Review of: "Mucosal immunization with DTaP confers protection against Bordetella pertussis infection and cough in Sprague-Dawley rats"

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Potential competing interests: The author(s) declared that no potential competing interests exist.

The manuscript titled Mucosal immunization with DTaP confers protection against Bordetella pertussis infection and cough in Sprague-Dawley rats is an important study for development of vaccines for humans. Further, authors used intranasal, oral gavage and intramuscular routes for vaccine-immunization for their studies. Immunization with IM-wP, IM-aP, IN-aP, and OG-aP immunization protected against Bp induced cough. Mucosal immunization (IN-aP and OG-aP) also protected against acute inflammation and decreased bacterial burden in the lung. Scientific questions are relevant and use of a coughing rat model for studying pertussis vaccine-effects seems novel. Overall, the manuscript is well organized.

However, there are some concerns/questions with the data that need to be addressed.

Comment 1. It might be interesting to have a kinetics of the antibody response. Did the authors observe a difference in the longevity / persistence of the antibody response related to the immunization route?

Comment 2. Fig. 2A It seems that 2 out of 4 rats really have a heavy cough. What is the reproducibility of the model? How did the authors verify if the rats were indeed infected? Did cough correlate with the injected Bp dose?

Comment 3. At day 1 post-challenge there was a significant 98.5% reduction in bacterial burden in the lung of IM-aP immunized rats compared to MVC. Could the authors observe a difference between IM-aP and other groups?

Comment 4: Fig 8 Could the authors clarify how the results are calculated and how the normalization is carried out? What did the max cytokine represent: total cytokine per rat per group?

The cytokine profile in the lung of IM-wP immunized rats on day 9 appeared to be like the cytokine profile of IN-aP immunized rats on day1. is it a similar mechanism but shifted in time?

Comment 5: line290 rats OG-aP immunized did have a slight increase in IL-17, though not significant. IL-17 is significantly increased in OG-aP immunized rats as compared to MVC control group (Fig 8).

Comment 6: line 303 significant increase in blood lymphocytes in the IN aP vaccinated rats compared to
MVC post-challenge. Serum cytokines appeared to be lower in IN aP vaccinated rats as compared to post-challenge MVC rats. Do the authors have an explanation?

**Comment 7:** how did the authors prepare lung homogenates?