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Letter to the Editor

Bordetella pertussis Polymorphism and Pertussis Vaccines

The topic of the article by Bottero et al. (1) is an important one, a bacterial vaccine for a preventable disease, pertussis. Many articles have been published on the subject in the last 10 years (for a review, see reference 6). In their study, Bottero et al. neither followed the recommendations of consensus meetings nor used published nomenclature. Furthermore, they compared vaccines that cannot be compared.

In the abstract and throughout the manuscript, the authors claim that the vaccines are not protecting against new circulating isolates. How can they say that during the clinical efficacy trials, the isolates circulating in the countries where these trials were performed differed from the vaccine strains (at least in Europe) and then say that the vaccines were still found to be efficacious? We all agree that the actual isolates "escape" the herd immunity, but with the high level of vaccination coverage and the efficacy of whole-cell vaccines, these isolates can be controlled in many countries.

According to the description in Materials and Methods (1), Bottero et al. also did not use standardized techniques. For the PCR diagnosis, they did not use the agreed-upon reference technique published in 2005 (7). For the pulsed-field gel electrophoresis (PFGE) analysis, they cite the reference paper of Mooi et al. (5) but did not follow all of its conditions, which are now used by all other teams in Europe and the United States (3). Further, they use numbers to differentiate the isolates which are similar to, but not the same as, those used by Mooi et al., and this is very confusing for all readers. Also, they write that the Tohama strain is a vaccine strain, which is not the case in their country. For the animal assay, they cite the reference paper authored by myself and my colleagues (4) but do not follow the same protocol. We did not use intraperitoneal administration and did not conclude that 1/10 of the human dose of the vaccine is the right dose for an animal. Furthermore, as this test is now one of the World Health Organization's assays for licensing vaccines (8), it is important to follow the procedures for this assay exactly. Also, how can anyone dare to compare an unidentified combined diphtheria-tetanus-whole-cell pertussis vaccine (no source, manufacturer, or other information provided) with a noncombined, apparently homemade vaccine with no description of the technique used to detoxify the bacteria?

In Results and Discussion of their paper (1), the authors omit any comparison or discussion of the data from Argentina that my colleagues and I obtained and published recently about the same isolates that they used (2). Nor do they refer to the many studies performed in Europe and elsewhere on the same topic. The data presented concerning the animal model include the data at day 5 but no data for day 8. They neither discuss nor cite data previously published on whole-cell pertussis vaccine-induced immunity against infection due to different isolates, but they do present a superficial analysis of one clinical isolate without any confirmation of the data for at least two or three other isolates of the same PFGE groups.

For these many and various reasons, I advise caution in interpreting the paper of Bottero et al., which sits uncomfortably with much of the already available literature on pertussis strains.

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Author's Reply

First, I want to clarify for Dr. Guiso that in no part of our paper (1) did we state that "the vaccines are not protecting against new circulating isolates." Therefore, I recommend a more careful reading of our work in order to determine the true subject under discussion. My colleagues and I will not argue about what is not stated in the article. On the other hand, it is important to point out that all protection results presented in our paper were obtained using mice, and thus, care in making extrapolations to humans must be taken.

It remains difficult to prove that changes observed in *B. pertussis* populations affect vaccine efficacy in humans. To prove this requires at least a clinical trial with two vaccines, one derived from old strains and one from current isolates. To our knowledge, this kind of study has not yet been done.

As for Dr. Guiso's claim that we did not use standardized techniques, I want to point out that there are no universal standardized PCR protocols for *Bordetella pertussis*. Various single-target PCR assays are currently utilized, but none is universally considered to be the "gold standard." The paper on the agreed-upon reference technique for PCR diagnosis mentioned by Dr. Guiso (6) was written by M. Riffelmann, C. H.

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Wirsing von König, V. Caro, and N. Guiso for the Pertussis PCR Consensus Group. The protocol described in the article is very sensitive but lacks specificity in terms of *Bordetella* species. In fact, this PCR assay also detects *Bordetella holmesii*, *Bordetella petrii*, and *Bordetella bronchiseptica* isolates. Moreover, after publication of the article, additional different assays were proposed (3, 5). The important point is to avoid the use of the PCR as a unique method, separate from other diagnostic techniques and the clinical characteristics of the case. We have several years of experience in molecular diagnostic methodology, and good correlation between the PCR method we used and other diagnostic methodologies was observed. For the PFGE analysis, we followed the conditions reported by Mooi et al. (4), but as usual, we had to introduce minor changes to set up the technique in our lab. We do not think that the numbers used to differentiate the isolates were "very confusing for all readers." Comparison of the data from Argentina with those obtained by Dr. Guiso's group has already been done, so we considered it redundant to repeat it. For the animal assay, we followed the intranasal challenge model reported by Guiso et al. (2). As for the conditions reported in Materials and Methods, we consistently observed differences in the protection levels, which had to be communicated. Throughout her letter, Dr. Guiso criticizes not the validity of our experiments but whether we used standardized techniques. The important point here is that by applying all rigorous controls and reproducibility tests to our methodologies, we found coherent results on divergence from immunogen sequencing, PFGE, proteomics, and animal assays. Data for days 5 and 8 were included when mice were immunized with the commercial vaccine and challenged with the different strains. We do not understand why Dr. Guiso claims the contrary. In the case of the mice immunized with wPBp106, we presented the data obtained on days 0 and 5, since differences in protection at such times were already evident.

The Tohama strain was used in these experiments because it was isolated during the same period as the other vaccine strains, and because of that, it is considered an old strain, which is the important point: it is used for acellular pertussis vaccine production, and its genome is completely sequenced. It is important to emphasize that we are convinced that the current vaccines are effective but not the best. However, it will be important to keep analyzing the relevance that divergences between circulating bacteria and strains used in vaccine production have on protection. Our results show that circulating bacteria differ at the genome and proteome levels from strains used in vaccine production and, moreover, that divergence between strains affects vaccine efficacy in an intranasal challenge mouse model. These results do not mean that pertussis vaccines are not protecting humans.

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