Potency of field samples of oral poliovirus vaccine

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The transport and storage facilities and the potency of oral poliovirus vaccines currently administered in 108 centres in India were investigated. Storage and distribution practices in many of the centres were far from ideal. There was no significant loss of potency in the vaccine samples collected from a few centres, while samples from other centres showed a 60-90% loss of virus particles per dose. A national monitoring system has since been established to check the potency of every batch of oral poliovirus vaccine imported and to streamline the transport, storage, and administration of the vaccine. Constant vigilance as regards the quality of vaccines should ensure the success of any poliomyelitis immunization campaign.

The detection of a measurable circulating serum antibody is a valuable index of the effect of a programme of immunization with oral poliovirus vaccine. Cabasso et al. (3) demonstrated circulating antibodies against all three polioviruses in 99% of vaccinated children one month after they had received two doses of trivalent vaccine. Live vaccine, in countries with a warm climate, is known not to produce seroconversion in such a high proportion of children (5, 8). Interference from other enteroviruses present in the gut, the presence of antibody in breast milk, and the development of cellular resistance owing to previous exposure to natural polioviruses and/or perhaps to related viruses or protein deficiency, have all been mentioned as factors responsible for low seroconversion rates (4). Montefiore (7) reported that, when reasonable care was taken at all stages, low seroconversion rates could not be attributed only to faulty administration, while Brito Bastos et al. (2) emphasized the need for evaluating vaccine potency in terms of virus particles per dose.

To determine whether the potency of the vaccine played a role in the low seroconversion rates found in a country with a warm climate—India—vaccine samples were obtained from importers, distributors, and immunization centres in various parts of the country. The potency of the individual samples was determined in terms of virus particles per dose so as to calculate any loss of vaccine potency.

MATERIALS AND METHODS

Collection of vaccine samples

Vaccine samples were obtained from Delhi, Jaipur, Kanpur, Bhopal, Calcutta, Madras, Bombay, Jagadhari, Mathura, Meerut, Ferozepur, Ambala, Kasganj, and Chandigarh. As far as possible, amounts representing 10-20 doses of a particular vaccine lot were collected aseptically in a sterile universal container. The labelled containers were stored on solid carbon dioxide and shipped to the laboratory with the least possible delay. The samples were stored in the laboratory at −65°C to −70°C in a deep-freeze. Whenever a sample was obtained from a distributor or from an immunization centre, the supplier was asked to answer the questions indicated in Table 1.

The storage facilities were inspected and, as far as was practicable, suggestions were made for remediating any defects.

Determination of virus content of vaccine samples

The vaccine samples were titrated for the total live virus content in primary rhesus monkey kidney cell cultures. The kidneys, from adult monkeys that had been kept in quarantine for different periods, were trypsinized overnight at 4°C (I). The culture medium was Hanks balanced salt solution containing 0.5% lactalbumin hydrolysate (Difco),

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5% goat serum (obtained from the Delhi slaughterhouse), and antibiotics. The cells were washed with phosphate-buffered saline (pH 7.3) before being transferred to the maintenance medium (tissue culture medium TC 199 \(^a\) with 0.2% bovine albumin and antibiotics).

Serial 10-fold dilutions of different samples were made in the maintenance medium and 0.2-ml volumes of the appropriate dilutions—usually 10\(^{-6}\), 10\(^{-5}\), and 10\(^{-7}\)—were inoculated into 10 culture tubes and incubated at 35 ± 1°C in a roller drum incubator. The final readings were made on the seventh day and the virus content of the vaccine was determined in terms of TCID\(_{50}\) per ml by means of the Reed & Muench (12) formula. As a check on the sensitivity of the kidney cell cultures, a reference virus was titrated simultaneously with the vaccine samples.

Methods of computing loss of vaccine potency and of determining the significance of differences in potency are described in the Annex.

**RESULTS**

**Storage and distribution**

In 21 of the 108 centres visited, vaccines were stored in a deep-freeze at −20°C; in 68 centres, in the freezing chamber of the refrigerator; and in 26 centres, in the main storage compartment of the refrigerator. In one centre, the vaccine was kept at room temperature, while in another it was stored inside an earthen pitcher. Only 8 centres had installed a stand-by generator to prevent any interruption in power supply to the deep-freeze or refrigerator containing the poliovaccine. Maintenance of the deep-freeze or refrigerator appeared to be satisfactory in only 42 of the centres visited.

In none of the centres was there any awareness of the effects of cycles of freezing and thawing on the vaccines. Sixty-nine of the samples were from vials from which the rubber stoppers had been removed before the contents were used. However, 42 centres reported that the rubber stoppers were never removed and that the contents were removed with a sterile syringe and needle. At 62 centres the vaccine was delivered direct into the recipient’s mouth by means of a syringe or plastic pipette, while 33 centres employed plastic or metal spoons. In only 2 centres was the vaccine diluted in 5–10 ml of water before administration; disinfectant, soap, or potassium permanganate was used for washing the spoons in a few centres. In one centre the desired amount of vaccine was injected into a cube of desiccated semi-porous sugar, which was fed to the person to be vaccinated. In only 38 centres were mothers instructed to avoid breast-feeding for a time after vaccination. Food of any kind was prohibited during the 1/2–2-hour post-vaccination period in 23 centres.

**Loss of potency during storage**

Of the 191 samples tested, 113 did not show any reduction in titre (Table 2). There was a 0.1–0.3-log reduction in the titre of the individual dose in 22 samples. Statistical analysis with the Pizzi

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\(^a\) Difco Laboratories, Detroit, MI 48232, USA.
Table 2. Results of potency testing of 191 vaccine samples

<table>
<thead>
<tr>
<th>Manufacturer or supplier of the vaccine</th>
<th>Samples tested</th>
<th>Samples showing no loss of potency</th>
<th>Samples showing loss of potency (in log scale and percentages)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>20–50 % or 60–90 % or 92–99 % or more than 99 % &gt; 2.0 log</td>
</tr>
<tr>
<td>Institute of Poliomyelitis, Moscow (liquid vaccine)</td>
<td>154</td>
<td>103</td>
<td></td>
</tr>
<tr>
<td>Institute of Poliomyelitis, Moscow (drages)</td>
<td>4</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Lederle Laboratories, Pearl River, NY</td>
<td>3</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Institute of Immunology and Virology, Belgrade</td>
<td>2</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Haffkine Institute, Bombay a</td>
<td>27</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Pasteur Institute, Coonoor</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>191</td>
<td>113</td>
<td></td>
</tr>
</tbody>
</table>

a Vaccine imported in bulk was bottled at this Institute.

formula (10) did not always reveal a significant loss of potency in such samples, although the loss may well have been biologically significant. The centres from which these 22 samples were obtained were informed that the loss of potency in their samples was marginal. In 56 samples (30% of the total), the titre of the individual dose showed a reduction of at least 0.4 log. This loss was always found to be statistically significant. The centres that supplied samples of such low-titre vaccine were informed of the results immediately and advised to destroy the remaining lots of the vaccine from which the representative samples had been drawn. In 6 centres, the sample drawn from the stored vaccine was found to be satisfactory, while the vaccine that was actually given to children showed a significant reduction in potency.

Remedial measures

In the initial phase of this study it was found that neither the main importers nor the regional or peripheral distributors took care to despatch the vaccine at sub-zero temperatures. They were using wet ice with or without insulating material. The peripheral immunization centres were obtaining their supplies in vacuum flasks with or without ice. When the preliminary results of the survey were known, it was made obligatory for the main importers to distribute all vaccine supplies on solid carbon dioxide, and this has since been adopted as standard practice in India. It is recommended by the Indian national drug control authorities that, once the vaccine has reached the regional or peripheral distributors, it should be stored only in a deep-freeze at −20°C. Supplies are subsequently distributed to the field on solid carbon dioxide or in a freezing mixture, so that the vaccine is transported at sub-zero temperatures.

A constant check is now being maintained on all imported batches of poliovirus vaccines. Samples are taken at the port of entry and are flown to the testing laboratory on solid carbon dioxide, thus ensuring that only fully potent vaccines are released from the importer’s premises. A periodic check is also made at the premises of the regional distributors located in various parts of the country.

DISCUSSION

The incidence of poliomyelitis is increasing in many countries (4) and the low seroconversion rates that have been reported indicate low efficacy of oral poliovirus vaccine. In addition to interference from other enteroviruses, other factors—antibody in breast milk, protein deficiency, cellular resistance to poliovirus or related viruses, and even inhibitors in the saliva and in throat swabs from antibody-free children—have been incriminated (13). Surprisingly, no systematic work appears to have been done to assess the potency of the vaccines being administered in developing countries, although Brito Bastos et al. (2) have stressed the need to test vaccine potency in terms of the number of virus particles present per dose.
The poliovirus titre for each filling lot should be determined routinely before the vaccine (14) is distributed. However, the fact that any particular lot is fully potent before distribution is no guarantee that every dose from that lot will be fully potent when it is administered, and this is crucial to the success of any vaccination campaign.

Storage in an opened or loosely capped vial increases the pH and together with other factors may accelerate significantly the loss of vaccine titre (6). The storage conditions in a refrigerator or deep-freeze must also be checked regularly and care is required to ensure that the vaccines are used before the expiry date. Vaccines should not be exposed to light or to ambient temperatures for long periods before administration.

When cases of poliomyelitis occur after vaccination, a serious attempt should be made to ascertain the potency of the vaccine that was administered. There appears to be ample justification for re-examining the criteria for a causal association with vaccination (9). Undoubtedly, the potency of the vaccine should be the most important factor in this regard in areas where wild viruses are endemic.

This study has emphasized the need for the national control authorities to exercise control over all batches of vaccines used in the country. Vaccine samples for the testing of potency should be obtained regularly at the port of entry and from indigenous manufacturers, wholesale and regional distributors, local storage depots, and vaccination centres. Dealers, health administrators, and parents should be made aware of their respective roles. Frequent surprise inspections at immunization centres are also valuable.

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RÉSUMÉ

Afin de déterminer dans quelle mesure l’activité du vaccin antipoliomyélique (buccal) est le facteur responsable de la médiocre efficacité de ce vaccin dans les climats chauds, on a prélevé des échantillons à partir de lots de vaccins utilisés sur le terrain, et mesuré l’activité de ces échantillons. On a inspecté un total de 108 centres situés dans diverses parties de l’Inde afin d’évaluer les moyens de transport et de stockage du vaccin dont ils disposaient, et l’on y a recueilli des échantillons de vaccin. Il est apparu que les pratiques de stockage et de distribution de plusieurs centres étaient loin d’être idéales. Les échantillons de vaccin provenant d’un petit nombre de centres ne présentaient aucune perte d’activité notable, alors que dans ceux d’autres centres, il y avait une baisse de 60% à 99% du nombre de particules virales présentes dans les doses individuelles.

Dans les cas où une poliomyélite survient après vaccination, il faut s’efforcer de vérifier l’activité du vaccin qui avait été administré. Il semble bien qu’à cet égard l’activité du vaccin soit le facteur le plus important dans des régions où des virus sauvages sont endémiques. Un contrôle constant de la qualité du vaccin devrait assurer le succès de toute campagne d’immunisation contre la poliomyélite, même dans les climats chauds.

REFERENCES


**Annex**

**COMPUTATION OF LOSS OF VACCINE POTENCY AND TEST OF SIGNIFICANCE REFERENCES**

The virus content of each dose is calculated from its TCID₅₀/ml value on the basis of the volume recommended by the manufacturer for each dose. Since the virus content of a sample of trivalent vaccine containing 10⁵.₇ or 10⁶.₀ of type 1, 10⁵.₀ of type 2, and 10⁴₅ of type 3 may be expected to be 10⁴₅₃ TCID₅₀, the exact loss of potency is computed on the assumption that a sample with a virus content of 10⁴ has a potency of 100%. Thus two samples with a total virus content of 10⁵ and 10⁴ may be considered to have lost 90% and 99% of their potency, respectively.

The standard error of the individual titre is calculated with the formula developed by Pizzi (10), i.e.,

(SE) standard error of ID₅₀ = \sqrt{(0.79 hR)/n}

where 0.79 is a constant, \( h \) is the log of the dilution factor, \( R \) is the interval on the log scale between the cumulative 25% and 75% end-points, and \( n \) is the number of tubes per dilution.

To test the significance of the difference between any two virus titres, the following calculation is made.

\[ T_1 - T_2 \sqrt{S_e_1^2 + S_e_2^2} \]

where \( T_1 \) is the first titre, \( T_2 \) is the second titre, \( S_e_1 \) is the standard error of the first titre, and \( S_e_2 \) is the standard error of the second titre. If this calculation yields a value of 2 or more, the difference between virus titres is regarded as statistically significant with 95% limits of confidence.