Electronic Supplementary Material (ESI) for Environmental Science: Water Research & Technology.

Electronic Supplementary Information (ESI)

Molecular survey of *Legionella* and *Naegleria fowleri* in private well water and premise plumbing following the 2016 Louisiana flood

Dongjuan Dai,[1] William J. Rhoads,[1] Adrienne Katner,[2] Laurel Strom,[1,3] Marc A. Edwards,[1] Amy Pruden,[1*] Kelsey J. Pieper[1*]

Section ESI.1 Community-wide sampling instructions (Basic kit)

These sampling materials were developed based on the Virginia Household Water Quality Program's citizen science sampling for well users in Virginia (www.wellwater.bse.vt.edu).

1. After the water has not been used for at least 6 hours, unpack the plastic bag and remove the caps from the bottles. Set the caps on the counter upside down, trying not to touch the inside of the caps or bottles. Collect water samples from your kitchen tap.
2. With bottle 1 held under the kitchen tap, open the cold water tap and fill the bottle completely at full flow (as if you were filling a glass of water). Once filled, leave the water running and set bottle 1 aside.
3. Immediately fill bottle 2 to the top. Continue to let the water run, and put the caps on bottles 1 and 2. Take care not to touch the inside of the caps or the bottles.
4. Allow water to run for 5 minutes at full flow.
5. At 5 minutes, fill bottles 3. Once all the bottles are filled, turn water off and place the caps back on the bottles. Take care not to touch the inside of the caps or the bottles.
6. Make sure the cap is securely tightened on all bottles so they do not leak during transport.
7. Place bottles 2 and 3 in the separate bags provided.
8. If you cannot drop the samples off immediately after collection, place the bottles into your refrigerator or on ice until you can drop them off. Do not freeze any samples.
9. Complete the two-page questionnaire.
10. Place bottles and questionnaire back into the plastic bag. Bring your sample bag to: [location].

[1] Virginia Tech, Via Civil and Environmental Engineering, Blacksburg, VA 24061, USA
[2] Louisiana State University Health Science Center, New Orleans, LA 70112, USA
[3] Present address: 11510 S. Baltimore Rd. Spokane, WA 99223, USA
[*] Corresponding authors, apruden@vt.edu, kpieper@vt.edu

Section ESI.2 Community-wide sampling instructions (Advanced kit)

These sampling materials were developed based on the Virginia Household Water Quality Program's citizen science sampling for well users in Virginia (www.wellwater.bse.vt.edu).

1. After the water has not been used for at least 6 hours, unpack the plastic bag and remove the caps from the bottles. Set the caps on the counter upside down, trying not to touch the inside of the caps or bottles. Collect water samples from your kitchen tap.
2. With bottle 1 held under the kitchen tap, open the cold water tap and fill the bottle completely at full flow (as if you were filling a glass of water). Once filled, leave the water running and set bottle 1 aside.
3. Immediately fill bottle 2 to the top. Continue to let the water run, and put the caps on bottles 1 and 2. Take care not to touch the inside of the caps or the bottles.
4. Allow water to run for 5 minutes at full flow.
5. At 5 minutes, fill bottles 3, 4 and 5 back-to-back. Once all the bottles are filled, turn water off and place the caps back on the bottles. Take care not to touch the inside of the caps or the bottles.
6. With bottle 6 held under the kitchen tap, open the hot water tap and fill the bottle completely at full flow. Once filled, turn off the hot water and put the cap on the bottle.
7. Make sure the cap is securely tightened on all bottles so they do not leak during transport.
8. Place bottles 2, 4, and 6 in the separate bags provided.
9. If you cannot drop the samples off immediately after collection, place the bottles into your refrigerator or on ice until you can drop them off. Do not freeze any samples.
10. Complete the two-page questionnaire.
11. Place bottles and questionnaire back into the plastic bag. Bring your sample bag to: [location].

Section ESI.3. Selected questions from the questionnaire whose results were used for data analysis in this study

Q: What water treatment devices are currently installed? Check all that apply.

( ) none
( ) acid neutralizer
( ) sediment filter
( ) ultraviolet (UV) light
( ) water softener (conditioner)
( ) reverse osmosis
( ) iron removal
( ) activated carbon (charcoal) filter
( ) chlorinator
( ) other:_____

Q: Did you test your well water before the flood?

( ) no
( ) yes
If yes, what did you test for
( ) bacteria
( ) nitrate
( ) pesticides
( ) VOCs/sVOCs
( ) metals

Q: Did you test your well water after the flood?

( ) no
( ) yes
If yes, what did you test for
( ) bacteria
( ) nitrate
( ) pesticides
( ) VOCs/sVOCs
( ) metals

Q: Was your well or septic system damaged during the flood?

( ) no
( ) yes
If yes, please explain:_____

Q: About your private well where you draw samples from

Is it a: ( ) dug or bored well ( ) drilled well ( ) don't know
What is the depth of the well? _____feet ( ) don't know
What year was well constructed? _____ ( ) don't know
How many homes are supplied? _____ ( ) don't know

Section ESI.4. Inorganic aspects of water quality problems

Method: Detection limits of the inorganics analysis were 0.5 µg/L for arsenic, 1 µg/L for copper and lead, 5 µg/L for zinc, and 10 µg/L for iron. Water chemistry below detection limit was treated as half of the detection limit. Water conductivity was used as a proxy for total dissolved solids.

Results and discussion: Primary and secondary regulations for drinking water contaminants by United States Environmental Protection Agency (U.S. EPA) for public drinking water were applied as the reference standard for assessing inorganic levels in private wells (Table S2). Out of the 113 wells, only 1 or 2 wells exceeded the enforceable standards of three primary contaminants (arsenic, copper, and lead). Among the non-mandatory aesthetic-based standards, manganese, iron, and aluminum are the top three concerns as 47.8%, 19.5%, and 7% of the 113 wells had higher levels than the non-mandatory standards, respectively. Another aesthetic concern for most wells is the high levels of sodium (>20 mg/L), while high sodium in drinking water may aggravate chronic congestive heart failure in humans (WHO 1996). These secondary contaminants of concern (manganese, iron, and sodium) are naturally occurring elements in groundwater, and are likely borne to the specific geological aquifers rather than being affected by flood.

Reference:

WHO, (1996), Sodium in drinking-water
http://www.who.int/water_sanitation_health/dwq/chemicals/sodium.pdf

Table S1. Microbial assessment of five homes sampled during the neighborhood-scale sampling campaign.

		August 27, 2016						September 3, 2016				
Well	Sample*	pH	TC[α] (MPN /100mL)	EC[β] (MPN/ 100mL)	*Legionella* spp. (gc/mL)	*Lp*[γ]	*Nf*[δ]	TC (MPN /100mL)	EC (MPN /100mL)	*Legionella* spp. (gc/mL)	*Lp*	*Nf*
1[#]	First draw	7.81	-	-	3.0E+03	ND[ε]	ND	-	-	4.0E+02	ND	ND
	Second draw	7.74	14.72	ND	1.1E+03	ND	ND	9.80	ND	ND	ND	ND
	Well water	7.76	14.87	ND	3.9E+02	ND	ND	9.70	ND	4.6E+02	ND	ND
	Hot draw	-	-	-	1.6E+03	ND	ND	-	-	1.9E+02	ND	ND
	Hot flushed	8.00	-	-	1.5E+03	ND	ND	-	-	ND	ND	ND
2	First draw	7.59	-	-	1.1E+03	ND	ND	-	-	4.7E+03	ND	ND
	Second draw	7.55	22.66	ND	ND	ND	ND	ND	ND	8.5E+03	ND	ND
	Well water	7.53	16.2	ND	3.3E+01	ND	ND	ND	ND	6.9E+01	ND	ND
	Hot draw	-	-	-	3.2E+03	ND	ND	-	-	2.2E+04	ND	ND
	Hot flushed	7.45	-	-	1.4E+03	ND	ND	-	-	5.9E+02	ND	ND
3	First draw	7.82	-	-	ND	ND	ND	-	-	6.4E+01	ND	ND
	Second draw	7.77	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Well water	7.82	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Hot draw	-	-	-	ND	ND	ND	-	-	ND	ND	ND
	Hot flushed	7.92	-	-	ND	ND	ND	-	-	ND	ND	ND
4	First draw	7.23	-	-	4.6E+01	ND	ND	-	-	1.8E+03	ND	ND
	Second draw	7.21	ND	ND	ND	ND	ND	ND	ND	-	ND	ND
	Well water	7.00	1.01	ND	ND	ND	ND	ND	ND	7.2E+01	ND	ND
	Hot draw	-	-	-	ND	ND	ND	-	-	9.7E+01	ND	ND
	Hot flushed	7.27	-	-	ND	ND	ND	-	-	1.8E+03	ND	ND
5	First draw	-	-	-	1.6E+02	ND	ND	-	-	-	-	-
	Second draw	7.42	ND	ND	ND	ND	ND	-	-	-	-	-
	Well water	7.38	ND	ND	ND	ND	ND	-	-	-	-	-
	Hot draw	-	-	-	2.3E+02	ND	ND	-	-	-	-	-
	Hot flushed	7.47	-	-	4.4E+01	ND	ND	-	-	-	-	-

[α]TC: total coliform; [β]EC: *E. coli;* [γ]*Lp: L. pneumophila;* [δ]*Nf: N. fowleri;* [ε]ND: not detected.

*Samples collected: First draw: first draw cold water; Second draw: second draw cold water; Well water: 5-min flushed cold water; Hot draw: first draw hot water; Hot flushed: 5-min flushed hot water. All water samples were collected from

their kitchen taps.

[#]This house was resampled later during the community-wide sampling campaign and its TC/EC reduced to be not detectable.

Table S2. Summary of sampled private wells (homes) during the community-wide sampling campaign

Geological locations (total 113 private wells)		
Ascension parish	n= 45 (40%)	
Livingston parish	n= 62 (55%)	
Others	n= 6 (5%)	
Sampling kits (total 113 private wells)*		
Basic kits (n=73, 65%)	First draw (250 mL) for inorganics analysis Well water (250 mL + 125 mL) for inorganics, total coliform and *E. coli* analysis	
Advanced kits (n=40,35%)	First draw (250 mL + 1 L) for inorganics and OPs analysis Well water (250 mL +125 mL + 1 L) for inorganics, total coliform and *E. coli,* and OPs analysis Hot draw (1 L) for OPs analysis	
Flood-induced damage reported by well-users	Out of all 113 private wells	Out of the 40 private wells with OPs measured
Flood-induced damage observed	n= 43 (38%)	n= 15 (38%)
No flood-induced damage	n= 59 (52%)	n= 20 (50%)
Data unavailable	n= 11 (10%)	n= 5 (13%)
In-house treatment	Out of all 113 private wells	Out of the 40 private wells with OPs measured
With a treatment	n= 22 (20%)	n= 10 (25%)
Without a treatment	n= 75 (66%)	n= 24 (60%)
Data unavailable	n= 16 (14%)	n= 6 (15%)
Location with regard to flood zone	Out of all 113 private wells	Out of the 40 private wells with OPs measured
Within flood zone	n= 99 (88%) (65 basic + 34 advanced kits)	n= 34 (85%)
Outside flood zone	n= 14 (12%) (8 basic + 6 advanced kits)	n= 6 (15%)
Construction type of private wells	Out of all 113 private wells	Out of the 40 private wells with OPs measured
Drilled	n= 75 (66%)	n= 26 (65%)
Dug/bored	n= 9 (8%)	n= 2 (5%)
Unknown	n= 29 (26%)	n= 12 (30%)

*First draw: first draw cold water; Well water: 5-min flushed cold water; Hot draw: first draw hot water, from kitchen taps.

Table S3. Summary statistics of inorganic parameters in well water (5-min flushed cold water) (n=113)

Inorganic parameters	Standard[1]		Median	90th percentile	Maximum	% of wells exceeding standard
Arsenic[a]	MCL[2]	10	BDL[c]	0.5	27.4	0.9%
Cadmium[a]		5	BDL[d]	BDL[d]	2.7	0.0%
Chromium[a]		100	BDL[d]	BDL[d]	1.5	0.0%
Copper[a]	Action Level	1300	2.3	28.0	1418.0	1%
Lead[a]		15	BDL[d]	4.4	66.2	2%
Aluminum[a]	SMCL[3]	50-200	0.5	6.2	798.0	7%
Chloride[b]		250	7.5	36.2	150.9	0.0%
Copper[a,5]		1000	19.7	196.6	2387.0	1.8%
Iron[a]		300	88.6	556.1	2425.0	19.5%
Manganese[a]		50	47.7	174.0	393.2	47.8%
pH		6.5-8.5	7.8	8.1	8.5 (5.2)	3.5% (3.5%)
Silver[b]		100	BDL[d]	BDL[d]	BDL[d]	0.0%
Sulfate[b]		250	2.9	5.0	11.0	0.0%
TDS[b,6]		500	195.7	291.9	523.9	2.7%
Zinc[a]		5000	65.6	548.2	3836.0	0.0%
Sodium[b]	DWEL[4]	20	61.4	89.5	161.7	94%

[a] in the unit of µg/L; [b] in the unit of mg/L; BDL[c]: below detection limit of 0.5 µg/L; BDL[d]: below detection limit of 1 µg/L.
[1]Reference standards for public water by US. EPA; [2] MCL: maximum contaminant level; [3] SMCL: secondary maximum contaminant level; [4] DWEL: drinking water equivalent level; [5] Additional copper measurement from first draw of cold water; [6] TDS: total dissolved solids.

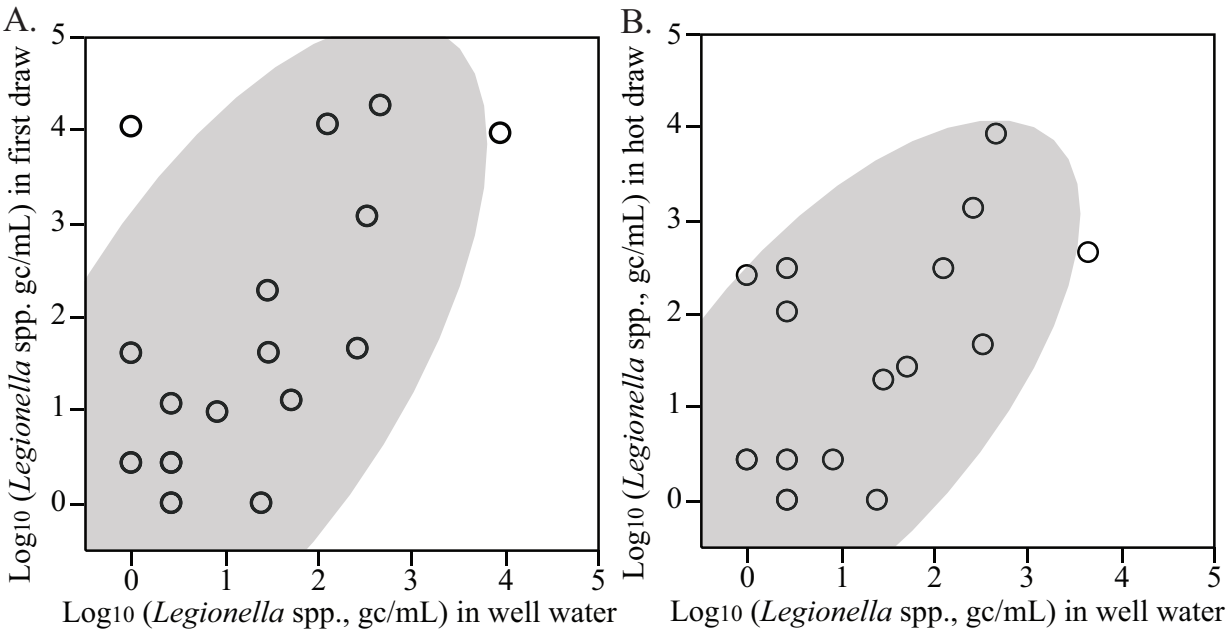

Fig. S1. *Legionella* spp. gene copy numbers in A) first draw cold water and B) first draw hot water positively correlated with the *Legionella* spp. level in well water (Spearman ρ=0.54 and 0.48, p=0.011 and 0.02, n=21 and 23). Shaded oval indicates the 95% ellipse to assist visualization of positive correlations.

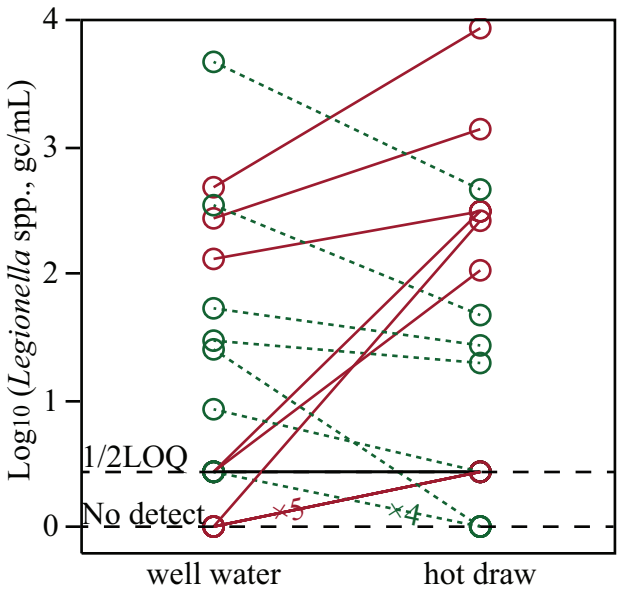

Fig. S2. A diverging pattern in the *Legionella* spp. levels between hot draw and well water. Gene copy numbers of *Legionella* spp. increased (red line) or decreased (green dashed line) in first draw hot water (hot draw) in comparison to corresponding well water (5-min flushed cold water) from the same household. Samples with no detectable *Legionella* spp. are shown as zero. Samples with detectable *Legionella* spp. yet below the limit of quantification are shown as the value of 1/2LOQ, and samples with no detects of *Legionella spp.* are shown as 0. Notes "×5" and "×4" represent the number of homes having the same trends and thus overlapped in the plot.

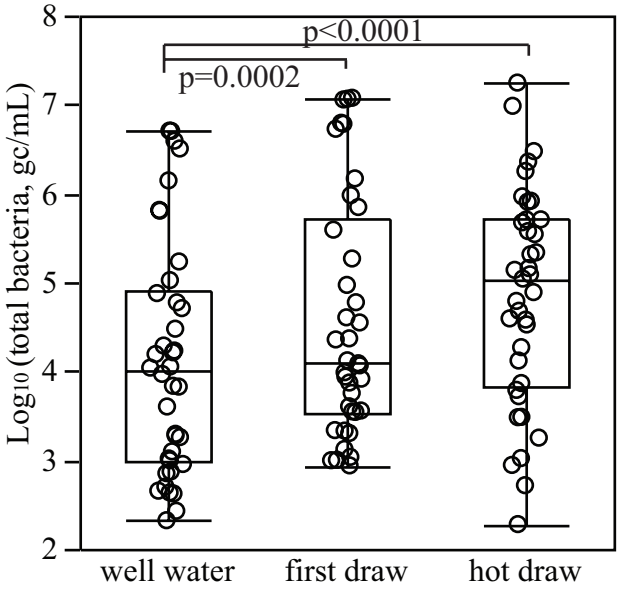

Fig. S3. Total bacterial numbers in three types of water. Total bacterial numbers significantly increased in first draw cold water (first draw) and first draw hot water (hot draw) in comparison to those in well water (5-min flushed cold water). The p-values are from matched pair Wilcoxon signed rank tests.

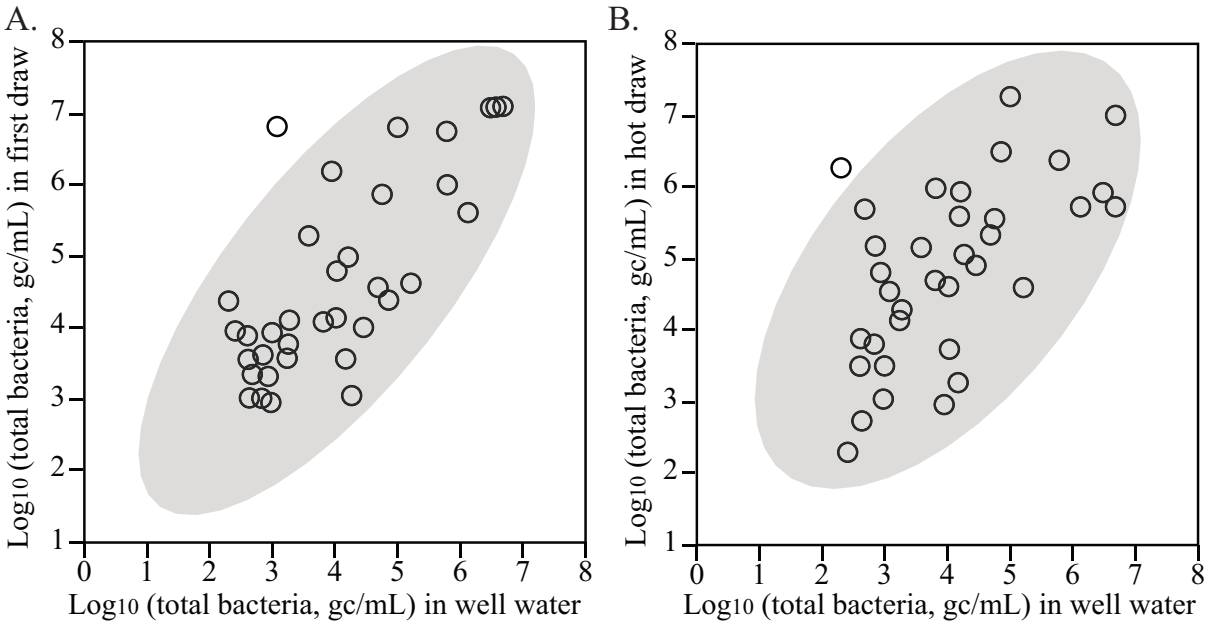

Fig. S4. Total bacterial numbers in A) first draw cold water (first draw) and B) first draw hot water (hot draw) positively correlated with total bacterial numbers in well water (5-min flushed cold water) (Spearman ρ=0.70 and 0.58, p<0.0001). Shaded oval indicates the 95% ellipse to assist visualization of positive correlations.

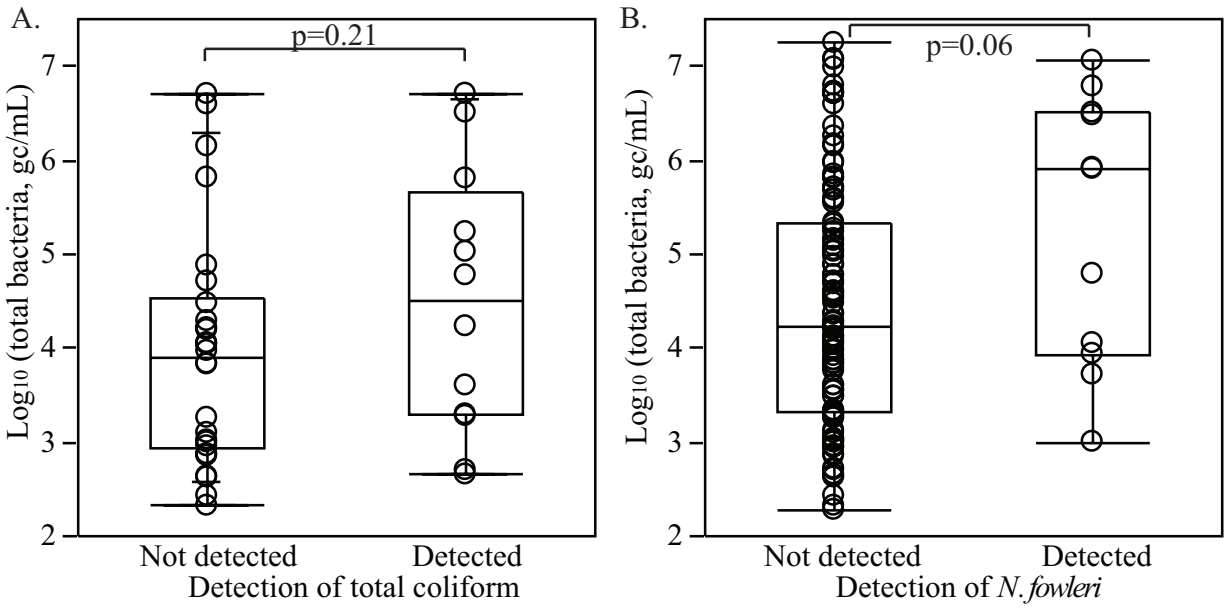

Fig. S5. Total bacterial numbers in well water showed no association with total coliform and *N. fowleri.* Total bacterial numbers in well waters (5-min flushed cold water) with positive detection of A) total coliform or B) *N. fowleri* were not different from those with no detection.

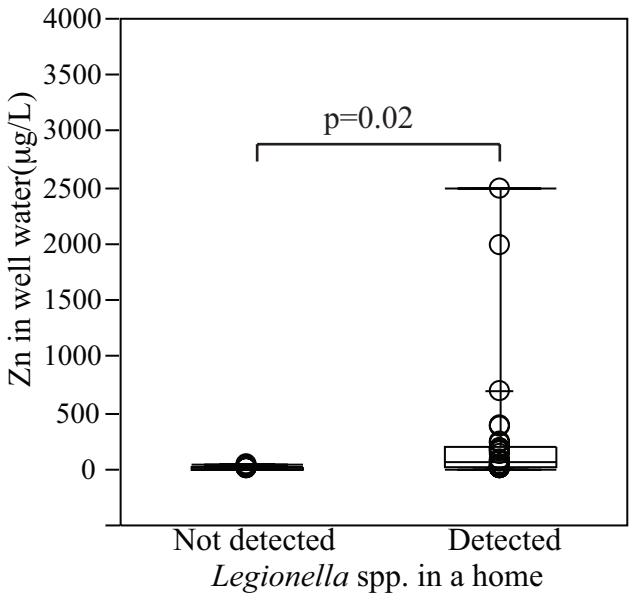

Fig. S6. Higher zinc levels in well water in homes with the detection of *Legionella* spp. than in homes with no detection of *Legionella* spp. The p-value is from Wilcoxon test.