

Etiologic features of diarrheagenic microbes in stool specimens from patients with acute diarrhea in Thailand

Kazuhisa Okada^{1,2*}, Warawan Wongboot^{1,3}, Watcharaporn Kamjumphol¹, Namfon Suebwongsa¹, Piyada Wangroongsarb³, Pipat Kluabwang⁴, Nuttagarn Chuenchom⁵, Witaya Swaddiwudhipong⁶, Thanee Wongchai⁷, Weerawat Manosuthi⁸, Norrathep Assawapatchara⁹, Patchanee Khum-on¹⁰, Patpong Udompat¹¹, Chareeya Thanee¹², Suwatthiya Kitsaran¹³, Lakkana Jirapong¹⁴, Charoen Jaiwong¹⁵, Supalert Nedsuwan¹⁶, Chotipong Siripipattanamongkol¹⁷, Pilailuk Akkapaiboon Okada³, Siriporn Chantaroj³, Sho Komukai¹⁸, Shigeyuki Hamada^{1,2}

¹Thailand-Japan Research Collaboration Center on Emerging and Re-emerging Infections, Nonthaburi, Thailand; ²Research Institute for Microbial Diseases, Osaka University, Osaka, Japan; ³National Institute of Health, Department of Medical Sciences, Nonthaburi, Thailand; ⁴Department of Pediatrics, ⁵Department of Medicine, ⁶Department of Community and Social Medicine, and ⁷Department of Clinical Laboratory, Maesot General Hospital, Tak, Thailand; ⁸Department of Medicine, Bamrasnaradura Infectious Diseases Institute, Nonthaburi, Thailand; ⁹Department of Medicine, Ranong Hospital, Ranong, Thailand; ¹⁰Department of Medical Technology, Chum Phae Hospital, Khon Kaen, Thailand; ¹¹Department of Community and Social Medicine, Prapokklao Hospital, Chanthaburi, Thailand; ¹²Department of Pediatrics and ¹³Department of Medicine, Sunpasitthiprasong Hospital, Ubon Ratchathani, Thailand; ¹⁴Department of Radiology, Samutsakhon Hospital, Samutsakhon, Thailand; ¹⁵Department of Pediatrics, ¹⁶Department of Preventive and Social Medicine, and ¹⁷Department of Medicine, Chiangrai Prachanukroh Hospital, Chiang Rai, Thailand; and ¹⁸Department of Integrated Medicine of Graduate school of Medicine, Osaka University, Osaka, Japan

***Corresponding author:** Kazuhisa Okada

E-mail Address: kazuhisa@biken.osaka-u.ac.jp

Supplemental material

Table S1

Table S2

Table S3

Table S4

Figure S1

Figure S2

Figure S3

Methods

Figure S1. Box and whisker plot showing quantitation cycle (Cq) values of qPCR-positive samples for 24 target genes among 370 diarrheal and 370 non-diarrheal control subjects. The bottom and top of each box represent 25th and 75th percentiles of the data, respectively. The median value is displayed inside the box by the line. The maximum and minimum values are displayed with vertical lines connecting the points to the center box, along with the outliers represented herein indicated by closed and open circles. Numbers indicate the number of positive specimens.

Figure S2. Detection of enteropathogens by month based on qPCR assays and distribution of Cq-values. The blue dots represent Cq values from the non-diarrheal control subjects and the red dots represent Cq values from the diarrheal patients. The red line indicates the cut-off value shown in Table 1.

Figure S3. Detection of enteropathogens by age group using qPCR assays and distribution of Cq-values. The blue dots represent Cq values from the non-diarrheal control subjects and the red dots represent Cq values from the diarrheal patients. The red line indicates the cut-off value shown in Table 1.

Table S1. Clinical symptoms caused by each enteropathogen as determined based on quantitative PCR results and patient data

	All cases				Cases under Cq-cutoff value			
	Yes	No	OR	P-value	Yes	No	OR	P-value
Rotavirus								
Fever	90 (32.6)	11 (12.9)	3.14 (1.61-6.14)	< 0.001	***	66 (23.9)	11 (12.9)	2.05 (1.04-4.04)
Nausea	19 (22.4)	82 (29.7)	0.69 (0.39-1.22)	0.215		18 (21.2)	59 (21.4)	1 (0.56-1.81)
Vomiting	74 (34.3)	27 (18.6)	2.25 (1.37-3.72)	0.001	**	62 (28.7)	15 (10.3)	3.41 (1.86-6.22)
Diarrhea (≥ 10 times/day)	30 (31.6)	69 (27.1)	1.25 (0.75-2.08)	0.425		21 (22.1)	54 (21.2)	1.07 (0.61-1.88)
Abdominal pain	18 (19.8)	83 (30.7)	0.57 (0.32-1)	0.058		14 (15.4)	63 (23.3)	0.61 (0.33-1.14)
Fecal WBC	3 (18.8)	25 (23.8)	0.82 (0.23-2.88)	0.762		1 (6.2)	18 (17.1)	0.46 (0.08-2.63)
Fecal RBC	6 (35.3)	19 (26.8)	1.52 (0.51-4.54)	0.553		4 (23.5)	14 (19.7)	1.32 (0.39-4.44)
Hospital stay			-0.66 (-1.45, 0.13)	0.105				-0.57 (-1.47, 0.32)
Norovirus GII	Yes	No	OR	P-value	Yes	No	OR	P-value
Fever	44 (15.9)	15 (17.6)	0.87 (0.46-1.65)	0.738		20 (7.2)	9 (10.6)	0.64 (0.29-1.45)
Nausea	11 (12.9)	48 (17.4)	0.73 (0.36-1.46)	0.403		3 (3.5)	26 (9.4)	0.4 (0.13-1.26)
Vomiting	47 (21.8)	12 (8.3)	2.99 (1.54-5.81)	0.001	**	24 (11.1)	5 (3.4)	3.25 (1.26-8.41)
Diarrhea (≥ 10 times/day)	11 (11.6)	45 (17.6)	0.63 (0.31-1.26)	0.192		5 (5.3)	22 (8.6)	0.63 (0.24-1.65)
Abdominal pain	7 (7.7)	52 (19.3)	0.37 (0.17-0.83)	0.009	**	2 (2.2)	27 (10.0)	0.25 (0.07-0.92)
Fecal WBC	3 (18.8)	21 (20.0)	1.02 (0.29-3.62)	1		1 (6.2)	10 (9.5)	0.88 (0.15-5.29)
Fecal RBC	1 (5.9)	20 (28.2)	0.23 (0.04-1.31)	0.062		1 (5.9)	10 (14.1)	0.53 (0.09-3.21)
Hospital stay			-0.61 (-1.58, 0.37)	0.223				-0.28 (-1.62, 1.06)
Shigella/EIEC	Yes	No	OR	P-value	Yes	No	OR	P-value
Fever	32 (11.6)	9 (10.6)	1.07 (0.5-2.31)	1		16 (5.8)	5 (5.9)	0.93 (0.34-2.51)
Nausea	10 (11.8)	31 (11.2)	1.08 (0.51-2.28)	0.847		8 (9.4)	13 (4.7)	2.14 (0.87-5.24)
Vomiting	25 (11.6)	16 (11.0)	1.05 (0.54-2.02)	1		13 (6.0)	8 (5.5)	1.07 (0.44-2.6)
Diarrhea (≥ 10 times/day)	8 (8.4)	32 (12.5)	0.67 (0.3-1.48)	0.347		5 (5.3)	15 (5.9)	0.94 (0.35-2.57)
Abdominal pain	14 (15.4)	27 (10.0)	1.66 (0.83-3.29)	0.182		10 (11.0)	11 (4.1)	2.91 (1.21-6.96)
Fecal WBC	7 (43.8)	9 (8.6)	8.02 (2.5-25.75)	0.001	**	7 (43.8)	2 (1.9)	32.68 (6.75-158.38)
Fecal RBC	5 (29.4)	9 (12.7)	2.89 (0.86-9.74)	0.134		5 (29.4)	4 (5.6)	6.6 (1.65-26.35)
Hospital stay			-0.31 (-1.41, 0.79)	0.580				-0.74 (-2.23, 0.74)
Campylobacter	Yes	No	OR	P-value	Yes	No	OR	P-value
Fever	36 (13.0)	4 (4.7)	2.75 (1-7.55)	0.031	*	16 (5.8)	2 (2.4)	2.12 (0.55-8.18)
Nausea	9 (10.6)	31 (11.2)	0.97 (0.45-2.09)	1		4 (4.7)	14 (5.1)	1 (0.34-2.96)
Vomiting	20 (9.3)	20 (13.8)	0.64 (0.33-1.23)	0.231		9 (4.2)	9 (6.2)	0.66 (0.26-1.66)
Diarrhea (≥ 10 times/day)	3 (3.2)	36 (14.1)	0.23 (0.07-0.7)	0.003	**	1 (1.1)	17 (6.7)	0.22 (0.04-1.17)
Abdominal pain	7 (7.7)	33 (12.2)	0.63 (0.27-1.44)	0.334		2 (2.2)	16 (5.9)	0.43 (0.11-1.67)
Fecal WBC	1 (6.2)	14 (13.3)	0.61 (0.1-3.57)	0.690		1 (6.2)	9 (8.6)	0.98 (0.16-5.97)
Fecal RBC	3 (17.6)	6 (8.5)	2.43 (0.59-10.05)	0.367		3 (17.6)	5 (7.0)	2.92 (0.68-12.51)
Hospital stay			0.96 (-0.13, 2.06)	0.086				0.72 (-0.76, 2.21)
EAEC (aggR)	Yes	No	OR	P-value	Yes	No	OR	P-value
Fever	74 (26.8)	27 (31.8)	0.78 (0.46-1.32)	0.408		9 (3.3)	2 (2.4)	1.19 (0.29-4.88)
Nausea	22 (25.9)	79 (28.6)	0.88 (0.51-1.52)	0.680		3 (3.5)	8 (2.9)	1.34 (0.38-4.77)
Vomiting	52 (24.1)	49 (33.8)	0.62 (0.39-0.99)	0.055		5 (2.3)	6 (4.1)	0.56 (0.18-1.77)
Diarrhea (≥ 10 times/day)	23 (24.2)	70 (27.5)	0.85 (0.5-1.46)	0.588		1 (1.1)	10 (3.9)	0.37 (0.07-2.09)
Abdominal pain	27 (29.7)	74 (27.4)	1.12 (0.67-1.89)	0.687		1 (1.1)	10 (3.7)	0.41 (0.07-2.31)
Fecal WBC	5 (31.2)	25 (23.8)	1.51 (0.5-4.58)	0.541		1 (6.2)	4 (3.8)	2.18 (0.32-14.94)
Fecal RBC	6 (35.3)	17 (23.9)	1.76 (0.58-5.3)	0.366		1 (5.9)	4 (5.6)	1.36 (0.2-9.35)
Hospital stay			-0.41 (-1.19, 0.38)	0.311				-1.07 (-3.28, 1.13)
EAEC (astA)	Yes	No	OR	P-value	Yes	No	OR	P-value
Fever	163 (59.1)	54 (63.5)	0.83 (0.51-1.37)	0.527		7 (2.5)	3 (3.5)	0.66 (0.18-2.39)
Nausea	52 (61.2)	165 (59.8)	1.06 (0.64-1.73)	0.899		2 (2.4)	8 (2.9)	0.95 (0.23-3.96)
Vomiting	129 (59.7)	88 (60.7)	0.96 (0.63-1.48)	0.913		7 (3.2)	3 (2.1)	1.46 (0.4-5.28)
Diarrhea (≥ 10 times/day)	56 (58.9)	152 (59.6)	0.97 (0.6-1.56)	1		1 (1.1)	8 (3.1)	0.46 (0.08-2.66)
Abdominal pain	61 (67.0)	156 (57.8)	1.48 (0.9-2.42)	0.138		1 (1.1)	9 (3.3)	0.46 (0.08-2.6)
Fecal WBC	10 (62.5)	59 (56.2)	1.26 (0.44-3.61)	0.788		0 (0.0)	4 (3.8)	0.68 (0.04-13.29)

Fecal RBC	10 (58.8)	38 (53.5)	1.22 (0.43-3.46)	0.790		1 (5.9)	3 (4.2)	1.78 (0.24-12.98)	1
Hospital stay			-0.65 (-1.36, 0.06)	0.072				0.65 (-1.95, 3.26)	0.624
<i>Aeromonas</i>	Yes	No	OR	P-value		Yes	No	OR	P-value
Fever	44 (15.9)	19 (22.4)	0.65 (0.36-1.19)	0.192		4 (1.4)	4 (4.7)	0.3 (0.08-1.13)	0.092
Nausea	25 (29.4)	38 (13.8)	2.61 (1.47-4.64)	0.002	**	5 (5.9)	3 (1.1)	5.34 (1.37-20.86)	0.020 *
Vomiting	38 (17.6)	25 (17.2)	1.02 (0.59-1.77)	1		6 (2.8)	2 (1.4)	1.77 (0.41-7.74)	0.483
Diarrhea (≥ 10 times/day)	27 (28.4)	34 (13.3)	2.58 (1.46-4.56)	0.001	**	6 (6.3)	2 (0.8)	7.36 (1.68-32.3)	0.006 **
Abdominal pain	25 (27.5)	38 (14.1)	2.32 (1.31-4.09)	0.006	**	5 (5.5)	3 (1.1)	4.86 (1.24-18.97)	0.027 *
Fecal WBC	3 (18.8)	27 (25.7)	0.74 (0.21-2.59)	0.758		0 (0.0)	5 (4.8)	0.55 (0.03-10.49)	1
Fecal RBC	4 (23.5)	17 (23.9)	1.04 (0.31-3.43)	1		1 (5.9)	3 (4.2)	1.78 (0.24-12.98)	1
Hospital stay			0.16 (-0.76, 1.09)	0.731				-0.16 (-2.77, 2.44)	0.903
<i>Sapovirus</i>	Yes	No	OR	P-value		Yes	No	OR	P-value
Fever	11 (4.0)	4 (4.7)	0.78 (0.26-2.4)	0.759		7 (2.5)	2 (2.4)	0.93 (0.22-3.97)	1
Nausea	7 (8.2)	8 (2.9)	3.02 (1.1-8.32)	0.055		5 (5.9)	4 (1.4)	4.14 (1.16-14.74)	0.037 *
Vomiting	11 (5.1)	4 (2.8)	1.76 (0.58-5.34)	0.421		6 (2.8)	3 (2.1)	1.26 (0.34-4.69)	0.745
Diarrhea (≥ 10 times/day)	1 (1.1)	13 (5.1)	0.29 (0.05-1.57)	0.124		1 (1.1)	7 (2.7)	0.53 (0.09-3.08)	0.688
Abdominal pain	2 (2.2)	13 (4.8)	0.53 (0.14-2.1)	0.373		2 (2.2)	7 (2.6)	0.98 (0.23-4.19)	1
Fecal WBC	0 (0.0)	3 (2.9)	0.89 (0.04-17.98)	1		0 (0.0)	2 (1.9)	1.25 (0.06-27.31)	1
Fecal RBC	1 (5.9)	1 (1.4)	4.27 (0.42-43.73)	0.351		1 (5.9)	0 (0.0)	13 (0.51-333.64)	0.193
Hospital stay			-0.33 (-2.28, 1.63)	0.744				-0.37 (-2.97, 2.24)	0.783
<i>Astrovirus</i>	Yes	No	OR	P-value		Yes	No	OR	P-value
Fever	9 (3.3)	2 (2.4)	1.19 (0.29-4.88)	1		6 (2.2)	2 (2.4)	0.8 (0.18-3.52)	1
Nausea	2 (2.4)	9 (3.3)	0.84 (0.2-3.47)	1		2 (2.4)	6 (2.2)	1.25 (0.28-5.47)	1
Vomiting	7 (3.2)	4 (2.8)	1.13 (0.34-3.69)	1		5 (2.3)	3 (2.1)	1.06 (0.27-4.11)	1
Diarrhea (≥ 10 times/day)	4 (4.2)	7 (2.7)	1.63 (0.49-5.37)	0.498		3 (3.2)	5 (2.0)	1.72 (0.44-6.72)	0.452
Abdominal pain	2 (2.2)	9 (3.3)	0.77 (0.19-3.16)	0.737		2 (2.2)	6 (2.2)	1.14 (0.26-4.98)	1
Fecal WBC	1 (6.2)	3 (2.9)	2.83 (0.39-20.65)	0.437		1 (6.2)	1 (1.0)	6.74 (0.66-68.96)	0.248
Fecal RBC	0 (0.0)	1 (1.4)	1.34 (0.05-34.4)	1		0 (0.0)	1 (1.4)	1.34 (0.05-34.4)	1
Hospital stay			-0.93 (-3.13, 1.28)	0.412				-1.02 (-3.93, 1.88)	0.49
<i>Salmonella</i>	Yes	No	OR	P-value		Yes	No	OR	P-value
Fever	34 (12.3)	9 (10.6)	1.15 (0.53-2.46)	0.848		7 (2.5)	0 (0.0)	4.76 (0.27-84.19)	0.206
Nausea	8 (9.4)	35 (12.7)	0.75 (0.34-1.65)	0.565		2 (2.4)	5 (1.8)	1.48 (0.32-6.72)	0.670
Vomiting	22 (10.2)	21 (14.5)	0.67 (0.36-1.26)	0.247		4 (1.9)	3 (2.1)	0.86 (0.21-3.54)	1
Diarrhea (≥ 10 times/day)	20 (21.1)	22 (8.6)	2.82 (1.47-5.41)	0.003	**	3 (3.2)	4 (1.6)	2.11 (0.51-8.72)	0.395
Abdominal pain	12 (13.2)	31 (11.5)	1.2 (0.59-2.41)	0.709		5 (5.5)	2 (0.7)	6.83 (1.5-31.05)	0.013 *
Fecal WBC	3 (18.8)	11 (10.5)	2.13 (0.57-8.02)	0.396		0 (0.0)	3 (2.9)	0.89 (0.04-17.98)	1
Fecal RBC	4 (23.5)	8 (11.3)	2.49 (0.69-9)	0.236		0 (0.0)	1 (1.4)	1.34 (0.05-34.4)	1
Hospital stay			-0.59 (-1.66, 0.48)	0.282				-0.01 (-2.92, 2.9)	0.995
<i>Plesiomonas</i>	Yes	No	OR	P-value		Yes	No	OR	P-value
Fever	18 (6.5)	14 (16.5)	0.35 (0.17-0.74)	0.008	**	3 (1.1)	3 (3.5)	0.3 (0.07-1.35)	0.145
Nausea	10 (11.8)	22 (8.0)	1.57 (0.72-3.42)	0.281		1 (1.2)	5 (1.8)	0.88 (0.14-5.42)	1
Vomiting	21 (9.7)	11 (7.6)	1.29 (0.61-2.72)	0.573		2 (0.9)	4 (2.8)	0.37 (0.08-1.75)	0.224
Diarrhea (≥ 10 times/day)	9 (9.5)	22 (8.6)	1.14 (0.51-2.53)	0.833		0 (0.0)	6 (2.4)	0.2 (0.01-3.6)	0.196
Abdominal pain	18 (19.8)	14 (5.2)	4.45 (2.14-9.28)	< 0.001	***	4 (4.4)	2 (0.7)	5.52 (1.16-26.41)	0.037 *
Fecal WBC	1 (6.2)	15 (14.3)	0.57 (0.1-3.29)	0.692		0 (0.0)	4 (3.8)	0.68 (0.04-13.29)	1
Fecal RBC	1 (5.9)	9 (12.7)	0.6 (0.1-3.64)	0.679		0 (0.0)	2 (2.8)	0.79 (0.04-17.31)	1
Hospital stay			-0.19 (-1.43, 1.04)	0.759				-0.98 (-3.58, 1.63)	0.462
ETEC (<i>elt</i>)	Yes	No	OR	P-value		Yes	No	OR	P-value
Fever	27 (9.8)	7 (8.2)	1.15 (0.49-2.69)	0.832		4 (1.4)	2 (2.4)	0.55 (0.12-2.64)	0.629
Nausea	8 (9.4)	26 (9.4)	1.04 (0.46-2.34)	1		3 (3.5)	3 (1.1)	3.32 (0.74-14.89)	0.145
Vomiting	21 (9.7)	13 (9.0)	1.08 (0.53-2.21)	0.856		2 (0.9)	4 (2.8)	0.37 (0.08-1.75)	0.224
Diarrhea (≥ 10 times/day)	10 (10.5)	24 (9.4)	1.16 (0.54-2.49)	0.839		3 (3.2)	3 (1.2)	2.73 (0.61-12.24)	0.350
Abdominal pain	7 (7.7)	27 (10.0)	0.79 (0.34-1.83)	0.678		2 (2.2)	4 (1.5)	1.65 (0.35-7.91)	0.644
Fecal WBC	3 (18.8)	8 (7.6)	2.97 (0.76-11.69)	0.161		0 (0.0)	1 (1.0)	2.11 (0.08-54.04)	1
Fecal RBC	3 (17.6)	5 (7.0)	2.92 (0.68-12.51)	0.180		0 (0.0)	0 (0.0)	4.09 (0.08-213.19)	1
Hospital stay			-0.37 (-1.56, 0.82)	0.540				-1.28 (-4.18, 1.62)	0.389

*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Table S2. Detection of enteropathogens in patients under and over five years of age using qPCR assays

	Number of positive (%) in all cases				Number of positive (%) in cases under Cq-cutoff value					
	< 5 yrs	≥ 5 yrs	OR	P -value	< 5 yrs	≥ 5 yrs	OR	P -value		
Rotavirus	83 (34.4)	21 (16.3)	0.38 (0.22-0.64)	< 0.001	***	64 (26.6)	16 (12.4)	0.4 (0.22-0.72)	0.001	**
Norovirus GII	50 (20.7)	11 (8.5)	0.37 (0.19-0.73)	0.003	**	26 (10.8)	3 (2.3)	0.22 (0.07-0.7)	0.004	**
<i>Shigella /EIEC</i>	21 (8.7)	20 (15.5)	1.92 (1.01-3.67)	0.056		6 (2.5)	15 (11.6)	4.9 (1.91-12.59)	0.001	**
<i>Campylobacter</i>	30 (12.4)	10 (7.8)	0.61 (0.29-1.27)	0.218		16 (6.6)	2 (1.6)	0.27 (0.07-1.03)	0.040	*
EAEC (<i>aggR</i>)	64 (26.6)	39 (30.2)	1.2 (0.75-1.92)	0.467		10 (4.1)	1 (0.8)	0.26 (0.05-1.44)	0.106	
EAEC (<i>astA</i>)	133 (55.2)	88 (68.2)	1.73 (1.11-2.71)	0.019	*	8 (3.3)	2 (1.6)	0.54 (0.13-2.24)	0.504	
<i>Aeromonas</i>	34 (14.1)	32 (24.8)	2 (1.17-3.43)	0.015	*	2 (0.8)	7 (5.4)	5.87 (1.38-24.95)	0.010	*
Sapovirus	13 (5.4)	2 (1.6)	0.33 (0.08-1.3)	0.097		7 (2.9)	2 (1.6)	0.61 (0.14-2.61)	0.505	
Astrovirus	8 (3.3)	5 (3.9)	1.21 (0.41-3.63)	0.774		5 (2.1)	3 (2.3)	1.19 (0.31-4.62)	1	
<i>Salmonella</i>	31 (12.9)	12 (9.3)	0.71 (0.36-1.42)	0.395		3 (1.2)	4 (3.1)	2.44 (0.59-10.04)	0.244	
Plesiomonas	11 (4.6)	22 (17.1)	4.2 (1.99-8.85)	< 0.001	***	1 (0.4)	5 (3.9)	7.08 (1.15-43.64)	0.021	*
ETEC (<i>elt</i>)	29 (12.0)	6 (4.7)	0.38 (0.16-0.91)	0.024	*	4 (1.7)	2 (1.6)	1.03 (0.22-4.93)	1	

*, P < 0.05 ; **, P < 0.01; ***, P < 0.001.

Table S3. Number of pathogens detected in each month or season based on qPCR in 370 diarrheal cases

	Hot				Rainy				Dry and cool				<i>P</i> -value
	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	
Rotavirus	9	3	4	4	2	3	1	0	6	9	14	49	< 0.001 ***
Rotavirus –Cut-off*	7	3	4	3	1	2	1	0	5	5	11	38	< 0.001 ***
Norovirus GII	6	0	1	2	6	2	0	0	4	13	12	15	0.004 **
Norovirus GII –Cut-off	4	0	1	1	3	0	0	0	2	5	5	8	0.093 -
<i>Shigella/EIEC</i>	1	0	3	2	9	3	2	2	1	7	3	8	0.134 -
<i>Shigella/EIEC</i> –Cut-off	0	0	1	1	7	2	1	1	0	3	2	3	0.026 *
<i>Campylobacter</i>	1	3	2	2	5	5	0	5	2	5	4	6	0.207 -
<i>Campylobacter</i> –Cut-off	1	2	1	1	1	1	0	4	0	3	1	3	0.421 -
EAEC (<i>aggR</i>)	11	4	3	6	12	7	8	8	7	10	8	19	0.031 *
EAEC (<i>aggR</i>) –Cut-off	1	1	1	1	2	0	0	1	0	1	1	2	0.355 -
EAEC (<i>astA</i>)	18	9	7	6	20	14	11	16	18	27	25	50	0.270 -
EAEC (<i>astA</i>) –Cut-off	0	0	1	1	0	0	0	0	1	3	3	1	0.112 -
<i>Aeromonas</i>	7	4	4	4	4	5	1	9	6	4	8	10	0.100 -
<i>Aeromonas</i> –Cut-off	1	1	1	0	1	0	0	2	2	0	1	0	0.391 -
Sapovirus	0	0	0	1	5	2	0	0	2	2	3	0	0.138 -
Sapovirus –Cut-off	0	0	0	1	2	1	0	0	2	1	2	0	0.822 -
Astrovirus	0	1	0	0	0	1	0	0	0	5	4	2	0.100 -
Astrovirus –Cut-off	0	0	0	0	0	1	0	0	0	4	1	2	0.193 -
<i>Salmonella</i>	5	1	0	0	5	2	1	9	2	2	3	13	0.092 -
<i>Salmonella</i> –Cut-off	0	0	0	0	1	0	0	1	1	0	0	4	0.515 -
<i>Plesiomonas</i>	4	2	1	3	5	0	2	5	3	3	3	2	0.045 *
<i>Plesiomonas</i> –Cut-off	0	0	0	0	3	0	0	0	2	1	0	0	0.299 -
ETEC (<i>elt</i>)	2	1	0	0	5	4	2	0	3	7	4	7	0.154 -
ETEC (<i>elt</i>) –Cut-off	0	1	0	0	3	0	0	0	0	0	0	2	0.414 -

*, Pathogen positives under cut-off values (see Table 1).

Table S4. Basic information of the 370 diarrheal and 370 non-diarrheal control subjects

Characteristics	Diarrhea (%)	Control (%)	OR	P-value
No. of subject	370 (100)	370 (100)		
Age group in years				
< 5	241 (65.1)	232 (62.7)	ref	-
5-15	38 (10.3)	46 (12.4)	0.80 (0.5-1.27)	0.337
16-59	53 (14.3)	56 (15.1)	0.91 (0.6-1.38)	0.662
≥ 60	38 (10.3)	36 (9.7)	1.02 (0.62-1.66)	0.949
Sex				
Male	224 (60.5)	189 (51.1)	1.44 (1.08-1.94)	0.014
Female	146 (39.5)	181 (48.9)	ref	-
Three periods (season)				
Mar-Jun (hot)	77 (20.8)	108 (29.2)	-	-
Mar	29 (37.7)	60 (55.6)	0.71 (0.4-1.29)	0.264
Apr	18 (23.4)	20 (18.5)	1.33 (0.63-2.81)	0.456
May	12 (15.6)	12 (11.1)	1.48 (0.6-3.61)	0.393
Jun	18 (23.4)	16 (14.8)	1.66 (0.76-3.63)	0.203
Jul-Oct (rainy)	96 (25.9)	97 (26.2)	-	-
Jul	32 (33.3)	21 (21.6)	2.25 (1.15-4.43)	0.019
Aug	22 (22.9)	19 (19.6)	1.71 (0.82-3.55)	0.15
Sep	15 (15.6)	32 (33.0)	0.69 (0.33-1.44)	0.323
Oct	27 (28.1)	25 (25.8)	1.60 (0.82-3.12)	0.173
Nov-Feb (cool and dry)	197 (53.2)	165 (44.6)	-	-
Nov	27 (13.7)	26 (15.8)	1.53 (0.79-2.99)	0.208
Dec	38 (19.3)	24 (14.5)	2.34 (1.23-4.45)	0.01
Jan	44 (22.3)	65 (39.4)	ref	-
Feb	88 (44.7)	50 (30.3)	2.60 (1.54-4.38)	0
Disease profile				
Frequency of defecation				
≤ 5 times	176 (50.3)	-	-	-
6-9 times	79 (22.6)	-	-	-
≥10 times	115 (31.1)	-	-	-
Stool condition				
Semi solid	9 (2.4)	-	-	-
Watery	189 (51.1)	-	-	-
Liquid mucus	131 (35.4)	-	-	-
Mucus	24 (6.5)	-	-	-
Bloody mucus	17 (4.6)	-	-	-
Fecal WBC (abnormal)	16 (13.2)	-	-	-
Fecal RBC (presence)	17 (19.3)	-	-	-
Systemic syndrome				
Abdominal pain (Yes)	91 (25.1)	-	-	-
Vomiting (Yes)	216 (59.8)	-	-	-
Nausea (Yes)	85 (23.5)	-	-	-
Fever (Yes)	276 (76.5)	-	-	-

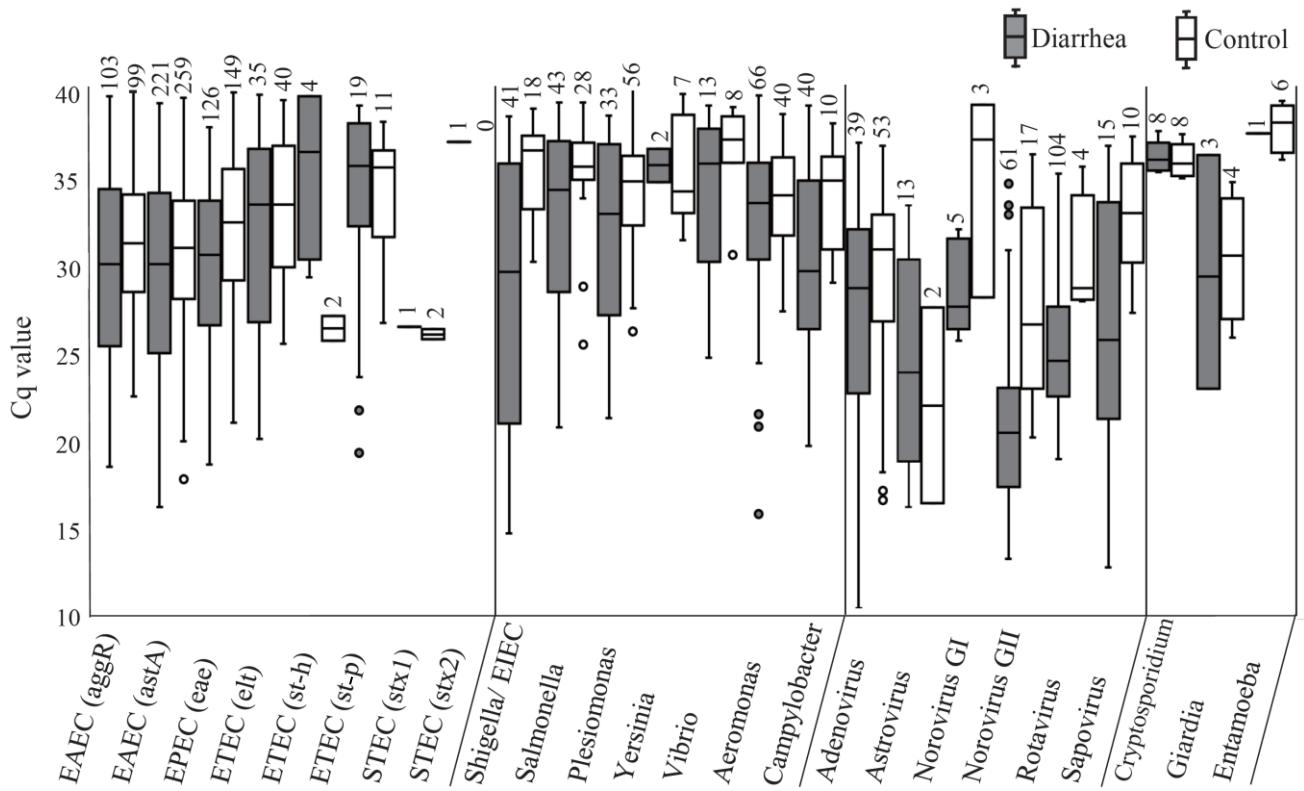
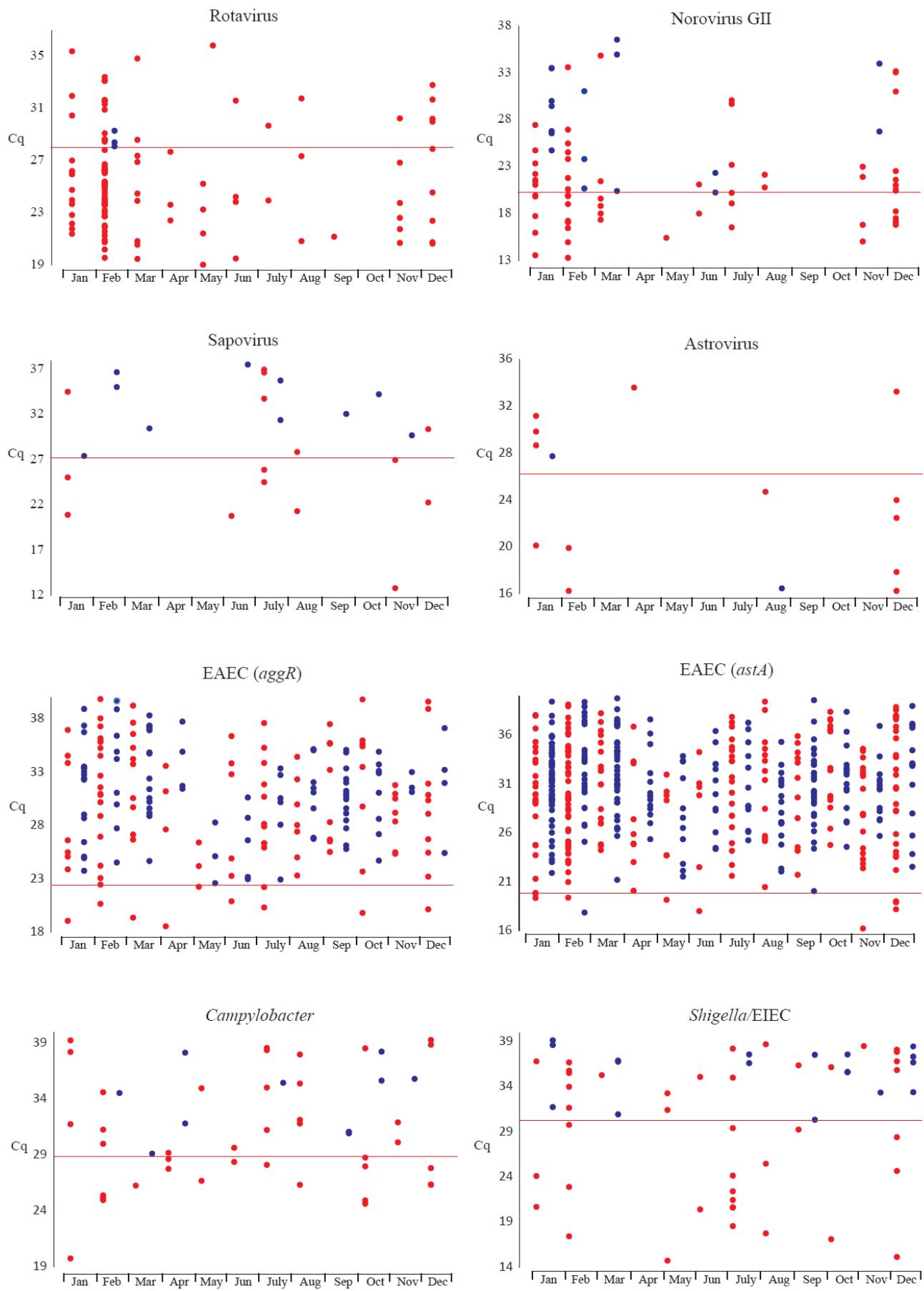


Figure S1. Box and whisker plot showing quantitation cycle (Cq) values of qPCR-positive samples for 24 target genes among 370 diarrheal and 370 non-diarrheal control subjects. The bottom and top of each box represent 25th and 75th percentiles of the data, respectively. The median value is displayed inside the box by the line. The maximum and minimum values are displayed with vertical lines connecting the points to the center box, along with the outliers represented herein indicated by closed and open circles. Numbers indicate the number of positive specimens.



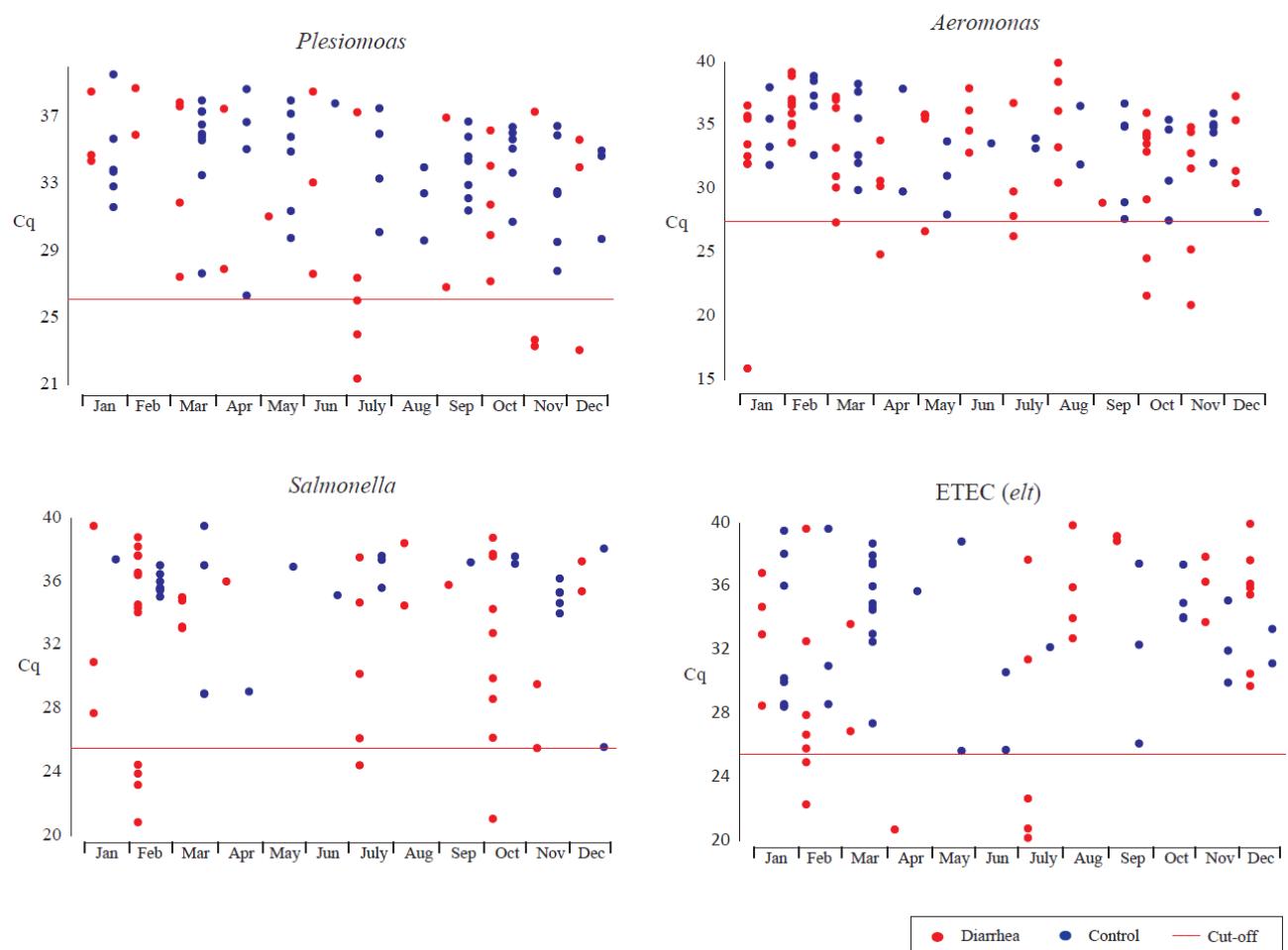
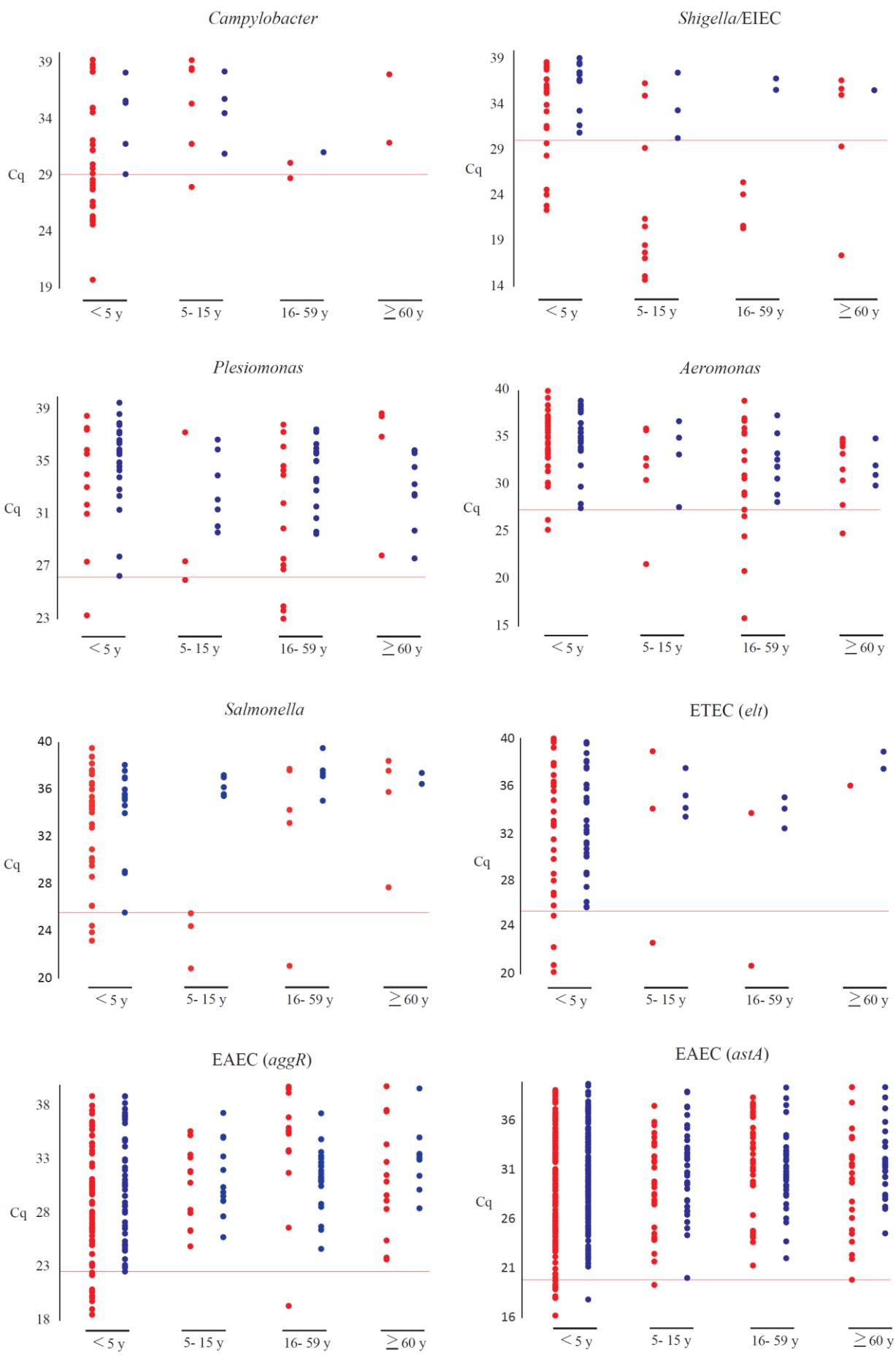


Figure S2. Detection of enteropathogens by month based on qPCR assays and distribution of Cq-values. The blue dots represent Cq values from the non-diarrheal control subjects and the red dots represent Cq values from the diarrheal patients. The red line indicates the cut-off value shown in Table 1.



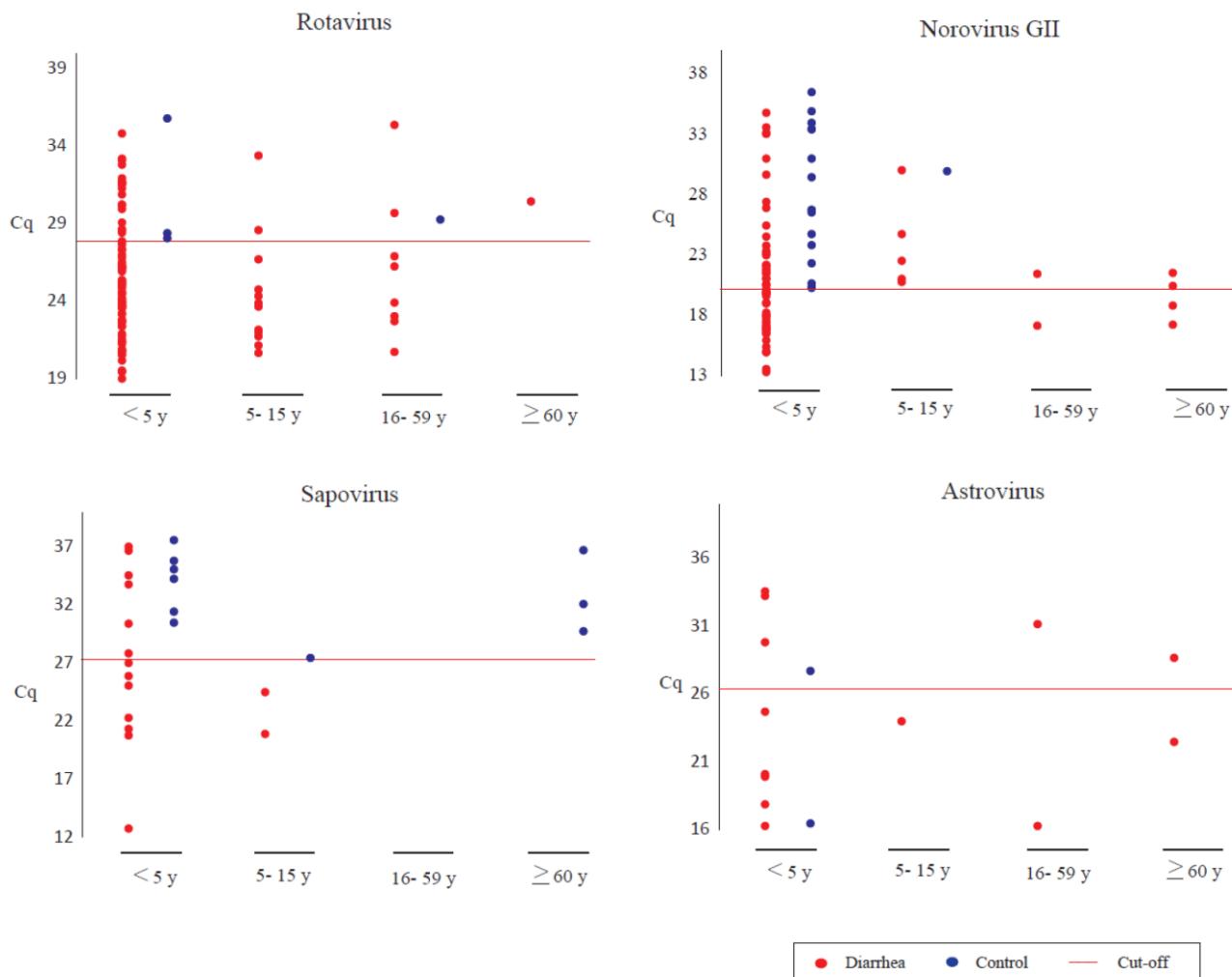


Figure S3. Detection of enteropathogens by age group using qPCR assays and distribution of Cq-values. The blue dots represent Cq values from the non-diarrheal control subjects and the red dots represent Cq values from the diarrheal patients. The red line indicates the cut-off value shown in Table 1.

Supplementary methods

We reported the patient baseline characteristics and disease profile with proportions, stratified by the groups. The relationships between disease profile and pathogens detected/not detected in the cases were presented as ORs and *P*-values were determined using the Fisher's exact test, as shown in Table 1. Similarly, we also reported the relationships between disease profile and Cq-value (positive/negative).