SUPPLEMENTARY MATERIAL

Detection of potentially pathogenic enteric viruses in environmental samples from Kenya using the bag-mediated filtration system

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Methods

Study permissions

This study was approved by the Kenya Medical Research Institute (KEMRI) Scientific and Ethics Review Unit (Non-SSC Protocol #464, KEMRI/RES/7/3/1), National Commission for Science, Technology, and Innovation (NACOSTI/P/15/2731/4929), and Research Ethics Committee, Faculty of Health Sciences, University of Pretoria (UP) (Ethics Reference #119/2017). Ethics approval was also received by PATH (RDC-0772) and the University of Washington (UW) (HSD Study #48465) Institutional Review Boards/Human Subject Committees.

Study design

Sampling sites included four sites in Nairobi: the Motoine River bordering the Kibera informal settlement (Kibera), a sewer conveyance line outlet connecting to the Mathare River (Starehe), and sewer conveyance lines in the Eastleigh neighborhood (Eastleigh A and B). Samples were also obtained from a pumping station in Mombasa (Kipevu), and sewer conveyance lines in Garissa (Bullah Sheikh) and Kisumu (Kisumu Polytechnic).

Sample collection and processing

Samples were collected in a collection bag with pre-screen mesh (249-µm pore size). Samples were filtered onsite immediately or stored (4ºC, <24 hours) until filtration at a separate, secure location utilizing the previously described "bucket" modification (Zhou et al., 2018). To filter, the collection bag was hung on a tripod stand, a positively charged ViroCap™ filter (Scientific Methods, Granger, IN, USA) was attached to the bag outlet port, and the sample was filtered by gravity. The filter was transported on cold packs to KEMRI in Nairobi, where a preservative mixture (2% sodium benzoate [Alfa Aesar, Ward Hill, MA, USA] and 0.2% calcium propionate [TCI America, Portland, OR, USA]) was added, held for 20 minutes, and recovered through the filter outlet (Fagnant et al., 2017).

Viruses were eluted from filters via a single 30-minute elution with a 1.5% beef extract (BBL™ Beef Extract powder; Becton, Dickinson and Company, Sparks, MD, USA) and 0.05 M glycine (Merck, Darmstadt, Germany) solution, pH 9.5. Samples were secondary concentrated using polyethylene glycol (PEG) 8000 (AMRESCO, Solon, OH, USA) precipitation and resuspended in 10-mL phosphate-buffered saline.

RT-PCR

At UP, samples were analyzed for EV, HAstV, HAV, NoV GI, NoV GII, and HRV by direct real-time RT-PCR using CeeramTools® kits (bioMérieux), which contain positive, negative, and internal PCR inhibition controls. Primers and probes for HAdV and SaV analyses were synthesized by Applied Biosystems (Thermo Fisher Scientific Inc., Waltham, MA, USA) (Table S1). HAdV-2 (ATCC VR 846) and SaV from a clinical specimen (Murray et al., 2013) were used as positive controls for the respective in-house real-time PCR assays, and nuclease-free water (Promega Corp., Madison, WI, USA) as negative controls. Real-time RT-PCR analyses were performed using a Lightcycler 2.0 (Roche Diagnostics, Mannheim, Germany). If inhibition was observed, a 1:10 nucleic acid dilution was re-analyzed and these Cq values were not reported.

Primers for PMMoV analysis were synthesized by Integrated DNA Technologies (Coralville, IA, USA) and probes by Applied Biosystems (TaqMan™ MGB Probe, Thermo Fisher Scientific Inc.) (Table S1). RT was completed using SuperScript™ IV VILO™ Master Mix (Thermo Fisher Scientific Inc.), and qPCR using an ABI StepOnePlus™ Real-Time PCR System (Thermo Fisher Scientific Inc.) with TaqMan® Environmental Master Mix 2.0 no UNG (Thermo Fisher Scientific Inc.). Nuclease-free water (IBI Scientific, Dubuque, IA, USA) was used as negative controls. Standard curves were determined via analysis of a dilution series in duplicate of a purified recombinant plasmid containing the PMMoV assay amplicon. qPCR inhibition was not observed when samples were diluted 1:10. To determine the average PMMoV gene copies per reaction, linear regression analysis of the duplicate Cq and gene copy numbers was executed, and average PMMoV gene copy numbers were divided by two, accounting for differences in nucleic acid type (PMMoV gene target is single-stranded, while the purified recombinant plasmid is double-stranded [Bustin et al., 2009]).

Table S1. Primers and probes used for detection of enteric viruses

Assay target	Sequence (5' to 3')		Citation
Human adenovirus	Forward	GCC ACG GTG GGG TTT CTA AAC TT	Heim et al., 2003
	Reverse	GCC CCA GTG GTC TTA CAT GCA CAT C	Heim et al., 2003
	Probe (dye)	[FAM]-TGC ACC AGA CCC GGG CTC AGG TAC TCC GA-[TAMRA]	Heim et al., 2003
Sapovirus	Forward1	GAC CAG GCT CTC GCY ACC TAC	Chan et al., 2006
	Forward2	TTG GCC CTC GCC ACC TAC	Chan et al., 2006
	Reverse	CCC TCC ATY TCA AAC ACT AWT TTG	Chan et al., 2006
	Probe (dye)	[FAM]-TGG TTY ATA GGY GGT AC-[MGB-NFQ]	Chan et al., 2006
Pepper mild mottle virus	Forward	GAG TGG TTT GAC CTT AAC GTT TGA	Haramoto et al., 2013
	Reverse	TTG TCG GTT GCA ATG CAA GT	Zhang et al., 2006
	Probe (dye)	[FAM]-CCT ACC GAA GCA AAT G-[MGB-NFQ]	Zhang et al., 2006

Table S2. Real-time RT-PCR results, reported as the quantification cycle, from the direct analysis of sequentially collected BMFS samples

Date (dd/mm/yyyy)	Site	EV		HAdV		HAstV		HAV		NoV GI		NoV GII		SaV		HRV		PMMoV	
14/04/2015	Kibera	24.2	23.9	26.0	26.3	26.6	25.0	27.6	25.6	28.9	28.6	30.9	29.6	28.8	28.8	22.8	23.0	25.4	24.8
14/04/2015	Starehe	23.2	23.8	26.7	26.6	27.4	29.6	25.5	23.7	30.5	30.8	31.6	30.0	29.1	30.6	26.5	26.9	25.5	23.9
28/04/2015	Starehe	25.3	25.1	28.2	27.8	30.7	28.4	29.2	28.3	30.4	30.2	31.5	32.0	27.4	29.8	25.8	25.2	24.3	24.3
28/04/2015	Eastleigh A	25.2	24.6	25.8	20.2	35.0	36.0	32.4	32.5	29.2	32.4	32.0	32.6	33.5	32.7	32.1	32.1	25.7	24.0
28/04/2015	Eastleigh B	25.9	26.1	32.8	32.1	30.5	35.6	32.9	33.8	31.3	29.6	30.3	32.0	33.2	32.7	29.5	28.1	26.4	27.3
04/05/2015	Kibera	23.9	24.3	27.8	27.4	24.4	24.6	28.9	29.6	27.6	28.0	28.8	29.7	28.6	28.8	24.8	24.2	25.3	26.2
13/05/2015	Kibera	30.0	29.7	33.7	33.8	34.4	35.3	34.5	35.3	34.1	33.9	32.1	32.7	38.4	38.5	26.6	26.7	31.5	31.8
13/05/2015	Starehe	28.0	26.6	29.9	27.9	33.6	31.5	29.9	29.0	37.5	32.2	35.6	31.2	33.0	31.3	31.6	28.5	27.7	26.5
13/05/2015	Eastleigh A	26.4	27.7	31.8	31.3	32.6	30.7	35.9	34.4	29.5	30.3	26.6	26.8	31.0	31.6	29.8	29.2	26.5	26.9
13/05/2015	Eastleigh B	24.7	25.7	33.3	33.1	34.6	n.d.	27.3	27.0	32.4	34.5	30.2	31.5	31.9	33.8	28.7	33.2	26.9	26.3
26/05/2015	Kibera	26.3	26.7	32.2	32.2	30.1	30.5	31.0	30.4	35.5	33.6	33.8	32.8	31.3	31.6	26.8	25.7	28.6	28.6
26/05/2015	Starehe	25.5	26.7	28.8	29.1	31.3	31.2	26.6	26.8	31.2	32.1	28.0	29.8	30.8	31.4	30.1	29.6	24.7	25.0
26/05/2015	Eastleigh A	25.7	26.1	26.1	24.0	30.9	34.3	28.5	28.1	31.7	33.0	31.3	32.1	29.5	28.6	31.2	31.3	24.9	26.1
26/05/2015	Eastleigh B	23.6	23.5	32.0	32.2	27.3	26.2	31.8	32.8	31.2	32.6	28.9	27.6	31.9	31.9	30.8	28.1	26.4	26.2

RT-PCR, reverse transcription polymerase chain reaction; BMFS, bag-mediated filtration system; EV, enterovirus; HAdV, human adenovirus; HAstV, human astrovirus; HAV, hepatitis A virus; NoV GI, norovirus GI; NoV GII, norovirus GII; SaV, sapovirus; HRV, human rotavirus; PMMoV, pepper mild mottle virus; n.d., not detected

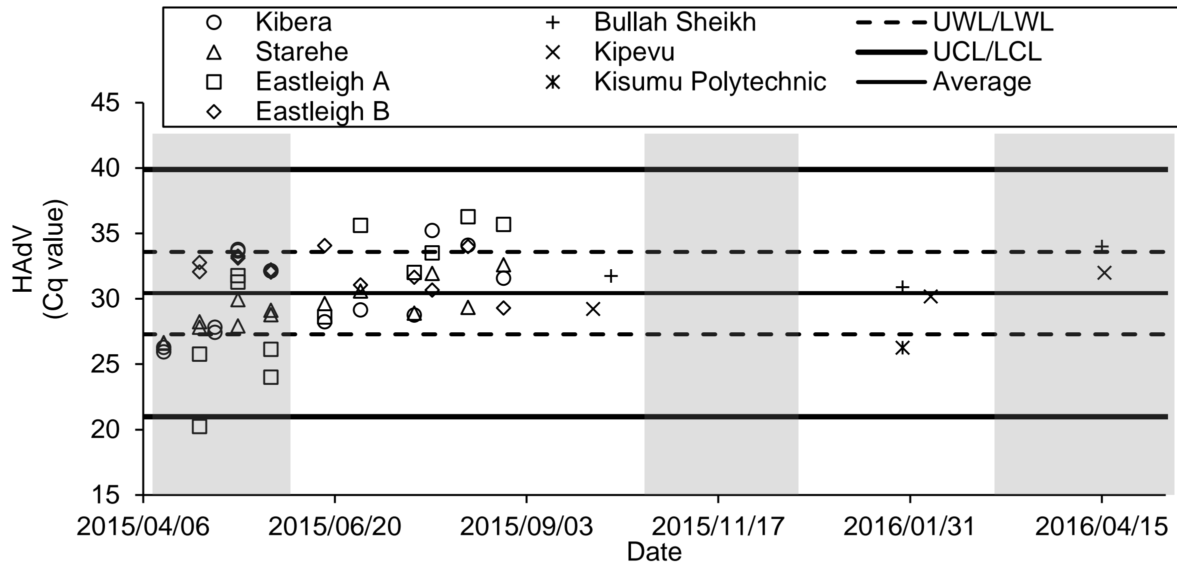

Figure S1. Detection of human adenovirus (HAdV) at the seven sites sampled in Kenya. Shaded areas show the rainy season. UWL and LWL are the upper and lower warning levels (average ± one standard deviation). UCL and LCL are the upper and lower control levels (average ± three standard deviations).

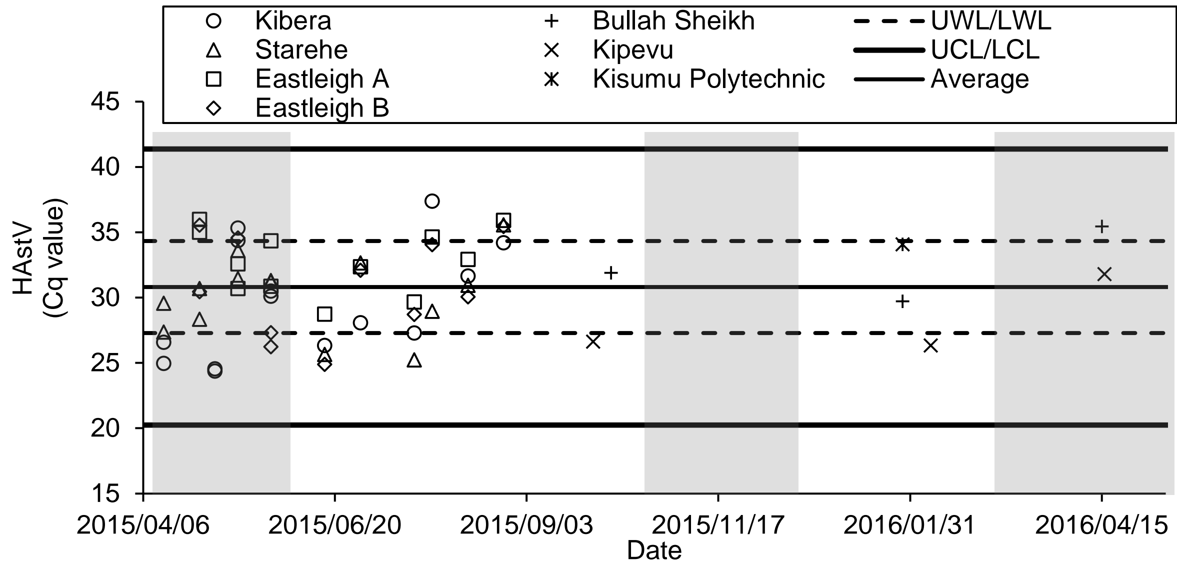

Figure S2. Detection of human astrovirus (HAstV) at the seven sites sampled in Kenya. Shaded areas show the rainy season. UWL and LWL are the upper and lower warning levels (average ± one standard deviation). UCL and LCL are the upper and lower control levels (average ± three standard deviations).

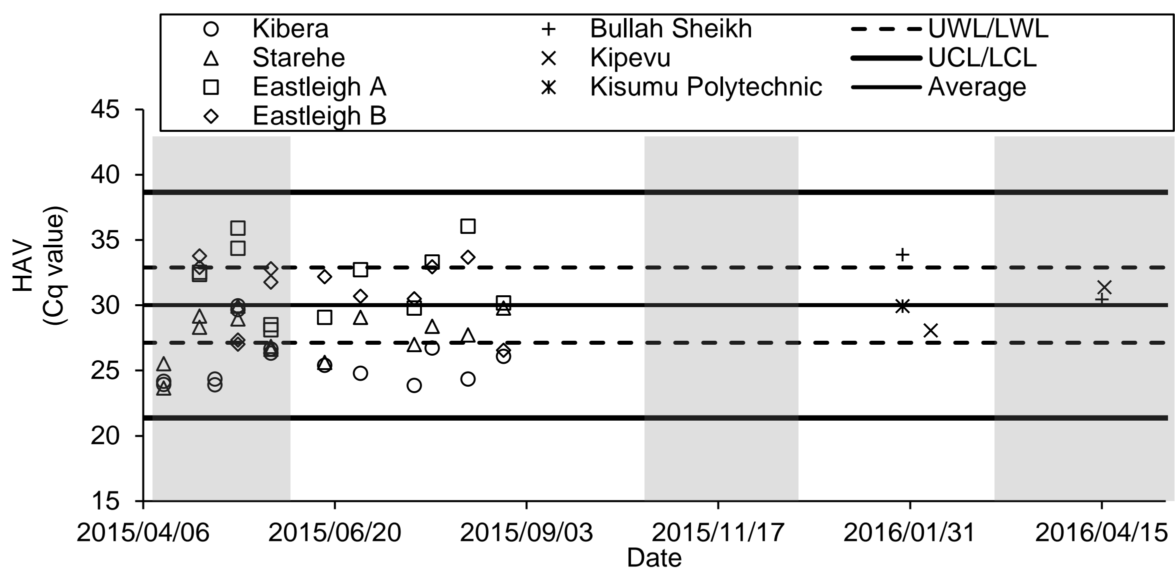

Figure S3. Detection of hepatitis A virus (HAV) at the seven sites sampled in Kenya. Shaded areas show the rainy season. UWL and LWL are the upper and lower warning levels (average ± one standard deviation). UCL and LCL are the upper and lower control levels (average ± three standard deviations).

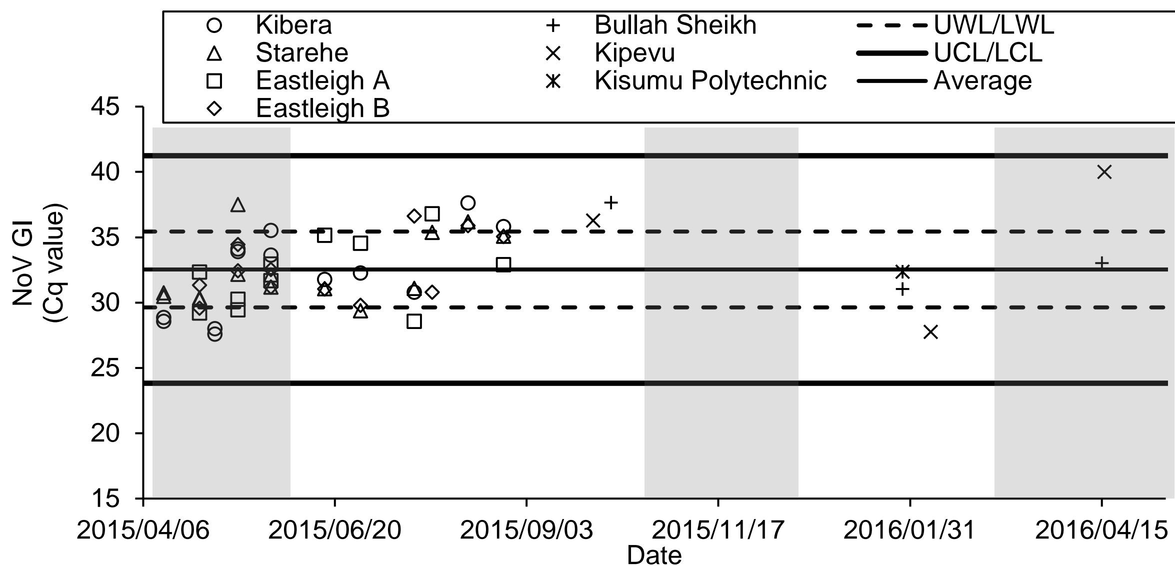

Figure S4. Detection of norovirus GI (NoV GI) at the seven sites sampled in Kenya. Shaded areas show the rainy season. UWL and LWL are the upper and lower warning levels (average ± one standard deviation). UCL and LCL are the upper and lower control levels (average ± three standard deviations).

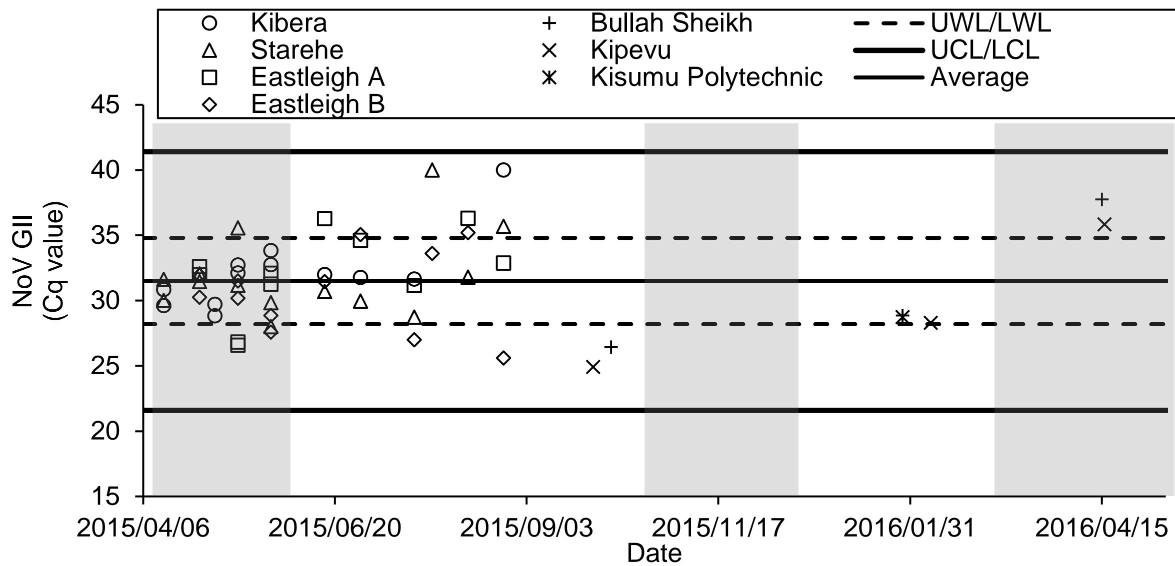

Figure S5. Detection of norovirus GII (NoV GII) at the seven sites sampled in Kenya. Shaded areas show the rainy season. UWL and LWL are the upper and lower warning levels (average ± one standard deviation). UCL and LCL are the upper and lower control levels (average ± three standard deviations).

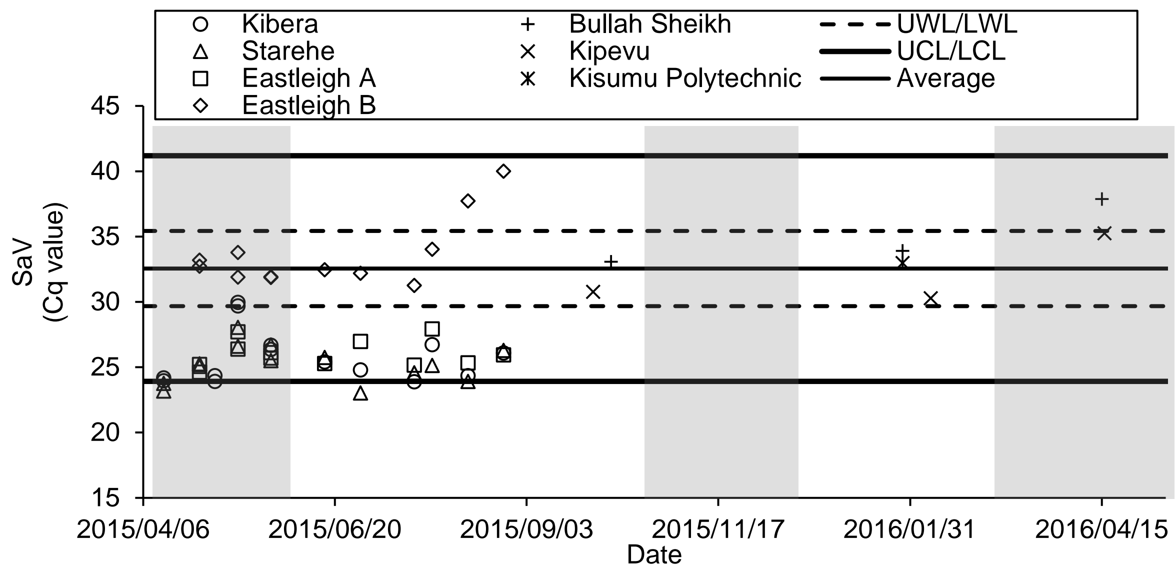

Figure S6. Detection of sapovirus (SaV) at the seven sites sampled in Kenya. Shaded areas show the rainy season. UWL and LWL are the upper and lower warning levels (average ± one standard deviation). UCL and LCL are the upper and lower control levels (average ± three standard deviations).

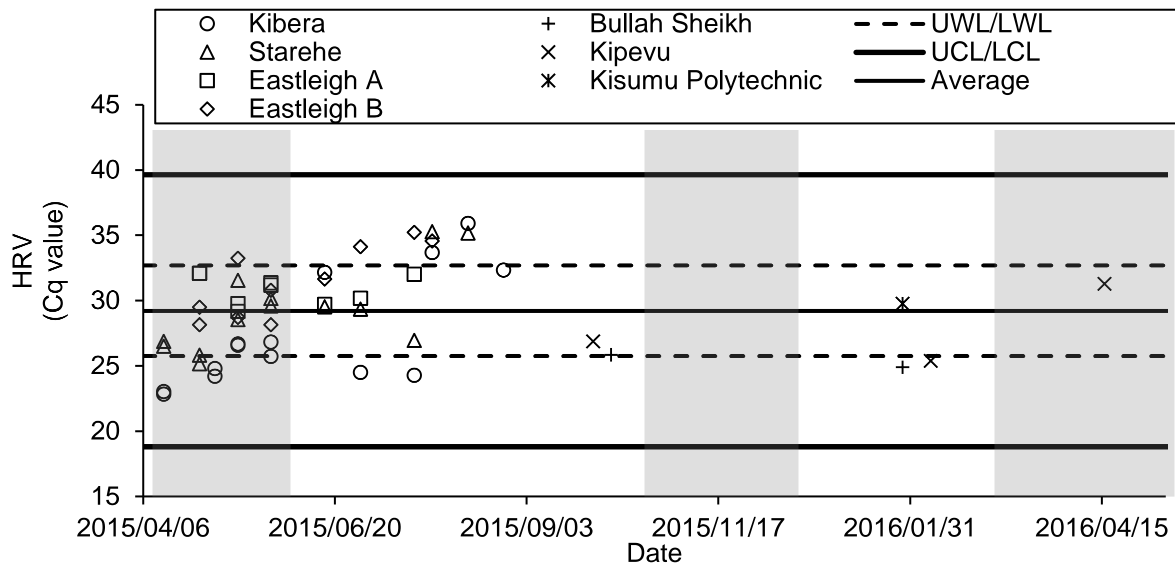

Figure S7. Detection of human rotavirus (HRV) at the seven sites sampled in Kenya. Shaded areas show the rainy season. UWL and LWL are the upper and lower warning levels (average ± one standard deviation). UCL and LCL are the upper and lower control levels (average ± three standard deviations).

	14 Apr	28 Apr	4 May	13 May	26 May	16 Jun	30 Jun	21 Jul	28 Jul	11 Aug	25 Aug	29 Sep	6 Oct	28 Jan	8 Feb	15 Apr	16 Apr
14-Apr	1																
28-Apr	1.0	1															
4-May	1.0	1.0	1														
13-May	1.0	1.0	1.0	1													
26-May	1.0	1.0	1.0	1.0	1												
16-Jun	1.0	1.0	1.0	1.0	1.0	1											
30-Jun	1.0	1.0	1.0	1.0	1.0	1.0	1										
21-Jul	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1									
28-Jul	0.02	0.01	0.18	0.01	0.003	0.03	0.02	0.02	1								
11-Aug	0.02	0.01	0.18	0.01	0.003	0.03	0.02	0.02	1.0	1							
25-Aug	0.71	0.56	0.93	0.77	0.46	0.71	0.71	0.71	0.99	0.99	1						
29-Sep	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.63	0.63	0.99	1					
6-Oct	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.63	0.63	0.99	1.0	1				
28-Jan	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.18	0.18	0.93	1.0	1.0	1			
8-Feb	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.63	0.63	0.99	1.0	1.0	1.0	1		
15-Apr	0.91	0.88	0.96	0.95	0.86	0.91	0.91	0.91	1.0	1.0	1.0	0.99	0.99	1.0	0.99	1	
16-Apr	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.63	0.63	0.99	1.0	1.0	1.0	1.0	0.99	1

Figure S8. Statistical significance of human enteric virus detection between sampling days. Blue shading indicates less frequent detection on the sampling day provided in the column, and red shading indicates less frequent detection on the sampling day provided in the row.

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