plausible' relationships were not statistically significant.

Two statistically significant factors that were positively related to an increased yield were the number of bacteria and parasites included on a routine stool test. Laboratories testing less than five bacteria on a routine culture had a lower yield than laboratories testing five or more (2.7% vs 5.1%, significant at the 0.1 level). Likewise, laboratories testing for one or two parasites had a lower yield than those testing for 3 or more (5.1% v 8.2%, significant at the 0.05 level).

Some of the variations noted between laboratories, which could potentially influence the laboratories pathogens yields, are as follows:

- ***The proportion of stool specimens rejected:*** the percentage of stools *specimens rejected* varied from 0% to 50%. Most laboratories indicated rejecting 5% of specimens or less. The proportion of specimens tested onsite did not influence the likelihood of rejecting a specimen. On a provincial basis, the average proportion of specimens rejected ranged from 1.3% to 10%.

- ***Transport media:*** the frequency with which laboratories *receive specimens with and without transport media* varied as did policies on how to deal with specimens received without transport media. Of all laboratories receiving stool, less than 10% indicated *always* receiving stool without transport media, ice or refrigeration. Five percentage of labs indicated rejecting such specimens if received from an outpatient. Lack of transport media is known to decrease pathogen viability, especially when immediate testing is not available (Wang *et al*, 1983, Wasfy *et al*, 1995).

- ***Testing of repeat specimen:*** the laboratory protocol for *testing repeat specimens* over a set time period varied considerably. The majority of labs had a *one specimen per day* policy. Current research suggests the increase in yield from repeat testing is low (Torres *et al,* 2001).

- ***Limiting testing based on length of*** **hospitalisation*:*** the proportion of laboratories within each province/territory having policies in place for rejecting stool based on the length of hospitalisation varied from 0% to 58%. Of all laboratories testing stool from inpatients, 22% rejected specimens if hospitalisation was over 3 days in duration. Only 0.4% of laboratories reject specimens if hospitalised for greater than 7 days. These figures are similar to US laboratories where 21% of surveyed laboratories indicated rejecting specimens from patients hospitalised for more than 3 days and 3% rejected specimens from patients hospitalised for more than 7 days (Morris *et al* 1996).

- ***Testing fully formed stool:*** the average proportion of laboratories within each province/territory that rejected fully formed stool specimens varied from 0 to 22% with an average of 8%. This compares to 5% reported in a US survey of 67 laboratories (Morris *et al*, 1996). The majority (64%, provincial/territorial range 22 – 100%) of laboratories indicated testing the stool as usual.

4.4 Conclusions and Future research

This study is one in a series of national studies on AGI that will contribute to the quantification and description of under-reporting occurring within Canada's national enteric disease surveillance systems. This study found that at the laboratory interface, 91.2% of all tests for bacterial, parasitic or viral enteric pathogens were negative. This is just one of a number of key places in Canada's national enteric surveillance system where cases are lost. This accumulative loss affects both the efficiency and sensitivity of the surveillance system.

Knowing where and how many cases are lost assists in an accurate interpretation of passive surveillance data. Correction factors can be developed to adjust surveillance figures so they more accurately reflect the actual situation in the community. To further refine these correction factors at the laboratory interface, the following is needed:

- Research to define the fraction of AGI cases that are attributable to an infectious cause, and
- Pathogen specific under-reporting rates.

To improve the efficiency and sensitivity of the system itself, future work needs to concentrate on improving the pathogen yield. The high number of negative results means the cost per positive identification is over $450 (based on an estimated $40 per test). Figures in the US range from US$952 to US$1200 per positive result (Koplan *et al*, 1980; Guerrant *et al*, 1987, Guerrant *et al*, 1985). Improving pathogen yield will not only improve the efficiency of the surveillance system, it will provide physicians with the information required to prescribe specific treatments and also improve the sensitivity of AGI surveillance enabling outbreaks to be identified more frequently and rapidly.

Ideally, laboratory practices that improve the likelihood of identifying enteric pathogens should be harmonized across the country. Further work is required to understand what pathogens are causing AGI in the community and what adjustments to stool requisition and testing policies can be made to improve pathogen yield.

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