Author’s response to reviews

Title: The Epidemiology of Varicella Zoster Virus Infection in Italy

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Author’s response to reviews: see over
Sir,

please find enclosed the answers to each point raised by the Reviewers. Also note that I have written out the term VZV in our title in full.

Regarding the name of the ethics committee which approved the study, we followed the enrolling criteria already adopted for all studies performed during the European Seroepidemiology Network (ESEN)) project. In detail, specimens were collected anonymously and only age, sex and date of sampling were recorded. Sera from individuals known to be affected by an immunodepressive condition or by an acute infection or to have recently undergone a blood transfusion were excluded. No other information about health status or symptoms was recorded at the time of blood sampling. All individuals who provided serum samples gave verbal informed consent; consent for minors was provided by parents. These explanations are already described in the Methods section, and for this reason we would prefer not to add any sentence on this specific point.

I hope that the comments and the changes included in the text will make the revised paper suitable for publication in Vaccine. Please do not hesitate to contact me if you need further explanation.

Yours sincerely,

Giovanni Gabutti

Reviewer: Andrew Vyse

Reviewer's report: This is an interesting paper presenting some valuable data.

I have 6 main comments, four of which I think it very important that the authors consider and address

Minor essential revisions:

# The authors should discuss the strengths and weaknesses of each of the data sets used.

In the first part of Discussion section, we have highlighted the fact that the notification system for infectious diseases is mandatory. However, the weakness of the statutory notification system lies in the high level of undernotification. This fact is discussed in depth, explaining how the SPES sentinel system provides a more realistic
picture of the epidemiological impact of VZV in Italy. The hospital discharge database (SDO), used to evaluate hospital admissions, allows the evaluation of the more clinically serious infections that require hospitalizations. The SDO database is also important to estimate part of the costs related to an infectious disease. We think that all these points were already discussed in the different sections of the paper.

However, we have added this sentence at the beginning of the Discussion section:

“Active and passive surveillance systems are extremely important for the evaluation of the epidemiological impact of an infectious disease. However, data collected through these systems have both strengths and weaknesses. For example, the national routine notification system adopted in Italy, being mandatory, is useful to describe the epidemiology of a specific infectious disease and allows the evaluation of historical temporal trends but is certainly affected by undernotification and underdiagnosis. Sentinel surveillance systems, such as SPES, being active surveillance systems, are certainly more precise, but their implementation is difficult and expensive. On the other hand, hospital discharge databases have poor sensitivity and a limited potential for clinical interpretation; however, they allow the evaluation of the more clinically serious infections. For all these reasons it is important, when possible, to evaluate different data sources to study the epidemiology of an infectious disease.”

# The regional differences seem particularly pronounced using the incidence data but much less so with the antibody prevalence data - this should be discussed. For example, might this perhaps be reflecting the quality of the data sets used rather than indicating important geographical differences in the epidemiology of VZV in Italy?

In another study we have estimated the degree of underreporting both as the ratio between seroprevalence and life-time cumulative incidence on the basis of statutory notification, and as the ratio between annual incidence according to the SPES database and annual statutory notifications. The degree of underreporting at the national level was 7.7, while annual underreporting, in 2000, was 3.3. Underreporting was greater in older age groups and in southern Italy (Ciofi Degli Atti M.L. et al.
Assessment of varicella underreporting in Italy. Epidemiol. Infect., 128: 479-484, 2002). This trend has been observed for similar infectious diseases (i.e. measles). According to these previous data, we believe that the reason regional differences are particularly pronounced using the incidence data but much less so with the antibody prevalence results is related to the high degree of undernotification in southern Italy. Besides, the higher seroprevalence registered in northern Italy with respect to southern Italy could be explained with the higher spread of VZV in northern Italy, where a large part of the population lives in large urban and industrial areas.

For all these reasons, we have added, in the Discussion section, the following sentence:

"Regional differences were particularly pronounced using the incidence data but much less so with the antibody prevalence results. These differences may be explained by the high degree of undernotification in southern Italy [12]. Also the higher seroprevalence registered in northern Italy may be related to the higher spreading of VZV in northern regions where a large part of the population lives in large urban and industrial settings."

We have also deleted the following sentence from the Discussion section: "although underreporting is known to be greater in southern Italy [12]."

# The authors need to carry out a multivariable analysis (e.g. logistic regression) that includes age group, gender and region (and year of sample collection for the antibody prevalence data) as independent variables. This will be particularly useful for exploring more thoroughly any geographical effect on the epidemiology of VZV. (I would be surprised if region remained a significant factor for the antibody prevalence data after a multivariable analysis).

# 95% confidence intervals need to be put around all key estimates presented.

These points have been addressed in the Methods, in the Results, and in the Discussion sections.
In the Materials and methods section we have added these parts or sentences:

**Statistical analysis**

Direct standardized incidence rates were calculated by using national 1991 census data as the reference population.

Seroprevalence data were summarized as percentages with 95% confidence interval (95%CI) and positive........

A multiple logistic regression model was used to determine the relationship between antibody titres (positive versus negative) and a set of explanatory variables. The following variables were included in the model: sex, age group, geographical area and year of sample collection. The likelihood ratio test was used to compare different models. Statistical analysis was performed by Stata software version 9.2.

In the Results section we have modified some p and added a new sentence as follows:

- 5-9 years, northern vs. southern Italy, $\chi^2 = 8.70$ (p=0.003)
- 10-14 years, northern vs. southern Italy, $\chi^2 = 4.49$ (p=0.026)
- 15-19 years, northern vs. central Italy, $\chi^2 = 5.82$ (p=0.016)
- 15-19 years, northern vs. southern Italy, $\chi^2 = 4.68$ (p=0.043)
- ≥ 40 years, northern vs. southern Italy, $\chi^2 = 4.34$ (p=0.037)

However, multivariate analysis showed that the only variable associated with antibody titre is age group.

In the Discussion section we have changed a sentence as follows:

The trend in seroprevalence was similar for males and females and for geographical areas. It was also similar when comparing the three geographic areas, although some significant geographical differences were found for certain age classes.

Discretionary Revisions:
The age stratified antibody prevalence graphs are very similar for both the 1996 and 2004 data. This invites some comment about how the epidemiology of VZV may or may not have changed since the end of the 20th century.

The author’s present antibody prevalence data from 2 separate time points (1996 and 2004). More could be made of this valuable feature of these data. For example, assuming both sample collections to be comparable, when stratifying by common birth cohorts sera from 2004 represents an additional 8 years of opportunity for exposure to VZV compared to sera collected in 1996. Therefore prevalence using 2004 sera should be higher or at least remain the same (but not be lower) than prevalence in the same birth cohort using samples collected in 1996. This characteristic could be used to estimate an incidence to complement the other incidence data presented.

Reviewer’s points are certainly interesting. However, we think that these two points are already well explained and discussed in the paper and we would prefer not to change the text.
Reviewer: Peter Wutzler
Reviewer's report:

Major points:
The manuscript addresses the epidemiology of varicella and zoster in Italy by different data sources. Furthermore, the seroprevalence of VZV in child and adult population of northern, central and southern Italy was determined and compared with data obtained in 1996. However, the results are not sufficiently new for the readership of an internationally renowned journal since these findings confirm data published previously by the same author in 2001. Nevertheless, the data can be of interest for the national vaccination policy in Italy. Therefore, the paper can be considered for publication in a national medical journal after it was shortened considerably.

We think that this paper could be of interest even for an international readership as it analyzes the epidemiology of important infectious diseases, such as Varicella and Zoster, comparing data obtained 8 years apart in a country where universal vaccination has not yet been adopted. As an international and national debate is under way on the opportunity to adopt mass vaccination, we think that our data could be useful at the national level, but certainly help to confirm a different pattern of VZV virus spreading in Italy with respect to other European countries. We believe that this particular aspect is extremely important to better understand the epidemiology of this infection and to better define vaccination strategies at the national and international level. Besides, we think that these specific points have already been included in the text and/or in the revised form of the paper. For these reasons we hope that the revised version of our paper, taking into consideration the reviewers’ comments, could be suitable for publication in Vaccine.

Minor points:
The statistical analysis does not consider samples with equivocal results that have to be re-tested using FAMA. In addition, the 95% confidence intervals were not added.

The 95% confidence intervals have been added (please see answer to Reviewer’s 1 specific point). The equivocal results have been defined on the basis of the ELISA test.
adopted. We agree that equivocal sera could be re-tested using FAMA, but this was not a specific objective of our study; besides, being that only 1.7% of the total samples analyzed were equivocal, we have chosen not to consider equivocal results, in order to analyze data in the same way performed in the previous survey.

Results confirmed a clear north-south gradient with the highest incidence of varicella in northern Italy followed by central and then southern Italy. The authors need to discuss the reasons for these differences.

This specific point has been addressed in reply to Reviewer n. 1’s comments (please see answer to Reviewer’s 1 specific point and the parts modified or newly included in the paper).