**Bordetella pertussis**: the intersection of genomics and pathobiology

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**Abstract**

There has been much recent concern over an increasing incidence of pertussis despite high levels of vaccine coverage of infants. Many reports have documented that much of the increased incidence is due to infection in adolescents and adults. This renewal of interest in pertussis comes at a time when the findings of the Bordetella genome project have led to a quantum leap forward in our understanding of the biology, evolution and pathogenesis of the bacterium responsible for the disease. The impact of this basic research on current clinical problems posed by *B. pertussis* infection is discussed.

CMAJ 2005;173(1):55-62

DOI:10.1503/cmaj.050105

CMAJ • JULY 5, 2005; 173 (1) 55

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the human host was very recent. Little research has been conducted on *B. parapertussis*, and its true host range has therefore not been conclusively determined. However, because this review focuses on *Bordetella* disease in humans, only the human-adapted *B. parapertussis* strain will be discussed, and in the remainder of this article, “*B. parapertussis*” refers to that strain.

In the *Bordetella* genome project, which was undertaken to further investigate the relations between different *Bordetella* species and to gain new insight into the genetic bases for the differing host ranges and pathogeneses of these bacteria, the genome sequences of a representative strain of each of *B. pertussis*, *B. parapertussis* and *B. bronchiseptica* were generated and analyzed. The enormous amount of information arising from this project will take many years and much basic research to understand. The main findings have been reviewed thoroughly and will be only summarized here.

Importantly, the genomes of the 3 species differ considerably in size, and their size correlates with their gene coding capacities: *B. bronchiseptica* encodes 1191 more genes than *B. pertussis* (Fig. 2, Table 3), and is therefore expected to have a greater functional repertoire. The difference in genome size, however, is compounded because *B. bronchiseptica* has only 18 pseudogenes, whereas *B. pertussis* has 358. Pseudogenes are remnants of once active genes that have been silenced by mutation (Fig. 3). In most cases, although the mutations render the gene non-functional, the gene is sufficiently intact that a clear picture of its identity remains. Many pseudogenes were formed when an insertion sequence (IS) element was inserted into the gene. IS elements are simple mobile genetic elements that do not carry any information other than that required for their mobility. The *B. pertussis* genome is remarkable in that it contains 261 copies of just

| Species          | Host range   | Diseases                      | Comments                                                                 | References |
|------------------|--------------|-------------------------------|--------------------------------------------------------------------------|------------|
| *B. pertussis*   | Humans       | Whooping cough (infants), persistent cough (adults) | Classic illness lasts 6–8 wk; complications rarely arise, but include pneumonia, seizures and encephalopathy, which can be fatal | 1–7        |
| *B. parapertussis* | Humans, sheep | Whooping cough, chronic pneumonia | Often considered to cause a milder disease than *B. pertussis*. Human and ovine hosts form 2 distinct lineages, so ovine hosts are unlikely to be reservoirs for human infection | 8–11       |
| *B. bronchiseptica* | Mammals    | Kennel cough (dogs), atrophic rhinitis (swine) | Asymptomatic carriage in many animal hosts; infection in humans in rare | 12–15      |
| *B. avium*       | Birds        | Rhinotracheitis               | The *B. avium* genome sequencing project is in progress                  | 16         |
| *B. trematum*    | Humans?      | Wound infections, otitis media | Infection in humans is rare                                                | 17         |
| *B. holmseii*    | Humans?      | Septicemia                    | Rare but increasing number of infections in humans; may cause a pertussis-like illness | 18,19      |
| *B. hinzii*      | Humans, domestic fowl | Asymptomatic | Infection in humans is rare; probably a commensal of the respiratory tract of fowl. | 20–22      |
| *B. petrii*      | Environment  | Not known                     | Recently isolated from river sediment in a bioreactor                     | 23         |

*Adapted, with permission, from Preston A, Parkhill J, Maskell DJ. The Bordetellae: lessons from genomics. Nat Rev Microbiol 2004;2:179-90.
3 IS elements; 238 are copies of IS481. The evolution of B. pertussis from B. bronchiseptica (or a B. bronchiseptica-like ancestor) was marked by the acquisition and rapid multiplication of IS elements. B. parapertussis has also undergone a similar evolution. It contains 588 more genes than B. pertussis but 603 fewer than B. bronchiseptica and 112 copies of 2 IS elements (90 of which are IS1002). In addition to their role in pseudogene formation, the IS elements have had a major impact on the organization of the B. pertussis and B. parapertussis genomes. Recombination between different copies of the identical IS elements has led to large-scale reordering of and deletions in their respective chromosomes compared with B. bronchiseptica (Fig. 2) and resulted in the different genome sizes.

Having evolved recently from a common ancestor, the 3 species contain a largely identical core genome. Indeed, only 11 genes in B. pertussis have been found to be species-specific (genes found only in that species and not in the other bordetellae),26 and no genes have been identified as unique to B. parapertussis. The genome sequence project identified 114 genes as being unique to B. pertussis, excluding IS elements, but genome-wide analysis (by microarray-mediated genomic typing) revealed that 103 of the 114 were in fact found in other strains of B. bronchiseptica.26 It is likely that the B. pertussis gene set merely reflected differences between the B. bronchiseptica strain whose genome was sequenced and the B. bronchiseptica strain from which B. pertussis evolved. The 11 species-specific genes have not been previously characterized, and their functions are not obvious from their DNA sequences. The same study showed that all 50 genes identified by the genome sequence project as being unique to B. parapertussis were present in other strains of B. bronchiseptica.26

### Table 2: Bacterial components important to the ability of Bordetella to cause disease

| Bacterial component | Expressed by | Function | Comment | Acellular vaccine component |
|---------------------|-------------|----------|---------|-----------------------------|
| Filamentous hemagglutinin | BP, BPa, BB | Adhesin (mediates adherence of bacteria to host structures) | Immunosuppressive activity may reduce lifespan of immunity | Yes |
| Pertactin | BP, BPa, BB | Adhesin | Several different fimbrial types expressed by the different species | Yes: type 2 & 3 |
| Fimbriae | BP, BPa, BB | Adhesin | | |
| Pertussis toxin | BP | Catalyzes ADP ribosylation of host G-proteins, which causes a number of effects | Genes present in BPa and BB but not expressed. Precise role in disease is unclear | Yes: inactivated toxin |
| Adenylate cyclase | BP, BPa, BB | Cytotoxin. Synthesizes cAMP in host cells, which disrupts host cell physiology. Anti-inflammatory effect through action on host immune cells | | No |
| Type III secretion system | BB, BPa<sub>ov</sub> | Alters of host immune cell function. Important for chronic infection? | Genes present in BP and BPa<sub>ov</sub> but expression has not been detected in these species | No |
| Dermonecrotic toxin | BP, BPa, BB | Toxin. Activates host GTP binding protein Rho. Causes changes in host cell morphology | Role in virulence is unclear | No |
| Tracheal colonization factor | BP | Adhesin? | | No |
| BrkAB system | BP, some BB | Resistance to serum-mediated killing | | No |
| Lipopolysaccharide | BP, BPa, BB | Structural component of bacterial outer membrane. Proinflammatory activity. Resistance to host defence molecules | Complex glycolipid. Structure varies between species | No |
| Tracheal cytotoxin | BP, BPa, BB | Cytotoxin. Contributes to damage to the respiratory epithelium | Cell wall breakdown product | No |
| BvgAS system | BP, BPa, BB | Global regulator of expression of most Bordetella virulence factors | | No |

Note: BP = B. pertussis, BPa = B. parapertussis, BPa<sub>ov</sub> = ovine-adapted B. parapertussis, BPa<sub>hu</sub> = human-adapted B. parapertussis, BB = B. bronchiseptica.
Adaptation of B. pertussis and B. parapertussis to the human host

The lack of species-specific genes in B. pertussis and B. parapertussis suggests that the evolution of these species from B. bronchiseptica and their adaptation to humans has occurred primarily through gene loss and recombination of their chromosomes. The acquisition of their human host niche does not appear to have arisen from the acquisition of new genes encoding factors that promote interaction between these bacteria and humans.

Many of the genes that are present in B. bronchiseptica but absent (and thus presumably lost during evolution) from B. pertussis and B. parapertussis appear to encode surface proteins (those located in the bacterial membrane and thus exposed to the bacterium's environment), including fimbrial adhesins (molecules on the surface of the bacterium that help it adhere to host structures during infection). Others encode proteins involved with small-molecule uptake and utilization; for example, a large number of proteins enable iron uptake. Both kinds of proteins are important for colonization of and persistence in a mammalian host, and it is likely that B. bronchiseptica has the ability to utilize a wide range of nutrients and to adhere to a wide repertoire of different molecules. This might explain the broad host range of B. bronchiseptica. Furthermore, it has long been assumed, but never directly proven, that B. bronchiseptica can exist in the environment.

Since there are no genes associated with adaptation to humans in B. pertussis or B. parapertussis, then it is possible that these species express certain genes differently than B. bronchiseptica and that this difference is responsible for infection in humans. Most of the genes that are present in all 3 species but expressed only in B. bronchiseptica are pseudogenes in B. pertussis or B. parapertussis and are presumably responsible for functions required for existence in the environment or non-human host. Several genes are present in all 3 species but expressed only in B. pertussis, and it is possible that these and presumably other, as yet unidentified, genes confer on B. pertussis the ability to infect humans. For example, the genome sequence project confirmed that the genes responsible for pertussis toxin synthesis and secretion are present in B. pertussis, B. parapertussis and B. bronchiseptica. However, only B. pertussis is known to express the toxin. The lack of expression of the toxin in B. parapertussis and B. bronchiseptica was previously thought to be a result of mutations in the regulatory regions of the associated genes. However, genome sequence analysis showed both that one of the pertussis toxin genes in B. parapertussis is a pseudogene, which might explain the lack of toxin expression in this species, and that, when the regulatory regions from all 3 species are compared, many of the putative mutations (often base pair substitutions) are conserved in B. parapertussis and B. bronchiseptica. This suggests that these 2 species contain the ancestral promoter sequence and that differences in expression are a result of mutations in the B. pertussis sequence. Several of these mutations are in regions that dictate the level of gene expression and would be expected to increase this level in B. pertussis. At one time pertussis toxin was thought to be responsible for much of the pathology associated with whooping cough, but the fact that B. parapertussis does not express the toxin yet causes

Fig. 2: A diagram depicting the colinearity of the genomes of B. bronchiseptica (BB) and B. parapertussis (BPa) and B. bronchiseptica and B. pertussis (BP). Regions of the 2 genomes that contain identical DNA are joined by red lines. Thus, vertical red lines represent regions that are present at the same point in both genomes. Slanted red lines represent regions that are present in both genomes, but at different locations. Black triangles show the locations of IS elements in each genome. Note that many genomic regions that have moved location in the B. pertussis or B. parapertussis genomes when compared with B. bronchiseptica are flanked by IS elements, indicating that these repeats are important foci of genomic reorganization in B. pertussis and B. parapertussis. The diagram was generated using the Artemis Comparison Tool (www.sanger.ac.uk/Software/ACT/) and is reproduced, with permission, from Parkhill J, Sebaihia M, Preston A, Murphy LD, Thomson N, Harris DE, et al. Comparative analysis of the genome sequences of Bordetella pertussis, Bordetella parapertussis and Bordetella bronchiseptica. Nat Genet 2003;35:32-40.

Table 3: Features of the Bordetella genomes*

| Feature               | B. pertussis | B. parapertussis | B. bronchiseptica |
|-----------------------|--------------|------------------|-------------------|
| Genome size           | 4 086 186    | 4 773 551        | 5 338 400         |
| (no. of base pairs)   |              |                  |                   |
| Genes, no.            | 3 816        | 4 404            | 5 007             |
| Pseudogenes, no.      | 358 (9.4)    | 220 (5.0)        | 18 (0.4)          |
| (% of total genes)    |              |                  |                   |
| % GC content of genome| 67.72        | 68.10            | 68.07             |
| IS element: no.       | IS481: 238   | IS1001: 22       | 0                 |
|                       | IS1002: 6    | IS1002: 90       |                   |
|                       | IS1663: 17   |                  |                   |

Note: % GC = % of total DNA that comprises guanosine and cytidine residues, IS = insertion sequence.

*Adapted, with permission, from Preston A, Parkhill J, Maskell DJ. The Bordetellae: lessons from genomics. Nat Rev Microbiol 2004;2:379-90.
a disease very similar to that caused by B. pertussis argues against a major role for the toxin in damaging the host. In fact, the only difference in infection profile between B. pertussis and B. parapertussis that may possibly be ascribed to pertussis toxin expression is the increased lymphocytosis observed in B. pertussis-infected patients.8,31 Thus, although it is possible that the progenitor of B. pertussis expressed pertussis toxin (albeit at low levels) and that B. pertussis evolved to express the toxin at higher levels, the role of the toxin in disease and thus adaptation to humans is still unclear.

Although there are probably a number of genes that are present in all 3 species but only expressed in B. pertussis and B. parapertussis that might contribute to their adaptation to humans, no genes that are present and functional in both B. pertussis and B. parapertussis but absent or non-functional in B. bronchiseptica have yet been identified.28 This suggests
that \textit{B. pertussis} and \textit{B. parapertussis} adapted to humans independently, each evolving its own distinct repertoire of functions that enabled exploitation of this niche. Because little research has been conducted on \textit{B. parapertussis}, the specific mechanisms of its pathogenesis remain largely unknown. As the information generated by the \textit{Bordetella} genome sequences is explored, a better understanding of the specific molecular mechanisms underlying \textit{B. pertussis} and \textit{B. parapertussis} infection will develop.

The current \textit{B. pertussis} problem

Waning immunity

As we have seen, both infection- and vaccine-induced immunity to \textit{B. pertussis} wane in adolescence, resulting in a population that is largely vulnerable to reinfection and that is a source of infection for susceptible infants. Why immunity is short-lived is unknown. As with most pathogens, research into \textit{B. pertussis} has focused on its ability to cause disease, and thus most of the bacterial factors that have been investigated are those associated with virulence. This has biased selection of components for acellular \textit{B. pertussis} vaccines such that they are composed of the adherence molecules filamentous hemagglutinin, pertactin and fimbriae, and inactivated pertussis toxin. It is now evident that some of these factors (e.g., filamentous hemagglutinin) not only cause damage to tissues but also modify the human host’s immune response to favour persistence of the bacterium.\textsuperscript{32–34} Thus, it is possible that using these factors as vaccine components results in the same immunomodulation that occurs during infection and affects the nature and thus the duration of the resulting immunity. Research into the effects of immunosuppression by \textit{B. pertussis} components on immunity is ongoing.

Antigenic variation

A controversial hypothesis is that strains of \textit{B. pertussis} currently circulating in the population have diversified from the very limited number of strains used to construct vaccines, so that vaccine-induced immunity does not fully recognize and protect against current disease-causing strains.\textsuperscript{35–37} Although evidence suggests that there is divergence between the pertactin and pertussis toxin protein sequences in vaccine strains compared with those found in strains in current circulation, some experts argue that the use of multiple components in acellular vaccines means either that divergence in just 1 or 2 of these components is insufficient to compromise vaccine-induced immunity or that these variations do not undermine vaccine-induced immunity.\textsuperscript{38,39}

\textbf{B. parapertussis}

Another potential confounding issue regarding the increasing incidence of \textit{B. pertussis} infection is that of infection by \textit{B. parapertussis}. Disease due to \textit{B. parapertussis} infection is often regarded as milder than that due to \textit{B. pertussis}, although it is clear that many symptoms appear similar.\textsuperscript{40} Several tests are used in diagnostic laboratories to confirm \textit{B. pertussis} infection: direct fluorescent antibody staining of organisms in respiratory tract fluids, culture of the organism and polymerase chain reaction (PCR). Direct antibody staining is thought to be of low sensitivity and is not widely used. Bacterial culture is considered the “gold standard” of diagnosis, but the organism requires particular media for culture and in most cases will be recovered only during the first few weeks of illness.\textsuperscript{41} However, \textit{B. parapertussis} will grow on any of the media used to culture \textit{B. pertussis} and, because there is no discernable difference between the 2 species in terms of colony appearance on agar, unless a specific test is employed for \textit{B. parapertussis}, straightforward culture is unlikely to differentiate between them. PCR is considered more sensitive than bacterial culture and is able to detect both viable and nonviable organisms within a sample.\textsuperscript{42} Several PCR-based tests that detect \textit{B. pertussis} and \textit{B. parapertussis} in a discriminatory fashion have been developed,\textsuperscript{43–45} but it is unclear whether \textit{B. parapertussis}-specific PCR is routinely used in diagnostic laboratories. Certainly the genome sequence information has identified many more targets for discriminatory PCR diagnostic tests. However, unless specific measures are taken, it may be difficult for laboratories to distinguish between \textit{B. pertussis} and \textit{B. parapertussis} infection. It has thus been proposed that the incidence of \textit{B. parapertussis} disease may be underestimated.\textsuperscript{46}

Furthermore, by several different analyses, it appears that \textit{B. pertussis} vaccines offer little, if any, protection against \textit{B. parapertussis} even though this bacterium expresses filamentous hemagglutinin, pertactin and fimbriae.\textsuperscript{41} Differences between epitopes (the specific region of a molecule to which antibodies bind) of these factors in the 2 species likely account for this lack of protection, and genome sequence analysis identification of several differences between the \textit{B. pertussis} and \textit{B. parapertussis} components supports this argument.\textsuperscript{47} At present, both \textit{B. pertussis} and \textit{B. parapertussis} appear to occupy the same niche in humans. It is possible that further decreases in \textit{B. pertussis} infection over time (if booster vaccines in adults become widespread) will increase the opportunity for \textit{B. parapertussis} infection. Because the contribution of \textit{B. parapertussis} to pertussis-like illness is not properly understood, specific tests will need to be implemented as routine in diagnostic laboratories to identify the pathogen.

Clinical link: Can the use of current \textit{B. pertussis} vaccines eradicate pertussis disease?

Early \textit{B. pertussis} vaccines were composed of inactivated whole cells. Although these vaccines were efficacious, public concerns over a perceived link with adverse reactions led to the development of a second generation of acellular vaccines, composed of the purified pertussis toxin, filamentous hemag-

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glutinin, fimbriae and pertactin protein antigens of B. pertussis, which displayed improved safety profiles over whole-cell vaccines. Acellular pertussis vaccines have been in use in Canada since 1997. The realization that vaccine-induced immunity is not lifelong has led to the development of a formulation of the acellular pertussis vaccines that can be administered as booster vaccinations to adolescents and adults. Booster vaccinations of adolescents are recommended in Canada. Although adult booster vaccinations appear to increase titres of antibodies against the vaccine components, the duration of the antibody response is still unknown, and insufficient data are available to warrant a proper evaluation of the protective effects of these vaccinations. A recent report from the Global Initiative on Pertussis working group formulated a series of suggestions for giving booster vaccinations to various groups of adults, ranging from booster vaccination of the general adult population to vaccination of specific groups. It certainly makes good sense for workers who are in regular contact with susceptible infants or for parents of infants to be given boosters. However, the same report suggested that such boosters might need to be given every 10 years to provide lifelong protection against B. pertussis infection and to eliminate the adult reservoir of B. pertussis. Although this is certainly a possible scenario, it remains to be seen whether many countries will adopt such a strategy, or if sufficiently high vaccination coverage can be achieved to provide herd immunity to susceptible infants.

An alternative strategy to using repeated boosters of current vaccines is to develop new vaccines that provide lifelong immunity to B. pertussis infection. Unfortunately, but perhaps understandably, there is no enthusiasm for (and in fact probably resistant to) this idea among vaccine-producing pharmaceutical companies. The cost of developing and licensing a novel vaccine is enormous. In addition, it would mean replacing a vaccine that has the prospect of being used in infants and multiple times in adults with one that would be used for just a single course in infants. However, the availability of genome sequence information means that the entire antigen repertoire of this organism is available and that B. pertussis is amenable to the genomics-driven vaccine design that has been pioneered for type B Neisseria meningitidis and applied to many other pathogens. As more information comes to light on the limited duration of immunity from current vaccines, on the role of adult infection in disease in infants and the role of B. parapertussis in disease, the pressure to develop new and better B. pertussis vaccines that also protect against B. parapertussis may grow. Genome sequence information will be instrumental in identifying bacterial components that are highly conserved between species and might be used as cross-protective antigens.

Conclusion

The short-lived immunity to B. pertussis generated by current acellular vaccines means that, unless the introduction of widespread booster vaccination programs in adults are successful, whooping cough among infants will remain a problem. Evidence suggests that current B. pertussis vaccines will not protect against B. parapertussis infections and thus, even if such programs are introduced, it is possible that B. parapertussis will fill the B. pertussis-vacated niche. The Bordetella genome project contains a wealth of information about the basic biology of B. pertussis, and it promises a dramatic increase in our understanding of this organism and its interaction with the human host. The question remains of whether this will translate into improved intervention strategies to curb the apparently increasing frequency of B. pertussis infection. This may in turn come down to a choice between continued and expanded use of existing vaccines, with the acceptance that immunity will be short-lived, and the enormously costly process of developing new and improved vaccines that also protect against B. parapertussis, with the long-term view of eradicating this disease. Comparisons of the long-term cost-effectiveness of each strategy will be interesting.

This article has been peer reviewed.

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Competing interests: None declared.

Acknowledgements: Research in Dr. Preston’s laboratory is funded by a Natural Sciences and Engineering Research Council of Canada Discovery Grant, a Canadian Bacterial Diseases Network Young Investigator Award and a Poultry Industry Council Research Grant.

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