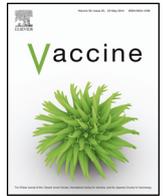




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A cluster randomized non-inferiority field trial on the immunogenicity and safety of tetanus toxoid vaccine kept in controlled temperature chain compared to cold chain

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ARTICLE INFO

Article history:

Received 19 May 2014

Received in revised form 20 August 2014

Accepted 8 September 2014

Available online xxx

NCT01559597

Keywords:

Controlled temperature chain

Cold chain

Tetanus toxoid

Immunogenicity

Maternal and neonatal tetanus elimination

ABSTRACT

Background: In resource-poor settings, cold chain requirements present barriers for vaccine delivery. We evaluated the immunogenicity and safety of tetanus toxoid (TT) vaccine in “Controlled Temperature Chain” (CTC; up to 40 °C for <30 days before administration), compared to standard cold chain (SCC; 2–8 °C). Prior to the study, stability parameters of TT–CTC were shown to meet international requirements.

Methods: A cluster randomized, non-inferiority trial was conducted in Moïssala district, Chad, December 2012–March 2013. Thirty-four included clusters were randomized to CTC or SCC. Women aged 14–49 years, eligible for TT vaccination and with a history of ≤ 1 TT dose, received two TT doses 4 weeks apart. Participants were blinded to allocation strategy. Tetanus antibody titers were measured using standard ELISA at inclusion and 4 weeks post-TT2. Primary outcome measures were post-vaccination seroconversion and fold-increase in geometric mean concentrations (GMC). Non-inferiority was by seroconversion difference ($TT_{SCC} - TT_{CTC}$) <5% and ratio of GMCs (TT_{SCC}/TT_{CTC}) <1.5. Adverse events were monitored at health centers and at next contact with participants.

Results: A total of 2128 women (CTC = 1068; SCC = 1060) were recruited. Primary intention to vaccinate analysis included 1830 participants; 272 of these were included in the seroconversion analysis. Seroconversion was reached by >95% of participants; upper 95%CI of the difference was 5.6%. Increases in GMC were over 4-fold; upper 95%CI of GMC ratio was 1.36 in the adjusted analysis. Few adverse events were recorded.

Conclusions: This study demonstrates the immunogenicity and safety of TT in CTC at <40 °C for <30 days. The high proportion of participants protected at baseline results in a reduction of power to detect a 5% non-inferiority margin. However, results at a 10% non-inferiority margin, the comparable GMC increases and vaccine's stability demonstrated in the preliminary phase indicate that CTC can be an alternative strategy for TT delivery in situations where cold chain cannot be maintained.

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1. Introduction

Effective immunization with tetanus toxoid (TT) requires a cold chain system to store and transport vaccines at 2–8 °C from manufacturer to beneficiaries. The maintenance of the cold chain ensures quality of all types of vaccines. However, it can be an obstacle to vaccine delivery, especially in resource-poor countries where

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cold chain infrastructure and electricity are not always available [1,2]. Several studies have shown the feasibility of using specific vaccines under controlled temperature chain (CTC) [3–11], where vaccines are maintained outside the standard 2–8 °C recommendation for a defined duration and temperature, depending on the vaccine's particular heat-stability profile [12]. The possibility of using specific vaccines outside storage recommendations started with the introduction of vaccine vial monitors (VVM) [13,14]. A VVM is a small sticker attached to the vaccine vial that contains a time-temperature sensitive square and an outer circle. When the square reaches the color of the circle, it indicates potential degradation and the vial should be discarded [15].

Immunization of women with TT is a central strategy of the Maternal and neonatal tetanus elimination (MNTE) initiative [16]. This initiative aims to achieve the elimination goal of <1 neonatal tetanus (NT) case per 1000 live births per year in all districts of each country by end 2015. By December 2013, 25 countries [17] had not reached the elimination goal and others may be at risk of increased NT cases if efforts to maintain high TT coverage in women of child-bearing age do not continue [16]. One of the pillars of the MNTE initiative is to conduct TT supplementary immunization activities (SIA) targeting women of reproductive age in high-risk areas [16]. Delivering TT vaccine in CTC could remove one of the important barriers to reaching underserved and marginalized populations considered mostly affected by tetanus.

This study was designed to assess immunological non-inferiority of TT kept in CTC compared to standard cold chain (SCC) when administered to women of childbearing age. Additionally, the safety of TT kept in CTC was assessed. A non-inferiority design was based on the expectation that CTC would help increase vaccination coverage by facilitating activities. Allocation to CTC or SCC was done at cluster level to avoid potential confusion and administration errors if individual randomization were used, as well as to replicate actual implementation strategies.

2. Materials and methods

2.1. Study design

This study was a cluster randomized, non-inferiority field trial conducted in three health zones of Moïssala district, Chad between December 2012 and March 2013. Clusters, corresponding to a village or group of neighboring villages with an estimated population of 600–800 residents, were identified. Clusters were stratified according to distance to health centers (\leq or $>$ 5 km) and to infant vaccination activities taking place at village level. Clusters were assigned to receive TT kept in CTC or SCC with equal probability and by stratum (Stata, College Station, TX, USA). All women aged 14–49 years residing in study clusters were invited to participate and were allocated to CTC or SCC according to the predefined random allocation. While vaccinators and health personnel conducting the study were aware of allocation group, village heads,

participants and laboratory personnel analyzing samples were blinded to the allocation.

In this study, CTC vaccines were kept outside the cold chain, at $<40^{\circ}\text{C}$, from district to participant level for a maximum of 30 days.

2.2. Objectives

The primary objective of the study was to demonstrate the non-inferiority of TT kept in CTC compared to that kept in SCC in terms of seroconversion and increase in antibody titers. Non-inferiority of CTC vaccine could be claimed if, one month after vaccination, the difference ($\text{TT}_{\text{SCC}} - \text{TT}_{\text{CTC}}$) in percentage of participants reaching seroconversion was $<5\%$ and the ratio of geometric mean anti-tetanus antibody concentrations (GMCs) ($\text{TT}_{\text{SCC}}/\text{TT}_{\text{CTC}}$) was <1.5 . The study also evaluated adverse events (AEs) following administration of TT kept in CTC and SCC.

2.3. Vaccine

In May 2012, prior to the study, TT in 10 dose-vials (Serum Institute of India Limited, Hyderabad, India) from three different batches (018B2001A, 018L1008B and 018L1024D) were exposed to CTC conditions in Moïssala district, Chad. This vaccine has a VVM 30, reaching discard point after 30 days at 37°C . Following this, 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. Teams were instructed to perform daily duties normally. A maximum ambient temperature of 43.1°C was registered during this period. Exposure temperatures were monitored using electronic temperature recorders (LogTag[®] TRID30-7). Exposure temperatures in the three vaccine carriers used ranged from 24.6°C to 40.1°C (mean 31.2°C ; with $30 \leq 35^{\circ}\text{C}$ for 50% of the time and $\geq 35^{\circ}\text{C}$ for 14%). 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 (4.8 – 13.2°C , with 3% of the time $>8^{\circ}\text{C}$).

Exposed and control vaccines were tested for potency, pH, toxicity and adsorption following standard testing procedures [18–20] at the Belgian Scientific Institute of Public Health (WIV-ISP) in Brussels. The WIV-ISP is authorized to perform the required in-vivo tests; care of the animals was in accordance with institutional guidelines. After exposure period, laboratory results showed that vaccines still met specifications required for use and were considered stable (Table 1). The same vaccine batches were used for immunization of study participants.

2.4. Participants

Eligible participants were women 14–49 years of age living in the study area who had received a maximum of one previous TT

Table 1
Results of potency, pH and flocculation tests for vaccines kept in controlled temperature chain (CTC) and standard cold chain (SCC).

Batch	CTC			SCC		
	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 (23 min)
018L1008B	222 (161–308) ¹	6.63	18.4 (19 min)	147 (108–200) ²	6.66	18.4 (24 min)
018L1024D	135 (98–185) ²	6.53	19.2 (20 min)	92 (63–131) ²	6.59	15.2 (30 min)

Note: The variability of potency results is related to an in-vivo testing. All batches meet international requirement. No statistical differences were observed between CTC and cold chain batches.

¹ 1st run, reference value ED50 = 118.

² 2nd run, reference value ED50 = 125.

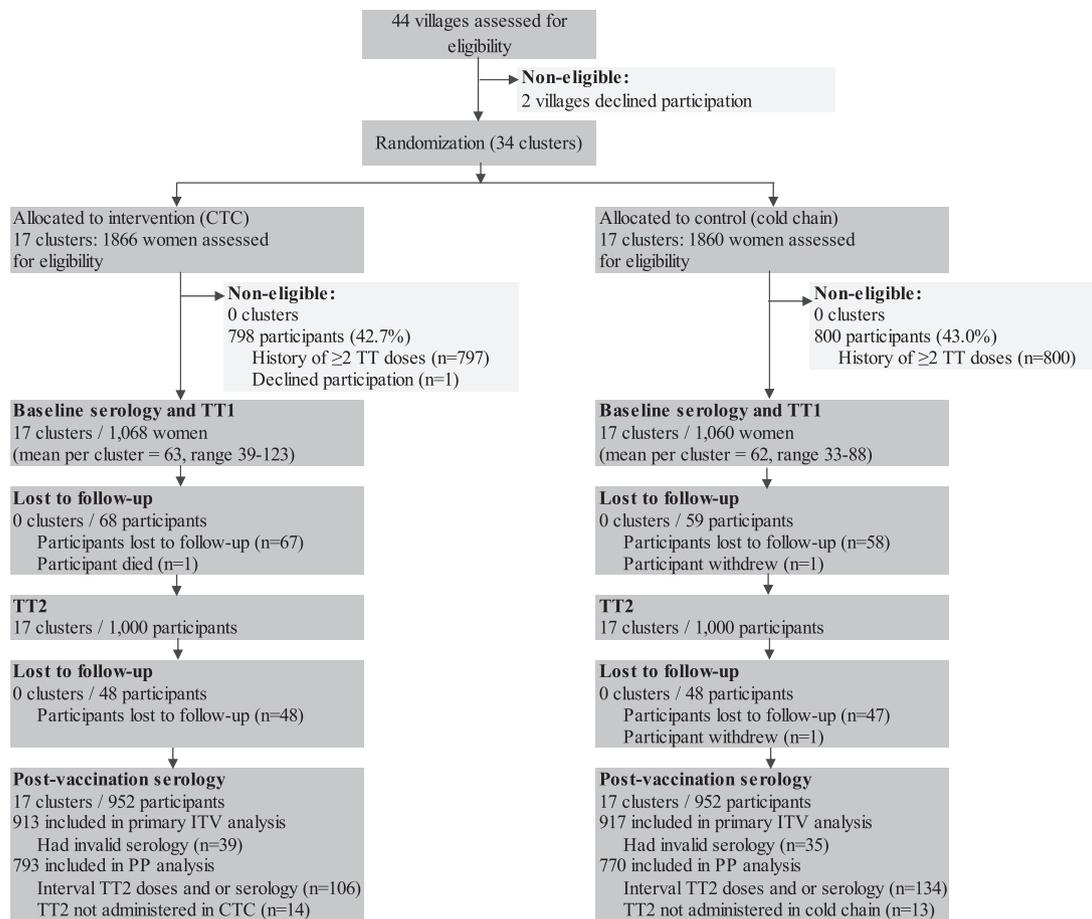


Fig. 1. Participants' flow chart.

dose as determined by vaccination history, who were eligible for vaccination according to the national schedule and who had no contraindications to TT vaccination. Exclusion criteria included previous vaccine allergic reactions, pregnancy within two weeks to term, traveling before the end of the study and unwillingness to participate. The vaccination history questionnaire was based on the Multiple Indicators Cluster Survey (MICS) TT questionnaire previously used in Chad [21]. Participants' vaccination cards/records, when available, were used to confirm participants' vaccination history. The questionnaire was pre-tested and administered by trained interviewers in the local languages. Eligibility for the study was assessed by a study nurse.

2.5. Intervention

Study teams performed three planned visits to the villages. On the day of inclusion into the study, five drops of fingertip blood from each participant were collected on filter paper (Protein Saver™ Card, Whatman 903). After blood sampling, the vaccinator administered the 1st dose of TT vaccine intramuscularly into the left deltoid muscle. Four to six weeks later study teams returned to the villages to administer the 2nd TT dose. After 4 weeks, when antibody concentrations are considered to peak [22], a third visit was conducted to obtain a second blood sample. Participants received two TT doses kept in CTC or SCC according to the strategy randomly assigned to their cluster.

CTC vaccines were placed in vaccine carriers without ice-packs for a maximum of 30 days. Number of days in CTC and VVM status were registered daily. Exposure temperatures were monitored continuously using LogTag® TRID30-7.

Participants were observed for 30 min after vaccination to manage and record immediate AEs. AEs occurring 7 days post-vaccination were evaluated at the next contact with study team or at a local health center if participant sought medical assistance.

2.6. Outcomes

The main study outcomes were the proportion of participants protected against tetanus and the fold-increase in antibody level after two doses of TT vaccine. AEs were also analyzed.

Dried whole blood absorbed on filter paper was used to determine anti-tetanus antibodies. Samples were dried at ambient temperatures for 4 h and placed in individual plastic bags with a silica sachet. Samples were kept at ambient temperatures (<25 °C) in an air-conditioned room. Once in the laboratory, samples were kept at -15 to -25 °C for long-term storage.

Anti-tetanus IgG levels were determined using an indirect endpoint ELISA test validated by the WIV-ISP: 30 µl of standard TT solution (PhEur. Biological Reference Preparation, 0.03 IU/ml) and in-house positive control anti-tetanus antibody solution (0.05 IU/ml) were spotted onto filter paper. Standardized discs were punched using an office paper puncher (Harris Uni-Core I.D. 6.0 mm) from the center of the dried blood spots and from blank paper discs used as negative controls. Standard, control and participants' discs were added in duplicate in a flat-bottomed 96-well microtiter plate (NUNC, TC microwell). The discs were eluted with 200 µl of ELISA compatible buffer (PBS) and incubated for 90 min. Eluted standard, controls and patient samples were diluted with PBS buffer and loaded into TT-antigen pre-coated wells of an ELISA plate (NUNC MaxiSorp™). The incubation of standard, control and

samples was followed by successive additions of biotinylated rabbit anti-hIgG (Thermo Fisher Scientific), streptavidine-peroxidase and Tetramethylbenzidin (TMB). Optical density was measured with the Softmax PRO software (Molecular Devices) at 450 nm and 650 nm. Anti-tetanus antibody concentrations were quantified by comparison with the standard curve (4-parameter fitting).

2.7. Sample size

The sample size was calculated based on anticipated seroconversion frequency. We assumed that after 2 TT doses kept at 2–8 °C as recommended, 90% of participants would have a protective antibody level. To detect a difference of not more than 5% in the CTC group compared to the cold chain group, with a one-sided α of 2.5% and 90% power, we aimed to enroll 1050 participants per group. This considered a possible 10% loss to follow-up. Due to the small geographical area of the study site, stratification and randomization, the intra-cluster correlation coefficient was considered small (<0.005). The 5% non-inferiority margin was chosen based on both statistical and clinical considerations and was considered acceptable and conservative in terms of the public health relevance of CTC.

2.8. Statistical methods

Immunological responses evaluated include seroconversion, seroprotection and increase in GMC. As recommended by World Health Organization (WHO), an anti-tetanus IgG level of 0.16 IU/ml was considered protective [22]. Because protective antibody is overestimated by standard indirect ELISA at values <0.20 IU/ml when compared to neutralization assay [23,24], an additional analysis was conducted using 0.20 IU/ml as the cutoff. For the analysis of the increase in GMCs, pre- and post-vaccination antibody concentrations and their differences were log₁₀-transformed to obtain a more closely normal distribution. Differences in seroconversion percentages and increase in GMCs were analyzed using the upper limit of the Wilson-type 95% confidence interval (CI). Inverse cumulative distribution curves were also compared.

An additional analysis of the ratio of GMCs was computed using analysis of covariance to adjust for baseline characteristics and cluster. Differences between the groups regarding post-vaccination reactions were analyzed using Fisher’s exact test. Immunogenicity analysis was conducted both for intention-to-vaccinate (ITV) and per-protocol (PP) populations. Safety analysis included all study participants. Data were double entered by independent data clerks and analyzed with STATA software, version 11.2.

2.9. Ethical considerations

The study was designed and performed in accordance with the principles of the Declaration of Helsinki and with Good Clinical Practice Guidelines established by the International Conference on Harmonization. The study was approved by the Committee

Table 2
Participants and clusters characteristics in the controlled temperature chain (CTC) and standard cold chain (SCC) groups^a.

	CTC	SCC
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)
Clusters’ characteristics		
Number of clusters	17	17
Participants per cluster (range)	63 (39–123)	62 (33–88)
Clusters >5 km from health facility	10 (62.5%)	11 (64.7%)
Clusters with routine vaccination activities	5 (29.4%)	6 (35.3%)

^a Data are means (SD) or numbers (%).

for the protection of persons in France (St. Germain en Laye) and discussed at Chad’s National Vaccination Technical Committee before approval by the Ministry of Health in Chad. The head of each participating village provided permission for their village to participate and written informed consent was obtained before enrollment from all participants. All participation was voluntary and no identifying information encoded. The trial was registered at clinicaltrials.org with registry number NCT01559597.

3. Results

3.1. Study population

A total of 2128 participants residing in 42 villages grouped in 34 clusters were enrolled in this study (1068 in CTC; 1060 in SCC) (Fig. 1). A total of 952 participants completed the study in each group. The primary ITV analysis included 1830 participants with pre- and post-vaccination antibody level results (913 in CTC; 917 in SCC). The PP population (n = 1563) includes all participants who received 2 TT doses 21 to 42 days apart according to the allocated strategy, had blood sampling 21 to 42 days post TT2 and had pre- and post-vaccination serological results. The reasons for exclusion from the PP analysis were an incorrect interval between TT doses and/or blood sampling (n = 240) and receiving TT doses kept in different strategies (n = 27). Baseline demographics were similar in both arms (Table 2).

3.2. Administered vaccines

Administered CTC vaccines were exposed to temperatures between 21.4 and 38.3 °C (25 ≤ 30 °C during 71.4% of time and

Table 3
Exposure temperatures in the controlled temperature chain (CTC) and standard cold chain (SCC) and vaccine vial monitor (VVM) status of administered vaccines.

TT dose and strategy	Temperatures		Duration of CTC		VVM (%) ¹
	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	1.5–11.2 ²	–	–	10
TT2-SCC	5.9	1.7–9.3 ³	–	–	10

¹ VVM read on a percentage scale with 100% indicating discard point.
² >8 °C for 35 min registered after opening the refrigerator for the provision of vaccines; <2 °C registered in 3 vaccine carriers (from 20 used) during a maximum of 1.5 h.
³ >8 °C for 10 min registered after opening the refrigerator for the provision of vaccines; <2 °C registered in 1 vaccine carrier (from 34 used) during 1 h.

≥30 °C for 20%) for 5 to 27 days with a median of 16 and 14 days for first and second dose (Table 3). Cold chain vaccines were kept between 1.5 and 11.2 °C (<2 or >8 °C for 0.2% of the time). At the time of use, all VVMs indicated that vaccine could be used.

3.3. Immunogenicity

At baseline, 272 participants (14.9%), had anti-tetanus IgG levels of <0.16 IU/ml (142 in CTC; 130 in SCC). Among susceptible participants, 95.77% (95%CI=91.09–98.05) in CTC and 96.15% (95%CI=91.31–98.35) in SCC had protective antibody levels following two doses of TT (Table 4). The upper limit of the 95%CI for the difference in seroconversion was 5.6 in the ITV analysis and 4.4 in the PP analysis. If a protection cutoff of 0.20 IU/ml is used, there were 512 susceptible participants at baseline (259 in CTC; 253 in SCC); the difference in seroconversion was 1.48 (95%CI=−2.8 to 5.7).

Following vaccination, overall seroprotection was equal in both groups: 99.34% in the CTC and 99.45% in the SCC groups (Table 4).

Pre-vaccination GMC was 0.35 IU/ml in both groups ($p=0.82$). After vaccination, GMCs were 1.47 IU/ml (95%CI=1.40–1.54) in the CTC group and 1.55 IU/ml (95%CI=1.48–1.62) in the SCC. Inverse cumulative distribution curves of GMCs pre and post-vaccination by group are presented in Fig. 2.

After vaccination, there was a 4.21-fold increase in GMC in the CTC group (95%CI=4.00–4.43) and a 4.51-fold (95%CI=4.31–4.73) in the SCC group. The upper limit of the 95%CI for the ratio of GMCs was 1.16. The regression model adjusting for GMC at baseline and previous vaccination showed a GMCs ratio of 0.99 (95%CI=0.72–1.36). The PP analysis did not show any significant differences (Table 4).

3.4. Safety

Almost all participants (97.3%) were observed for the full 30 min after vaccination. No AEs were observed during this period. A small number of participants ($n=25$) self-reported AEs occurring 7 days after vaccination (2 in CTC, 23 in SCC, $p<0.000$). These were characterized by a local reaction at the injection site with pain and swelling accompanied by fever in 13 cases and headache in 8. No AEs were reported by health centers.

4. Discussion

This study demonstrates the stability and immunogenicity of TT kept in CTC at temperatures <40 °C for up to 30 days. Laboratory results showed that TT in CTC retained adequate potency levels. Seroprotection results and cumulative distribution curves showed similar immunological responses in CTC and SCC groups. In this study, the high proportion of participants already protected at baseline resulted in a reduction of power to detect the non-inferiority in seroconversion in the CTC group at a 5% margin as intended. However, previous CTC studies have used 10% non-inferiority margin [25]. In this study, a 10% margin with a protection threshold of 0.20 IU/ml results in 96.3% power to establish non-inferiority of TT in CTC. Seroconversion results, comparable increases in GMC and vaccine's stability demonstrated in the preliminary study phase indicate that TT in CTC does not result in a significant loss of vaccine effectiveness.

The possibility of using TT in CTC is a major advantage for countries where maternal and neonatal tetanus continues to be a public health problem. WHO recommends immunization against tetanus with the combined tetanus and diphtheria toxoids [26]. However, TT continues to be used in most countries aiming to achieve MNTE goals [27]. The implementation of SIAs in CTC

Table 4 Seroconversion and seroprotection rates and post-vaccination GMCs in the controlled temperature chain (CTC) and standard cold chain (SCC) groups, intention-to-vaccinate (ITV) and per-protocol (PP) populations.

Population	CTC		SCC		Difference in rates		Ratio of GMCs*	
	N	% (95%CI)	N	% (95%CI)	(TT _{SCC} - TT _{CTC}) (95%CI)	(TT _{SCC} /TT _{CTC}) (95%CI)	(TT _{SCC} /TT _{CTC}) (95%CI)	(TT _{SCC} /TT _{CTC}) (95%CI)
Unprotected at baseline (<0.16 IU/ml)								
ITV	142	95.77 (91.09–98.05)	130	96.15 (91.31–98.35)	0.38 (−5.0 to 5.6)	0.97 (0.72–1.31)	0.97 (0.72–1.31)	0.97 (0.72–1.31)
PP	128	96.88 (92.24–98.78)	103	96.12 (90.44–98.48)	−0.76 (−6.7 to 4.4)	0.99 (0.72–1.36)	0.99 (0.72–1.36)	0.99 (0.72–1.36)
All participants								
ITV	913	99.34 (98.57–99.70)	917	99.45 (98.73–99.77)	0.11 (−0.7 to 0.9)	1.06 (0.92–1.23)	1.06 (0.92–1.23)	1.06 (0.92–1.23)
PP	793	99.50 (98.71–99.80)	770	99.48 (98.67–99.80)	−0.02 (−0.9 to 0.8)	1.07 (0.92–1.26)	1.07 (0.92–1.26)	1.07 (0.92–1.26)

* Adjusted analysis.

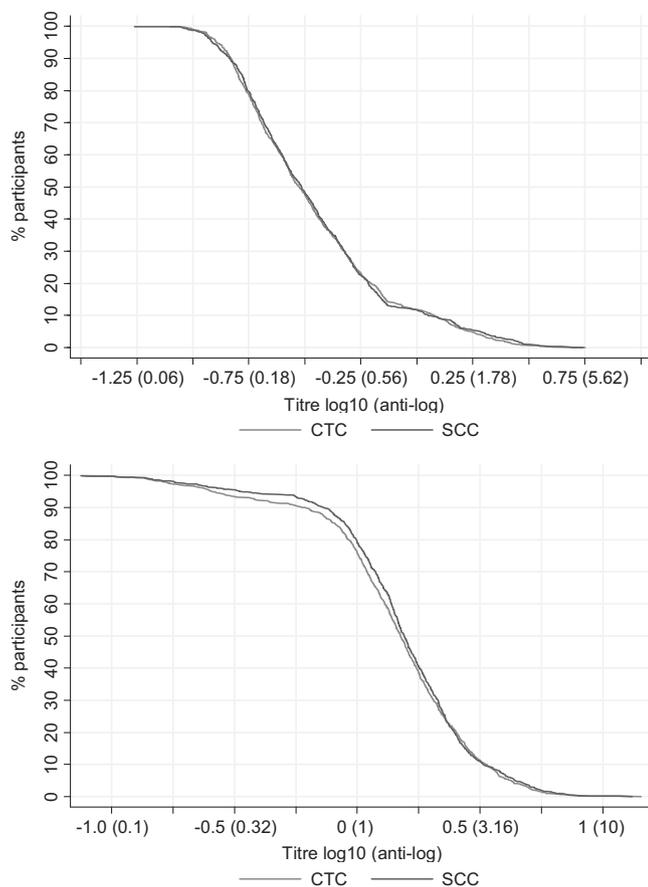


Fig. 2. Inverse cumulative distribution curves, pre- and post-vaccination GMCs in the controlled temperature chain (CTC) and standard cold chain (SCC) group.

presents an opportunity to reach populations that are inaccessible by “traditional” strategies.

Registration of AEs occurring after vaccination relied on self-reporting. Previous studies have shown that spontaneous reporting of AE after TT administration is infrequent [28]. A larger number of women might have experienced reactions that were not reported; there was no indication that any serious unreported AE occurred.

In this study, baseline tetanus protection was higher than anticipated. It is possible that despite the use of a structured questionnaire by trained interviewers, not all previous TT doses were captured. TT vaccination history can be difficult to determine, especially among women vaccinated a long time ago [29] and those with low awareness of the purpose of vaccination [30]. Nonetheless, we found that 74.5% of young (<21 years) nulliparous women without previous TT vaccination opportunities were protected at baseline according to our ELISA technique and 0.16 IU/ml cut-off.

Antibody levels obtained from standard indirect ELISA overestimate protection at low antibody levels; use of that assay may have limited the detection of participants with insufficient neutralizing anti-tetanus antibodies for protection. The use of a modified ELISA technique, such as double-antigen or inhibition ELISA or toxin-binding inhibition assay (ToBI) would have provided antibody level results that correlate better with those obtained with *in vivo* neutralization assays [23]. The use of a 0.20 IU/ml cut-off probably provides a more accurate assessment of the protection in the study population. Use of different assays and lack of standardization between laboratories limit the comparison of results across studies. Agreement on an internationally recognized methodology would facilitate comparison and interpretation of results [22].

In addition, in response to a meningitis epidemic, a campaign using meningococcal serogroup A polysaccharide-TT conjugate vaccine (PsA-TT) was conducted in the study area 7 months before study initiation. 69.6% of participants reported receiving the vaccine. The anti-tetanus immunizing effect of PsA-TT [31] likely contributed to the high baseline protection.

This study demonstrates that TT manufactured by Serum Institute of India Limited can be used in CTC in settings with high ambient temperatures. The use of TT produced by other manufacturers in CTC needs to be evaluated. To date the only vaccine licensed for use in CTC is PsA-TT (MenAfriVac). The adoption of CTC strategies requires political engagement that facilitates licensure of vaccines in CTC by manufacturers and regulators and supports its implementation by countries. The use of CTC can help increase vaccination coverage by reaching people living in remote areas and increasing availability of vaccines in places where cold chain is extremely difficult to maintain. It can also reduce logistical demands and cost of SIAs [32]. These are major advantages for the countries that are still striving to achieve MNTE.

Competing interest statement

The authors declare no competing interests.

Acknowledgements

We wish to thank the population of Ngalo, Biri and Kaba 6 for their participation in the study. Many thanks also to health and administrative authorities in Ngalo, Biri, Kaba 6, Moïssala, Mandoul and N'Djamena for their support and engagement. We are also grateful to the Médecins Sans Frontières teams in the field for their hard work and enthusiasm in the conduct of the study. We also thank Médecins Sans Frontières headquarters staff involved in the study for their support and advice. Thanks also to Serum Institute of India Ltd for their advice and recommendations. Many thanks for their huge work to all staff involved in the *in vivo* and *in vitro* assays at the WIV-ISP, especially to Isabelle Hansenne, Fabrice Ribaucour and Geneviève Waeterloos.

This study was financed by MSF and the project Optimize, a WHO-PATH collaboration funded by the Bill & Melinda Gates Foundation.

This study was conceived by FF, RFG, SZ and AJG. All authors provided substantial contributions to the design of the study. AJG, PB, PG and MT were involved in the study implementation. CL, CD and MHR were involved in the interpretation of the results. The first draft of the manuscript was written by AJG and RFG. All authors contributed to the writing of the manuscript and agree with the results and conclusions.

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