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No demonstrable association between the Leningrad–Zagreb mumps vaccine strain and aseptic meningitis in a large clinical trial in Egypt

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Abstract

To address the claim that the Leningrad–Zagreb (L-Z) mumps vaccine strain is causally associated with aseptic meningitis, a prospective, post-marketing safety study was conducted with a measles-mumps-rubella vaccine (MMR) (TRESIVAC®; Serum Institute of India Ltd., Pune, India), which uses the L-Z strain as its mumps component in Egypt. In all, 453 119 children (65 423 children aged 16–24 months and 329 211 children aged 5–7 years) received MMR. The control groups which, as a result of local health regulations, were slightly younger than vaccinees, comprised 12 253 and 46 232 children, respectively. Using questionnaires, the parents recorded solicited local, systemic and neurological adverse events for up to 42 days post-vaccination. All data were analysed externally on an intention-to-treat basis by individuals not participating in the study. Local and/or systemic reactions were reported in a small percentage of participants, with pain, fever and parotitis being the most common signs among vaccinees in both age groups. No case of aseptic meningitis, encephalitis, anaphylaxis or convulsions was observed in any participant. Thus, in this series of more than 450 000 Egyptian children, the L-Z mumps vaccine strain in this vaccine did not cause aseptic meningitis. The vaccine is considerably cheaper than Western competitors and a valid alternative to other MMR vaccines.

Keywords: Aseptic meningitis, Leningrad–Zagreb strain, MMR vaccine, mumps vaccine, vaccination
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Introduction

Immunization against measles, mumps and rubella is normally carried out using a combined live virus measles-rubella (MR) or measles-mumps-rubella (MMR) vaccine. However, questions have been raised regarding the safety and effectiveness of the different mumps components. One strain may be safe and immunogenic but not sufficiently protective [1–3], whereas another can be very effective but may have a potential to cause aseptic meningitis [3–6]. Around ten mumps vaccine strains have been developed of which Jeryl-Lynn, RIT 4385, Leningrad–Zagreb (L-Z) and Urabe are the best known.

An Indian manufacturer, Serum Institute of India Ltd (Pune, India), has developed its own MMR vaccine (TRESIVAC®) in which the L-Z strain is used for mumps protection. The vaccine was licensed in 1993, and is prequalified by the WHO. More than 150 million doses have been used in different countries across five continents.

The experience with this vaccine was positive until three studies [7–9] from Brazil dealing with a local mass vaccination campaign raised some doubts over its safety. Estimates from retrospective analyses in India [10] and Bahamas [11] suggested that one case of aseptic meningitis is causally associated with 57 000–100 000 doses of the L-Z strain. However, the Brazilian study [7] calculated a rate of one case per 6200–19 000 vaccine doses. Several factors discussed elsewhere [12] may explain such discrepancy but, subsequently, the Indian vaccine has been regarded as possibly less safe than other MMR vaccines. The Western competitors are considerably more expensive, and so the prevention of mumps has often been neglected in poorer countries because MMR has been 'replaced' by MR or even monovalent measles vaccine.

To clarify the question of the incidence of the post-L-Z strain aseptic meningitis [5–7], we conducted a large prospective study in Egypt where MMR could be incorporated in the existing vaccination programme.

Materials and Methods

Set-up

Under the auspices of the Ministry of Health and Population, Egypt, two open phase IV studies were carried out in 2002–2004. The objective was to assess adverse events with the Indian-made MMR vaccine (TRESIVAC®) in two paediatric age groups, paying special attention to aseptic meningitis. Because Egyptian law mandates compulsory MMR vaccination at age 18–24 months and 6–7 years, the best feasible control groups comprised children aged 16–17 months and 5–6 years, respectively. Once the follow-up ended, these children also received MMR vaccine. Because the scheduled paediatric immunizations are compulsory in Egypt, most children received DPT and Hib vaccines during the study period.

The two age groups involved comprised younger children aged 16–24 months in nine governerates, and older children aged 5–7 years in eight governerates of the country. The study protocol was approved by the Steering Committee on Vaccines (ethical committee) and consent in writing or by thumb impression was obtained from a legal guardian. The study was conducted in accordance with the Declaration of Helsinki, good clinical practice guidelines, and local regulatory requirements.

A child of either gender was included in the study if he or she was healthy, of appropriate age, available for the entire follow-up period (see below) and consent was obtained. The exclusion criteria comprised any acute infectious disease, febrile seizures, previous cerebral injury, history of anaphylaxis, hypersensitivity, anti-cancer medication, radiotherapy, haemophilia or known thrombocytopaenia.

Vaccination

One dose of 0.5 mL of MMR (TRESIVAC®) was administered subcutaneously in the antero-lateral aspect of the thigh (young children) or in the deltoid region (school children). Each dose contained at least 1000 $CCID_{50}$ of live attenuated measles viruses of the Edmonston–Zagreb strain propagated on human diploid cell culture. A dose of the mumps component had at least 5000 $CCID_{50}$ of the L-Z strain propagated on chick fibroblast cell culture. For rubella, the amount of the Wistar RA 27/3 strain was at least 1000 $CCID_{50}$, propagated on human diploid cell culture. Other ingredients were 2.5% of (partially hydrolyzed) gelatin, 5% of sorbitol, and residual ($\leq$15 μg) of neomycin. The diluent was water. A concomitant vaccination was allowed, if needed.

Twenty-four different lots of the vaccine (EU 615V, EU 618V to EU 640V) were used, all released by National Control Authority, India, and National Organization for Drug Control and Research, Egypt.

Collection of data

Bilingual questionnaires (Fig. 1) were used to collect information in writing. If the parents were illiterate, a person who was able to write was asked for help. The solicited adverse events were classified as local, systemic or neurological events. The local reactions were pain, swelling or redness at injection site. For systemic reactions, we specifically asked for fever measured by thermometer (given to all) from the axilla in young children and orally from school children, parotitis, rash, cervical or axillary lymphadenopathy, or arthralgia.

Because the neurological manifestations were our special interest, all suspected cases of encephalopathy or encephalitis, or any type of meningitis, were to be reported. Parents were advised of the suggestive symptoms and signs by the vaccinating physician. The questionnaires (Fig. 1) had an extra space for potential unlisted events. For serious adverse events, a special form was used. This was completed by the principal investigator who was informed within 24 h by e-mail, fax, or telephone.

In light of the existing information on MMR vaccines [13,14], reactions were checked by parents for 6 weeks post-vaccination, divided into days 0, 2, 7–14, 15–28 and 29–42. Because aseptic meningitis develops within 6 weeks [6], usually 2–3 weeks post-vaccination, the follow-up lasted for 42 days. When needed, the parents' information was augmented by the vaccinating physicians during a visit to hospital or the staff visits to home. The Adverse Events Following Immunization surveillance system, organized by WHO Expanded Programme on Immunization, was utilized. School doctors contributed to the follow-up of the older children.

Analysis of data, and statistical methods

The case report forms were in duplicate, with a copy being submitted to an external data analyser (iGATE Clinical Research International, now DiagnoSearch Life Sciences Pvt. Ltd., Mumbai, India). The severity of adverse events was scored from 0 (no reaction) to 3 (severe).

For all events, the dates of immunization, onset of event, resolution of event and the outcome were recorded. Investigators assessed the possible causal relationship to vaccination as not related, unlikely, suspiciously or probably related. In addition, reasons for drop-out from the study were recorded.

Statistical analysis was performed using SAS, version 8.2 (SAS Institute, Cary, NC, USA). Quantitative analysis included the number, mean, standard deviation, and the range of the findings, whereas qualitative analysis included

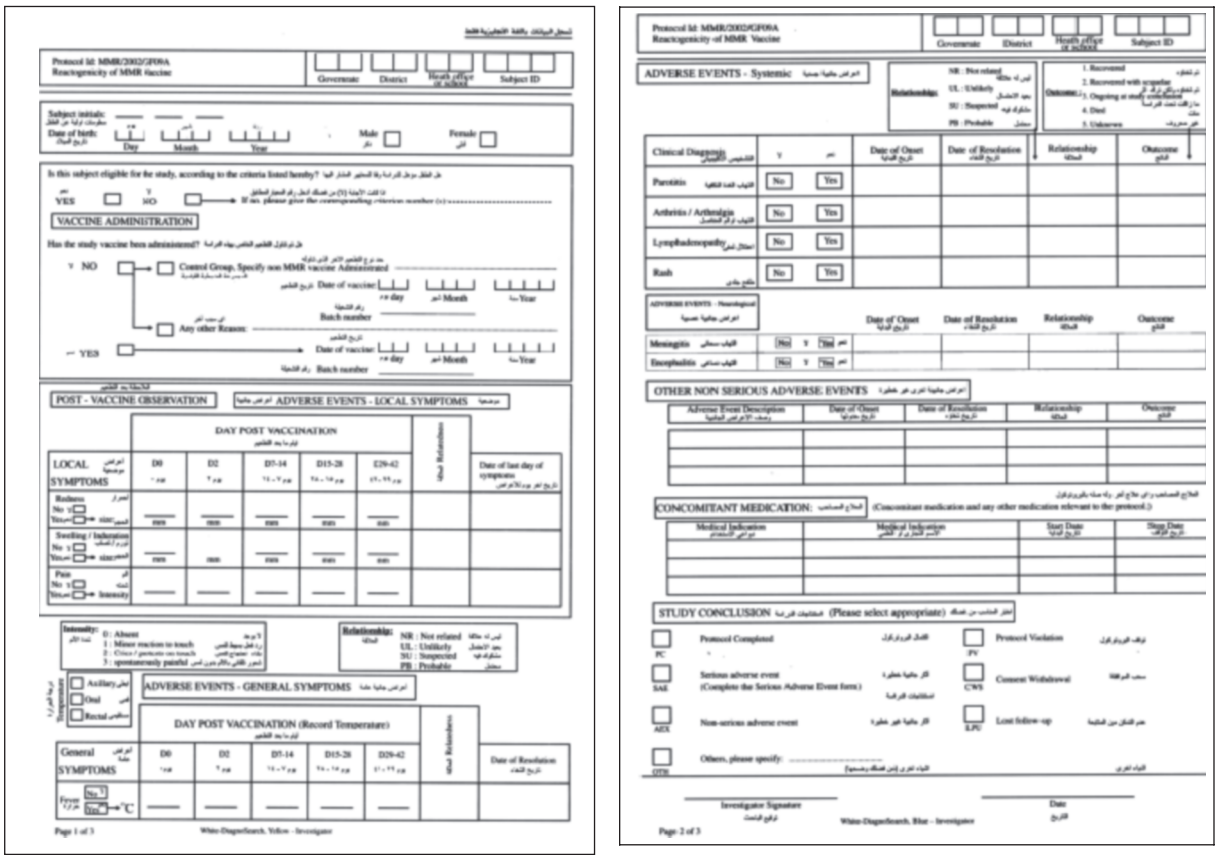

FIG. 1. The data were collected with bilingual questionnaires, specifically designed for the study.

the number and the percentage of events, and 95% CI where applicable.

The incidence of adverse events were compared between the test and control groups using the 'two sample test for proportion' [15]. The hypothesis for incidence of events among vaccinees was tested using the 'one sample test for proportion' [15] by formulating null and alternative hypotheses as per the maximum percentages quoted in the literature as: fever ≤15% [14], rash ≤5% [16], parotitis ≤1.6% [17], meningitis ≤0.11% [18], encephalitis ≤0.00004% [19], thrombocytopaenia ≤0.0033% [20] and arthralgia ≤0.0003% [21].

Randomly selected vaccination sites and the case record forms were audited periodically by an external agent (Wider-perspectives Ltd, Reading, UK). In addition, the study was monitored by the sponsor's clinical trial monitors in association with doctors from the Ministry of Health and Population, Egypt.

Results

Data were obtained from 453 119 children (Fig. 2). Intention-to-treat analysis comprised 77 676 younger children (16–24 months) of whom 65 423 were vaccinees and 12 253 were controls. Of the 375 443 older children (5–7 years), 329 211 were vaccinees and 46 232 were controls.

In per-protocol analysis, the corresponding numbers for the younger children were 73 745 (total) of whom 61 895 were vaccinees and 11 850 were controls. Of the 371 184 older children, 325 204 were vaccinees and 45 980 were controls. There were no drop-outs as a result of adverse events. Table 1 shows the results of intention-to-treat analysis. The per-protocol analysis did not differ significantly in any respect.

Younger children

The results of vaccinees vs. controls are listed in Table 2. Among the 16–24 month old vaccinees, the only adverse events exceeding a frequency of 1% were fever (2.5% vs. 1.6% in non-MMR vaccinees, $p < 0.0001$), local pain (2.3% vs. 0.08%, $p < 0.0001$) and local redness (1.7% vs. 0.08%, $p < 0.0001$). Parotitis was very rare and less frequent in vaccinees (0.04%) than nonvaccinees (0.17%; $p < 0.0001$).

The local events usually occurred on days 0–3, post-vaccination. No child in either group was reported as developing meningitis or encephalitis, or with signs or symptoms suggesting involvement of the central nervous system.

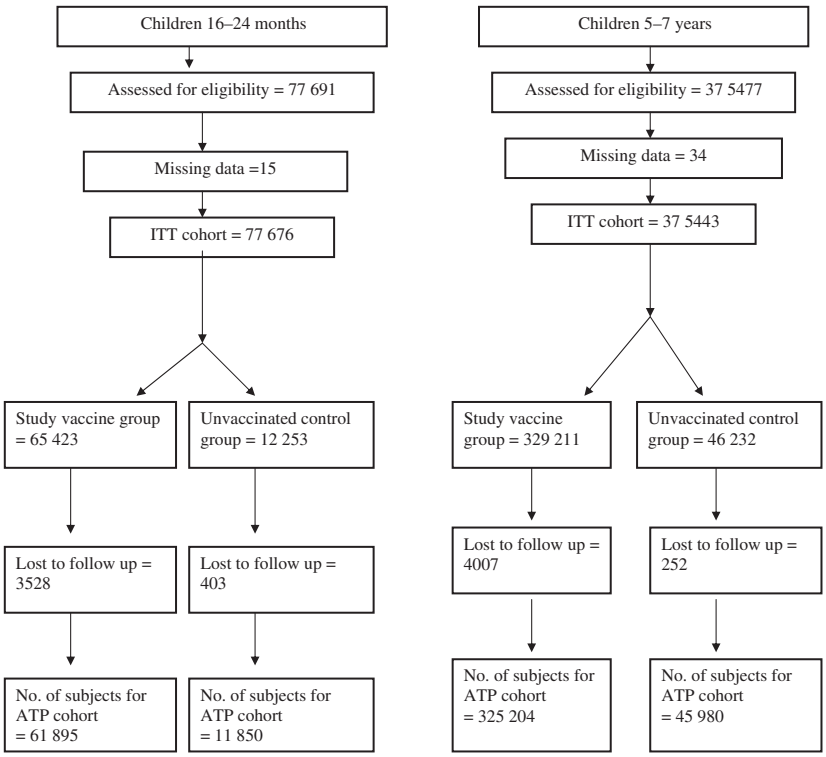

FIG. 2. Trial profile. ATP, according to protocol; ITT, intention-to-treat.

TABLE 1. Demographic characteristics of the vaccinees vs. nonvaccinees, intention-to-treat analysis

	16–24 months		5–7 years	
Parameter	**Study group**	**Control group**	**Study group**	**Control group**
Number (*n*)	65 423	12 253	329 211	46 232
Age (mean ± SD)	18.66 ± 0.98	16.75 ± 0.72	6.46 ± 0.31	5.42 ± 0.26
Sex				
Male	33 504	6399	166 180	24 621
Female	30 990	5745	160 281	21 191
Unknown[a]	929	109	2750	420

[a]Allocated to study or control group, but gender not defined.

Older children

There were also few adverse events in the 5–7 years age group (Table 2). The adverse events exceeding 1% in frequency were fever (2.5% vs. 2.9% in non-MMR vaccinees, p 0.0001), parotitis (2.5% vs. 0.9%, p 0.0001), local pain (1.32% vs. 0%, p 0.0001) and local redness (1.1% vs. 0%, p 0.0001).

Local pain was understandably at its highest on day 0 (59%), whereas fever usually rose during days 15–28 post-vaccination. The median time for onset of parotitis was 18 days. Once parotitis developed, the median resolution time was 8.4 days.

No child in this group either was reported to have symptoms or signs suggesting aseptic meningitis or encephalitis. One case of *Haemophilus influenzae* type b meningitis was detected in a 6.7-year-old girl on day 19 post-vaccination, although no causal association to vaccination was found.

Ninety-three cases of clinical mumps were observed among vaccinees (0.03%) vs. 29 cases (0.06%) in controls. Measles was diagnosed clinically in four vaccinees and three controls. A causal association with MMR vaccination was unlikely.

Discussion

The central finding of the present study was that no aseptic meningitis or other involvement of the central nervous system was detected in a series of 394 634 vaccinated Egyptian children aged 18–24 months or 6–7 years. The finding

TABLE 2. Incidence of solicited local and systemic reactions

	Children aged 16–24 months				Children aged 5–7 years			
	Study group (*n* = 65 423)		Control group (*n* = 12 253)		Study group (*n* = 329 211)		Control group (*n* = 46 232)	
Parameters	***n* (%)**	**95% CI**	***n* (%)**	**95% CI**	***n* (%)**	**95% CI**	***n* (%)**	**95% CI**
Pain	1548 (2.37)	2.30–2.50	10 (0.08)	0.00–0.10	4356 (1.32)	1.30–1.40	0	
Redness	1157 (1.77)	1.70–1.90	10 (0.08)	0.00–0.10	3728 (1.13)	1.10–1.20	0	
Swelling	688 (1.05)	1.00–1.10	12 (0.10)	0.00–0.20	2745 (0.83)	0.70–0.80	0	
Fever	1640 (2.51)	2.40–2.60	197 (1.61)	1.40–1.80	8184 (2.49)	2.40–2.50	1344 (2.91)	2.80–3.10
Rash	113 (0.17)	0.10–0.20	20 (0.16)	0.10–0.20	391 (0.12)	0.10–0.14	11 (0.02)	0.00–0.04
Parotitis	25 (0.04)	0.00–0.10	21 (0.17)	0.10–0.20	8208 (2.50)	2.40–2.50	433 (0.94)	0.90–1.0
Arthralgia	11 (0.02)	0.00–0.02	0		200 (0.06)	0.01–0.11	0	
Lymphadenopathy	6 (0.01)	0.00–0.01	4 (0.03)	0.00–0.10	430 (0.13)	0.10–0.16	2 (0.004)	0.00–0.008

supports the view, held prior to the Brazilian reports [7–9], that the Indian MMR vaccine is a safe product. Our data support an earlier study suggesting that the risk of aseptic meningitis following the use of the L-Z strain is 0.9 cases per 100 000 doses [10]. These figures place the L-Z strain in the same category as the Jeryl-Lynn strain with one case per 800 000 doses [22]. These two strains are considerably safer than the Leningrad-3 or the Urabe strains, which cause aseptic meningitis once per 1000 doses [23] or up to 900 doses [24], respectively.

It may not always be in the interests of the community to use the vaccine that is deemed the safest [25]. In its position paper [23], the WHO rightly states that, in mumps vaccination, strain-specific differences in adverse events exist but are not strong enough to form the basis of a recommendation; all current strains except Rubini are valid alternatives. Now that even the Jeryl-Lynn strain has failed [26], we think it is time to reconsider the value of all potential vaccine strains [3]. The low price of Indian MMR vaccine adds to its value in a large scale use. It should also be kept in mind that the 'same' strain used by different manufacturers might have changed over the years. This view is supported by the great variation in the reported incidence of post-L-Z aseptic meningitis: from one case per 900 doses (one prefecture in Japan) [24] through 1:62 000 (Canada) [27] up to 1:120 000 (France) [28].

Although convinced of the safety of the Indian vaccine, we are aware of limitations with respect to the present study. It was not double-blind, but open, and thus prone to errors in reporting. However, a double-blind study large enough to detect very rare cases of aseptic meningitis in rural Egypt would have required insurmountable resources. The control groups were not ideal because the ages were slightly dissimilar; this selection had to be made to fit with the legal vaccination requirements of Egypt. The adverse events were not checked daily, although, optimally, they should have been [14,29]. Instead, the checking was grouped in pre-set time periods and even this was not easy to achieve. Finally, the vaccinees had been immunized against measles at age 9 months (a legal requirement). This undoubtly reduced the reactions (such as fever) but is highly unlikely to have affected our main outcome variable: aseptic meningitis associating with the L-Z strain.

Therefore, despite these limitations, we believe that valuable information was obtained from this very large (*n* > 450 000) study. The L-Z mumps vaccine strain was not found to be associated with aseptic meningitis. Because the Indian-made measles and rubella components have shown good safety and effectiveness, this MMR combination poses an appropriate and inexpensive alternative to the widely used Western competitors.

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Transparency Declaration

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R. M. Dhere are employees, and Dr S. Bhardwaj was a former employee. Drs S. A. Oun and S. S. Abu Bakr have been working at the Ministry of Health & Population, Egypt.

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