3rd Inter. Conf. Virol., Cairo Univ. Centrer, Nov. 24-25, 2010
Egyptian J. Virol, SP. Issue, 21-34, 2010

POST-DISTRIBUTION SURVEY FOR POTENCY OF LIVE ATTENUATED ORAL POLIO VACCINES (OPV) USED IN SUDAN NORTHERN STATES PERIPHERAL CHILDREN IMMUNIZATION CLINICS

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ABSTRACT

National immunization days (NIDs) started in 1994 in Sudan by using trivalent OPV (tOPV) and in the beginning of 2010 bivalent OPV (bOPV) was permitted to be use. Since OPV is manufactured from a highly thermolabile vaccine strain of poliovirus, it should be stored under the recommended temperature of -20°C or below. A vaccine vial monitor (VVM) is a label containing a heat sensitive material which is placed on a vaccine vial to register cumulative heat exposure over time. Despite of several polio vaccine coverage campaigns in the last two years, four of five children poliomyelitis cases reported in March 2009 in northern Sudan States, came from the Red Sea State. The aim of this study is to investigate the quality of OPV used for children immunization clinics in Red Sea State, to ensure whether the vaccine is still keeping its potency after being exposed to storage and transportation conditions. Several fixed, out reach and mobile immunization clinics were selected from all Red Sea State localities. Fifty seven bOPV samples and Fifty seven tOPV samples were taken during NIDs campaign in February 2010 and June 2010 respectively, and their potency were tested by the determination of a cell culture infective dose 50% ($CCID_{50}$) in Hep-2 Cincinnati cell line and using the

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Karber's Formula. All tOPV (type 1, 2 and 3) samples were found to be potent, within World Health Organization (WHO) normal limits. However, type-3 poliovirus in most of the bOPV (type 1 and 3) samples showed a titer lower than the WHO normal limits although all samples have at least grade II VVMs. These data indicate that type-3 poliovirus in case of bOPV needs more investigations in spite of cold chain and grade II VVM appear to be efficient.

Key words: bOPV, NIDs, OPV, Polio eradication, tOPV, VVM

INTRODUCTION

In 1988, the World Health Assembly adopted resolution WHA41.28 on global eradication of poliomyelitis by the year 2000. By 2008, all but four countries had interrupted indigenous transmission of wild polioviruses (Afghanistan, India, Nigeria and Pakistan) **(WHO, A63/27, 2010)**.

National immunization days (NIDs) are organized as part of the global goal of poliomyelitis eradication, targeting all children less than 5 years of age **(WHO, 2008; Ariane *et al.*, 2010)**.

As in a number of developing countries, NIDs were started in 1994 in Sudan by using trivalent OPV (tOPV) and in the beginning of 2010 bivalent OPV (bOPV) was permitted to be use.

In developing countries, immunity induced following OPV is very low, about 30%. Failure of OPV has risen from 5% in 1960s to an alarming 30% currently **(Poliomyelitis, 1995)**. The factors for decreased vaccine take rate may be due to interference by antibodies in breast milk, intercurrent non polio enteroviruses preventing colonization by the vaccine virus strains, helminthic infestation or presence of non-specific inhibitors in saliva of infants. Decreased potency of the vaccine could be another probable reason **(Cockburn and Drozdrov, 1970; Shiva *et al.*, 2003)**.

Like other biological products, vaccines have to be of sufficient stability under the conditions of transport and storage to maintain their potency at the point of use **(Tydeman and Kirkwood, 1984)**. The widely used OPV is a non lyophilized live attenuated vaccine, and is also the most unstable vaccine in the WHO Expanded

Program of Immunization (EPI) (**Sokhey *et al.*, 1988; Pipkin and Minor, 1998**). Several studies have reported the stability of OPV at ambient temperatures with regard to various stabilizers used in its formula (**Mirchamsy *et al.*, 1978; Mauler and Gruschkau, 1978**). OPV is known to retain its potency over a long period when stored at -20°C or below (**Zaffran, 1996; Der-Yuan *et al.*, 2000**).

The common childhood vaccines are usually transported under 2–8°C conditions from the main stores to the peripheral immunization sites, so that some OPV must face an extreme variation in storage temperature and could enter a freezing-thawing cycle before use.

Ideally, during vaccination activities, the vaccinators should use cool boxes with ice packs for transporting the OPV to prevent the vaccine's exposure to heat. Countries where polio transmission and import still occur often face challenges in securing enough vaccine carriers and ice packs to support the campaign outreach activities. WHO and UNICEF recommended flexible polio vaccine management and guidance for this approach has been published (**WHO, 2000; WHO, 2007; Ariane *et al.*, 2010**).

The addition of 1 M $MgCl_2$, to several enteroviruses, including the three poliovirus serotypes, was shown to reduce the loss of infectivity at high ambient temperatures (**Wallis and Melnick, 1962; Newman *et al.*, 1995**)

A vaccine vial monitor (VVM) is a label containing a heat sensitive material which is placed on a vaccine vial to register cumulative heat exposure over time. Different types of VVMs are available in order to match the varying stability profiles of vaccines. Oral Polio Vaccine (OPV) is the most heat-sensitive of the EPI vaccines and is equipped with a VVM_2, which reaches its endpoint after a cumulative exposure to 37°C for up to 2 days (**WHO, 2000; Ariane *et al.*, 2010**). The inner square of the VVM is made of heat sensitive material that is light at the starting point and becomes darker with exposure to heat.

VVM applied directly to the vaccine by the manufacturers enables the health worker to verify at the time of use whether the vaccine is in usable condition/or has lost its potency due to temperature abuse. Concerns are sometimes raised about that VVM may not accurately reflect the status of OPV in the vial to which they are attached (**WHO, 1999**)

and transported to various transient levels (**Jain *et al.*, 2003**).

Despite of routine immunization and several polio vaccine coverage campaigns in the last two years, four of five children poliomyelitis cases reported in March 2009 in northern Sudan States, came from the Red Sea State.

To the best of our knowledge this was the first study done in Sudan with the objective to investigate the quality of OPV used for children immunization clinics in Red Sea State, to ensure whether the vaccine is still keeping its potency after being exposed to storage and transportation conditions.

MATERIALS & METHODS

Materials:

Minimum essential medium (MEM) containing Earle's salts and L-glutamine from Applichem (Germany), heat-inactivated and qualified fetal bovine serum (FBS) from Invitrogen (Newzeland), sodium bicarbonate, antibiotics and trypsin/EDTA were all supplied by Sigma (Germany). The reference vaccine and neutralizing antiserum of three-serotypes of poliovirus were kindly provided by GSK (Belgium). The microtiter plates, tissue culture flasks, and other plastic accessories were from Nunc (Denmark).

OPV Samples:

Sudan Red Sea State is one of the 15 Sudan northern states, found in the East of Sudan. This state occupies a 212,000 square kilometers and its climate is dry hot in summer and warm rainy in winter.

The red sea state is subdivided into 10 localities (Port Sudan, Suwakin, Gunub and Oleeb, Sinkat, Dordeeb, Haya, Tokar, Ageag, Gabiet and Halaib), 19 administrative units and it consists of 582 villages. Samples collection was covering all three types of vaccination sites (30 fixed, 86 out reach & 16 mobile sites) distributed over the 10 localities.

During NIDs campaigns, a number of 57 bOPV samples were taken in February 2010 and 57 tOPV samples were taken in June 2010 from all Red Sea State localities, by Multistage Stratified Simple Random Sampling technique adopting proportionate sampling.

Cell and Cell Culture:

The Hep-2 (Cincinnati) cells were purchased from VACSERA, Egypt,

and they were maintained in MEM supplemented with 10% FBS in a humidified incubator at 36°C under 5% CO_2 condition. The confluent Hep-2 cells were subcultured with the aid of 0.25% trypsin/EDTA solution.

Potency Test:

The potency test was conducted according to the methods for potency testing of vaccine used in the WHO EPI (**WHO, 1997**). Serial dilutions of the samples and Reference Vaccine were inoculated in rows of 8 wells of microtiter plates, together with a highly specific antiserum to neutralize the polioviruses of the other serotypes in OPV. After 3 hours incubation, a trypsinized Hep-2 cell suspension was added. The microtiter plates were incubated at 36°C at 5% CO_2 for 5 to 7 days. At the end of the incubation period, the plates were read microscopically using an inverted microscope (Wilovert_Germany) for cytopathic effect (CPE), wherein infected cells were rounded up, refractile and detaching from the plate surface. The cell culture infective dose 50% ($CCID_{50}$) per human dose was calculated using the Karber's formula. 5% MEM and trypsinized Hep-2 cell suspension were added to the cell control and antiserum mixture rows.

RESULTS

A total of 57 representative tOPV samples were collected from areas covered by fixed, out reach and mobile immunization sites in the 10 localities of the red sea state during NIDs in June 2010. With the exception of one sample obtained from Gabiet and another one sample from Halaib that were not complying with the WHO potency limit for serotype-3 and serotype-1 respectively, all other samples were found to comply with the WHO potency limit for the three serotypes, type1, 2 and 3 ($10^{6.0}$, $10^{5.0}$ and $10^{5.8}$ respectively), as shown in **Table (I).**

A number of 57 representative bOPV samples were similarly collected from areas covered by fixed, out reach and mobile immunization sites in the 10 localities of the red sea state during NIDs in February 2010. The results presented in **Table (II)** showed that 13.6% of type-1 (from Haya, Gabiet and Dordeeb localities) and 73.6% of type-3 were found to not comply with the WHO potency limit for the two serotypes, type-1 and 3 ($10^{6.0}$ and $10^{5.8}$, respectively). Samples that proved to comply with the WHO potency limit for type- 3 were obtained from Haya, Ageag and Middle Port Sudan localities.

Table I. Potency titres of trivalent oral poliovirus vaccine samples type 1, 2 and 3 collected from Red Sea State in June 2010.

No.	Locality	Sample	Type 1	Type 2	Type 3
1	Haya	Fixed*	6.4	5.3	6.3
2		Pool**	6.3	5.3	6.0
3	Halaib	Fixed	5.5	5.0	6.3
4		Pool	6.2	5.5	6.1
5	Sinkat	Fixed	6.0	5.1	6.2
6		Pool	6.1	5.3	6.1
7	Gabiet	Fixed	6.3	5.5	6.2
8		Pool	6.0	5.1	5.4
9	Ageag	Fixed	6.3	5.5	6.3
10		Pool	6.3	5.4	6.0
11	Tokar	Fixed	6.2	5.1	6.2
12		Pool	6.2	5.5	5.9
13	Suwakin	Fixed	6.1	5.2	6.0
14		Pool	6.4	5.5	6.3
15	Dordeeb	Fixed	6.2	5.5	6.2
16		Pool	6.1	5.0	6.0
17	Gunub and Oleeb	Fixed	6.3	5.6	6.2
18		Pool	6.1	5.2	6.2
19	Middle Port Sudan	Fixed	6.4	5.3	6.4
20	Southern Port Sudan	Fixed	6.2	5.2	6.1
21	Eastern Port Sudan	Fixed	6.3	5.4	6.2
22	All Port Sudan	Pool	6.3	5.2	6.3
23	Port Sudan	State Store	6.2	5.3	6.4
24	Khartoum	Federal Store	6.5	5.5	6.4

* Main immunization site act as main store in specific locality.

** All immunization sites other than the main store (fixed site) in specific locality.

Table II. Potency titres of bivalent oral poliovirus vaccine samples type 1 and 3 collected from Red Sea State in February 2010.

No.	Locality	Sample	Type 1	Type 3
1	Haya	Fixed*	5.9	6.0
2		Pool**	6.3	5.8
3	Halaib	Fixed	6.2	5.1
4		Pool	6.6	5.3
5	Sinkat	Fixed	6.2	5.2
6		Pool	6.1	5.3
7	Gabiet	Fixed	5.7	5.1
8		Pool	6.0	5.8
9	Ageag	Fixed	6.3	6.7
10		Pool	6.0	6.7
11	Tokar	Fixed	6.1	5.3
12		Pool	6.5	5.6
13	Suwakin	Fixed	6.2	5.5
14		Pool	6.6	5.1
15	Dordeeb	Fixed	5.9	5.6
16		Pool	6.4	5.5
17	Gunub and Oleeb	Fixed	6.2	5.3
18		Pool	6.2	5.2
19	Middle Port Sudan	Fixed	6.2	6.9
20	Southern Port Sudan	Fixed	6.5	5.6
21	Eastern Port Sudan	Fixed	6.3	5.5
22	Port Sudan All	Pool	6.1	5.5

* Main immunization site act as main store in specific locality.
** All immunization sites other than the main store (fixed site) in specific locality.

DISCUSSION

Preservation of vaccine potency through proper storage and maintenance of the cold chain during shipping or transit seems to be more pertinent to developing countries of the world especially the tropical countries where refrigeration is not readily available and exposure of stored vaccine product to extreme environmental conditions can be expected (**Galazka, 1989**). A successful immunization program requires vaccines to be stored in conditions that maximize their potency and a continuous temperature monitor that helps to identify a break in the cold chain. Weak links in the cold chain have been attributed to improper storage and there are reports where monitoring the vaccine cold chain was done through temperature recording (**Thakker and Woods, 1992; Cheriyan, 1993; Jain *et al.*, 2003**). Current regulations require that for maintenance of potency, the vaccine must be stored and shipped frozen and that after thawing, it must be stored in the refrigerator at not more than 10°C for a period not exceeding 30 days after which time it must be discarded (**WHO, 1999; Muhammad *et al.*, 2010**). Thermostability requirements were defined by WHO as OPV that loses less than 0.5 log 10 of titre of each of the vaccine strain after exposure to 37°C for 2 days (**WHO, 1999; Muhammad *et al.*, 2010**).

In this study data indicate that 73.6% and 13.6% of type-3 and type-1 respectively, from bOPV samples were found to not comply with the WHO potency limit. In accordance with this observation, **Hanjeet *et al.* (1996)**, found that 78.6% and 28.6% out of 42% dissatisfactory tOPV from 33 samples in a study in Malaysia were due to type-3 and type-1 respectively. **Der-Yuan *et al.* (2000)**, found that only 3.8% of type-3 from 79 tOPV samples of study done in Taiwan were not satisfactory. However, the WHO lower limit for type-3 at that time was $10^{5.5}$ and now it changes to $10^{5.8}$ (**WHO, 1997**). However, in case of tOPV samples collected in June, the data showed that tOPV samples obtained from main federal and Red Sea State stores, in Khartoum and Portsudan respectively, were comply with the WHO potency limit. These results indicated that the cold chain may be efficient in Sudan main stores at that period of time.

The significant potency drop of type-3 poliovirus strain in bOPV samples is most probably due to defects in cold chain at that time, defects in VVM sensitivity, type-3 inherent thermostability and/or the vaccine stabilizer used in the vial may be not efficient.

In case of the tested samples of bOPV a significant potency drop of type-3 poliovirus strain were obtained although the VVM status did not reach the discard point.

The obtained results agreed with **Der-Yuan *et al.* (2000),** who reported that some OPV face an extreme variation in storage temperature and could enter a freezing-thawing cycle before use. Under these conditions, it can not be guaranteed that all polio vaccines are able to maintain effective titers to induce a satisfactory immune response.

Also **Miller and Harris, (1994); Kendall *et al.* (1997),** reported that vaccines during transport and storage at rural and urban health center's can be also easily exposed to temperatures beyond the recommended range (2–8°C). In addition **Bishai *et al.* (1992); Gradon and Lutwick, (1999); Jain *et al.* (2003),** reported that exposure at 10°C, 21% of OPV samples are still at risk of deterioration.

It is to be considered that immunization strategy during NIDs is based upon house to house immunization (from door to door) by different teams of volunteers, which may lead to exposure of vaccine vial to direct sun light. Moreover during NIDs, OPV vaccine kept whole day in thawing form and unopened vials were retained back to freeze in storage cold chain to be used in the next day.

In case of VVM sensitivity **(Arya and Agarwal, 2007)** reported that the use of VVM is based on chemical changes induced by heat, and thus they are thermal and not biological indicators, making them fallible. For instance, they do not reflect evaporative and radiative transfer of heat from the atmosphere. Furthermore, VVM do not record spikes in temperature variation, exposure to sunlight, humidity or prolonged exposure to lower temperatures.

It has been documented by WHO that time evaluated for VVM to

reach discard point varies at different temperature exposures (**WHO, Geneva, 1996; Jain *et al.*, 2003**). Concerns are sometimes raised that VVM may not accurately reflect the status of OPV in the vial to which they are attached (**WHO, Geneva, 1999**) and transported to various transient levels.

Pipkin and Minor, (1998), reported that Preliminary studies suggested that the type-3 component of the live oral polio vaccine is the most labile. Most attention therefore is focused on the type-3 strains. For the type-3 poliovirus, **Der-Yuan *et al.* (2000),** found that degradation of the type-3 poliovirus seemed to be correlated with the storage temperature in each storage area. However, the exact cause of type-3 poliovirus degradation is still unknown. It is suggested that it could be due to the higher level of the minimal storage temperature of the OPV. The results obtained for type-3 in bOPV may comply with this suggestion.

High concentrations of $MgCl_2$, were shown to improve the poliovirus thermostability (**Wallis, and Melnick, 1962**), and are now systematically used to stabilize all OPV preparations (**WHO, 1990**). However, the thermostabilization due to $MgCl_2$, still less efficient than that required in some endemic areas of the world, notably south Asia and Africa (**Rong *et al.*, 1995**).

This suggestion agreed with the data obtained from study on 57 samples of bOPV may indicate that the thermostabilization due to $MgCl_2$ in the vaccines still less efficient than that required in Africa.

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