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Evaluation of poliovirus antibody titers in orally vaccinated semi-captive chimpanzees in Uganda

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Abstract

Background: To understand immunological responses in chimpanzees vaccinated with live-attenuated vaccine (oral polio vaccine; OPV), serum neutralizing antibodies against poliovirus types 1, 2, and 3 were investigated over time.

Methods: The neutralizing antibody titers against poliovirus types 1, 2, and 3 were determined by microneutralization test using 100 ID₅₀ of poliovirus types 1, 2, and 3 (Sabin strains).

Results: Neutralizing antibodies against poliovirus types 1, 2, and 3 were detected in 85.7%, 71.4%, and 65% of the serum from 42 chimpanzees tested 9 years post-vaccination. The neutralizing antibody titers in chimpanzees were similar to the documented levels in human studies as an indicator of vaccine efficacy.

Conclusions: This study reveals persistence of neutralizing antibodies in chimpanzees for at least 9 years after vaccination with OPV. This first study in chimpanzees provides useful information for the evaluation of the success of vaccination with OPV in other captive apes.

Introduction

Poliomyelitis is a life-threatening acute paralytic disease caused by poliovirus (PV) [2, 3, 19, 32, 33, 39]. Poliovirus is highly infectious belonging to the genus Enterovirus in the family Picornaviridae with three distinct serotypes (type 1, 2, and 3) [3]. Poliovirus is mainly transmitted through the fecal-oral route as the virus replicates efficiently in the intestinal tract. In humans, it is typically shed with the stool for 2–4 weeks. Feces can serve as a source of contamination of water, milk, and food. Houseflies can passively transfer poliovirus from feces to food [13]. Virus transmission is facilitated by poor sanitation and factors such as crowding, low levels of hygiene, water quality, and sewage-handling facilities. Humans are considered as the sole reservoir for poliovirus. The three serotypes are able to cause human infection with incubation periods of 5–35 days. Poliomyelitis is an acute viral infection, which ranges in severity from a non-specific illness to paralysis with permanent disability and death. Historically, poliovirus paralyzed and killed high numbers of people before the licensing of inactivated poliovirus vaccine (IPV) in 1955 and Oral Polio Vaccine (OPV) in 1962 and introduction of vaccination worldwide [33]. The IPV is prepared by growing the poliovirus in monkey kidney tissue culture (vero cell line) and inactivated with formaldehyde [6]. It contains 2-phenoxyethanol, neomycin, streptomycin, polymyxin B (used to prevent bacterial and fungal growth), and all three serotypes of poliovirus [7]. The vaccine is effective in inducing circulating antibodies in blood, thus preventing polio virus in the gut from entering and replicating in the central nervous system [39]. Whereas trivalent OPV is a live-attenuated vaccine containing all three serotypes of poliovirus in a ratio of 10:1:6 [7, 26].

These attenuated PV strains replicate in the human gut inducing mucosal immunity that inhibits replication of the virus in the gastrointestinal tract [7, 26]. The OPV produces long-lasting mucosal immunity by stimulating the formation of IgA antibodies in the intestine and also serum antibodies in the bloodstream [35].

Although humans are the only known natural reservoirs of poliovirus, non-human primates, especially apes (chimpanzees and gorillas), are susceptible [10, 11]. Macaques, African green monkeys and *Cebus* spp. can be experimentally infected with poliovirus but do not generally develop a paralytic disease when infected by the peripheral route [36]. However, paralytic disease because of poliovirus infections via human contacts has been reported in captive chimpanzees, orangutans, gorillas, and colobus monkeys [1, 3, 10, 17, 20, 21]. Antibodies and shedding of virus have been found in imported animals [10], and some chimpanzees may act as symptomless carriers [4]. A suspected poliovirus outbreak in wild apes was reported in Kaskela Community of the Gombe chimpanzee population research group in Tanzania in 1966 where six chimpanzees died from the disease and at least six others were paralyzed lifelong [14–16, 22]. A similar outbreak among the free-ranging chimpanzees was reported in Beni, Zaire, with at least seven of 48 chimpanzees in the study group handicapped by limb paresis [27]. Analysis of chimpanzee skeletons of two adult females with longterm, partial paralysis and a group of unaffected adult Gombe chimpanzees revealed that poliovirus caused considerable asymmetries in the skeleton [31]. It was not clarified whether the outbreak was part of a natural cycle within the great ape population or a result of poliovirus transmission from humans. Chimpanzees in Gombe National Park (25 km²) inhabit a potentially contaminated environment from human communities living close to the park in the north and south sharing the same resources such as water [16, 37]. Poliovirus was widespread in the local human population during the 1960s and therefore transmission from humans was discussed as the most likely route when the chimpanzee community was habituated for research [22, 37]. Chimpanzees are susceptible to almost all human pathogens, so close proximity increases the risk of disease transmission [28]. Hence, it was recommended that chimpanzees and other apes in captive facilities should be vaccinated against poliovirus to avoid the risk of direct or indirect transmission from humans [25, 38, 40].

Therefore, primates in sanctuaries are generally vaccinated with the human poliovirus vaccine. However, as yet no long-term studies have been undertaken to evaluate immunological responses in vaccinated apes, and all recommendations on vaccination strategies are based on human studies. The American Zoo and Aquarium Association Species Survival Plan recommend vaccination of captive great apes with IPV at 12–13 months of age with a booster once at 1–2 years [25, 38]. Captive facilities and sanctuaries in Africa are using OPV in apes following human vaccination schedules, but variable regimes are used. To complement the Global Polio Eradication Program, vaccination programs of non-human primates in sanctuaries have to be adapted to the current state of art.

In this study, we show that OPV induces a long-lasting immunity against poliovirus in sanctuary chimpanzees in Uganda, and we discuss the future use of IPV for the vaccination of non-human primates in captivity.

Materials and methods

Study site

Ngamba Island Chimpanzee Sanctuary which started in 1998 is located on Ngamba Island (S 000 06/E 32°39', 0.46 km², 1160 m above sea level) and is part of the Koome group of islands in Mukono District, Uganda, lies 23 km off Entebbe in the northwest of Lake Victoria. Ngamba Island Chimpanzee Sanctuary currently cares for 44 rescued orphan chimpanzees on 100 hectares of secondary rain forest in a semi-captive management system with dietary supplements every day.

Study species

The study was conducted on 42 orphan chimpanzees that live in a semi-captive management system on Ngamba Island Chimpanzee Sanctuary. The group consisted of 23 females and 19 males with age group categorized as follows: four infants (1–5 years), six juveniles (6–8 years), 12 sub-adults (9–11 years), and 20 adults (12 years and more). They have lived on the island for varying lengths of time after being rescued from illegal traders and poachers since 1998. Rescued individuals are held in quarantine for 90 days during which period they are vaccinated against polioviruses using OPV (0.1 ml of Sabin Polio, Panacea Biotec Ltd, A-241 Okhla Indi.Area-1, Delhi-110020). Routine management of these rescued chimpanzees on the island exposes them to direct human contact by caregivers and veterinarians and indirectly to tourists, school groups, local community members, and researchers. They are fed on locally grown fruits and vegetables purchased from local markets.

Blood collection

Blood was collected from all chimpanzees during our annual medical checks in February, 2007. Individual animal welfare is of paramount importance and is part of our standard operating procedures; hand-held intramuscular injection of anesthetic drugs using a combination of ketamine (3 mg/kg) and medetomidine (0.03 mg/kg) is administered to minimize stress. Blood (7.5 ml) was taken from the femoral vein using EDTA Vacutainer tubes. Plasma/serum was extracted by centrifugation and stored at -80°C at the Uganda Virus Research Institute until transported on dry ice to Robert Koch-Institut, Berlin, Germany, for analysis. Retrospective serum collected in 2001 and 2005 and stored at -40°C was included in the assessment of antibody titers. This study was approved by Chimpanzee Sanctuary & Wildlife Conservation Trust as part of the veterinary preventive healthcare management, and research and export permits were issued by Uganda Wildlife Authority, Uganda National Council of Science and Technology, CITES Office in Uganda and Germany.

Serology

The methods for the determination of the neutralizing antibody titers against poliovirus types 1, 2, and 3 have been described elsewhere [41]. Briefly, the microneutralization test using 100 ID₅₀ poliovirus (Sabin strains) was performed according to WHO guidelines [41]. A positive control using an in-house reference serum (IHR) of known neutralizing activity was included in each test to control reproducibility of results. The international standard serum (a preparation of pooled human serum) containing 25 IU, 50 IU, and 5 IU for polioviruses 1, 2, and 3, respectively, was used to calibrate the potency of the IHR [9]. Before laboratory testing, serum was inactivated for 30 minutes at 56°C in a water bath. Afterward, twofold dilutions of serum starting from 1:4 to 1:512 were incubated for 3 hours at 36°C in duplicate with 100 TCID₅₀ of the corresponding Sabin-type poliovirus. Human rhabdomyosarcoma cell suspensions were added, and results were scored after 7 days of culture. A serum dilution of \geq 1:4 giving protection against 100 TCID₅₀ of poliovirus was considered to be positive.

Results

The vaccinated chimpanzees with OPV under this study were found with neutralizing antibodies against poliovirus types 1, 2, and 3 detected in 85.7%, 71.4%, and 65%, respectively, of the tested serum 9 years post-vaccination. Of the studied chimpanzees, five had no neutralizing antibodies (<1:4) against poliovirus types 1, 2, and 3, including the captive-born infant that had not been vaccinated. Percentages for neutralizing antibodies from 2001 to 2005 serum were not computed because of high levels of bacterial contamination in the samples. For non-contaminated serum samples (results not shown here), neutralizing antibody titers were comparable with those obtained from 2007. The three recent orphan arrivals (Kityo, Rutoto and Rambo) had multiple vaccinations (more than three with OPV) while in quarantine as shown in Table 1, but the neutralizing antibodies showed the same trend as those vaccinated once or twice.

Discussion

We present for the first time a vaccination success of captive chimpanzees with OPV. The neutralizing antibody titers found in chimpanzees are similar to those reported in human studies [9], indicating a high level of protection in the OPV-vaccinated chimpanzees. This study also reveals persistence of neutralizing antibodies for at least 9 years post-vaccination with OPV. The percentage of neutralizing antibodies detected against poliovirus types 2 and 3 (71.4% and 65%) was generally lower than those for poliovirus 1 (85.7%). This is in agreement with serological studies in humans, showing lower antibody titers for poliovirus types 2 and 3 [8, 9]. Four chimpanzees had no neutralizing antibodies (<1:4) against poliovirus types 1, 2, and 3 despite records showing they had been vaccinated at least once. This may be because of other viral or helminth intestinal infections prohibiting uptake of OPV as reported in developing countries where OPV has been shown to be less potent in inducing humoral immunity. Repeated vaccinations up to 5–10 times are hence required to protect all children [23, 30, 32, 34].

It is known that immunity to poliomyelitis is dependent on humoral neutralizing antibodies, both after natural infection and after vaccination. Hence, presence of antibodies beyond a threshold antibody level is an indication of protective immunity in case of poliomyelitis. It has been shown that enterovirus infections also induce T-cell immunity [24], but little information is available about the cellular immunity in great apes. It is not even clear whether these individual chimpanzees with low titers or no detectable antibodies are susceptible to infection as it is with humans.

Trivalent OPV is recommended by Expanded Program on Immunisation as a vaccine of choice for eradication of poliomyelitis because of its low cost, ease of administration, superiority in conferring intestinal immunity, and the potential to infect household and community contacts secondarily [12]. However, it has been reported that OPV is less potent in inducing humoral immunity in developing countries where the infection of the intestines by other viruses may prohibit the intake of OPV. Further, the continuous use of OPV may result in increase vaccine-associated paralytic polio, circulating vaccine-derived polioviruses (cVDPV) and those originating from immune-deficient vaccine-derived polioviruses (iVDPV) [26, 29]. Therefore, the IPV, which is already widely used in developed countries, should play the major role during the endgame of polio eradication and thereafter.

Hence, poliovirus vaccination of non-human primates, especially apes in captive facilities (zoos and sanctuaries), should be evaluated along with the global eradication program and follow the established WHO global action for laboratory containment of wild polioviruses [42, 43]. This study shows that OPV induces high levels of protective immunity in chimpanzees, but future use of OPV in captive apes should be assessed. As vaccine-derived strains (VDPV) may be excreted in feces for several weeks and even years (in the case of immunodeficient patients), it serves as a source of dissemination of polioviruses and the cause of poliomyelitis [18, 26, 29]. Sequence drift has been shown in VDPV as an indication of prolonged replication of the vaccine strain either in one individual or in the community [5, 26, 29]. Although it is not known for how long the polioviruses are excreted in feces of vaccinated apes, it may be risky to continue to use OPV in apes, and IPV may be safer to use at this point. The results presented in this study will serve as a baseline data for more studies to be undertaken in primates to estimate risks and advantages of OPV vs. IPV vaccinations for great apes in sanctuaries.

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Table

Table 1 Neutralizing poliovirus antibodies from 2007 chimpanzee serum

Chimpanzee name	Sex	Vaccination dates	Neutralizing antibodies, 2007		
			Poliovirus 1	Poliovirus 2	Poliovirus 3
Masiko	¹ C.male	21.11.01	1:4	>1:32	<1:4
Robbie	Male	27.11.01	>1:32	1:4	>1:32
Tumbo	Male	21.11.01	>1:32	>1:32	>1:32
Eddy	Male	21.11.01	>1:32	1:16	1:8
Sunday	¹ C.Male	27.11.01	>1:32	>1:32	1:16
Mika	Male	20.11.01	>1:32	1:16	>1:32
Megan	Female	03.10.01	>1:32	>1:32	>1:32
Kidogo	Female	26.09.01	>1:32	>1:32	>1:32
Peace	Female	21.11.01	>1:32	>1:32	1:16
Sophie	Female	26.09.11	>1:32	>1:32	1:8
Nagoti	Female	03.10.01	>1:32	>1:32	<1:4
Katie	Female	26.09.01	>1:32	1:16	>1:32
Connie	Female	26.09.01	>1:32	>1:32	<1:4
Bahati	Female	03.10.01	>1:32	>1:32	<1:4
Natasha	Female	21.11.01	>1:32	1:8	1:8
Becky	Female	27.11.01	>1:32	1:16	>1:32
Sally	Female	27.11.01	>1:32	1:4	>1:32
Cindy	Female	11.10.01	>1:32	1:4	1:4
Ikuru	Female	11.10.01	>1:32	1:4	<1.4
Nkumwa	Female	11.10.01	<1:4	1:4	<1:4
Billi	Female	20.11.01	>1:32	1:8	1:4
Yoyo	Female	20.11.01	1:4	<1:4	<1:4
Namukisa	Female	20.11.01	>1:32	>1:32	1:4
Pasa	Female	20.11.01	Ct	1.8	Ct
Ndyakira	Female	23.05.02/30.07.02	>1:32	1:8	1:16
Kazahukire	Female	20.08.02	1:4	1:4	<1:4
Nakuu	Female	31.05.02/30.07.02	>1:32	Ct	Ct
Nani	Female	2002	1:8	Ct	Ct
Mawa	Male	10.11.01	>1:32	>1:32	>1:32
Kalema	Male	10.11.01	1:16	1:4	>1:32
Umutama	Male	20.11.01	>1:32	1:4	1:8
Umugezi	Male	20.11.01	>1:32	<1.4	>1:32
Baluku	Male	20.11.01	>1:32	<1.4	>1:32
Asega	Male	10.11.01	>1:32	>1:32	<1.4
Kisembo	Male	11.10.01	<1.4	<1.4	<1.4
Indi	Male	20.11.01	>1:32	>1:32	<1:4
Okech	Male	19.04.02/21.05.02	>1:32	1:8	<1.4
Bwambale	Male	09.03.02	1:16	>1:32	<1:4
Kyewunyo	Female	Not vaccinated	<1.4	<1.4	<1.4
Kityo	Male	9/06; 11/06; 02/07	>1:32	1:16	<1:4
Rutoto	Male	06/06; 08/06; 02/07	>1:32	1:8	1:8
Rambo	Male	09/06; 10/06; 12/06; 02/07	>1:32	>1:32	<1:4

Cutoff at dilution of 1:4 in serum.

¹Castrated male; Ct: results could not be read because of bacterial contamination.