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Comment

Interactive comment on “Application of DVC-FISH method in tracking *Escherichia coli* in drinking water distribution networks” by L. Mezule et al.

L. Mezule et al.

linda.mezule@rtu.lv

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We thank for the comments.

P521, L15 and elsewhere: The authors state that no waterborne outbreak of disease has been reported in Riga in the last 10 years. Based on this statement, and their data clearly showing viable and sometimes cultivable *E. coli* in the Riga drinking water (Table 1 & P522, L15-23), the following questions: 1. Does this mean that *E. coli* is not a meaningful parameter for detecting/predicting outbreaks of waterborne disease? 2. Alternatively, does this mean that molecular methods (which detect much more *E. coli* than conventional methods) show organisms that are not relatable to disease (e.g., organisms that have lost their pathogenicity or their ability to divide sufficiently to cause disease)?

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Due to limited surveillance (no reporting system of sporadic or mild infections) in the presented case (Riga) the probability of detecting pathogens in the distribution system is rather low. Thus, separate sporadic contamination cases are not usually detected. Indeed, the presented data doubt the suitability of sole *E. coli* as an appropriate indicator for drinking water analyses. Due to this, two hypothesis can be given for the obtained results: (i) in drinking water the fate of *E. coli* does not correlate with other pathogens; (ii) the detected *E. coli* belong either to the group of “environmental” *E. coli* (Ishii, 2009) or have been adapted to the water-environment after long term survival in the drinking water. The second hypothesis could explain the very-low concentrations of non-culturable *E. coli* detected directly after the treatment. To determine the potential pathogenicity of these *E. coli* further studies must be performed. However, at the moment the tests are limited to the problem that these bacteria are not cultivable and current toxicity tests are based on animal feeding tests. Ref.: Ishii S., Sadowsky MJ. 2008. *Escherichia coli* in the Environment: Implications for Water Quality and Human Health. *Microbes Environ*,23(2): 101-108.

P523, L1-7: Is it possible to distinguish between *E. coli* accumulation and actual growth in the network? The authors analysed rather young biofilms (2 weeks old). Is there any indication that older biofilms would harbour either (a) more *E. coli* (due to accumulation and/or growth) or (b) less *E. coli* (due to increased competition and predation)?

Two week long biofilm growth was selected based on previous experience – this time was selected as the shortest period allowing to obtain recordable and more or less stable biofilm. Long term incubation (more than 1 year) was not evaluated due to simultaneous accumulation of organic and inorganic matter. The studies on determination whether the detected *E. coli* come from accumulation or growth are performed with our research group at the moment.

P523, L8-15: More *E. coli* was found further away from the treatment plant and several explanations for that are suggested. Is there any evidence to suggest that the higher

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numbers are due to growth of *E. coli* in the network? Is an alternative explanation that a longer distance in the network increases the risk/possibility of pipe-failure and leakage and thus external contamination, which would also explain increased numbers?

Similarly as with the previous comment, this question is being addressed in our research group at the moment.

P523, L15-17: This comment is very similar to the first comment above. Although these are indeed theoretical risks, the fact that no outbreaks have been reported in the network under investigation seems to suggest that a correlation between these organisms and disease is not evident.

Indeed there have been no major outbreaks, however, due to national and social reasons there is no tendency in reporting sporadic mild infections officially (people usually do not go to a doctor with mild diarrhea). Thus, it can be only suggested that the occurrence of this indicator (moreover, its fate in distribution networks) does not correlate to the occurrence of real pathogens (*Cryptosporidium*, *E. coli* O157:H7) which would cause severe health problems unsolvable by the individual itself.

P523, L19: It seems that this statement/paragraph requires a reference.

A reference indicating on the ability of pathogen growth after treatment will be added: Jjemba PK., Weinrich LA, Cheng W, Giraldo E, LeChevallier MW, 2010. Regrowth of potential opportunistic pathogens and algae in reclaimed-water distribution systems. *Appl Environ Microbiol*, 76(13): 4169-4178.

P523, L26-30: A positive correlation between HPC and total counts are reported and interpreted. However, it would be useful to place this in perspective: only about 1% of the total bacteria were cultivable – a number fairly typical for drinking water biofilms. It is not clear how this directly relate to “the formation of more favourable conditions for colonisation and growth”?

The results showed that both total bacterial counts and heterotrophic plate counts in-

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creased with water residence time and had a correlation. Indeed the percentage of cultivable from total bacteria was close to the reported 1 %, however, in the site G-NET2 (water residence time above 28 hours) it increased till more than 6% which is an indication in formation of more favourable growth conditions.

The percent of cultivable heterotrophic bacteria from total bacterial counts in 6 sampling sites (recalculated from data in Table 2 (Drink. Water Eng. Sci. Discuss., 5, 515–532, 2012): S-DW (0,72%); S-NET1 (2,65%); S-NET2 (2,42%); G-DW (0,7%); G-NET1 (0,41%); G-NET2 (6,16%).

P524, L10-11: The implication of this sentence seems to be that one should expect denser biofilms which would explain the higher cell concentrations in the water (L5-6). Although this seems logic, it contradicts the statement in L4-5 that “no correlation between TBC in biofilm and water was observed”. Can it be that two week old biofilms measured in this study are simply not representative of the actual biofilm situation in the network, to which the water is exposed continuously?

It has been described previously (see ref. Flemming 2002) that there is usually no correlation between cell counts in biofilms and water. This was the case in our study too. Only correlation between TBN and HPC in the biofilm and a correlation between TBN and HPC in water was observed. Since there are no universal recommendations for the age of the biofilm under the analysis, 2 weeks were chosen based on the previous experience, water residence time (not more than 48 hours) and elevated accumulation of organic and inorganic matter in older biofilms (more than 1 yr).

P524, L12-15: Although this is indeed similar to the data of Delahaye to some extent, it contradicts other data e.g. our from group (Hammes et al. 2010, Water Research) which suggest that a correlation between TBC and ATP should be expected. Can this be a result of chlorine disinfection that affects viability (thus ATP) but not TBC? Is there a logic explanation for the high variability in ATP values?

Yes, it could be connected to the fact that for Riga drinking water distribution system

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chlorination is applied. The recommended level of free chlorine in the distribution system is 0.2 mg/l, however, usually it is below the detection level. Our opinion is that in case of ATP correlation to TBN no generalization to all systems can be made. Not only because of the differences in distribution system chemical content (presence of chlorine), but also because of changeable ATP levels in the cells themselves.

TECHNICAL CORRECTIONS 1. Please add scale bars on Figure 3

The bar (10 μm) in Figure 2 has been added.

Interactive comment on Drink. Water Eng. Sci. Discuss., 5, 515, 2012.

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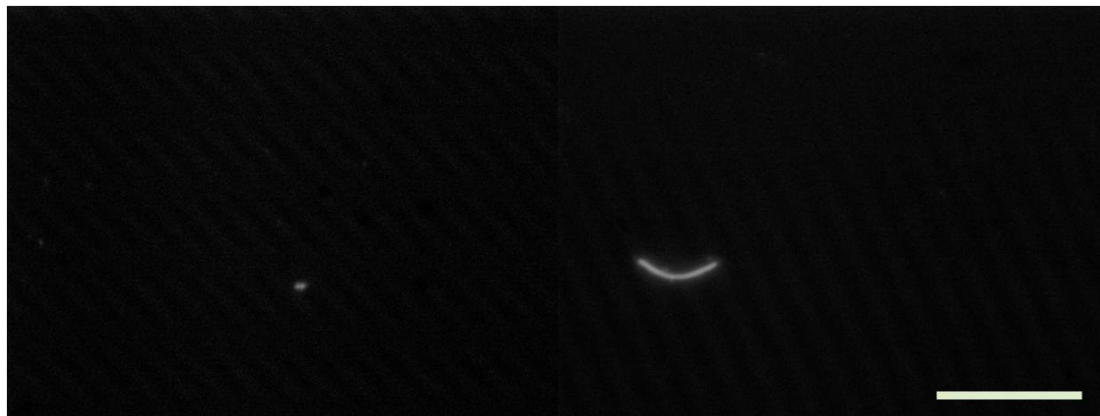


Fig. 1. Figure 2. Bar 10 μm

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