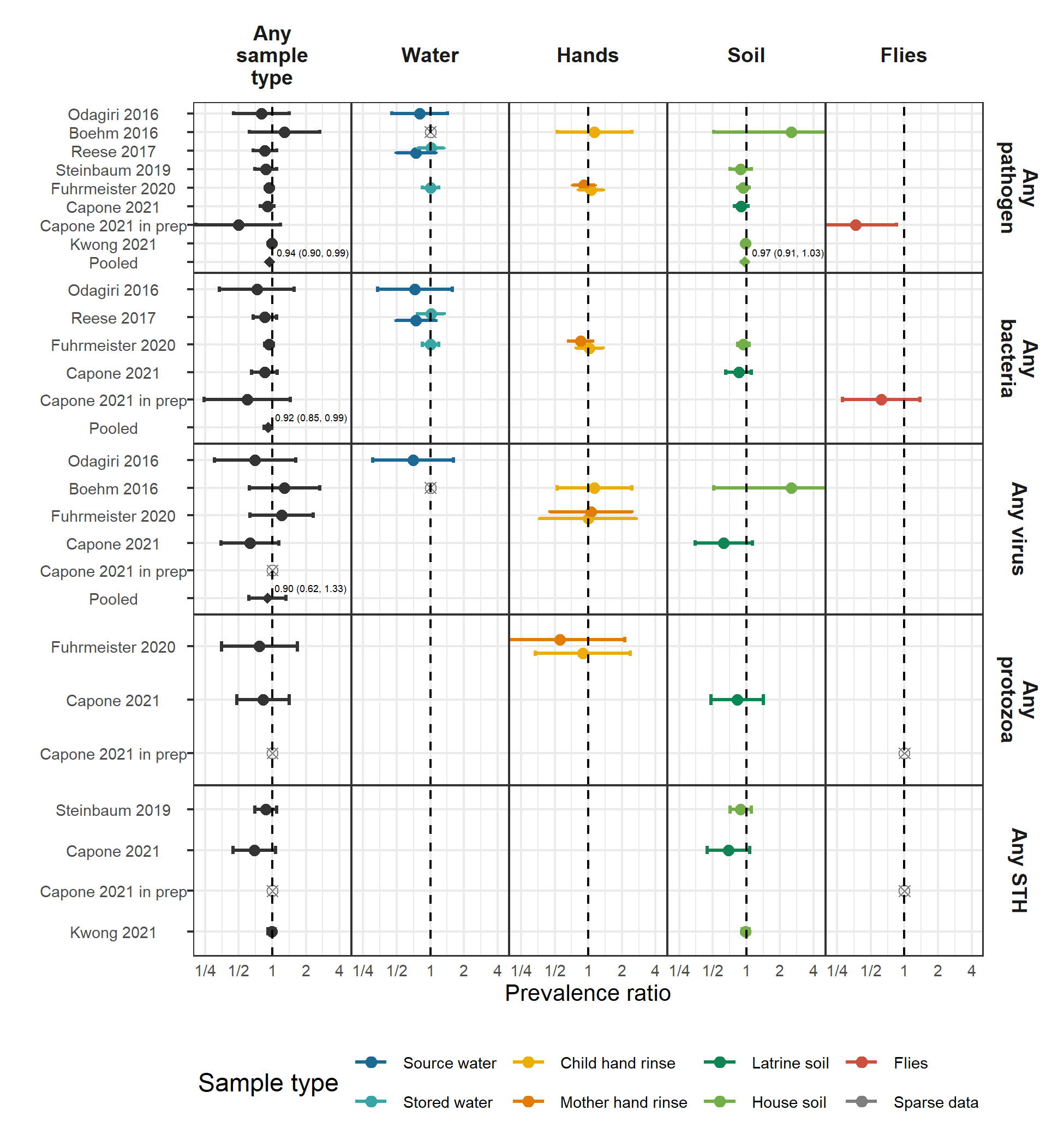
Effect of water, sanitation, and hygiene interventions on enteropathogen detection in the environment: an individual-data meta-analysis

## Figures



**Figure 1.** Forest plots of intervention effects on the prevalence of any enteropathogen or type of enteropathogen (any bacteria, any virus, any protozoa and any STH) in different types of environmental samples. Pooled estimates are presented when there are four or more study-specific estimates for a specific sample type and target combination and are denoted with diamond-shaped points. Grey crossed points denote data that were too sparse to estimate a prevalence ratio (i.e., <10 positive or negative observations). Samples of the same type from different locations (source vs. stored water, flies in kitchen vs. latrine, soil from courtyard vs. latrine) or different individuals (child vs. mother’s hands) are plotted separately. Point estimates and confidence intervals are printed next to pooled estimates. All estimates are adjusted for potential confounders.

Chart, scatter chart

Description automatically generated

**Figure 2.** Forest plots of intervention effects on the prevalence of any MST marker or type of MST marker (human or animal MST markers) in different types of environmental samples. Pooled estimates are presented when there are four or more study-specific estimates for a specific sample type and target combination and are denoted with diamond-shaped points. Grey crossed points denote data that were too sparse to estimate a prevalence ratio (i.e., <10 positive or negative observations). Samples of the same type from different locations (source vs. stored water, flies in kitchen vs. latrine, soil from courtyard vs. latrine) or different individuals (child vs. mother’s hands) are plotted separately. Point estimates and confidence intervals are printed next to pooled estimates. All estimates are adjusted for potential confounders.

## Tables

### Table 1. Characteristics of included publications

| **Parent study** | **Study design** | **Intervention** | **Time between intervention and environmental sampling** | **Location** | **Author/ year** | **Sample types** | **Targets** | **Analytic method** | **Number of samples** |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| WASH Benefits Bangladesh | Cluster-randomized trial | Latrine upgrades, child potties, scoops for feces disposal | 4 months | Rural Bangladesh | Boehm et al. 2016 | Stored drinking water, child hands, soil | Rotavirus, General, human, avian and ruminant fecal markers | qPCR | 1,497 |
| - | - | - | 16-35 months | - | Fuhrmeister et al. 2020 | Stored drinking water, child and mother hands, soil | Pathogenic E. coli, norovirus, Giardia | qPCR | 2,606 |
| - | - | - | ~2 years | - | Kwong et al. 2021 | Courtyard soil | Soil-transmitted helminths | Microscopy | 1,396 |
| WASH Benefits Kenya | Cluster-randomized trial | Latrine upgrades, child potties, scoops for feces disposal | ~2 years | Rural Kenya | Steinbaum et al. 2019 | Courtyard soil | Soil-transmitted helminths | Microscopy | 2,107 |
| MapSan | Controlled before-and-after study | Latrine upgrades | ~1 year | Urban Mozambique | Holcomb et al. 2020 | Source and stored water, household and latrine soil, food, flies caught in latrine and kitchen | Panel of 17 enteropathogens, human and avian fecal markers | qPCR | 1,081 |
| Gram Vikas | Matched cohort study | Latrine upgrades, piped water | ~6-10 years | Rural India | Reese et al. 2017 | Source and stored water | V. cholerae, Shigella | Slide agglutination serotyping | 3,452 |
| Total Sanitation Campaign | Cluster-randomized trial | Latrine upgrades | ~1 year | Rural India | Odagiri et al. 2016 | Source water | V. cholerae, rotavirus, adenovirus,general, human, and animal fecal markers | qPCR, microscopy | 60 |

### Table 2. Mean (SD) abundances by study arm and adjusted abundance differences between intervention and control arms. Means are log10 transformed concentrations for MST markers, and are mean egg counts for soil transmitted helminths (*Ascaris* and *Trichuris*).

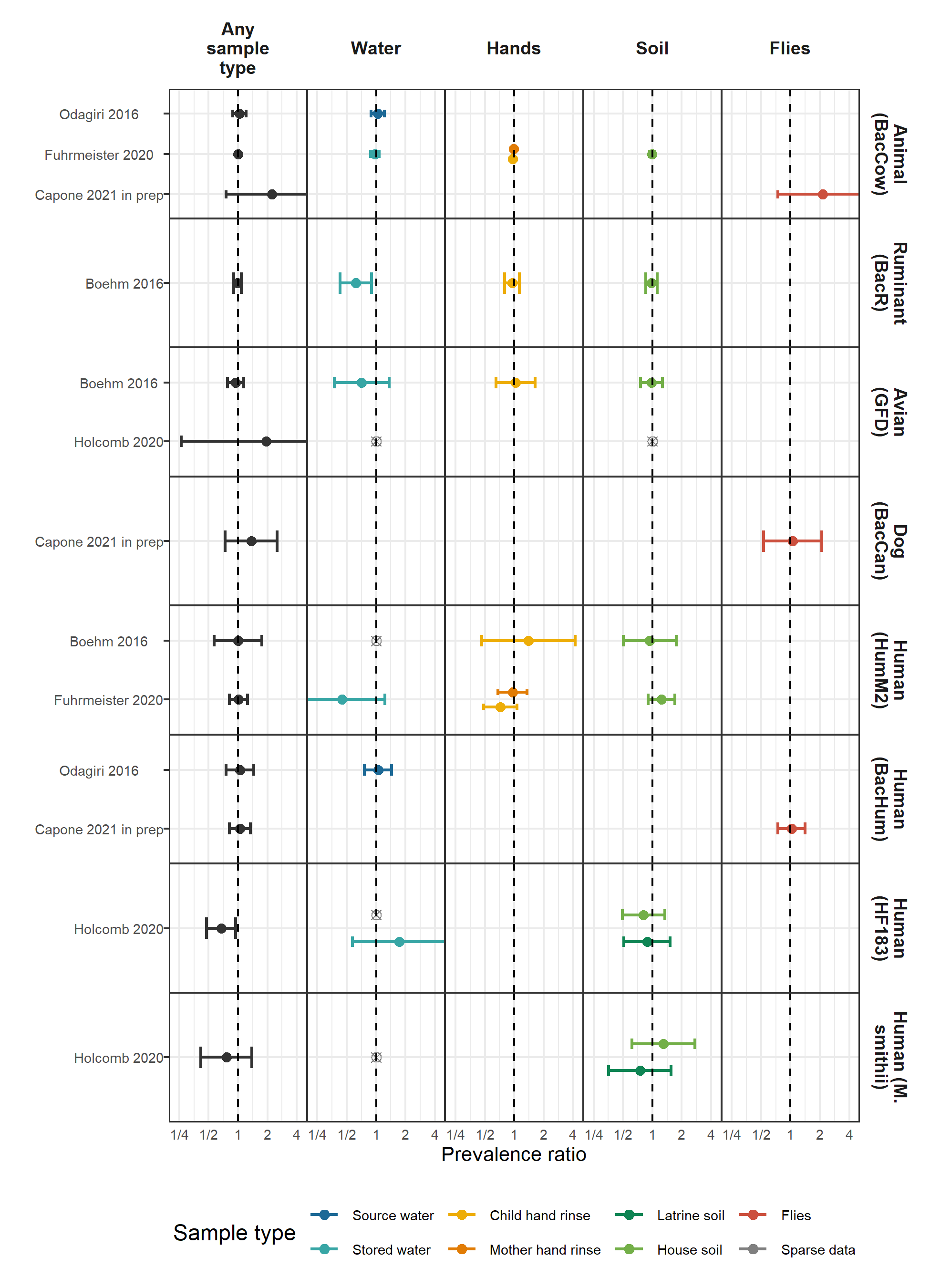
| **Study** | **Sample** | **Target** | **N** | **% in ROQ** | **Control mean, median (SD)** | **Intervention mean, median (SD)** | **Difference (95% CI)** | **P value** | **Wilcoxon P value** |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Fuhrmeister 2020 | Child hand rinse | Animal (BacCow) | 365 | 75.9 | 3.6, 3.9 (1.4) | 3.4, 3.8 (1.4) | -0.17 (-0.47 0.12) | 0.25 | 0.17 |
| - | Mother's hand rinse | Animal (BacCow) | 725 | 66.5 | 3.3, 3.8 (1.4) | 3, 3.7 (1.5) | -0.28 (-0.49 -0.07) | 0.01 | 0.01 |
| Holcomb 2020 | Latrine soil | Human (M. smithii) | 113 | 51.3 | 6.7, 6.5 (0.6) | 6.5, 6.3 (0.5) | -0.14 (-0.38 0.11) | 0.27 | 0.58 |
| Capone 2021 in prep |  | Human (BacHum) | 173 | 77.5 | 3.8, 3.8 (1.3) | 4, 4.2 (0.9) | 0.14 (-0.19 0.47) | 0.41 | 0.07 |
| Steinbaum 2019 | House soil | Ascaris | 2,101 | 100.0 | 2.2, 0 (18.8) | 1.4, 0 (9.3) | 0.65 (0.33 1.28)a | 0.21 | 0.33 |
| - | - | Trichuris | 2,102 | 100.0 | 0.2, 0 (1.8) | 0.2, 0 (1) | 0.73 (0.36 1.48)a | 0.38 | 0.39 |
| Kwong 2021 | House soil | Ascaris | 1,423 | 100.0 | 2.3, 0.7 (6.7) | 2.2, 0.6 (6.9) | 0.96 (0.68 1.37)a | 0.84 | 0.50 |
| - | - | Trichuris | 1,423 | 100.0 | 1.6, 0.4 (5) | 2, 0.4 (5) | 1.21 (0.86 1.71)a | 0.27 | 0.19 |

ROQ: Range of quantification; SD: Standard deviation; CI: Confidence interval; Wilcoxon P-value: Non-parametric Wilcoxon rank sum test P-value.

a Marks ratio estimates from negative binomial models

## Supplementary Figures

**Figure S1.** Forest plots of intervention effects on the prevalence of specific pathogens.

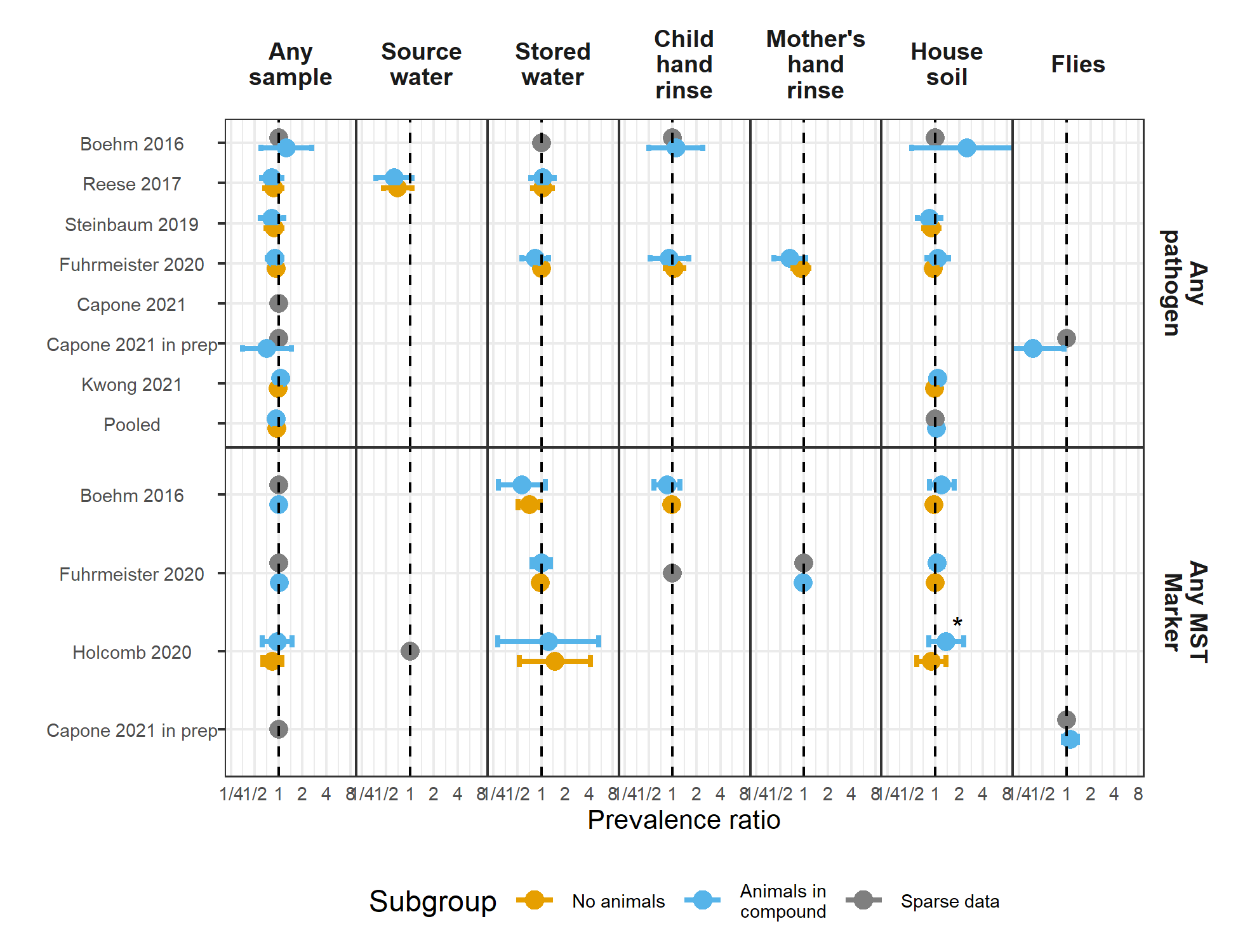


**Figure S2.** Forest plots of intervention effects on the prevalence of specific MST markers.

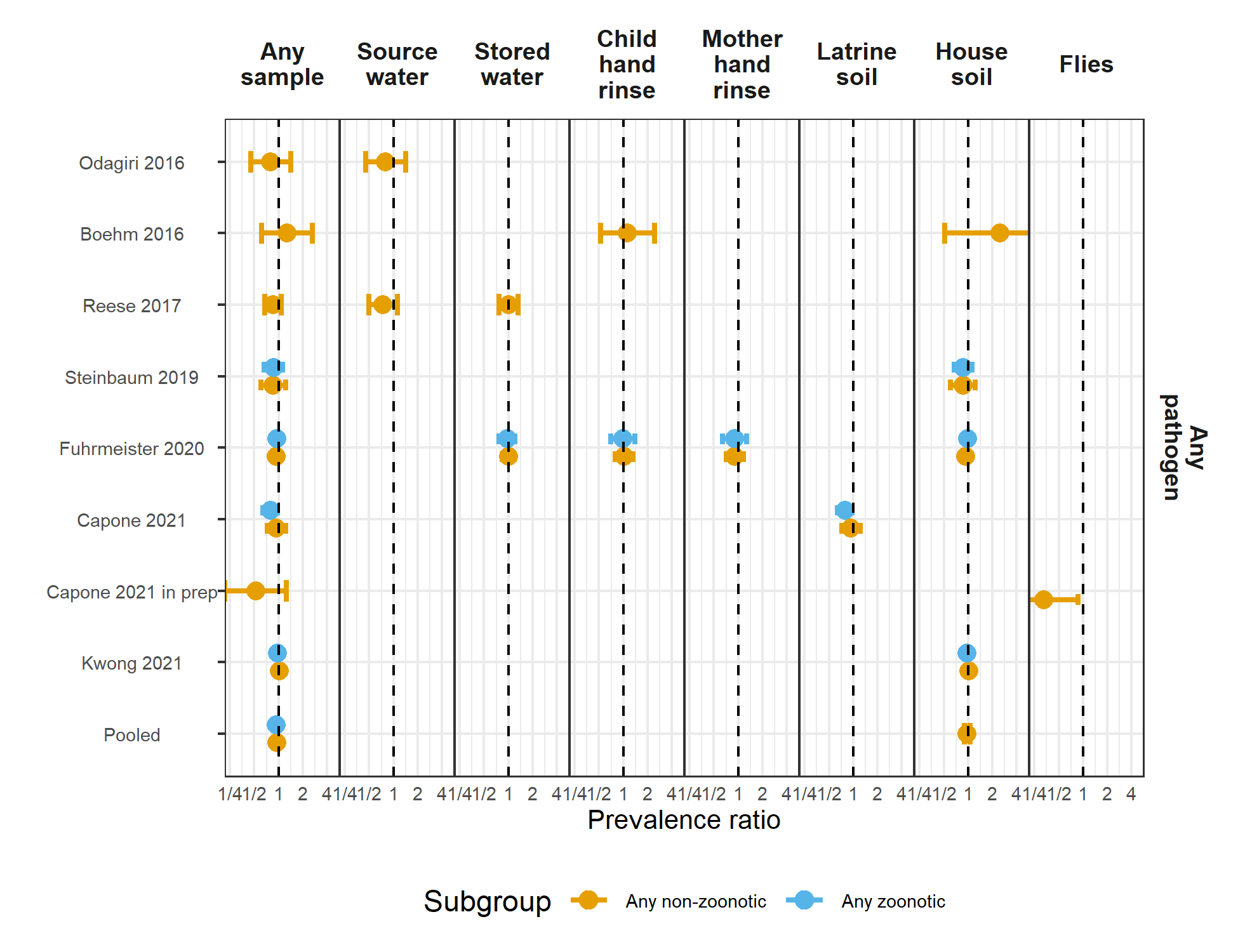
Chart, scatter chart

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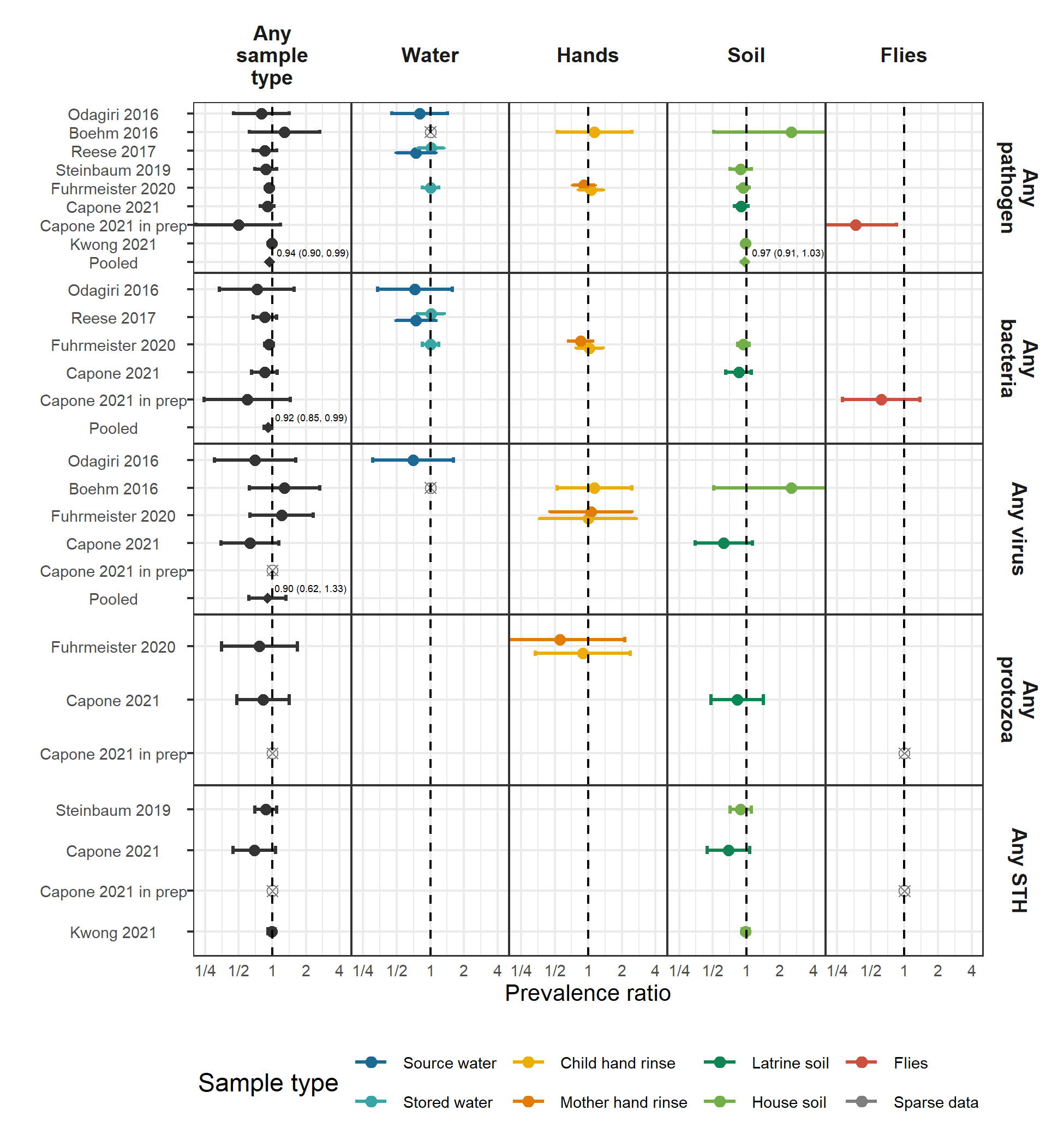
**Figure S3.** Forest plots of intervention effects on any enteropathogen, and any MST markers in different types of environmental samples, stratified by whether the sample was collected during the wet versus dry season (defined by the 6 months of highest average rainfall). Significant effect modification, as determined by the p-values on the regression model interaction term, is marked with asterisks (P < 0.05 = “\*”, P < 0.01 = “\*\*”, P < 0.001 = “\*\*\*”).



**Figure S4.** Forest plots of intervention effects on any enteropathogen, and any MST markers in different types of environmental samples, stratified by whether any animals were present in the compound. Significant effect modification, as determined by the p-values on the regression model interaction term, is marked with asterisks (P < 0.05 = “\*”, P < 0.01 = “\*\*”, P < 0.001 = “\*\*\*”).



**Figure S5.** Forest plots of intervention effects on any enteropathogen in any type of environmental samples , stratified by whether the pathogen is zoonotically transmitted.

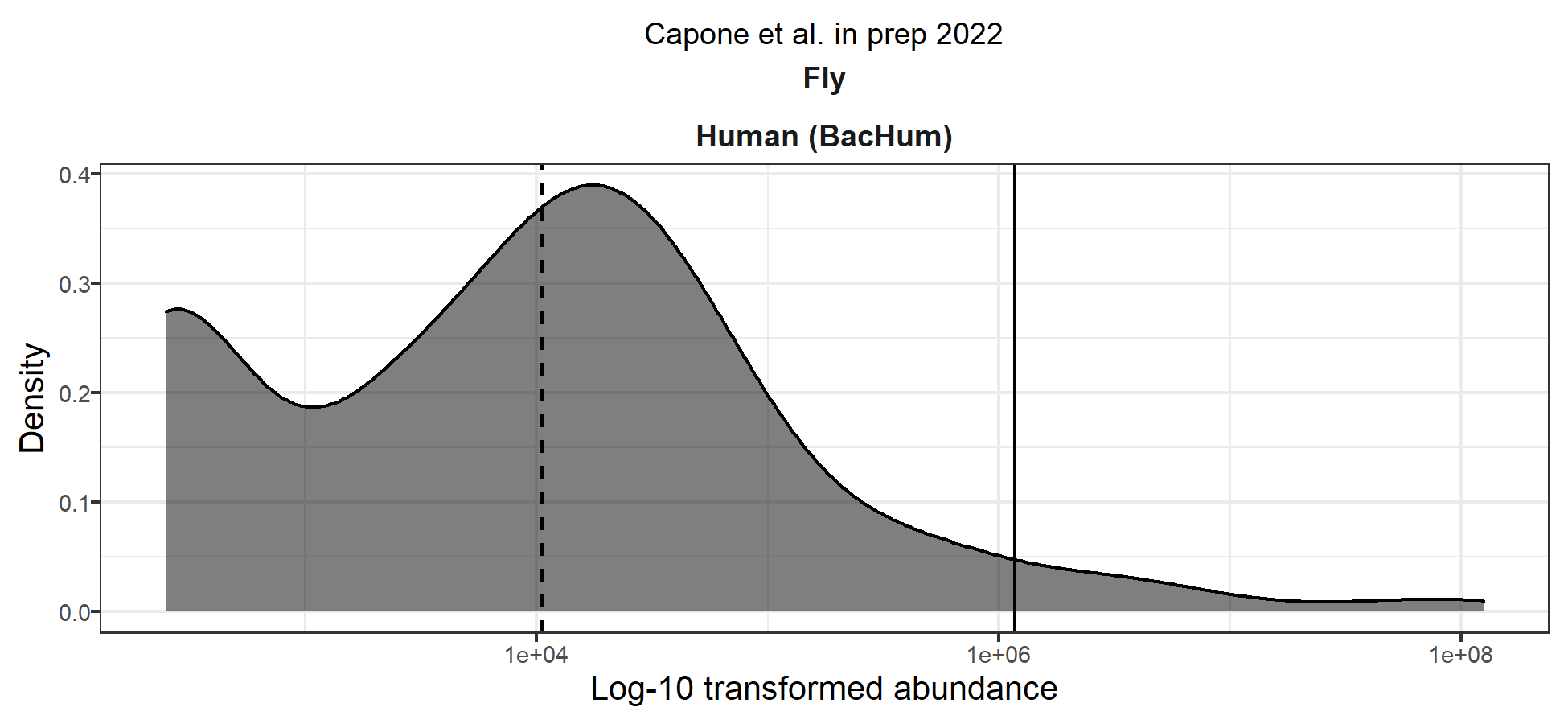


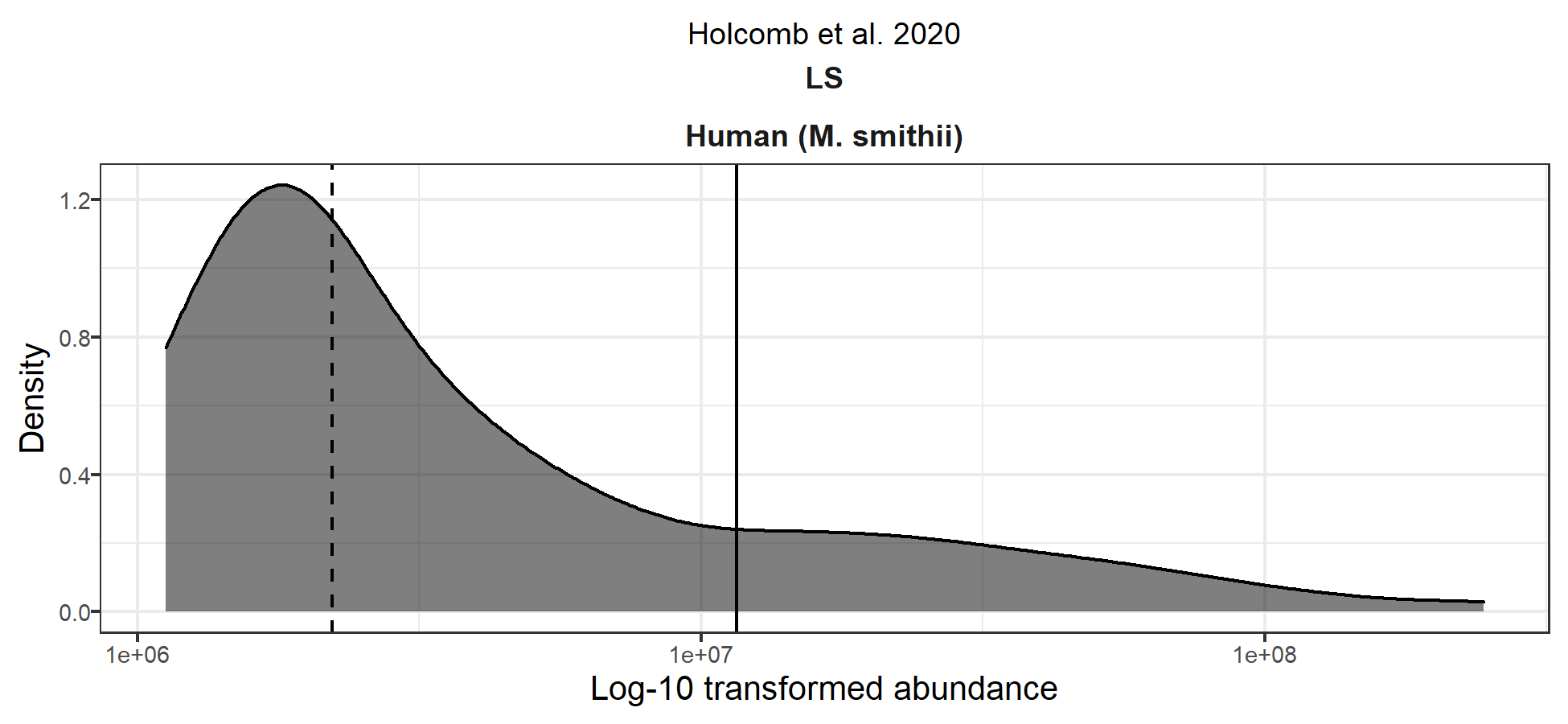
**Figure S6.** Forest plots of unadjusted intervention effects on the prevalence of any enteropathogen or type of enteropathogen (any bacteria, any virus, any protozoa and any STH) in different types of environmental samples. Point estimates and confidence intervals are printed next to pooled estimates.

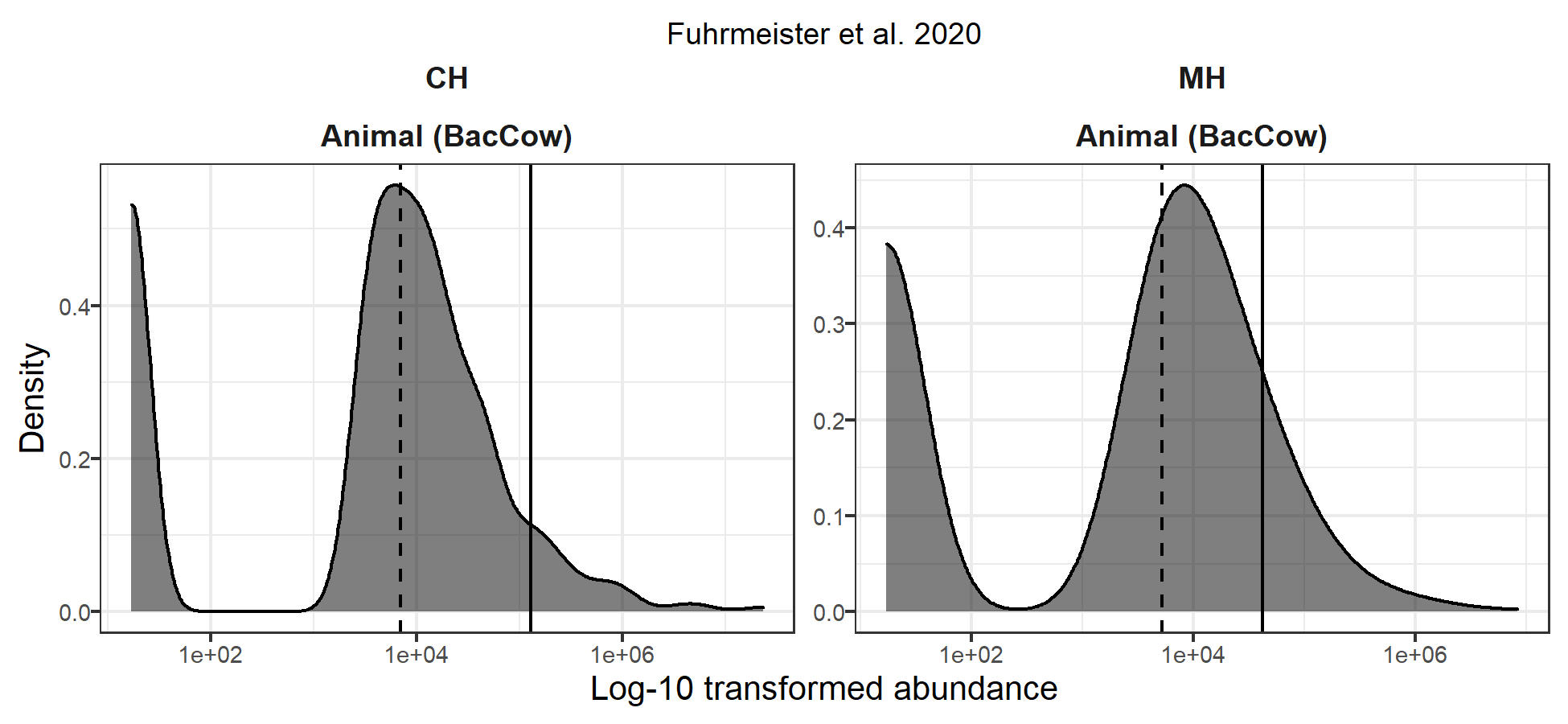
Chart

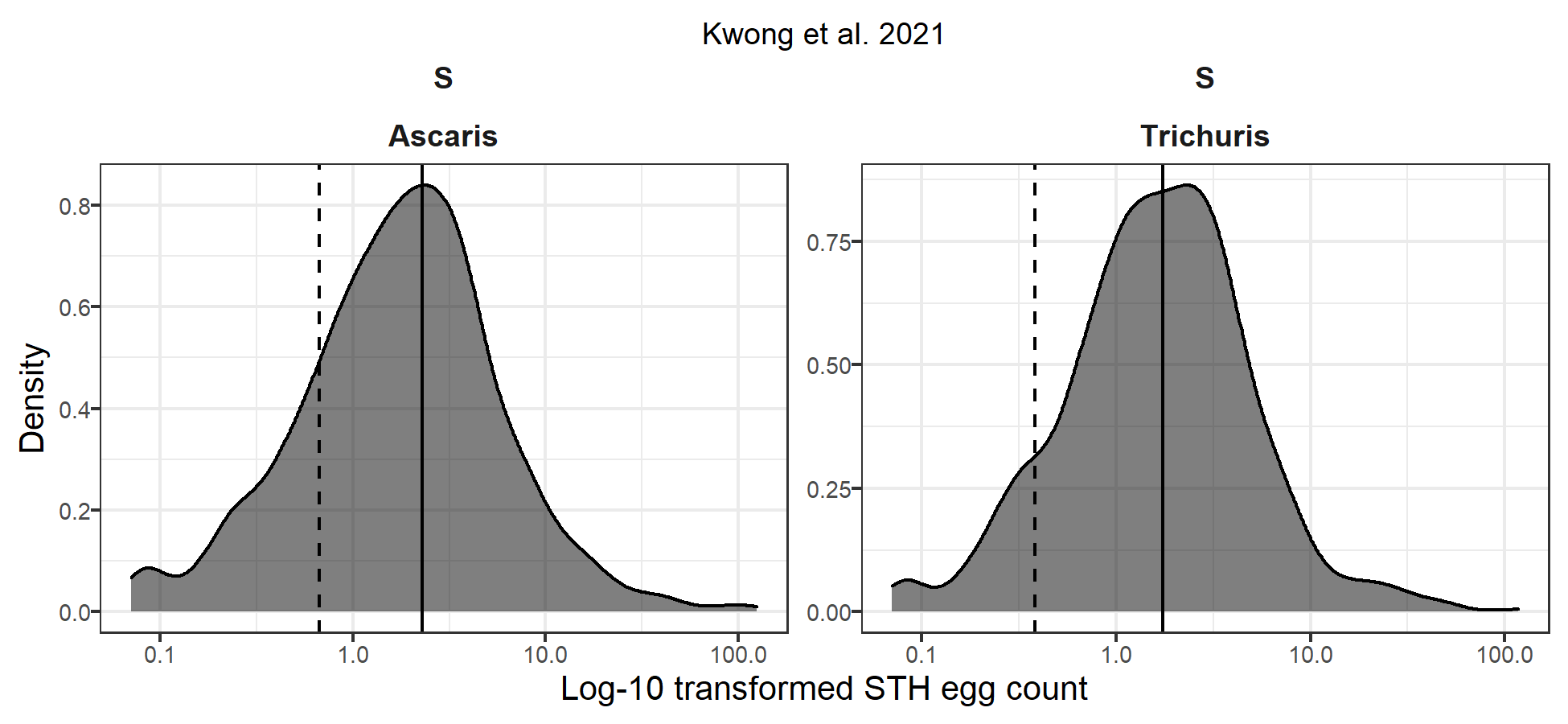
Description automatically generated

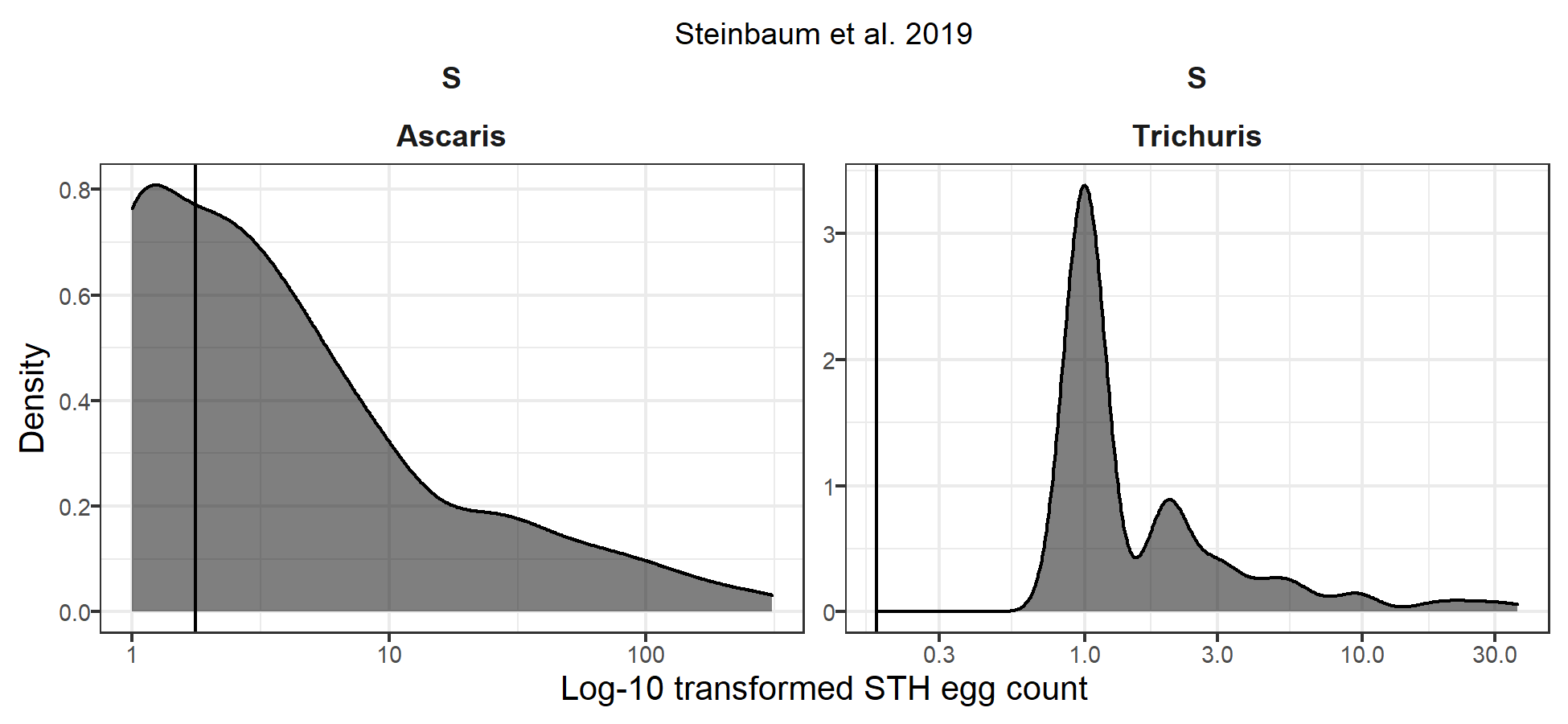
**Figure S7.** Forest plots of unadjusted intervention effects on the prevalence of any MST marker or type of MST marker (human or animal MST markers) in different types of environmental samples. Point estimates and confidence intervals are printed next to pooled estimates.











**Figure S8.** Distributions of log-10 transformed abundance outcomes. Black vertical lines mark the means, and dashed lines mark the medians.