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ORIGINAL ARTICLE

Human polyomavirus KI, WU, BK, and JC in healthy volunteers

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Abstract

Despite the growing importance of infections caused by the human polyomaviruses (HPyVs), information about their transmission, pathogenesis, and epidemiology is scarce. The objective of this work was to evaluate the excretion and distribution of HPyV (HPyV1-HPyV4 [former BKPyV, JCPyV, KIPyV, and WUPyV, respectively]) among asymptomatic individuals from different geographic regions in Brazil, in order to verify the existence of distinct epidemiologic patterns among the Brazilian population. Saliva samples from 889 healthy volunteers living in nine locations in Brazil were analyzed by real-time polymerase chain reaction (PCR) to detect HPyV1–4. Among 889 participants, 346 (39%) had evidence of infection with one or more HPyV species: 127 (14.3%) had HPyV1 only; 70 (7.9%) had HPyV3 only; 60 (6.7%) had HPyV4 only, and 25 (2.8%) had HPyV2 only. Coinfections were detected in 64 participants (7.3%). Although HPyV excretion was detected in samples from all locations, the frequency and distribution of viral species varied significantly. The epidemiologic findings presented demonstrate that the four HPyV species studied are circulating in five geographic regions of Brazil. Salivary excretion of these viruses appears common among healthy Brazilians. The distribution of viral species varies considerably between regions as well as within regions.

Keywords Polyomaviruses · Saliva · BKPyV · JCPyV · KIPyV · WUPyV

Introduction

Human polyomaviruses (HPyVs), of the *Polyomaviridae* family, are non-enveloped, double-stranded DNA viruses [1]. Recently, the International Committee on Taxonomy of Viruses (ICTV) renamed all species of polyomavirus, identifying them numerically according to the order of description [2]. Consequently, polyomaviruses species formerly known as BKPyV, JCPyV, KIPyV, and WUPyV are now named HPyV1, 2, 3, and 4, respectively [2].

Although the routes of transmission for HPyVs remain unknown, oral and respiratory routes of transmission have been proposed [1]. Primary infection usually occurs sub-clinically during childhood and establishes a persistent infection [1]. HPyV infection has been associated with disease in immunocompromised persons [1, 3, 4]. However, HPyV excretion has been observed in samples from immunocompetent persons, and the significance of these infections is unknown.

The objective of this work was to evaluate the excretion and distribution of HPyV (HPyV1-HPyV4) among asymptomatic individuals from various geographic regions in Brazil, in order to verify the existence of distinct epidemiologic patterns among the Brazilian population.

Materials and methods

Saliva samples were collected from healthy volunteers at nine locations in five geographic regions in Brazil (Table 1). Samples were collected during the period from August 2010 to September 2017. Each healthy volunteer provided a 5-mL saliva sample. Volunteers were recruited through posters distributed on college campuses, residential condominiums, and outpatient clinics. All volunteers of any age who agreed to participate and who stated that they did not have a history of immunocompromise or chronic disease were included in the study. No professional health survey was performed. All salivary samples collected were included in the study. After collection, samples were transported to the laboratory and stored

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Table 1 Distribution of saliva samples by location

Region	State	Gender		No. of samples
		Female	Male	
Southeast	Rio de Janeiro	169	86	255
	Minas Gerais	102	60	162
Northeast	Bahia	58	37	95
	Pernambuco	64	26	90
	Rio Grande do Norte	7	16	23
South	Santa Catarina	13	17	30
	Paraná	61	57	118
Midwest	Distrito Federal	49	22	71
North	Pará	31	14	45
Total		554	335	889

in a freezer at − 70 °C until processing time. Salivary samples were processed and analyzed for detection of HPyV within a maximum of 3 months after collection. Specimens were tested for the presence of HPyVs using a step-one real-time polymerase chain reaction (PCR) system (Thermo Fischer Scientific, Waltham, Massachusetts, USA), as previously described [5].

The protocol for this study was reviewed and approved by the Ethics Committee of the Hospital Universitário Clementino Fraga Filho (protocol number 891.574) of the Federal University of Rio de Janeiro, Brazil.

Statistical analysis was performed using Minitab for Windows Release 16.0 (Minitab Inc., State College, PA). Differences between groups were considered significant at P values < 0.05.

Results

Of the 889 saliva samples analyzed, 346 (39%) were positive for any of the four HPyVs screened for in this study: 127 (14.3%) had HPyV1 only; 70 (7.9%) had HPyV3 only; 60 (6.7%) had HPyV4 only; 25 (2.8%) had HPyV2 only. Coinfections were detected in 64 participants (7.3%): 21 had HPyV1 plus HPyV4; 20 had HPyV1 plus HPyV3; 12 had HPyV1 plus HPyV2; 4 had HPyV2 plus HPyV3; 1 had HPyV4 plus HPyV3; 6 had HPyV1 plus HPyV2 plus HPyV3. Overall HPyV1 was detected with the highest frequency (21%; $n = 187$ samples, including coinfections).

Of the 889 participants, 335 (37.7%) were male, and 554 (62.3%) were female. Among men, 130 (38.8%) were positive for HPyV; among women, 216 (39%) were positive for HPyV. Among male participants, HPyV1, 2, 3, and 4 were detected in 20.9%, 6.9%, 10.7%, and 7.8%, respectively (including strains detected in coinfections). Among female participants, HPyV1, 2, 3, and 4 were detected in 21.1%, 4.3%, 11.7%, and 10.1%, respectively (including strains detected in coinfections). No difference between male and female participants was observed for the shedding of any HPyV species. Volunteers were stratified into groups according to age: 0 to 10 years ($n = 13$), 11 to 20 years ($n = 187$), 21 to 30 years ($n = 299$), 31 to 40 years ($n = 142$), 41 to 50 years ($n = 114$), 51 to 60 years ($n = 87$), 61 to 70 years (n = 29), and 71 years or older ($n = 18$). The frequency of HPyV detection was similar for all age groups (mean, 39.5%). Participants aged 0 to 10 years and 61 to 70 years had higher detection rates than the other subgroups (53.8 and 48.3%, respectively; Fig. 1a), but this difference was not significant. When analyzing the frequency of distribution of each individual species, the excretion of HPyV1 remained constant (21.1–25.4%) among age groups, with a slight decrease among participants aged 11 to 20 or ≥ 71 years. HPyV2 was not detected among individuals aged 0–10 years. Excretion peaked among individuals aged 21–30 years and decreased after 41–50 years. Excretion of HPyV3 increased with increasing age, until the age of ≥ 71 years, after which excretion decreased. HPyV4 showed elevated excretion among participants aged 0–10 and ≥ 71 years (Fig. 1b).

Higher mean viral loads were observed for HPyV4 and HPyV2, compared with other HPyV species. The geometric mean number of DNA copies detected was 177,950.0 copies/mL for HPyV4, 5465.5 copies/mL for HPyV2, 1896.7 copies/mL for HPyV1, and 102.5 copies/mL for HPyV3 (Fig. 2).

Although salivary excretion of HPyV was detected in all geographic locations, the frequency and distribution of viral species varied significantly. The lowest detection levels were observed in the southern region (15.3% in Paraná and 16.7% in Santa Catarina). The highest detection levels were observed in the northern region (71.1% in Pará) (Fig. 3).

In the southeast region, Rio de Janeiro and Minas Gerais had detection rates of 27.5 and 59.3%, respectively. Of the 70 positive samples collected in Rio de Janeiro, HPyV4 was detected in 51.4%, HPyV1 in 25.7%, HPyV3 in 12.9%, HPyV2 in 2.9%, and coinfections in 7.1%. In Minas Gerais, the 96 positive samples contained HPyV1 (29.2%), HPyV4 (25%), and HPyV3 (16.6%). There was a higher rate of coinfection in this area (29.2%), compared with others. HPyV2 was only detected in two samples that were coinfected (Fig. 3).

In the northeast region, Bahia, Pernambuco, and Rio Grande do Norte had detection rates of 67.4%, 22.2%, and 60.9%, respectively. Of the 64 positive samples in Bahia, 89% had HPyV1, and 1.6% had HPyV3; 9.1% of samples were coinfected. HPyV4 was not detected; HPyV2 was only detected in one sample that also contained HPyV1 and HPyV3. Of the 20 samples from Pernambuco, 90% were positive for HPyV3, 5% for HPyV2, and 5% for HPyV1; HPyV4 was not detected. In Rio Grande do Norte, 52.2% of samples were positive for HPyV2, 4.35% for HPyV1, and 4.35% for coinfection with HPyV1 plus HPyV2. Neither HPyV3 nor HPyV4 was detected (Fig. 3).

In the southern region, Paraná and Santa Catarina had detection rates of 15.3 and 16.7%, respectively. Of the 18 samples in Paraná, 77.8% were positive for HPyV1, 16.7% for

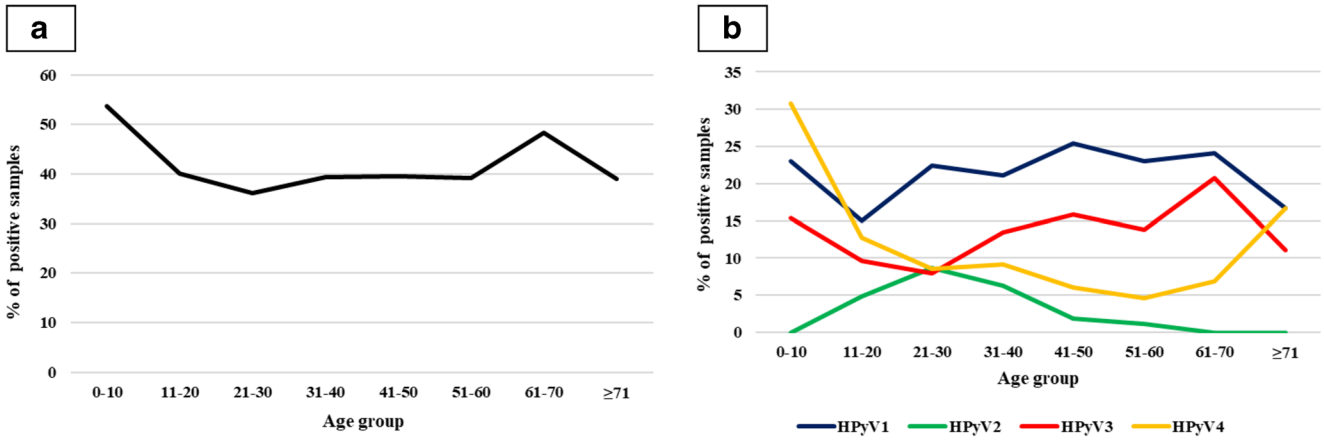

Fig. 1 Age distribution of healthy volunteers tested for salivary presence of human polyomavirus. **a** Percentage of detection of any HPyV species tested. **b** Percentage of detection of each HPyV species

HPyV2, and 5.5% for HPyV1 plus HPyV2 coinfection. HPyV3 and HPyV4 were not detected. In Santa Catarina, HPyV3 was detected in all five positive samples (Fig. 3).

Of the 27 positive samples collected in the Federal District of the Midwest region, HPyV3 was present in 63%, HPyV1 in 14.8%, HPyV2 in 11.1%, and coinfections with HPyV1 plus HPyV3 in 11.1%. HPyV4 was not detected in the Federal District (Fig. 3).

In the northern state of Pará, 71.1% ($n = 32$) of samples were positive for HPyV. Of these, HPyV1, HPyV2, and HPyV3 were each detected singly in 12.5% of samples; coinfection with these species was detected in 62.5% of samples. HPyV4 was not detected (Fig. 3).

Discussion

Asymptomatic excretion of HPyV has often been observed in respiratory, urine, and blood samples from immunocompetent asymptomatic individuals [5–13]. We found that 39% of participants in this study were asymptomatically excreting some of the HPyV species surveyed in their saliva. These individuals are potential sources of viral transmission to the immunocompromised population, which may develop severe disease [1, 3, 4].

In this study, there was no significant difference in viral excretion between male and female participants in the study. The literature does not yet define an association between male

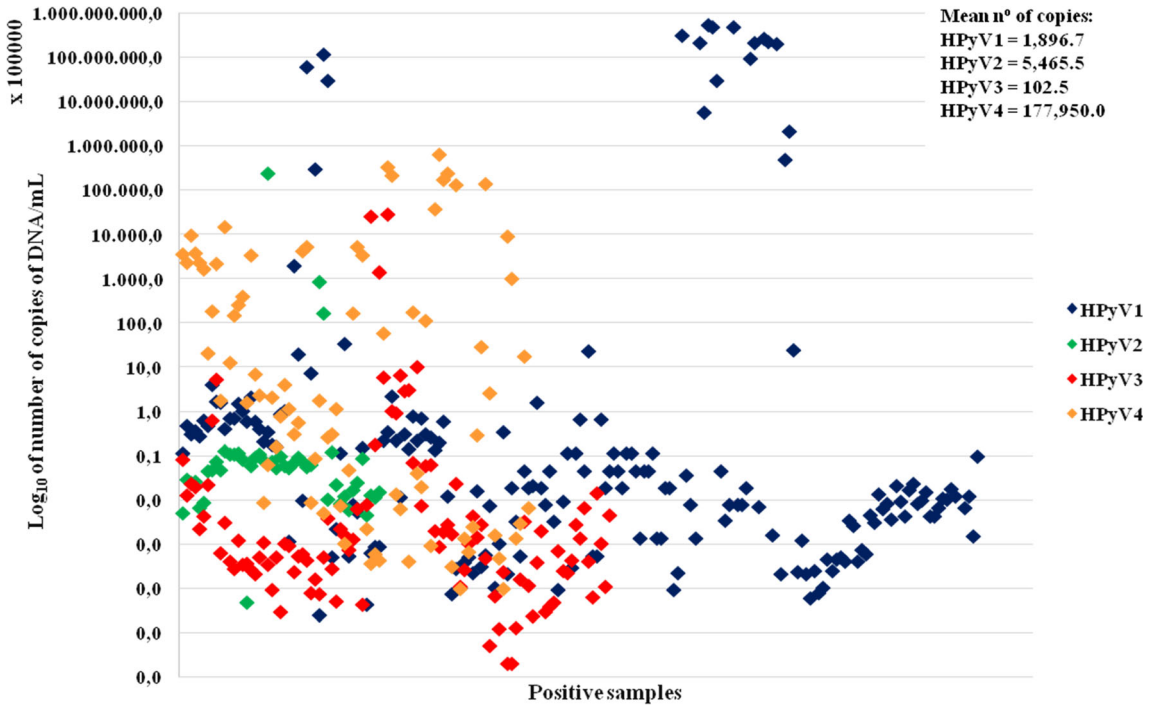

Fig. 2 Human polyomavirus loads in saliva samples collected from 889 healthy volunteers. Viral loads are shown for each HPyV species tested, with each point representing one sample. One sample from each donor was examined. Data are expressed as log of DNA copies per mL saliva

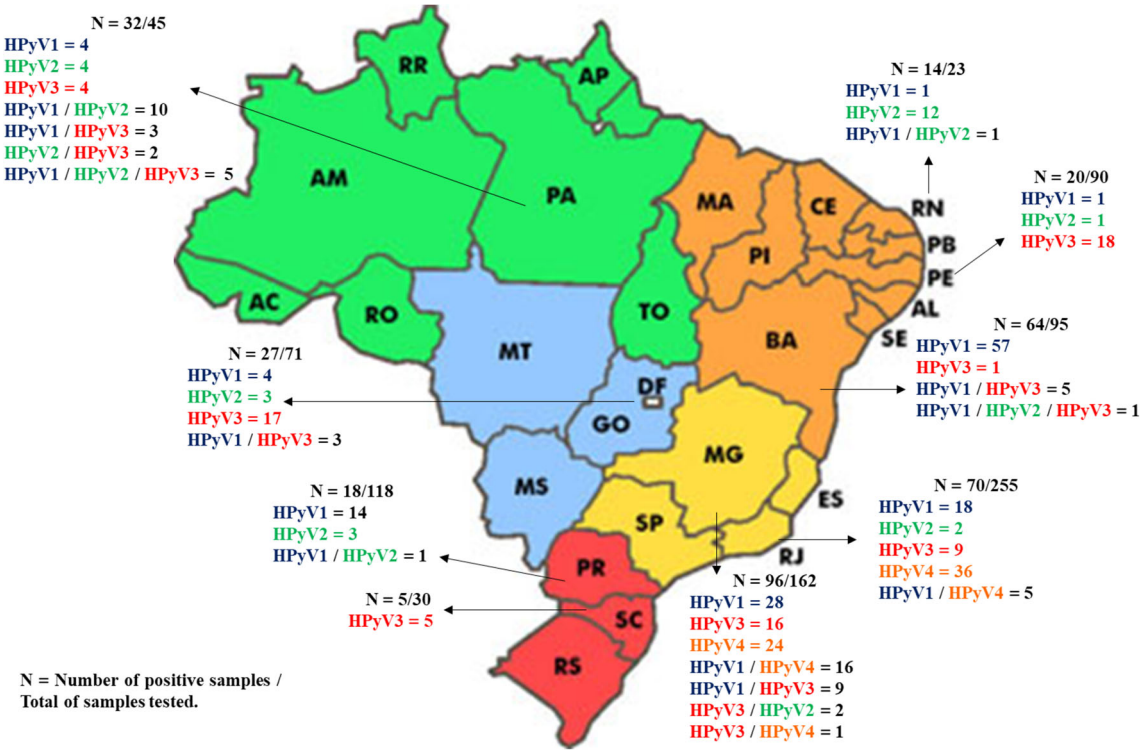

Fig. 3 Distribution of human polyomavirus species detected. Geographical regions are identified by distinct colors: north in green, northeast in orange, Midwest in blue, southeast in yellow, and south in red. BA Bahia, DF Federal District, MG Minas Gerais, PA Pará, PE Pernambuco, PR Paraná, RJ Rio de Janeiro, SC Santa Catarina

or female and increased excretion of HPyV. However, some studies have reported higher viral excretion among women [5, 14]. Pregnancy-associated HPyV1 and HPyV2 excretion has been documented and is thought to be related to the immunologic and hormonal changes of pregnancy [15]. The hypothesis that estrogen production during menstrual cycles could influence the urinary excretion of HPyV1 remains to be proven [16].

Higher detection rates were observed among participants aged 0 to 10 years and among those aged 61 to 70 years. Elevated rates of viral excretion among children are expected because primary infection usually occurs in childhood [1, 3]. The second peak observed among older individuals could reflect the occurrence of viral re-exposure or a decline in the immune response of these individuals, similar to that observed in immunocompromised individuals [1, 3, 4].

Diverse HPyV species were detected across geographic regions as well as within the same region. HPyV1 was detected in all geographic areas except Paraná; HPyV3 was detected in all geographic areas except Santa Catarina and Rio Grande do Norte. In contrast, HPyV4 was only detected in the southeast region (Rio de Janeiro and Minas Gerais), and HPyV2 was detected with high frequency in Pará but with low frequency in other localities.

DNA viruses have proven useful as markers that both corroborate and extend the population histories inferred from human DNA [17]. In the case of HPyV1 and HPyV2, several publications have reported that these viruses co-evolved with the human population and that they could be used as markers for human migration [18–20]. Although this characteristic has only been described for HPyV1 and HPyV2, it is reasonable to speculate that other HPyV species would be similarly useful. A multiplicity of nations has contributed to the formation of the Brazilian population, which comprises a genetically heterogeneous group of persons with European, African, and Amerindian origins [21]. The regional ethnic distribution of the Brazilian population has been strongly influenced by immigration throughout history. Therefore, it is possible that the observed variation in the frequency and distribution of HPyV species could be attributed to the genetic diversity of this population. However, this hypothesis must be tested by further studies involving population genetics.

Conclusions

In describing the circulation of four HPyV species in five Brazilian geographic regions, this study adds to the store of epidemiologic knowledge related to these viruses. The results also show that viral salivary excretion is common among the healthy Brazilian population and that the distribution of these species varies considerably by region as well as within regions.

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Compliance with ethical standards

The protocol for this study was reviewed and approved by the Ethics Committee of the Hospital Universitário Clementino Fraga Filho (protocol number 891.574) of the Federal University of Rio de Janeiro, Brazil.

Conflicts of interest The authors declare that they have no conflicts of interest.

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