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Epidemiology of Simian Polyomavirus SV40 in Different Areas of Russian Federation (RF)

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1. Introduction

DNA-containing polyomavirus SV40 was isolated in 1961 from poliovaccine prepared on kidney cell culture of *M.mulatta* naturally infected with this virus. Main part of poliovaccines (IPV and OPV) was contaminated with SV40. From 1955 till 1963 hundred millions of people in USA, Russia (USSR), and in some other countries were immunized with contaminated SV40 Salk and Sabin vaccine and a lot of people became carriers of SV40. This virus was detected also in some human tumors. Epidemiological researches were not carried out in Russia before 2009. By present time possibility and ways of SV40 excretion into environment and possibility of its horizontal spreading is established. From the beginning of 60s (in Russia a little bit later) vaccines were free from SV40 contamination. In this article the data about SV40 infection of population in Moscow, St. Petersburg, in some cities of the Black Sea coast of Caucasus, in Novosibirsk and in one area of Krasnoyarsk region are presented., Analysis of the source of SV40 people infection is performed.

Small size DNA virus was isolated from mice in 1953. The virus was called “polyomavirus” SV40 (Gross, 1951; Gross, 1953). because it was able to produce multiple malignant tumors in newborn rodents.

Simian SV40 was the second discovered polyomavirus. It was isolated in 1961 from poliovaccine propagated on *M.mulatta* kidney cell cultures naturally infected with SV40 as it was established later (Eddy et al., 1961; Sweet & Hilleman, 1960).

SV 40 is a small DNA virus which does not have envelope; its capsid consisted of 72 capsomers. The size of virion is 45 nm; the viral genome is represented by two-stranded DNA aprox. 5000 bp long. On character of the changes caused in cell cultures, the virus also has received the name “vacuolating virus”(Sweet & Hilleman, 1960; Butel et al., 1999).

During next years polyomaviruses were isolated from different animals including different monkey species and from humans with different pathology as well (Simon,2008; Gjoerup, 2010; Voevodin, 2009).).

In 1957 baboon polyomavirus was discovered (Gardner et al., 1989); polyovavirus of African green monkeys was discovered in 1979 (zur Hausen & Giosmann, 1979), after that polyomaviruses of *M.fascicularis* and chimpanzee were discovered (but not isolated). Two

human polyomaviruses BKV and JCV were firstly isolated in 1971: JCV from a patient with Progressive multifocal leukoencephalopathy (Padgett et al., 1971), BKV from patient with nephropathy (Gardner et al., 1971; Padgett et al., 1971).

Later 3 more polyomaviruses were isolated: KI, Wu from respiratory tract and MCV from rare malignant skin tumor – Merkel carcinoma (Feng et al., 2008)

Among human viruses such ubiquitous viruses as JCV and BKV are widely known and are determined from 60 to 90% of cases in humans according to data of different authors (Knowles et al., 2006; Maginnis & Atwood, 2009).

However many publications often contradictory were associated with simian polyomavirus SV40. The virus was unintentionally introduced into human populations together with poliovaccine contaminated with SV40 as it turned out later (Shah, 2007).

The middle 50s of the last century was marked by epoch-making event – creating of 2 types of poliovaccines: Salk formolvaccine (IPV) and live attenuated Sabin vaccine (OPV). More than 100 million people were immunized in USA beginning from 1956 till 1963 using the above mentioned 2 types of vaccines; more than 70 million people were immunized in Russia (USSR) in 1960 using Sabin vaccine (Fisher, 1999. Rizzo, 1999, Peden, 2008, Drozdov, 2005, Mironova et al., 2006). Significant amount of predominantly young people were immunized in different European countries (Shah & Nathanson, 1976).

It is necessary to look back to the history of poliovaccine creation by Salk and Sabin. Both vaccines (Salk inactivated formolvaccine and Sabin live attenuated vaccine) were poliovirus propagated on *M. mulatta* kidney cell culture. Soon it was shown that in high percent of cases *M. mulatta* had been already infected with SV40 in natural conditions (Sweet & Hilleman, 1960; Lapin et al., 1965). That is why many poliovaccine lots were contaminated with this virus. As it was found out later Salk method of vaccine preparation did not provide full inactivation of the vaccine (Carbone et al., 1997). As to the Sabin vaccine – it was initially contaminated with polyomavirus (Peden, 2008).

Zoonotic SV40 origin perhaps is beyond doubt but some authors advance an opinion in their publications about preexisting of this virus in humans (Levine, 1998; Geissler, 1985; Paracchini, 2005). This assumption could be supported if SV40 (or DNA sequences of this virus or antibodies to it) could be reliably detected in human materials obtained before mass poliovaccination. Some publications contain information about detection the antibodies to SV40 before beginning the vaccination against poliomyelitis (Geissler, 1985). It can be supposed that the virus could be introduced into human population from the countries of monkey natural habitat (Southeast Asia and India). Serological investigations revealed SV40 infection of Zoo workers and in workers of India companies exporting monkeys and contacting with animals. SV40 footprints were detected in their blood (Horvath, 1965; Engels et al., 2004; Shah, 1966). All this testifies the possibility of contact infection humans from animals carriers of the virus. (citation from: Vastad, JAMA, 2002, 288, 1337-1338). However the quantity of people infected with SV40 greatly increased particularly after beginning of mass poliovaccination which testifies this supposition.

Thus mass vaccination of millions of people with poliovaccine which made it possible to eradicate poliomyelitis as an epidemic disease simultaneously led to infection of people with simian polyomavirus SV40.

In 1961 as it was mentioned above USA investigators revealed contamination of poliovaccine with simian (*M.mulatta*) virus SV40.

Serological investigation and PCR revealed antibodies to SV40 and virus DNA sequences in polio immunized people. To the researcher's surprise the antibodies to SV40 were also detected in not vaccinated people. In connection with this fact the researchers came out with a suggestion that in human populations virus can spread horizontally (Knowles et al., 2006) It has been shown that virus can excrete into surrounding with excrements, breast milk, sperm, that supports the assumption about the possibility of horizontal spreading of the virus (David, 2001; Martini, 1996; Vastag, 2002).

Some investigators described discovery of polyomaviruses in sewage and in sea and river waters which undoubtedly could be the source of horizontal spreading of infection in regions with high SV40 prevalence (Vastag, 2002; Bofill-Mas et al., 2009).

Appearance of people infected with SV40 after immunization with polio vaccine caused anxiety of physicians due to virus ability to induce malignant tumors in laboratory rodents. However absence of apparent increase of tumors in polio vaccinated people reduced the interest to SV40 in people. A new wave of anxiety and long lasting discussions continuing up to now was caused by discovery of DNA sequencing of SV40 in some human tumors. As a result great many contradictory publications appeared describing infection of people with this virus, its origin, ways of spreading and possible connection with human pathology (Carbone, 1994, 1997,1999; De Rizzo, 1998, 2001; Arrington, 2000; Tognon, 2001; Lednicky,2001; Bergsagel, 1992; Vilchez, 2002; Klein, 2002; Bocchetta, 2001; Lapin & Chikobava, 2011; Cutrone et al., 2005; Carter et al., 2003; Hübner R. & Van Marck E.2002; Martini et al., 1998; Barbanti-Brodane et al., 2004).

Some researchers considered discovery of SV40 in human blood and in tumors as artifact (Shah, 1976; Lopez-Rios, 2004; Manfredi, 2005; Mayall, 2003, and many others). Disagreement was caused mainly by high homology of SV40 with ubiquitous human polyomaviruses JCV and BKV and consequently with their immunologic and molecular-biological reactivity (Testa, 1998; Lopez-Rios, 2004; Heinsohn, 2005; Shah & Nathanson, 1976; Shah, 2007; Carter et al., 2003). Some researchers denied infection people with SV40 and believed that discovered antibodies and DNA sequencing belong to human polyomaviruses JCV and BKV.

Because *M.mulatta* are carriers of polyomavirus SV40 in natural conditions WHO recommended to replace in manufacture poliovaccines kidneys of rhesus monkeys with kidneys of green monkeys not containing this virus. At the same time methods of vaccine decontamination were improved. It is possible to assert that in the period from 1960 till 1962 poliovaccine produced by USA, Great Britain, and some other European countries was free from SV40 contamination. However by that period of time hundred millions of people had been already immunized with contaminated SV40 vaccine in USA, Russia (USSR) and many other countries. In Russia (USSR) that produced vaccine for own needs and also for export to east European countries and countries of southeast Asia clearing vaccine from contamination happened some years later Chumakov et al., 1961;Chumarjvet al., 1963; Scovranek, 1961)

Vaccine produced in east countries was contaminated with SV40 till 1978 according to data of USA and English researchers who investigated SV40 contamination in poliovaccine

samples produced in different time in different countries. Authors of the publication note that method of heat inactivation of the vaccine in the presence of $MgCl_2$ inactivated SV40 not completely (Cutrone et al., 2005).

Epidemiological investigations carried out in different countries revealed infection of population with SV40. K. Leithner et al (2005) in Fig 4 of their article published a schematic map demonstrating data on infection of population with SV40 in European countries where contaminated SV40 vaccine was used and afterwards SV40 infection of population was revealed and also those countries where contaminated vaccine was also used but the population was not examined for SV40 infection. Such countries were Russia, Ukraine, Byelorussia, and some East European countries in which vaccination was carried out with OPV Sabin vaccine perhaps produced in Russia (USSR).

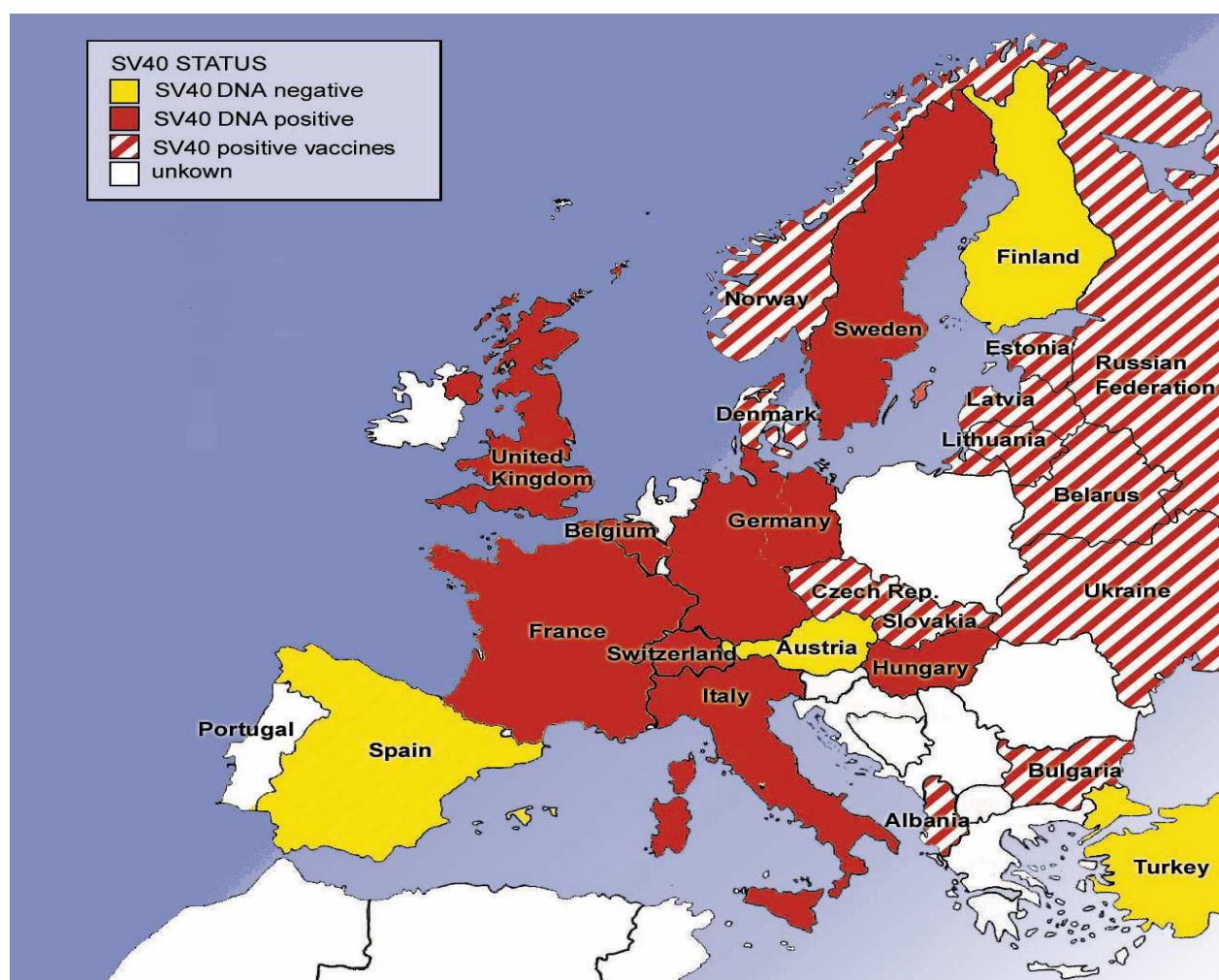


Fig. 1. Red color designates countries where contaminated SV40 vaccine was used and SV40 carriers were discovered (continuous coloring does not mean degree of prevalence). Shaded area designates countries where contaminated vaccine was also used but people were not tested for SV40 carriers. (Leithner 2005). See also Table 1.

Authors of this publication prepared a Table on the basis of literary data which helps to understand where the source of SV40 infection was.

Country	Rating of contamination of polio vaccines with SV40	Vaccines, vaccination programs and origin of vaccines
Albania	Positive	Contaminated Russian vaccine (OPV) used since 1960 (Chumakov et al, 1961; Chumakov et al, 1963; Shah & Nathanson, 1990; Levine et al, 1998).
Austria	Negative	Mass vaccinations with SV40-free British vaccine (OPV) since winter 1961/62 (Kundratitz, 1962; Friza, 1962)
Bulgaria	Positive	Contaminated Russian vaccine (OPV) used since 1960 (Chumakov et al, 1961; Chumakov et al, 1963; Shah & Nathanson, 1990; Levine et al, 1998).
CSSR	Positive	Since 1960: limited use of IPV, mass vaccinations with OPV, partly with contaminated Russian vaccine (Chumakov et al, 1961; Chumakov et al, 1963; Shah & Nathanson, 1990; Levine et al, 1998; Skovranek, 1961; Chron Wld Hlth Org, 1960)
Denmark	Positive	Vaccinations from 1955 with widely contaminated Danish vaccine (IPV), SV40-free from 1963 (Engels, 2003). A combined schedule was introduced in 1968 (Murdin et al, 1996).
Finland	Negative	Mass vaccinations since 1957 with SV40-free Belgium vaccine (IPV) (Hirvonen, 1999). Finland has never used OPV on a routine basis (Murdin et al, 1996)
Germany East	Positive	Contaminated Russian vaccine (OPV) used since 1960 (Geissler, 1983; Chumakov et al, 1963; Shah & Nathanson, 1976; Prog Med Virol, 1990; Levine et al, 1998, Belian & Rademacher, 1961).
Hungary	Positive	Since 1957: limited use of IPV, mass vaccinations with vaccines from the US, Canada, Hungary and Russia (also OPV) (Chumakov et al, 1963; Shah & Nathanson, 1976; Geissler, 1990; Levine et al, 1998)

Norway	Positive	Vaccinations started 1956 with Danish vaccine (IPV); since 1957 potentially contaminated U.S.vaccine (IPV) (Thu et al, 2006), change to OPV from 1967 to 1979, then back to IPV from 1979 onwards (Murdin et al, 1996).
Poland	Unclear	Mass vaccinations (OPV) since 1958 with Koprowski strain live vaccine [94]; vaccine was claimed to be Russian made (Minor et al, 2003), but Russian vaccines were derived from Sabin's strain (Chumakov et al, 1961)
Russia (USSR)	Positive	Mass vaccinations since 1959 with contaminated Russian vaccine (OPV). A small proportion of persons were vaccinated with IPV at the beginning of the mass vaccinations (Chumakov et al, 1961; Chumakov et al, 1963; Shah & Nathanson , 1976; Levine et al, 1998;Chron Wld Hlth Org 1960).
Spain	Unclear	Mass vaccinations since 1963 with British vaccine (OPV) [101]; British vaccines were SV40-free since 1962 (Sangar et al, 1999); in contrast some vaccines were later claimed to have been contaminated (De Sanjose et al, 2003).
Sweden	Positive	In 1957 potentially contaminated U.S. vaccine (IPV), from 1958 SV40-free Swedish vaccine (IPV). Sweden has never used OPV (Murdin et al, 1996)
Turkey	Negative	Vaccination was not started before 1970, at a time where polio vaccines were required to be SV40-free (De Rienzo et al, 2002). The type of the vaccine is unclear. In a global poliomyelitis eradication initiative starting in 1989, OPV was used.
United Kingdom	Positive	Vaccination started in 1956 with OPV (Chron Wld Hlth Org, 1958; Chron Wld Hlth Org, 1960). SV40-free since 1962 (Rev Sanid Hi Publica, 1965)

Table 1. (Leithner, 2005)

We were aimed at carrying out epidemiological investigation and determining infection of population with polyomavirus SV40 in different regions of Russia and also determine intensity and source of infection. Investigation was carried out mainly anonymously. Each sample was accompanied with information about the place from where the sample was delivered, sex and age of the person.

To determine SV40 infection of population in Russia we decided to determine virus DNA sequences in blood samples of persons of different sex and age delivered to us by different medical institutions from different regions of Russia. Investigation of biological samples for SV40 is rather difficult because standard PCR and immunological tests (IFA, western blotting) do not allow differentiation of SV40 from widely spread ubiquitous human polyomaviruses JCV and BKV which have cross-immunological and cross molecular-biological-reactivity with SV40 (Viscidi, 2003). That is why for SV40 detection we chose the recommended method Real Time PCR (RQ-PCR) with TaqMan probe addressed to the area with 9-nucleotid deletion distinguishing SV40 from JVC and BKV.

2. Methods

For PCR analysis 100 ul of blood was put into a test tube containing EDTA and kept at 4° C until it was delivered to the laboratory for DNA extraction. After extraction DNA samples were kept at -20° C.

Genome of SV40 can be present in the cell in 2 forms: episomal or integrated into genome of host cell. Because of few SV40 copies in the sample the best method allowing detecting episomal forms of SV40 DNA in RQ-PCR are methods based on DNA sorption by SiO₂. That is why we used modified GuSCN protocol (Boom et al., 1992, Testa et al., 1998) for extraction of DNA from whole blood.

Three hundreds microliters of solution containing 5 M GuSCN, 1% Triton X-100(v/v), 20 mM EDTA, 50 mM Tris-HCl (pH 6,4), 10 ul SiO₂ was added to the blood sample (100 ul) and incubated 15 min, centrifugated and the sediment was washed once in 5 M GuSCN, 50 mM Tris-HCl (pH 6,4) and twice in 10 mM Tris-HCl (pH 7,3), 50 mM NaCl, 50% ethanol. The sample was desiccated in thermostat and DNA was eluted by TE-buffer (10 mM Tris-HCl (pH 8,0), 1Mm EDTA). For PCR amplification 5 ul were used. Rest was kept at -20° C.

As it was mentioned above SV40 detection was carried out by RQ-PCR method with internal probe directed to SV40 genome region in 4517 position, characterized by deletion of 9 nucleotides distinguishing SV40 from viruses JCV and BKV. PCR was carried out on BioRad Thermocycler according to the following program: 5 min initial denaturation, then 8 sec at 94°C, 23 sec at 60°C, 30 sec at 72°C, during 50 cycles. DNA extracted from *M.mulatta* blood was used as positive control. Water 5 ul from opened test tube kept together with extracted DNA samples was used as negative control.

3. Results

In total 768 blood samples obtained from different cities and regions of Russian Federation (St. Petersburg - 56, Moscow - 50, Krasnodar region - 352, Novosibirsk - 108, Krasnoyarsk region - 202) have been investigated by RQ-PCR. Results of these investigations are presented in Table 2.

Region	Number of samples	Number of SV40 positive	
		Absolute number	%
Krasnodar region	100	49	49
Moscow	50	8	16
St. Petersburg	56	8	14
Novosibirsk	108	32	29.6
Krasnoyarsk region	202	64	26.7

Table 2.

Because this investigation revealed a rather high number of SV40 carriers in Krasnodar region we decided to check the situation in Sochi and Adler (Table 3).

Region	Number of samples	Number of SV40 positive	
		Absolute number	%
Sochi	102	44	43
Adler	150	48	32

Table 3.

To confirm the results of RQ PCR, and prepare amplicons for sequencing another sets (SV5/SV6) of primers directed to conservative region 173-bp length of SV40 T-ag were used. These PCR primers amplify a region of SV40 that could be distinguished from BK and JC viral DNA. (Testa *et al.* 1998).

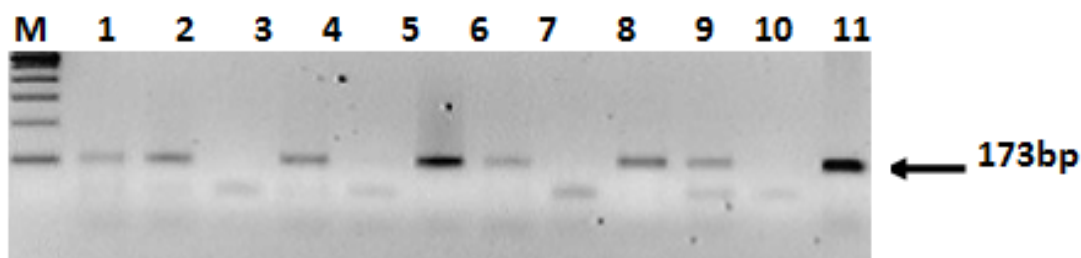


Fig. 2.

The ethidium bromide-stained 2.5% agarose gel shows the PCR products of 9 blood samples, some of which are SV40 positive. N10(-) control (water), 11 (+) control (DNA extracted from blood of SV40+ positive *M. mulatta*).

The obtained samples were sequenced on a ABI Prism 377 sequenator. Result of sequencing confirmed belonging of amplified regions to SV40.

We believe that the results can be corrected both towards the increase and towards reduction on the basis of age, sex and other factor analyzes of representative groups.

Nevertheless obtained material allows drawing some conclusions. High percent of SV40 infected children in Sochi and Krasnodar region at the age of 10 years who were immunized with poliovaccine reliably free from contamination with this virus definitely testifies horizontal way of contamination.

Numerous publications are devoted to polyomavirus problem and a great part of them to SV40 problem: reliability or unauthenticity of SV40 detection in healthy people, in human neoplasm, SV40 role in tumor emergence, SV40 origin and ways of spreading are discussed. In a great degree a lot of publications are connected with scandalous character of the problem - SV40 introduction into human population during immunization against poliomyelitis.

Despite the fact that preparing vaccines on rhesus kidney cell cultures was stopped about 30 years ago SV40 carrying is still determined in high percent of cases in healthy persons and also in some malignant human neoplasm (brain tumors, mesotheliomas, osteosarcomas etc.).

Polyomavirus SV40 has been detected practically on all continents (Fig. 2). After introduction it into human population it should be considered as human virus - man has become its reservoir. The virus circulates among people, spreading horizontally, excretes into surrounding, pollutes it and this is perhaps a very important factor of its epidemiology.

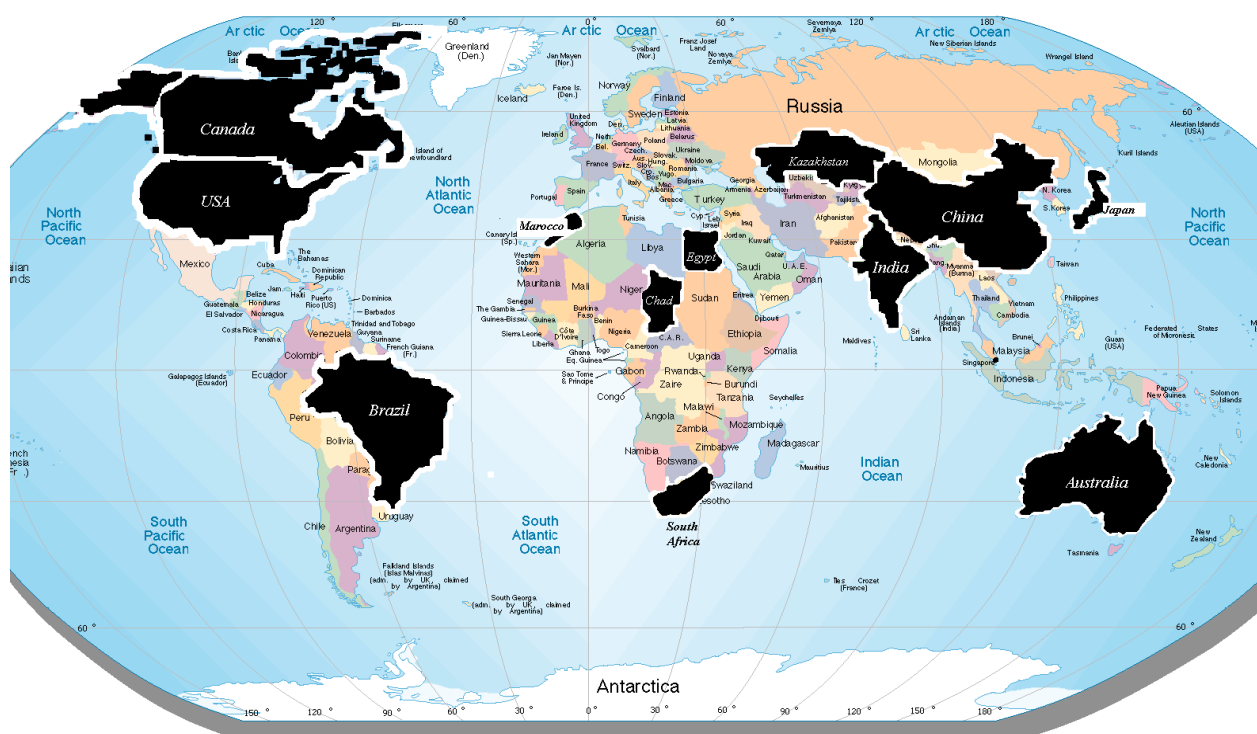


Fig. 3. SV40 infection of population was discovered on all Continents. Black color designates countries where SV40 carriers were discovered. However it does not mean the prevalence of SV40 infection.

4. Conclusion

In this article data on analysis of SV40 polyomavirus contamination of population in some regions of Russia are presented. Infection was detected by PCR in real time (RQ-PCR) with TaqMan probe directed to the site of 9 nucleotide deletion distinguishing SV40 from ubiquitous human viruses JCV and BKV, The source of infection was mainly vaccination

with Sabin OPV vaccine contaminated with SV40. Virus SV40 was detected in archive vaccine samples produced in East European countries before 1978 because heating with $MgCl_2$ did not free the vaccine from SV40 contamination. Infection rate was investigated in 5 Russian regions: Russia (Moscow, St. Petersburg, cities of the Black Sea coast, Novosibirsk and Krasnoyarsk region).

The highest values were detected on the Black Sea and the lowest in St. Petersburg. Presence of SV40 in children vaccinated with reliably decontaminated vaccine confirms possibility of horizontal spreading of the virus. According to reference data contamination of population was revealed on all Continents.

Despite the fact that preparing polio vaccine on culture of M.mulatta kidney cells was stopped 30 years ago SV40 carriage still exists in healthy people in rather high percent and also in human malignant neoplasms practically in all countries, on all Continents (Basetse, 2002; Leithner et al., 2006; Minor et al., 2003; Zekri et al., 2007; Dang-Tan et al., 2004). Polyomavirus SV40 was introduced into human population and should be considered as human virus. Man has become its reservoir. The virus circulates among people, spreads horizontally, excretes into environment and pollutes it and this is an important factor in its epidemiology.

Perhaps true value of SV40 prevalence in different regions can be established at examination of randomized groups generated on age, sex, place of residing, conditions of life and cultural skills.

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This book represents an overview on the diverse threads of epidemiological research, brings together the expertise and enthusiasm of an international panel of leading researchers to provide a state-of-the-art overview of the field. Topics include the epidemiology of dermatomycoses and *Candida* spp. infections, the epidemiology molecular of methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from humans and animals, the epidemiology of varied manifestations neuro-psychiatric, virology and epidemiology, epidemiology of wildlife tuberculosis, epidemiologic approaches to the study of microbial quality of milk and milk products, Cox proportional hazards model, epidemiology of lymphoid malignancy, epidemiology of primary immunodeficiency diseases and genetic epidemiology family-based. Written by experts from around the globe, this book is reading for clinicians, researchers and students, who intend to address these issues.

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