Author's response to reviews

Title: Prevalence study of Legionella spp. contamination in ferries and cruise ships

Enclosed please find our revisions according to reviewers’ indications concerning our manuscript "Prevalence study of Legionella spp. contamination in ferries and cruise ships".

Yours sincerely,
Andrea Piana

Reviewer's report (Christian Lueck)

1) ".........please give details how the water was sampled ...........":
   According to reference number 9, two water samples were collected from every sampling site in a single
time-point; the first sample was collected for qualitative evaluation, without flaming and immediately after
the tap was switched on; the second sample was collected for quantitative analysis, after the water ran for
at least 5-10 minutes, being more representative of the water flowing in the system.

2) "...............water was sampled twice.............":
   There might be a misunderstanding related to water sampling; in fact, a single survey was performed per
   ship.

3) "................how many samples were collected............. ":
   The total amount of water samples collected was 90: ten samples were collected from every ship, five for
   qualitative and five for quantitative evaluation.

4) "........how many samples per ship were positive......... ":
   An average of four samples (range, 0-10) was positive per ship.

5) "........how many samples contained ............ ":
   Of the positive water samples, 22.2% contained 1 x 103 - 1 x 104 Legionella per litre, while 50% 1 x 104 - 1
   x 105, 22.2% 1 x 105 - 1 x 106, 5.6% 1 x 105 - 1 x 106. Then, 27.8% contained > 100,000 Legionella per
   litre. Previously, we considered the cut-off "> 10,000 Legionella per litre", which is judged the critical level
   for environmental decontamination in absence of Legionnaires' disease, according to Italian guidelines.

6) "...........state clearly how the strains were differentiated......... ":
   We did forget to include in "Methods" section the other laboratory test Legionella Immune Sera "Seiken"
   (Denka Seiken Co. LTD), which let us to identify Legionella pneumophila serogroup 1, 2, 3, 4, 5, 6 and L.
   bozemanii, L. dumoffii, L. gormanii, L. micdadei. This test was used after the Legionella Latex test (Oxoid) ir
   order to better differentiate colonies.

7) "..............how many colonies were investigated..............":
   Suspected colonies with a mottled surface or an iridescent and faceted cut glass appearance, were counted
   from each sampling. All colonies from plates with 10 and 10-20 random colonies were subcultured on
   buffered charcoal yeast extract (BCYE) agar (with cysteine) and charcoal yeast extract agar (cysteine-free)
   media (Oxoid), blood-agar and McConkey agar plates for 2 days.

8) "..............comment on the fact that no serogroup 1 was detected........... ":
   However, we did not identify Legionella pneumophila serogroup 1, which is responsible for more than 90%
of clinical cases. This finding might be related to differences in environmental prevalence among Legionella
   pneumophila serogroup 1 and non-pneumophila species. Several reports, evidenced that the high
   frequency of Legionella pneumophila serogroup 1 isolation from clinical samples is not strictly linked to
   environmental predominance but might be due to higher infectivity or more efficient intracellular growth. The
The low incidence of non-pneumophila species among clinical isolates associated to their high environmental frequency implies that these species are less virulent and pathogenic than Legionella pneumophila serogroup 1 (Doleans A. et al. Clinical and environmental distributions of Legionella strains in France are different. J Clin Microbiol 2004, 42(1):458-460).

9) ".........there were no positive cultures in samples from 41-60 degreesC ............. ":
Of the 90 water samples, six (6.6%), collected in a single cruise-ship, had temperature ranging from 42.4 to 56.6 degreesC. Negative cultures of these water samples might be due to their residual free and total chlorine, ranging from 1.80 to 1.95 and from 1.90 to > 2, respectively.

10) ".........state clearly what kind of risk assessment- and/ or prevention .......... ":
We thank for this indication; we modified in "Discussion" section items concerning of prevention and risk of acquiring Legionella infection assessment, according to Italian and European guidelines (references n. 8, 10).

Reviewer's report (Joan Cayla)

1) ".........better with Results section and Discussion section......... ":
We modified sections of our paper as indicated by Reviewer.

2) ".........comment in discussion that serogroup 1 is more virulent............. ":
However, we did not identify Legionella pneumophila serogroup 1, which is responsible for more than 90% of clinical cases. This finding might be related to differences in environmental prevalence among Legionella pneumophila serogroup 1 and non-pneumophila species. Several reports, evidenced that the high frequency of Legionella pneumophila serogroup 1 isolation from clinical samples is not strictly linked to environmental predominance but might be due to higher infectivity or more efficient intracellular growth. The low incidence of non-pneumophila species among clinical isolates associated to their high environmental frequency implies that these species are less virulent and pathogenic than Legionella pneumophila serogroup 1 (Doleans A. et al. Clinical and environmental distributions of Legionella strains in France are different. J Clin Microbiol 2004, 42(1):458-460).

3) ".........implement the discussion about preventive measures......... ":
We thank for this indication; we modified in "Discussion" section items concerning of prevention and risk of acquiring Legionella infection assessment, according to Italian and European guidelines (references n. 8, 10, 13).