Author’s response to reviews

Title: Legionella spp. in Southeastern Italy: surveillance and epidemiology

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Author’s response to reviews: see over
Dear Editor,

on behalf of the authors of the manuscript "MS: 1648270166335694", I would like to thank you for the chance to consider the manuscript for publication on BMC Public Health after the major revisions. Moreover I thank your qualified Referees for their comments that permitted us to greatly improve the paper.

In my region, it is time for important decision in the field of prevention. By having the honor of publishing our experience on your prestigious journal, the other authors and I are sure to highlight the importance of investing on the public health problem of Legionella spp. control and prevention.

Please find in attachment the revised paper and the cover letters addressed to the Referees. The manuscript was rewritten following - point by point - all the reviewers’ comments. Moreover, as you suggested, much emphasis on the methodological shortcomings of the manuscript was put.

Some comments of Referee 2 concern the English language, we specified that a native English speaker translated the manuscript. Anyway, we have had the paper revised by another English mother tongue expert in bio-medical papers.

We hope that all our efforts will allow you to accept the article.

Thank you for your time and best regard.

Christian Napoli
Dear Referee 1,

thank you for your evaluation and for your important suggestions. We apologize for the “little confused” previous version of the manuscript and really appreciate your efforts for reviewing it. According to your comments, the manuscript underwent a major revision. Moreover, we updated the environmental surveillance until December 2009. We are now re-submitting the correct manuscript.

A point-by-point description of the changes follows (in black small letters your comments, in red capital letters our reply):

1. The manuscript includes useful information which may be published considering that the authors will provide a major revision to the manuscript. Unfortunately, the manuscript suffers from severe methodological shortcomings. **THE INFORMATION INCLUDED IN THE PREVIOUS VERSION OF THE MANUSCRIPT WAS VERY VAGUE AND FOR THIS REASON - ACCORDING TO YOUR COMMENTS – IT WAS REWRITTEN. IN PARTICULAR, MUCH EMPHASIS WAS PUT ON THE METHODOLOGICAL SHORTCOMINGS OF THE MANUSCRIPT.**

2. In the method section, the authors do not mention the watersides from which the samples were collected. They do not differentiate if the samples were collected from water supply systems (how many from hot and how many from cold water supply system) or from cooling towels or other watersides (fountains etc). **THIS FUNDAMENTAL INFORMATION WAS ADDED. NOW THE MANUSCRIPT REPORTS - IN THE METHOD SECTION - THE PRECISE NUMBER AND KIND OF WATERSIDES AND WATER SUPPLIES. MOREOVER, ALL THESE DATA ARE REPORTED IN TABLE N.2, WHICH INCLUDES THE NUMBER OF POSITIVE SAMPLE FOR EACH WATERSIDES.**
3. Moreover, they do not mention anywhere and they do not differentiate the third sampling of each building/item and repetitive sampling possibly after remedial actions. It is very important to analyse separately the first sampling of the buildings/items from the second and third sampling. **THE AUTHORS AGREE WITH THIS COMMENT: IT IS NOT RIGHT TO ANALYZE SECOND AND THIRD SAMPLING OF THE SAME BUILDING OR AFTER REMEDIAL ACTIONS. WE DID NOT DO THAT, BUT IN THE PREVIOUS VERSION IT WAS NOT SPECIFIED. THE SENTENCE “WATER SAMPLES COLLECTED DURING RE-INSPECTIONS OR AFTER TAKING CORRECTIVE ACTIONS WERE NOT INCLUDED IN THE ANALYSIS” WAS ADDED. EVERY BUILDING WAS CONSIDERED ONCE AND, FURTHERMORE IT WAS SPECIFIED THAT THE NUMBER AND CHOICE OF THE SAMPLED POINTS WAS DONE ON THE BASIS OF THE NATIONAL GUIDELINES SUGGESTIONS.**

4. They write generally risk assessment, do they mean hygiene inspection or something different? **IN THE PREVIOUS VERSION RISK ANALYSIS WAS ONLY MENTIONED WITHOUT ANY INFORMATION ABOUT METHODS AND RESULTS. IN THE NEW VERSION A SECTION WAS DEDICATED TO RISK ANALYSIS BOTH IN THE METHODS AND RESULTS.**

5. The authors should provide the results according to the above suggested changes in the methodology. **THANKS TO YOUR METHODOLOGICAL SUGGESTIONS THE RESULTS ARE NOW PRESENTED ACCORDING TO THE METHODS CHANGES. SOME TABLES ENABLE A BETTER RESULTS COMPREHENSION.**

6. Moreover, it is important to analyze their data according to time (yearly) to see if there is an increasing or decreasing trend until the period 2000-2009. **WE FULLY AGREE, THE TREND IS ONE OF THE BEST WAY TO UNDERSTAND THE DISTRIBUTION OF AN EVENT. A GRAPHIC SHOWING THE TREND OF POSITIVE SAMPLES YEAR BY YEAR WAS ADDED.**

Minor comments

- p. 2, line 33; p. 9, line 204: “incidence” in place of “frequency”. **DONE! THANK YOU VERY MUCH!**
- p. 2, line 38; p.8, line 180; p. 10, line 228: “concentration” or other in place of “emission”. **DONE! THANK YOU VERY MUCH!**
- p. 2. line 40; p. 10, line 231: consider another term, e.g. Health care facilities, if it is not only hospitals. **DONE! THANK YOU VERY MUCH!**
- p. 3, line 46-51: these lines would be better to be deleted. **DONE! THANK YOU VERY MUCH!**
- p. 3, line 59: please delete “and” before already. **DONE! THANK YOU VERY MUCH!**
- p. 3, line 63: it would be useful to be added the following sentence: “…… and currently by ELDSNet, which is coordinated by ECDC, carries out surveillance of the pneumonic form of legionellosis, involving all EU Member States, Iceland and Norway. It aims to identify relevant
public health risks, enhance prevention of cases and monitor epidemiological trends. DONE! THANK YOU VERY MUCH!

- p. 3, line 67: “distinguish” in place of “verify”. DONE! THANK YOU VERY MUCH!

- p. 4, line 96: “mL” in place of “lm”. DONE! THANK YOU VERY MUCH!


Do you consider for a better risk analysis procedure, if there is a failure to correlate Legionella positive microbiological test results and risk analysis procedures? ON THE BASES OF THE REFERENCES THAT YOU SUGGESTED A CORRELATION STUDY BETWEEN RISK ANALYSIS AND RESULTS OF MICROBIOLOGICAL TEST WAS PERFORMED. OUR RESULTS SHOWED A SIGNIFICANT ASSOCIATION BETWEEN MEDIUM OR HIGH RISK LEVEL AND LEGIONELLA SPP POSITIVE MICROBIOLOGICAL TEST RESULTS. THIS FINDINGS FIT WITH ONES SHOWED IN THE PREVIOUSLY MENTIONED PAPERS.

- p. 6, line 148: “and” in place of “e”. DONE! THANK YOU VERY MUCH!

- p. 8, line 182: “of” instead of “on”. DONE! THANK YOU VERY MUCH!

- p. 8, line 185: “suggest” instead of “suggests”. DONE! THANK YOU VERY MUCH!

- p. 8, line 187: please add … that subsequently underwent … and delete “to” before disinfection. DONE! THANK YOU VERY MUCH!

- p. 8, line 188: “thereafter” in place of “periodically”. DONE! THANK YOU VERY MUCH!

- p. 8, line 198: please add … that the pool of serogroups… DONE! THANK YOU VERY MUCH!

- p. 9, line 199: “be used for” instead of “be able to obtain”. DONE! THANK YOU VERY MUCH!

- p. 9, line 201: “incidence” in place of “level”. DONE! THANK YOU VERY MUCH!

- p. 9, line 202: “has” instead of “is”. DONE! THANK YOU VERY MUCH!

- p. 9, line 217: “reach” in place of “arrive at”. DONE! THANK YOU VERY MUCH!

- p. 10, line 235: Any suggestions for the need to conduct a more successful risk analysis?. YES, FINDINGS FROM THIS STUDY SHOW THAT A WELL DONE RISK ASSESSMENT EVALUATION COULD BE USEFUL TO PREDICT LEGIONELLA CONTAMINATION IN WATER SYSTEM. IN FACT, IN THE PRESENT STUDY EVEN IF THE STANDARDIZE QUESTIONNAIRE USED FOR THE RISK ASSESSMENT IS A SHORT REPORT INCLUDING JUST 18 ITEMS, IT WAS USEFUL FOR THE RAPID EVALUATION OF THE PRINCIPAL ENVIRONMENTAL RISK FACTORS AND TO SHOW THAT THERE IS
A CLEAR CORRELATION BETWEEN THE PRESENCE OF A MEDIUM OR HIGH LEVEL OF RISK (M/B GRADING) AND POSITIVE RESULT OF ENVIRONMENTAL ANALYSIS FOR *LEGIONELLA* SPP. OTHER AUTHORS DEVELOPED A MORE PRECISE STANDARDIZED SCORED INSPECTION SYSTEM, DEMONSTRATING A BETTER RELIABILITY IN PREDICTING *LEGIONELLA* PROLIFERATION IN THE WATER SYSTEMS AND IN PREVENTING LEGIONNAIRE DISEASE (HADJICHRISTODOULOU C, EPIDEMIOLOGY AND INFECTIONS 2006). IN LIGHT OF THESE DATA, EVEN IN SOUTHEASTERN ITALY, THE OER STAFF IS WORKING ON THE IMPROVEMENT OF RISK ASSESSMENT STANDARDIZED REPORT. A DISCUSSION IS PRESENT IN THE TEXT!

Thank very much you for your time.

Best regards

The Authors
Dear Referee 2,

thank you for your evaluation and for your important suggestions. We apologize for the “little confused” previous version of the manuscript and really appreciate your efforts for reviewing it. According to your comments, the manuscript underwent to a major revision. Moreover, we updated the environmental surveillance until December 2009. We are now re-submitting the correct manuscript.

A point-by-point description of the changes follows (in black small letters your comments, in red capital letters our reply):

1. This paper analyses the results of a large number of environmental samples and compares these results with epidemiological data and risk analysis results. However, the methods are not appropriately described and the results are not understandable and can be better and more clearly presented. The title and abstract are needed to be expressed more accurately. **THE INFORMATION INCLUDED IN THE PREVIOUS VERSION OF THE MANUSCRIPT WAS VERY VAGUE AND FOR THIS REASON - ACCORDING TO YOUR COMMENTS – IT WAS REWRITTEN. IN PARTICULAR, MUCH EMPHASIS WAS PUT ON THE METHODOLOGY AND ON THE RESULTS PRESENTATION. THE TITLE AND THE ABSTRACT WERE CHANGED ACCORDING TO YOUR SUGGESTIONS.**

2. The manuscript needs to be reviewed by a native English speaker. **WE APOLOGIZE FOR ENGLISH: A NATIVE ENGLISH SPEAKER TRANSLATED THE MANUSCRIPT, BUT SHE PROBABLY FAILED WITH SCIENTIFIC LANGUAGE. NOW WE HAVE HAD THE PAPER REVISED BY AN ENGLISH MOTHER TONGUE EXPERT IN BIOMEDICAL PAPERS AS YOU RECOMMEND.**

3. Lines 62-63: a more updated reference can be used by the authors, since the coordinating center of EWGLI is called now Health Protection Agency: (e.g. Ricketts KD, Yadav R,

4. Line 78: Authors should define what “surveillance of sanitary events” is. THE SENTENCE WAS CHANGED IN “IS RESPONSIBLE FOR THE EPIDEMIOLOGICAL SURVEILLANCE AND DISEASE CONTROL”.

5. The title of the paper should be more descriptive and indicate the types of buildings and water facilities. ACCORDING TO YOUR SUGGESTIONS IT WAS CHANGED TO “LEGIONELLA SPP. AND LEGIONELLOSIS IN SOUTHEASTERN ITALY: DISEASE EPIDEMIOLOGY AND ENVIRONMENTAL SURVEILLANCE IN COMMUNITY AND HEALTH CARE FACILITIES”.

6. ABSTRACT. Lines 26, 27: authors should describe better what surveillance included. For example: surveillance of legionellosis and environmental surveillance of Legionella spp. in water facilities? In addition, what clinical monitoring means. Methods should be better explained: from where the samples were taken (hotels, hospitals, homes, other buildings)? THE ABSTRACT WAS ENTERLY REWRITTEN FOLLOWING YOUR SUGGESTIONS. A GREAT ATTENTION WAS GIVEN TO A BETTER DESCRIPTION OF THE ENVIRONMENTAL AND CLINICAL SURVEILLANCE, TO THE SAMPLING WATERSIDES.

What was the sampling procedure, where the samples were analyzed? What case definitions were used for surveillance, who reported the cases? SINCE THE ABSTRACT WAS TOO LONG WE PREFERRED TO SPECIFY THESE CHARACTERISTICS IN THE METHOD SECTION. ANYWAY, IF YOU SUGGEST TO ADD THIS INFORMATION IN THE ABSTRACT TOO, IT WILL BE DONE.

Results do not give any numerical data, but a general statement. ALL THE NUMERICAL RESULTS WERE ADDED, IN THIS WAY THE READER IS ABLE TO GET ALL THE INFORMATION FROM THE ABSTRACT TOO. THANK YOU VERY MUCH.

In conclusions, risk analysis results are mentioned, but the methods and results do not give any information on what is “negative risk analysis”. THANKS TO YOUR SUGGESTIONS THE RISK ANALYSIS SECTION WAS IMPROVED BOTH IN THE ABSTRACT AND IN THE TEXT. ALL DATA ABOUT RISK FACTORS WERE RE-ANALYZED IN A MORE CORRECT WAY.

Methods and results do not indicate anything about clinical diagnosis criteria. AFTER YOUR COMMENTS WE SPECIFIED THAT WE ANALYZED THE SURVEILLANCE FORMS SENT TO THE OER BY PHYSICIANS ACCORDING TO THE NATIONAL SURVEILLANCE PROGRAM. UNFORTUNATELY ON THE FORM IS NOT INCLUDED THE CLINICAL DIAGNOSIS CRITERIA, BUT JUST THE LABORATORY DIAGNOSIS CRITERIA AND SYMPTOMS THAT THE PATIENT HAS SHOWED. BOTH SYMPTOMS AND LABORATORY TEST ARE NOW REPORTED IN THE ABSTRACT.

7. METHODS. Methods section can be divided into the following subsections: Environmental sampling, Microbiological examination, risk analysis etc. DONE! THANK YOU! NOW THE TEXT HAS BEEN TIDED UP. Lines 91-92: the number of health care facilities should be included. The types (e.g. hotels, homes etc) and numbers of community facilities should also be included. The number of samples collected of each type of facility should be
defined, as well as the water system type (water distribution system, pool, spa, etc.). THIS FUNDAMENTAL INFORMATION WAS ADDED. NOW THE MANUSCRIPT REPORTS - IN THE METHOD SECTION - THE PRECISE NUMBER AND KIND OF WATERSIDES AND WATER SUPPLIES. MOREOVER, ALL THESE DATA ARE REPORTED IN TABLE N.1 AND N.2, WHICH INCLUDES THE NUMBER OF POSITIVE SAMPLE FOR EACH WATERSIDES. The sentence in line 92-93 “the microbiological investigation was always conducted together with a risk analysis” should be elaborated more: for example, environmental sampling was always conducted together with a risk analysis”. DONE. The sampling should be described separately in a subsection. DONE. The risk analysis procedure and criteria should be described separately in a subsection of methods. DONE. What means positive and negative risk analysis should be defined. DONE. The laboratories that conducted the microbiological analysis should be mentioned in the manuscript, as well as who conducted the sampling and the risk analysis. DONE.

8. RESULTS. Lines 129-137: Results described in the first paragraph should be converted in a table format in order to include the denominators. ALL THE RESULTS WERE REPORTED IN TABLES INCLUDING THE DENOMINATORS AND ENABLING A BETTER RESULTS COMPREHENSION. How many samples were obtained from each facility? FOLLOWING YOUR SUGGESTIONS, IN TABLE N.1 WE SPECIFIED THE NUMBER OF SAMPLES OBTAINED FROM EACH “KIND” OF FACILITY; UNFORTUNATELY, IT IS VERY DIFFICULT INDICATE THE NUMBER OF SAMPLES FOR EACH FACILITY BECAUSE WE DID NOT GET ALWAYS THE SAME NUMBER OF SAMPLES FROM EACH FACILITY. IN FACT, AS DISCUSSED IN METHODS SECTIONS, THE SAMPLING FOLLOWED THE NATIONAL GUIDELINES INDICATIONS. FOR EXAMPLE, CONCERNING THE NUMBER OF ROOMS TO SAMPLE, AT LEAST THE 10% OF THE TOTAL NUMBER OF ROOMS WAS SAMPLED. The percentage 34.6% and the percentages in the following sentence refer to the number of samples or the number of facilities? What types of facilities were these (water distribution systems, spas)? Lines 138-148: Please refer to the previous comments. WE APOLOGIZE FOR THE CONFUSED DATA PRESENTED IN THE PREVIOUS VERSION OF THE MANUSCRIPT. THE RESULT PRESENTATION WAS CLARIFIED ACCORDING TO YOUR SUGGESTIONS.

9. DISCUSSION. Line 175-176: it is not clear if the percentages 12.5% and 21% represent numbers of buildings or samples. In the results section it is mentioned that 21% is the number of samples, while in the discussion that 21% is number of community buildings. THE DISCUSSION WAS GREATLY REWRITTEN AND NOW ALL THE PERCENTAGES REPORTED ALWAYS REFER TO THE PRECISE DENOMINATOR. Results of this study and level of contamination can be compared with other parts of Italy or with other countries findings. DONE! AN INTERESTING DISCUSSION RESULTED, THANK YOU! Lines 187-189: There is no description in the methods section or results about the efficacy of disinfection method, or the type of disinfection method used. If authors do such a statement about the efficacy of method, they should define the disinfection method, how it was evaluated and how and when the periodical risk assessment was conducted. ALL THE FACILITIES TESTED POSITIVE FOR LEGIONELLA (WITH BACTERIA COUNT LEVEL HIGHER THAN TRESHOLDS REPORTED IN THE NATIONAL GUIDELINES) UNDERWENT TO DISINFECTIONS, BUT WE DID NOT TESTED THE EFFICACY OF METHODS. FOR THIS REASON THE SENTENCECE WAS DELETED.
The role and importance of trained parsons in risk analysis and sampling can also be discussed. **WE AGREE ON THE IMPORTANCE OF TRAINING FOR PEOPLE INVOLVED IN RISK ANALYSIS AND SAMPLING BUT, SINCE THE NEW VERSION OF THE MANUSCRIPT IS QUITE LONG, WE JUST MENTION IT.**

Thank very much you for your time.

Best regards

The Authors