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Freezing temperatures in the vaccine cold chain: A systematic literature review

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Abstract

The dangers of accidental freezing of vaccines in the cold chain have prompted studies throughout the globe to better characterize the risk. To date, there has been no systematic review of these studies. This analysis highlights that accidental freezing is pervasive and occurs across all segments of the cold chain. Between 14% and 35% of refrigerators or transport shipments were found to have exposed vaccine to freezing temperatures, while in studies that examined all segments of distribution, between 75% and 100% of the vaccine shipments were exposed. More rigorous study designs were associated with higher levels of freeze exposure. As more expensive, freeze-sensitive vaccines are introduced into immunization schedules, freeze prevention will become increasingly critical for ensuring that the world's children are receiving fully potent vaccine.

Keywords: Freeze-sensitive vaccines; Accidental freezing; Cold chain

1. Introduction

Vaccines are powerful public health tools that save an estimated 3 million lives each year [1]. Recognizing their importance, the global health community has prioritized the increased availability of vaccines to all the world's children. Yet this commitment has also had the effect of placing additional stress on an already fragile cold chain, the distribution network of equipment and procedures used to maintain vaccine quality from the vaccine manufacturer to the vaccine recipient.

World Health Organization (WHO) guidelines and manufacturer product inserts recommend that all vaccines except oral polio vaccine be kept at 2–8 °C during in-country distribution. However, a poorly functioning cold chain may deviate from this target range and expose vaccines to freezing temperatures. Damage from accidental freezing can result in potency loss for freeze-sensitive vaccines such as diphtheria, tetanus, pertussis, liquid *Haemophilus influenzae* type b (Hib), hepatitis B, and inactivated polio virus [2–6]. However, cold chain practices tend to prioritize protecting vaccine from heat damage, often at the risk of exposure to freezing temperatures. As a result, accidental freezing of vaccines is a largely overlooked problem, yet freeze-sensitive vaccines represented over 31% of the US$ 439 million UNICEF spent on all vaccines in 2005 [7].

Increased awareness of this danger has prompted studies of the cold chain, designed to characterize the risks of vaccine exposure to freezing temperatures. However, to date there has been no systematic review or cross-comparison of these

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studies. This paper attempts to review and analyze the current global data on freezing temperatures within the vaccine cold chain. The objective is to identify the settings in which freezing occurs and explore common factors among these studies and settings. It is our hope that this review will help raise further awareness of the extent to which vaccines are being exposed to freezing temperatures worldwide as well as encourage immunization program managers to design studies to investigate the state of their own cold chains and institute programmatic actions to prevent potential freeze exposure.

2. Methods

2.1. Identification of cold chain studies

To capture the broadest range of cold chain studies for inclusion within our analysis, a search for studies published between January 1985 and June 2006 was performed using four major electronic databases: PubMed, Popline, Embase, and Biosis. The term "vaccine" coupled with one of the following terms was searched in all databases: thermostability, stability, refrigerator, cold chain, storage, and temperature. In addition, we searched several public health websites for unpublished studies, including WHO, TechNet21, Epivac, and BASICS II. Finally, we solicited unpublished studies from the immunization community through a posting on the TechNet21 listserv. A total of more than 1200 articles were identified; the majority of articles were excluded based on subject matter inferred by the titles, while 117 warranted further review against our established inclusion criteria:

- Studies collecting primary data on the temperature conditions within existing cold chain infrastructures (during either transport or storage), using consistent procedures across the study.
- Studies using temperature-measuring devices that register freezing temperatures.

The resulting 35 articles that met the inclusion criteria are listed in Table 1. Of the 35 articles, 25 were published, while 10 were unpublished. Additionally, 3 of the 35 articles were from non-English sources.

2.2. Data extraction methods

For each of the studies that met the inclusion criteria, the following information was compiled: the number of samples measured (number of refrigerators or shipments), the percentage of samples that registered temperatures that were too cold, the temperature that was used as the cutoff for defining "too cold" in each study (freeze-exposure threshold), the period and frequency of temperature measurement, the type of measuring equipment used, the country in which the study was conducted, and the year in which the study was published or reported. From articles in which both transport and storage temperature data were collected, information about each segment was noted separately. Thus, some studies provided multiple data points.

2.3. Design of analysis

Transport and storage data were analyzed separately to accommodate for differences in endpoints across the various studies and also because the factors that could potentially influence temperature variations could be expected to differ between transport and storage situations. The data were also categorized as developed-country versus developing-country studies to facilitate comparisons across presumed differences in cold chain infrastructure [8]. To complement descriptive statistics, a series of multivariate meta-regression analyses was pursued to identify factors that might be associated with the level of exposure to freezing temperatures found in either transport or storage studies.

2.4. Study limitations

To date, a substantial proportion of the worldwide studies of freezing temperatures in the cold chain have been conducted in the Asia-Pacific region, with this bias reflected in the study set (13 out of the 35 studies included in the analysis). Furthermore, almost half of the developed-country studies were conducted in Australia (7 out of 16 studies). Therefore, although the study findings may be indicative, they prevent full characterization of the study outcomes as representative of the worldwide problem.

In addition to geographic clustering, publication bias – the potential for studies with only certain types of results to be published – could also prevent the study results from being generalized. For this reason, much effort went into finding unpublished studies and resulted in 28% of the study set (10 out of 35 articles) coming from unpublished sources. A large percentage of unpublished sources may also better reflect the likely universe of studies on this topic, as vaccine freeze-exposure studies are more often conducted internally to guide systems improvements. Still, the findings from this study may not be generalized for all subcategories of analysis. For instance, we were only able to find two transport studies in developed countries, making it difficult to draw conclusions about vaccine temperature exposure under that particular scenario [9,10].

3. Results

3.1. Identified studies

Of the 35 articles selected, 17 articles reported on research that was conducted in developed countries and 18 in developing countries. Each subset was then further divided into studies evaluating the temperatures of storage versus transport segments of the cold chain (Table 1). Two articles were dropped prior to the comparative analysis due to an inabil-

Table 1
Cold chain studies evaluating vaccine storage or transport temperatures below the recommended range

	Authors	Year	Country	Unit of analysis	Sample size	Occurrence of temperatures below freeze threshold (%)
1	Ford and Gibbs [23]	1990	New Zealand	Refrigerator	27	26
2	Lugosi and Battersby [24]	1990	Hungary	Transport shipment	166	38
3	Thakker and Woods [25]	1992	Great Britain	Refrigerator	8	50
4	Bass [26]	1993	Papua New Guinea	Refrigerator	17	6
5	Cheriyan [9]	1993	UK	Transport shipment	7	0
6	Miller and Harris[a] [27]	1994	Australia	Vials	59	48
7	Woodyard et al. [28]	1995	USA	Refrigerator	27	26
8	Yuan et al. [29]	1995	Canada	Refrigerator	135	6
9	Finnegan and Howell [30]	1996	Ireland	Refrigerator	111	7
10	Hanjeet et al.[a] [31]	1996	Malaysia	Vials	234	99
11	Jeremijenko et al. [32]	1996	Australia	Refrigerator	50	34
12	Guthridge and Miller [10]	1997	Australia	Transport shipment	5	40
13	Kone [33]	1997	Mauritania	Refrigerator	108	4
14	Steinglass [34]	1997	Kazakhstan	Refrigerator	15	67
15	Wawryk et al. [35]	1997	Australia	Refrigerator	40	53
16	Gold et al. [36]	1998	Australia	Refrigerator	1	100
17	Reimer and Lewis [37]	1998	Australia	Refrigerator	52	23
18	Gold et al. [38]	1999	Australia	Refrigerator	32	22
19	Battersby and Feilden [39]	2000	Ukraine	Refrigerator	1	100
20	Berhane and Demissie [40]	2000	Ethiopia	Refrigerator	60	2
21	Bell et al.[b] [41]	2001	USA	Refrigerator	262	15
22	Lewis et al. [42]	2001	Australia	Refrigerator	102	36
23	Munck [43]	2001	Kyrgyzstan	Refrigerator	19	0
24	TechNet 21 New Delhi Consultation Report [44]	2001	Nepal	Refrigerator	1	100
25	Gazmararian et al. [45]	2002	USA	Refrigerator	641	7
26	Ortega Molina et al. [46]	2002	Spain	Refrigerator	43	5
27	PATH [47]	2002	E. European Country	Refrigerator	185	25
28	PATH/University of Melbourne [11]	2003	Southeast Asian Country	Refrigerator Transport shipment	11 8	36 75
29	Edstam et al. [48]	2004	Mongolia	Transport shipment	240	15
30	Glele Kakaï [12]	2004	Benin	Refrigerator Transport shipment	16 6	56 50
31	Nelson et al. [13]	2004	Indonesia	Refrigerator Transport shipment	14 16	29 44
32	PATH, Village Reach [14]	2004	South African Country	Refrigerator Transport shipment	10 8	80 75
33	PATH/UNICEF [15]	2005	Bolivia	Refrigerator Transport shipment	25 11	60 100
34	McGuire [49]	2006	Kenya, Liberia, Pakistan	Transport shipment	90	60
35	Wirkas et al. [16]	2006	Papua New Guinea	Refrigerator Transport shipment	16 12	31 100

[a] These studies met our original inclusion criteria; however, the reported data points could not be compared on the same basis as the other studies. In the case of study #6 and #10, the percent of freeze-exposed vials could not be compared to freeze-exposed refrigerators or transport shipments since some of the vials may have been stored together, thereby obscuring the independence of the reported data points. Therefore, these studies do not appear in the comparative analysis.

[b] Additional data was acquired through e-mail correspondence with the author to resolve questions about the reported data such as what temperatures were measured during transport versus storage legs of cold chain assessment [13,15] how many unacceptable temperature readings were in the freezing range[32] or how many refrigerators were included per physician practice [41].

ity to compare the reported data points on the same basis as the other studies (marked with an asterisk in Table 1). Six of the remaining 33 articles collected data on both transport and storage segments. These data were divided and grouped with their respective transport and storage study subsets, resulting in a total of 39 data points for the comparative analysis.

The majority of transport studies analyzed all transport segments of the cold chain, from central storage to clinics. Most of the storage studies examined the lower levels of the

Table 2
Summary of studies

Cold chain segment	Developing countries	Developed countries
Transport		
Number of studies	9	2
Study sample size[a] (mean, standard deviation [S.D.])	61.9 (86.2)	6 (1.4)
Freeze threshold temperature (°C) (mean, S.D.)	−0.4 (1.0)	0 (0)
Year study conducted (mean, S.D.)	2003 (4.9)	1995 (2.1)
Rigorous monitoring[b] (duration and frequency)	78%	50%
% shipments found below freeze threshold weighted mean (95% CI)	35.3% (14.8–55.8%)	16.7% (0–44.9%)
Storage		
Number of studies	14	14
Study sample size[a] (mean, S.D.)	35.6 (51.3)	113.6 (168.3)
Freeze threshold temperature (°C) (mean, S.D.)	0 (0)	0.4 (1.0)
Year study conducted (mean, S.D.)	2002 (4.1)	1997 (3.8)
Rigorous monitoring[b] (duration and frequency)	50%	29%
% refrigerators found below freeze threshold, weighted mean (95% CI)	21.9% (10.3–33.6%)	13.5% (6.4–20.7%)

[a] The units of analysis are shipments for the transport studies and refrigerators for the storage studies.
[b] Rigorous monitoring was calculated by assigning either a 0 or a 1 to both the duration and frequency of monitoring checks and then multiplying the two assignments to arrive at a single indication (0 or 1) to label the study as having rigorous monitoring or not. For duration of monitoring, anything more than 1 week was assigned a 1. For frequency of monitoring, any study with continuous monitoring was given a 1; spot checks or one time point only measurements were given a 0.

cold chain, focusing on clinic refrigerators. The size of the studies varied, ranging from 1 to over 600 refrigerators or from 5 to over 200 transport shipments. There were also differences in the definition of the freeze-exposure threshold. While the majority of studies used 0 °C for this threshold, some considered only temperatures below −3 °C, while others deemed anything below 2 °C as too cold. In some cases, the definition also included a time element to determine an unacceptable level of exposure. Another notable variation in design across the studies was the frequency and duration of the temperature monitoring.

3.2. Comparison of studies and outcomes

Key study design parameters (sample size, freeze-exposure threshold, year the study was conducted, and level of monitoring rigor) were compared across four study scenarios (developed versus developing country, transport versus storage). These figures are summarized in Table 2.

4. Discussion

4.1. Analysis of key findings

- During transport, the occurrence of freezing temperatures was found to be 16.7% in developed countries compared to 35.3% in developing countries. This difference was not significant, potentially indicating that the current transport practice common to all countries – vaccines placed with frozen ice packs inside of insulated carriers – is placing vaccines at risk, regardless of the resource setting in which it is conducted. Observations indicate that the WHO-recommended practice of "conditioning" ice packs (allowing them to begin melting before placing them in transport cold boxes) is not routinely followed. Alternatively, the lack of statistical significance could also be driven by the relatively small number of included studies for the transport analysis in developed countries.
- During storage, the occurrence of exposure to freezing temperatures was found to be 13.5% in developed countries versus 21.9% in developing countries. Again, this difference was not found to be statistically significant, underscoring the fact that vaccine exposure to freezing temperatures in the cold chain appears to be a truly global problem, occurring in resource-rich as well as resource-limited settings.
- The monitoring rigor of the study was found to be a significant predictor of freezing temperatures, implying that studies designed to gauge freezing temperatures with more frequent or continuous monitoring were more likely to report occurrences of vaccine freezing. Studies in both developed and developing countries with less rigorous monitoring reported significantly fewer occurrences of vaccine exposure to freezing temperature than studies in developing countries with scheduled or continuous monitoring (55% less [$p < 0.001$], and 40% less [$p = 0.001$], respectively). Another important finding was that among the studies with more rigorous monitoring, there was no significant difference between developed- and developing-country status in the occurrence of vaccines exposed to freezing temperatures ([$p = 0.23$]).
- In both the transport and storage analyses, the larger studies had less exposure of vaccines to freezing temperatures. However, after controlling for monitoring rigor in the storage analysis, it appears that the size of the study makes no difference in the observed proportions of vaccine exposed to freezing temperatures ([$p = 0.18$]). In fact, monitoring rigor was the only variable that carried significance, implying that study methods matter more than location or study

size in terms of the proportion of vaccine freeze exposure found.

- In the six studies that analyzed the exposure of vaccine shipments to freezing temperatures as they traveled through both shipment and storage segments of the cold chain from either national or regional stores all the way to health clinics, the findings were even more striking: between 75% and 100% of the vaccine shipments were exposed to freezing temperatures at least once during the distribution process [11–16]. These comprehensive studies suggest that the risk of damaging freeze-sensitive vaccines is present in virtually every stage of the cold chain.

4.2. Guidance for future studies

Although this review cannot reach a conclusion about the proportion of vaccine that is actually damaged by exposure to freezing temperatures or actually results in moderated immunogenicity, we do know that potency loss does occur when freeze-sensitive vaccines undergo the phase change to a frozen state. Many epidemiological studies have also pointed to vaccine freezing as a possible contributor to low immune response in vaccinated individuals and the existing literature relating freeze exposure to potency loss is compelling enough to suggest some degree of impact on immune response [2–6,17–19]. More lab-based studies are needed to augment the literature base in order to gain a more precise understanding of the degree of potency loss associated with common field conditions. Detailed cold chain studies that monitor both time and temperature exposure across the entire length of the cold chain would provide the richest source of exposure conditions for such purposes.

It is also important to note that only 35 studies could be found in the global literature to date, highlighting the lack of attention to conditions which may be undermining immunization efforts worldwide. The sheer paucity of literature is a call to action for more rigorous and comprehensive studies that examine the exposure of vaccines to freezing temperatures through all transport and storage segments of the cold chain.

4.3. Recommendations

Freeze avoidance will become even more critical in the future as more expensive freeze-sensitive vaccines such as DTP-hepatitis B, liquid Hib, pneumococcal, and influenza are introduced into developing-country immunization programs. Fortunately, accidental exposure to freezing temperatures can be prevented. The following is a list of simple and proven tools available today that could help program managers minimize the potential for freeze damage to their vaccines:

- *Investigate vaccine freezing in cold chains*. WHO recommends that immunization programs use temperature-monitoring studies to identify where problems are occurring so that corrective action can be taken. A WHO protocol has been developed and successfully applied in several countries [20,11,13–15].
- *Apply innovative cold chain practices*. Using cool water packs instead of frozen ice packs during cold box transport to avoid the freezing commonly associated with improper ice-pack conditioning [13], storing the more heat-stable vaccines in air-conditioned rooms, and conducting limited transport at ambient temperatures are possible freeze-prevention approaches.
- *Improve training*. Recommended changes in training include educating vaccine managers and handlers about freeze-sensitive vaccines, providing clear guidelines on vaccine handling procedures, and increasing the focus on freeze prevention during supervision.
- *Update cold chain infrastructure*. Improved cold chain equipment is an important aspect of freeze prevention. New refrigerators are being developed that automatically control temperatures and eliminate the possibility of inadvertent vaccine freezing. At a minimum, min/max thermometers can help users better understand the range of temperatures that vaccines are exposed to over time and can prompt mitigation strategies. Electronic temperature-monitoring devices are also available to accompany vaccines during transport and storage, including low-cost devices that signal exposure to an established freeze-exposure temperature [21].
- *Take advantage of vaccines' heat stability*. Freezing temperatures damage freeze-sensitive vaccines more quickly than ambient temperatures. Vaccine vial monitors, heat-exposure indicators required on all vaccines distributed by UNICEF, allow vaccine programs to utilize the natural heat stability of some vaccines to expose them to temperatures warmer than 8 °C for limited time without risk of heat damage. It may also be possible to allow a slight warming of the recommended cold chain temperatures to reduce freezing without causing heat damage [22].
- *Create system-wide policy to avoid freeze damage*. Policy changes (at the global and country level) are needed to prioritize the prevention of freezing in the cold chain and apply resources to raising awareness, training, and equipment infrastructure.

5. Conclusion

This analysis highlights that exposure of vaccines to freezing temperatures is pervasive, occurring in both developed- and developing-country settings, as well as within both the storage and transport segments of the cold chain. Across the four scenarios analyzed, the average proportion of exposure ranges from 14% to 35%. In the six studies that measured temperatures longitudinally through multiple sections of the cold chain, between 75% and 100% of the vaccine shipments were exposed to freezing temperatures. As vaccine availability expands and more expensive, freeze-sensitive vaccines are introduced into immunization programs, freeze preven-

tion will become even more essential, both for making the most productive use of immunization dollars and for ensuring that the world's children are receiving fully potent and effective vaccines.

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