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# A new, rapid, and promising approach to aerosol immunization: Inflatable bags and valved masks

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ABSTRACT

Booster doses of MMR vaccine equal in dosage to injected doses were aerosolized into a 3/4 l bag that inflated in 4 s. The bag was then attached to valved masks, and its contents rapidly inhaled in one or two deep breaths by preschool Mexican children. Antibody responses in the children exposed to the aerosolized measles component were superior to those noted after injection, while responses to the mumps and rubella components were equivalent. The new method appears to be effective, safe, and has several advantages over previously used methods. Further explorations of the approach seem merited.



## 1. Introduction

In all of the recent aerosol studies in children with measles vaccine [1–5], subjects have been exposed to aerosol for 30 s through a loose fitting mask using aerosol generated by the Classic Mexican Device (CMD), a system designed by one of us (JFC) which has been described in previous publications [1–3,6,7]. This approach is inherently wasteful of vaccine, since aerosol doses only enter the subject during inspiration, and there is visible escape of aerosol into the ambient air during exhalation. With the CMD, only about one-half of the aerosol generated in 30 s (about 5 l) is inhaled with tidal breaths by a schoolchild, and only one-fifth by a 12-month-old child, thus implying wastage of 50% and 80%, respectively. Despite vaccine wastage, boosting responses to aerosolized measles vaccines have nonetheless been consistently better than injected vaccine in equivalent doses in schoolchildren [1–3]. One study showed superior antibody boosting responses compared to customary larger doses of injected vaccine when only about 1000 plaque forming units (pfus) were estimated to have been administered by aerosol [3], with perhaps only 500 pfus actually being inhaled and even less retained. In contrast, aerosolized vaccines have induced poorer responses than injected vaccine in recent studies of primary immunization in 9-month-old and 12-month-old children [4,5].

Single use, disposable, and inflatable plastic bags offer a potentially improved method of delivering vaccine aerosols with compressed air systems. The nebulizer in such a system interfaces with the bag rather than the child, thus minimizing prospects of infections being transmitted from child-to-child by aerosol generating devices. By providing a "contained" system, contamination of ambient air with aerosolized vaccine is reduced compared with previous methods. The actual amount of aerosol inhaled when a child is exposed to continuously generated aerosols is uncertain, and inhaled doses are likely to be highly variable from child-to-child. The proposed system with valved mask helps in assuring that all children are exposed to similar inhaled doses, which is visually confirmable by noting the full collapse of the reservoir bag. If vaccine loss in such a system is less than current wastage in schoolchildren or infants, then its higher efficiency may permit even more aerosol doses from an injected dose than with previous systems [3].

To evaluate the viability of this approach, we arranged for pilot, proof-of-principle studies in Mexican preschool children using MMR vaccine from Serum Institute of India. Booster doses of MMR vaccine are mandated upon elementary school entry in Mexico.

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MMR aerosol has been shown to be effective and safe in a recent study in adults [6]. This is the first MMR aerosol study in children.

While interested in outcomes for all three MMR components, the main objective was to determine whether the new approach yielded serologic booster responses to measles that were superior to injected vaccine while yielding non-inferior responses to the two other components; otherwise, the new approach could not be recommended as an alternative to earlier aerosol approaches.

## 2. Materials and methods

### 2.1. Study population

Parents of children attending preschools in two communities on the outskirts of Toluca, Mexico were informed of the MMR vaccine trial by local health staff, and 143 signed consent forms and had their child randomly assigned to receive a booster dose of either injected ($n = 70$) or aerosolized vaccine ($n = 73$). The principal of one of the schools decided at the scheduled morning for immunization that vaccine would only be given to children whose parents were present for the immunization, which was inconvenient for many parents just arriving with their child at the school. Additionally, a few children did not come to school on immunization day either because of illness or other reasons. Consequently, 23 of the 72 children randomly assigned to treatment at this school did not participate vs. 12 of 71 at the other preschool. The 108 remaining participants were given a booster dose and had baseline blood samples collected, including 50 of the 70 initially assigned to receive injections and 56 of the 73 initially assigned to aerosol treatment; 2 children assigned to injections were switched to the aerosol group upon the insistence of their parents. Only 92 of the 108 students returned for collection of convalescent blood samples, 46 having received injections and 46 receiving aerosol treatments. These 92 paired samples were subjected to PRN testing for measles (see below), but only 88 paired sera remained, 44 from each group, in sufficient quantity for subsequent ELISA testing for measles, mumps, and rubella.

The average age of participating children was 68 months (range 62–73), half were boys and half girls, and their previous MMR injections had taken place on average 54 months (27–60) earlier.

Respiratory infections were uncommon in these children at the time of immunization. Only eight children, six in the aerosol group, had rhinorrhea at the immunization session. However, children who did not attend school on the immunization day may have been ill at home with respiratory or other infections. No child was excluded because of infections.

### 2.2. Vaccine

10 dose vials of MMR vaccine from Serum Institute of India were used, batch number 1339-X. The potencies in CCID50/dose for measles, mumps and rubella were $\log_{10}$ 3.717, $\log_{10}$ 4.5, and $\log_{10}$ 3.65, respectively. The specific vaccine strains were Edmonston-Zagreb, RA27/3, and L-Zagreb, respectively.

### 2.3. Equipment

We used a portable, electrically powered air compressor to produce compressed air in an attached metal tank of three-gallon capacity (DeVilbiss Pro-Air II, model PAFAC 153-1). The tank was equipped with a toggle switch, which allowed quick initiation and curtailment of airflow from the tank. The pressure of air in the tank was monitored with a pressure gauge, which was kept at 40 psi, the same operating pressure as used by the CMD, although flow from the tank in the present application was continuous rather than pulsatile.

An IPI Medical Products nebulizer as used in the CMD was connected to the air tank by plastic tubing. The "T" piece was attached to the outlet port of the nebulizer, and one of the outlets of the "T" was occluded by a cork and the other fitted with a cork with a bored out hole having a diameter equal to the outer diameter of the plastic tube inserted into the reservoir bag (see below).

We modified commercially available, pediatric, non-rebreathing kits designed to administer oxygen (Hudson RCI latex free, low resistance, non-rebreathing mask systems with Safety-Vent, approximately $2 each) into a two-part disposable system for each child.

One part consisted of an inflatable plastic bag of 3/4 l capacity, the mouth of which was fitted with a short piece of polyethylene plastic tubing that was secured in place by duct tape. Aerosol was delivered into the bag through this tube. The neck of the bag was occluded after the bag was filled with aerosol, and released a few seconds later after the tube in the mouth of the bag was inserted into the stem of the altered mask.

The oxygen supply tube to the mask was cut close to its entrance, and its entrance port was then rotated to the side. The perforated Safety-Vent holes in the mask were occluded with 1 inch square pieces of duct tape. A short piece of polyethylene plastic tubing was tightly inserted into the stem of the mask, the tube having an internal diameter equal to the outer diameter of the tube in the 3/4 l bag. The latter tube with its accompanying bag filled with aerosol remained securely attached to the mask after insertion without the need for any further manipulation. The mask, as provided, contained a one-way intake valve in its stem and a one-way exhaust valve in the mask.

The two parts of each system were assembled in Mexico in advance of the trial, and stored in zip-lock plastic bags until use.

Repeated simulations with this system showed that the 3/4 l bag filled with aerosol in 4 s, and that 0.053 ml of vaccine was aerosolized during this time. To assure equal doses were administered by aerosol and by injection, 10 dose vials of vaccine were therefore dissolved in 0.53 ml chilled diluent supplied by the producer before being placed in the nebulizer, thus giving 10 aerosol doses of 0.053 each for every 10 dose vial for injection. Preliminary tests revealed ready reconstitution in this smaller volume, and satisfactory aerosol generation using the concentrated vaccine. 10 dose vials were reconstituted with 5.0 ml diluent for subcutaneous injection, resulting in 10 injected doses of 0.5 ml.

### 2.4. Aerosol administration

Children in small groups of about 5 were fitted with the altered masks, which were secured by tightening the elastic strap of the mask around the neck below the ears, and by compressing the pliable metal bridge inserted in the mask over the nose to ensure a tight fit. Masks were observed for proper valve function during normal breathing. The desired technique to empty the bag filled with aerosol by rapid, deep inhalation was then demonstrated to the group. Preliminary experiments showed that the fit of the mask to the face could be improved by having an assistant apply light pressure on the exterior surface of the mask during inspiration of aerosol.

Separate classrooms were dedicated for aerosol and injection groups. As a precaution, the room where aerosol was given had an exhaust fan situated in a window, and bags were filled with aerosol in a location between the window and the children.

Vaccine was administered to children at one of the schools on February 11, 2008 and to children in the second school on the following day. All persons assisting in vaccine delivery either had histories of measles, mumps and rubella or had received prior vaccination with MMR vaccine.