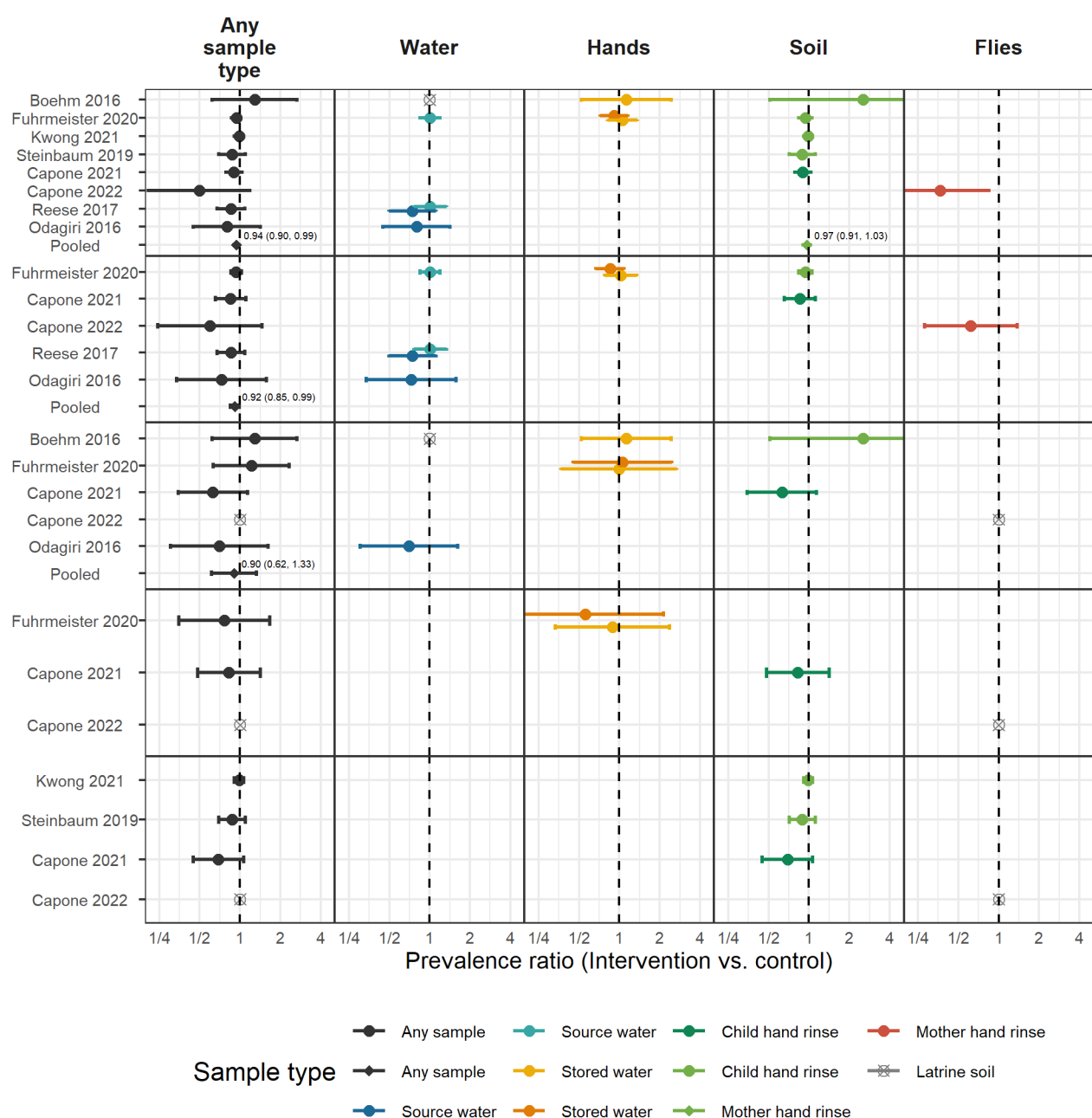


# **Effects of water, sanitation, and hygiene interventions on detection of enteropathogens and host-specific faecal markers in the environment: an individual-participant data meta-analysis**

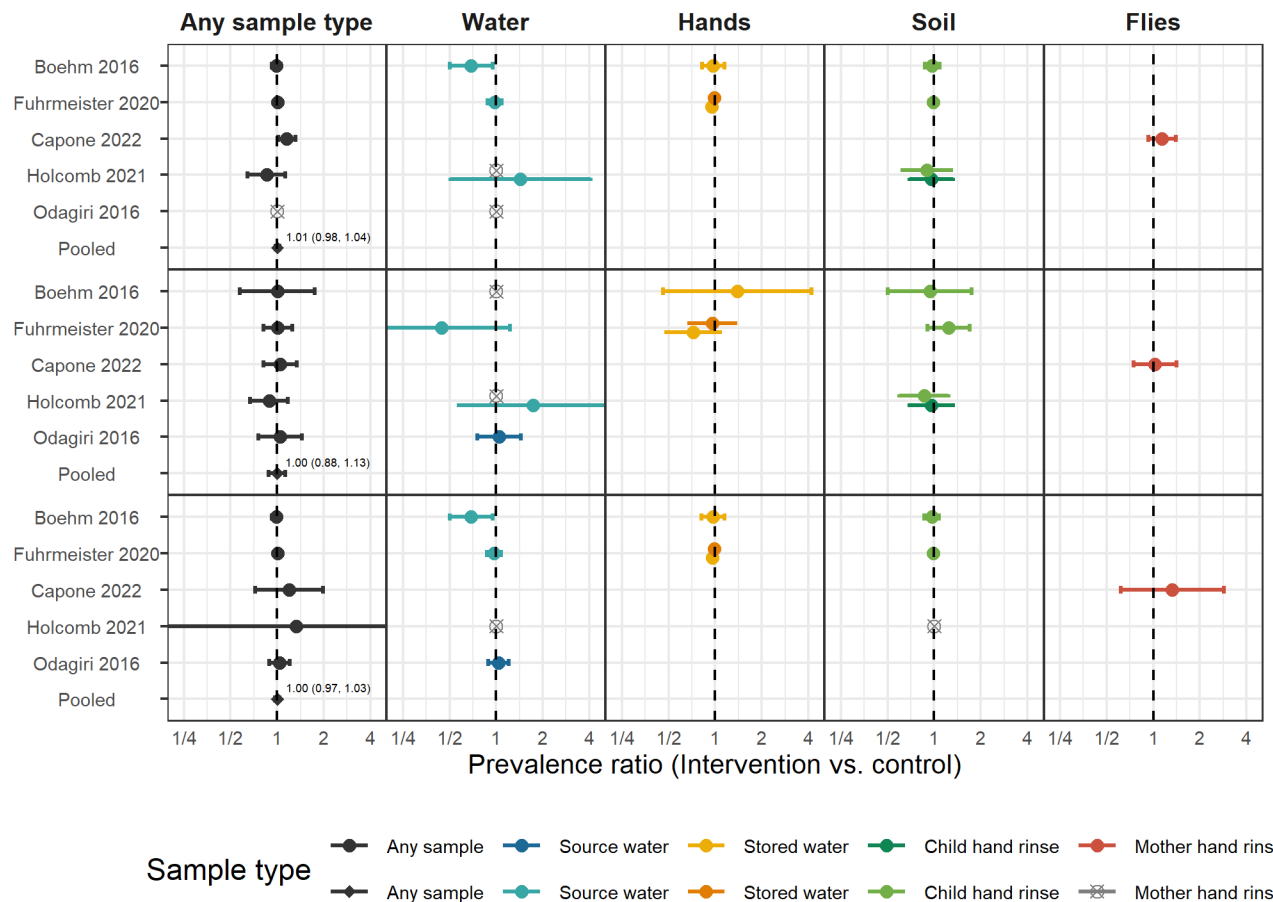
Andrew Mertens PhD, Benjamin F. Arnold PhD, Jade Benjamin-Chung PhD, Prof Alexandria B. Boehm PhD, Joe Brown PhD, Drew Capone PhD, Prof Thomas Clasen PhD, Erica Fuhrmeister PhD, Jessica A. Grembi PhD, David Holcomb PhD, Jackie Knee PhD, Laura H Kwong PhD, Audrie Lin PhD, Prof Stephen P. Luby MD, Rassul Nala MPH, Prof Kara Nelson PhD, Sammy M. Njenga PhD, Clair Null PhD, Amy J. Pickering PhD, Mahbubur Rahman MBBS, Heather E. Reese PhD, Lauren Steinbaum PhD, Prof Jill Stewart PhD, Ruwan Thilakaratne MPH, Oliver Cumming PhD, Prof John M. Colford Jr., Ayse Ercumen PhD

## **Figures**

**Figure 1.**



**Figure 2.** Forest plots of WASH 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 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.



**Figure 3.** Forest plots of WASH 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 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	1,482
-	-	-	16-35 months	-	Fuhrmister et al. 2020	Stored drinking water, child and mother hands, soil	Pathogenic E. coli, norovirus, Giardia	qPCR	2,601
-	-	-	~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	2,149
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	General, human and avian fecal MST markers	qPCR	353
-	-	-	~1 year	-	Capon e et al. 2021	Household and latrine soil	Panel of 18 enteric pathogens	qPCR	88
-	-	-	~2 years	-	Capon e et al. 2022 in prep.	Flies caught in latrine and kitchen	Panel of 16 enteric pathogens and MST markers	qPCR	86
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

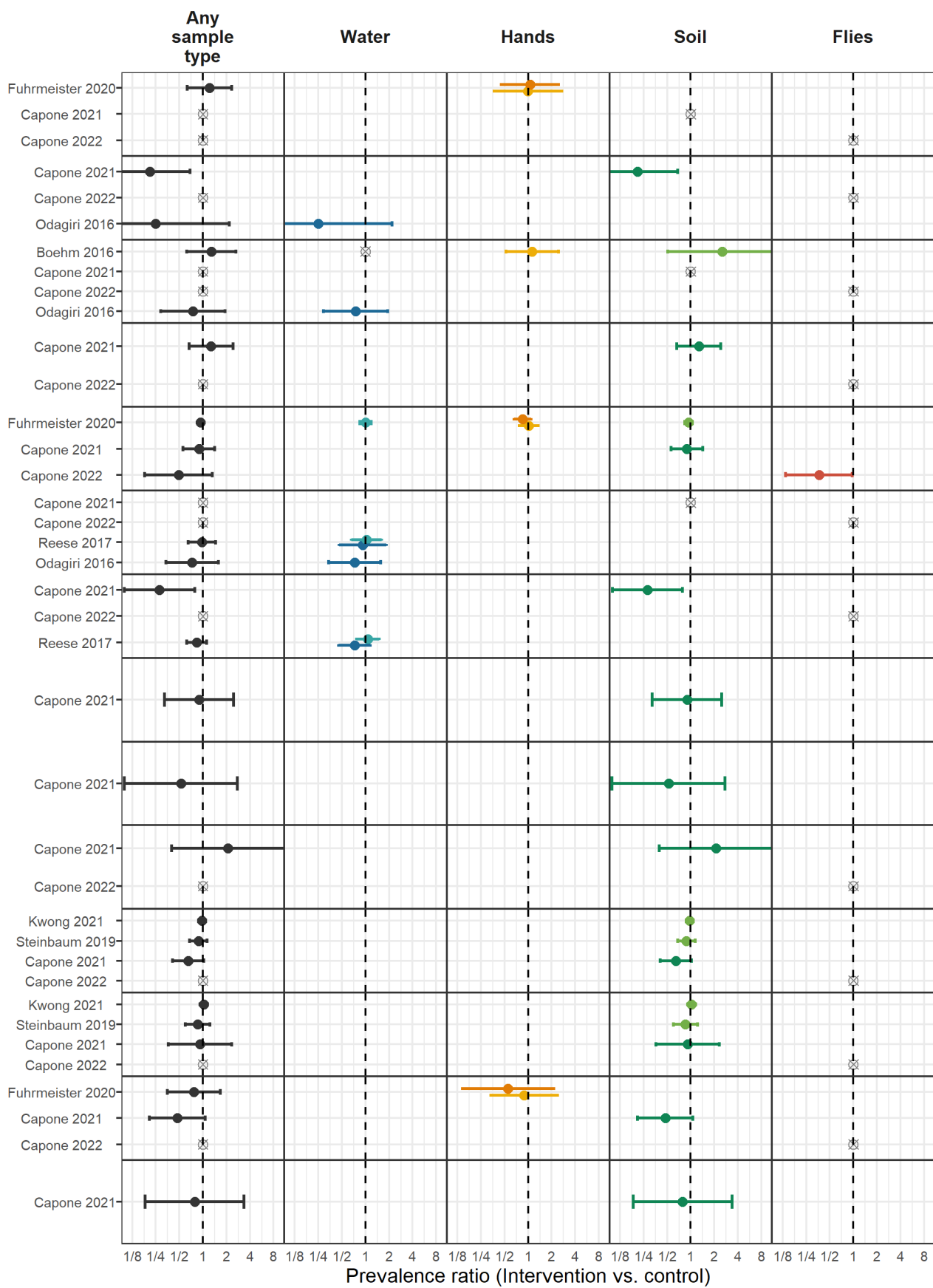
**Table 2. Mean (SD) abundances of enteropathogen and MST targets by study arm. Means are log<sub>10</sub>-transformed gene copies for MST markers and mean egg counts for soil transmitted helminths (*Ascaris* and *Trichuris*). Intervention effects are shown as adjusted differences in log<sub>10</sub>-transformed gene copies and ratios of helminth egg counts between the intervention and control arms.**

Study	Sample	Target	N	% in ROQ	Control mean, median (SD)	Intervention mean, median (SD)	Intervention effect (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 2021	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 2022 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) <sup>a</sup>	0.21	0.33
-	-	Trichuris	2,102	100.0	0.2, 0 (1.8)	0.2, 0 (1)	0.73 (0.36 1.48) <sup>a</sup>	0.38	0.39
Kwong 2021	House soil	Ascaris	1,426	100.0	2.3, 0.7 (6.7)	2.2, 0.6 (6.9)	0.97 (0.68 1.38) <sup>a</sup>	0.85	0.54
-	-	Trichuris	1,426	100.0	1.6, 0.4 (5)	2, 0.4 (5)	1.22 (0.87 1.71) <sup>a</sup>	0.26	0.17

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

<sup>a</sup> Marks ratio estimates from negative binomial models.

## **Supplementary Figures**

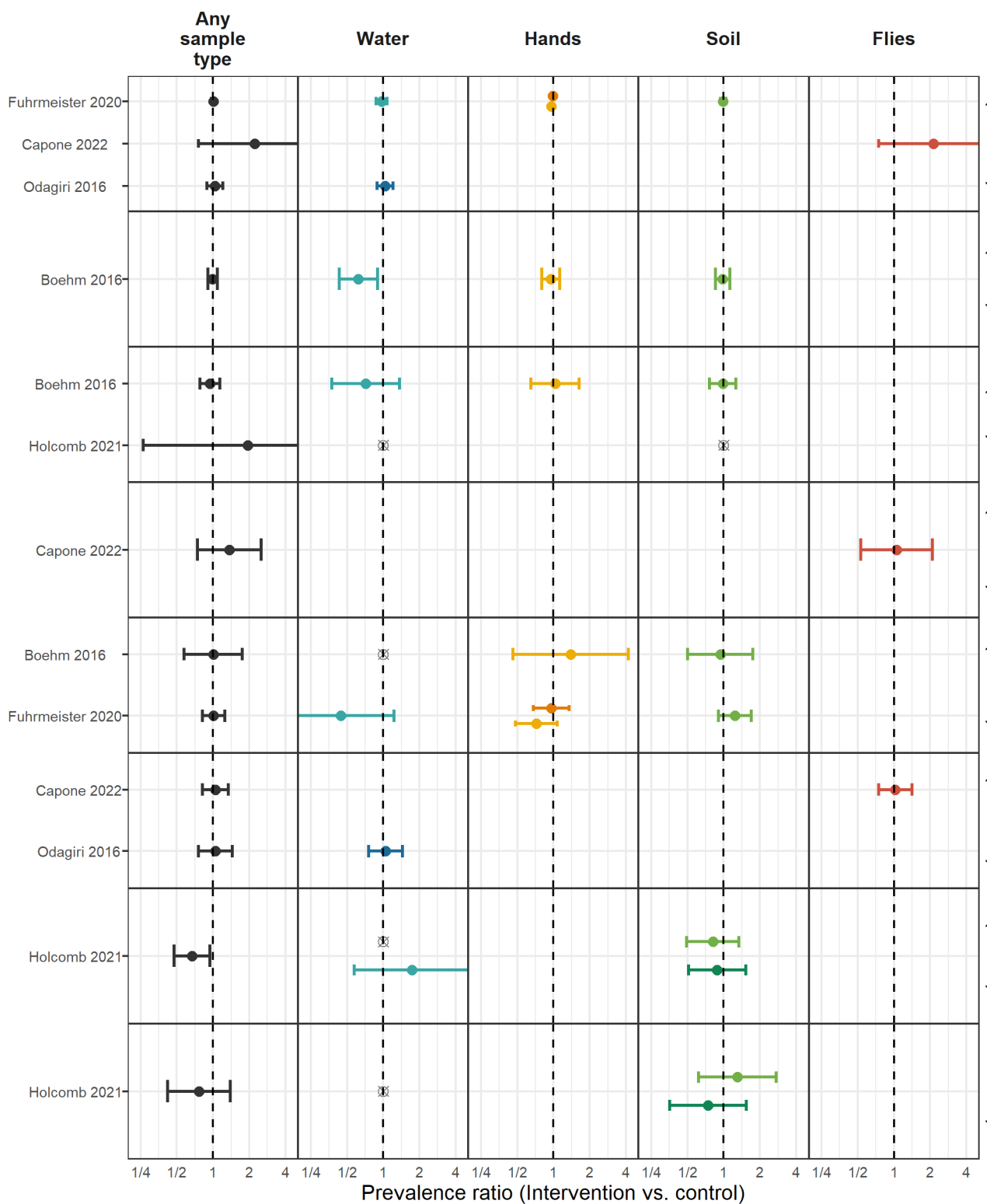


Sample type

- Any sample
  Source water
  Stored water
  Child hand rinse
  Mother hand rinse
- Any sample
  Source water
  Stored water
  Child hand rinse

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

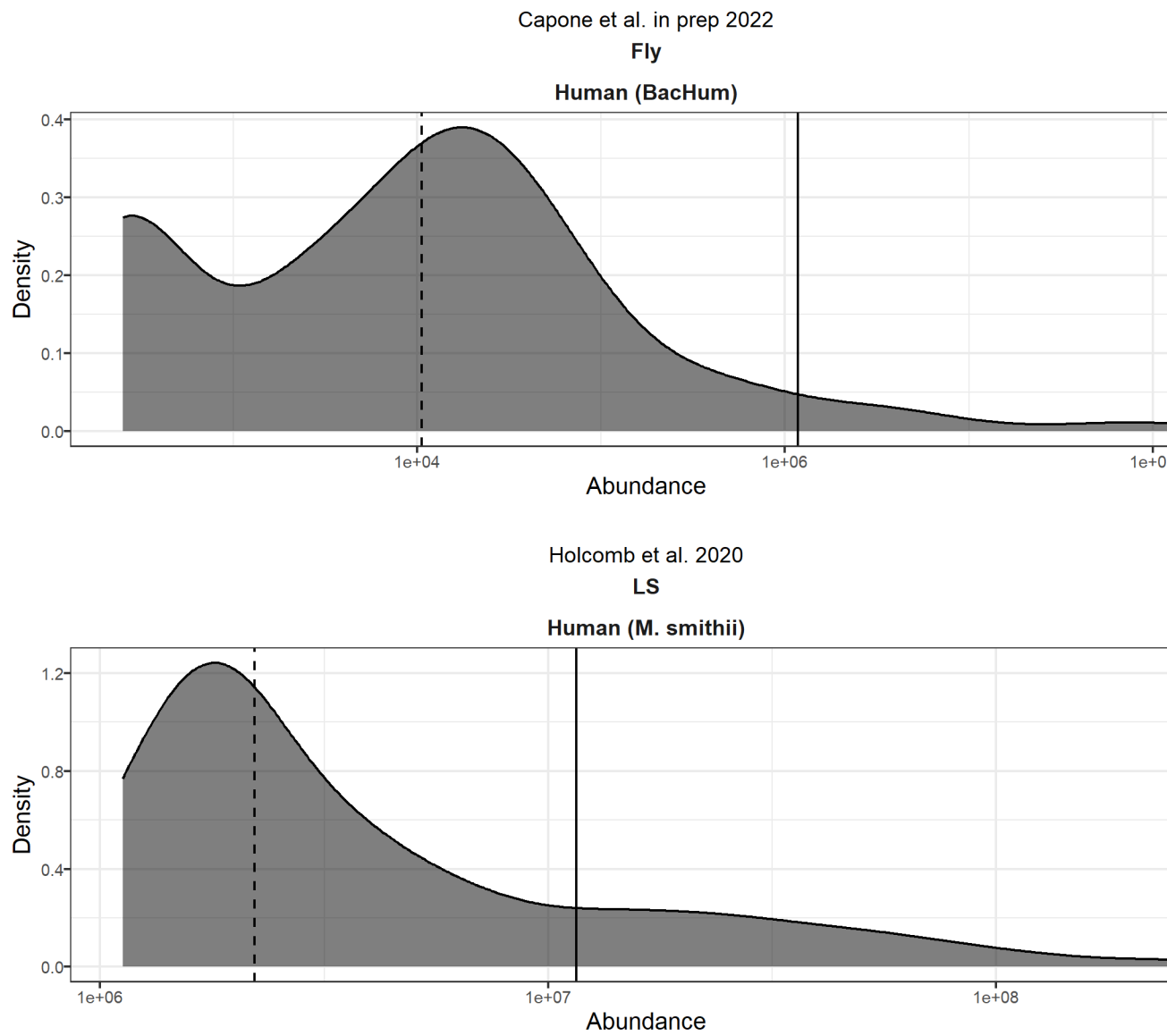


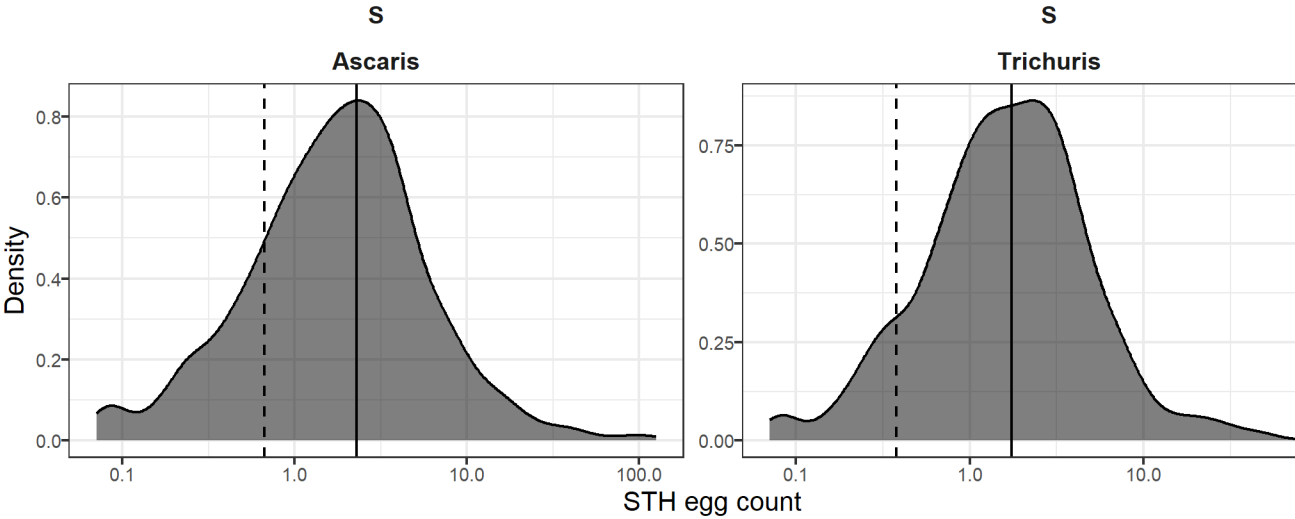
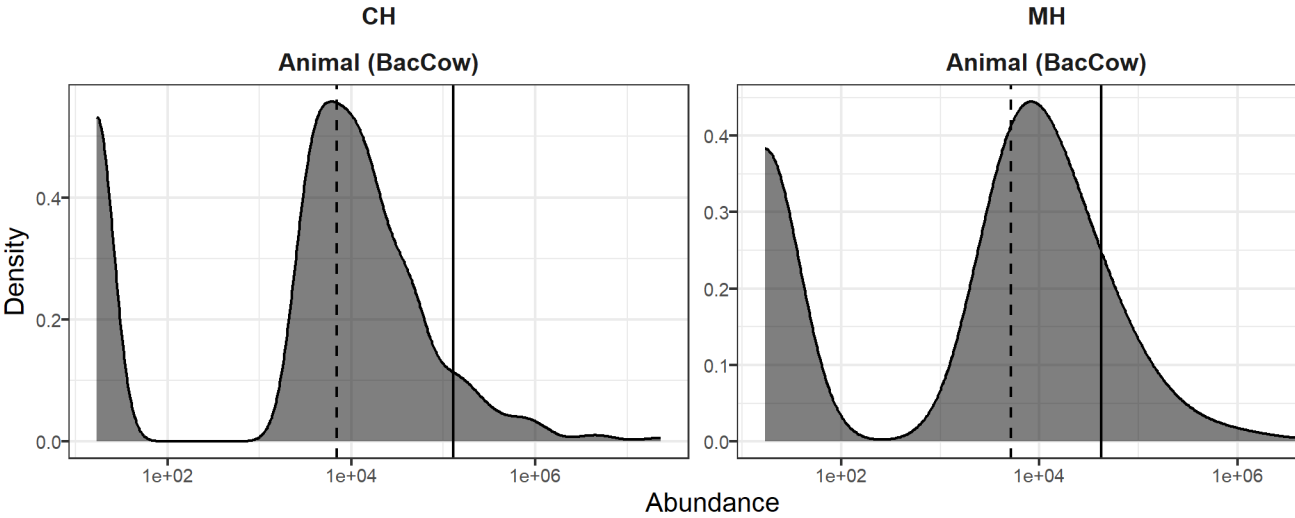


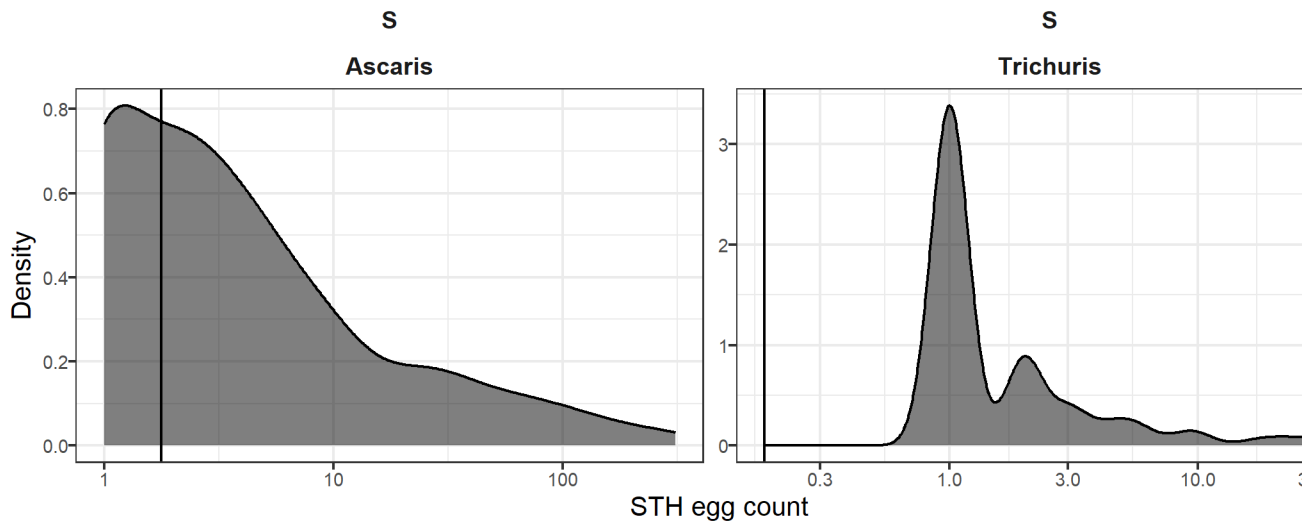
**Sample type**

- Any sample
- Source water
- Stored water
- Child hand rinse
- Mother hand rins
- Any sample
- Source water
- Stored water
- Child hand rinse

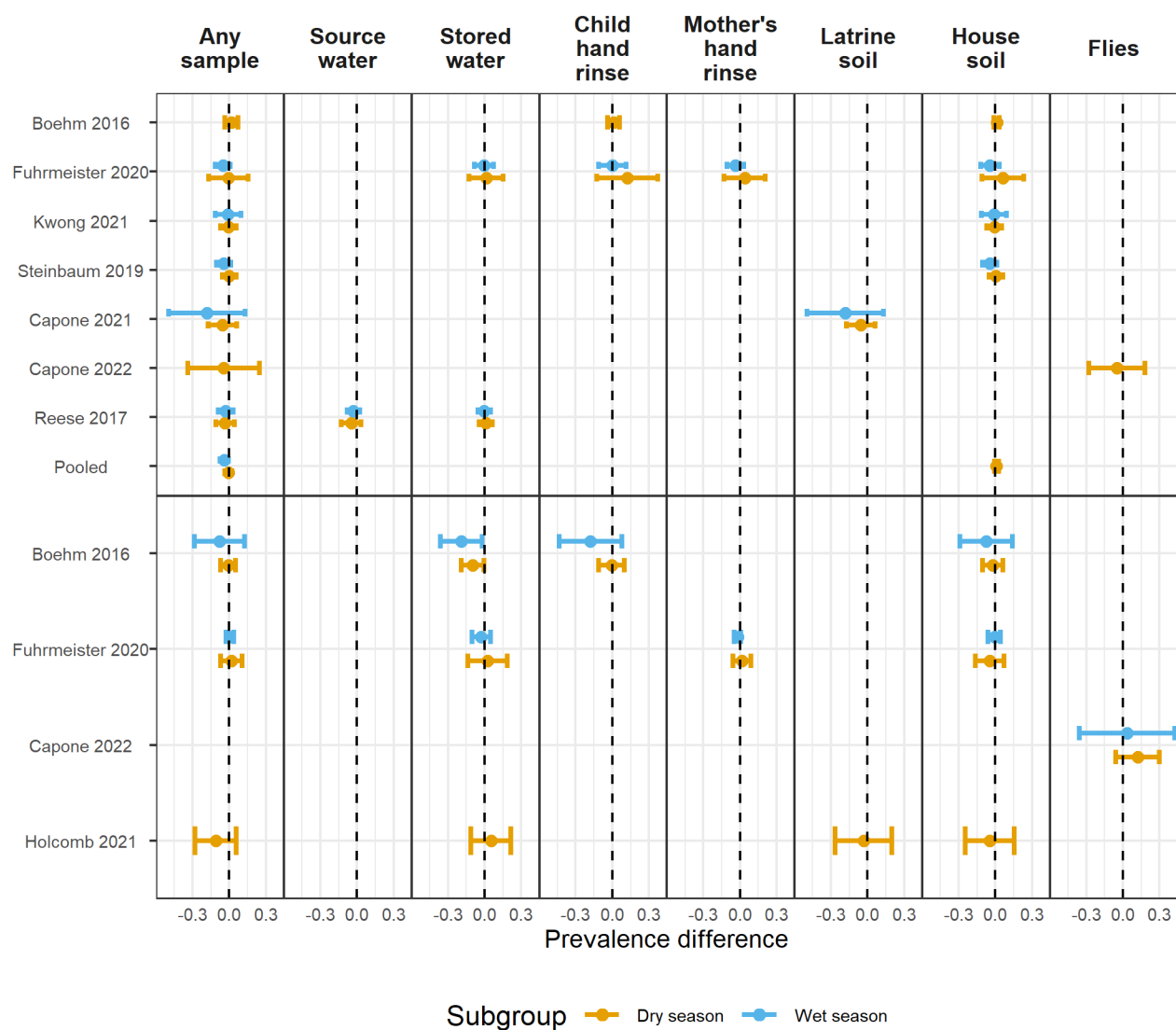
**Figure S2.** Forest plots of intervention effects on the prevalence of specific MST markers.





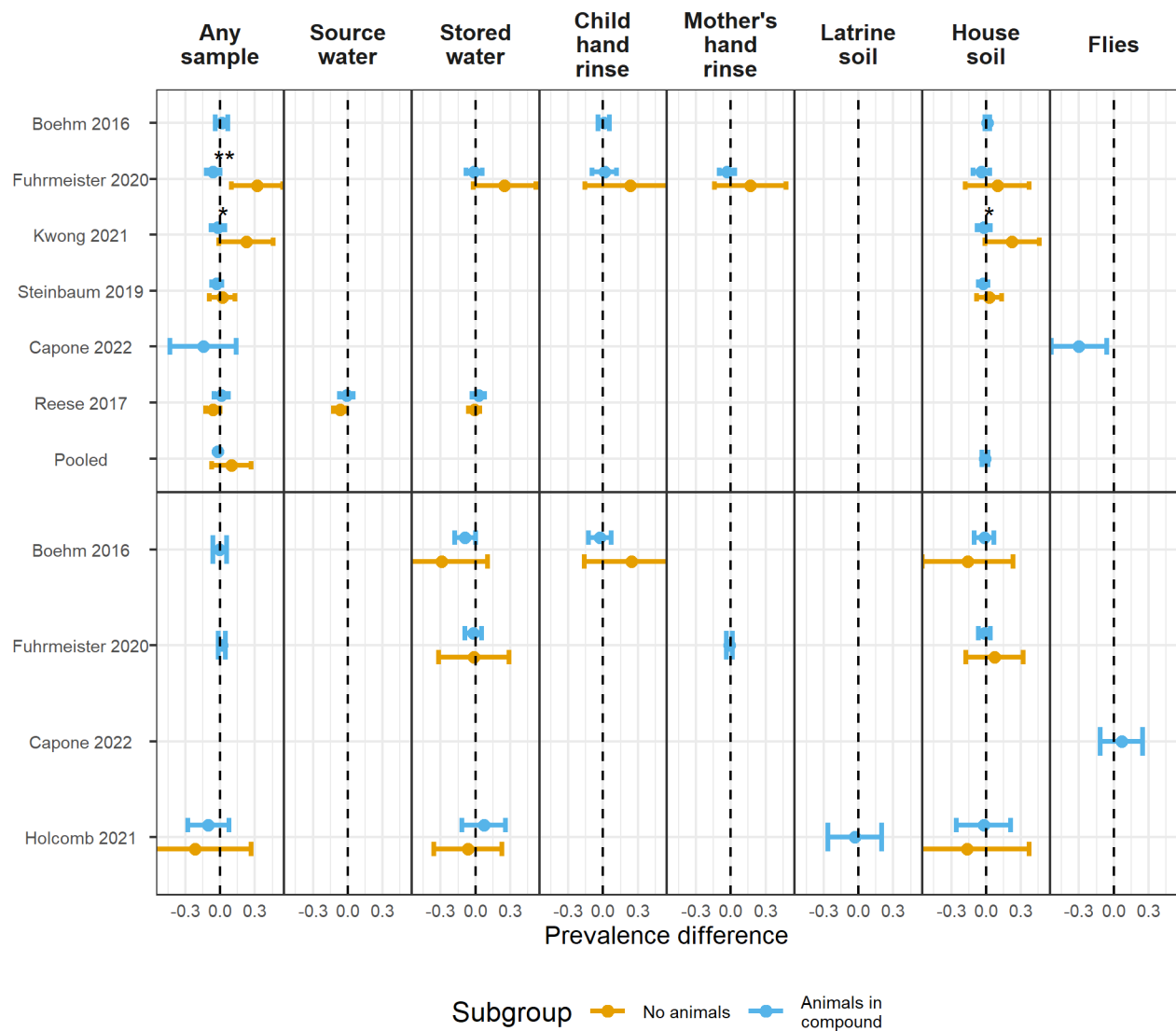


**Figure S3.** Distributions of abundance outcomes. The X-axes are displayed on the log-10 scale. Black vertical lines mark the means, and dashed lines mark the medians. Values below the limit of detection were imputed with half the limit of detection and values below the limit of quantification were imputed with the midpoint between the limits of detections and quantification, leading to some bimodal distributions.

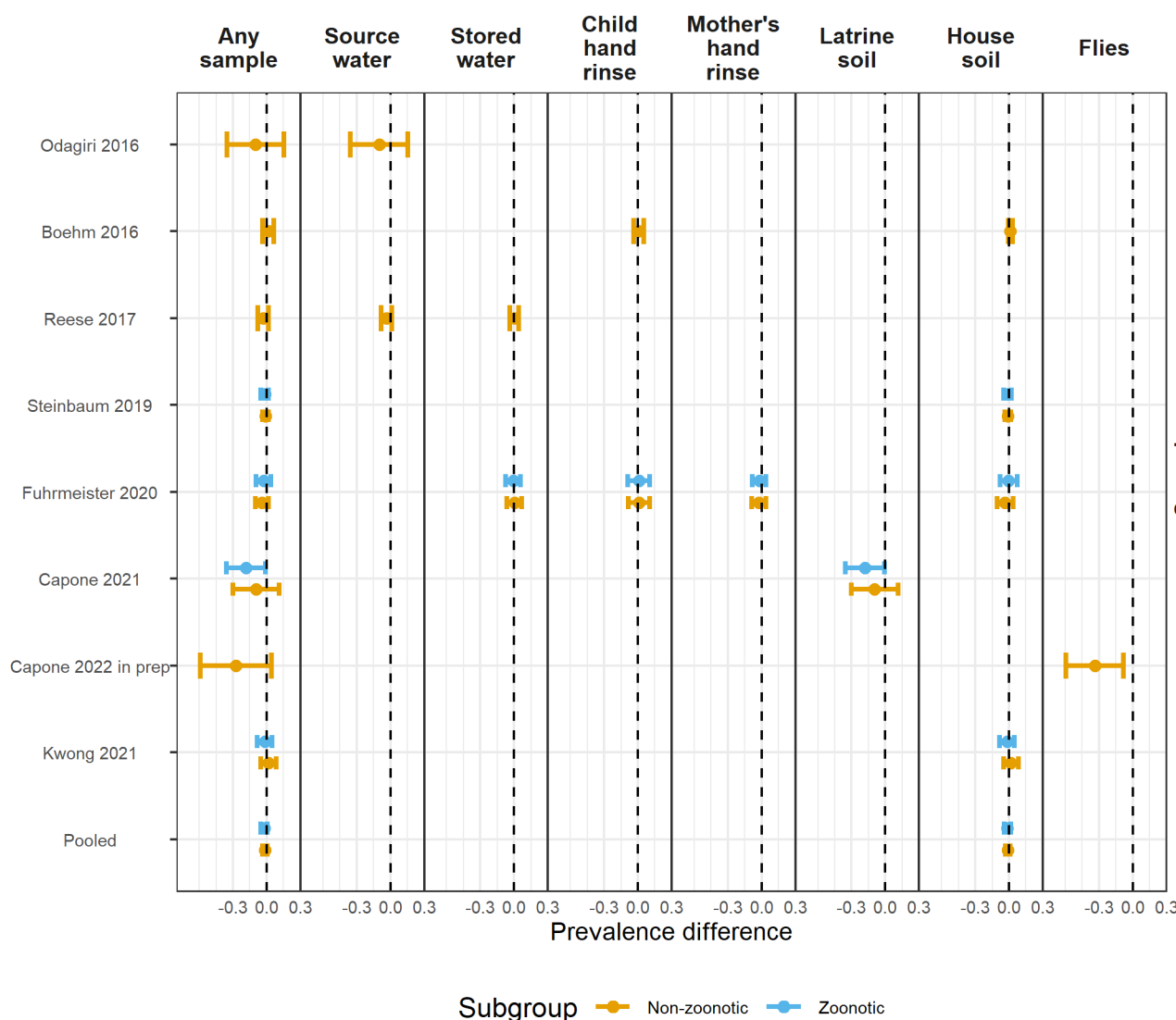


**Figure S4.**

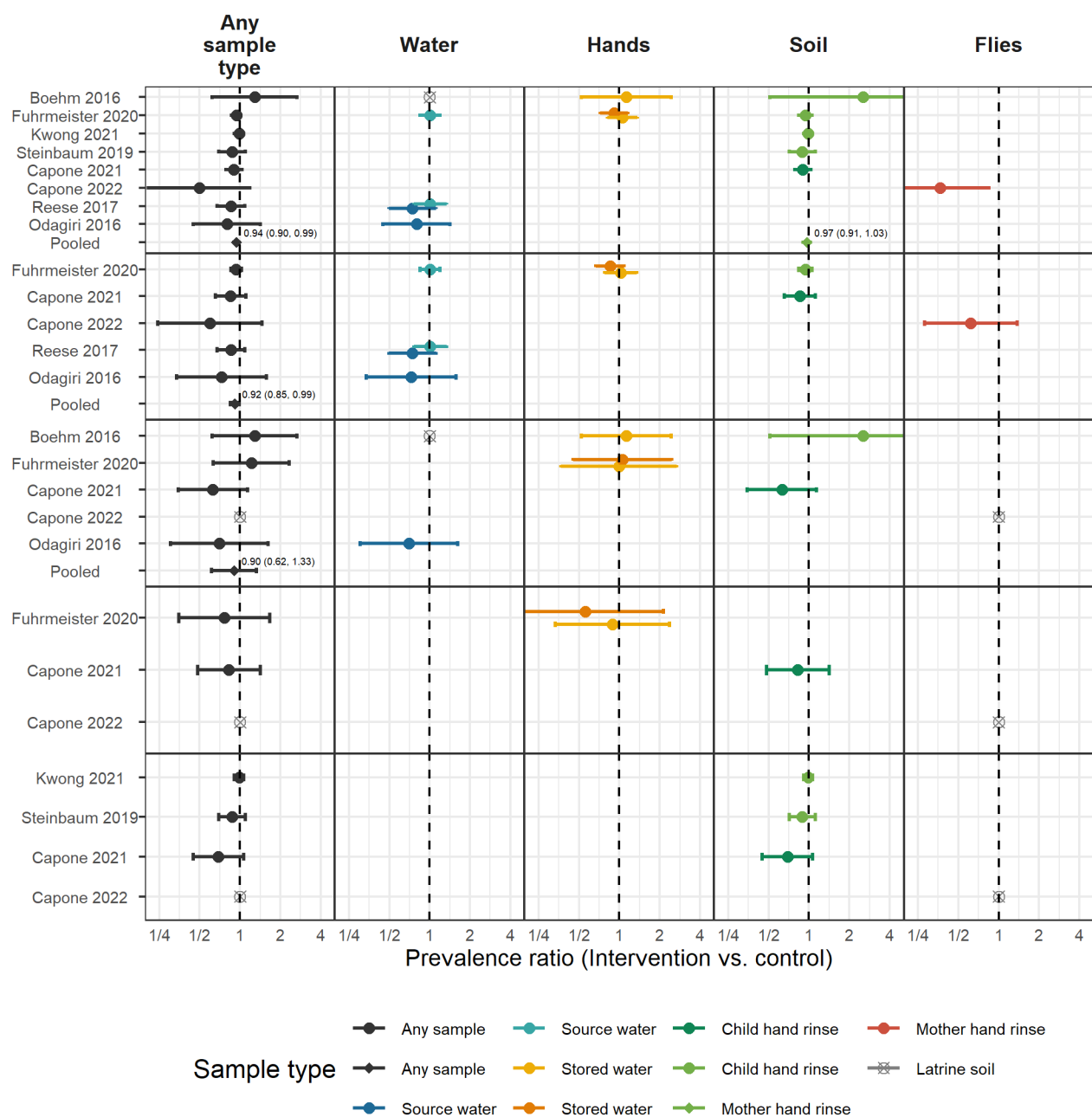
Forest plots of any enteropathogen prevalence differences or any MST prevalence differences between intervention and control arms, stratified by whether the sample was collected during the wet versus dry season (defined by the 6 months of highest average rainfall). Significant effect modification, as determined by the p-values on the regression model interaction term, is marked above points with asterisks ( $P < 0.05 = *$ ,  $P < 0.01 = **$ ,  $P < 0.001 = ***$ ). Grey crossed points denote data that were too sparse to estimate a prevalence ratio (i.e.,  $<10$  positive observations).



**Figure S5.** Forest plots of any enteropathogen prevalence differences or any MST prevalence differences between intervention and control arms, 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 above points with asterisks ( $P < 0.05 = *$ ,  $P < 0.01 = **$ ,  $P < 0.001 = ***$ ). Grey crossed points denote data that were too sparse to estimate a prevalence ratio (i.e.,  $<10$  positive observations).

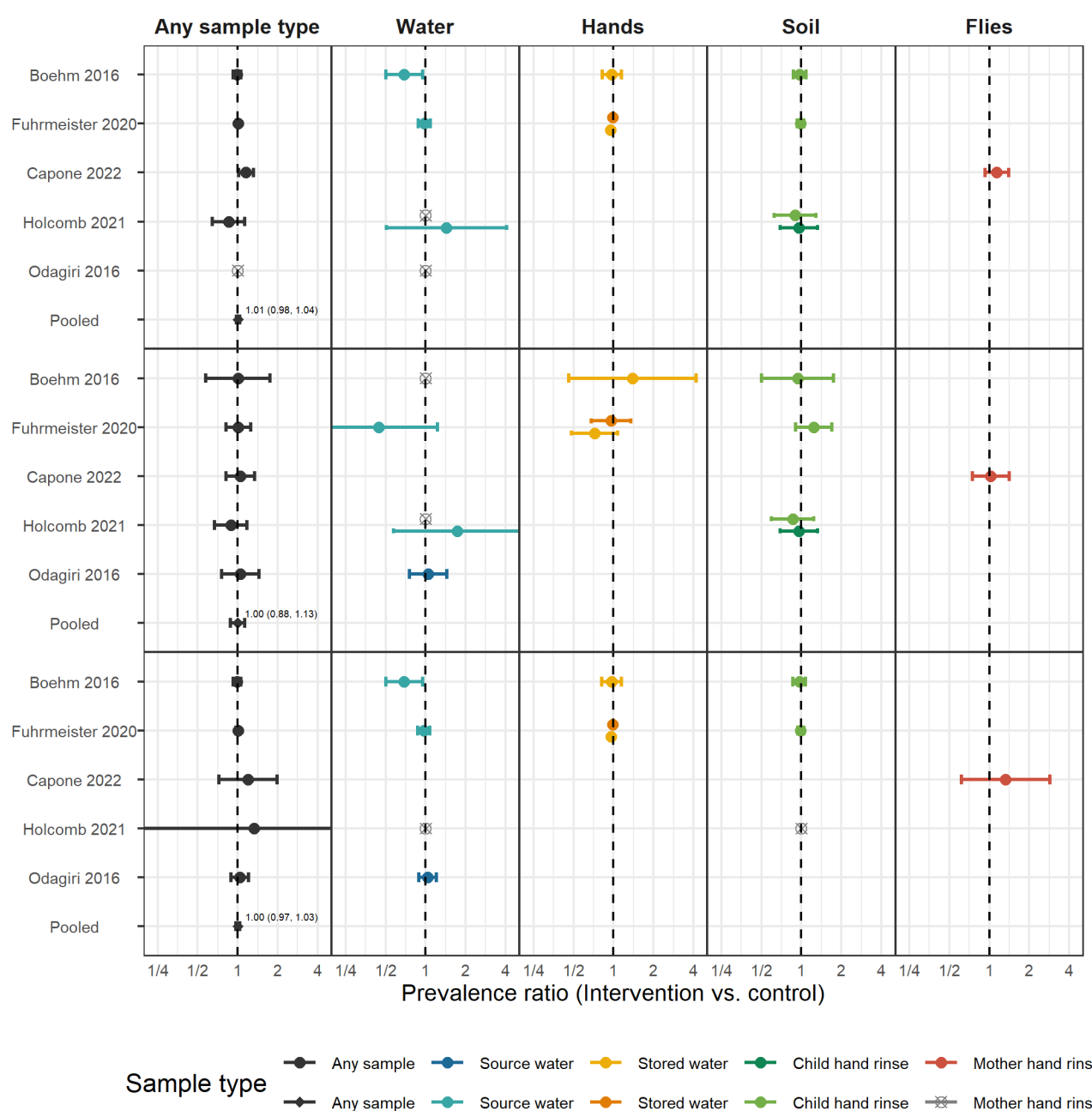


**Figure S6.** Forest plots of any enteropathogen prevalence differences or any MST prevalence differences between intervention and control arms, stratified by whether the pathogen is zoonotically transmitted. Grey crossed points denote data that were too sparse to estimate a prevalence ratio (i.e., <10 positive observations). Significant effect modification, as determined by the p-values on the regression model interaction term, is marked above points with asterisks ( $P < 0.05 = *$ ,  $P < 0.01 = **$ ,  $P < 0.001 = ***$ ). Grey crossed points denote data that were too sparse to estimate a prevalence ratio (i.e., <10 positive observations).



**Figure S7.** 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. Grey crossed points denote data that were too sparse to estimate a prevalence ratio (i.e., <10 positive observations).





**Figure S8.** 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. Grey crossed points denote data that were too sparse to estimate a prevalence ratio (i.e., <10 positive observations).