Immunogenicity and safety of measles–mumps–rubella vaccine delivered by disposable-syringe jet injector in healthy Brazilian infants: A randomized non-inferiority study

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This study aimed to determine if immunogenicity to measles–mumps–rubella vaccine delivered to infants via a disposable-syringe jet injector (DSJI) was non-inferior to that administered by needle and syringe (NS). Vaccination safety was evaluated, as were the use, performance, and acceptability of each delivery method. The DSJI was the PharmaJet® 2009 generation-1 device (G1) and the vaccine was measles–mumps–rubella vaccine from Bio-Manguinhos. Five hundred eighty-two healthy Brazilian infants were randomized to receive vaccine via G1 or NS. Seroconversion rates against measles and mumps viruses in the G1 treatment group did not meet non-inferiority criteria when compared with the NS group; however, responses in the G1 group to rubella virus were non-inferior to those of NS vaccinees. Most adverse events were mild or moderate. Crying after injection was more frequent in the NS group, and local skin reactions were more common in the G1 group. Five serious adverse events were judged causally unrelated to treatment and all resolved. Parents/guardians expressed a strong preference for G1 over NS for their children. Vaccinators found the G1 easy to use but noted incomplete vaccine delivery in some cases. Although the G1 has been superseded by an updated device, our results are important for the continued improvement and evaluation of DSJIs, which have the potential to overcome many of the challenges and risks associated with needle-based injections.

Abbreviations: DSJI, disposable-syringe jet injector; G1, PharmaJet® 2009 generation-1 device; NS, needle and syringe; MMR, measles–mumps–rubella vaccine; AE, adverse event; SC, subcutaneous; FDA, (United States) Food and Drug Administration; ANVISA (from Portuguese abbreviation), Brazilian Health Surveillance Agency; CONEP (from Portuguese abbreviation), Brazilian National Research Ethics Commission; ITT, intention-to-treat; PP, per-protocol; ELISA, enzyme-linked immunosorbent assay; NT, neutralizing titer; PRNT, plaque reduction neutralization test; IgG, immunoglobulin G; GMC, geometric mean concentration; SeroC, seroconversion; CI, confidence interval; CRF, case report form; OR, odds ratio.

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1. Introduction

Disposable-syringe jet injectors (DSJIs) are needle-free devices that employ a sterile, single-use syringe to administer vaccines with a fine stream of pressurized, high-velocity liquid that penetrates the skin [1,2]. The liquid typically is propelled by release of a piston powered by a compressed spring or gas. DSJIs were developed to address risks associated with a type of device used earlier, the multiple-use nozzle jet injector (MUNJI), after evidence of cross-contamination between patients [1,3-5]. Prior to this, MUNJIs had been widely used in mass immunization campaigns [6,7] and in the military.

Several DSJIs have been approved and marketed in the United States and Europe [1]. They are capable of delivering all injectable vaccines used in immunization programs, whether into intradermal, subcutaneous, or intramuscular tissues, and have the potential to overcome many of the challenges and risks associated with needle-based injections and sharps waste. DSJIs that are particularly attractive for use in developing-country programs are low cost and use manually compressed springs rather than compressed gas.

Antibody responses to vaccines administered by DSJIs generally have been reported as comparable or superior to those induced by needle and syringe (NS). Vaccines shown to induce immunity when given by DSJI include typhoid, diphtheria, pertussis, hepatitis A [8,9], influenza [10–13], and measles–mumps–rubella (MMR) [14]. To date, no immunogenicity data have been published for MMR vaccine administered with the DSJI tested in this study.

The reported rates of local adverse events (AEs) (e.g., edema, erythema, tenderness) have been higher for DSJI delivery than for NS [15], but events are generally mild [8,16,17]. Some studies have found the pain during jet injection to be equivalent to or less than that associated with injection using a conventional NS [15], although other studies reported higher levels of pain with DSJIs [10,12].

For DSJI technology to be adopted globally, data demonstrating immunogenicity and safety of vaccination are important. Another consideration includes acceptability of DSJIs by patients or their parents/guardians and by vaccinators. To provide an initial clinical evidence base for the potential use of DSJIs in immunization programs, we conducted a randomized, controlled trial in which healthy Brazilian infants aged 12 to 18 months received MMR vaccine via either DSJI or NS. The primary aim of the study was to determine if the immunogenicity of the vaccine delivered via DSJI was non-inferior to that administered by NS. The comparison was made for each vaccine antigen separately. Secondary aims were to collect safety data and to survey parents and vaccinators for their perceptions of the specific DSJI evaluated. We also recorded insights on operational aspects of this study that will be useful in future clinical trials of DSJIs.

2. Methods

2.1. Vaccine

MMR vaccine from Bio-Manguinhos used in this study was formulated according to procedures transferred by GlaxoSmithKline to Bio-Manguinhos. Each reconstituted 0.5-mL dose contained the following:

- ≥ 1000 CCID₅₀ of measles live attenuated virus (Schwarz strain),
- ≥ 5000 CCID₅₀ of mumps live attenuated virus (RIT 4385 strain, derived from the Jeryl Lynn strain),
- ≥ 1000 CCID₅₀ of rubella live attenuated virus (Wistar RA 27/3 strain).

2.2. Injection devices

The DSJI used for subcutaneous (SC) vaccination in this study was the first-generation PharmaJet system (PharmaJet; Golden, CO, USA) shown in Fig. 1 and referred to hereafter as the G1. The system consisted of two injectors: a blue device described by the manufacturer’s instructions as suitable for adults and children aged two years and older, and a purple injector suitable for infants and for children up to two years old. The G1 had the United States Food and Drug Administration (FDA) (510(k) number K081532, 26 February 2009) and Brazilian Health Surveillance Agency (Agência Nacional de Vigilância Sanitária [ANVISA]) regulatory clearance at the time of the study. The blue injector was used for two pilot studies and the purple injector was used for the infant study reported here. These injectors are spring-powered. Both G1 injectors (blue and purple) were superseded by the PharmaJet Stratis device in late 2011 and are no longer available.

The vaccine in the NS treatment group was administered with sterile, single-use, disposable needles (13 × 4.5, Brazilian scale; 26 gauge, 5/8 in., US scale) and 3-mL-capacity, sterile, single-use, disposable syringes (BD).

2.3. Study populations and setting

The study was conducted at three public health immunization clinics operated by the Health Secretary of Rio de Janeiro: Guadalupe, Irajá, and Rocinha. Adult and pediatric pilot studies

2 This vaccine is currently used exclusively in the Brazil National Immunization Program.
were conducted to train vaccinators on the use of the G1 and to assess immediate injection-site results. These studies included ten healthy adult males aged 18 to 50 years and 15 healthy children aged four to six years (data not shown). The study reported here was conducted on healthy infants aged 12 to 18 months from August 2010 through March 2011.

Eligibility criteria required that participants be in good health and not enrolled in another research study. They were not to have received their first dose of MMR vaccine and were required to be up to date on all other routine vaccines included in Brazil’s Basic Child Vaccination Schedule. They were not to have received any other injectable vaccines within 28 days prior to the study. Written informed consent was obtained from all participants or parents/guardians. The study was approved by the research ethics committee of the Municipal Health Department of Rio de Janeiro, the Brazilian National Research Ethics Commission (Comitê de Ética em Pesquisa [CONEP]), and the PATH Research Ethics Committee, and was registered as International Standard Randomized Controlled Trial 4280032 [18].

The intention-to-treat (ITT) population included all enrolled infants who received an SC injection of MMR vaccine and had safety data recorded immediately and 60 min following injection. All members of the ITT population were included in safety and tolerability analyses.

The per-protocol (PP) population comprised those infants in the ITT population (thus meeting all eligibility criteria listed above) who received an SC injection of MMR vaccine and had a post-vaccination blood sample taken within 35 to 56 days after vaccination. A subject who had not previously received MMR and was baseline-positive for antibody against the measles antigen (due to prior exposure) but negative for the other two antibodies was excluded from the PP population for the analysis of measles antibody. However, that individual was still a member of the PP population for the analysis of mumps and rubella antibody responses.

2.4. Vaccinations and study visits

The treatment consisted of a 0.5-mL dose of MMR vaccine administered SC in the left deltoid area, using either the G1 or NS. Infants were randomized 2:1 to receive vaccine via G1 or NS, respectively. This allocation ratio emphasizes the experimental group, allowing better use of resources to generate more data for the G1 [19]. There were three clinic visits. The first visit included a baseline blood draw, vaccination, and monitoring of AEs immediately and at 60 min following vaccination. The second visit occurred within days 8 to 28 after the day of vaccination and included the collection of AEs recorded in diaries, plus a review of delayed local and systemic AEs. The third visit occurred within days 35 to 56 after the day of vaccination and included the post-vaccination blood draw and evaluation of delayed local or systemic AEs.

2.5. Immunogenicity assessment

Enzyme-linked immunosorbent assays (ELISAs) were performed at the Respiratory Virus Laboratory of Instituto Oswaldo Cruz (Fiocruz, Rio de Janeiro). The plaque reduction neutralization test (PRNT) was performed at the Virologic Technology Laboratory of Bio-Manguinhos (LATEV, Fiocruz, Rio de Janeiro). The geometric mean concentration (GMC) for antibodies to each antigen was also calculated for the two treatment groups. Parents/guardians of infants in either study arm who failed to seroconvert to any of the three vaccine antigens were contacted after the study and offered an additional MMR dose delivered by NS.

The immunogenicity of the MMR vaccine was assessed primarily as a percentage of baseline-negative infants who seroconverted for antibodies against each of the three vaccine components—measles, mumps, and rubella viruses. Seroconversion (SeroC) was calculated separately for each vaccine antigen as the percentage of baseline-negative vaccinees having a post-vaccination antibody level greater than or equal to the following cutoff levels:

- Anti-measles neutralizing titer (NT): ≥ 200 milli-international units per mL (mIU/mL) by PRNT (methods described in reference [26]).
- Anti-mumps Immunoglobulin G (IgG): ≥ 231 units/mL by ELISA, or if < 231 units/mL by ELISA (Enzymost® anti-parotitis-virus/IgG, Siemens-Behring) and retested by PRNT, then a positive test at a dilution ≥ 1:10.
- Anti-rubella IgG: ≥ 4 IU/mL by ELISA (Enzymost® anti-rubella-virus/IgG, Siemens-Behring).

Non-inferiority was defined a priori as a difference of less than 10% on the upper limit of the 95% confidence interval (CI) for the difference in SeroC rates between the two treatment groups (SeroCNS – SeroCG1). The sample size was calculated with Power Analysis & Sample Size Software (PASS) 2008 (Number Cruncher Statistical Systems, Kaysville, Utah). Data were analyzed using SPSS predictive analytics software, version 16.0 (SPSS, Inc., Chicago, Illinois). With an allocation ratio G1: NS of 2:1, it was calculated that the sample should be 348 G1

![Fig. 1. The first-generation PharmaJet system (G1) for vaccine delivery with charging station, vial adapter, and syringe.](image-url)
recipients plus 174 NS recipients to equal 522 total subjects. With these group sizes, if both true proportions were 85%, then the power to find the G1 statistically non-inferior to NS would be 88%. To allow for 10% loss of data due to causes such as subject withdrawal and some subjects having detectable pre-vaccination titers, the targeted sample size was increased to a total of 388 plus 194 to equal 582. We also calculated a 95% CI for the ratio of the GMCs (separately for antibody to each of the vaccine antigens) among subjects in the NS and G1 treatment groups (GMC$_{G1}$/GMC$_{G2}$). If the upper limit of the 95% CI for the ratio was <1.5, then the null hypothesis of inferiority of the G1 treatment group would be rejected.

For post hoc analyses on immunogenicity, a series of univariate and multivariate regression analyses were done to assess the importance of several independent variables (e.g., age and gender of vaccinees, duration of injector use, or loss of vaccine at the injection site) regarding SeroC rates or log$_{10}$ of the titer of antibodies against measles, mumps, and rubella viruses as dependent variables. For analyses of incomplete delivery or loss of vaccine, data were gathered from vaccinators’ qualitative observations of the injection as prompted by specific fields and open-ended comment sections in the case report form (CRF).

2.6. Safety assessment

The safety and tolerability of vaccination was assessed in terms of the following AEs: 1) local injection-site reactions and systemic AEs observed immediately upon vaccination as well as 60 min later by a clinic physician blinded to the method of injection, 2) local injection-site reactions and systemic AEs recorded by parents on a diary card for days 1 through 10 or otherwise ascertained by study staff during the second clinic visit, and 3) delayed local injection-site reactions and systemic AEs ascertained by study staff during the third clinic visit. The possible, probable, or definite relationship of AEs to treatment (vaccine, injection device, or other aspect of treatment) was determined by the principal investigator. Parents/guardians and study staff were aware of the injection method. Pearson’s chi-square test or Fisher’s exact test was used, as appropriate for the comparison (Fisher’s exact test was used when an observed cell was <5), to evaluate the statistical significance of the differences in AEs between the treatment groups, with \( p \leq 0.05 \) defined as significant. \( p \)-Values were not adjusted for multiple comparisons and were calculated for reference purposes only.

Pre-specified local injection site signs and symptoms included pain, laceration, bruising, induration, swelling, erythema, warmth, and pus or drainage. Pre-specified systemic AEs included anaphylaxis, swelling under the jaw line, rash, irritability/crying, loss of appetite, sleepiness, and fever (axillary temperature \( \geq 37.5 \) °C).

2.7. Performance, acceptability, and usability of injection devices

Qualitative information regarding the use, performance, and acceptability of the two methods of injection—by G1 or conventional NS—was collected, with emphasis on the following assessments: 1) the incomplete delivery of vaccine, which could have implications for immune response; 2) the perceptions of the provider and parent/guardian of the subject regarding use and acceptability; and 3) ease of use and human factors. Injection performance and human factor data were recorded by vaccinators in the CRF after each vaccination to document delivery of the vaccine. Following each injection, parents/guardians of infants were asked by the vaccinator to rate qualitatively the injection experience (poor, acceptable, or excellent) and indicate whether they would like to have their child receive a future vaccination using the same mode of injection. Monitoring of vaccinators during the study showed that they were employing the recommended techniques for G1 and NS injections.

3. Results

3.1. Study populations

The ITT population consisted of 582 healthy infants. There were no significant differences between subjects in the two treatment groups with respect to the ratio of males to females, age, weight, height, or skin color. Median age was 13 months (range 12.0–18.8; four subjects from 18.1 to 18.8 months of age, all in the G1 group, were considered to meet eligibility requirements); median weight was approximately 10 kg (range 6.6–17.0). Of the 582 subjects, 573 had a blood sample of sufficient volume for determination of antibodies against the three viral antigens. However, 21 of these had the sample taken outside the pre-specified window of days 35 to 56 and so were not eligible for inclusion in the PP population for the analysis of antibody responses by treatment group, leaving a PP population of 552. In addition, five pre-vaccination blood samples contained antibodies at a level above the designated cutoff for antibodies against measles virus (\( \geq 200 \) mIU/mL); antibody levels in samples from four individuals were above the designated cutoff for antibodies against mumps virus (\( \geq 231 \) units/mL); and one sample was too small to permit necessary retesting for antibodies against mumps virus. Thus, 547 infants met the criteria for inclusion in the PP population for analysis of antibodies against measles and mumps viruses; for rubella the PP population was 552.

3.2. Immunogenicity assessment

Table 1 shows by treatment group the SeroC rates for antibodies to measles, mumps, and rubella viruses among baseline-negative infants in the PP population 35 to 56 days after receiving an injection of MMR vaccine. For antibodies to rubella virus, the upper limit of the 95% CI for the difference SeroC$_{G1}$–SeroC$_{G2}$ was <10%, meeting the criterion for non-inferiority. For antibodies against measles and mumps viruses, the upper limits of the 95% CI were >10%; thus, the responses in the G1 treatment group did not meet non-inferiority criteria. GMCs of serum antibodies against the three vaccine components also were estimated. For antibodies to both measles and mumps viruses, the upper limit of the 95% CIs for the ratio GMC$_{G1}$/GMC$_{G2}$ for subjects in the PP population exceeded the protocol-defined limit of 1.5, while for antibodies against rubella virus it was less than 1.5 (Table 1). Thus, the GMCs for measles and mumps vaccine components delivered by the G1 did not meet the non-inferiority definition for comparison with NS, but those for rubella virus were non-inferior, mirroring the results of the SeroC rates. Families of all infants who did not mount an adequate response were notified, and all of these infants subsequently were re-vaccinated.
Because the post-vaccination SeroC rates for antibodies against measles and mumps viruses among subjects in the G1 treatment group did not meet non-inferiority criteria compared with those of infants in the NS treatment group, we conducted a number of post hoc analyses to identify factors that may have contributed to the diminished antibody responses in the G1 treatment group. Several variables were found to have a significant effect on SeroC rates among subjects in the G1 treatment group for antibodies against measles and/or mumps virus but not for antibodies against rubella virus.

In the G1 group, female gender (p = 0.032, Pearson’s chi-square) and children 12 to <13 months (p = 0.016, Pearson’s chi-square), were associated with a lower SeroC rate for measles antibody, and in univariate regression analysis female gender was marginally associated with a lower SeroC rate for mumps antibody (odds ratio [OR] 0.656, p = 0.052). Lower body weight also exhibited a lower, but not statistically significant, SeroC rate for measles antibody (OR 0.498, p = 0.063).

In a further multivariable regression analysis, we looked for evidence of possible incomplete delivery of vaccine, noted by vaccinators on the CRF as “failure to inject 0.5 mL,” “liquer or vaccine at the injection site,” or “spray at injection.” These observations were reported for 13% of vaccinations in the NS treatment group and 58% in the G1 treatment group. Incomplete delivery was significantly associated with a reduced SeroC rate for mumps antibody (p = 0.044) and lower measles GMCs in the G1 treatment group (p = 0.047).

### 3.3. Safety assessment

Vaccination was generally well tolerated in infants in both treatment groups, but there were statistically significant differences in the frequency of local and systemic adverse events (AEs) observed immediately, 60 min, and 1 to 10 days following injection of measles-mumps-rubella vaccine (per-protocol [PP] population).

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#### Table 1

Seroconversion (SeroC) rates and geometric mean concentrations for antibodies against measles, mumps, and rubella viruses by treatment group among baseline-negative subjects following an injection of measles-mumps-rubella vaccine (per-protocol [PP] population).

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Treatment group (Total N subjects*)</th>
<th>Subjects sero-converting N (%)</th>
<th>SeroCNS−SeroC&lt;sub&gt;G1&lt;/sub&gt; (95% CI)</th>
<th>Geometric mean concentration (GMC)</th>
<th>GMC&lt;sub&gt;NS&lt;/sub&gt;/GMC&lt;sub&gt;G1&lt;/sub&gt; (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-measles NT</td>
<td>NS (182)</td>
<td>182 (100.0)</td>
<td>9.3 (5.9, 12.7)</td>
<td>4996.75 mIU/mL</td>
<td>1.40 (1.19, 1.64)</td>
</tr>
<tr>
<td></td>
<td>G1 (365)</td>
<td>331 (90.7)</td>
<td>14.4 (6.1, 22.7)</td>
<td>3561.20 mIU/mL</td>
<td>1.57 (1.27, 1.92)</td>
</tr>
<tr>
<td>Anti-mumps IgG&lt;sup&gt;b&lt;/sup&gt;</td>
<td>NS (183)</td>
<td>140 (76.5)</td>
<td>0.3 (−1.5, 2.1)</td>
<td>43.05 IU/mL</td>
<td>1.01 (0.87, 1.18)</td>
</tr>
<tr>
<td></td>
<td>G1 (364)</td>
<td>226 (62.1)</td>
<td></td>
<td>42.47 IU/mL</td>
<td></td>
</tr>
<tr>
<td>Anti-rubella IgG</td>
<td>NS (184)</td>
<td>183 (99.5)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>G1 (368)</td>
<td>365 (99.2)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* The number of subjects for the anti-rubella PP population was 552. Because four subjects had high pre-vaccination antibody blood levels and one had an inadequate blood sample, the number for anti-measles and anti-mumps PP populations was 547.

<sup>b</sup> Includes two subjects in the G1 group negative by ELISA but positive by PRNT retest.

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#### Table 2

Number of subjects with local and systemic adverse events (AEs) observed in at least 4% of vaccinees in either treatment group immediately, 60 min, and 1 to 10 days following an injection of measles-mumps-rubella vaccine (intention-to-treat [ITT] population).

<table>
<thead>
<tr>
<th>Time of observation</th>
<th>Type of AE</th>
<th>NS</th>
<th>G1</th>
<th>p-Value&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immediately</td>
<td>Injection-site AE</td>
<td>9/194 (4.6%)</td>
<td>1/194 (0.5%)</td>
<td>0.082</td>
</tr>
<tr>
<td></td>
<td>• Blood at injection site</td>
<td>8/388 (2.1%)</td>
<td>36/388 (9.3%)</td>
<td>-0.001</td>
</tr>
<tr>
<td></td>
<td>• Papules</td>
<td>153/194 (78.9%)</td>
<td>21/194 (10.8%)</td>
<td>-0.001</td>
</tr>
<tr>
<td></td>
<td>• Short cry</td>
<td>174 (89.7%)</td>
<td>183 (47.2%)</td>
<td>-0.001</td>
</tr>
<tr>
<td></td>
<td>• Inconsolable cry</td>
<td>10/194 (5.2%)</td>
<td>92/388 (23.7%)</td>
<td>-0.001</td>
</tr>
<tr>
<td></td>
<td>Systemic AE</td>
<td>9/194 (4.6%)</td>
<td>24 (12.4%)</td>
<td>-0.001</td>
</tr>
<tr>
<td></td>
<td>• Erythema</td>
<td>25/193 (13.0%)</td>
<td>33/384 (8.6%)</td>
<td>0.100</td>
</tr>
<tr>
<td></td>
<td>• Swelling</td>
<td>16/192 (8.3%)</td>
<td>80/383 (20.9%)</td>
<td>-0.001</td>
</tr>
<tr>
<td></td>
<td>• Fever</td>
<td>17/192 (8.9%)</td>
<td>80/383 (20.9%)</td>
<td>-0.001</td>
</tr>
<tr>
<td>Total with AEs&lt;sup&gt;b&lt;/sup&gt; 60 min</td>
<td>Systemic AE</td>
<td>85/153&lt;sup&gt;a&lt;/sup&gt; (55.6%)</td>
<td>109/277 (39.4%)</td>
<td>0.001</td>
</tr>
<tr>
<td>Total with AEs at 60 min&lt;sup&gt;b&lt;/sup&gt; Days 1 10</td>
<td>Any (&lt;span class=&quot;degree&quot;&gt;≥&lt;/span&gt;37.5 °C)</td>
<td>22/153&lt;sup&gt;a&lt;/sup&gt; (14.4%)</td>
<td>23/277 (8.3%)</td>
<td>0.049</td>
</tr>
<tr>
<td></td>
<td>• Loss of appetite</td>
<td>81/193 (42.0%)</td>
<td>153/384 (39.8%)</td>
<td>0.624</td>
</tr>
<tr>
<td></td>
<td>• Irritability</td>
<td>37/193 (19.2%)</td>
<td>75/384 (19.5%)</td>
<td>0.918</td>
</tr>
<tr>
<td></td>
<td>• Rash</td>
<td>41/193 (21.2%)</td>
<td>72/384 (18.8%)</td>
<td>0.476</td>
</tr>
<tr>
<td></td>
<td>&quot;Spray at injection.&quot;</td>
<td>12/193 (6.2%)</td>
<td>24/384 (6.3%)</td>
<td>0.988</td>
</tr>
<tr>
<td></td>
<td>&quot;Liquid or spray at injection.&quot;</td>
<td>137 (80.6%)</td>
<td>264 (78.8%)</td>
<td>0.640</td>
</tr>
</tbody>
</table>

<sup>a</sup> Numbers in “Total” rows refer to the total number of subjects with adverse events, and some subjects experienced more than one AE; in this table we included only those AEs reported in ≥ 4% of subjects.

<sup>b</sup> Note that denominators are lower in some cases. This is because a smaller number of parents/guardians recorded this information.

<sup>c</sup> Pearson's chi-square test or Fisher's exact test was used, as appropriate for the comparison.
differences in the frequencies of certain AEs. Table 2 shows the frequencies of pre-specified and other local and systemic AEs noted immediately, at 60 min, and 1 to 10 days after injection. Overall, the percentage of subjects with any AE immediately following vaccination was significantly greater among subjects in the NS treatment group compared with the G1 treatment group (89.7% versus 47.2%, \( p < 0.001 \)). Local AEs (mainly papules) were more frequent among G1 vaccinees, while crying following injection was more frequent in the NS treatment group.

By 60 min, the proportion of subjects in the NS and G1 treatment groups with an AE declined to 12.4% and 28.9%, respectively (Table 2). The difference between groups after 1 h was largely due to the higher rate of local AEs (mostly erythema at the injection site) in the G1 treatment group.

During days 1 through 10 (Table 2), the proportions of subjects with one or more pre-specified AEs were similar in both treatment groups (78.8% in the G1 group; 80.6% in the NS group). G1 vaccinees continued to experience more local AEs (mostly erythema and swelling at the injection site), while NS vaccinees had slightly more systemic AEs, such as fever. No AE reported during this time was rated as serious. One NS vaccinee had a local AE rated as severe (Grade 3 pain), while a small proportion of subjects in both treatment groups (9.0% G1 to 13.5% NS) experienced at least one severe systemic AE. All other local and systemic AEs were graded as mild or moderate (Grade 1 or 2).

By the second clinic visit, the overall frequency of any delayed AEs was 13.5% for the G1 group, compared with 15.0% for the NS group, and by the third clinic visit, the frequency declined to 6.5% compared with 4.2%, respectively. In addition to these AEs, persistent injection site stigmata (e.g., scars, hypochromia, macula, and papules) were noted as minor events more prevalent among G1 compared with NS treatment group subjects at both the second clinic visit (9.9% G1 versus 0.5% NS, \( p < 0.001 \), Fisher’s exact test) and third clinic visit (3.5% G1 versus 0.0% NS, \( p = 0.006 \), Fisher’s exact test). Five subjects in the study experienced a serious AE but all events resolved and none were judged to have been related to treatment. In the G1 treatment group, two were cases of pneumonia and one was thought to be dengue. In the NS treatment group, there was one case of a subgaleal hematoma and one of meningitis. Among nine other significant AEs (including five cases of pneumonia), two cases of pneumonia with onset ten days after G1 vaccination were described as possibly related and probably related to treatment; the other events were judged not related.

### 3.4. Acceptability assessment

In the G1 treatment group 90.2%, 9.5%, and 0.3% of parents/guardians rated the injection experience as excellent, acceptable, and poor, respectively. The ratings for NS were 7.2%, 62.9%, and 29.3% for these descriptors. When asked about future injections, 96.1% of families of infants vaccinated with the G1 indicated they would prefer it when their child needed an injection, and 92.3% of families of infants injected with NS indicated they would prefer an alternate mode of injection.

At each of the three study sites, one primary vaccinator administered most of the injections. Filling the needle-free syringe from the vaccine vial using the vial adapter was rated by the vaccinators as easy for 99% of the injections. In approximately 4% of cases, the vaccinator had to obtain another syringe package or reattach/realign the syringe to the device. The G1 was rated as easy to use for 99% of injections, and less than 5% of the injections were noted as causing slight hand/arm strain for the vaccinator. There were no reports of any vaccinator injury related to the G1.

### 4. Discussion

Our study compared the immunogenicity of a MMR vaccine administered via the G1 with that of the vaccine administered via NS. While the results showed non-inferiority of SeroC rates for G1 delivery of the rubella component of the vaccine, SeroC and GMCs for the measles and mumps components did not meet non-inferiority criteria. Our post hoc analyses showed that some characteristics such as female gender and younger age (12 to <13 months) were associated with a lower SeroC rate for measles antibody, although the mechanisms by which female gender or infant age might lead to lower SeroC are not known. Incomplete delivery of vaccine was associated with lower SeroC rates for mumps and lower measles GMCs. A small amount of liquid on the surface of the skin is common following DSJI injections; however, the high rate of incomplete injections observed in our study and the relationship with lower immune responses suggests that the G1 was not optimized for this age group. Incomplete delivery was observed visually as wetness on the skin, a spray in the air at the time of injection, or reflux of vaccine from the puncture site. Visual observation is a subjective method; use of a quantitative method for measuring liquid not injected might have strengthened the correlation between volume of vaccine delivered and immune responses.

In the NS group, the performance of the measles and rubella vaccine components was excellent, and the SeroC and GMCs were comparable to previous reports [20–24]. In contrast, mumps immunogenicity by NS was poor, although the results of this study are consistent with the immunogenicity variation observed in several studies using the same MMR vaccines and laboratory methods [20–26]. The reasons for these variations in immunogenicity have not yet been explained. It should be noted that in one of these studies [27], vaccines from two different MMR producers were used, and both had similar mumps SeroC rates of around 70%.

The PharmaJet G1 used in this study has been superseded by the PharmaJet Stratis and is no longer available. The Stratis was designed to improve injection quality by reducing incomplete injections and simplifying operation, helping to reduce training requirements. The Stratis and another DSJI, the Lectrajet® from D’Antonio Consultants International, Inc., have recently been evaluated for delivery of trivalent inactivated influenza vaccine, and results showed that vaccination with DSJIs in these studies produced immune responses non-inferior to those from vaccination with NS [11,13]. Bench testing of both the G1 and Stratis to assess whether jet injection affects the viability of the live measles, mumps, and rubella viruses in the vaccine found minimal loss of vaccine potency (written communication, April 25, 2014: Melissa Coughlin, Marcus Collins, and Paul Rota, all of United States Centers for Disease Control and Prevention). Studies of MMR vaccine delivery to infants with the Stratis device are needed to assess non-inferiority to NS for this vaccine.
Vaccination was generally safe and well tolerated by infants who were administered MMR vaccine using either the G1 or NS; G1 vaccinees had more minor local injection-site AEs while NS vaccinees had more crying, irritability, and fever. Vaccinators found the G1 very easy to load and use but noted a problem with incomplete delivery of vaccine in a significant proportion of vaccinees. Parents/guardians expressed a strong preference for the G1 over NS as a mode of injection for their children, which may be related to the significantly lower degree of crying observed during and after injection with the G1.

Although the G1 system has been discontinued, our study demonstrates the importance of evaluating new DSJIs and provides insights for future studies. We suggest including the following activities for any trial evaluating immunizations via DSJIs:

- Include an NS control group receiving the same vaccine at the same dose and depth of delivery as the DSJI group.
- Work closely with the DSJI manufacturer to train vaccinators on the use of the device and to monitor the performance of the devices used.
- Include quantitative measurement of loss of vaccine during immunization. Methods for quantifying the volume of liquid on the exterior of the skin and the device have been developed and used in other clinical studies, including weight-based and absorption-based procedures [27,28], but qualitative observation is the only known method for reporting vaccine sprayed in the air.
- Conduct interim analyses of injection performance to identify and correct any device malfunctions or additional training needs as they occur.
- Create a plan for re-immunization of subjects in any study arm who do not exhibit an adequate immune response.

5. Conclusions

The DSJI is a promising technology with potential for use in mass immunization campaigns and for routine immunization programs in low- and middle-income countries. The use of a sterile, single-dose, disposable, non-reusable syringe in these devices eliminates the risk of blood-borne infections that can be associated with the use of a needle and syringe, and the use of a spring to power the injection makes the DSJI attractive for settings that lack access to other power sources. Parents found the G1 highly acceptable and vaccinators considered it easy to use. While the specific DSJI used in this study cannot be endorsed for use in mass immunization programs, and has been discontinued, our experiences and recommendations may inform future evaluations of newer DSJIs for routine infant immunizations.

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