



# SV40 seroprevalence in two Latin American countries involved in field trials of candidate oral poliovaccines

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## SUMMARY

**Objectives:** This study sought to determine SV40 seroprevalence in residents of two Latin American countries, Colombia and Nicaragua, which were sites of prelicensure oral poliovaccine (OPV) trials.

**Methods:** Archival sera were tested for SV40 neutralizing antibody using a virus-specific plaque-reduction assay. Samples included 517 sera from Colombia and 149 sera from Nicaragua.

**Results:** Overall SV40 seroprevalence was 22.8% for Colombian subjects and 12.8% for Nicaraguans. Subgroups of Colombian subjects ranged in frequency of SV40 seropositivity from 10.0% to 38.6%. Birth cohorts both older and younger than the age cohort that contained potential OPV vaccinees from both countries had SV40 antibodies. Gender and ethnicity had no significant effects on SV40 seropositivity.

**Conclusions:** Inhabitants of both Colombia and Nicaragua had detectable SV40 neutralizing antibody, including those of ages presumably not recipients of potentially SV40-contaminated OPV. This observation provides support for the concept that transmission of SV40 human infections can occur. Frequency of SV40 antibody positivity was elevated over that reported for the US where there was limited use of contaminated OPV. This investigation indicates also that study results of SV40 infections in humans will reflect whether subject populations had probable exposures to contaminated poliovaccines and to environmental conditions favoring cycles of viral transmission.

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## Introduction

Polyomavirus simian virus 40 (SV40) has been reported to cause infections in humans, although the prevalence and distribution of such infections are unknown. The natural host for SV40 is the rhesus macaque and the origin of cross-species human infections dates from 1954 and the use of contaminated poliovaccines.<sup>1,2</sup> Vaccine lots of both inactivated (IPV) and live attenuated oral (OPV) poliovaccine were potentially contaminated before the discovery of SV40 in 1960. The virus was an unrecognized agent present in many of the primary rhesus monkey kidney cell cultures used for vaccine production. In the case of IPV, some infectious SV40 survived the vaccine inactivation process.<sup>2</sup> It was early 1963 before all vaccine preparations were considered to be virus-free. Notably, individuals with evidence of SV40 infections are sometimes too young to have received contaminated poliovaccine directly, indicating more recent sources of virus exposure. Reports of experimental evidence of current infections in humans have been based

on assays of seroprevalence, detection of viral DNA in tissues, expression of viral antigens in tissues, and/or recovery of infectious virus.<sup>1,3</sup> SV40 is recognized to have oncogenic properties in laboratory animals and viral markers have been detected in some human cancers. In contrast, other reports have failed to detect evidence of SV40 human infections.<sup>1</sup>

A model has been developed to explain the discrepant reports regarding SV40 and human infections.<sup>1,3</sup> This model predicts that human infections were initiated primarily by the use of contaminated OPV, rather than IPV, because the titer of infectious SV40 was much higher in the oral vaccine and the route of exposure was arguably more natural (oral vs. intramuscular).<sup>2</sup> Those conditions would have increased the chances that an SV40 infection would be established in a vaccinee. This scenario predicts that SV40 infections were limited geographically, as they were dependent on the use of prelicensure contaminated OPV. Such field trials were carried out in Central and South America and in Russia, but only on a very limited scale in the US.<sup>1,3</sup>

Polyomaviruses are thought to establish long-term persistent infections. This would extend the duration for possible transmission by an infected individual. Cycles of SV40 infections theoretically could be maintained in exposed populations by horizontal

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infection of unvaccinated contacts by transmission of the virus via the fecal/urine–oral route.<sup>1,3</sup> This would occur most frequently in regions with poor sanitation, resulting in a higher prevalence of infections and higher seroprevalence. In contrast, in areas with good sanitation, viral transmission would be interrupted, resulting over time in a very low prevalence of infection and very low seroprevalence. In support of a fecal/urine–oral route of transmission, SV40 has been detected in stool and urine samples from humans<sup>4–9</sup> and in cage waste (feces, urine) of monkeys.<sup>10</sup> In addition, human polyomaviruses have been detected in sewage and contaminated waters.<sup>11–13</sup> A pattern of decreasing infection rates concomitant with increasing standards of living for agents spread by the fecal–oral route has been well-established (e.g., poliovirus, *Helicobacter pylori*, hepatitis A).<sup>14–16</sup> Thus, the model predicts that the outcome of studies of possible human infections by SV40 will differ, reflecting the particular characteristics of the populations surveyed.

A recent analysis of 400 archival sera collected from 1993 to 1995 detected ethnic differences in SV40 seroprevalence in women in Houston, Texas.<sup>17</sup> Neutralizing antibody prevalences among Caucasian and African-American women were 5% and 6%, respectively, similar to other US and UK reports.<sup>18–20</sup> In contrast, Hispanic women had a seroprevalence of 23% ( $p=0.01$ ). There is a large immigrant population from Latin America in Houston and it is known that potentially contaminated pre-licensure OPVs were used in several of those countries.

To follow up on our observation involving Houston Hispanics, we carried out studies using archival sera from two Latin American countries. We report here the frequency of SV40 neutralizing antibody in sera collected from residents in Colombia and Nicaragua. Both countries conducted mass immunization programs from 1958 to 1960 using a candidate prelicensure OPV. As predicted by the SV40–human infection model and suggested by the Houston study, SV40 seroprevalence rates were elevated in subjects from both Latin American countries compared to the US and UK rates.

## Materials and methods

### *Vaccination histories in Colombia and Nicaragua*

In early 1958, an outbreak of paralytic poliomyelitis occurred in the county of Andes, a mountainous rural area in Colombia. In response, a community vaccination program was carried out from May to August 1958 centered in the town of Andes, 80 miles south of Medellin. About 7000 children under the age of 7 years were vaccinated with the candidate live, attenuated OPV provided by Lederle Laboratories of the American Cyanamid Company.<sup>21</sup> This was followed by a campaign to vaccinate children under the age of 10 years in the city of Medellin. That program covered September 1958–April 1959 and vaccinated about 133,000 children using the same OPV from Lederle Laboratories.<sup>22</sup> The three poliovirus types were administered singly in capsules 3–4 weeks apart. The following year (July–September 1960) a mass immunization program in the city of Bogota vaccinated more than 187,000 children under the age of 7 years. This program used the single-dose Lederle oral, live-attenuated trivalent poliovirus vaccine.<sup>23</sup>

An epidemic of paralytic poliomyelitis also occurred in Nicaragua in 1958, with a large number of cases reported in the city of Managua. A vaccination program was started in Managua in September 1958 targeting children less than 10 years of age. The same live attenuated oral vaccine from Lederle Laboratories was employed that had been used in Colombia. The three vaccine strains were administered orally as fluid doses, with 3 weeks between each dose. This program was then extended to the surrounding rural area, followed by a maintenance program in Managua to vaccinate newborn infants. By May 1959, nearly 60,000 children had been vaccinated.<sup>24</sup> Vaccinations continued using the

Lederle trivalent vaccine. By April 1960, about 73,000 children under 10 years of age had received the oral vaccine.<sup>25</sup>

The production and testing of live, attenuated OPV strains by Lederle Laboratories was described.<sup>26</sup> The report included the history of vaccine lots used in the field studies in Colombia and Nicaragua. However, extensive tests of the vaccine preparations to rule out contamination by bacterial, fungal, or viral agents did not include assays for SV40 as that virus had not yet been discovered.

### *Serum samples*

The Colombian serum samples were collected in 1968–1970 in the city of Cali for a study of herpesvirus type 2 infection and had been stored at Baylor College of Medicine. Subjects included hospitalized cervical cancer patients (Group A) and matched cancer controls (Group B), members of the general population of the city (Group C), and prostitutes/sex workers (Group D). The Nicaraguan samples were collected in 2010 in the city of Managua for a study of *H. pylori* infection and had been stored in Managua. Subjects were patients undergoing endoscopy at the Hospital Escuela Lenin Fonseca (Group E). Studies were approved by the Institutional Review Board for Human Subject Research for Baylor College of Medicine, Houston, Texas, and by the Human Research Ethics Committee at the University of Toronto, Canada. Some of these sera had been used in previous studies.<sup>27–30</sup> Selection of sera for this study depended on sample availability and adequacy of quantity.

### *Serum neutralization assay*

A specific plaque reduction neutralization assay was used to detect and titer neutralizing antibodies against SV40 in the human sera. This assay was performed as previously described.<sup>17</sup> The test is specific for SV40 and does not detect cross-reacting antibodies against human polyomaviruses BK virus (BKV) and JC virus (JCV).<sup>18,31</sup>

### *Statistical analysis*

The standard Chi-square test was used to test differences between percentages. Fisher's Exact test was used when values were less than 5. Statistical differences were determined based on the traditional statistical significant level of a  $p$ -value of  $<5\%$ . All analyses were performed using the statistical software SAS version 9.4.

## Results

### *SV40 seroprevalence among Colombian and Nicaraguan subject groups*

Overall SV40 antibody positivities for the different Colombian and Nicaraguan subject groups are shown in Table 1. The frequency of SV40 neutralizing antibody for the total Colombian subjects (Groups A–D) was 22.8% (118/517). Group D (sex workers) had the highest antibody positivity at 38.6% (22/57), Group C (general population) had a positivity rate of 24.1% (77/320), and the other two Colombian groups (A and B) ranged in seropositivity from 10.0% to 17.1%. The difference in seropositivity between Group D and Group C was statistically significant ( $p=0.0185$ ), as was the difference between Group C and Groups A+B ( $p=0.0131$ ). When Group C omitted subjects in the birth cohort that contained potential vaccinees (vbc) (birth dates 1949–1960) and was compared to Groups A+B (which contained no (vbc) members), the difference in seropositivity remained significant ( $p=0.0039$ ). The Nicaraguan subjects (Group E) showed a seropositivity frequency of 12.8% (19/149). The difference in seroprevalence between the total Colombian (Groups A–D) and Nicaraguan (Group E) subjects

**Table 1**  
SV40 neutralizing antibody positivity among Colombian and Nicaraguan subject groups.

Country	Group <sup>a</sup>	Group criteria	Total subjects	No. SV40 antibody positive (%) <sup>b</sup>	SV40 neutralizing antibody titers (median)
Colombia	A	Cervical cancer patients	70	12 (17.1)	1:20–1:200 (1:20)
	B	Matched cancer controls	70	7 (10.0)	1:20–1:1000 (1:80)
	C	General population	320	77 (24.1)	1:10–1:1000 (1:20)
	D	Sex workers	57	22 (38.6)	1:10–1:100 (1:40)
	Total (Groups A–D):		517	118 (22.8)	1:10–1:1000 (1:20)
Nicaragua	E	Endoscopy patients	149	19 (12.8)	1:10–1:200 (1:20)

<sup>a</sup> Sample collection dates: Groups A–C, 1968; Group D, 1970; Group E, 2010.

<sup>b</sup> Statistical significance: Group C vs. Groups A + B,  $p = 0.0131$ ; Group D vs. Group C,  $p = 0.0185$ ; Groups A–D (Total) vs. Group E,  $p = 0.0143$ ; Group C vs. Group E,  $p = 0.0099$ ; Group C-(vbc) vs. Groups A + B,  $p = 0.0039$ . (vbc) = Birth cohort that contains potential vaccinees (birth dates 1949–1960).

**Table 2**SV40 neutralizing antibody positivity related to age among Colombian and Nicaraguan subjects.<sup>a</sup>

Country	Group	Group criteria	Date of birth (age in yrs at time of sample) <sup>b</sup>	No. SV40 antibody positive/no. tested (%) <sup>c</sup>
Colombia	A	Cervical cancer patients	≤1929 (≥39)	7/44 (15.9)
			1930–1948 (20–38)	5/26 (19.2)
			1949–1960 (8–19) <sup>d</sup>	0
	B	Matched cancer controls	≤1929 (≥39)	6/43 (14.0)
			1930–1948 (20–38)	1/27 (3.7)
			1949–1960 (8–19) <sup>d</sup>	0
	C	General population	≤1929 (≥39)	15/68 (22.1)
			1930–1948 (20–38)	20/87 (23.0)
			1949–1960 (8–19) <sup>d</sup>	30/142 (21.1)
			>1961 (<7)	4/11 (36.4)
	D	Sex workers	≤1929 (≥41)	3/8 (37.5)
			1930–1948 (22–40)	9/28 (32.1)
1949–1960 (10–21) <sup>d</sup>			10/21 (47.6)	
Combined Groups A–D:			31/163 (19.0)	
			35/168 (20.8)	
Nicaragua	E	Endoscopy patients	≤1929	40/163 (24.5)
			1930–1948	4/11 (36.4)
			1949–1960 <sup>d</sup>	110/505 (21.8)
			>1961	1/10 (10.0)
			Total:	7/34 (20.6)
			3/29 (10.3)	
			6/75 (8.0)	
	Total:		17/148 (11.5)	

<sup>a</sup> Samples that lacked date of birth information were not included.

<sup>b</sup> Age estimates based on dates of birth and years of sample collections: 1968 (Groups A–C), 1970 (Group D), and 2010 (Group E).

<sup>c</sup> Statistical significance: Groups A + B (≤1929–1948) vs. Group C (≤1929–1948),  $p = 0.1086$ ; Group D (1949–1960) vs. Group C (1949–1960),  $p = 0.0208$ ; Group D (≤1929–1948) vs. Group C (≤1929–1948),  $p = 0.1997$ ; Groups A–D (Total) (1949–1960) vs. Group E (1949–1960),  $p = 0.2381$ ; Group C (1949–1960) vs. Group E (1949–1960),  $p = 0.4124$ ; Group E (1949–1960) vs. Group E (1961–1970),  $p = 0.1324$ ; Group E (1949–1960) vs. Group E (1961–1969),  $p = 0.5260$ .

<sup>d</sup> Birth cohort that contains potential vaccinees (vbc, birth dates 1949–1960).

was significant ( $p = 0.0143$ ), as was Group C compared to Group E ( $p = 0.0099$ ). SV40 neutralizing antibody titers ranged from 1:10 to 1:1000, with median antibody titers of 1:20 to 1:80 for the individual groups from the two countries.

#### Effect of age on SV40 seroprevalence

Age estimates for the subjects were based on reported dates of birth and the years that samples were collected (Table 2). The subjects in each group were then subdivided, reflecting age at time of sampling. The birth cohort that contained potential vaccinees in each group (vbc, birth dates 1949–1960) is marked. In Groups D and E, the potential vaccinee age subgroup cohort displayed the highest SV40 seropositivity within the group. That is not surprising, as some members of those subgroups presumably had been vaccinated as children. SV40 antibody prevalence between Colombian Group D, birth cohort 1949–1960, and Group C, 1949–1960, differed significantly ( $p = 0.0208$ ). Other comparisons were not statistically different (Table 2). It is noteworthy that subjects in birth cohorts both older and younger than the potential vaccinees had detectable SV40 neutralizing antibodies. This seropositivity across age groups is supportive of the concept that SV40 transmission can occur among humans.

#### Effects of gender and ethnicity on SV40 seroprevalence

The effect of gender on SV40 seropositivity was examined (Table 3). Females predominated among the groups, representing 67% of Colombian subjects and 74% of the Nicaraguans. Antibody positivity was similar between females and males in the Colombian general population (24.3% vs. 23.4%), whereas males were more seropositive than females among the Nicaraguan patients (18.4% vs. 10.8%). Neither of those differences was statistically significant. However, comparisons of females in Group C and Group D ( $p = 0.033$ ) and of females in Group C and Group E ( $p = 0.0089$ ) were significantly different.

Three ethnic categories were reported for the Colombian subjects and SV40 seropositivity was similar among all three (Table 4). There were no statistical differences in antibody positivity among the groups. No ethnicity information was available for the Nicaraguan subjects.

#### Discussion

This study revealed serologic evidence of SV40 infections in populations in two Latin American countries, Colombia and Nicaragua. In both countries, children had been vaccinated with

**Table 3**  
SV40 seropositivity related to gender among Colombian and Nicaraguan subjects.<sup>a</sup>

Country	Group	Group criteria	No. SV40 antibody positive/no. tested (%) <sup>b</sup>		
			Female	Male	Total
Colombia	A + B	Cancers + controls	19/140 (13.6)	0	19/140 (13.6)
	C	General population	34/140 (24.3)	39/168 (23.4)	73/308 (23.2)
	D	Sex workers	22/57 (38.6)	0	22/57 (38.6)
		Total (Groups A–D):	75/338 (22.2)	39/167 (23.4)	114/505 (22.6)
Nicaragua	E	Endoscopy patients	12/111 (10.8)	7/38 (18.4)	19/149 (12.8)

<sup>a</sup> Samples that lacked donor gender information were not included.

<sup>b</sup> Statistical significance: Group C (female vs. male),  $p=0.7270$ ; Group E (female vs. male),  $p=0.1099$ ; Group C (female) vs. Group D (female),  $p=0.033$ ; Group C (female) vs. Group E (female),  $p=0.0089$ ; Group C-(vbc) (female) vs. Groups A + B,  $p=0.0757$ . (vbc)=Birth cohort that contains potential vaccinees (birth dates 1949–1960).

**Table 4**  
SV40 seropositivity among Colombian ethnic groups.<sup>a</sup>

Group	Group criteria	Ethnic group [No. SV40 antibody positive/no. tested (%) <sup>b</sup>			
		Mixed	White	Black	Unknown
A	Cervical cancer	6/49 (12.2)	5/16 (31.2)	0/3 (0)	1/2 (50.0)
B	Cancer controls	5/36 (13.9)	1/19 (5.3)	1/13 (7.7)	0/2 (0)
C	General population <sup>c</sup>	29/101 (27.7)	28/111 (25.2)	7/43 (16.3)	10/51 (21.6)
D	Sex workers <sup>d</sup>	—	—	13/41 (31.7)	9/16 (56.2)
	Total:	40/186 (21.0)	34/146 (23.3)	20/100 (20.0)	20/71 (29.6)

<sup>a</sup> No ethnic categories were reported for Nicaraguan subjects.

<sup>b</sup> Statistical significance: Mixed only, Group C-(vbc) vs. Groups A + B,  $p=0.0867$ ; White only, Group C-(vbc) vs. Groups A + B,  $p=0.1384$ ; Black only, Group C-(vbc) vs. Groups A + B,  $p=0.3801$ ; Black only, Group D vs. Group C,  $p=0.097$ . (vbc)=Birth cohort that contains potential vaccinees (birth dates 1949–1960).

<sup>c</sup> Samples that lacked ethnic group information were not included.

<sup>d</sup> Only two ethnic categories were reported for Group D.

prelicensure candidate live attenuated OPV at a time when such vaccinees were frequently contaminated with yet-to-be-discovered SV40.<sup>21–25</sup> Overall seroprevalences of SV40 neutralizing antibody were 22.8% and 12.8% for the Colombian and Nicaraguan subjects, respectively (Table 1). The Colombian seroprevalence was similar to that of Hispanic women (23%) in Houston in an earlier study.<sup>17</sup>

It is noteworthy that SV40 antibody-positive individuals were identified in age groups both older and younger than the potentially vaccinated cohorts (Table 2). This indicates that human transmission of SV40 apparently had occurred among the populations analyzed. This observation adds support to the concept that human-to-human transmission of SV40 can occur. The highest SV40 seroprevalence rates were among the Colombian sex workers (Group D) (Tables 1–3). The reason for this is not known, but one possible explanation is that unsanitary conditions were more often encountered by this subject group than others, increasing the chances for virus transmission.

There appeared to be a potential gender difference in SV40 seropositivity among the Nicaraguan patients, with males more likely to have SV40 antibodies than the females (18.4% vs. 10.8%), although this difference was not statistically significant. Such a difference was not apparent for the Colombian subjects (Table 3). The higher positivity among the Nicaraguan males could not be explained by potential vaccine exposure as only 1 of the 7 positive males was in the vaccinee age birth cohort. Environmental working conditions for the males might have contributed to higher virus exposures. Observations of gender differences for SV40 seropositivity have not been consistent among earlier studies. No gender differences in SV40 seropositivity were observed among ethnic Kazakhs or Russians in a study of SV40 infections in Kazakhstan.<sup>32</sup> SV40-contaminated oral poliovaccines prepared in Moscow, USSR, were used in Kazakhstan and some European and Asian countries.<sup>32</sup> In contrast, higher rates of SV40 neutralizing antibody were found in females of certain age groups in Hungary and the Czech

Republic.<sup>33</sup> Both of those Central European countries had used oral poliovaccines potentially contaminated with SV40 for vaccination of children.<sup>33</sup> Explanations suggested for the serological differences observed between females and males in those countries were that mothers probably had increased exposures to an infectious agent from their children, plus females were more likely than males to be employed at places with contacts with young vaccinees, such as nurseries, kindergartens, and hospitals.

The results from an earlier study of tissue samples from Costa Rica are compatible with findings described here based on analyses of sera of Colombian and Nicaraguan origin.<sup>34</sup> Costa Rica, another Central American country, had a nationwide campaign in 1959–1960 to vaccinate children under age 11 using the prelicensure OPV from Lederle Laboratories.<sup>34–36</sup> Lymphoma and control tissues collected from 1999 through 2003 were tested in a blinded fashion. After decoding, it was found that 24% (30/125) of lymphomas were SV40 DNA positive, whereas none of the control tissues were positive, and that 64% of the B cell lymphomas that contained SV40 DNA expressed SV40 T-antigen protein detectable by immunohistochemistry (whereas none of the control tissues gave positive staining). It was noted that 20% of the SV40-positive lymphomas had occurred in patients born after the use of contaminated vaccines.<sup>34</sup>

A recent study of archival stool samples from one-year-old infants in Mexico City supports the concept of possible transmission of polyomaviruses, including SV40, via fecal excretion.<sup>9</sup> Serial stool samples had been collected between 1999 and 2001 in a long-term study of diarrheal pathogens and breast-feeding.<sup>37</sup> There had been a large-scale field trial of Sabin's prelicensure live oral poliovaccine in 1959 in four Mexican cities—Mexico City, Guadalajara, Monterrey, and Puebla.<sup>38,39</sup> In the polyomavirus excretion study, virus shedding in the stool was detected by PCR in 64% of the 39 infants tested between 11 and 13 months of age. BKV was shed by 21 of the infants, SV40 by 6, and both BKV and SV40 by 3 of the infants. Some infants shed virus sporadically

**Table 5**  
Studies of SV40 seroprevalence in humans using archival sera.

Study reference	Country	Serum donor group <sup>a</sup>	Dates of serum collection	Type of antibody assay <sup>b</sup>	No. SV40 positive/ no. tested (%)	Positive donors born after 1963
Morris et al. (1961) <sup>40</sup>	USA, Maryland	Volunteers given RSV + SV40 by respiratory route	Approx. 1960	Neut-SV40 CPE	22/35 (62.8)	NA
Horvath (1972) <sup>41</sup>	Hungary	Contact with monkeys or monkey kidney cells	1969	Neut-SV40 CPE	15/37 (40.5)	NA
Shah et al. (1972) <sup>48</sup>	USA, Maryland	Children	1969	Neut-SV40 CPE	(a) 28/141 (19.8) (b) 9/278 (9)	+ +
Zimmerman et al. (1983) <sup>49</sup>	Germany	(a) Lab workers with contact with SV40 (b) Cancer patients	NR	ELISA (SV40 virions)	(a) 6/11 (54.5) (b) 42/143 (29.4)	+ +
Jafar et al. (1998) <sup>31</sup>	USA, Texas	Patients at VAMC hospital (a) HIV-positive (b) HIV-negative	1990–95	Neut-PR	(a) 38/236 (16.1) (b) 21/180 (11.7)	+ +
Butel et al. (1999) <sup>18</sup>	USA, Texas	Patients at children's hospital	1995	Neut-PR	20/337 (5.9)	+
Butel et al. (2003) <sup>33</sup>	Hungary, Czech Republic	(a) Samples sent to State Public Health Service (Pecs) (b) Surveillance program by State Institute of Health (Prague)	1995 1985	Neut-PR Neut-PR	(a) 17/589 (2.9) (b) 7/350 (2.0)	+ +
Rollison et al. (2003) <sup>50</sup>	USA, Maryland	Study of brain tumors	1974, 1989	Neut-PR	15/132 (11.4)	NR
Minor et al. (2003) <sup>19</sup>	United Kingdom, Poland	Blood donors (UK)	(a) 1985 (b) 1989–94 (c) 1992–97	Neut-SV40 CPE	(a) 34/800 (4.5) (b) 47/1000 (4.7) (c) 26/923 (2.8)	NR + NR
Knowles et al. (2003) <sup>51</sup>	England	Healthy people (Poland) Serological surveillance (Public Health Laboratory Service)	1991	Neut-SV40 CPE	79/2435 (3.2)	+
Carter et al. (2003) <sup>43</sup>	USA, Washington	Cancers + controls	1987–99	ELISA (VLPs)	0/699 (0)	0
Engels et al. (2004) <sup>52</sup>	USA	Collaborative Perinatal Project-Mothers	1959–66	(a) EIA (VLPs) (b) Neut-PR	(a) 82/500 (16.4) (b) 99/495 (20)	NA
Engels et al. (2004) <sup>42</sup>	USA	Zoo workers (a) Primate contacts (b) Other workers	1997	EIA (VLPs)	(a) 25/109 (23) (b) 15/145 (10)	NR
Engels et al. (2004) <sup>53</sup>	USA	NCI Seer Study (cancer + controls)	1998–2000	EIA (VLPs)	117/1346 (8.7)	+
Nurgalieva et al. (2005) <sup>32</sup>	Kazakhstan	Healthy volunteers (a) Kazakhs (b) Russians	1999	Neut-PR	(a) 7/154 (4.5) (b) 8/153 (5.2)	+ +
Rollison et al. (2005) <sup>54</sup>	USA, Maryland	Serum bank (cancer + controls)	1974	ELISA (VLPs)	11/510 (2.2)	NA
Lundstig et al. (2005) <sup>46</sup>	Sweden, Finland	(a) Children (b) Pregnant women	NR NR	EIA (VLPs)	(a) 22/288 (7.6) (b) 16/141 (7.1)	NR NR
Kean et al. (2009) <sup>20</sup>	USA, Colorado	(a) Blood donors (b) Children's hospital	2007	ELISA, soluble VP1 capsid proteins ELISA (VP mimo)	(a) 32/1501 (2.1) (b) 16/721 (2.2)	+ +
Corallini et al. (2012) <sup>44</sup>	Italy	Healthy blood donors (ages 18–65)	2005–10	ELISA (VP mimo)	154/855 (18)	+
Mazzoni et al. (2012) <sup>55</sup>	Italy	(a) Mesothelioma patients (b) Asbestos exposure (c) Healthy blood donors (d) Pregnant women	2006–12	ELISA (VP mimo)	(a) 25/97 (25.8) (b) 8/90 (8.9) (c) 49/299 (16.4) (d) 12/94 (12.8)	NR
Wong et al. (2013) <sup>17</sup>	USA, Texas	Patients at public hospital (a) Pregnant, White + African American (b) Not pregnant, White + African American (c) Not pregnant, Hispanic	1972–73 1993–95 1993–95	Neut-PR Neut-PR Neut-PR	(a) 15/212 (7.0) (b) 16/299 (5.4) (c) 23/101 (23)	NA + +
Taronna et al. (2013) <sup>56</sup>	Italy	Healthy children (ages ≤17)	2009–12	ELISA (VP mimo)	54/328 (16.5)	+
Comar et al. (2014) <sup>57</sup>	Italy	Healthy pregnant women	2004–05 2006–11	(a) Neut-PR (b) ELISA (VP mimo)	(a) 13/123 (10.6) (b) 14/110 (12.7)	+ +
Mazzoni et al. (2014) <sup>58</sup>	Italy	(a) Glioblastoma patients (b) Healthy controls (c) Breast cancer patients	NR	ELISA (VP mimo)	(a) 15/44 (34) (b) 15/101 (15) (c) 12/78 (15)	NR
Tognon et al. (2015) <sup>59</sup>	Italy	(a) Non-Hodgkin lymphoma patients (b) Matched controls	NR	ELISA (VP mimo)	(a) 62/150 (41) (b) 33/213 (15)	NR
Mazzoni et al. (2015) <sup>60</sup>	Italy	(a) Osteosarcoma patients (b) Matched controls	2006–13	ELISA (VP mimo)	(a) 24/55 (44) (b) 20/114 (17)	+ +
Tognon et al. (2016) <sup>45</sup>	Italy	Healthy subjects	2014	ELISA (SV40 T-ag mimo)	138/704 (20)	+

(continued on next page)

Table 5 (continued)

Study reference	Country	Serum donor group <sup>a</sup>	Dates of serum collection	Type of antibody assay <sup>b</sup>	No. SV40 positive/no. tested (%)	Positive donors born after 1963
Mazzoni et al. (2017) <sup>61</sup>	Italy	Healthy elderly (ages 66–100)	2014–15	ELISA (SV40 T-ag mimo)	60/273 (22)	NR
Mazzoni et al. (2017) <sup>62</sup>	Italy	(a) Pregnant women (ages 15–48) (b) Nonpregnant (ages 18–40)	NR	ELISA (SV40 T-ag mimo)	(a) 23/134 (17) (b) 36/180 (20)	NR
Mazzoni et al. (2018) <sup>63</sup>	Italy	(a) Osteosarcoma patients (mean, 21) (b) Healthy controls (mean, 21)	2002–12	ELISA (SV40 T-ag mimo)	(a) 87/249 (35) (b) 56/247 (23)	+ +
This study	Colombia	(a) Cancer patients + matched controls (b) General population (c) Sex workers	1968 1968 1970	Neut-PR	(a) 19/140 (13.6) (b) 77/320 (24.1) (c) 22/57 (38.6)	NA + +
This study	Nicaragua	Endoscopy patients at hospital	2010	Neut-PR	19/149 (12.8)	+

<sup>a</sup> Abbreviations: RSV = respiratory syncytial virus; VAMC = Veterans Affairs Medical Center; NCI = National Cancer Institute; NA = not available; NR = not reported.

<sup>b</sup> Type of assay: Neut-SV40 CPE = neutralization of development of SV40 cytopathic effect (CPE); Neut-PR = neutralization of SV40 plaque formation (plaque reduction); ELISA/EIA (VLPs) = enzyme-linked immunosorbent assay/enzyme immunoassay using SV40 virus-like particles (VLPs), competition assays sometimes included; ELISA (VP mimo) = ELISA based on peptides representing specific epitopes of SV40 virus particle (capsid) proteins (mimotopes); ELISA (T-ag mimo) = ELISA based on peptides representing specific epitopes of SV40 viral oncoprotein T-antigen (mimotopes).

for up to 8 weeks (including one infant excreting SV40). As these infants were born decades after the 1959 field trial, environmental exposure to SV40 must have occurred.

This report adds a new geographic region to previous studies of SV40 seroprevalence in humans (Table 5). Early reports had established that humans can be infected with SV40 and mount an immune response, based on evidence from infected volunteers<sup>40</sup> and workers in contact with monkeys and/or monkey kidney cells.<sup>41,42</sup> Many of the subsequent studies involved samples collected in the US or Europe, with reported collection dates ranging from 1959 to 2015. Donor groups have included healthy adults, cancer patients, and children, with observed seropositivity rates ranging from 44% to 0%. With the exception of the one negative study,<sup>43</sup> it is noteworthy that all others detected SV40 antibodies in some of the donors, and often in individuals born after 1963 (by which time poliovaccines were believed to be free from contaminating SV40). Healthy donors generally displayed SV40 seroprevalence rates of 10% or less.

There are several limitations to the reported studies, including the current one. The explanation for a difference in seroprevalence among donor groups, such as with the Colombian and Nicaraguan subjects, is unknown. There is usually no information known about how many of the subjects analyzed had been vaccinated as children or were from households in which another person had been vaccinated. Plus, prelicensure contaminated OPV was rarely used in some countries, including the US, historical circumstances which suggest a greatly reduced likelihood that SV40 infections would have been established in those populations. In addition, there is usually no knowledge of the sanitary conditions of the subjects' living environment, which would likely have impacted human-to-human spread of the virus.

Results of seroprevalence studies are dependent on the conditions of the assay used. Neutralization of SV40 infectivity (by either plaque reduction or inhibition of SV40 CPE) is considered to be highly specific. We previously showed that high-titer rabbit antisera against human polyomaviruses BKV or JCV had no neutralizing activity against SV40<sup>18</sup> and that human sera with high anti-BKV titers were negative for detectable SV40 neutralizing activity.<sup>31</sup> ELISA/EIA tests based on SV40 virus-like particles (VLPs) can detect some cross-reactive antibodies against BKV or JCV, whereas competitive-inhibition steps with BKV or JCV VLPs used in such assays might compete out some SV40-specific antibodies and, theoretically, reduce apparent SV40 seroprevalence rates. A recently

developed new ELISA approach based on two synthetic peptides that mimic specific epitopes on SV40 viral capsid proteins (VP mimotopes) appears to specifically detect SV40 antibodies.<sup>44</sup> Another newly described ELISA uses two synthetic peptides designed to mimic specific epitopes on SV40 T-antigen, the viral replication protein and oncoprotein. That test can detect antibodies against SV40 T-antigen and, thereby, provide evidence that SV40 had replicated in those infected human hosts.<sup>45</sup>

Another factor in studies that employ archival sera is that it is not known how many times the stored sera had been thawed, which could have affected antibody titers and reduced some below the level of detection. In the current study, there was a difference in the timing of serum collections—about one decade after vaccinations in Colombia versus five decades later for the Nicaraguans. It has been reported that SV40 antibody may wane over time,<sup>46</sup> perhaps reducing some to undetectable levels. Whereas it is known that individuals with contacts with Asian rhesus monkeys can develop SV40 antibodies,<sup>42,47</sup> those monkeys would not be a possible exposure source in the current study, nor in many of the other reported studies (Table 5), as their natural geographic distribution is mainland Asia.

Little is known about the human immune response to natural infections by SV40. It is unknown what conditions drive a robust response, including the level of viral replication needed to elicit a detectable immune response and to ensure long-duration neutralizing antibody. It is possible that SV40 does not replicate to high enough titers in some infected humans to yield a long-lasting response. There also may be some cross-reactivity with another (unknown) virus, although as described above the plaque-reduction neutralization test and peptide mimotope assays are thought to be specific for SV40. Future studies are necessary to explore the human immune response to SV40 and to determine current infection rates in targeted populations and whether any health consequences are related to such infections.

In conclusion, this study showed that residents of Colombia and Nicaragua often possess serological evidence of SV40 infections. It confirms previous indications that certain populations exposed to potentially contaminated poliovaccines reveal evidence of SV40 infection and transmission within the population. It also emphasizes that studies designed to address SV40 infections in humans must consider the history and characteristics of the subjects being evaluated.

## Author contributions

C.W. performed antibody assays and summarized data. S.Z. analyzed data. E.A. and L.P. provided sera and data analysis and interpretation. J.S.B. was responsible for study design, data analysis and interpretation, and manuscript preparation.

## Conflict of interest

The authors have no conflicts of interest to declare.

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