Airborne Transmission of *Bordetella pertussis* Demonstrated in a Baboon Model of Whooping Cough

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(See the brief report by Warfel et al, on pages 902–6.)

Pertussis is a vaccine-preventable respiratory disease caused by *Bordetella pertussis* [1]. Globally, it is estimated that pertussis vaccines prevented approximately 700,000 deaths in 2008, attesting to the ostensible success of the vaccines [2]. However, despite vaccine coverage approaching 90% [3], 16 million cases of pertussis occur annually [2], resulting in 195,000 deaths in children <5 years of age [4]. Most of these cases are in low- and middle-income countries.

Although the morbidity and mortality numbers are lower in high-income countries, pertussis remains endemic in the face of high vaccination coverage [5, 6]. For example, in the United States, despite a 92.2% reduction in the number of cases and a 99.3% reduction in deaths since the introduction of pertussis vaccines in the 1940s [7], *B. pertussis* continues to circulate [8], causing outbreaks such as the 2010 epidemic in California, which resulted in 7,824 cases and 10 deaths in infants [9, 10]. Outbreaks occur every 2–5 years, and this cyclical nature of pertussis, which has not changed since the introduction of vaccines [8], is attributed to the immune status of the population. Immunity lasts 4–20 years after infection and 4–12 years after vaccination [11]. When immunity is high as a consequence of natural infection or vaccination, the number of cases dips; when immunity wanes, the number of cases rises. Although other factors may play a role [8, 10, 12, 13, 14], the epidemiology of pertussis is ultimately framed by the transmission dynamics of *B. pertussis*, which is influenced by waning immunity and the ability of the immune status of the population to be boosted either by vaccination or by natural infection [11, 15, 16, 17].

The reason why immunity to pertussis wanes even after natural infection is probably linked to the ability of *B. pertussis* to evade and modulate both innate and adaptive immune responses [18], although the mechanisms by which it does so are incompletely understood. There are also many questions regarding how *B. pertussis* is transmitted and whether specific virulence or survival factors are involved in transmission. *B. pertussis* is restricted to humans and has no known environmental reservoir [1]; thus, survival of this pathogen relies on strategies that aid transmission between those who are infected and those who have no or low levels of protective immunity.

Pertussis is highly contagious [1]. Direct inoculation with as few as 140 bacteria is sufficient to cause disease in naive children [19], and epidemiological studies have shown that transmission is associated with close contact, primarily among family members [20, 21, 22]. Because pertussis is a respiratory illness with distinctive symptoms [1], airborne transmission via respiratory droplets has been postulated, but, surprisingly, such transmission has not been formally demonstrated, in part owing to the difficulties in ruling out contact between infectious and uninfected individuals.

Studies to address airborne transmission are more tractable in animal models; animals can be kept segregated more easily, and experimental parameters can be better controlled. Mice, rabbits, rats, and pigs are some of the animals used to study pertussis [23]; however, transmission has not been demonstrated in these models. The Merkel laboratory thus revisited the idea of using a nonhuman primate to model pertussis, reasoning that a model using a species more closely related to humans would be more likely to reproduce all aspects of pertussis pathogenesis, including disease and transmission [24]. They found that monkeys had been used previously to study pertussis, but that the results were inconsistent. It was noted that the Taiwan macaque (*Macaca cyclopsis*) was more susceptible to pertussis compared to the rhesus macaque (*Macaca mulatta*); however, experiments with *M. cyclopsis* were not feasible because they are endangered.
Warfel and colleagues instead reevaluated rhesus macaques [24]. They discovered that only 25% of inoculated animals showed symptoms of pertussis, in line with previous observations [24].

The authors traced this attenuated response to the higher core body temperature of the rhesus macaques, which ranges between 38.7°C and 39.8°C. At this temperature, B. pertussis grew more slowly than it did at 37°C, and the amount of adenylylcyctase toxin, a key virulence factor, was dramatically reduced. These limitations may have contributed to the mild degree or absence of overt clinical symptoms, even though all of the rhesus macaques were infected.

Dissatisfied with the rhesus macaque model, the Merkel laboratory turned their attention to another nonhuman primate, Papio anubis—the olive baboon, an animal with a body temperature of 37°C–39°C. Using an inoculum of $10^9$–$10^{10}$ colony-forming units/mL of strain D420 (a recent clinical isolate), weanling baboons were infected by delivering 1 mL to the top of the trachea and 0.5 mL to the back of each naris. All infected baboons were infected by delivering 1 mL to the top of the trachea and 0.5 mL to the back of each naris. All infected baboons contracted disease that looked remarkably like human pertussis. Notable clinical manifestations included the production of the characteristic cough that lasted 2 weeks, elevated white blood cell counts, and a mild fever, suggesting that the olive baboon could serve as a good model for pertussis. The model used a high bacterial dose to establish the infection, and it is not clear whether a smaller inoculum size would also suffice. Nevertheless, this model made it possible to address whether airborne transmission of B. pertussis could occur.

The airborne transmission study, the results of which are described in this issue of The Journal of Infectious Diseases, was conducted in a specialized biocontainment unit. In this unit, the input and exhaust air is filtered with a high-efficiency particulate air filter, and the unit is engineered to have air flowing unidirectionally from the side housing the inoculated baboons to the side housing the naive ones. Air also flows through the wire mesh sides of the baboon cages, and the distance between the cages at either end is 7 feet. The cages housing the naive animals (at the distal, exhaust air side) have stainless steel barriers on opposite sides to prevent physical contact between animals but still allow for air flow through the mesh. Index animals were infected and placed in the cage near the input air source. Twenty-four hours later, naive animals were placed either in the same cage or in the cage(s) at the distal end. The results of these experiments which were conducted in this highly controlled setting, showed that all of the naive baboons got pertussis. Because pertussis was seen even in naive animals housed where physical contact was prevented, this demonstrated that airborne transmission of pertussis can indeed occur. Interestingly, the time to infection differed between the animals who were housed together and the ones in the cages on the other side of the room. Naïve animals housed in the same cage as the index animals developed pertussis in 10 days, whereas it took 19 days for animals in the distal cages to display similar clinical manifestations. As the authors point out, these results probably reflect a decreased efficiency of transmission because of the dilution of the inoculum size as a function of distance from the source of infection, something that mimics human epidemiological data, in which generally higher attack rates are seen in household settings, where there is a high likelihood of close contact, than in school settings, where the probability of close contact is lower.

The establishment of the baboon model is an important advance in B. pertussis research. The disease in baboons resembles clinical whooping cough, and baboons infected with pertussis have developed immune responses that have protected them from subsequent infection [24]. Furthermore, because transmission of pertussis in baboons is not only possible but also reflects the epidemiology seen in human disease (albeit using a small sample size), this model could be used to better understand pertussis pathogenesis and to test future vaccine formulations, therapeutics, or other control measures that might influence the complex dynamics between waning immunity, boosting, transmission, and the mechanisms of infection.

**Notes**

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**References**


