Research article

Variation in Storage Temperatures for Foot and Mouth Vaccine in Cambodia

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Abstract Vaccine efficacy may be influenced by pre-use storage condition. This study assesses vaccine cold storage management and vaccine handling practices at 30 veterinary drug stores spread across the provinces of Pursat (n=10), Kampong Cham (n=9) and Phnom Penh (n=11) in Cambodia. Electronic data loggers were used to record the temperature in each cold storage facility every thirty minutes for a period of thirty days. The findings indicated that vaccines were exposed to freeze temperature for 24-100 hrs (3%-14%) and more than 100 hrs (15%-87%) of time recordings in 8 and 9 facilities respectively. Vaccines were exposed to heat for 254-327 hrs (35%-45%) of time recordings in 3 facilities and between 468-7200 hrs (65%-100%) in 4 facilities. Most of the refrigerators also contained food and/or drinks, leading to the frequent door opening. Vaccines were often stored in the bottom drawers and door shelves, which were the warmest locations within refrigerators in the study. The vast majority of the study refrigerators (93.3%) were not equipped with a maximum-minimum thermometer. Daily refrigerator temperature recording was not practiced in any of veterinary drugstores. This study also highlighted the considerable temperature fluctuations to which vaccines were exposed to a number of refrigerators. The frequent power failures which occur in Cambodia contribute to these temperature fluctuations. This study consequently also investigated the speed and duration of temperature rise in a cold storage facility during a typical power failure in Phnom Penh. The results suggested that corrective training for vaccine wholesalers/retailers and relevant government staff may be a useful first step in attempting to improve vaccine storage conditions, hence, improved potency. Maintenance of vaccine potency is likely to improve the success of vaccination programs in Cambodia. This critical but neglected issue requires improved practices and ongoing monitoring. The results also highlight the need for improvement and solutions to avoid ongoing future exposure of vaccines to freezing, too cold and too hot temperatures, particularly in hot tropical countries like Cambodia.

Keywords data logger, veterinary drug store, vaccine, vaccine cold storage, Cambodia

INTRODUCTION

Vaccination is a key control method for foot and mouth disease (FMD) in countries where the disease is endemic as is the situation in Cambodia. Immunization is a highly effective way of protecting

individuals and communities from infectious disease. However, successful vaccination campaigns require proper storage, transportation and handling of vaccines, including inactivated FMD vaccines (El-Sayed, El-Din, Rizk, & El-Aty, 2012). The FMD vaccines currently used in Cambodia are inactivated, oil adjuvant purified antigen preparations. Vaccine shelf life is always indicated by the manufacturer and is usually six to twelve months under the specified conditions of storage. Typically these include storage between 2-8 ˚C protected from light and freezing. Typically freezing the vaccine or heating the vaccine will promote emulsion breakdown and destroy vaccine integrity/efficacy over time (S. Seneque, Merial (Asia), personal communication, September 5, 2014). A study by Bell et al., (2001) revealed that failure to keep the thermometer in the vaccine storage facilities was associated with vaccine storage temperatures outside recommended range (2-8 ^{o}C). Cortese and Smith (2004) reported freezing of vaccine will disrupt the integrity of the antigens and degrade the adjuvant and overheating can have the same effect. Incorrect handling or storage of vaccine may result in an ineffective vaccine being administered and failure of protection (Rashid, Rasheed, & Akhtar, 2009). To remain potent, FMD vaccines should be stored under refrigeration usually at 4±2 ^{o}C for the optimal retention of antigenic potency (Garland, 1999) and should not be used if they have frozen or exposed to high temperatures or are outside the use by date (Cortese & Smith, 2004). Weir and Hatch (2004) suggested that never store vaccines on refrigerator-door shelves, where they are often exposed to warm air every time the door opens. The vaccine is thought to lose immunogenic potency progressively as the storage temperature increases above these levels. Thawing frozen vaccines or re-cooling over-heated vaccines does not restore vaccine integrity (Cortese & Smith, 2004) and damages their immunogenicity (Garland, 1999).

When an inactivated oil adjuvant FMD vaccine was stored at 4 ^{o}C for 15 months, no appreciable vaccine potency loss could be detected by the direct challenge testing of vaccinated cattle and specific antibody assay (Doel, 2003; Garland, 1999). Recent research has confirmed that FMD vaccine may keep their potency for two years at 4 ^{o}C, three weeks at 25^{o}C and one week at 37 ^{o}C with full protection against challenge with FMDV O1/Aga/EGY/93 (El-Sayed et al., 2012). Protection was decreased to 80% when vaccines were stored at 25 ^{o}C for 4 weeks and at 37 ^{o}C for 2 weeks. The efficiency of the cold chain is, therefore, a critical factor for optimal vaccine storage (Garland, 1999). Farmer interviews during the 2010 FMD outbreak in Cambodia suggested very poor protection of cattle in responses to vaccination administered by government authorities in Kampong Cham province. The major reasons for this failure were thought to be poor planning, timing and implementation of the vaccination program, as well as improper vaccination technique (under-dosing) and weaknesses in the vaccine cold chain (Sieng & Kerr, 2013). The donated FMD vaccine used was reported not to have been stored at the proper temperatures recommended by the manufacturer (District veterinarians and village animal health workers, personal communication, October 30, 2010). These allegations require proper testing as temperature in vaccine cold storage facilities in Cambodia have not previously been investigated and reported. Consequently, this study represents is first attempt to measure the performance of government and commercial vaccine cold storage facilities in three regions of Cambodia.

OBJECTIVE

In this study we aimed to investigate vaccine storage temperatures in veterinary drug stores in two Cambodian provinces and in the capital city of Phnom Penh.

METHODOLOGY

The main study (Study 1) design involved continuous monitoring of temperatures for 30 days at 30 veterinary drugstores (VDs) spread across the provinces of Pursat (PS, n = 10), Kampong Cham (KC, n = 9),

and the capital Phnom Penh (PP, n = 11). All known eligible VDs in the 3 areas was invited to participate based on selection criteria including significant vaccine sales and willingness to participate. The study sites included predominantly vaccine retailers but also government vaccine stores of the study sites. In KC almost all vaccine stores in Prey Chhor district (3) and Kampong Cham provincial town (6) participated in the study. In PS province, six (6) VDS participated in Sampov Meas district, as well as four (4) from Bakan district. Eleven (11) stores in PP participated in the research study. A total of thirty refrigerators in 30 VDs located in 3 areas in Cambodia were thus selected for the study. Of these thirty VDs, 3 were government cold storage facilities, and 27 were vaccine retailers. Of the 27 VDs, 26 used a domestic type refrigerator and one used a cold-box. Two out of 3 government cold storage facilities used domestic type refrigerator and one (PP) involved a large refrigerated vaccine storage facility. Temperature recording was via electronic data loggers[1] (Thermochron®), programmed to record temperatures at 30 minute intervals for 30 days, a total of 1,440 readings for each refrigerator. The recording accuracy was ±1 ^{o}C. The performance of the data loggers was tested in a refrigerator with a known temperature as demonstrated by a thermometer. The precise date of placement of the data loggers was unknown to participants until immediately before placement. A single data logger was placed centrally in the refrigerator or cold box next to vaccines but not placed immediately beside or on an ice pack or ice. The VDs were visited every week by research assistants to ensure that the data logger was still in the same position. Each half-hourly temperature recording was classified as 'freezing' (≤ 0 ˚C), 'too cold' (> 0 but < 2 ˚C), 'recommended range' (2-8 ˚C) or 'too hot' (> 8 ˚C) and the proportion of samples in each category determined for each data logger.

These data were analyzed within temperature categories by one way analysis of variance (AOV) to test the effect of province (and the reliability of its power supply) on the proportion of samples in each category. A separate dataset was created in which the duration of each period spent within each temperature category over the 30 days experimental period was recorded. This enabled analysis of the mean time spent in each temperature category. This was repeated measures analysis so a mixed restricted maximum likelihood (REML) model was fitted with data logger as a random effect and province, temperature category and their interaction fitted as fixed effects. Analyses were performed using JMP 12[2] with a statistical significance level of $P < 0.05$. Because power failures are a frequent and sometimes prolonged event in Cambodia, an additional small study was carried out to test the effect of a power failure on temperatures within a vaccine storage facility (Study 2). The study used the data loggers and ran for a period of 18 days. The government vaccine cold storage facility in Phnom Penh was chosen for this study because the presence of a 24 hour guard at that facility allowed accurate recording of the time that the electricity blackout began and ended, so that these times could be matched with the temperatures recorded by the data loggers inside the vaccine cold storage facility during the same period. The effect of location within a refrigerator on temperature variability was also investigated in a VD in Phnom Penh (Study 3). The study was carried out for a period of 30 days by using the same data loggers.

RESULTS AND DISCUSSION

At the completion of study period for Study 1, we received 30 completed data loggers, giving an overall successful recording rate of 100. Table 1 summarizes the performance of each refrigerator during the 30 day study period, including mean, median, maximum and minimum temperatures recorded and time spent in each of the four temperature categories: 'freezing' (≤ 0 ^{o}C), 'too cold' (> 0 but < 2 ^{o}C), 'recommended range' (2-8 ^{o}C) and 'too hot' (> 8 ^{o}C). The final column in table 1 titled

[1] *Thermochron® DS1921, Dallas Identification/ALFA-TEK Australia, 7/42-50 Stud Road, PO Box 882, Bayswater, VIC. 3153, Australia*
[2] *JMP version 12 (SAS Institute Inc., NC, USA, 2015)*

'No. of changes' refers to the number of times that refrigerator moved between temperature categories during the recording period.

Table 1 Details of each of the vaccines storage refrigerators including the cumulative percentage of temperature recordings in each of four temperature categories, and number of changes between these categories over a 30 day period

	Site	vaccines in door shelf	Food/drinks also stored	Mean temp (°C)	Median temp (°C)	Freezing (%)	Too cold (%)	Correct (%)	Too hot (%)	Temp. range max-min (°C)	No. of changes
Phnom Penh (PP)	[a]PP1	no	no	6.40	6.5	0.0	0.0	98.9	1.1	4.5 - 16.0	6
	PP2	yes	no	13.60	14.0	0.0	0.0	0.0	*100.0*	8.5 - 26.5	1
	PP3	yes	yes	4.04	4.0	0.0	3.0	96.5	0.5	1.0 - 10.5	23
	PP4	yes	yes	5.89	5.0	0.6	6.5	71.5	*21.4*	0.0 - 25.0	127
	PP5	yes	yes	0.92	0.5	*40.1*	35.1	24.4	0.5	-2.5 - 11.5	212
	PP6	yes	yes	-1.30	-1.5	*86.9*	11.2	1.7	0.1	-4.0 - 13.0	32
	PP7	yes	yes	2.33	2.5	4.3	23.4	71.9	0.4	-1.0 - 15.0	318
	PP8	yes	yes	4.18	4.0	1.4	8.3	84.7	5.6	-1.0 - 24.5	171
	PP9	yes	yes	6.94	6.5	3.2	10.3	51.2	*35.3*	0.0 - 15.0	224
	PP10	yes	yes	8.14	8.0	0.1	0.1	60.1	*39.8*	0.0 - 15.0	230
	PP11	yes	yes	3.54	4.5	*15.1*	10.5	67.9	6.5	-9.5 - 14.5	61
Kampong Cham (KC)	KC1	yes	yes	-1.30	-2.0	*76.4*	7.1	11.4	5.1	-8.5 - 20.0	121
	KC2	yes	yes	3.28	3.0	7.7	25.5	63.7	3.2	-1.5 - 14.5	160
	KC3	yes	yes	3.17	2.5	*26.9*	16.7	43.4	*13.0*	-8.0 - 22.5	190
	KC4	yes	yes	2.28	2.0	7.2	30.8	61.4	0.7	-1.5 - 12.5	676
	KC5	yes	no	1.70	1.5	*12.3*	45.1	42.4	0.3	-2.0 - 13.5	213
	[b]KC6	no	yes	1.51	-1.0	*73.6*	5.1	6.8	*14.5*	-4.0 - 24.5	45
	KC7	yes	yes	4.56	3.5	6.5	17.1	59.2	*17.2*	-3.5 - 20.5	242
	KC8	yes	yes	5.17	5.0	8.0	6.7	65.9	*19.4*	-3.0 - 19.5	344
	KC9	yes	yes	5.63	5.5	4.2	7.2	66.4	*22.2*	-2.0 - 17.5	187
Pursat (PS)	[b]PS1	yes	yes	1.10	1.0	*37.8*	21.2	40.9	0.1	-3.5 - 10.5	1165
	PS2	yes	yes	6.34	6.5	0.0	0.0	94.0	6.0	4.0 - 12.0	26
	PS3	no	yes	9.24	9.5	0.0	0.0	35.0	*65.0*	2.0 - 19.5	59
	[c]PS4	yes	yes	8.56	7.5	0.0	0.7	53.9	*45.4*	1.0 - 24.5	80
	PS5	yes	yes	1.77	2.0	*26.5*	22.7	50.7	0.1	-3.5 - 9.0	141
	PS6	yes	yes	3.56	3.5	0.0	9.4	90.1	0.5	0.5 - 11.5	185
	PS7	yes	yes	4.20	3.0	0.1	17.5	71.7	10.7	0.0 - 22.0	236
	PS8	yes	yes	5.94	6.5	*19.0*	0.3	35.8	*44.9*	-9.0 - 13.0	58
	PS9	yes	yes	9.85	9.5	0.0	0.0	23.7	*76.3*	6.5 - 14.5	164
	PS10	yes	yes	1.96	2.0	3.7	45.2	50.8	0.3	0.0 - 10.0	926

[a]Department of Animal Health and Production vaccine cold storage, [b]Provincial Office of Animal Health and Production vaccine cold storage, [c]Cold box rather than refrigerator

In Phnom Penh, eleven refrigerators were monitored for a median 743 (range 716-750) hours. Although two of the eleven PP refrigerators were equipped with a maximum and minimum thermometer, in neither of them was the internal temperature of the refrigerator regularly monitored. In fact, one of the two refrigerators that was equipped with a maximum and minimum thermometer nevertheless had 100% of its data-logger temperature recordings at > 8 °C (too hot). Three other refrigerators in PP also registered a significant proportion of recordings (21.4%, 35.3% and 39.8%) in the 'too hot' temperature range (> 8 °C), while other 3 refrigerators vaccines were exposed to too cold (> 0 but < 2 °C) and freezing temperature (≤ 0 °C) in 25.6%, 75.2% and 98.1% of recordings. Of the 11 PP refrigerators, 9 were also used to store food and/or drinks. In all refrigerators, vaccines were stored on door shelves.

In Kampong Cham, nine refrigerators were monitored for a median 743 (range 769-793) hours. None of the refrigerators were equipped with a maximum and minimum thermometer. Vaccines in one refrigerator were exposed to both heat temperatures (> 8 °C) for 13.0% of recording and too cold and freezing temperatures for 43.6% of recordings (Tabel 1). In four refrigerators, vaccines were exposed to too cold (> 0 but < 2 °C) and freezing temperature (≤ 0 °C) for 33.2%, 57.4%, 38% and 83.5% of the temperature recordings. The results for the POAHP's refrigerator are especially significant because large quantities of donated and government FMD and other types of vaccines are stored there periodically. The temperatures in that facility were freezing (≤ 0 °C) for approximately three quarters of time (78.6%) and too hot (> 8 °C) in 14.5% of recordings. Of the nine refrigerators, 8 refrigerators were also used to store food and/or drinks. In 8 of the 9 KC refrigerators, vaccines were stored in the door shelves.

In Pursat, nine refrigerators and an ice box were monitored for a median 725 (range 668-743) hours. None of the vaccine storage facilities were equipped with a maximum and minimum thermometer. In two refrigerators vaccines were exposed to heat (> 8 °C) for 65.0%, and 76.3% of the temperature recordings. Vaccines were exposed to too cold (> 0 but < 2°C) and freezing temperatures (≤ 0 °C) in 2 refrigerators for 48.9% and 49.2% of the temperature recordings. The most pronounced example of temperature variability in Pursat was recorded in refrigerator PS8, which recorded 44.9% of reading in the 'too hot' range (> 8 °C) and 19.0% of readings in the 'freezing' category (≤ 0 °C). The results from the single set of cold box recordings (PS4, Table 1) showed that vaccines were exposed to heat > 8 °C for 45.4% of the temperature recordings. As in Kampong Cham province, the temperatures in that government facility (PS1) were freezing (≤ 0 °C) for 37.8% of recordings and too cold (> 0 but < 2 °C) for 21.2% of recordings. All 10 monitored refrigerators in Pursat were used to store food and/or drinks. In all nine refrigerators, vaccines were found to be stored on door shelves.

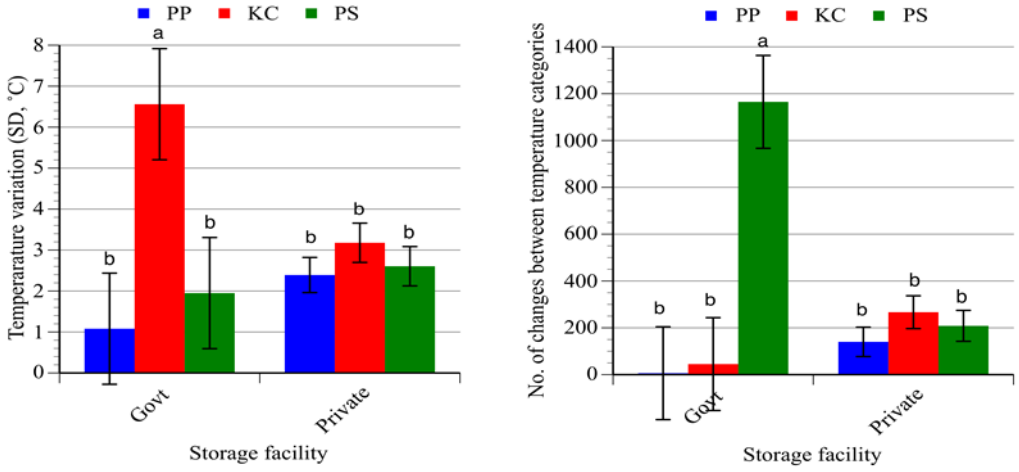

Fig. 1 Significant interaction between the effects of study area and ownership on temperature variation (SD, Left) and number of changes between temperature categories (Right)

The number of episodes between temperature ranges that each site encountered in all study areas was described in Table 1. The results from the analysis of the variables showed that there was no significant effect of Province, Government/Private status or interaction between these two for mean or median temperature, or the percentage of time spent in different temperature categories ($p > 0.05$). However, for the standard deviation of temperature, and the number of temperature episodes in different categories while there was no overall effect of the province (Table 1) there was significant interaction between the effects of Province and Government/Private status ($p < 0.05$, Fig 1).

Given the very small number of Government facilities, this should be interpreted with caution. This demonstrates that most of the variation in these was due to large variation between the Government sites included in the study. In particular, the vaccine storage facility in POAHP of KC recorded very high variability in temperature, and the POAHP of PS a high number of transitions between temperature categories. On the other hand, the POAHP in PP showed very consistent maintenance of temperature. Indeed, although the study was small, it provides clear evidence of serious problems with vaccine storage that could jeopardize the success of a vaccination program. Vaccine damage depends on the ambient temperature and the duration of exposure to adverse temperatures (Wawryk, Mavromatis, & Gold, 1997) and should not be used if they have been frozen or exposed to high temperatures (Cortese & Smith, 2004). The vaccine cold storage is critical point for the successful or failure of the vaccination program (Thakker & Woods, 1992). Those vaccine refrigerators can no longer maintain temperatures in recommended range (2-8 ^{o}C) have to be brought for the services or replaced (Grasso, Ripabelli, Sammarco, Manfredi Selvaggi, & Quaranta, 1999). Weir and Hatch (2004) suggest that never store vaccines on the refrigerator-door shelves. Failure to keep the thermometer in the vaccine refrigerators was associated with vaccine storage temperatures outside recommended range (2-8 ^{o}C) (Bell et al., 2001). None of the cold storage facilities were routinely monitored by the owners/managers. Storing vaccines contrary to the manufacturers' recommendation is likely to adversely affect their potency and consequent efficacy in the field, reducing the effectiveness of disease prevention programs which rely heavily on vaccination. Even though the study was small, it highlighted serious problems with 53.3% (n = 16) of the study vaccine cold storage facilities where 56.3% (n = 9) set to freeze and too cold temperatures from 43.6%-98.1% and 43.7% (n = 7) set to too hot temperatures from 35.3%-100% of the recordings. Moreover, the study results suggest that the majority of wholesalers/retailers and government officials in charge of cold storage facilities would benefit from a brief and specific training session on vaccine and cold storage management in order to ensure all vaccines are stored at the correct temperature range or avoid the damage of vaccines while storing inside their refrigerators. The Study 2 investigating the consequences of power failure on temperatures within the DAHP's Phnom Penh vaccine storage facility revealed that the temperature spike on the day 8 coincided with a 6-hours electricity blackout recorded by the DAHP guard. Ninety minutes after the blackout began the storage facility temperature had risen above acceptable refrigeration range (8 ^{o}C), and after 6 hours without electricity, the temperature had risen to 16 ^{o}C. After the electricity supply was restored, it took two hours for the temperature within the cold storage facility to return to the refrigeration range 2-8 ^{o}C. The effect on temperature of location within a refrigerator (Study 3) was investigated using the best performing commercial study refrigerator owned by a private VD in PP (PP3). The refrigerator registered 96.5% of temperature recording the correct refrigeration range (2-8 ^{o}C) during the 30 days study period. Marked variation was recorded depending on the location within the refrigerator. The mean temperatures of 0.7 (-2.5 - 4.5), 1.5 (-0.8 - 5.0), 0.0 (-2.3 - 5.5), 6.8 (4.0 - 8.8) and 5.3 (1.3 - 8.8) were recorded at the top shelf, 2nd shelf, 3rd shelf, bottom drawer and door shelf within the refrigerator respectively. The temperatures at the bottom drawer and door shelf were much warmer than other parts of the fridge.

CONCLUSION

Most of the vaccine cold storage facilities in all 3 study areas failed to maintain the recommended temperature range (2-8 ˚C) where vaccines in 8 facilities were exposed to freeze temperature, ranging between 24-100 hrs (3%-14%) and 9 facilities (including two from the POAHP) spent more than 100 hrs (15%-87%) of the temperature recordings. In three and four facilities, vaccines were exposed to above the recommended max temperature for 254-327 hrs (35%-45%) and 468-7200 hrs (65%-100%) of recordings. The practical implications of this on vaccine efficacy cannot be determined with certainty and are the subject of a separate study. However, they are unlikely to be positive and some simple measures could be attempted in order to improve vaccine storage conditions in both governmental and commercial facilities. SEACFMD reliance on vaccine to control FMD (FAO control of Avian Influenza) needs to consider these results and their importance on success of vaccination programs. The results from this study suggest that training program for government vaccine responsible staff (DAHP and POAHP) must be developed and recommend that each POAHP holds training for vaccine retailers. Education of vaccine distributors is a logical step, as lack of understanding about the effects of heating, freezing and temperature fluctuations on vaccine potency is doubtless part of the problem. The Cambodian government is aware that farmer uptake of vaccination will be discouraged if vaccines damaged by cold chain failures fail to provide expected protection. Licensing of vaccine retailers is an option being considered by the Cambodian government so that government officers can audit commercial vaccine storage conditions. In the interim, vaccine distributors should be offered training to improve cold storage standards. At a bare minimum, these operators should be encouraged to use thermometers to check and record the operating temperatures twice daily in their refrigerators. The effects of repeated door opening should be presented to them, together with recommendations to store vaccines separately from food and/or drink, or at least to store them in the colder shelves. Likewise, the effect of power outages on refrigerator temperatures should be demonstrated, together with the need for them to have an alternative source of power (generator) or ice in order to keep the vaccine refrigerated in such an event. While it is difficult to supervise conditions in commercial vaccine outlets, it is within the government's power to ensure that better temperature monitoring and management is applied at the government storage facilities responsible for managing the large quantities of donated and government funded vaccines which underpin disease control programs against FMD, HPAI and other animal diseases of national significance. All government vaccine cool rooms or refrigerators should be equipped with max-min thermometers in order that temperatures can be monitored and controlled. Protocols should be developed whereby remedial action is initiated by a temperature trigger point during power outages. It is also important that critical experimentation to better define the detrimental consequences of improper vaccine storage on vaccine efficacy be supported. This will assist with the development of critical thresholds triggering discarding of vaccine if they are breached.

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