

MINIREVIEW

Bordetella pertussis transmission

Elizabeth A. Trainor¹, Tracy L. Nicholson² and Tod J. Merkel^{1,*}

¹Division of Bacterial, Parasitic and Allergenic Products, Center for Biologics Evaluation and Research, FDA, Bethesda, MD 20892, USA and ²National Animal Disease Center, Agricultural Research Service, USDA, Ames, IA 50010, USA

*Corresponding author: Laboratory of Respiratory and Special Pathogens, DBPAP/CBER/FDA, Building 72, Room 3308, 10903 New Hampshire Ave., Silver Spring, MD 20993, USA. Tel: +240-402-9746; E-mail: tod.merkel@fda.hhs.gov

One sentence summary: Transmission of the respiratory pathogens *Bordetella pertussis* and *Bordetella bronchiseptica* can be studied using the newly developed baboon, mouse and swine animal models.

Editor: Nicholas Carbonetti

ABSTRACT

Bordetella pertussis and *B. bronchiseptica* are Gram-negative bacterial respiratory pathogens. *Bordetella pertussis* is the causative agent of whooping cough and is considered a human-adapted variant of *B. bronchiseptica*. *Bordetella pertussis* and *B. bronchiseptica* share mechanisms of pathogenesis and are genetically closely related. However, despite the close genetic relatedness, these *Bordetella* species differ in several classic fundamental aspects of bacterial pathogens such as host range, pathologies and persistence. The development of the baboon model for the study of *B. pertussis* transmission, along with the development of the swine and mouse model for the study of *B. bronchiseptica*, has enabled the investigation of different aspects of transmission including the route, attack rate, role of bacterial and host factors, and the impact of vaccination on transmission. This review will focus on *B. pertussis* transmission and how animal models of *B. pertussis* transmission and transmission models using the closely related *B. bronchiseptica* have increased our understanding of *B. pertussis* transmission.

Keywords: Animal models; *Bordetella pertussis*; *Bordetella bronchiseptica*; Whooping cough; aerosol transmission

BACKGROUND

Bordetella pertussis and *B. bronchiseptica* are Gram-negative bacterial respiratory pathogens. *Bordetella pertussis* is the causative agent of whooping cough and is considered a human-adapted variant of *B. bronchiseptica* (Goodnow 1980; Parkhill et al. 2003; Preston, Parkhill and Maskell 2004; Diavatopoulos et al. 2005).

Some mechanisms of pathogenesis are shared between the *Bordetella* species. For example, in both *B. pertussis* and *B. bronchiseptica*, transcriptional activation of most virulence factors is controlled by a two-component system encoded by the *bvg* locus. This locus encodes a histidine kinase sensor protein, BvgS, and a DNA-binding response-regulator protein, BvgA. In response to environmental cues, BvgAS controls expression of a spectrum of phenotypic phases transitioning between a virulent (Bvg⁺) phase and a non-virulent (Bvg⁻) phase, a process referred to as phenotypic modulation. During the virulent Bvg⁺ phase, the BvgAS system is fully active and many of the known

virulence factors are expressed, such as filamentous hemagglutinin, pertactin, fimbriae, adenylate cyclase-hemolysin toxin and dermonecrotic toxin, as well as a type III secretion system (TTSS/T3SS) (Cotter and Jones 2003; Melvin et al. 2014). Conversely, BvgAS is inactive during the Bvg⁻ phase, resulting in the maximal expression of motility loci, virulence-repressed genes (*vrg* genes) and genes required for the production of urease (Akerley et al. 1992; Akerley and Miller 1993; McMillan et al. 1996). Previous studies involving phase-locked and ectopic expression mutants demonstrated that the Bvg⁺ phase promotes respiratory tract colonization by *B. pertussis* and *B. bronchiseptica* (Cotter and Miller 1994; Akerley, Cotter and Miller 1995; Cotter and Miller 1997; Martinez de Tejada et al. 1998; Merkel et al. 1998), while the Bvg⁻ phase of *B. bronchiseptica* promotes survival under conditions of nutrient deprivation (Cotter and Miller 1994, 1997).

Despite the close genetic relatedness, *B. pertussis* and *B. bronchiseptica* differ in several classic fundamental aspects of

Received: 10 July 2015; Accepted: 17 August 2015

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bacterial pathogens such as host range, pathologies and persistence. *Bordetella pertussis* is the causative agent of pertussis (commonly called whooping cough), a highly contagious infection of the respiratory tract. Despite sustained vaccination rates exceeding 95% in the United States, the disease remains endemic in the population. Only humans are naturally infected with *B. pertussis* and infection leads to an acute disease with no evidence of a prolonged carrier state (Hewlett et al. 2014). Due to the severe host restriction, the lack of prolonged carriage and lack of an animal reservoir and the ability to survive in the environment, maintenance of *B. pertussis* in the population requires an unbroken chain of transmission. The disease is characterized by paroxysmal coughing spasms that are thought to contribute to transmission. High numbers of bacteria can be isolated from the airway early in infection, but the ability to isolate bacteria wanes as the infection progresses and bacteria are rarely isolated from patients in the paroxysmal coughing stage of the disease. The early stages of infection, following the onset of cough and while bacterial counts in the airway are still high, are considered the most contagious (Gordon and Hood 1951). Early epidemiological studies of whooping cough identified direct, prolonged contact with an infected individual as the source of infection to a naïve host (Luttinger 1916; Culotta, Dominick and Harrison 1938). Reported attack rates in unvaccinated children within household contact studies ranged between 58 and 100% (Mertsola et al. 1983; Isomura 1991; Schmitt 1997; Simondon et al. 1997; Storsaeter and Gustafsson 1997; Heining et al. 1998). A recent household study in New South Wales showed that secondary attack rates were lower in vaccinated household contacts and in primary cases who received antibiotic treatment within 7 days of disease detection (Terry et al. 2015). Studies investigating attack rates among school settings have reported rates between 0 and 36%, which supports the requirement of prolonged close contact for transmission (Culotta, Dominick and Harrison 1938; Rodman, Bradford and Berry 1946; Etkind et al. 1992). It is worth noting that at the time of these studies no evidence existed to support airborne transmission of pertussis between hosts (Gordon and Hood 1951; Schellekens, von König and Gardner 2005). The earliest documented hypothesis that pertussis may be spread via respiratory droplets occurred in 1916 and originated from the observation that cases of whooping cough could be traced back to the exposure of uninfected individuals in a movie theatre prompting the author to hypothesize that the 'sputum from infected children was carried on air currents to an unlimited number of individuals' (Luttinger 1916). It would be almost 100 years before this author's hypothesis was validated in well-controlled studies in which transmission by contact could be ruled out (Warfel, Beren and Merkel 2012).

The development of the baboon model for the study of *B. pertussis* transmission, along with the development of the swine and mouse model for the study of *B. bronchiseptica*, has enabled the investigation of different aspects of transmission including the route, attack rate, role of bacterial and host factors, and the impact of vaccination on transmission. This review will focus on how each of these models has led to an increase in our understanding of the mechanisms of transmission for *B. pertussis*.

THE MOUSE MODEL OF *B. BRONCHISEPTICA* TRANSMISSION

Bordetella bronchiseptica is closely related to *B. pertussis* and causes respiratory illness in a wide range of mammalian species including mice, dogs, cats, poultry and livestock animals such as

pigs (Parkhill et al. 2003; Mattoo and Cherry 2005). *Bordetella bronchiseptica* rarely causes infections in humans; however, its genetics are related to that of *B. pertussis* therefore transmission studies in *B. bronchiseptica* may shed light on the possible host and bacterial molecular mechanisms involved in *B. pertussis* transmission (Diavatopoulos et al. 2005; Park et al. 2012). Important caveats should be noted, however, *B. bronchiseptica* has a larger genome than *B. pertussis*, it causes a chronic infection in a broad range of mammalian species and it has been shown to be capable of surviving in the environment. *Bordetella pertussis* causes an acute infection only in humans and does not survive in the environment. Although individual effectors may have similar functions in *B. bronchiseptica* and *B. pertussis*, there are clearly differences in host range and pathogenesis suggesting there may be differences in mechanisms of transmission.

Mice can become colonized with *B. bronchiseptica* and shed the bacteria from the nares; however, they do not display the characteristic cough of a human *B. pertussis* infection. Transmission of *B. bronchiseptica* is not observed between wild-type mice. In order to observe transmission of *B. bronchiseptica* between mice, it is necessary to use mice with defective innate immune responses (Rolin et al. 2014). Transmission is observed between mice lacking a functional TLR4 receptor. The TLR4 receptor mediates recognition of the *B. bronchiseptica* LPS. This recognition triggers innate immune responses and the downstream development of adaptive immune responses (Mann et al. 2005). The transmission observed between TLR4-deficient mice is due to both increased susceptibility of these mice to infection and increased bacterial load in the nares or upper respiratory tract that ultimately results in increased shedding from the infected mice (Rolin et al. 2014). Additionally, mice are not a natural host for *B. bronchiseptica*, so it is plausible that the increased shedding combined with increased susceptibility facilitates transmission in immunocompromised mice that is not observed in wild-type mice. Using mice as a model for studying transmission of *B. bronchiseptica*, Harvill et al. have demonstrated that mice vaccinated with a whole cell vaccine exhibit reduced bacterial shedding and reduced transmission relative to mock-vaccinated mice (Smallridge et al. 2014). Additionally, the same study demonstrated that vaccination with an acellular pertussis (aP) vaccine resulted in reduced transmission, but not to the same degree as the reduced transmission rate resulting from the whole cell pertussis (wP) vaccine.

THE *B. BRONCHISEPTICA* SWINE MODEL

In some instances it may be beneficial to use an infection system that utilizes an isolate and its natural host when evaluating the role of specific factors involved in host-pathogen interactions. *Bordetella bronchiseptica* is highly contagious among many poultry and livestock species, including swine. *Bordetella bronchiseptica* is widespread in swine populations and is a significant contributor to respiratory disease in pigs. Additionally, experimental direct and airborne transmission of *B. bronchiseptica* has been documented. Using a virulent *B. bronchiseptica* strain originally isolated from a swine herd exhibiting atrophic rhinitis, Nicholson et al. have observed both direct and indirect or airborne transmission of *B. bronchiseptica* between pigs (Brockmeier and Lager 2002; Nicholson et al. 2012, 2014).

This model does not require the use of any genetically modified strains of *B. bronchiseptica* or immune-deficient animals. It does however require the facilities to house and care for sows and piglets as well as appropriate containment facilities to work

with infected pigs. Additionally, significant effort is required to obtain *B. bronchiseptica*-free animals. In both *B. pertussis* and *B. bronchiseptica*, the majority of virulence gene expression is regulated by a two-component sensory transduction system encoded by the *bug* locus. BvgAS controls expression of a spectrum of phenotypic phases transitioning between a virulent (Bvg⁺) phase and a non-virulent (Bvg⁻) phase, a process referred to as phenotypic modulation.

The hypothesis that phenotypic modulation by the BvgAS signal transduction system is required for transmission was tested by Nicholson et al. using the swine model. Both the wild-type isolate and a Bvg⁺ phase-locked mutant transmitted to naïve piglets housed in the same cage as well as to naïve piglets in cages across the room (Nicholson et al. 2012). The results from this study demonstrated that in a natural host, phenotypic modulation from the fully virulent Bvg⁺ phase was not required for respiratory infection and host-to-host transmission of *B. bronchiseptica*.

Among the *bug*-activated genes are those that encode the type III secretion system (T3SS) that mediates persistent colonization of the lower respiratory tract in the mouse *B. bronchiseptica* model (Yuk, Harvill and Miller 1998; Yuk et al. 2000). However, these genes are not required for transmission demonstrated by the fact that wild type and a T3SS mutant are both capable of direct and indirect transmission among swine (Nicholson et al. 2014). The requirement for *bug*-repressed genes in swine infection and transmission has not been tested because a Bvg⁻ phase-locked strain could not be recovered from directly challenged piglets (Nicholson et al. 2012).

THE BABOON MODEL OF PERTUSSIS TRANSMISSION

The hypothesis that pertussis is spread via respiratory aerosols and also transmitted via airborne droplets was confirmed using the recently described baboon model of *B. pertussis* pathogenesis (Warfel et al. 2012). Directly challenged baboons develop symptoms of whooping cough similar to humans, exhibiting elevated white blood cell counts, *B. pertussis* colonization of the respiratory tract and the characteristic paroxysmal coughing spasms (Warfel et al. 2012). This is the first *B. pertussis* animal model to recapitulate the coughing observed in humans. *Bordetella pertussis* transmission is observed from directly challenged baboons to cohoused baboons as well as baboons housed in separate cages across the room (Warfel, Beren and Merkel 2012). The rate of transmission and development of symptomatic pertussis was faster for cohoused animals than for those in separate cages (Warfel, Beren and Merkel 2012). The time to develop disease in each case mimicked that observed in humans where much closer household contacts have a higher rate of transmission than more casual contacts in a school classroom, hospital or nursery setting.

The number of pertussis cases dropped greater than 90% in the industrialized world according to the World Health Organization with the advent of the wP vaccine in the 1950s. However, due to adverse side effects the wP vaccine was replaced with an aP vaccine in the 1990s. However, in spite of the high vaccination rates there has been a steady increase in the number of pertussis cases observed. This increase began before the introduction of the aP vaccine but the rate of increase has climbed in the years following introduction of the aP vaccine. In 2012, there were over 48 000 cases of pertussis reported to the Centers for Disease Control and Prevention in the United States alone

(Clark 2014). One hypothesis is that the aP vaccine does not protect against transmission as well as the wP vaccine. In fact, epidemiological vaccine efficacy trials monitoring the attack rate of aP-vaccinated individuals exposed to household cases of primary pertussis indicate that on average 13% are diagnosed with clinical pertussis (Miller et al. 1949; Lambert 1965; Mortimer et al. 1990; Isomura 1991; Schmitt et al. 1996; Storsaeter and Gustafsson 1997; Heininger et al. 1998). These observations suggest that aP-vaccinated individuals are susceptible to pertussis infection. Recent comparative studies indicate that wP-vaccinated children are less susceptible to pertussis diagnosis during outbreaks relative to aP-vaccinated children (Sheridan et al. 2012; Liko, Robison and Cieslak 2013).

The baboon model provides a controlled and unique system in which to test how vaccination with the aP vaccine impacts transmission of pertussis. Acellular-vaccinated baboons challenged with pertussis do not show clinical signs of disease but do have *B. pertussis* colonization of the airways comparable to naïve animals (Warfel, Zimmerman and Merkel 2014). Using the baboon model, the authors were able to demonstrate that transmission of pertussis can occur to and from an aP-vaccinated animal. An increasingly popular hypothesis is that infected, aP-vaccinated individuals could serve as asymptomatic carriers of pertussis capable of transmission to unvaccinated individuals. In fact, PCR and serological data in vaccinated children and adults suggest that asymptomatic pertussis can occur (Deen et al. 1995; Heininger et al. 2004; Ward et al. 2006). These observations highlight the importance of understanding the mechanisms involved in *B. pertussis* transmission including bacterial factors, host factors and how immune response contributes.

CONCLUSION

Each of the animal models discussed provides advantages for studying transmission of *Bordetella* species and therefore may provide insights into the transmission of *B. pertussis*. The mouse model is small, inexpensive and can be genetically manipulated. The swine model offers the ability to study bronchiseptica disease and transmission in a natural host. The baboon model closely mimics critical aspects of human disease such as elevated white blood cells and coughing. Additionally, humans, non-human primates and swine share the same anatomical structure of lymphoid tissues in the upper respiratory tract (Perry et al. 1997; Pracy et al. 1998), while rodents lack the pharyngeal and palatine tonsils that are known to function as inductive sites for secretory antibody responses in these species (Kuper et al. 1992; Wu, Nguyen and Russell 1997; Lugton 1999; Debertain et al. 2003). In addition, human tonsils have deep antigen-retaining crypts and tonsils express germinal centers shortly after birth, whereas the rodent nasal-associated lymphoid tissues have a plain surface and requires an external stimulus to induce the expression of germinal centers (Brandtzaeg 2010).

A combined look at the data from epidemiological and experimental studies begins to shape a picture of what factors are critical for *B. pertussis* transmission as well as those that do not play a significant role. Human and baboon studies indicate that symptomatic disease is not required for transmission. For infected individuals with asymptomatic disease, prolonged close contact appears to be sufficient for transmission to a naïve person. Airborne transmission from individuals with cough is likely to be enhanced. Data from both the mouse *B. bronchiseptica* model and the baboon *B. pertussis* model suggest that

factors that increase the susceptibility of the host or increase the bacterial load in the host increase rates of transmission. Studies in *B. bronchiseptica* suggest that Bvg-induced modulation is not required for direct host-to-host transmission (Nicholson et al. 2012). However, the *bvg*-repressed genes have been shown to allow survival of *B. bronchiseptica* in the environment suggesting that they are likely important for transmission that occurs through environmental sources (Coote 2001). No function for these genes has been described to date in the obligate human pathogen *B. pertussis*. However, since *B. pertussis* must survive in aerosolized respiratory droplets to colonize new hosts, it is possible that Bvg-mediated phenotypic modulation is important for *B. pertussis* transmission by prolonging survival in the air. Studies are currently underway to test this hypothesis. Identifying factors required for pertussis transmission will improve treatment strategies and possibly lead to development of improved vaccines to control the spread of pertussis within communities.

Conflict of interest. None declared.

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