CCDR
Canada Communicable Disease Report
ISSN 1188-4169
Volume : 32S4 November 2006

Supplement

Proceedings of the National Microbiology Laboratory Pertussis Workshop

Winnipeg, Manitoba
7 March, 2006

Public Health Agency of Canada
Agence de santé publique du Canada
Canada

Suggested citation: Public Health Agency of Canada. *Proceedings of the National Microbiology Laboratory Pertussis Workshop*. CCDR 2006;32S4:1-22.

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PROCEEDINGS OF THE NATIONAL MICROBIOLOGY LABORATORY PERTUSSIS WORKSHOP

Winnipeg, Manitoba
7 March, 2006

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Summary

A National Consensus Conference on Pertussis was held in 2002, and a number of recommendations were made for laboratory diagnosis and surveillance. The purpose of the National Microbiology Laboratory Workshop on 7 March, 2006, was to gather Canadian experts in pertussis in order to examine the recommendations made in 2002 and discuss issues related to laboratory diagnosis and strain characterization for surveillance. Participants included representatives from academia, federal and provincial laboratories, hospital laboratories and industry. Twenty-one people attended the workshop, representing Nova Scotia, Newfoundland, Quebec, Ontario, Manitoba, Saskatchewan, Alberta, British Columbia and the United States.

The objectives of the workshop were to (1) discuss and establish recommendations for a diagnostic laboratory system for pertussis; (2) discuss and establish recommendations for a laboratory surveillance system for pertussis; and (3) develop an action plan to implement these recommendations. An initial series of presentations provided a background on pertussis in Canada and consisted of an overview of the national surveillance of the disease; serologic and PCR (polymerase chain reaction) diagnosis; laboratory characterization of pertussis; and an investigation into recent pertussis cases in Toronto. Further presentations described the National Microbiology Laboratory (NML) activities with respect to vaccine preventable bacterial diseases, and the local and global challenges in laboratory public health, discussed by the Canadian Public Health Laboratory Network (CPHLN). The format of the workshop consisted of presentations and full group discussions.

Recommendations

The following is a summary of the recommendations identified during the workshop:

1. Recommendation for a diagnostic laboratory system for pertussis

    1.1 PCR diagnosis for pertussis should be encouraged and made widely available in Canada. The NML should provide a laboratory proficiency program for PCR diagnosis of pertussis.

    1.2 Culturing of the *Bordetella pertussis* organism should continue to allow the bacteria to be archived for future reference, or at least appropriate specimens (such as nasopharyngeal aspirates) should be stored frozen such that *B. pertussis* bacteria may be recovered from the specimen as required.

    1.3 The national case definition of pertussis should be revised to reflect the fact that laboratory test results should be interpreted in the context of the clinical presentation of the patient.

    1.4 A sero-epidemiology study of pertussis in the Canadian adult population should be considered. This study/project would fulfill two objectives: (i) to understand the level of protective immunity against pertussis in the adult population, which may provide evidence for future recommendation of pertussis vaccination in this population; (ii) to define the role of routine serology for the diagnosis of pertussis in adolescents and adults.

2. Recommendation for a laboratory surveillance system for pertussis:

    2.1 A working group with representatives from British Columbia, Alberta, Ontario, Nova Scotia, the NML and the Centre for Infectious Disease Prevention and Control (CIDPC) should be established to examine how to implement a national program to study and characterize strains.

    2.2 CIDPC should study effective ways to utilize existing resources or programs to implement sentinel sites for collection of not only *B. pertussis* strains but also epidemiologic data, such as vaccination history.

3. Action plan to implement the above recommendations:

    3.1 CPHLN will initiate a survey to find out which Canadian hospital laboratories are providing PCR diagnosis of pertussis, to identify sites where culture for *B. pertussis* is being done, and/or to identify potential sites where suitable nasopharyngeal aspirate specimens may be stored frozen for future culture work.

    3.2 NML will study the feasibility of providing a PCR proficiency program for pertussis.

    3.3 NML will set up a working group to discuss implementing a national strain characterization program.

3.4 CIDPC will study ways to enhance the national surveillance of pertussis by incorporating the laboratory strain characterization component.

3.5 CIDPC will take the lead in revising the national case definition of pertussis to reflect the importance of interpreting laboratory results in the context of compatible clinical and epidemiologic information.

3.6 NML will work with interested parties to examine the feasibility of implementing a standardized enzyme-linked immunosorbent assay (ELISA) for measuring serum antibodies to pertussis as well as the feasibility of carrying out a sero-epidemiology study in the Canadian adult population.

Vaccine Pressure and Immune Selection on Vaccine Preventable Bacterial Disease Agents

Dr. Raymond Tsang

Selection on vaccine preventable bacterial disease agents can result from immunity developed in the population as a result of vaccination (vaccine pressure) and/or natural immunity that develops as a result of the endemic nature of the disease.

Immune selection from vaccine pressure

Pathogens like *Streptococcus pneumoniae* and *Haemophilus influenzae* have been reported to respond to vaccine pressure by either "capsule switching" or "capsule replacement". For example, in *S. pneumoniae* causing invasive pneumococcal disease, introduction of the heptavalent pneumococcal conjugate vaccine (PCV7) has led to the emergence of diseases due to either vaccine-related pneumococcal serotypes[1] or non-vaccine serogroups[2]. Capsule switching has also been demonstrated in disease-causing pneumococci[3]. Changes in the epidemiology of invasive *H. influenzae* disease have been reported from different countries, including Canada, in the post-vaccination (*H. influenzae* serotype b [Hib]) era[4-6].

In *Bordetella pertussis*, many genetic polymorphisms have been described, involving such diverse genes as fimbriae, pertussis toxin (PT) and pertactin, which encode for the virulence factors (and hence are vaccine components)[7-9]. Analysis of genomic DNA from *B. pertussis* strains by pulsed-field gel electrophoresis (PFGE) is able to differentiate between recent clinical isolates and strains used in the manufacture of pertussis vaccines[10,11]. These genetic changes have been suggested to be responsible for the resurgence of pertussis in countries with high rates of vaccine coverage[8].

Immune selection from natural immunity

Serogroup C *Neisseria meningitidis* is endemic in Canada, and the currently licensed vaccines against it target the serogroup-specific capsule polysaccharide[12]. After more than a decade of endemic disease and many localized outbreaks, genetic and antigenic changes in the subcapsular protein components (the serotype and serosubtype antigens) were detected in the serogroup C ET-15 *N. meningitidis* strains recovered from cases of invasive meningococcal disease[13,14]. Since the vaccine induces serum antibodies that target the capsular polysaccharide antigens and the changes documented are in the subcapsular outer membrane proteins, it is reasonable to hypothesize that such changes are likely due to selection from natural immunity and are unrelated to vaccine pressure.

Role of the laboratory for surveillance of vaccine preventable bacterial diseases agents

Since bacteria change over time and some of these changes may lead to vaccine escape mutants, it is essential that public health laboratories have a system to monitor the evolving bacteria for their potential to cause vaccine breakthrough cases. The discussions of this workshop will focus on laboratory diagnostic and surveillance issues for the monitoring of pertussis in Canada.

Overview of National Surveillance for Pertussis

Dr. Scott A. Halperin

After the introduction of whole cell pertussis vaccine there was a remarkable decrease in the number of reported pertussis cases. Over the last 15 years, however, there has been a resurgence of pertussis, although to levels far below the pre-vaccine era[15]. Pertussis is a notifiable disease in all provinces and territories. Cases are reported provincially/territorially, and confirmed cases are reported nationally. The Notifiable Disease Surveillance System is the Public Health Agency of Canada's passive surveillance system, and the Immunization Monitoring Program, Active (IMPACT) is its active surveillance system. There is no formal system of *B. pertussis* strain characterization within these surveillance systems.

The Canadian case definitions for pertussis include both suspected and confirmed case definitions. A *confirmed case* includes laboratory confirmation of infection as defined by isolation of *B. pertussis* from an appropriate clinical specimen or positive polymerase chain reaction (PCR) assay for *B. pertussis*, or a person who is epidemiologically linked to a laboratory-confirmed case with one or more of the following for which there is no other known cause:

- paroxysmal cough of any duration,
- cough ending in vomiting or associated with apnea,
- cough with inspiratory whoop.

A probable case is defined as a cough lasting 2 weeks or longer in the absence of appropriate laboratory tests and not epidemiologically linked to a laboratory-confirmed case and with one or both of the following with no other known cause:

- paroxysmal cough,
- inspiratory "whoop".

The 10 to 14 year age group has demonstrated the greatest increase in the number of cases. The progression of the peak age group has shifted away from infants and preschool-aged children to these young adolescents. In fact, the incidence in the 10 to 14 year age group is now second only to that among infants < 1 year of age. This shifting epidemiology has been most graphically demonstrated by evaluating sequential, cyclical outbreaks of pertussis in British Columbia[16]. Beginning in the year 2000, there was a large shift in the peak age group of cases to involve young adolescents. There is a continual marching cohort of peaks from 1993 to 2003. This marching cohort effect has been blunted by implementation of the adolescent pertussis vaccine program.

In 1994 in Newfoundland and Labrador, the peak incidence of pertussis was among children 1 to 4 years old. The peak in this age group disappeared with the implementation of the acellular pertussis vaccine. Similar diminutions are now being seen in the adolescent age cohorts that are routinely receiving the adolescent formulation of acellular pertussis vaccine.

IMPACT is a national surveillance system based in tertiary care pediatric centres across Canada. IMPACT comprises 12 centres accounting for 75,000 pediatric admissions annually with over 90% of the tertiary care pediatric beds in Canada. Hospitalized cases of pertussis have been a surveillance target of IMPACT for over a decade. There has been a gradual reduction in the annual number of hospital-admitted cases with the introduction of acellular vaccine. We are seeing fewer vaccine failures and increased effectiveness in preschool-aged children. There has been a shift in hospitalized pertussis to those infants who have not completed their primary immunization series[17].

In summary, the epidemiology of pertussis in Canada is shifting from preschool-aged young children to adolescents and adults. Infants too young to have completed the primary series of immunization are at greatest risk of morbidity and mortality. Differences in disease burden across jurisdictions are more likely related to the adequacy and intensity of surveillance. Except during outbreaks, laboratories continue to be the greatest source of pertussis reporting. Laboratory diagnosis of pertussis depends on the age of patients, lower detection rates being reported in adults and adolescents. This results in underreporting and underestimation of disease in adolescents and adults. Strain epidemiology is not widely known.

Serologic Tests for the Diagnosis of Pertussis

Dr. Scott A. Halperin

The diagnosis of pertussis by serology has a long history, and a range of methods have been used. One of the oldest tests routinely employed was measurement of pertussis agglutinins; however, this test correlated best with population immunity and was less useful for diagnosing pertussis in an individual patient or for determining whether an individual was susceptible to infection by *B. pertussis*. As currently performed, pertussis agglutinins are measured by a microagglutination method whereby serial dilutions of a patient's serum is mixed overnight at 35 ºC with phase 1 *B. pertussis* organisms, and the agglutinin titre is the highest serum dilution demonstrating bacterial agglutination. Agglutinins have been shown to correlate best with antibodies against fimbriae, pertactin and lipo-oligosaccharide. Because of their lack of correlation with individual infection or protection, this assay has no current role in a clinical diagnostic laboratory.

Pertussis antibodies have also been measured by the Chinese hamster ovary (CHO) cell cytotoxicity neutralization assay. This assay is based upon the observation that CHO cells undergo a unique morphological change in the presence of PT that is neutralized by pertussis antitoxin antibodies. This assay measures biologically active antibodies that correlated well with protection in animal models. However, anti-PT antibodies measured by enzyme immunoassay (see below) have been shown to correlate well with CHO neutralization titres and are far less labour-intensive to perform. Therefore, CHO neutralization titres are only available in certain research laboratories and are not routinely used for the diagnosis of pertussis.

Enzyme immunoassay (EIA) is currently the most widely used serologic method for the diagnosis of pertussis. EIAs can use whole bacterial cells or specific antigens and can measure IgG, IgA, IgM or all antibody classes. There are no commercial EIA kits available for pertussis serology that perform adequately. Whole bacterial antigens have the advantage of being less costly to prepare but are less well-standardized and suffer from cross reaction with other *Bordetella* species and other organisms (such as *H. influenzae*). One such whole bacterial EIA kit is used for serologic diagnosis in Australia. All currently marketed EIA kits, whether based on whole bacteria or individual antigens, have problems with antibody quantification[18].

EIAs for pertussis are also performed in various reference laboratories using locally prepared reagents and methods. EIAs measuring anti-PT antibodies are the most widely available. PT is uniquely produced by *B. pertussis*, so anti-PT antibodies have the highest sensitivity for *B. pertussis* infection. There are no known cross-reacting antibodies to PT, but the antibody response to it is variable, particularly in young infants. PT is also part of all currently available acellular pertussis vaccines, so differentiating between recent vaccine-induced or infection-induced antibodies is not possible.

Filamentous hemagglutinin (FHA) is a constituent of the cell wall of *B. pertussis* and is a protective antigen in animal models of pertussis. No correlation between anti-FHA antibodies and protection was demonstrated in the human efficacy studies with acellular pertussis vaccines; however, FHA is a component of most acellular pertussis vaccines. FHA induces a more consistent immune response than does PT (increased sensitivity), although anti-FHA antibodies crossreact with antibodies to other organisms (other *Bordetella* species, *H. influenzae*, *Mycoplasma pneumoniae*), resulting in decreased specificity.

Pertactin is an outer membrane adhesin protein (69 kilodalton) that is a protective antigen in animal models and correlates with protection in human clinical trials. It induces a somewhat less consistent antibody response than FHA but is more specific to *B. pertussis* (although other *Bordetella* species produce a similar protein). It is part of all of the acellular pertussis vaccines currently marketed in North America. The assay is more variable than the PT and FHA antibody assays. Fimbriae are also bacterial surface attachment factors, which elicit antibodies less consistently than FHA. The anti-fimbriae assays are also less well-standardized than the PT or FHA antibody assays. Fimbriae 2 and 3 are part of the vaccine currently in widespread use in Canada.

EIAs that measure IgG antibodies are the most commonly used, best-standardized and most available assays. IgG antibodies are detectable 2 to 3 weeks after primary immunization and 5 to 7 days after booster immunization. Anti-PT antibodies are slower to rise than antibodies against FHA, pertactin or fimbriae. Antibody levels drop rapidly during the first year after immunization or infection but remain detectable for 1 to 3 years. IgM assays are poorly standardized and only occasionally reported. There is little known about the natural history of IgM antibodies after pertussis infection. It is suspected that because of the wide-spread circulation of *B. pertussis* in the population, IgM antibodies are not generally elicited after exposure to the organism, since most of the population has been previously exposed.

Measurement of IgA antibodies to pertussis antigens has the potential to differentiate infection from immunization. IgA response is less common after immunization than after infection so has been proposed as a measure of infection. However, the IgA response is demonstrated only after 20% to 50% of infections. Although less commonly induced after immunization, IgA antibodies are measurable in some vaccinees, particularly against the bacterial surface antigens. Overall, IgA assays have low sensitivity, particularly in young infants. IgA titres do persist for up to 1 year and thus are suggestive of recent infection (or, in some, recent immunization).

Using multiple antigen/antibody combinations increases the sensitivity of the serologic diagnosis of pertussis but at the expense of specificity. Diagnostic algorithms have been employed that use either the presence of antibody against PT alone as a diagnostic level of pertussis or the presence of antibody against at least two of FHA, pertactin or fimbriae. Multiple antigens/antibodies increase the cost and complexity of serologic testing.

Standardization of pertussis serology has been attempted. In 1995, there was a collaborative international study involving 33 laboratories. Panels of sera were distributed to various industry, public health and academic laboratories, and the results from laboratories performing the tests were compared. PT and FHA antibody tests had the best inter-laboratory correlation; fimbriae and pertactin assays were the most variable[19].

There is a growing consensus among pertussis researchers and laboratories that for serologic diagnosis in the clinical diagnostic laboratory, the IgG anti-PT assay provides the best compromise between sensitivity and specificity. Paired serologic tests (samples from the acute and convalescent stages) is ideal but is rarely obtainable. Single serum diagnosis is possible based on comparisons with levels in the general population or using statistically generated diagnostic titre thresholds.

Although there have been a number of research reports employing such methodology, the Massachusetts Public Health laboratory has led the way in routinely using single serum anti-PT antibody levels for the diagnosis of pertussis[20]. In its assay, a positive result is defined as a titre > 99% of the upper tolerance limit of a population of uninfected controls. This assay has been instrumental in providing laboratory confirmation of pertussis in older children, adolescents and adults in whom cultures are frequently negative. Massachusetts initially accounted for the overwhelming majority of adolescent and adult cases in the United States; however, recently confirmed cases have been reported from other jurisdictions as well, as the assay becomes more widely available.

A US Centers for Disease Control and Prevention/ Food and Drug Administration study evaluated pertussis serology using sera available from the National Health and Nutrition Examination Survey. Anti-PT antibodies were measured in over 6,000 US residents from 6 to 49 years of age. Using a population modeling approach, they proposed adopting a threshold of ≥ 94 EU (ELISA units)/mL as a diagnostic level of pertussis[21]. The European Sero-Epi Network is standardizing results of a variety of serologic tests from laboratories across Europe using different methodologies; anti-PT antibody assays are included. This network has established a threshold of ≥ 125 EU/mL as a diagnostic level for pertussis[22,23].

In summary, the literature suggests that for routine, clinical diagnostic serologic testing, anti-PT IgG EIA is the assay of choice. Given the current state of the art, pertussis serology should likely be limited to select reference laboratories until standardized kits are available. For national reportable disease statistics, laboratory-confirmed cases by serology should be restricted to cases with serology results from these laboratories. However, even for these laboratories, proficiency testing is needed. Ideally, serologic assays with single serum pertussis should be correlated with the US or European standards as a collaborative program. Reference sera should also be correlated to establish results that are interpretable and comparable among laboratories.

The Use of PCR in the Diagnosis of Pertussis

Dr. Susan Richardson

At the time of the 2002 National Consensus Conference on Pertussis, it was recognized that PCR was a very useful tool for the diagnosis of pertussis, demonstrating enhanced sensitivity of detection of the organism over traditional culture techniques. *B. pertussis* is a slowly growing bacterium that is fastidious with respect to its growth requirements. Despite the most careful handling of specimens and quality control of media, the viability of this organism can be reduced by toxic factors in the media and factors in specimen collection and transport. Thus, *B. pertussis* is an ideal candidate for detection by PCR, and indeed most assays have demonstrated a 2- to 4-fold increase in sensitivity by this technique.

Despite this obvious advantage, diagnosis most commonly relies on culture or clinical diagnosis without verification. This is largely because there are no commercially available pertussis PCR assays and relatively few laboratories that perform in-house assays. Although there are eight different *Bordetella* species, only *B. pertussis* commonly causes infection in humans and is the only species associated with outbreaks of infection. Of the other species, only *B. parapertussis* and *B. holmesii* cause pertussis-like illness in humans, and *B. bronchiseptica*, *B. hinzii* and *B. trematum* have been reported to cause rare cases of human respiratory infection.

The most commonly described PCR assays target sequences that detect *B. pertussis* (and/or *B. holmesii*) or *B. parapertussis* (Table 1). Insertion sequence IS*481* is by far the most frequently used target for *B. pertussis*, although it is also present in *B. holmesii*. Other sites for *B. pertussis* include the PT promoter gene, the porin gene, pertactin gene and adenylate cyclase gene. Insertion sequence IS*1001* differentiates *B. parapertussis* from *B. pertussis*, although *B. holmesii* may also contain the gene sequence and may therefore give a positive signal[24].

PCR methodology varies from conventional single-step, block-based PCR to nested PCR and real-time PCR. Nested PCR can, in theory, provide greater sensitivity but is also more time-consuming and prone to contamination. The advantages of real-time PCR are a reduced time to positivity and a decreased risk of contamination. There are very little comparative data about the optimal method of extraction, but most methods seem to be successful using specimens collected from the nasopharynx on dacron or rayon swabs (not calcium alginate swabs) or as aspirates, transported in Amies medium with or without charcoal or in Regan Lowe medium.

Table 1. PCR gene targets used to detect *Bordetella* species

Organism	PCR target (genome copies)					
	IS*481*		Pertussis toxin promoter		IS*1001*	
B. pertussis	+	(80-100)	+	(1)	-	
B. parapertussis	–/(+)		-		+	(20)
B. holmesii	+	(8-10)	-		–/(+)	
B. bronchiseptica	–/(+)		-		-	

Detection of the amplicon can be carried out by gel electrophoresis and ethidium bromide staining or probe-based detection, including FRET (fluorescence resonance energy transfer) probes, TaqMan probes, molecular beacons and SYBR Green (non-specific).

The sensitivity of most PCR assays is excellent with an analytic sensitivity of 1 to 10 cfu/mL and a clinical sensitivity that exceeds culture by 2 to 4 times. Inhibition is not uncommon, especially with mucoid specimens, and occurs in 1% to 22% of specimens. It can, however, be overcome with dilution. Pertussis PCR assays should be monitored with an internal control.

Despite the excellent achievable analytic sensitivity, in practice, as evidenced by results from a multi-centre proficiency panel, the interlaboratory variation is high[25]. Of six laboratories using seven assays to study dilutions of three test isolates, the range of detection was from 4 to 30,000 cfu/mL, showing an unacceptable performance in some laboratories. A second proficiency panel that addressed both sensitivity and specificity found that nested PCR showed no advantage and that results were appropriate for the primers chosen[25].

Issues of specificity are important for pertussis PCR assays. For those using the most common primer target (IS*481*), it is important to recognize that there will be cross-reactivity with *B. holmesii* and some *B. bronchiseptica*. Therefore, some have advocated using a second primer set for confirmation (such as those targeting the PT promoter or the pertactin gene) or IS*1001* for *B. parapertussis*. The use of IS*481* and IS*1001* should differentiate *B. pertussis*, *B. parapertussis* and *B. holmesii* from one another. However, this is expensive and time-consuming to do on a routine basis and is probably not necessary in outbreaks, which appear to be confined to *B. pertussis*. An alternative is to confine verification of specimens with a second *B. pertussis*-specific PCR to early in the course of an outbreak.

Quite apart from specificity issues with respect to other *Bordetella* species, the specificity of pertussis PCR assays has been compromised by laboratory contamination in some highly visible situations in the past. In the course of two large pertussis outbreaks in New York State, it was found that 60% of positive PCR tests performed by a private laboratory using an in-house test were not confirmed by a reference PCR[26]. A proficiency panel performed by the laboratory in question showed contamination of the negative controls. Thus, prevention of contamination is critical. This is true for any molecular testing, although the potential public health implications of the incorrect detection of a reportable communicable illness like pertussis are of particular concern. A helpful rule of thumb is that if the rate of positivity of pertussis PCR increases in relation to culture or greatly exceeds the usual 2- to 4-fold increase in sensitivity over culture, contamination should be suspected. In the New York outbreak, a 100-fold increase in sensitivity of PCR over culture had been observed. This supports the maintenance of culture, in at least some settings, for verification of PCR results in addition to supporting strain typing, anti-

microbial susceptibility testing and identification of *B. parapertussis* and *B. holmesii*.

To prevent contamination, laboratories must observe the usual stringent requirements of a molecular diagnostics laboratory, i.e. they must have the appropriate space, staff, training, professional expertise and tight quality control. Sample processing should be kept to a minimum, uracil-N-glycosylase (UNP) can be included to decrease carryover contamination, and appropriate negative and positive controls must be included in each run[27].

A European consensus panel developed recommendations in 1994 for PCR detection of pertussis. These include using specific probes for added sensitivity and specificity; confirming questionable results with alternative targets; and ensuring that all culture-positive specimens are also PCR positive, that other commensals and pathogens test negative in the system, that healthy controls test negative and that an internal control is used to detect inhibition[27].

At the present time, there is a need to survey the current status of molecular testing for *B. pertussis* in Canada and to develop up-to-date Canadian guidelines for such testing. Culture should be retained, at least in some centres, as an important benchmark for the validity of PCR results and for other testing that may be required, such as strain typing and antimicrobial susceptibility testing. A national proficiency testing program is a priority, given the variety of testing methods available and the lack of standardization or availability of commercial kits. A Canadian pertussis laboratory working group could have useful interactions with other groups outside Canada that are interested in a collaborative approach to issues surrounding molecular and other testing for *B. pertussis*.

A Large Cluster of Pertussis Cases in Toronto, Ontario – Laboratory Investigation

Dr. Frances Jamieson

The Central Public Health Laboratory (CPHL), Ontario Ministry of Health and Long-Term Care, has provided nucleic acid amplification testing (NAAT) for the diagnosis of *B. pertussis* and *B. parapertussis* since 1999, using standard PCR based on the method of Van der Zee et al[28]. Culture for *B. pertussis* and *B. parapertussis* was continued and is set up in the laboratory in conjunction with the PCR assay. In May of 2005, the laboratory implemented (after a 4-month comparison and validation process) a real-time PCR method for the detection of *B. pertussis* and *B. parapertussis* as a replacement for the standard PCR assay that was in use[29]. As part of the validation process, using serial end-point dilution it was determined that the detection limit of the real-time assay is less than one organism per reaction, an increase in sensitivity over the standard, gel-based PCR assay. In the real-time assay an internal control (for specimen integrity and inhibition) human ribonuclease protein (RNP) was used, and UNP was incorporated to prevent carry-over contamination during product analysis. The use of probes as the detection method for real-time PCR assays, as well as the measures described, greatly decreases the risk of false-positives. Other standard protocols, such as routine environmental surveillance for contamination control, are also in use to decrease the risk of false-positive results.

In October 2005, the laboratory noticed a large increase in the number of specimens submitted for pertussis (2- to 3-fold increase over specimens submitted during the same period in 2004-2005). A large percentage of these specimens were found to be NAAT positive, culture negative (the percentage doubling from October 2004 to October 2005). The pertussis assay runs were reviewed, and no issues were identified (all controls performed as expected and environmental surveillance swabs were negative). Further analysis of the specimens submitted indicated that one particular clinic in Toronto accounted for approximately 35%, and most of those (54%) were from patients in the age group 1 to 4 years. Compared with previous years, the number of positive pertussis cases had increased significantly; almost all of these were NAAT positive alone, most of which were “late-cycle” positives using the real-time PCR assay. These late cycle positives correspond to a very low organism load (as low as ≤ 1 organism) and would not be likely to grow in culture.

A blinded comparison panel of NAAT negative, positive and “weak” positive specimens was exchanged with the molecular laboratory of the Division of Microbiology, Department of Pediatric Laboratory Medicine, at the Hospital for Sick Children (Dr. Susan Richardson). The results from the CPHL and the Hospital for Sick Children

correlated 100%. RSV (respiratory syncytial virus) culture material was also tested using the real-time pertussis PCR assay and was negative (no false-positive results). The PCR products from the real-time assay were analyzed by gel electrophoresis. Positive specimens were found to have bands that were at the appropriate molecular weight (~120 bp), and sequencing of these specimens determined that they were 100% homologous to *B. pertussis*.

Therefore, the positive real-time PCR assay results on the specimens received during the investigation were not false-positive laboratory results, but they have generated a number of questions:

- Is there a transient carrier state (low numbers of organism present)?
- Was the assay detecting dead organism (e.g. patients had received antibiotics at the time of specimen sampling)?
- What is the clinical utility of molecular assays for pertussis detection, given the high sensitivity of the real-time PCR assay?
- What role does vaccine efficacy play?
- These questions and others generated from the discussion will be investigated further with a case-control study to be conducted jointly by the CPHL, the Hospital for Sick Children, the Toronto Public Health Unit and other researchers.

Laboratory Characterization of *B. pertussis* Strains

Dr. Mark Peppler

The utility was demonstrated of laboratory characterization of strains using the epidemiology of northern Alberta clinical isolates from 1995 to 2002 and monitoring changes in strains over time with the use of PFGE and allelic typing of PT S1 subunit and pertactin. A *B. pertussis* isolate possessing both "old" *ptxS1* and *prn* alleles is termed "old"; possessing both "new" *ptxS1* and *prn* alleles is termed "new"; and possessing one "old" and one "new" *ptxS1* and *prn* allele is termed "transitional" (Table 2). The distribution from old to transitional to new strain types has changed. The majority of isolates are in the "new" category (Table 3). The most prominent PFGE type during this period was BpeXba008 (Table 4). No difference in distribution was noted among those aged > 21 as compared with < 21-year-olds.

Despite this shift in strain types over time, no significant difference could be seen in the strains that caused clinical disease in vaccinated versus unvaccinated patients. There were 1,300 cases from 1995 onward, 758 of which were culture-positive, resulting in 278 complete records for which there were strains to analyze. The majority of cases were in the infant group. The data support the conclusion that *B. pertussis* has not escaped from vaccine-induced immunity by antigenic drift.

Continued surveillance of strains across Canada to monitor future changes over time is recommended. A national commitment to support and standardize these studies is required, and allelic typing and serotyping of fimbriae should be added to the profiles.

Table 2. Vintage of pertactin (prn) and pertussis toxin S1 (ptxS1) alleles

	"Old" (present before 1970)	"New" (emerged since 1985)
ptxS1	B, D, E	A
prn	1	2, 3, 9

Table 3. Estimated total numbers (percentages) for each combination of *prn* and *ptxS1* alleles found in the top 30 PFGE types combined from Alberta

	prn2 ptxS1A "transitional"	prn2 ptxS1B "transitional"	prn1 ptxS1B "old"	prn2 ptxS1A "new"	pr3 ptxS1A "new"	prn9 ptxS1A "new"	Totals
1985-1994	301 (15.8)	0 (0)	593 (31.2)	867 (45.6)	109 (5.7)	31 (1.6)	1,901
	16%		31%	54%			100%
1995-2002	60 (21.6)	2 (0.7)	0 (0)	178 (64.0)	6 (2.2)	32 (11.5)	278
	22.3%		0%	77.7%			100%

Table 4. Distribution of PFGE and *ptxS1/prn* types, 1995-2002

	ptxS1A and *prn* types				*ptxS1B* and *prn* types				Totals by PFGE type
PFGE type	1	2	3	9	1	2	3	9	
BpeXba001				5					5
BpeXba002	16		1						17
BpeXba004				2					2
BpeXba007						1			1
BpeXba008	1	178	3	1		1			184
BpeXba010				2					2
BpeXba011				4					4
BpeXba020				2					2
BpeXba024				1					1
BpeXba051				5					5
BpeXba072				1					1
BpeXba101				2					2
BpeXba102				3					3
BpeXba106				1					1
BpeXba107	43		2						45
BpeXba109				1					1
BpeXba116				2					2
Total 278 (%)	60 (21.6)	178 (64.0)	6 (2.2)	32 (11.5)	0 (0)	2 (0.7)	0 (0)	0 (0)	278

The Canadian Public Health Laboratory Network: Meeting Local and Global Challenges in Laboratory Public Health

Dr. Greg Horsman

The Canadian Public Health Laboratory Network

The Canadian Public Health Laboratory Network (CPHLN) is a nationally minded, proactive forum of public health laboratories. Since its formation, CPHLN has forged strong functional bonds linking federal and provincial public health laboratories. The CPHLN has been a voice of laboratory advocacy, and its federal and provincial member laboratories have been providing front-line response to naturally occurring infections and deliberately introduced bioterror-related agents.

The CPHLN was established in 2001. In response to the terrorism events of 11 September, 2001, and the subsequent anthrax attacks in Washington DC in 2001, the CPHLN had also elected to include bioterror response as one of its responsibilities. In addition to addressing concerns about the increased frequency and potential lethality of bioterrorism agents, the scope of the Network includes other aspects of public health, such as water and food safety in response to water-borne outbreaks in Walkerton, Ontario, and North Battleford, Saskatchewan. As well, it has expanded to develop strategies to advance laboratory standardization of diagnostic testing across Canada and an initiative to enhance reference testing. Moreover, the CPHLN recognizes the impact of infectious diseases on chronic disease and has taken steps to enhance programs that will address infectious diseases that have an impact on chronic disease.

The CPHLN recognizes the responsibility of public health laboratories to safeguard public health and to provide surveillance and response capabilities that serve as a cornerstone for all public health professionals.

The role of CPHLN is to provide a forum for public health laboratory leaders to share best practices and lessons learned in an atmosphere of trust. The role of the CPHLN is also to leverage its combined strength to champion for the sustainability and support of public health laboratories in their efforts to provide rapid and coordinated laboratory response to communicable disease agents.

The Pan-Canadian Public Health Network

The CPHLN does not stand alone. CPHLN is part of a national framework for local, national and global public health. It is part of the Pan-Canadian Public Health Network federal/provincial/territorial environment.

Under the reorganization within Health Canada and the formation of the new Public Health Agency of Canada (PHAC), the CPHLN is one of six other expert groups that create the framework for public health activities in Canada. These expert groups are as follows:

- Communicable Disease Control
- Canadian Public Health Laboratories
- National Emergency Preparedness and Response
- Surveillance and Information
- Non-communicable Disease and Injury Prevention
- Health Promotion

The groups are accountable to the F/P/T Public Health Network Council, which collates their findings and reports to the F/P/T Conference of Deputy Ministers of Health.

The membership of the CPHLN consists of provincial public health laboratories (10), the PHAC, Defence Research and Development Canada, the Council of Chief Medical Officers of Health, Canadian Blood Services, Héma-Québec and the Canadian Food Inspection Agency.

The CPHLN core comprises medical and scientific laboratory directors but has access to a broad spectrum of diagnostic and technical expertise from within each of Canada's public health laboratories.

The PHAC has representation in CPHLN from the following;

- Laboratory for Foodborne Zoonoses
- Centre for Emergency Preparedness and Response
- Centre for Infections Disease Prevention and Control
- National Microbiology Laboratory
- HIV/AIDS/Retrovirology

Cross-cutting issues

There has been an expansion in laboratory focus over time, and the linkage of the nation's public health laboratories continues to grow with cooperation and collaboration on cross-cutting issues. We are building laboratory linkage to epidemiology and other emergency response centres for infectious disease response. We are in the process of developing strategies that link management of infectious disease with prevention of chronic disease, where applicable. Our plans include the inter-jurisdictional development of integrated approaches to address cross-cutting public health issues. The development of a national command and control structure with cooperative interfacing and integration with local hierarchical reporting structures and protocols is part of our emergency planning.

The CPHLN has subcommittees that are generally jointly co-chaired by federal and provincial/territorial representatives. The subcommittees are the

- Laboratory Standardization Subcommittee
- Bioterror Response Subcommittee
- Food & Water Safety Subcommittee
- Reference Centre Advisory Subcommittee.

There are working groups set up periodically. There is a working group that advises on Pulse Net Canada. This group plays an important role in implementing the standards that allow inter-laboratory comparison of molecular fingerprints of various pathogens. Recently, the Laboratory Influenza group was rolled into the larger Pandemic Influenza Preparedness Network, which expanded into areas of epidemiology and viral diagnostics, and worked with Canadian Blood Services and the Office of Laboratory Security to develop the laboratory annex to the National Influenza Pandemic Plan. There is a Lyme Disease Working Group developing guidelines on laboratory testing for Lyme disease in Canada. The Working Group on Laboratory Self-Assessment will develop a template that will be used to create a database of public health testing in Canada.

The Laboratory Standardization Subcommittee of CPHLN reviews the nationally notifiable diseases, of which pertussis is one. This is to ensure that the cases reported across Canada can be evaluated by epidemiologists properly. The PulseNet Canada Group is involved in standards for PFGE for various enteric and nosocomial pathogens. The Reference Centre Advisory Subcommittee reviews the need for public health defence tests. Each of these groups could have a role in implementing recommendations developed at the Pertussis Workshop.

In summary, the CPHLN develops partnerships within a national framework. These partnerships involve collaboration on laboratory standards for nationally notifiable diseases, molecular diagnostics in public health, Web-driven business and communication tools, ownership documents and authorship documents. More information on these activities can be accessed at www.CPHLN.ca.

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