Supplementary material: High prevalence of SARS-CoV-2 detection and prolonged viral shedding in stools: A Systematic Review and Cohort Study

1. Supplementary methods

RNA extraction

Viral nucleic acid extraction from nasopharyngeal samples was carried out with the High Pure Viral Nucleic Acid kit (Roche Molecular Biochemicals, Germany). For feces samples, TrIzol LS Reagent (Invitrogen, USA) was used to isolate RNA from the sample supernatants. RNA extraction was performed according to the manufacturer's instructions. Stool samples, stored in RNAlater solution, were vigorously vortexed and spanned for 5 minutes at 5000g before the nucleic acid extraction. Additionally, for RNAs extracted in feces, a PCR inhibitor clean-up was carried out with OneStep PCR Inhibitor Removal Kit (ZYMO Research, USA) following manufacturer instructions.

Viral genome detection

For follow-ups SARS-CoV-2 RNA was detected using real-time one step RT-QPCR targeting Orf1b with dual-labeled hydrolysis probe as previously reported by Chu et.al.[1], with some modifications. Briefly, 0.5 μM of forward and reverse primers (HKU-ORF1b-nsp14F and HKU-ORF1b-nsp14R) and 0.25 μM of probe (HKU-ORF1b-nsp141p) were mixed with 12.5 μL AgPath-ID™ One-Step RT-PCR 2X master mix (Thermo Fisher Scientific), 1 μL of 25X RT-PCR enzyme mix, and 5 μL of extracted RNA in a final volume of 25 μL. The reaction was conducted in a StepOnePlus™ thermal cycler (Applied Biosystems) with the following thermal profile: a reverse transcription step for 30 minutes at 50°C, an initial denaturation

for 10 minutes at 95°C followed by 45 cycles of 15 seconds at 95°C and 1 minute at 60°C. Fluorescence acquisition was conducted at annealing/extension step and the PCR cycle at which the fluorescence level meets the threshold (threshold cycle; C_T) was recorded for each sample. The limit of detection (LOD) of the assay was established at C_T 39.02, so all samples with fluorescent signal at or before this cycle were considered positive for the presence of SARS-CoV-2 RNA. Positive and negative controls were included in each run. As an amplification control we used the human RNase P (RP) housekeeping gene. RP gene primers, probe and the amplification reaction conditions were described previously (https://www.fda.gov/media/134922/download). The amplification reaction was prepared using 2 μM of each primer and 0.5 μM of dual-labeled probe, and an annealing-extension step was performed at 55°C for 1 minute. RP positive at or before 35 cycles indicated the presence of enough nucleic acid from human RNase P gene and the sample was considered of acceptable quality.

Search strategy and selection process

We conducted an electronic search from December 1, 2019, to June 3, 2020 in MEDLINE (via PubMed) database. We used the following terms in the MEDLINE search: ("2019 nCoV" OR “2019nCoV” OR "2019 novel coronavirus" OR "COVID 19" OR COVID19 OR "new coronavirus" OR "novel coronavirus" OR "SARS CoV-2" OR (Wuhan AND coronavirus) OR "COVID-19" OR "SARS-CoV" OR "2019-nCoV" OR "SARS-CoV-2" OR “coronavirus disease 2019” OR “coronavirus disease-19”) AND (“Stools" OR "Feces" OR "gastro" OR "gastrointestinal" OR "gastro-intestinal" OR "digestive"). References of studies searched

were also included. We hand searched (up to June 3, 2020) preprint servers (bioRxiv, medRxiv, and Social Science Research Network First Look) and coronavirus resource centers of The Lancet, JAMA, and New England Journal of Medicine. We did not limit our search by language.

REFERENCE

1. Chu DKW, Pan Y, Cheng SMS, et al. Molecular Diagnosis of a Novel Coronavirus (2019-nCoV) Causing an Outbreak of Pneumonia. *Clinical chemistry* 2020; **66**(4): 549-55.

2. Risk of bias assessment

Table S1.- Completed IHE checklist for case-series studies.

First Author	Xiong XL	Zhang J	Wang W	Zhang W1	Zhang W2	Xiao F 1	Xiao F2	Wei XS	Pan Y
Study objective									
1. Was the hypothesis/aim/objective of the study clearly stated?	Y	Y	Y	U	U	N	Y	Y	N
Study design									
2. Was the study conducted prospectively?	N	N	N	N	N	N	N	N	N
3. Were the cases collected in more than one centre?	N	N	Y	N	N	N	N	N	N
4. Were patients recruited consecutively?	N	N	N	N	N	N	N	N	N
Study population									
5. Were the characteristics of the patients included in the study described?	N	Y	N	N	N	N	Y	Y	N
6. Were the eligibility criteria (i.e. inclusion and exclusion criteria) for entry into the study clearly stated?	N	N	N	N	N	N	N	N	N
7. Did patients enter the study at a similar point in the disease?	U	N	N	N	N	N	N	N	N
Intervention and co-intervention									
8. Was the intervention of interest clearly described?	-	-	-	-	-	-	-	-	-
9. Were additional interventions (co-interventions) clearly described?	-	-	-	-	-	-	-	-	-
Outcome measure									
10. Were relevant outcome measures established a priori?	U	U	U	Y	Y	N	N	N	N
11. Were outcome assessors blinded to the intervention that patients received?	N	N	N	N	N	N	N	N	N
12. Were the relevant outcomes measured using appropriate objective/subjective methods?	Y	Y	Y	Y	Y	N	Y	Y	N
13. Were the relevant outcome measures made before and after the intervention?	N	N	N	N	N	N	N	N	N
Statistical analysis									
14. Were the statistical tests used to assess the relevant outcomes appropriate?	Y	N	N	Y	Y	N	Y	Y	N
Results and conclusions									
15. Was follow-up long enough for important events and outcomes to occur?	-	-	-	-	-	-	-	-	-
16. Were losses to follow-up reported?	-	-	-	-	-	-	-	-	-
17. Did the study provided estimates of random variability in the data analysis of relevant outcomes?	-	-	-	-	-	-	-	-	-
18. Were the adverse events reported?	-	-	-	-	-	-	-	-	-
19. Were the conclusions of the study supported by results?	Y	Y	Y	Y	Y	Y	Y	Y	Y
Competing interests and sources of support									
20. Were both competing interests and sources of support for the study reported?	Y	Y	Y	Y	Y	N	Y	Y	Y

Y: Yes; N: No; U: Unclear

Table S2.- Completed Newcastle-Ottawa scale checklist for cohort studies.

Author	Date	Selection	Comparability	Outcome
Chen C	March 2020	💣💣💣		💣💣
Chen Y	April 2020	💣💣💣		💣💣
Chen W	February 2020	💣💣💣		💣💣
Cheung KS	April 2020	💣💣💣		💣
Dreher M	April 2020	💣💣💣		💣
Han C	April 2020	💣💣💣		💣
Huang JT	June 2020	💣💣💣		💣
Kujawski S	March 2020	💣💣💣		💣💣
Li H	February 2020	💣💣💣		💣💣
Xu Y	April 2020	💣💣💣		💣💣
Zeng L 2	March 2020	💣💣💣		💣💣
Wu Y	March 2020	💣💣💣		💣💣
Zautner AE	April 2020	💣💣💣		💣
Zheng S	February 2020	💣💣💣		💣

Table S3.- Detection of SARS-CoV-2 after onset of symptoms in nasopharyngeal swabs and stools by qRT-PCR, and CT value. Rows in green represent the patients with at least one positive detection of SARS-CoV-2 in stools.

Patient number	Gender	Sample type	Number of samples	Positive samples	Day after onset of symptoms (presence and CT value)																																																			
					0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52
1	Female	STOOL	4	1				NEG								36.3									NEG															NEG																	
			NPS	4	2		38.9									27.8								NEG													NEG																						
2	Male	STOOL	4	1					35.6						NEG					NEG																			NEG																			
		NPS	1	1			22.3																																																		
3	Female	STOOL	1	0					NEG																																																
		NPS	4	1			24.2		NEG				NEG																NEG																											
4	Female	STOOL	5	1											38.2									NEG					NEG													NEG						NEG									
		NPS	4	1										29.5									NEG					NEG													NEG																
5	Male	STOOL	4	1						NEG						32.7				NEG							NEG																														
		NPS	4	0					NEG						NEG					NEG							NEG																														
6	Male	STOOL	5	0								NEG				NEG						NEG							NEG							NEG																					
		NPS	5	3							NEG					31.7			24.3						37.6								NEG																							
7	Male	STOOL	4	0														NEG		NEG									NEG						NEG																						
		NPS	4	4									37.1						31.3								35.1							37.9																							
8	Female	STOOL	2	0																									NEG										NEG																		
		NPS	2	2																			34.61														21.3																				
9	Female	STOOL	2	0											NEG												NEG																														
		NPS	4	2										NEG					NEG								23.9								31.9																						
10	Male	STOOL	3	0									NEG									NEG									NEG																									
		NPS	4	4								26.5				30.9							33.6									36.9																								
11	Female	STOOL	3	0										NEG									NEG									NEG																								
		NPS	4	3									28.3				31.6							35.82									NEG																							
12	Female	STOOL	1	0																																		NEG																			
		NPS	2	0																																		NEG																			NEG
13	Female	STOOL	3	0														NEG							NEG											NEG																					
		NPS	3	3									25.8						30.4							33.2																														
14	Male	STOOL	1	0																												NEG																									
		NPS	2	1																		35.9													NEG																					
15	Male	STOOL	1	0																																				NEG																	
		NPS	2	0																							NEG						NEG																							
16	Male	STOOL	1	0																																												NEG									
		NPS	2	0																		NEG													NEG																					
17	Male	STOOL	1	0																NEG																																				
		NPS	4	1											NEG			NEG									NEG								33.4																					
18	Male	STOOL	1	0																												NEG																									
		NPS	4	1												27.6		NEG							NEG							NEG																									
19	Female	STOOL	3	0											NEG										NEG										NEG																					
		NPS	3	1											30.3		NEG							NEG																																
20	Male	STOOL	1	0											NEG																																									
		NPS	3	1									NEG		NEG							34.0																																		
21	Female	STOOL	2	1																			37.8										NEG																							
		NPS	2	1																			NEG									28.7																								
22	Female	STOOL	1	0													NEG																																							
		NPS	5	4							19.3						36.0		32.2							NEG							33.5																							
23	Female	STOOL	1	0								NEG																																													
		NPS	4	3								15.4							33.5								30.8						NEG																							
24	Male	STOOL	4	1						NEG										36.1					NEG							NEG																								
		NPS	3	2																33.1					36.5							NEG																								
25	Male	STOOL	2	0																					NEG														NEG																	
		NPS	3	2														28.0							NEG														34.6																	
26	Male	STOOL	1	0																												NEG																								
		NPS	1	1																												33.7																								
27	Male	STOOL	1	1								36.1																																													
		NPS	5	5							20.6					21.3			22.4						31.8										31.0																					
28	Male	STOOL	1	0															NEG																																					
		NPS	3	1												35.2				NEG																NEG																					
29	Male	STOOL	1	0																NEG																																				
		NPS	3	3																26.9				26.4										35.1																						
30	Male	STOOL	1	0																		NEG																																		
		NPS	1	1																		35.2																																		
31	Female	STOOL	1	0																							NEG																													
		NPS	1	1																							28.7																													
32	Male	STOOL	2	1											37.24																				NEG																				
		NPS	2	1											35.73			NEG																																						

3. Figures

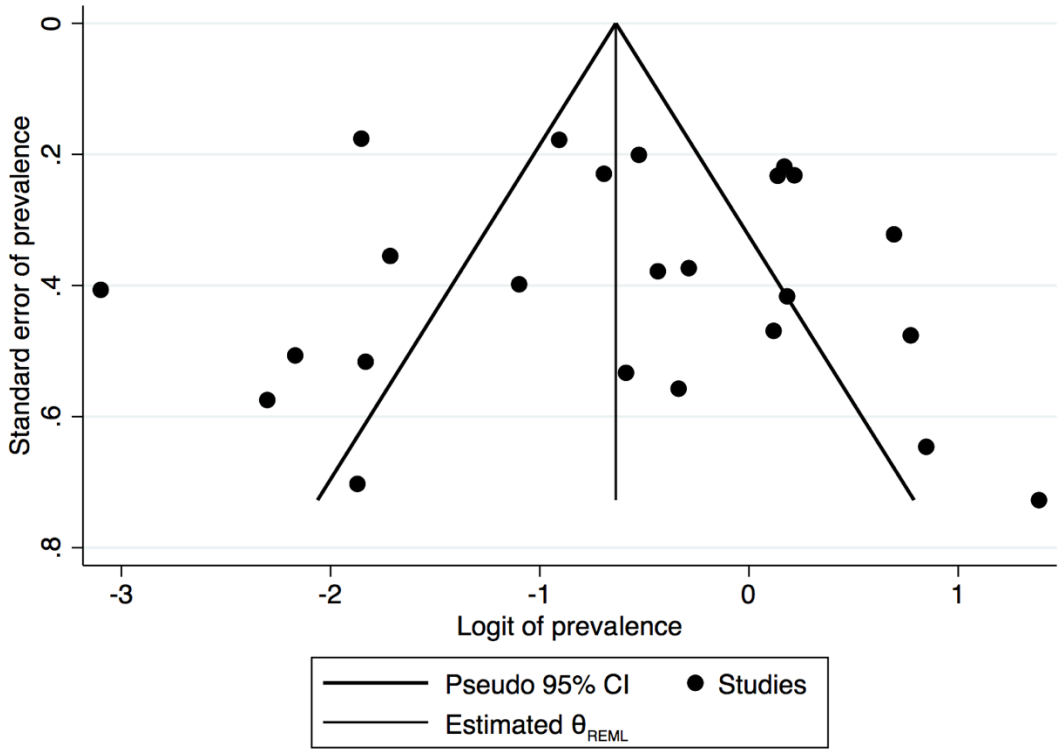

Figure S1.- Funnel plot of the studies included for SARS-CoV-2 detection in stools.

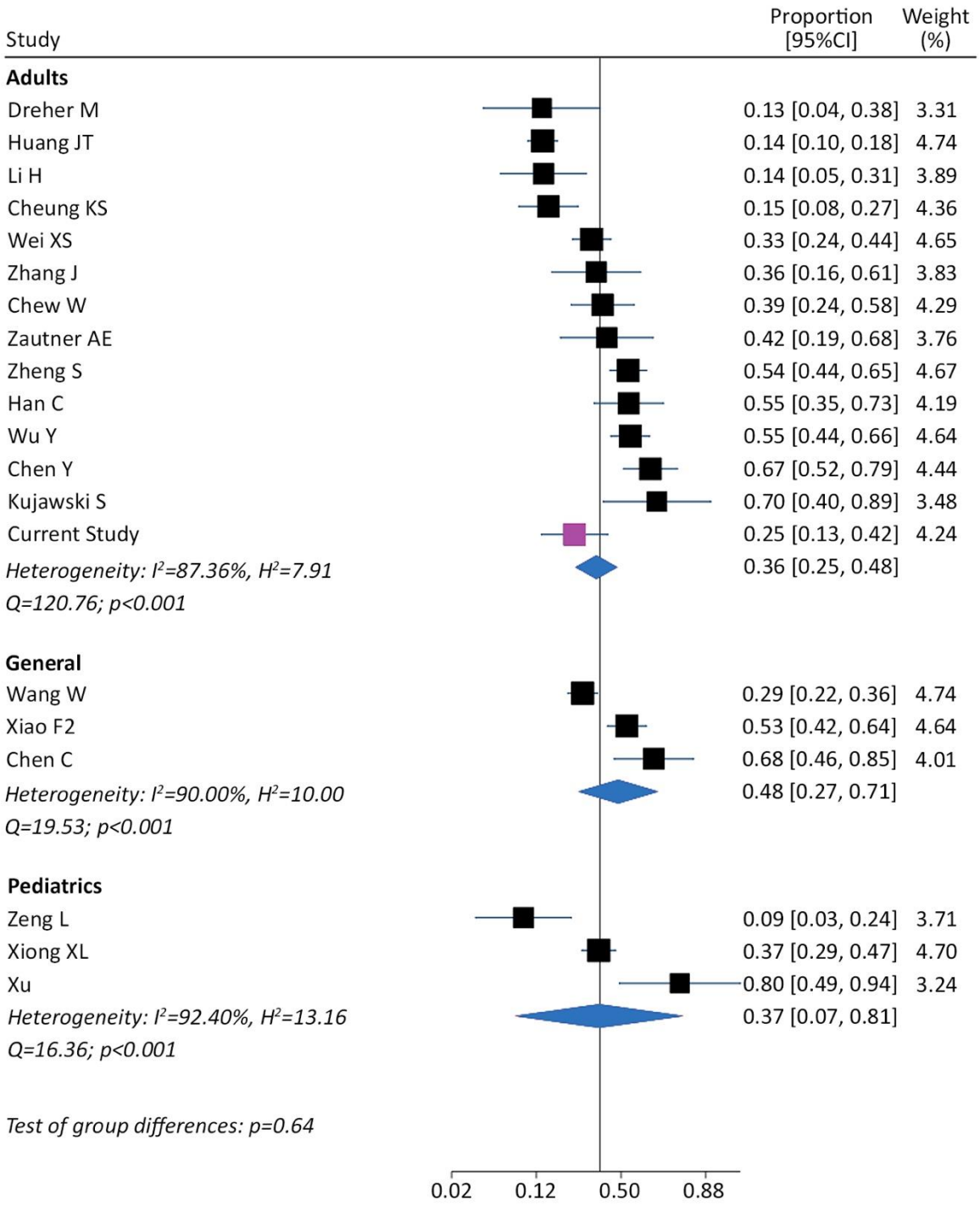

Figure S2.- Forest plots of prevalence of viral detection in stools from SARS-CoV-2 infected patients according to age (subgroup analysis).

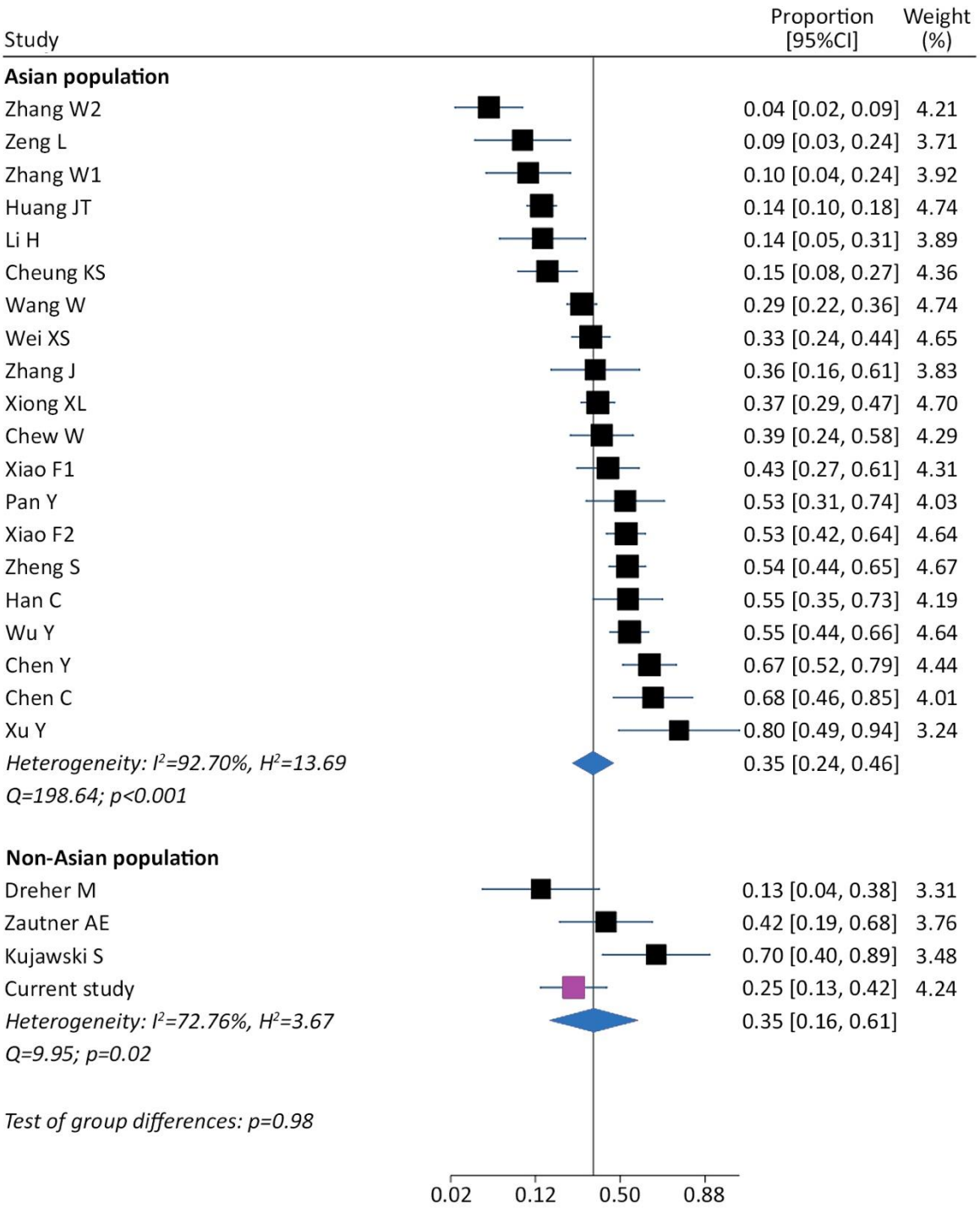

Figure S3.- Forest plots of prevalence of viral detection in stools from SARS-CoV-2 infected patients according to ethnicity (subgroup analysis).

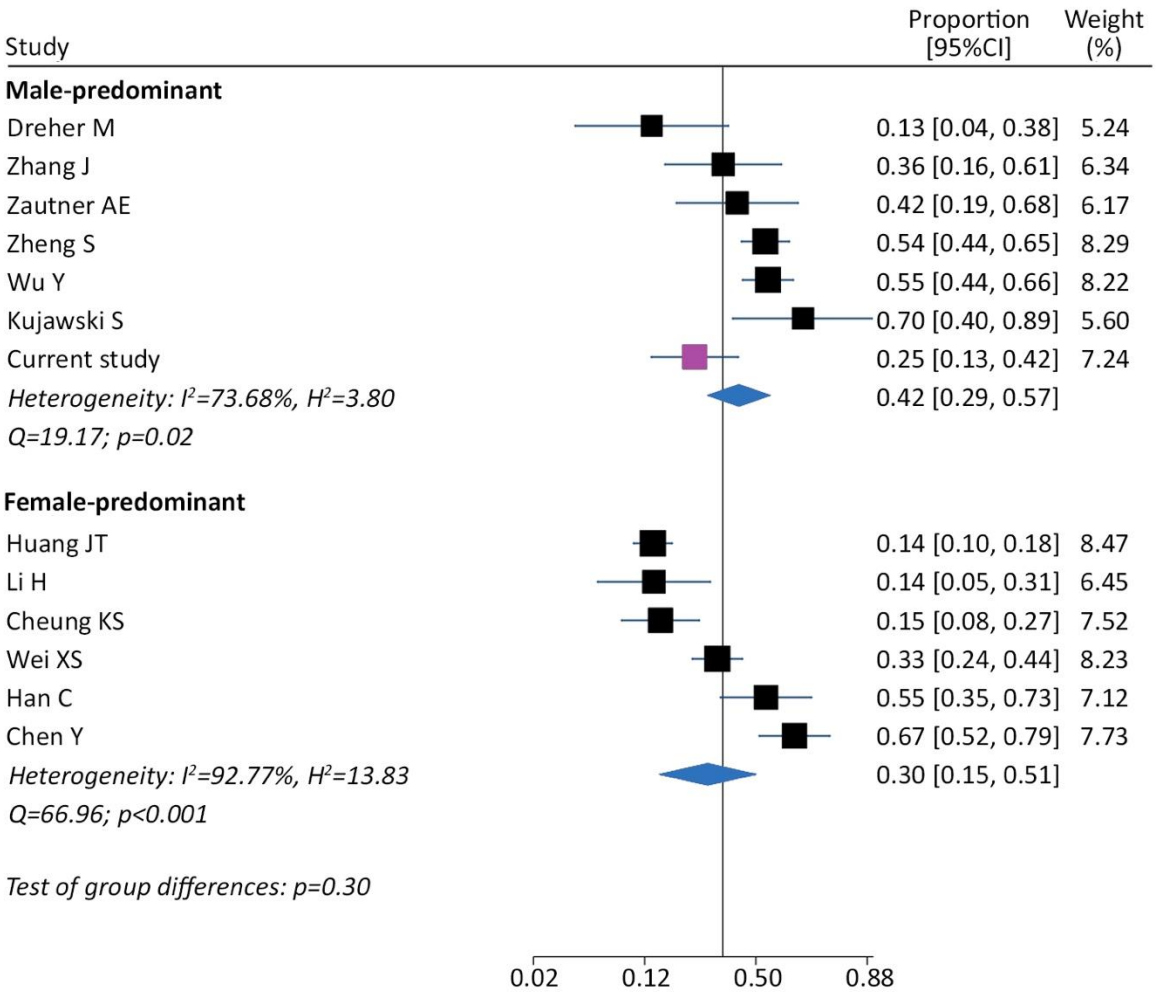

Figure S4.- Forest plots of prevalence of viral detection in stools from SARS-CoV-2 infected patients according to gender (subgroup analysis).

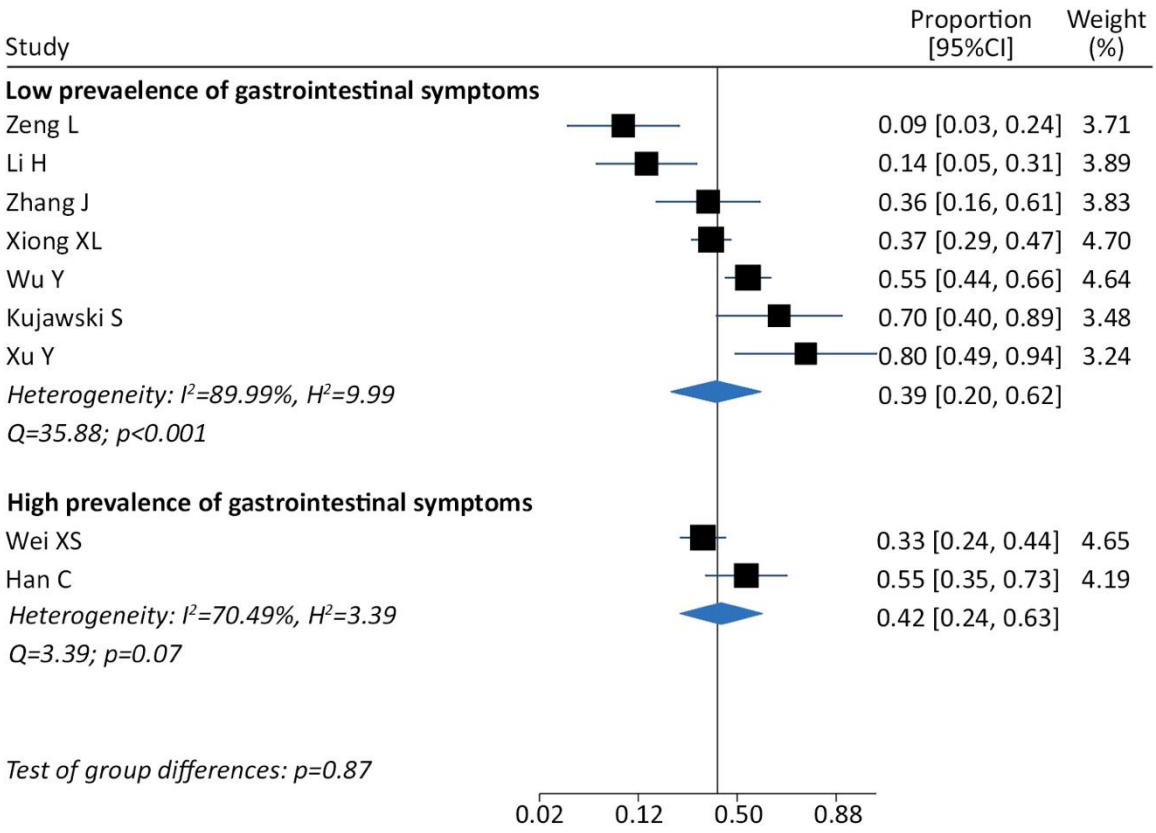

Figure S5.- Forest plots of prevalence of viral detection in stools from SARS-CoV-2 infected patients according to gastrointestinal symptoms (subgroup analysis).

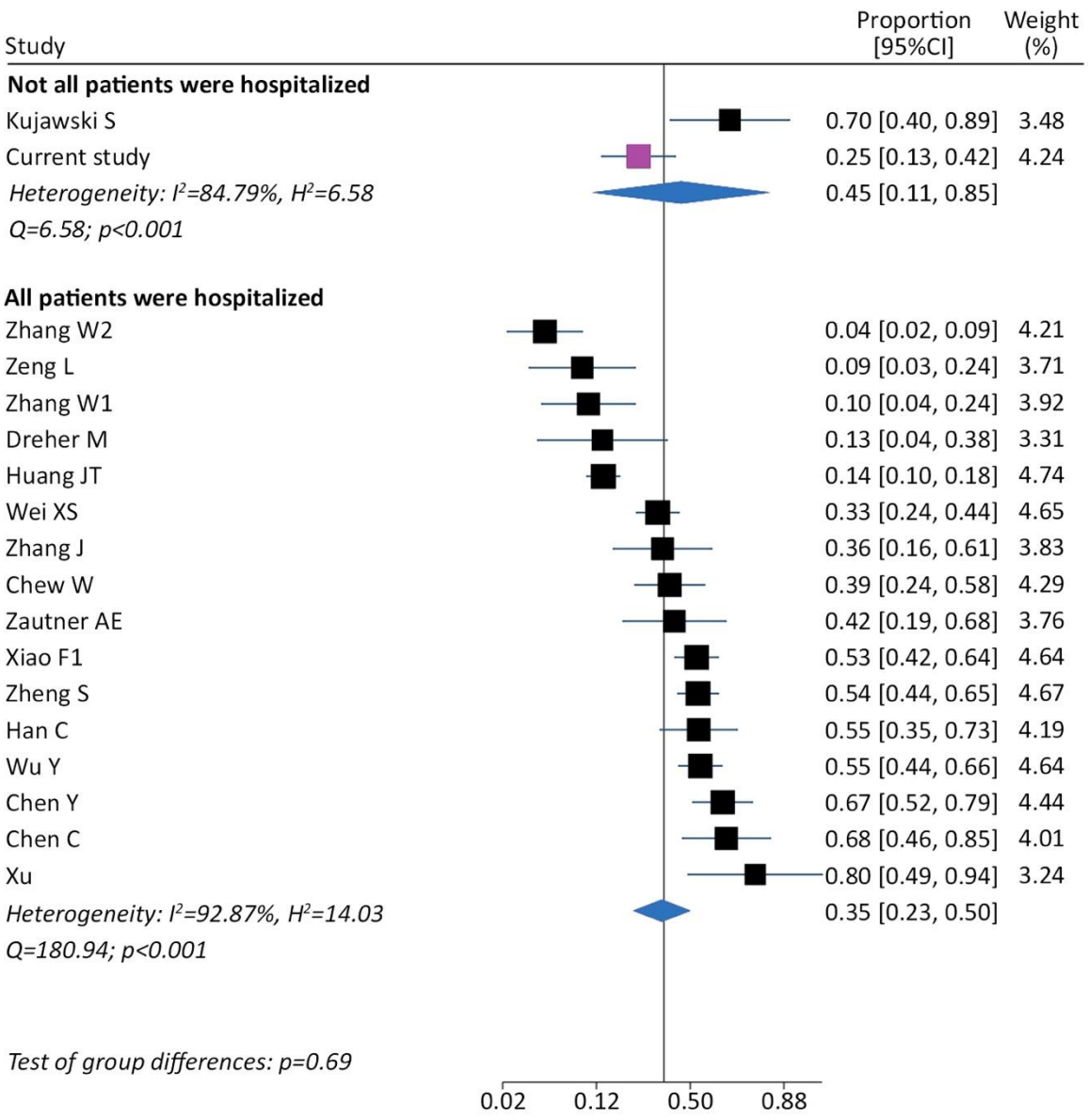

Figure S6.- Forest plots of prevalence of viral detection in stools from SARS-CoV-2 infected patients according to hospitalization (subgroup analysis).