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Article

Double-Stranded DNA Virus Assemblages in Groundwater in Three Informal Urban Settlements in Sub-Saharan Africa Differ from Each Other

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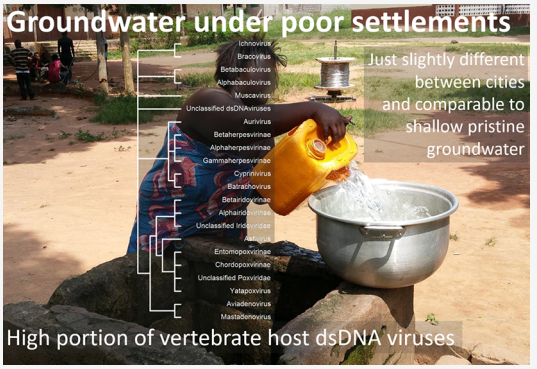
ABSTRACT: We mapped the double-stranded DNA (dsDNA) virus assemblage in groundwater below sub-Saharan urban poor settlements in Arusha (Tanzania), Dodowa (Ghana), and Kampala (Uganda). Our results indicated that ∼80% of dsDNA virus sequences matched the order of Caudovirales, i.e., indigenous bacteriophages; 1.8% of the dsDNA virus sequences matched those of viral pathogens that infect humans and larger animals, which we defined as so-called above-ground hosts. Within this group, the relative abundances of the genera Chordopoxvirinae, Alphaherpesvirinae, and Betairidovirinae were the highest. Culturable *Escherichia coli* bacteria were found even in deeper wells, indicating that all water was fecally contaminated. The community assemblages sampled in the cities were statistically significantly different from each other. Dissolved ions, population density, and sanitary status had no significant influence, but pH and latitude did. We concluded that the transport of dsDNA virus in groundwater was location specific but was not determined by input concentrations (i.e., related to population density) or related to groundwater chemistry. We hypothesize that other parameters, like the presence of macropores, cause these variations in these shallow, highly populated, heavily polluted terrestrial groundwater systems. Approximately 34% of Africa has similar hydrogeology, so this may affect many urban areas across the continent.



KEYWORDS: *dsDNA viruses, viral community composition, groundwater, poor urban settlements, sub-Saharan Africa, above-ground hosts*

1. INTRODUCTION

In Africa, 43% of the total population, or 547 million people, live in an urban environment, and this percentage is expected to grow to 60% in 2050, resulting in 1.5 billion urban dwellers.[1] Because public utilities cannot cope with high population growth percentages, a large part of the urban population lives in areas that are not serviced. On the basis of a GIS approach, Chavez Garcia Silva et al.[2] estimated that in 2015, ∼35% of the African urban population used groundwater obtained via self-supply, i.e., sourcing their own water supplies from where it can be found beyond the public water supply networks.[2,3] Many urban dwellers lack not only adequate drinking water supplies but also a sewerage system and off-site wastewater treatment. Usually, wastewater is disposed of on site where it ends up in the groundwater. In a previous study, we observed the systemic presence of viral fecal indicators human adenovirus and rotavirus and bacterial fecal indicator *Escherichia coli* below Dodowa, a peri-urban settlement near Accra, Ghana.[4]

Knowledge about viral diversity and abundance in the subsurface is limited. Recent studies have shown that large reservoirs of viral particles are present in deep systems below ocean floors[5,6] and in deep terrestrial systems.[7,8] Cai et al.[9] concluded that active and diverse viruses persist in the deep subseafloor sediments over thousands of years. In a shallow terrestrial system, Costeira et al.[10] were the first to report on bacteriophages and their bacterial hosts associated with a hydrocarbon contaminant plume. The bacteriophages were presumed to have significant effects on prokaryotic production and horizontal gene transfer and to impact biodegradation at the boundaries of the plume. To begin to understand the

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Table 1. Overview of Sample Types per Area

	site properties					
city	ID	UTM easting	UTM northing	UTM zone	type	depth (m)
Arusha	A01	239615	9625789	37	surface water	0
	A06	238596	9623202	37	dug well	5
	A11	239621	9625733	37	borehole	unknown
	A15	239811	9623795	37	piezometer	16
	A30	241906	9626145	37	spring	0
	A38	241850	9626157	37	spring	0
	A42	239811	9623795	37	piezometer	9.5
	A48	239811	9623795	37	piezometer	9.5
Dodowa	D01	820990	650905	30	dug well	5
	D08	821194	651456	30	dug well	5
	D17	821732	650596	30	borehole	unknown
	D29	821787	650618	30	dug well	5
	D30	820435	651034	30	dug well	5
	D31	820435	651034	30	dug well	5
	D32	820435	651034	30	dug well	5
	D33	820435	651034	30	dug well	5
	D35	820435	651034	30	dug well	5
	D36	821787	650618	30	dug well	5
	D37	820435	651034	30	dug well	5
	D39	820435	651034	30	dug well	5
	D43	820435	651034	30	dug well	5
	D58	821608	649979	30	surface water	0
Kampala	K03	450848	38193.8	36	piezometer	22
	K04	450848	38193.8	36	piezometer	11
	K05	450848	38193.8	36	piezometer	3
	K06	450841	38602.8	36	piezometer	24
	K07	450969	38337.4	36	spring	0
	K08	450583	38887.9	36	spring	0
	K11	450841	38602.8	36	piezometer	10
	K19	451112	38715.4	36	surface water	0
	K21	451604	37682.7	36	piezometer	50

factors governing virus distribution in terrestrial subsurface environments, Pan et al.[11] assessed total microbial cell and virus particle abundance (but not the composition of the assemblage) in groundwater concurrent with groundwater chemistry in a uranium-impacted alluvial aquifer. They concluded that microbial cell and virus abundances were correlated to geochemical conditions in the aquifer in the sense that geochemical conditions likely control microbial host cell abundance, which in turn controls viral (bacteriophage) abundance and likely also the community composition. All of the studies mentioned above focused on resident or "autochthonous" virus abundance and its role in controlling autochthonous bacterial host abundance.

The presence and abundance of viruses in the subsurface of terrestrial systems that originate from above-ground hosts (AGH), i.e., warm-blooded animals, including humans, cold-blooded vertebrates, and larger invertebrates, have not been studied frequently. Usually, the focus of research is on selected human viruses in relation to groundwater safety[4,12,13] in the vicinity of a well field or groundwater abstraction facility. To address this gap, Smith et al.[14] used a nontargeted approach and observed plant and animal viral pathogens in 1500-year-old water situated 80 m below the surface in a confined aquifer, which suggested the potential for long-term survival of viruses originating from AGH.

Today, approaches based on communities are more common than targeting single species (with PCR or specific virus tests). The methods follow a sequencing library preparation protocol, which usually includes a DNA amplification step,[7] because the amount of DNA in non-amplified samples is generally (too) low. Using next-generation sequencing and metagenomic analyses to assess viral diversity, several human and vertebrate viruses have already been detected in polluted surface water samples in Kampala, Uganda.[15]

The objective of this work was to assess the viral community composition, with an emphasis on viruses from AGH, in groundwater below three different urban poor settlements in three different areas across sub-Saharan Africa: Dodowa in Ghana, Bwaise slum in Kampala, Uganda, and Unga, Sombetini, and Osunyai wards in Arusha, Tanzania. Given that these areas not only are in three different countries but also are characterized by different population densities, different types of aquifers, and different groundwater compositions, we expected great differences.

1.1. Study Areas. Dodowa is located in the southeastern part of the Greater Accra Region, Ghana, and receives a mean annual rainfall of ∼900 mm per year, and with an average temperature of 27 °C,[16] the climate is tropical.[17] Groundwater in the area is found at shallow depths in the predominantly unconfined weathered basement [Togo Structural Unit (TSU)] and in fractured gneiss of the Dahomeyan Structural Unit (DSU).[18] Dodowa is peri-urban, and with a population of

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~15,000 people, with a population density of 2300 inhabitants per km^2 in 2016,[2] most inhabitants are engaged in agriculture.

Arusha is the third largest city in Tanzania, located on the foothills of the volcanic plain of Mount Meru near the East Africa rift in the northeastern part of the country. At 1100 meters above sea level, the average annual rainfall amounts to 794 mm, while potential evapotranspiration is 924 mm/year.[19] The temperature is highest in February with averages of 25 and 13 °C in the coolest month of July. In 2012, it had a population of 416,000.[20] In the Unga ward, most of the area is occupied by slums and industrial complexes, while in Sombetini and Osunyai, in the addition to the urban fabric, private gardens can exist and also small plantations (e.g., tea and bananas) are present. The population density in these neighborhoods was 11,100 inhabitants per km^2 in 2016.[2] Shallow groundwater is found in the predominantly basaltic volcanic extrusive and sedimentary (fluvial, colluvial, and eolian) deposits.[21]

Kampala, the capital of Uganda, has an average precipitation of 1450 mm/year and an evapotranspiration of 1150 mm/year.[22] Bwaise and Makerere urban settlements, our study area in Kampala, are located in the Lubigi catchment, northwest of Kampala. Groundwater is found shallow in weathered primarily Precambrian basement rocks of Archaean granitoids and orthogneiss with some metasedimentary rocks of the Proterozoic Buganda Group.[23] The estimated population of the entire catchment with a size of 65 km^2 is ~395,000; the majority has an income of less than 2 USD per person per day.[24,25] The population density in these neighborhoods was 11900 inhabitants per km^2 in 2016.[2] Most of Bwaise and Makerere is occupied by small shacks and houses. However, cropland, scattered trees, and, in the lowest parts, papyrus swamps are present.

2. EXPERIMENTAL METHODS

2.1. Virus Sampling, Concentration, and DNA Isolation. Most of the samples were taken from existing dug wells, boreholes and springs, and piezometers that were drilled specifically for this work, all tapping groundwater. Samples were taken in Dodowa (Accra, Ghana), in Bwaise and Makerere communities in Kampala (Uganda), and in Osunyai, Sombetini, and Unga Wards in Arusha (Tanzania) (Table 1). Dug wells are large diameter (2−4 m) shallow wells with a total depth of 5−10 m and relatively shallow groundwater tables (around 5 m below the surface). Some of the wells were closed with a concrete slab; sometimes they were without cover. Boreholes were preexisting, drilled by authorities or NGOs active in the area. Depths ranged from 10 to 50 m. Screening of the wells was unknown, but usually the wells were regularly screened less than 5−10 m from the bottom. Boreholes for piezometers for this project reached depths of 50 m. The time between piezometer borehole construction and sampling was 3 months to 1 year for the piezometers to equilibrate with their surroundings. Every month, and also just before sampling, the piezometers were purged with 2−3 well volumes with a 37 mm diameter pump (Gigant, Ekotechnika, Prague, Czech Republic) to sample fresh groundwater originating from the aquifer. Furthermore, in each area, a surface water sample was taken from a nearby river or canal draining the area. Because we expected a lower level of viruses in groundwater and a higher level in river water, 100 L samples were taken from groundwater, and from river water, we took 10 L samples.

In the field lab, of which one was set up in each city, samples were concentrated using a glass wool protocol.[4,26,27] Briefly, after being cleaned with HCl and NaOH, the glass wool was rinsed to near neutral pH and loaded with sample, conditioned with HCl to pH 3.5.[28] The viruses that were adsorbed to the glass wool were eluted with glycine-beef extract (pH 9.5). Flocculation by decreasing the eluate pH to 2.8 and centrifugation were used to concentrate the viruses into 10 mL of phosphate-buffered saline (pH 7.0). To avoid cross contamination, all equipment was cleaned in diluted bleach and rinsed with water, and buffers were autoclaved. Glass wool for concentration of the virus particles was used once and replaced with fresh glass wool for each sample. The 10 mL viral concentrates were stored at −20 °C and kept frozen during transportation from the field to IHE Delft (Delft, The Netherlands), where they were stored at −80 °C until further use. DNA was extracted from all concentrates by using the Boom protocol, using silica beads and a chaotropic agent, guanidinium thiocyanate, to release the DNA from the beads.[29,30] The resulting DNA concentrations were measured using a Qubit 3.0 Fluorimeter (Life Technologies, Bleiswijk, The Netherlands).

2.2. Sequencing. Two separate MiSeq (Illumina Inc., San Diego, CA) runs were carried out for samples from Arusha (8), Dodowa (14), and Kampala (9). DNA was prepared for sequencing using the Nextera XT DNA Library Preparation Kit (Illumina). Template length and size distributions were assessed with a Bioanalyzer (Agilent Technologies, Santa Clara, CA), and only samples with average fragment lengths of 500−1100 bp, and with a concentration of >850 pg/μL, were used for processing. The paired-end sequencing method was used, with 300 bp per end. Index-tagged paired-end Illumina sequencing libraries were prepared by using 17 unique indexing tags for the first run and 14 in the second run [Combinatorial Dual (CD) indexing, Illumina]. The tagged libraries were combined into pools and sequenced according to the manufacturer's protocols to generate tagged 300 bp paired-end reads.

2.3. Sequence Data Processing. Output sequence data for MiSeq were quality checked with FastQC (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/), using automatic mode. The MiSeq read pairs were trimmed with Trimmomatic (version 0.38)[31] in paired-end mode, with Illumina specific sequences removed, the leading and trailing three bases removed, sliding window 4:20, minimum length of 36, and converted to fasta format. The fasta read sequences were submitted to blastn (dc-megablast, version 2.2.31) and paired blastn, and single reads (i.e., only one of the pairs, R1) were submitted to tblastx, using default scoring matrix BLOSUM62 and standard genetic code (1). The tblastx method permitted more variation of the DNA level than blastn while conserving the sequence on the protein level, i.e., without an effect on the functioning of the organism. This is likely the case, because even in places that have been extensively studied, novel virus genes were discovered that lack homologues in the sequence database.[32] The searches were performed against the virus RefSeq database that was downloaded from NCBI (May 29, 2017, 7782 sequences),[33,34] with a cutoff e value set to 10^{-7}. Blast output data were processed with Megan version 6.8,[35] using nucl_acc2tax-May2017.abin for mapping of the NCBI sequence identifiers to taxonomic classes. The MiSeq read pair raw sequences were submitted to the European

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Nucleotide Archive (https://www.ebi.ac.uk/ena) under Accession Number PRJEB39013.

2.4. Double-Stranded DNA (dsDNA) Viral Groups. The total number of reads for dsDNA viruses was taken to normalize the numbers of reads of the viral groups found in the different samples. Special attention was given to viruses that had AGH: pathogens for large animal hosts, including humans, that are not supposed to be present in a sample taken from (deep) groundwater sources. Within those viruses, we distinguished three groups: warm-blooded animals, including humans, cold-blooded vertebrates, and larger invertebrates. The sequence counts of AGH viruses were pooled into their respective dsDNA virus genera.

2.5. Statistical Analyses. At three taxonomic levels, family, genus, and species, the virus abundance data were square-root transformed prior to constructing a Bray−Curtis resemblance matrix. A one-way analysis of similarities (ANOSIM) routine was run to test for significant differences among virus assemblages in the different cities. Before inclusion in analysis, the environmental data pH, electroconductivity (EC), temperature, population density, sanitation score, sample depth, and concentrations of Na^+, K^+, Mg^{2+}, Ca^{2+}, NH_4^+, Cl^-, HCO_3^-, SO_4^{2-}, NO_3^-, Fe^{2+}, Mn^{2+}, and Al^{3+} (Supporting Information and ref 18) were checked for collinearity. Highly correlated variables ($-0.75 < r > 0.75$) were not used in further analyses. The relationship between the abundance data and environmental variables was investigated using the DISTLM routine on the Bray−Curtis resemblance matrix.[36] The model was built using a stepwise selection of the environmental variables, and results were visualized with distance-based redundancy analysis ordination (dbRDA). All univariate and multivariate analyses were performed using the PRIMER 7 statistical software with the PERMANOVA+ add-on.[36,37]

3. RESULTS

3.1. Overall Viral Assemblage Composition. Two runs with Illumina MiSeq were used to sequence the 31 samples, i.e., Dodowa (14), Kampala (9), and Arusha (8). The MiSeq run also included 6 cDNA samples, but these appeared to contain barely any RNA virus sequences and many dsDNA sequences. This observation made us decide to discard the cDNA runs. In the presequencing Bioanalyzer test for MiSeq sequencing, 13 samples from Dodowa did not yield DNA of sufficient quality and could therefore not be used for analysis.

The total number of read pairs in the DNA samples was 42,295,906 (Table 2). The average length of the reads was 244 bases.

Because of the computing time needed for tblastx, only the first of the two read pair sets was analyzed. The blastn and tblastx searches against the RefSeq virus database yielded different percentages of hits. The relative number of Caudovirales (the largest group with dsDNA viruses) against the total number of dsDNA virus sequences was larger for the blastn run than for the tblastx run (Table 2). The genus Chordopoxvirinae had high numbers due to the high apparent abundance of two species, BeAn 58058 and Orf virus, indicating that the blastn and tblastx run resulted in different assemblage compositions. Only a few Orf virus sequences (<0.1‰) were found in the tblastx data, while BeAn 58058 virus sequences were found in both blastn and tblastx data (see Discussion and Conclusions). In the analyses, only the reads that matched with dsDNA viruses were used. Matches with ssDNA and RNA viruses were not considered because of the used DNA isolation and library preparation procedures and the failure of the cDNA step, respectively. For dsDNA families, blastn with all single reads (numbers for both ends added) resulted in 671,930 matches in total (Table 2). Pairing of the blastn reads yielded 231,488 matching reads (70% of one subset of single reads), while tblastx for one subset of single reads yielded a total number of 2,147,784 matching reads, i.e., ∼6.4 times as many as one subset of single reads with blastn. For all further comparisons, the tblastx data were used.

Table 2. Overview of Blast Data[a]

		blastn	tblastx
sequencing results	no. of samples	31	31
	no. of fasta reads (pairs)	84,591,812	42,295,906
	no. of sequences in the RefSeq virus database	7782	7782
	no. of hits in blast output	671,930 (total)	2,147,784
percentage of virus dsDNA reads	AGH	3.6	1.8
	Caudovirales	82 (paired reads)	77
	other non-AGH	14 (paired reads)	21

[a]The number of read pairs is half of the total number of reads in blastn. The number of tblastx hits is 6.9 times higher than the number of blastn hits. The dsDNA reads were analyzed further.

The most abundant group of dsDNA viruses consisted of bacteriophages belonging to the order Caudovirales (77% of all database hits).

Because the samples were taken from groundwater, special emphasis was placed on sequences that matched with sequences of pathogens for large animals, including humans, that are not supposed to be endogenous in a groundwater sample, indicated as AGH. Bacteriophages like in Caudovirales were regarded as autochthonous, i.e., not necessarily coming from AGH. Nine families that contained AGH dsDNA viruses were identified: Adenoviridae (4 species, all with a warm-blooded host), Asfarviridae (African swine fever virus), Baculoviridae (28 species with invertebrates as the multicellular host), Herpesvirales families (55 species, of which 47 had warm-blooded hosts, 7 had other vertebrate hosts, and one had an invertebrate host), Hytrosaviridae (Musca hytrovirus, one invertebrate host), Iridoviridae (20 species, of which 1 had a warm-blooded host, 9 had other vertebrate hosts, and the remaining 9 had invertebrate hosts), Polydnaviridae (2, with invertebrate hosts), Poxviridae (35 species, of which 27 had warm-blooded hosts, 3 had other vertebrate hosts, and 5 had invertebrate hosts), and unclassified dsDNA viruses (2 species with invertebrate hosts). From those families, a total of 22 genera were detected. On a genus level, the most abundant genera in the majority of the samples were Chordopoxvirinae (27 species), Alphaherpesvirinae (24 species), and Betairidovirinae (8 species, all with invertebrate hosts), with averages of 0.4‰, 1.7‰, and 2.9‰ of dsDNA virus reads, respectively (Figure 1). In the genus Chordopoxvirinae, reads matching rodent virus BeAn 58058[38] were found everywhere, up to 299 reads per sample. As worked out in the Discussion and Conclusions, these sequences were likely present due to contamination, which made us decide not to include these reads in the analyses.

For warm-blooded AGH virus species, the most abundant reads belonged to either Herpesvirales (Alphaherpesvirinae

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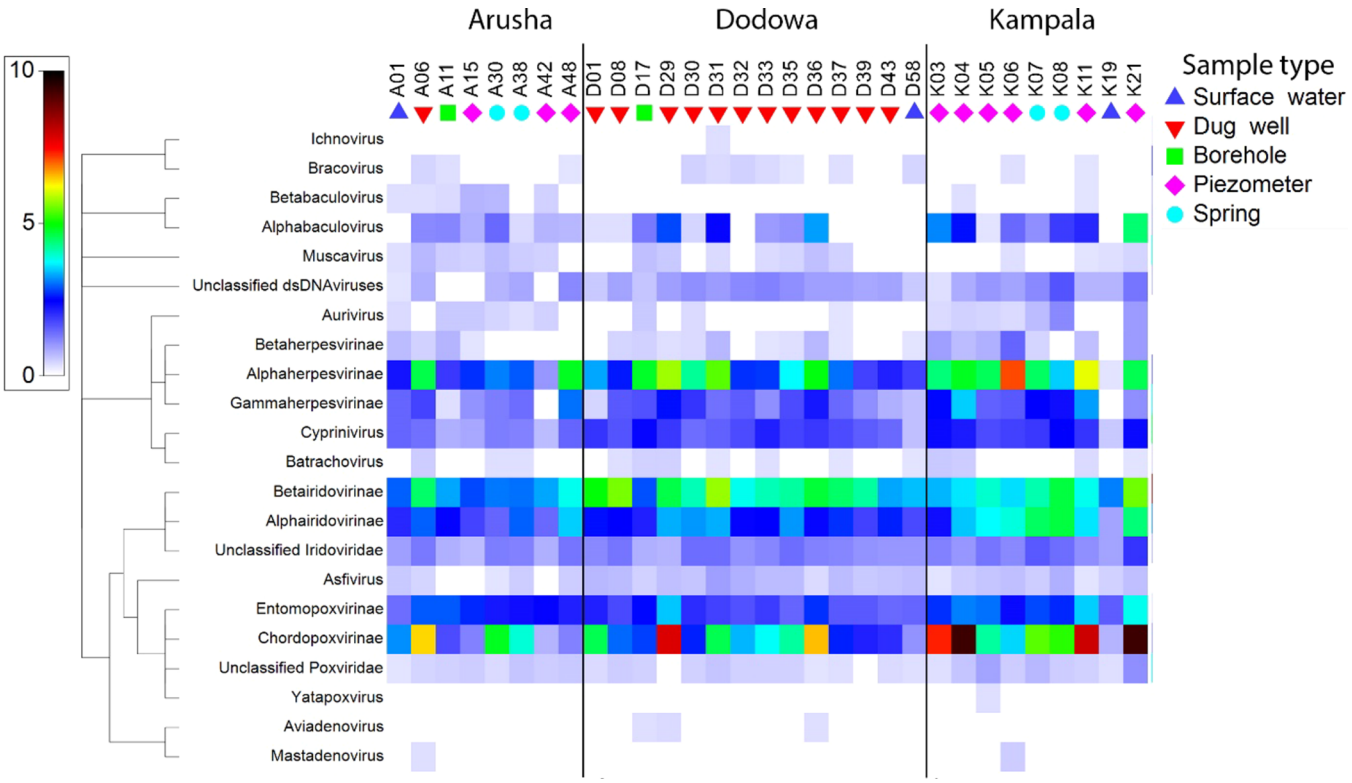

Figure 1. Shade plot of normalized above-ground host (AGH) virus abundances at the genus level in surface and groundwater samples from urban poor settlements in the cities of Arusha, Dodowa, and Kampala. The dendrogram is based on the taxonomic relatedness of the genera. In cases in which genera could not be distinguished, the next higher taxonomic level was used. The color bar indicates normalized abundances of AGH in permille of the total number of dsDNA reads. Chordopoxvirinae, Alphaherpesvirinae, and Betairidovirinae (invertebrate host) were the most abundant genera in a majority of the samples.

Table 3. Top-Ranked dsDNA Virus Species for Two AGH Groups, Warm-Blooded Vertebrate and Other Vertebrate Hosts[a]

	Arusha	Dodowa	Kampala
warm-blooded hosts	canid alphaherpesvirus 1	African swine fever virus	canarypox virus
	suid alphaherpesvirus 1	suid alphaherpesvirus 1	pigeonpox virus
	ateline gammaherpesvirus 3	bovine alphaherpesvirus 5	penguinpox virus
	saimiriine alphaherpesvirus 1	skunkpox virus	skunkpox virus
other vertebrate hosts	Singapore grouper iridovirus	Singapore grouper iridovirus	anguillid herpesvirus 1
	scale drop disease virus	infectious spleen and kidney necrosis virus	Singapore grouper iridovirus
	anguillid herpesvirus 1	anguillid herpesvirus 1	scale drop disease virus
	infectious spleen and kidney necrosis virus	scale drop disease virus	infectious spleen and kidney necrosis virus
	European catfish virus	cyprinid herpesvirus 3	European catfish virus

[a]The numbers after the species names indicate specific RefSeq NCBI database sequences.

and Gammaherpesvirinae) or Poxviridae (Chordopoxvirinae) (Table 3). For cold-blooded vertebrates, all samples contained most abundant viruses that had fish as hosts, e.g., including infectious spleen and kidney necrosis virus that can be found in tilapia fish.

3.2. *E. coli* Concentrations. Like in our previous study,[4] *E. coli* bacteria were found in almost all locations. The bacteria were not quantified in numerical detail, and their occurrence was used as an indicator for recent fecal contamination.

3.3. Overall Potential Mammalian Pathogens in Suburban Groundwater. Sequences most related to human herpes virus species sequences[39] were mostly detected in Dodowa, less commonly in Kampala, and in a few cases in Arusha. In Dodowa, all 10 samples of the dug wells (DW6 and DW7[4]) contained higher numbers of bovine alphaherpesvirus and especially bovine gammaherpesvirus (average of 14.3 reads per sample) than sampling points in other locations (13 of 19 of these did not contain any matching reads, and the remaining samples contained an average of 8.4 reads matching bovine gammaherpesvirus).

Thirteen reads of orf virus (a zoonotic parapox virus causing ecthyma contagiosum) were found in three samples in Kampala, but not in Dodowa and Arusha. Not with a mammalian host, but noteworthy, in Kampala we also found 29 reads for Nile crocodilepox virus in four samples, while we found none in other places.

3.4. Correlation between AGH Viral Genera and Other Parameters. On a genus level, a difference (ANOSIM) in the virus assemblages between cities was found (global $R = 0.32$; $= 0.001$). The ANOSIM R statistic can range from 0 to 1, where 0 indicates that there are no differences between cities and 1 means that the differences

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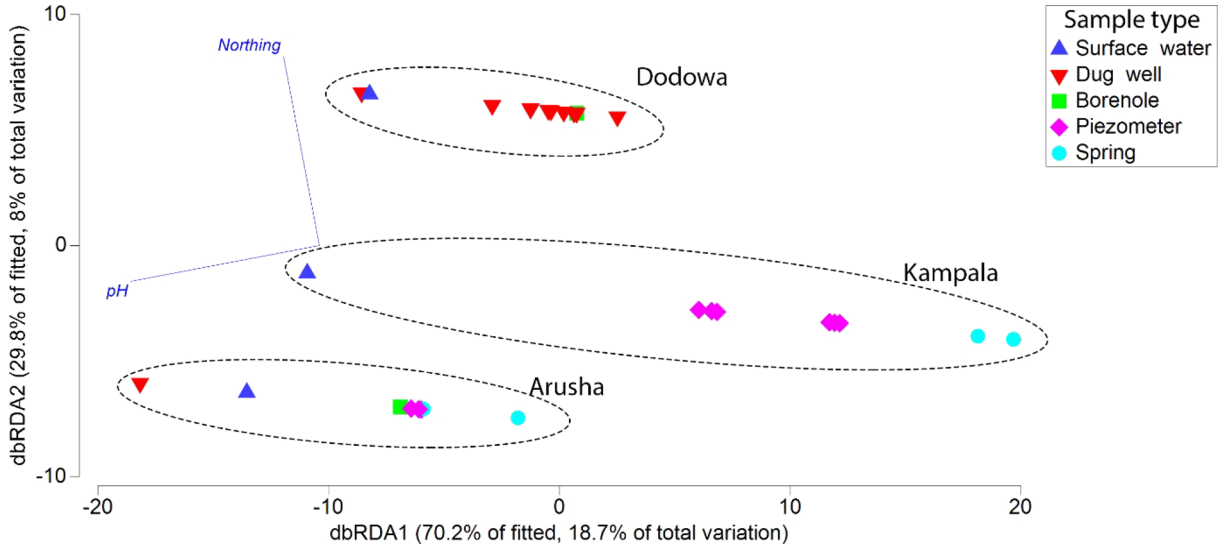

Figure 2. Relationship between geographic location and water quality parameters (blue vectors) with the type of surface and groundwater samples (colored shapes). Samples from the same cities (Arusha, Dodowa, and Kampala) are shown in the drawn-in dotted ellipsoid shapes. The plot represents a distance-based redundancy analysis (dbRDA) ordination of AGH virus genera on a Bray−Curtis similarity matrix with environmental variables chosen by stepwise selection. The pH and latitude (northing) of the cities explain the variation between the samples.

between cities are greater than the differences between samples. Pairwise tests confirmed that the virus assemblages between all comparisons differed. Samples from Arusha and Dodowa (R = 0.28; p < 0.01) were more similar to each other than samples from Arusha and Kampala (R = 0.34; p < 0.01) or Dodowa and Kampala (R = 0.33; p < 0.01). Only three variables were used: sanitation score, pH, and northing (location). Ion composition data were discarded after collinearity analysis.

A distance-based redundancy analysis ordination (dbRDA) with read data, grouped by genus, showed a clear clustering of samples for the different cities [with northing (latitude) as the most important geographical variable and pH as the most important water parameter (Figure 2)]. The sample types appeared in a fixed order: surface water, dug well, borehole or piezometer, and spring.

4. DISCUSSION AND CONCLUSIONS

This study is the first on the composition of the viral assemblages in groundwater under urban poor settlements in the tropical zone of sub-Saharan Africa.

From Figure 2, we concluded that the virus assemblages grouped per city. On a family level and a genus level, two of the surface water samples clustered separately outside the other samples (Supporting Information). Dissolved ion data co-correlated highly with other parameters and were therefore not included in the analysis. Furthermore, both Kampala and Arusha have population densities (11,900 and 11,100 inhabitants per km^2, respectively) more than 4 times that of Dodowa (2300 inhabitants per km^2),[2] but these data could not explain the differences in virus assemblage compositions, even if high-resolution data were used. Also, the sanitary risk inspection scores[4] did not have a significant effect on the viral assemblage composition. Pan et al.[11] observed strong correlations of total virus abundance (not the assemblage composition) with dissolved organic carbon (DOC) and groundwater uranium contamination; however, for the former, we did not have a complete data set, and the latter is not applicable.

Second, and despite these differences, we concluded that the AGH virus assemblages in the different urban poor settlements were not particularly diverse, which was apparent from the low correlation value of 0.211. Most virus species were found in all habitats, with small differences, indicating that they could survive under broad environmental conditions, but the populations were specialized to particular habitats.

Third, we concluded that in our "deep" samples (boreholes and piezometers), the average portion of AGH viruses was 19.9 ± 7.2‰ of all dsDNA virus reads, while in the "shallow" samples (springs and dug wells), that average was 17.1 ± 3.8‰; i.e., the difference was not significant. Also, *E. coli* bacteria were present in most of our samples, indicating widespread fecal contamination. From these and previous *E. coli* observations,[4] we concluded that there must be a rapid biocolloid infiltration and transport mechanism present to depths of at least 30 m in the case of Arusha and at least 50 m in the cases of both Kampala and Dodowa. Like bacteria, AGH virus particles could enter the subsurface through wastewater infiltration from on-site wastewater treatment facilities, mostly in the form of cesspits or infiltration fields from septic tanks, but they could also enter via recharge from precipitation recharging the aquifer, taking along diffusely present or perhaps even omnipresent AGH virus particles residing at the surface. We hypothesize that differences in hydrogeological characteristics, like the number of macropores, interconnections between macropores, and macropore dimensions have given rise to the small but significant variations in dsDNA viral assemblages. For unconsolidated layers overlying fresh basement rock, like in the case of Dodowa and Kampala, or for the predominantly basaltic volcanic extrusive and eolian and colluvial deposits, like in the case of Arusha, the overall relative importance of channel to matrix flow or a dual porosity or even multiporosity medium for groundwater flow is difficult to assess, although some studies suggest that the former predominates in all cases.[40−44] Crystalline basement rocks with a thick mantle of in situ weathered material underlie approximately 34% of Africa's land surface,[41,45−50] while basaltic volcanic extrusive rocks are mainly found in (parts

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of) east Africa. Various studies[49,51,52] have indicated that these types of rocks or aquifers are vulnerable to chemical and microbiological contamination.

Viruses have been found in the study locations before, either by targeting selected viruses in surface waters[4,28] or by carrying out a broad screening in selected surface waters.[15] Only 18‰ (permille) of all of our identified virus reads could indeed be linked to AGH virus species. Most of these species belonged to Herpesvirales, Iridoviridae, and Poxviridae, containing 3.9‰, 5.5‰, and 4.6‰, respectively, of all dsDNA virus sequences present; 54% of these virus sequences match with sequences of viruses that have a human or warm-blooded host. These hosts were not living in wastewater or wastewater infiltration. Also, a significant part (10.4‰ of all dsDNA) of the AGH appeared to be cold-blooded (vertebrates and invertebrates) without any link to wastewater. O'Brien et al.[15] also observed several possible human and vertebrate viruses in surface waters, including viruses belonging to Herpesvirales, Iridoviridae, and Poxviridae. They concluded that effluent from the Bugolobi wastewater treatment plant in Kampala appeared to impact the surface waters they had sampled. Apparently, even in these tropical groundwater environments, Herpesvirales, Iridoviridae, and Poxviridae seemed to be able to survive in such a way that they could be detected. O'Brien et al.[15] also found ssDNA viruses in most of their surface water samples, but we did not assess these.

The fact that a larger number of bovine viruses were found in dug wells in built-up areas could be explained by the location of one of the dug wells (DW6[4]), at the boundary of the built-up area, but another dug well (DW7) is situated in a built-up area at least 100 m from bush or livestock enclosures. Livestock was, however, not observed in both places.

The virus families observed in a pristine aquifer in the study of Kallies were comparable with our observations. We performed a quick blastn search with the online data from that study, taken from NCBI Sequence Read Archive (SRA) BioProject PRJNA530103. Like in our study, in Kallies et al.,[7] the majority of dsDNA virus sequences belonged to the order Caudovirales (60−70%). Furthermore, the aquifer in Germany contained relatively high but comparable abundances of sequences belonging to Mimiviridae and Phycodnaviridae families, which was comparable to our study [5.1 ± 1.2% and 9.3 ± 0.5% (n = 3), respectively, vs values in this study of 4.9 ± 1.7% and 8.9 ± 3.1% (n = 31), respectively, of all viral dsDNA sequences].

Among the most abundant in this study are sequences that match with BeAn 58058. Also for the blastn with the data from Kallies et al.,[7] the number of hits for the BeAn 58058 virus was among the highest. This indicates that matches with these sequences are very commonly found in groundwater samples. In many studies, a sequence match with this virus has come up as a contaminant that has a high percentage of nucleotide identities to human sequences (e.g., refs 53 and 54). Reads in our study that were assigned to BeAn 58058 were compared with the NCBI nr database and had indeed significant e values, down to 10^{-32}, with human and other primate sequences. These DNA sequences could therefore originate from human DNA contamination of the samples, which was why we removed them from the study results. Interestingly, when these sequences were removed from the results, the surface water samples did not cluster separately from the groundwater samples anymore. This difference may indeed have been due to the presence of human DNA contamination.

Because of the nature of this study, we could determine only relative abundances and could not assess the absolute concentrations of virus species. In our previous paper, we tested only the numbers of adenovirus and rotavirus (RNA virus) by qPCR.[4] In the study presented here, we could not find Adenovirus reads in all surface water samples, like in previous studies that found adenovirus in all samples with qPCR.[4,28] This can be explained by the fact that, compared to NGS sequencing, PCR is more sensitive and has a larger dynamic range.[55] Avian adenovirus sequences were found in our samples that were negative in the PCR tests in our previous study in Dodowa.[4] None of the samples, however, contained sequences that were most similar to human adenovirus, which implied we could not compare the adenovirus results in this study with the results of our qPCR-based work.

We conclude that although the AGH viral compositions between the cities were different at the genus level, at the family level the same viral families existed in all three cities. We hypothesize that rapid colloid transport played a major role in the infiltration of dsDNA virus particles in groundwater. One-third of Africa's land surface consists of rocks or aquifers that are vulnerable to chemical and microbiological contamination. Our work adds to that notion that a large part of urban and peri-urban subsurface of the African continent might also be vulnerable to virus material originating from AGH.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsestwater.0c00306.

Figures S1 and S2, Table S1, and additional references (PDF)

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Notes

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