Molecular epidemiology of human bocavirus infection in hospitalised children with acute gastroenteritis in South Africa, 2009-2015

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Highlights

- Human bocavirus was detected in 5.6% of hospitalized diarrhoea cases in children <5 years in South Africa between 2009 and 2015.
- Human bocavirus type 1 was the most common strain detected Human bocavirus may play a limited role in diarrhoeal disease burden in South Africa

Abstract

Human bocavirus (HBoV) is known to be associated with a variety of clinical manifestation including acute gastroenteritis (AGE). Despite their global prevalence, no data is available on the epidemiology of HBoV associated with AGE in South Africa (SA). Between April 2009 and April 2015, 3765 stool specimens were collected from children less than 5 years of age hospitalized with diarrhea. Specimens were screened for selected enteric viruses by enzyme immunoassay and quantitative polymerase chain reaction, bacteria by culture and parasites by staining and microscopy. HBoV was detected in 5.63% (212 of 3765) of cases, the majority of which were children ≤2 years (92%, 195 of 212), and were common in the summer and autumn months (60%; 128 of 212). Further investigations of coinfections showed that bacteria (adjusted odds ratio [aOR] = 2.20; 95% confidence interval [CI], 1.41-3.45; $P = .001$) and sapovirus (aOR = 2.05; 95% CI, 1.08-3.86; $P = .027$) were significantly associated with HBoV in multivariate analysis. HBoV genotyping was successful in 191 of the 212 samples with HBoV-1 being the most prevalent genotype observed (79.6%; 152 of 191) followed by HBoV-3 (13.6%; 26 of 191), HBoV-2 (5.2%; 10 of 191), and HBoV-4 (1.6%; 3 of 191). The high prevalence of HBoV-1, a virus known to be associated with respiratory infections, and the association between HBoV-positive specimens and already established AGE agents, suggests that HBoV may play a limited role in the observed AGE cases in SA.

KEYWORDS

Human bocavirus, diarrhoea, epidemiology, South Africa, genotypes, prevalence

1. INTRODUCTION

Diarrhoea is a leading cause of morbidity and mortality in children under 5 years of age, particularly in Africa and other low-income countries.[1,2,3] Diarrhoeal diseases can be transmitted through various routes, with about 90% of the estimated 2.2 million deaths attributed to poor sanitation and hygiene.[4] Although mortality due to diarrhoeal disease has significantly declined in high-income countries over the past two decades, the decline observed in low-income countries is moderate.[2,5] Among the causes of diarrhoeal disease, viruses are recognized as the major contributor and include rotavirus (RV), norovirus (NoV), sapovirus (SaV), astrovirus (AstV), and adenovirus (AdV).[6]

Human bocaviruses (HBoVs), first described in 2005[7], are members of the family *Parvoviridae*, subfamily *Parvovirinae*, genus *Bocaparvovirus* and have been reported worldwide in various studies as a potential cause of diarrhoea[8]. Since 2005, four HBoV genotypes (HBoV-1-HBoV-4) have been described and are characterised by a 5 kb single-stranded DNA genome, encapsulated in a non-enveloped icosahedral capsid protein coat.[9] The genome has three open reading frames (ORFs) with non-structural proteins NS1 nd NP1 regulating viral RNA processing, and nuclear phosphoprotein important in DNA replication and evasion of innate immunity encoded in the first and second ORFs, respectively.[8] The third ORF encodes the viral capsid proteins, VP1/VP2.[8]

Human bocavirus genotype 1 was the first genotype to be described and has a published prevalence rate of approximately 4.4–25% in symptomatic children patients with upper and lower respiratory tract infections.[7,8] The other genotypes (HBoV-2 to 4) have been detected in stool specimens of children with acute gastroenteritis (AGE) at a reported prevalence rate of 1.4–24.6%, 0.5-2.7% and 0-0.5%, respectively.[10,11] Reports on HBoV

in Africa have mainly focused on respiratory infections, with very few data on AGE. The few African studies on HBoV in AGE reveal detection rates of 2% (2/100) in children <2 years of age from Egypt[12] and 2.2% (7/317) in children <5 years of age from Gabon.[13] HBoV detection rates of 29% (28/96) in Nigeria and 33% (32/96) in Tunisia have also been reported in stool specimens from children with non-polio acute flaccid paralysis, aged between 4 months and 15 years.[14]

In South Africa (SA), HBoV have only been described in respiratory infections, with a reported prevalence of 11% (38/341) in children <2 years diagnosed with pneumonia and in 9.5% (49/517) of human immunodeficiency virus (HIV)-infected and 13.3% (125/943) of HIV-uninfected children hospitalised with respiratory infections.[15,16] Although the role of HBoV has been established in respiratory infections, HBoV significance in AGE is still unclear as the virus is frequently detected as a co-infection with other enteric pathogens and has also been detected in healthy individuals.[14,17] Additional evidence and studies are, therefore, needed to gain better national and global understanding of HBoV significance in AGE. In this study, 3765 diarrhoeal stool specimens from children <5 years of age hospitalised for the treatment of diarrhoea were collected in SA from April 2009 to May 2015. The specimens were examined for HBoV prevalence and molecular diversity and the associated patient data analysed to determine epidemiology and associated environmental factors.

2. Materials and methods

2.1 Ethical approval

Ethical approval for this study was granted by the Faculty of Health Sciences Research Ethics Committee, University of Pretoria, SA: Number (383/2015). The parent Rotavirus

Sentinel Surveillance Program has been approved by the University of Witwatersrand, SA (Approval No.M091018).

2.2 Study participants and sites

The study was conducted as a part of the South African Rotavirus Sentinel Surveillance Program (RSSP). Between April 2009 and April 2015, stool specimens were collected from children <5 years of age hospitalised for AGE, defined as the passing of three or more liquid or loose stools within 24 hours, for 7 days or less. Patients were recruited from four hospitals including Chris Hani-Baragwanath Academic Hospital (CHBAH) in an urban area of Gauteng Province, Edendale Hospital (EDH) in a peri-urban area of KwaZulu-Natal Province and, Matikwana (MKH) and Mapulaneng hospitals (MPH) in rural areas of Mpumalanga Province in SA. A standardised questionnaire was used to record demographic information, clinical presentation, medical history, socioeconomic and associated environmental factors for AGE cases. Stool specimens were collected within 48 h of admission and dried blood spots were collected from patients who gave consent for HIV testing. Specimens were transported to the Centre for Enteric Diseases (CED) at the National Institute for Communicable Diseases (NICD) for further screening.

2.3 Specimens processing and nucleic acid extraction

Faecal suspensions (10% (w/v) in distilled water) were prepared and clarified by centrifugation at 5000 *x g* for 5 minutes (Eppendorf centrifuge 5430, Merck, USA). Viral nucleic acid (both DNA and RNA) was extracted using QIAamp® Viral RNA Mini Kit (Qiagen, Hilden, Germany) according to manufacturer's instructions for automatic extraction in QIAcube extractor (Qiagen) and eluted in 60 µℓ of buffer before storage at -40˚C.

2.4 Enteric pathogen screening

All specimens were screened for HBoV by real-time PCR targeting the highly conserved NS1 region.[18] Specimens were also tested for the presence of five other enteric viruses: namely RV (enzyme immunoassays, conventional reverse transcription (RT)-PCR), NoV (real-time RT-PCR), SaV (real-time RT-PCR), human AstV (HAstV; real-time RT-PCR) and human AdV (HAdV; real-time PCR) as previously described.[19] Enteropathogenic bacteria were screened using standardised culture methods and parasites were screened using standardised staining and microscopy protocols as previously described.[19] Dried blood spots were tested for HIV RNA using the AmpliPrep automated extraction and COBAS TaqMan (Roche Diagnostics GmbH, Mannheim, Germany) automated real-time detection of HIV-1.[20]

2.5 Nucleotide sequencing, genotyping, and phylogenetic analysis

Genotyping of HBoV was performed by nucleotide sequence analysis of the VP1/VP2 region (576bp) using previously published methods.[14] For nucleotide sequence analysis, amplicons were purified either directly using ExoSAP-it (USB Corporation, Cleveland, OH) or QIAquick PCR purification kit (Qiagen). Purified PCR products were sequenced on an ABI 3500 Genetic Analyzer (Applied Biosystems/Life Technologies, Foster City, CA). The forward and reverse sequences were assembled by DNA Baser Sequence Assembler v5.15 (Heracle BioSoft SRL Romania, http://www.DnaBaser) and subjected to BLAST search (http://www.ncbi.nlm.nih.gov) to confirm identity. The sequences were submitted to GenBank and accession numbers MK387148 to MK387169 were assigned. Reference nucleotide sequences of HBoV strains obtained from GenBank (Accession numbers. AB763342; GQ243652, FJ973563, GQ867666, FJ973561, GQ506568, KU667151, JQ267789, JX257046, FJ170279, JQ734543, DQ340570, HQ152962, KJ634207, KU667128,

JQ513498, KC823115) were aligned with South African strains using MAFFT version 6.[21] Phylogenetic relationships among strains were inferred with the maximum-likelihood statistical method and the Hasegawa-Kishino-Yano model as determined by j-Model test program and implemented in MEGA7 (software version 7.0.14)[22] with 1000 bootstrap replicates.

2.6 Statistical analysis

All statistical analyses were performed using Stata software package, version 11.1 (Stata Corp., College Station, TX). Demographic data, clinical and environmental factors associated with HBoV detection were compared in children with or without HBoV infection, and in HBoV-positive cases; HIV-positive and HIV-negative children were compared. Categorical variables were described using frequency and percentages. Chi-square and Wilcoxon rank-sum tests were used to compare means and medians, respectively, while univariate and multivariate logistic regression were used to identify characteristics that were associated with HBoV infections or HIV status. Factors with p-values ≤ 0.2 resulting from univariate pairwise analysis were used in multivariate regression model adjusting for age, gender, site and year of collection. Differences within groups were calculated as odds ratios (OR) and p-values <0.05 were considered statistically significant.

3. RESULTS

3.1 Detection and epidemiology of HBoV

Between April 2009 and April 2015, 3765 stool specimens were collected from hospitalised children with AGE and 5.63% (212/3765) were positive for HBoV. Annual detection rates from 2009 to 2014 were between 4.16% and 7.62%, with the highest rate

observed in 2015 (10.52%; Figure 1, Table 1). Human bocavirus detection was similar across the sites throughout the study period (data not shown). Detection of HBoV increased from 4.69% (66/1407) in the 0-6 month age group to 6.61% (74/1119) in the 7-12 month age group, 6.38% (36/564) in the 13-18 month age group and 6.55% (19/290) in the 19-24 month age group (Table 1). Human bocavirus detection declined in older children (≥25 months; 4.47%; 17/380). The median age of children with HBoV was 10 months [interquartile range ([IQR]: 5.7-15). Univariate analysis showed that children ≤ 6 months had a lower prevalence of HBoV infection compared to children >7 months (OR = 1.34; 95% confidence interval (CI) 1.00-1.81; p=0.052). In addition, the HBoV prevalence by age group and year also showed differences (Figure 2).

Human bocavirus circulated throughout the year with a median monthly prevalence rate of 5.00% (IQR: 2.56-7.69) although prevalence spiked dramatically in December 2013 (30.77%; 4/13), February 2014 (17.24%; 5/29) and February 2015 (14.29%; 4/28; Figure 1). In addition, HBoV was detected more often in summer (adjusted OR (aOR)= 1.60; 95% CI 1.07-2.39; p=0.023) and autumn (aOR=1.60; 95% CI 1.09-2.34; p=0.017) compared to winter (Table 1). Univariate analysis showed that attending nursery school and increased numbers of people in the household were associated with HBoV infection. (Table 1). However, only nursery school attendance (aOR=1.68; 95% CI 1.08-2.61; p=0.021) was a significant predictor of HBoV among children when adjusted for age, site and year of collection (Table 1).

No differences in specific AGE symptoms were noted in HBoV positive compared to negative cases or in HIV-positive compared to HIV-negative children who were positive for HBoV (data not shown).

3.2 Human bocavirus co-infections with other enteric pathogens

In specimens fully screened for enteric viruses, bacteria and parasites (n=1654), HBoV was the only pathogen detected in 0.91% (15/1654) of cases and in 4.47% (74/1654) of cases with mixed pathogen infections. In 20.80% (344/1645) of fully screened cases, no enteric pathogens were detected. Only 42% (89/212) of the HBoV positive specimens were fully screened and HBoV prevalence in specimens without full screening was slightly higher (6.19%; 123/1988) compared to fully screened specimens (5.38%; 89/1654) although the difference was not statistically significant (p=0.57).

The enteric pathogens most often identified in HBoV co-infections were bacteria (n=43), HAdV (n=24) and RV (n=22). In univariate analysis, bacteria (OR=2.09; 95% CI 1.36-3.21; p=0.001), HAdV (OR=1.70; 95% CI 1.05-2.77; p=0.032), parasites (OR=1.82; 95% CI 1.05-3.17; p=0.032) and SaV (OR=2.33; 95% CI 1.25-4.33; p=0.007),) were significantly associated with HBoV (Table 1). However, only bacterial (aOR=2.20; 95% CI 1.41-3.45; p=0.001) and SaV (aOR=2.05; 95% CI 1.08-3.86; p=0.027) co-infections were significantly associated with HBoV in multivariate analysis.

3.3 Distribution of human bocavirus genotypes

Human BoV strains were successfully genotyped in 90% (191/212) of cases. Among the genotypes identified, HBoV-1 was the most frequently observed and accounted for 79.6% (152/191) of all typed HBoVs. The three other genotypes appear to circulate at lower frequency, and included HBoV-3 in 13.6% (26/191), HBoV-2 in 5.2% (10/191) and HBoV-4 in 1.6% (3/191) of cases. No significant associations were found between individual HBoV-2, -3 or -4 strains and demographic, clinical or environmental factors (data not shown).

Of the 191 strains sequenced, only 122 had sequence of adequate length (565bp) and quality to investigate divergence in the HBoV genome. All sequences clustered into the concordant genotypes (HBoV-1 to -4) assigned by the capsid gene (Figure 3). Multiple sequence alignments showed high nucleotide identity (97.9–100%) within the four genotypes with most of the nucleotide variations conserved at the amino acid level (99.5–100% sequence identity). In HBoV-1 strains (n=98), nucleotide differences were observed at 12 sites, while HBoV-2 (n=3) strains showed 15 differences, HBoV-3 (n=17) had 17 differences and HBoV-4 (n=2) only displayed two nucleotide differences. However, amino acid substitutions were similar among HBoV genotypes with HBoV-1 showing three and HBoV-2-4 displaying two amino acid substitutions in each genotype cluster. The mean diversities within the four genotypes, calculated using MEGA7 (maximum composite likelihood model) ranged from 0.002 to 0.007 base substitutions per site.

4. DISCUSSION

Human BoV, first described in 2005, has been associated with upper and lower-respiratory tract infections and gastrointestinal illness throughout the world.[10] However, the aetiological role of HBoV in gastrointestinal illness has not been fully elucidated and remains uncertain. Moreover, no studies to our knowledge have ever been conducted on HBoV and AGE in the South African population. Evaluation of HBoV circulation over a six-year period in children <5 admitted to hospital for AGE treatment showed a prevalence rate of 5.63%, similar to previous reports.[8] This was higher than earlier studies in Africa including one by EL-Mosallamy et al.[12] that detected HBoV in 2% of children <2 years in Egypt and Lekana-Douki et al.[13] in 2.2 % in children <5 years in Gabon.

Human BoVs were mostly detected in children <2 years with a peak prevalence observed between 6-12 month of age, similar to previous reports.[23,24,25] It is thought that children are protected from HBoV infections by maternal antibodies, which decline at 6 months, with a rebound in immunity after 24 months due to exposure.[26] Although our findings showed a similar trend, the significance of the associations between HBoV detection and age could not be confirmed with multiple logistic regression. While the impact of declining diarrhoeal cases may play a role in the variable HBoV prevalence by age group and year, additional study related to co-infections and strain circulation may also be warranted.

The seasonality of HBoV have been reported to vary among different countries and regions, with most studies suggesting higher detection rates in winter.[23,27,28,29]. Although a previous SA study on HBoV in children with respiratory tract infections observed higher incidences in the autumn-winter period, our study showed increased HBoV circulation in summer and autumn (December to May) of each year in AGE cases.[15]

Human bocavirus genotype distribution in children with AGE has been shown to vary by country, study period, type of specimen and study population.[25] Genotype 1 strains are primarily associated with respiratory infections, and therefore, the frequent detection in stools specimens would seems to indicate a concurrent respiratory tract infection rather than AGE.[8,25,30,31] However, the clinical presentation of HBoV-1 has been reported to include diarrhoea, and AGE is cited as one of the reasons for seeking health care services in 10% of children with acute respiratory infections due to HBoV-1.[32] This has led to a suggestion that HBoV-1 may be a pneumoenteric virus, with infection starting on

respiratory surfaces before spreading to the intestines.[3,30] Further research will be required to establish the association with diarrhoeal episodes.

Human bocavirus genotype 3 was the second most frequent strain detected in this study, contrary to previous reports describing HBoV-2 as more common.[21,25] In contrast to HBoV-1, HBoV-2-4 are considered enteric viruses because of their detection in AGE stools but not in respiratory specimens.[10,33] Few studies have reported the detection of HBoV-4 in children with AGE, and prevalence usually ranges from 0-0.5%, lower than seen in the current study.[25,33] One other study in Western India has reported higher HBoV-4 prevalence rates of 12% (3/24) in children ≤5 years hospitalised with AGE. [25]

The high rate of co-infection observed in this study is in accordance with previous reports which cite rates as high as 83% for respiratory specimens and 100% for faecal specimens.[31,34,35] Similarly, HBoV co-infections were high in this study, with only 15 cases detected alone in children with fully screened specimens. It has been suggested that the high rate of co-infection is because HBoV is either a helper virus, facilitating replication of other pathogens or that it requires a helper virus to facilitate a productive infection.[36] Our findings show bacteria and SaV, known AGE agents, were common in co-infections and significantly associated with HBoV, making it challenging to demonstrate the clinical importance of HBoV.[37] Moreover, the effect of co-infection on disease severity could not be established, similar to previous reports.[38]

Phylogenetic analysis in this study showed HBoV-1 strains were highly conserved in the VP1/2 capsid region demonstrating a low degree of genetic variability (i.e. 80 of the 98 HBoV-1 strains were identical). All the sequences clustered closely to reference HBoV

genotypes from GenBank although HBoV-2-4 were less similar than HBoV-1 strains. Previous studies have reported higher diversity in HBoV-2-4 compared to HBoV-1 which is comparable to our findings.[7,18]

Numerous studies that have examined the role of HBoV in diarrhoeal diseases have either supported or dismissed the significance of HBoV as a pathogen in AGE.[20,33,39] In the current study, no statistically significant association was observed between HBoV detection and any gastrointestinal symptoms. Our findings confirm the suggested notion that, in children hospitalised with severe AGE, the presence of HBoV is of minor importance and may not be related to AGE, especially when HBoV-1 is detected. The association of HBoV and AGE was not clear in this study due to absence of an appropriate diarrhoeal-free control group and the high levels of co-infections with other gastroenteritis pathogens.

Author's contribution

NAP: conceived, designed, supervised the study and edited manuscript

RN: performed laboratory tests & data analysis and wrote original draft of manuscript

SN: review of the manuscript

LC: performed data and phylogenetic analysis and finalised manuscript

MBT: reviewed, revised and edited manuscript

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Competing interests

The authors declare that they have no competing interests.

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Table 1: Univariate and multivariate analysis of demographic, clinical characteristics and environmental features associated with HBoV detection in children with AGE. Only variables with p-values <0.2 in the univariate analysis were reported and included in the multivariable model.

Parameter	**Bocavirus prevalence n/N (%)**	**Univariate analysis**		**Multivariate analysis**	
		Odds Ratio (OR; 95% Confidence Interval (CI)	**p-value**	**§Adjusted OR (aOR; 95% CI)**	**p-value**
Age (in months); unknown (n=5)					
0-6	66/1407 (4.69%)	ref	ref		
7-12	74/1119 (6.61%)	1.43 (1.02-2.02)	0.037		
13-18	36/564 (6.38%)	1.38 (0.91-210)	0.127		
19-24	19/290 (6.55%)	1.42 (0.84-2.41)	0.127		
≥25	17/380 (4.47%)	0.95 (0.55-1.64)	0.858		
Gender; unknown (n=1)					
Male	81/1639 (4.94%)	ref	ref		
Female	131/2125 (6.16%)	1.26 (0.95-1.68)	0.107		
Year; only years with data collected for a full 12 months were included in analysis					
2009 (April – December)	41/635 (6.46%)	**Not included in the analysis**			
2010	50/919 (5.44 %)	ref	ref		
2011	23/553 (4.16%)	0.75 (0.45-1.25)	0.274		
2012	20/459 (4.36%)	0.79 (0.47-1.35)	0.389		
2013	41/538 (7.62%)	1.43 (0.94-2.20)	0.098		
2014	22/519 (4.24%)	0.77 (0.46-1.29)	0.317		
2015 (January-April)	15/142 (10.52%)	**Not included in the analysis**			
Maximum number of stools per 24 hours; unknown (n=247)					
≤3	35/742 (4.72%)	ref	ref		
4-5	106/1820 (5.82%)	1.24 (0.84-1.84)	0.266		
≥6	60/956 (6.28%)	1.35 (0.88-2.07)	0.167		
Supplementary oxygen given; unknown (n = 1673)					
No	112/1992 (5.62%)	ref	ref		

Yes	1/100 (1.00%)	0.17 (0.02-1.23)	0.079		
Child's HIV status; unknown (n= 482)					
Negative	157/2921 (5.37%)	ref	ref		
Positive	26/362 (7.18%)	1.36 (0.89-2.09)	0.159		
Electricity in the house; unknown (n=171)					
No	90/1379 (6.53%)	ref	ref		
Yes	114/2215 (5.15%)	0.77 (0.58-1.03)	0.083		
Number of people that sleep in the same house as child; unknown (n= 1214)					
≤2	55/1224 (4.49%)	ref	ref		
3-6	68/1182 (5.75%)	1.30 (0.90-1.87)	0.162		
≥7	12/145 (8.28%)	1.92 (1.00-3.67)	0.050		
Nursery school attendance; unknown (n = 1223)					
No	97/2101 (4.62%)	ref	ref	ref	ref
Yes	35/441 (7.94%)	1.78 (1.9-2.66)	0.005	1.68 (1.08-2.61)	0.021
Outcome; unknown (n = 136)					
Died	2/92 (2.17%)	ref	ref		
Discharged	201/3537 (5.68%)	2.71 (0.66-11.08)	0.165		
Co-infecting pathogens; only fully screened specimens were included in the analysis (n=1654)					
HBoV only	15/1654 (0.91%)	ref	ref	ref	ref
HBoV + Bacteria	43/1654 (2.60%)	2.09 (1.36-3.21)	0.001	2.20 (1.41-3.45)	0.001
HBoV + Adenovirus	24/1654 (1.45%)	1.70 (1.05-2.77)	0.032		
HBoV + Parasites	17/1654 (1.03%)	1.82 (1.05-3.17)	0.032		
HBoV + Sapovirus	13/1654 (0.79%)	2.33 (1.25-4.33)	0.007	2.05 (1.08-3.86)	0.027
HBoV + 1 pathogen	28/1310 (2.13%)	3.43 (1.81-6.50)	0.000	**Not included in the analysis**	
HBoV + 2 or more pathogens	46/1310 (3.51%)	16.53 (8.96-30.47)	0.000		
No pathogen detected	344/1654 (20.80%)				
Seasonal detection					
Winter (June-August)	52/1163 (4.47%)	ref	ref	ref	ref
Spring (September-November)	32/737 (4.34%)	0.97 (0.62-1.52)	0.894	1.00 (0.64-1.59)	0.968
Summer (December-February)	58/892 (6.50%)	1.49 (1.01-2.18)	0.044	1.60 (1.07-2.39)	0.023

Autumn (March-May)	70/973 (7.19%)	1.66 (1.14-2.40)	0.007	1.60 (1.09-2.34)	0.017
Monthly detection					
January	18/311 (5.79%)	ref	ref		
February	29/440 (6.59%)	1.15 (0.63-2.11)	0.655		
March	25/385 (6.49%)	1.13 (0.60-2.11)	0.701		
April	23/253 (9.09%)	1.63 (0.86-3.09)	0.136		
May	22/335 (6.57%)	1.44 (0.60-2.18)	0.681		
June	27/473 (5.71%)	0.99 (0.53-1.82)	0.963		
July	15/379 (3.96%)	0.67 (0.33-1.35)	0.379		
August	10/310 (3.23%)	0.54 (0.25-1.20)	0.129		
September	9/235 (3.83%)	0.65 (0.29-1.47)	0.299		
October	12/235 (5.11%)	0.88 (0.41-1.86)	0.730		
November	11/267 (4.12%)	0.70 (0.32-1.51)	0.362		
December	11/141(7.80%)	1.38 (0.63-3.00)	0.420		

§Adjusted for age, gender, site and year

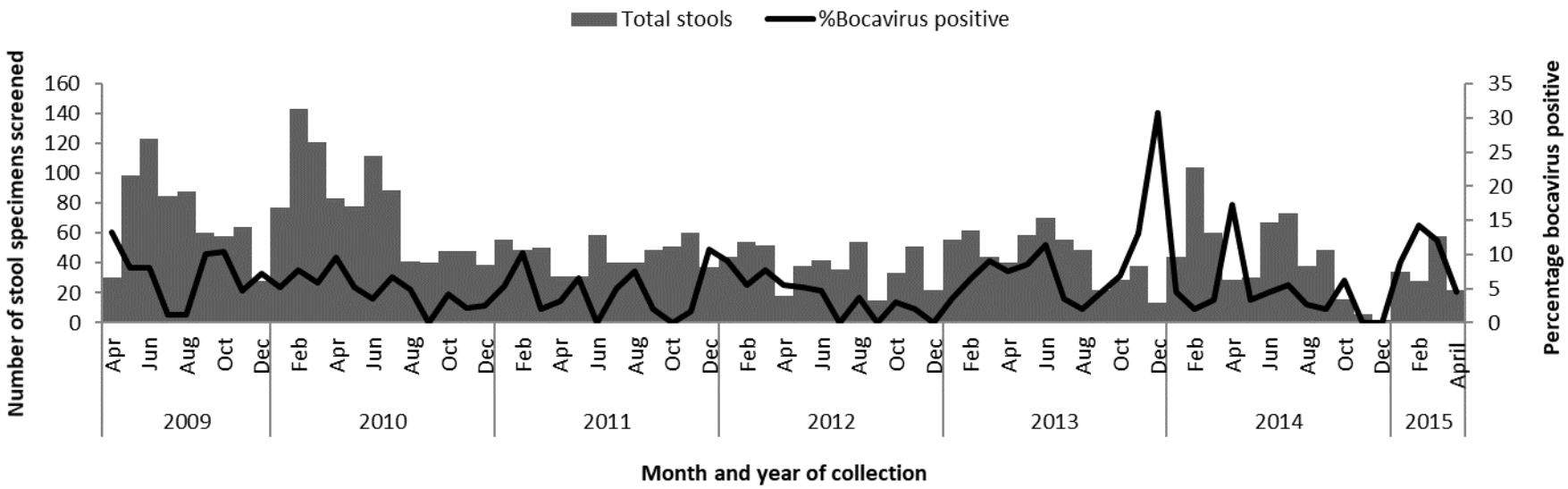

Figure 1: Monthly stool collection and percentage human bocavirus positive in children <5 years with acute gastroenteritis, April 2009–April 2015.

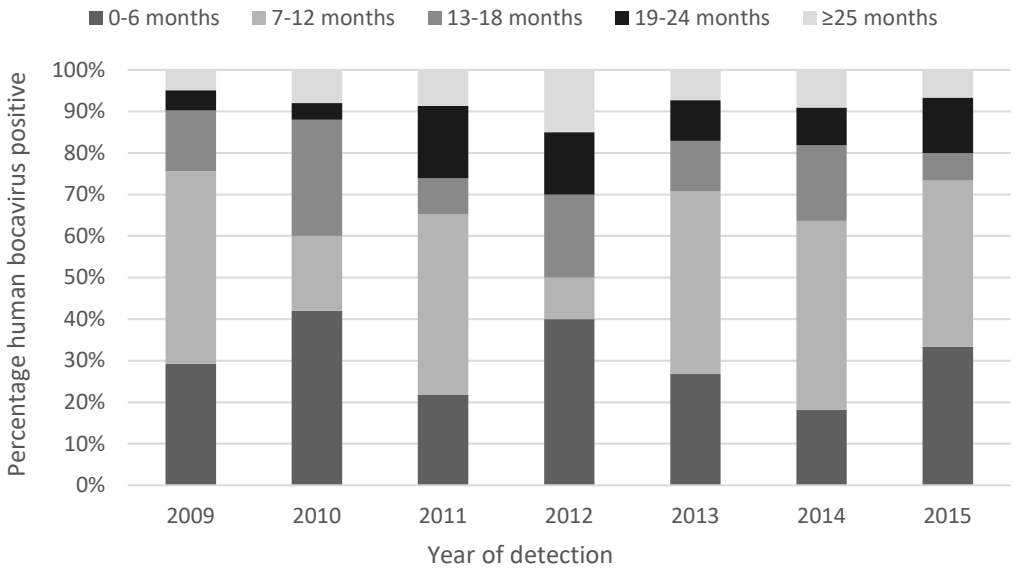

Figure 2. The age distribution of human bocavirus detection by year, 2009-2015. Collections for 2009 only spanned 9 months (April to December) and collections for 2015 only spanned 4 months (January to April).

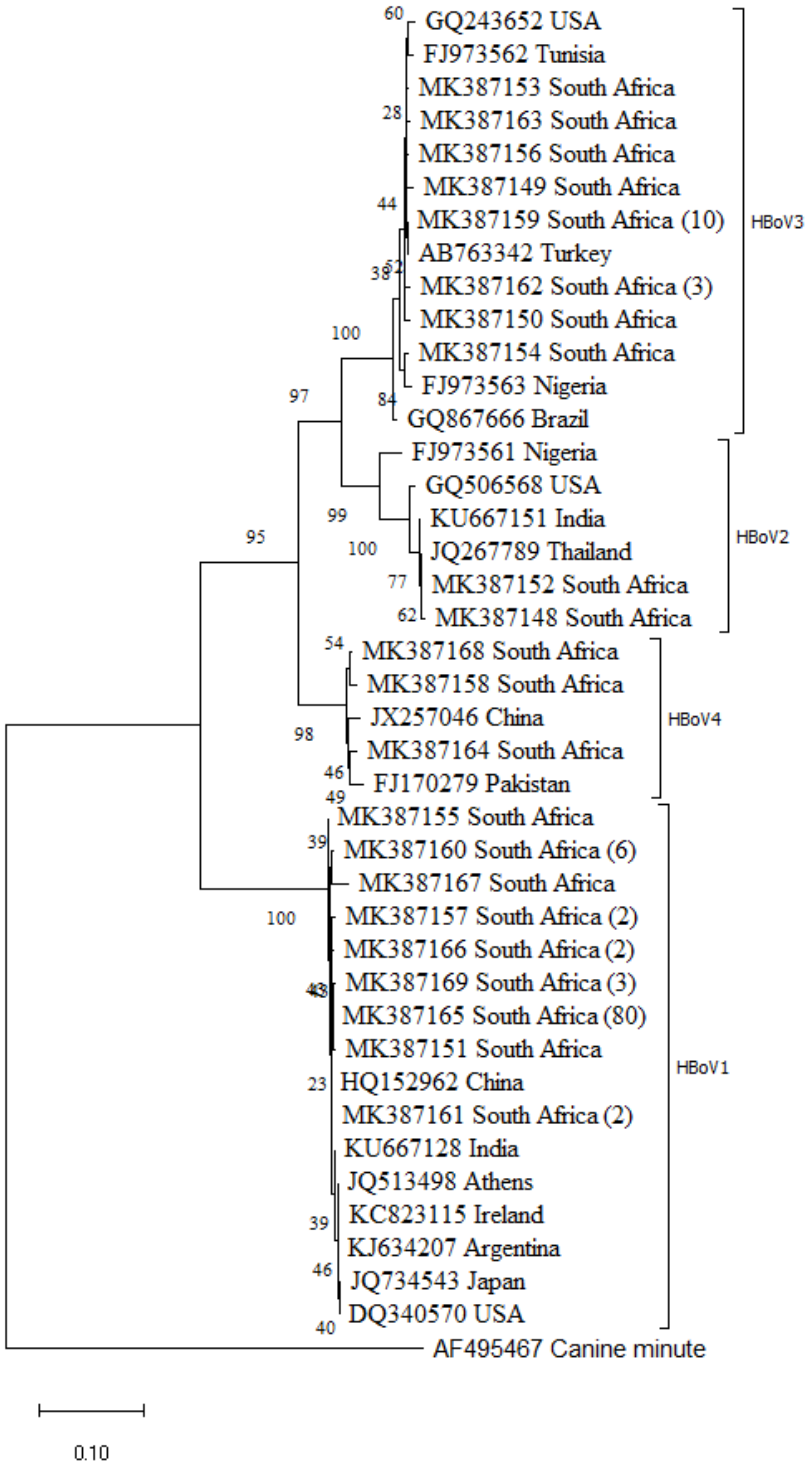

Figure 3. Phylogenetic analysis of partial VP1/VP2 gene sequences from South African and reference human bocavirus (HBoV) strains. Phylogeny was constructed using the maximum-likelihood statistical method with 1000 bootstrap replicates and the Hasegawa-Kishino-Yano model as determined by j-Model test program and implemented in MEGA7 (software version 7.0.14). The percentage of trees in which the associated taxa clustered together is shown next to the branches. Genotypes indicated are human bocavirus (HBoV) 1 to 4.

Note: SA-MKH-4417 = South African (SA) HBoV strain collected from/at Matikwana hospital (MKH), stool specimen 4417. Other hospital abbreviations Chris Hani-Baragwanath Academic Hospital (CHBAH), Edendale Hospital (EDH) and Mapulaneng hospitals (MPH).

NCBI accession numbers for the isolates include SA-CHBCH-3661 (MK387148), SA-CHBCH-4185 (MK387149), SA-CHBCH-4800 (MK387150), SA-CHBCH-4832 (MK387151), SA-CHBCH-6929 (MK387152), SA-CHBCH-8466 (MK387153), SA-CHBCH-8478 (MK387154), SA-CHBCH-8793 (MK387155), SA-CHBCH-9678 (MK387156), SA-MKH-4417 (MK387157), SA-MKH-4552 (MK387158), SA-MKH-4790 (MK387159), SA-MKH-5760 (MK387160), SA-MPH-4402 (MK387161), SA-NGH-6449 (MK387162), SA-NGH-6693 (MK387163), SA-RCCH-10093 (MK387164), SA-RCCH-13107 (MK387165), SA-RCCH-6745 (MK387166), SA-RCCH-6875 (MK387167), SA-RCCH-6919 (MK387168) and SA-RCCH-8891 (MK387169)