A cluster randomized non-inferiority field trial on the immunogenicity and safety of tetanus toxoid vaccine kept in controlled temperature chain compared to cold chain

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INTRODUCTION

Most vaccines are required to be kept in cold chain (2 - 8°C) from manufacturer to beneficiaries. The cold chain, however, can be an obstacle to vaccines delivery, especially in countries with limited cold chain infrastructure and electricity [1,2]. Several studies have shown the possibility of using specific vaccines outside the 2-8°C, under controlled temperature chain (CTC) [3,11].

CTC allows vaccines to be kept outside the cold chain for a defined duration and temperature, depending on the vaccine's particular heat-stability profile [12].

The possibility of using vaccines in CTC started with the introduction of Vaccine Vial Monitors (VVM) [13,14]. The VVM is a small sticker attached to the vaccine vial that contains a time-temperature sensitive square and an outer circle. The square changes color with exposure to heat indicating whether the vaccine is likely to have been damaged [15].

Immunization of women with TT is a central strategy of the Maternal and Neonatal Tetanus Elimination Initiative [16]. TT supplementary immunization activities (SIAs) target women of reproductive age in high-risk areas. The delivery of TT in CTC could remove one of the important barriers to reaching underserved and marginalized populations considered mostly affected by tetanus.

MATERIALS AND METHODS

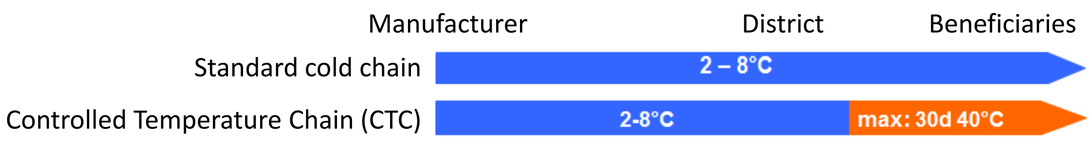
Study design

Cluster randomized, non inferiority trial conducted in three health zones of Moïssala district, Chad, between December 2012 and March 2013.

Clusters corresponding to a village or group or villages with 600-800 residents, were stratified according to distance to health centers (≤ o > 5km) and to infant vaccination activities taking place at village level.

All women 14-49 years of age were invited to participate. Eligible participants had received a maximum of one previous TT dose as determined by vaccination history, were eligible for vaccination according to national schedule and had no contraindications to TT vaccination.

Clusters were assigned to received TT kept in cold chain or CTC with equal probability and by stratum.



On inclusion into the study, five drops of fingertip blood were collected on filter paper from each participant. After blood sampling, the 1st dose of TT was administered intramuscularly into the left deltoid muscle. Four to six weeks later, study teams returned to the villages to administer the second TT dose. After 4 weeks, when antibody concentrations are considered to peak [17], a third visit was conducted to obtain a second blood sample. Participants received two TT doses kept in CTC or cold chain according to the strategy randomly assigned to their cluster.

Objective

Primary objective is to demonstrate the non-inferiority of TT kept in CTC compared to TT kept in standard cold chain in terms of seroconversion and increase in antibody titers. Non-inferiority of CTC vaccine could be claimed if the difference in seroconversion was <5% and the ratio of geometric mean anti-tetanus antibody concentrations (GMC) was <1.5.

Anti-tetanus IgG levels were determined using an indirect endpoint ELISA test. In the main analysis, an anti-tetanus IgG level of 0.16IU/ml was considered protective. Additional analysis used a 0.20IU/ml cutoff to consider the overestimation of antibody levels by standard ELISA [18,19]. Pre- and post-vaccination antibody concentrations and their differences were $\log_{10}$ transformed to obtain a more closely normal distribution. Differences in seroconversion and increase in GMCs were analyzed using the upper limit of the Wilson-type 95% confidence interval (CI).

The study also evaluated adverse events following administration of TT in CTC and standard cold chain. Differences in post-vaccination reactions were analyzed using Fisher's exact test.

Vaccine

Prior to the study, TT in 10 dose-vials (Serum Institute of India Limited, Hyderabad, India) from three different batches were exposed to CTC conditions in Moïssala district, Chad. CTC vaccines were kept inside vaccine carriers without ice-packs for 30 days and carried by teams during a mass vaccination campaign and outreach activities.

A maximum ambient temperature of 43.1°C was registered during the exposure period.

Exposure temperatures were monitored using electronic temperature recorders. These ranged from 24.6°C to 40.1°C (mean 31.2°C)

After the 30 days, a VVM percentage-based color intensity scale previously used [3,11], with 100% indicating discard point, showed 50% change in color suggesting that exposure to heat had not damaged the product. Control vaccines remained in the refrigerator in Moïssala district.

Table 1: Results of potency, pH and flocculation tests for vaccines kept in CTC and standard cold chain

	CTC			SCC		
Batch	Potency IU/dose (95%CI)	pH	Flocculation (Total Lf/ml)	Potency IU/dose (95%CI)	pH	Flocculation (Total Lf/ml)
018B2001A	95 (73-124)(1)	6.56	19.2 (21 min)	94 (71-124)(1)	6.50	15.2 (23min)
018L1008B	222 (161-308)(1)	6.63	18.4 (19 min)	147 (108-200)(2)	6.66	18.4 (24min)
018L1024D	135 (98-185)(2)	6.53	19.2 (20min)	92 (63-131)(2)	6.59	15.2 (30 min)

(1) 1st run, reference value ED50=118 (2) 2nd run, reference value ED50=125

RESULTS

Administered vaccines

Administered CTC vaccines were exposed to temperatures between 21.4°C and 38.3°C for 5 to 27 days with a median of 16 and 14 days for 1st and 2nd TT dose. Cold chain vaccines were kept between 1.5 and 11.2°C.

Table 2: Exposure temperatures and VVM status of the CTC and cold chain administered vaccines

TT dose and strategy	Temperatures		Duration of CTC		VVM (%)
	Mean (°C)	Range (°C)	Median (days)	Range (days)	
TT1 – CTC	27.7	21.4-37.5	16.0	5-27	40
TT2 – CTC	29.0	21.9-38.3	14.0	7-26	40
TT1 – SCC	5.6	4.7-11.2(1)	-	-	10
TT2 – SCC	5.9	5.2-9.3(1)	-	-	10

(1) >8°C for a total duration of 3h – registered after opening the refrigerator for the provision of vaccines

Study population

A total of 2128 participants residing in 42 villages grouped in 34 clusters were enrolled into the study. 952 participants completed the study in each group. The primary intention to vaccinate analysis included 1830 participants with pre- and post-vaccination antibody level result.

Table 3: Participants characteristics in the CTC and cold chain groups

	CTC	cold chain
Participants' characteristics		
Participants	1068	1060
Age (years)	25.25 (11.23)	24.97 (11.06)
Pregnant at inclusion	64 (5.97%)	79 (7.48%)
Number of pregnancies	2.69 (3.14)	2.55 (3.07)
Never been pregnant	392 (36.6%)	392 (37.1)
Years last pregnancy	4.65 (6.30)	4.27 (6.00)
Received a TT dose before inclusion	530 (49.5%)	554 (52.4%)
Years last TT dose	5.23 (5.94)	4.94 (5.30)
Baseline GMC in IU/ml (95%CI)	0.35 (0.33-0.36)	0.35 (0.33-0.37)

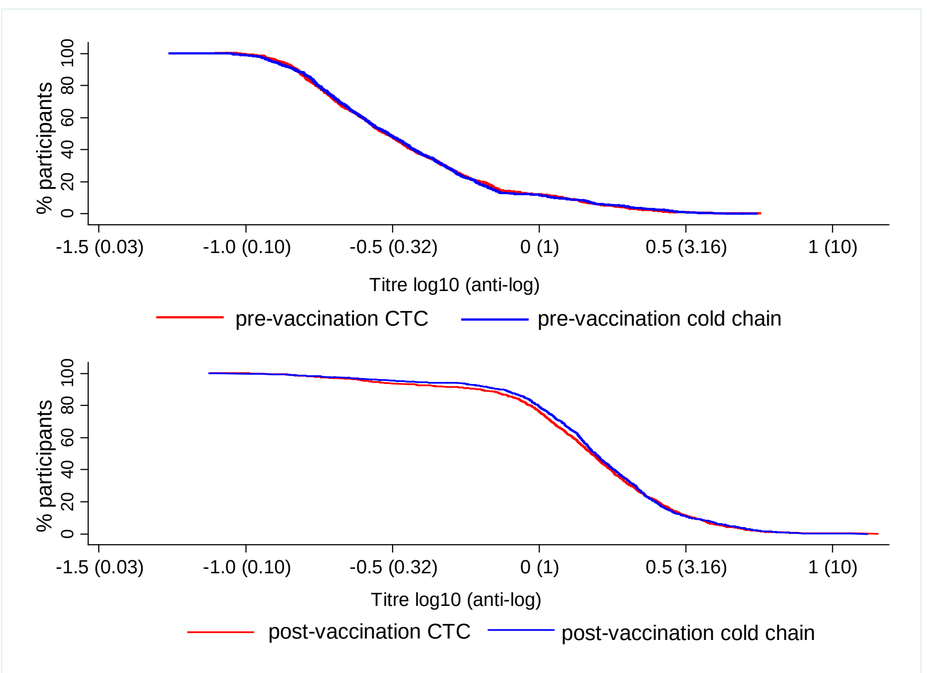
*Data are means (SD) or numbers (%)

Immunogenicity

A total of 272 participants (14.9%) had anti-tetanus IgG levels <0.16IU/ml at baseline. Among susceptible participants, 95.77% (91.09-98.05) in CTC and 96.15% (95%CI=91.31-98.35) on the cold chain group, had protective antibody levels following two doses of TT. The upper limit of the 95%CI for the difference in seroconversion was 5.6 in the intention to vaccinate analysis and 4.4 in the per-protocol analysis. If a protection cutoff of 0.20IU/ml was used, there were 512 susceptible participants at baseline and the difference of seroconversion was 1.48 (95%CI=-2.8 to 5.7)

After vaccination there was a 4.21 fold increase in GMC in the CTC (99%CI=4.00 to 4.43) and a 4.51-fold (95%CI=4.31-4.73) in the cold chain group. The upper limit of the 95%CI for the ratio of GMCs was 1.16.

Figure 2: Inverse cumulative distribution curves, pre- and post-vaccination GMCs, CTC and cold chain groups



Safety

No adverse events were observed 30 min post-vaccination. A small number of participants (n=25) self-reported an adverse event occurring 7 days after vaccination (2 in CTC and 23 in cold chain group). These were characterized by a local reaction at the injection site with pain and swelling in occasions accompanied by fever and headache.

DISCUSSION

This study demonstrates the stability and immunogenicity of TT manufactured by Serum Institute of India Limited kept in CTC at temperatures <40°C for up to 30 days. TT in CTC retained adequate potency levels. Moreover seroprotection results and cumulative distribution curves showed similar immunological responses in CTC and cold chain groups.

The high proportion of participants protected at baseline resulted in a reduction of power to detect the non-inferiority in seroconversion in the CTC group at a 5% margin. However, previous CTC studies have used a 10% non-inferiority margin [20]. In this study, a 10% margin with a protection threshold of 0.20IU/ml results in 96.3% power to establish non-inferiority of TT in CTC.

The possibility of using TT in CTC is a major advantage for countries where maternal and neonatal tetanus continues to be a major public health problem.

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