Evolution and emergence of Bordetella in humans  [Preprint]

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Abstract

Two highly infectious bordetellae, B. pertussis and B. parapertussis, have emerged in historical times as co-dominant in human populations. Both of these cause acute disease ('whooping cough'), whereas their progenitor, B. bronchiseptica, is of variable virulence in a wide variety of animals. The remarkably close phylogenetic relatedness of these three bordetellae and the two independent jumps to humans provide a unique opportunity to examine the evolution and genetics involved in the emergence of acute human pathogens. We hypothesize that the more virulent strains in humans reflects how acutely infectious pathogens may be favored in communities with large contact networks. We furthermore suggest that the differential expression of the various virulence factors by the two human pathogens can be explained by immune-mediated competition between the strains. The evolutionarily favored strategies of both of the human bordetellae result in immunizing infections and acute epidemics.

Keywords: Critical community size, Cross-immunity, trade-offs, Whooping cough.

Introduction

The term 'whooping cough' refers to the sound of the desperate inspiration of air between paroxysmal coughing episodes of children infected with Bordetella pertussis or B. parapertussis. Although the symptoms are highly distinctive and diagnostic, the first medical chronicle of whooping cough was recorded 1578[1]. Since this disease description is relatively recent in the history of respiratory diseases, it has been hypothesized that whooping cough has recently emerged or increased in virulence. B. pertussis and B. parapertussis are highly transmissible, gram-negative coccobacilli that colonize human respiratory tracts and transmit through the aerosolized droplets produced by coughing. The disease is acute, with severe coughing that can progress to vomiting, convulsions, coma and death [2]. B. bronchiseptica -- their close evolutionary progenitor -- infects and can cause diseases in a wide range of mammals from marsupials through ungulates, rodents to carnivores [3]. Surveys of domestic animals have revealed very high (25%-100%) seroprevalence in cats[4], dogs[5] and pigs[6]. Although human infections are rare, clinical records are steadily accumulating. Risk groups to B. bronchiseptica appear to be infants[7], individuals in close contact with animals [8] or that are immunologically compromised [9]. A fascinating aspect of Bordetella biology is how virulent, acute pathogens have evolved at least twice from the animal progenitor. Phylogenetic analyses (Box 1) reveal that B. pertussis and B. parapertussis represent independent lineages that cause similar illness (except for the lack of lymphocytosis associated with the pertussis toxin that is expressed only by B. pertussis). A B. parapertussis-like strain has been isolated from sheep, however this appears to represent a separate evolutionary clade [1, 10] (Box 1). The two 'whooping cough'-causing strains, thus, appear to be specialized on humans.

There are several testaments to the success of the bordetellae as pathogens of humans. First, current estimates places the annual number of infected children at approximately 50 million[11]. Second, the basic reproductive ratio (the average number of secondary infections directly resulting from one infection in a completely susceptible population) of whooping cough is approximately 15, making it one of the most contagious directly transmitted human pathogens[12]. Third, before the successful development of a vaccine, it was one of the most important childhood infections with a mean age of infection of 5 years[13]. From an epidemiological point of view, however, the great success in terms of transmissibility may have come with a cost in terms of reduced infectious period. B. bronchiseptica persists, sometimes for life, in the nasal cavity of its wildlife hosts[14]. The human bordetellae, in contrast, can only infect humans transiently with a latent period of 7-10 days and an infectious period of 3 weeks or more. Although B. pertussis and B. parapertussis cannot persist within an individual, the severe cough that result from disease provides for efficient transmission that allows epidemiological persistence within human populations. The benefit of heightened transmissibility at the cost of shortened infectious period may reflect the recently identified trade-off between invasion speed and persistence [15].

Epidemiology: invasion -- persistence trade-offs

There are two fundamental epidemiological quantities; the transmission rate (the per time unit rate at which susceptibles are being infected by the infectious) and the length of the infectious period (the time available for transmission). These, in turn, determine the basis
reproductive ratio, $R_0$ -- the number of secondary cases that results from a single infection in a completely susceptible population. Recent epidemiological theories identifies a key trade-off[15]: All else being equal -- that is identical basic reproductive ratios -- strains with short infectious period and high transmission rate, like $B. pertussis$, have the evolutionary advantage that they will spread more rapidly. Yet, in the face of adaptive immunity, these more transmissible strains suffer a heightened risk of extinction because they result in violent epidemic fluctuations. The short-term evolutionary advantage thus comes at the cost of heightened intermediate-term extinction rate (fig. 1a). This effect is illustrated in the historical incidence data that reveal a critical community size for whooping cough persistence [16, 17]. Cities above this threshold can sustain transmission through the inter-epidemic period, while smaller communities experience epidemics that are punctuated by periods of zero incidence (Fig 1b). Even when making allowance for the reporting rates of whooping cough, the periods of prolonged punctuations are likely to represent local extinction. As a consequence, acutely infectious strains such as $B. pertussis$ and $B. parapertussis$ will have a strong short-term advantage over any less acute $B. bronchiseptica$-like pathogens. However, the acute strains face epidemic extinction in populations of hosts that are sparsely distributed. Epidemiology, therefore, offers the prediction that acute strains can only persist in densely clustered host populations.

**Adaptation to humans**

$B. pertussis$ and $B. parapertussis$ are viewed as either species or subspecies within the $B. bronchiseptica$-complex. Given the scant historical records and the relative genetic homogeneity, particularly in the case of $B. parapertussis$, a current view is that they relatively recently emerged in humans[1]. We can erect two contrasting hypotheses about the emergence in humans. First, bordetellae may not have infected humans until the two strains acquired the new host tropism. This superficially fits the phylogenetic data. However, we deem this to be unlikely because sequencing do not reveal acquisition of any new genes within the $B. pertussis$ and $B. parapertussis$ genomes relative to that of $B. bronchiseptica$[18] (Box 1). In fact, $B. bronchiseptica$ has been observed to infect and cause disease in humans repeatedly[7-9]. What is more, $B. bronchiseptica$ efficiently infects a broad range of mammals[2, 3], so it does not seem probable that humans would be innately excluded from this list. The alternative hypothesis is that the acute pathogens in humans resulted from evolution within an increasingly urbanized human context. This idea involves (i) a population-level trade-off between rapid transmission and loss of endemicity (above) and (ii) the host-level competition and exclusion of ancestral strains with which new strains elicit cross-reacting adaptive immunity (below). We hypothesize that several aspects of the bordetellae can be understood within the evolutionary context of such community level ('ecological') trade-offs. Among all the host species of these three bordetellae, an unusual though not necessarily unique, feature of the human host is its density and virtually nonseasonal reproduction. This
allows for efficient transmission and a steady influx of new susceptible children. In contrast, the sparse populations of many mammalian hosts may favor long infectious periods. This alternative hypothesis sees humans, like most other mammals as susceptible to *B. bronchiseptica*. The absence of *B. bronchiseptica*, then, represents its ecological displacement by more acute and competitively superior strains (given human contact networks). General theory shows that only one strain will tend to persist in a host population given a shared immune response[19] – the epidemiological equivalent to Gause's competitive exclusion principle.

**Box 1: Phylogeny and Comparative Genomics**

Although other related species exist, three bordetellae, recently reclassified as subspecies, are considered the “classical” members of the genus *Bordetella* based on multilocus enzyme electrophoresis and insertion sequence typing[3]. *B. bronchiseptica* appears across this phylogenetic tree as a common commensal/pathogen of a very long list of mammals (Fig. 2). The two subspecies, *B. pertussis* and *B. parapertussis*, have independently adapted from *B. bronchiseptica*-like progenitors to become acute human pathogens. *B. parapertussis* is particularly closely related to *B. bronchiseptica* and isolates from different years and regions of the world are of the same electrophoretic type and are indistinguishable by a variety of genetic criteria. This suggests that they arose from a single clone relatively recently [3]. Despite the apparent similarities between *B. pertussis* and *B. parapertussis*, each of these human pathogens is more closely related to *B. bronchiseptica* than they are to each other (Fig. 2) [3].

The Sanger Centre has sequenced and annotated the genomes of three strains within the *B. bronchiseptica* complex [18]. The genome of *B. pertussis*, contains 4,086,186 base pairs. That of *B. parapertussis* is substantially (16%) larger, with 4,773,551 base pairs, and that of *B. bronchiseptica* is larger still (by 30%) with 5,339,179 base pairs. The differences are due to the loss of numerous sizable multigenic regions [10, 18]. However, the genes that are involved in interactions with the host are mostly conserved in all three subspecies, although subsets are differentially expressed by each. The loss of numerous metabolic genes by both human pathogens suggests that the extraordinary rate of loss of genome may be the result of a commitment to a closed life cycle, with direct aerosol transmission from person to person.

**The B. parapertussis paradox**

The existence of *B. parapertussis* creates a paradox to the strain competition theory. Based on genomics and almost clonal identity between *B. parapertussis* isolates from anywhere on earth over the past 50
Box 2: Future directions
Several open questions need to be resolved with respect to evolution and epidemiology within the *Bordetella*-complex:
- What is the diversity of expression of virulence factors in the wild *B. bronchiseptica*-clade?
- How do different strains in the same host population interact either directly or in an immune-mediated fashion?
- How did a third subspecies, *B. holmesii*, successfully invade human populations?
- What is the role of adult subclinical *B. pertussis* infections in shaping epidemiological and evolutionary dynamics?
- How does *B. pertussis* succeed in reinfesting previously immunized hosts?

A combination immunological/bacteriological studies and theoretical models may be needed to resolve such questions.

years, this subspecies is likely to have emerged from a *B. bronchiseptica*-like strain more recently than *B. pertussis*[1, 18]. If *B. pertussis* and *B. parapertussis* induce cross-protective immunity, competition should cause exclusion of one of them. Yet *B. parapertussis* likely invaded a human population in which *B. pertussis* was endemic, and the two strains appear to coexist [20].

Theoretically, strain co-existence requires evolution towards reduced immune-mediated cross protection. A number of recent clinical and laboratory studies have focused on reciprocal protection, either natural or vaccine-induced, between the two human-restricted bordetellae[21, 22]. Apparently, *B. pertussis* infection confers little reciprocal protection to *B. parapertussis*; even the best *B. pertussis* vaccines have limited efficacy against *B. parapertussis*[23] and the two strains co-infects the same populations and occasionally the same hosts[24]. Experimentally, *B. pertussis*-induced antibodies bind very poorly to *B. parapertussis* and immunoblots show very few cross-reacting antigens (E.T.Harvill et al., unpublished). Thus, experimental, epidemiological and phylogenetic data support a model in which *B. parapertussis* invaded and persists within human populations by avoiding cross immunity to *B. pertussis*. This hypothesis leads to several predictions regarding how immune mediated pressure might affect the expression and/or variation of prominent antigens of the *Bordetella* subspecies.

Virulence factors and immune-mediated interactions
If one compares the virulence factors expressed by the two human pathogens to those of *B. bronchiseptica*, there is no single subset of genes that appear to confer a unique ability to infect humans; Some are expressed by all [1, 2]. Of the remaining, *B. pertussis* and *B. parapertussis* have contrary expression patterns, even of the factors that have been shown to be important to infection. The genes for pertussis toxin (PTX), for instance, are encoded in the *B. bronchiseptica* genome [18] and are widely expressed by *B. pertussis* and appear to be important in avoiding early neutrophil recruitment required for antibody-mediated clearance [25]. These genes are retained in the *B. parapertussis* genome but not expressed[18]. Conversely, *B. parapertussis* -- and very much *B. bronchiseptica* -- makes an O-antigen, a polysaccharide that covers the surface of the bacterium and may shield other surface antigens from immune recognition, that is important in their colonizing the respiratory tract [26]. The O-antigen is not expressed by *B. pertussis* and the genes required for its assembly have been lost from the genome [18].

From an infection point of view, one may wonder (i) why *B. parapertussis* would give up on the expression of a virulence factor that slows early clearance and (ii) why *B. pertussis* would give up on a factor that prevents antibody recognition. One possible answer relates to immune-mediated competition between strains and evolution to minimize cross-protection. The key, from this perspective, is that most 'virulence factors' both convey a pathogen advantage and elicit an antibody-mediated immune response. For example, if we use pre-vaccination, 20th century records as a guide, most children would be naturally immunized to *B. pertussis* by the age of 5 [2, 12, 13] and might harbor similarly high antibody titers also against PTX to create strong immunological pressures against PTX-expression in a subsequently emerging *Bordetella* strain. Perhaps, the historical emergence of *B. pertussis* was faced with a similar selection against O-antigen expression as *B. bronchiseptica* may have been circulating in prehistoric humans? In addition to explaining their differential expression of prominent antigens and virulence factors, this reasoning may explain why *B. pertussis*-specific vaccines have such limited effect on the infection and disease by the closely related *B. parapertussis*. Understanding the way these strains interact via cross immunity within human populations may provide the basis for more informed and effective strategies to control their spread and resulting disease.

Conclusions
A fascinating aspect of *Bordetella*-science is the plethora of studies at all levels of biological organization from the molecular [3, 18], the immunological [14, 21, 22] through to the epidemiological [16, 17], and involving diverse approaches from genomics [18] to mathematical ecology [27, 28]. Reviewing this large body of literature has led us to several questions for the future (Box 2) and two broad conclusions: First, evolutionary and ecological processes are tightly intertwined in shaping the disease dynamics. Second, population-level epidemic dynamics appear to be shaped by molecular interactions within individual hosts; and molecular dynamics and gene expression in
turn is shaped -- through cross-immunity and invasion/persistence trade-offs -- by population-level processes.

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References: