Author's response to reviews

Title: Bordetella pertussis in infants hospitalized for acute respiratory symptoms remains a concern

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Version: 3  Date: 22 October 2013

Author's response to reviews: see over
Dear Dr. Harris,

Following your suggestions of the last email, we have answered point by point to the reviewer concern. We are now re-submitting our paper to your journal, hoping that it could be now in your journal.

Best regards

Dr. Fabio Midulla
Reviewer's report:

The authors have provided an improved manuscript based on the reviewer's comments. Most comments were taken into consideration, but not all.

Thank you.

The new title is more appropriate but reading through the manuscript, the main objective of the study still needs to be clearly described (is it to describe the clinical and laboratory characteristics that would help a clinician consider pertussis in an infant in order to start early treatment?) and important aspects of the methodology need to be better described as well.

The main objective of our study was: Describe clinical and laboratory characteristics that would help a clinician consider pertussis in an infant during an RSV epidemics. It is implied that if you make a prompt diagnosis you can also rapidly start the specific treatment. Second objective was to describe the genetic characteristics of B. pertussis.

The authors continue to speculate that vaccine immunity may be affected by the findings of 2 isolates with different genetic make up in this series, however, this is not supported by their study and it is something that only prospective epidemiologic and vaccine effectiveness studies might be able to ascertain.

We agree with the reviewer that with only two cases we cannot lead to any conclusion. We think that these preliminary results were important enough to be described. We agree with the reviewer that epidemiologic and vaccine effectiveness studies should be done in order to study this aspect.

Other Essential Revisions:

ABSTRACT

The sentence used in the background is not a background statement, it is methodology. Please change.

Done

Methods paragraphs: the information in the first parenthesis is unnecessary.

Following the suggestion of the reviewer we have deleted it.

Update methods accordingly after making change in "background".

Done

Results paragraph: Why first sentence on breast feeding? Provide report based on study methodology starting with demographics, etc.

Thank you for your suggestion. We have provided report based on study methodology. The first sentence is about breast feeding because among demographic characteristics is the only one that is significant.
Sentence in the middle: "Infants with pertussis more often had no chest sounds on auscultation." is this correct? Or are you referring to "abnormal chest sounds"?

Following the suggestion we have changed the sentence. We were referring to “abnormal chest sound.

Last sentence in results section is a good addition.

Thanks

Conclusion - not based on results - no incidence or rate of the disease given among all infants hospitalized for an acute respiratory infection. Are the authors concluding that "characteristics symptoms and laboratory findings of pertussis should be sought in infants with acute respiratory illnesses even during RSV season in order to start early antibiotic treatment"?

We agree with the reviewer that based on our results we cannot give incidence or rate of the disease. Yes we are concluding that “characteristics symptoms and laboratory findings of pertussis should be sought in infants with acute respiratory illnesses even during RSV season in order to start early antibiotic treatment”. According to our study we can only speculate that during an outbreak of RSV bronchiolitis, pertussis could be suspected on the base of clinical symptoms and laboratory findings.

MAIN DOCUMENT
Page 3 - First paragraph, third sentence - is pertussis resurgence attributed to incomplete immunization programs or incomplete immunization status of the patient? Also, the citations used in this sentence and this paragraph refer to European literature mostly, therefore this should be stated - that these variations are described "in Europe".

We believe that the resurgence of pertussis can be attributed to both: an incomplete immunization programs and an incomplete immunization status.
We have included “in Europe” in our sentence.

Second paragraph, first sentence - is "first booster dose at 3 months’ correct? A booster would imply completion of a primary series of vaccine - I am not aware of any country that considers a 3 month old ready for boosters...

You are right this was a mistake. We have changed the sentence and delete the word “booster”

Same paragraph, although pertussis infection may occur in a patient with RSV or in patients without RSV but at the same time as RSV is circulating, as the authors have shown, the clinical presentation of these two diseases is distinct and these two clinical syndromes "pertussis-like" and "bronchiolitis" can be differentiated by a knowledgeable clinician.

Yes, you are probably right. Therefore, we think that in some special situation the clinical presentation might be similar between “pertussis-like” syndrome and bronchiolitis making the diagnosis more difficult. We agree with the reviewer that a knowledgeable clinician can differentiate these two diseases. Yet, pertussis should always be suspected in infant with acute
respiratory symptoms.

**Last sentence of the second paragraph is unclear - what does "it" refer to?**

This sentence in our opinion means that in infants with acute respiratory symptoms the physician should always have in mind the possibility of pertussis infection and that a prompt diagnosis and treatment might prevent more severe cases of bronchiolitis. We have slightly changed the sentence.

**Third paragraph - Last sentence, not really true - the clinical and laboratory characteristics of pertussis have been well described, even in neonates, and therefore these are not lacking as suggested by the authors, rather, clinicians' awareness of these characteristics is necessary.**

Following the suggestion of the reviewer we have changed the sentence.

**Also, one would not consider it "unfortunate" that in clinical practice the diagnosis is usually made without microbiologic confirmation - again, most diagnoses rely on clinical and laboratory features and confirmation by PCR is achieved in places where resources are accessible.**

Following the suggestion of the reviewer we have changed “Unfortunately” with “Usually”

**Page 4 - The main purpose of this study needs to be better described. This is a descriptive study, so a description of the clinical and laboratory features of infants hospitalized with laboratory confirmed pertussis is provided and compared to those of infants with laboratory confirmed RSV. The second and third sentences of this paragraph are describing methods and should be moved to the methods section.**

Following the suggestion of the reviewer we have slightly changed the first sentence and moved the second and third sentence to the method section.

**Methods**

**Patients - first paragraph. Confusing. Mixes methodology with results. The sentence "no B. pertussis clustering was observed..." is a result, not a method.**

This sentence was added because a previous reviewer asked about this information and we have now moved it to the RESULTS section.

**It is not clear why was a 1:1 ratio chosen to identify controls to the cases. Usually the ratio in a retrospective study is higher so that at least two or three control are identified for every case. Please explain why 1:1 was chosen.**

We agree with your comment. We have decided to include one control for each case only because we thought that matching for age and sex was enough the rate 1:1. We agree with the reviewer that this could be a limit of our study and we should have included more controls for each case.

**Also, not clear as to whether patients were hospitalized or seen in the Emergency Department, or both, or Hospitalized after being seen in the ED. Please clarify this and**
also what the baseline population was.

The patients were all examined in the Emergency Department and then hospitalized in the ward that it is call “Pediatric Emergency Department”. The patients were enrolled during first 2 days of hospitalization.

Was it 164 infants that were hospitalized and were the 19 with pertussis among the 164? Can you please add the inclusion criteria for the study - how old did the infants need to be to be included in the study? Where there any other inclusion/exclusion criteria?

The 19 infants with pertussis were not included in the 164 infants. Since October 2004 we are conducting in our Department a cohort longitudinal study with all the consecutive infants that are hospitalized with a clinical diagnosis of bronchiolitis (first episode of acute lower airway infection, in infants less than 12 months of age). The design of this study considered that a clinical severity score is assigned to all the infants at admission in the hospital. Furthermore the parents of the infants give a written informed consent for the study and answer to a structured questionnaire on demographic characteristics of their child. The infant’s clinical information are obtained from the medical charts. All the information about the patients are baked up in a file in the computer. The 19 infants with pertussis and the 164 infants with RSV bronchiolitis belong to this group of patients. We have excluded all the infants with co-morbidity.

Page 5 - if this is a retrospective study, how was a clinical severity score assigned at admission? Are the authors meaning to say that " A clinical severity score was assigned to each child based on symptoms described at the time of admission"?.

See above.

Again, in this retrospective study, how was the study population selected (inclusion/exclusion criteria) and identified (based on discharge diagnostic codes, based on laboratory reports, both?) and how were parents of subjects asked to signed consent (by letter, in person, with a clinic visit?) one would expect that in a retrospective study some families might not be accessible anymore after some time has passed from their infant's initial hospitalization. Please describe how many potential subjects were identified and how many actually agreed to be in the study.

See above. As we said before, infants were selected only on the base of microbiological results obtained from the nasopharyngeal washes performed during the first two days of hospitalization.

The next two sections on testing need to indicate how patients are selected for testing (is this the decision of the clinician caring for them in the ED?, is there a protocol? is this for all hospitalized patients? is this routine for all ED patients, even if not hospitalized?)

See above. The design of the study considered that all infants included in the study performed nasopharyngeal washes during hospitalization for detecting 14 respiratory viruses and B. pertussis

Page 6 - were the "B.pertussis isolates" or "nasopharyngeal samples" cultured on charcoal agar?

The detection of B. pertussis was obtained from nasopharyngeal washes with the real time PCR.
B. pertussis isolated from nasopharyngeal washes were cultured on charcoal agar.

**Results**

Page 7 - please indicate if "schooling" for the study population refers to out of home care (daycare) given the age of the patients, or if indeed, they are in a school setting.

“Schooling” mean attending day care

second paragraph, sentence before last - "Finally, chest examination..." is not clear, please re-phrase. And in the sentence after that, please include the median and range data right next to each of the parameters it describes, not both together.

Following the suggestion of the reviewer we have rephrase the sentence and we have included the median and range data next to each parameters described.

**Discussion**

First paragraph - why starting with genetic information and vaccination? Again, the discussion should follow the order of the data results.

We really do not understand the comment. In the first paragraph of the discussion we have only summarized the main points that has been discussed in the discussion and we have followed the order of the results. In fact, the first sentence is about clinical and demographic results and the second is on genetic results.

In this same paragraph - second sentence, why "clinically" important that the strains found here differ from the ones in the vaccines - is there any difference in clinical presentation or management because of this? If there is, describe, if not, delete "clinically".

We agree with the reviewer and we have leave out “clinically”

Also Only 2 isolates were studied, there is no denominator, therefore the sentence "the B.pertussis strains circulating in our Pediatric emergency department seem to differ genetically from those ..." is not supported by the findings in this study.

We agree with the reviewer that one of the major limits of our study is the number of patients. In particularly, we have studied the genetic variability in only two isolates. In both the isolates we found a gene variation different from those currently used in DTaP vaccine in Italy. Our sentence was only a speculation.

When describing vaccination compliance (which would be better placed later in the discussion), it would be important to include a description of the vaccine recommendations in Italy and how many of the children in the study were eligible vs. not up to date.

Following the suggestion of the reviewer, we added a new sentence about this in the paper.
Third paragraph - third sentence describing pathogenesis mechanisms is unnecessary. Following the suggestion of the reviewer it was left out.

Page 10 - second paragraph - first sentence - lymphocytosis is not a confirmatory finding of pertussis. Also, the second sentence explaining pathogenesis is unnecessary.

Following the reviewer we have change slightly the first sentence and we have deleted the second one.

Page 11 - top paragraph, last sentence - consider "...because cultures were negative in 13 infants with PCR confirmed pertussis who were already receiving specific antibiotic...."

Following the reviewer comment we have changed the sentence.

Second paragraph - last sentence - is speculation.

Yes, we agree with the reviewer that this is just a speculation

page 12 - so, was nasal or nasopharyngeal washing done to establish a diagnosis?

Nasopharyngeal washing. We have changed this in the text.

Entire document: Check spelling and grammar throughout.

We have done it.

Tables 1 and 2 - some columns are shifted, please correct.

We have done it.

Food for thought: If the author's suggestion (not supported by this study) that the changes in the genetic make up of circulating strains results in diminished response/effectiveness of the available pertussis vaccines is correct, how will maternal immunization with these same vaccines result in better infant protection?

Thank you for the comment. We agree with you that also maternal immunization should be done with a new strain. We have slightly changed the sentence.