Vaccine 34 (2016) 5504–5511

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Thermotolerance of an inactivated rabies vaccine for dogs

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ARTICLE INFO

Article history:
Received 1 August 2016
Received in revised form 28 September 2016
Accepted 3 October 2016
Available online 8 October 2016

Keywords:
Canine-mediated human rabies
Vaccine
Thermo-tolerance
Non-inferiority trial
Cold-chain
Vaccine storage

ABSTRACT

This study provides the first robust data that the antibody response of dogs vaccinated with Nobivac® Rabies vaccine stored for several months at high temperatures (up to 30 °C) is not inferior to that of dogs vaccinated with vaccine stored under recommended cold-chain conditions (2–8 °C). A controlled and randomized non-inferiority study was carried out comparing the four-week post vaccination serological responses of Tanzanian village dogs inoculated with vaccine which had been stored at elevated temperatures for different periods of time with those of dogs vaccinated with the same product stored according to label recommendations. Specifically, the neutralizing antibody response following the use of vaccine which had been stored for up to six months at 25 °C or for three months at 30 °C was not inferior to that following the use of cold-chain stored vaccine. These findings provide reassurance that the vaccine is likely to remain efficacious even if exposed to elevated temperatures for limited periods of time and, under these circumstances, it can safely be used and not necessarily destroyed or discarded. The availability of thermotolerant vaccines has been an important factor in the success of several disease control and elimination programs and could greatly increase the capacity of rabies vaccination campaigns to access hard to reach communities in Africa and Asia. We have not confirmed a 3-year duration of immunity for the high temperature stored vaccine, however because annual re-vaccination is usually practiced for dogs presented for vaccination during campaigns in Africa and Asia this should not be a cause for concern. These findings will provide confidence that, for rabies control and elimination programs using this vaccine in low-income settings, more flexible delivery models could be explored, including those that involve limited periods of transportation and storage at temperatures higher than that currently recommended.

1. Introduction

Canine-mediated human rabies is a neglected tropical zoonotic disease [1] responsible for an estimated 59,000 deaths and 8.6 billion USD in economic losses annually [2]. The area with the highest incidence (3.6/100,000) is thought to be rural Africa [2,3]. This toll is made more tragic by the fact that highly effective vaccines, when used to protect domestic dogs in endemic countries, are able to eliminate the disease at source and, when used as post-exposure prophylaxis (PEP), can protect humans following bites from rabid animals [4].

Where annual canine vaccination is implemented, modeling and empirical data suggests that a coverage of 70% of the domestic dog population during annual vaccination campaigns will be sufficient to control and eliminate canine rabies [5,6]. While reaching this target has been shown to be feasible in most demographic settings in Africa and Asia [7], challenges still remain. Even small gaps in vaccination coverage can significantly delay time to elimination [8]. Therefore, where vaccination strategies rely on delivery through annual campaigns, a low turnout in only a few communities (that can arise, for example, through ineffective advertising or poor timing [9]) can jeopardise the success of the wider programme.

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http://dx.doi.org/10.1016/j.vaccine.2016.10.015
0264-410X/

Despite their effectiveness, team-led mass dog rabies vaccination campaigns are expensive to implement, with high fixed costs, often associated with personnel and vehicle use, resulting in costs ranging from $1.18 to $6.36 for each dog vaccinated [9,10]. Furthermore, due to the requirement of storing vaccines according to label recommended 'cold-chain' conditions (2–8 °C), the reach of the campaigns is limited to areas within easy reach of facilities that can store veterinary vaccines under stable and reliable refrigeration. Consequently developments that increase the flexibility with which vaccines can be delivered to and stored in communities may result in improved coverage and are likely to reduce the rate at which the inter-campaign coverage declines.

The cold-chain was originally devised as a set of guidelines that could be implemented worldwide to ensure that vaccines distributed through the World Health Organisation's Expanded Programme on Immunization [11] were handled and stored in consistent conditions [12]. The approach was relatively simple: keep all vaccines within the temperature range 2–8 °C. However, what started as a means of protecting the potency of vaccines during distribution slowly led to the emergence of a dogmatic view of how vaccines should be stored. As a result the planning of campaigns rarely considers the thermotolerance of specific vaccines, which limits strategic options and the flexibility with which vaccines are handled in the field [12]. Moreover, immunization programs typically require significant investment in infrastructure and management, including widely distributed refrigeration units for storage and cooler units for transportation. In many parts of the world, where electricity provision is poor, refrigeration units powered by kerosene, gas or solar power may be expensive, unreliable, or not available, constraining the use of vaccines in remote communities and limiting how vaccination strategies can be designed [13].

The development of thermotolerant vaccines that can be stored at ambient temperatures for extended periods of time could alleviate some of these constraints. In addition, with studies estimating up to 18% of total vaccination costs can be eliminated by the use of thermostable vaccines [14], their deployment may result in considerable economic savings. Successful examples of thermotolerant formulations being used to deliver vaccines in hard-to-reach communities in Africa and Asia include meningitis A [13] and hepatitis B vaccines for children [15–19], and Newcastle disease vaccines for chickens [20]. Moreover, the development of thermotolerant formulations, that enabled novel storage solutions to be developed and that, critically, empowered local communities to deliver their own campaigns, played a pivotal role in the eradication of small pox and rinderpest [21,22]. A thermotolerant rabies vaccine could have the same transformative effect for the planned elimination of canine-mediated human rabies.

The aim of this study, therefore, was to investigate the thermotolerance of the Nobivac® Rabies vaccine, commonly used in mass dog rabies vaccination campaigns around the world. Our principle objective was to determine whether the immunological response elicited by doses stored under non-cold-chain conditions was not inferior to the response elicited by doses of the same vaccine stored under normal cold-chain conditions.

2. Methods

To achieve this objective a controlled and randomized non-inferiority trial was carried out. The study compared the serological response at four weeks post vaccination in Tanzanian village dogs inoculated with a vaccine (Nobivac® Rabies, MSD Animal Health, Boxmeer, The Netherlands) which had been stored at elevated temperatures for different periods of time, with the response in similar dogs vaccinated with the same product stored according to label recommendations (at 2–8 °C).

2.1. Sample size calculations

The trial comprised seven groups of dogs each receiving Nobivac® Rabies vaccine stored under group specific conditions (described below). From a previous study, which involved western European pet dogs [23], relatively comparable to the Tanzanian pet dogs used in this study, forty 21-day post-vaccination titres were compiled. The geometric mean of the titres, which appeared log-normally distributed, was 3.7 IU/ml (1.89 $\log_2$; SD of 2.02 $\log_2$). Based on this, sample size calculations estimated that 40 dogs per group would give a power of more than 80% to establish non-inferiority to a margin of -1.2 $\log_2$. This test (based on the lower limit of the one-sided 95% confidence interval) is equivalent to requiring for each comparison that the difference between log titres of the elevated storage group and the cold-chain reference will be >-1.2 $\log_2$. Titre values occurring at this limit will be approximately 1.89 (the 'normal' level of historical data) minus 1.2 $\log_2$. This value equals 0.69 $\log_2$ (or 1.6 IU/ml), still well above the defined minimum protective level of 0.5 IU/ml [24]. With an assumed loss-to-follow-up of approximately 20%, the number of dogs recruited into the first phase of the study (day-zero) was increased to 50 per group (total dogs = 350). In summary the trial was powered to detect a difference of more than 1.2 $\log_2$ titre units between dogs receiving Nobivac® Rabies vaccine stored under cold-chain conditions and those receiving vaccine stored outside of the cold-chain.

2.2. Preparation of stored vaccines

The vaccine storage conditions for each of the seven groups were as follows:

1. Vaccine stored at 2–8 °C (normal cold-chain conditions)
2. Vaccine stored at 25 °C for three months (90 days)
3. Vaccine stored at 25 °C for six months (180 days)
4. Vaccine stored at 30 °C for three months
5. Vaccine stored at 30 °C for six months
6. Vaccine stored at 37 °C for three months
7. Vaccine stored at 37 °C for six months

Warehouse storage at 2–8 °C was used before and after all incubation steps and as the storage for group 1 vaccines. For the 90 and 180 day incubation at elevated temperatures a Hielkema room was used for storage at 25 °C (±3 °C) and 30 °C (±3 °C), whilst a Binder CB150 incubator was used for storage at 37 °C (±2 °C). Sequential numbers from 1 to 392 were then each randomly assigned to one of the seven groups and, following storage, all of the 1 ml vials of vaccine within each group were labeled with one of the numbers that had been assigned to that particular group. In this manner the sequential numbers were randomly distributed among the groups.

2.3. Study location and enrollment of dogs – Day-zero

To ensure that most of the dogs recruited into the study were seronegative for rabies the trial focused on villages that had not previously been targeted by mass dog rabies vaccination campaigns and are not served by private veterinary services. These target villages, located within the district of Babati (north-western Tanzania, GPS −4.32 (lat), 35.60 (long)), were Duru, Endagwe, Gesbit, Hoshan and Yarotonik. To locate and recruit dogs for the study, two field teams travelled on foot from household to household enquiring at each house whether dogs were owned and, if so, requesting participation in the study. Owners were asked whether

any dogs had previously received rabies vaccination with only unvaccinated dogs being selected. Following the signing of an informed consent form by the head of the household, all available dogs were given a full clinical examination. In order to control for age and body condition only adult dogs (estimated to be between six months and five years of age) with a body condition score between two and four inclusive (1 = thin; 5 = fat) were selected [25]. Following examination, each dog was permanently marked with a microchip (HomeAgain®, Merck Animal Health), assigned a sequential number and inoculated subcutaneously in the scruff of the neck with 1 ml of Nobivac® Rabies vaccine from the vial with the same number. Dogs presented for vaccination by the owners, but that did not fulfill inclusion criteria, were immunized with standard cold-chain stored vaccine but were not enrolled in the study. To ensure that participating dogs were seronegative at the time of a trial, a blood sample was collected prior to vaccination and stored for post hoc serological analysis. Only dogs with a pre-vaccination titre of <0.5 IU/ml were included in the analyses.

All dog owners were given a contact telephone number to call if, following immunization, any adverse effects were seen that were possibly attributable to the inoculation. Owners were also informed that dogs could not be considered to have been properly vaccinated and protected against rabies until after the 4-week follow up when all study dogs would be vaccinated with a dose of cold-chain stored Nobivac® Rabies vaccine.

2.4. Follow up visit: Day-28

Twenty-eight days later the field teams returned to the same households and, following identification using a microchip scanner, each participating dog was given a clinical examination and a blood sample was collected. During this visit, a 1 ml dose of cold-chain stored Nobivac® Rabies vaccine was inoculated.

2.5. Sample processing

All blood samples collected on day zero and day-28 were centrifuged and serum extracted the following day. Sera were stored at minus 20 °C prior to being shipped to the rabies reference laboratory ANSES (France) where serological analyses took place.

2.6. Serological analyses

As one of the methods recommended by the OIE Terrestrial Manual, the Fluorescent Antibody Virus Neutralisation (FAVN) test [26] was used for detection of antibodies against rabies virus. In brief, each serum sample as well as the positive and negative controls were distributed in four consecutive wells and serially diluted. The challenge rabies virus (CVS-11) containing approximately 100 TCID50/50 μl (TCID50 = 50% tissue culture infective dose) was added to each well. After 60 min incubation, a volume of 50 μl of 4×10^5 BHK21cells/ml suspension was added to each well and the micro-titre plates were incubated, in a humidified incubator with 5% CO_2, for 48 h at 36 °C ±2 °C. After incubation, the plates were stained by adding 50 μl of an appropriate dilution of a fluorescein isothiocyanate (FITC) anti-rabies monoclonal globulin to each well. A qualitative reading was performed according to specific positive fluorescent signal. The Spearman Kärber formula was used to calculate the $LogD_{50}$ titres of sera. The titres were expressed in International Unit per milliliter (IU/ml) by comparison of results obtained with those of the positive standard. The sera were tested over eight dilutions which allowed the quantification of titres ranging from 0.04 IU/ml (logD50 = 0.24) to 218.26 IU/ml (logD50 = 3.95).

2.7. Assessment of a low-tech cooling system

To estimate the temperatures that non-cold-chain stored vaccines are likely to be exposed to during campaigns, and to assess the effectiveness of a low-tech cooling system, five digital temperature loggers (Sensormetrix, www.sensormetrix.co.uk) (loggers 1–5) were placed in a variety of locations in northern-western Tanzania and were programmed to record the temperature at midday for 31 consecutive days during the hottest period of the year (Feb – March). To replicate a low-tech method that might be used to keep vaccines cool when power and refrigerators are not available, loggers 1 and 2 were placed inside vehicles inside insulated vaccine storage boxes with bottles of water placed around them. The water inside the bottles was replaced every day using local tap water. Logger 3 was placed in a similar vaccine storage box inside a vehicle without water bottles. All of the vehicles were being used on rabies vaccination campaign duties in villages on the perimeter of the Serengeti - Maasai Mara ecosystem. Loggers 4 and 5 were placed inside cupboards in buildings in the Serengeti - Maasai Mara ecosystem. The data were downloaded using the software provided and the midday temperature recorded by each logger for each day of the study period was plotted.

2.8. Statistical analysis

In accordance with the WHO recommendations [24] 0.5 IU per ml of rabies antibodies is the minimum measurable antibody titre considered to represent a level of immunity in humans that correlates with the ability to protect against rabies infection. The same measure is used in dogs and cats to confirm a satisfactory response to vaccination. As neutralizing antibodies are considered a key component of the adaptive immune response against rabies virus [27] antibody titres measured at around 28 days after inoculation are often used as an indicator of the potency of rabies vaccination [28,29]. Titre measurements were statistically analyzed using ANOVA. Post-hoc estimates of the differences between the elevated storage conditions and the cold-chain stored vaccine were calculated with one-sided 95% confidence intervals. The null hypothesis, of a storage condition being inferior to the cold-chain storage, was being tested for each of the elevated conditions in a hierarchical testing procedure: any given storage condition was only tested if storage conditions at lower temperatures (but same duration), and for shorter duration (but at the same temperature), had been found statistically non-inferior to the cold-chain storage condition (i.e. the null hypothesis of being inferior had been rejected). This hierarchical testing procedure is comparable to certain dose finding studies with ordered hypotheses with increasing doses [30] and does not need alpha-level adjustment.

An additional exploratory analysis, examining whether storage conditions impact seroconversion ($\geqslant$0.5 IU/ml), was performed using a logistic regression model with group and seroconversion as the explanatory and dependent variables respectively. Finally, an analysis was performed, according to international regulatory criteria [31], to determine whether vaccination within each group was satisfactory. For a group to be determined satisfactory the group arithmetic mean titre following immunization must be $\geqslant$0.5 IU/ml and less than 10% of the animals immunized must have a titre lower than 0.1 IU/ml [31].

All statistical analyses were performed using R-statistical environment [32].

3. Results

3.1. Safety/clinical issues

Following inoculation there were no adverse events reported by the dog owners during the 28 day period after vaccination.

However, during the day-28 follow up visit, two dogs were reported to have died of unknown causes with non-specific signs. Although the dates of these deaths were not recorded, the owners stated that they had occurred closer to the day-28 visit than the day-zero inoculation. We conclude therefore that these deaths were not related to vaccination and, further, that any adverse events that might have been experienced by dogs in any of the groups were of a mild nature such that the vaccines within each group can be considered safe.

3.2. Lost to follow up and post-inclusion removal

Because more households were visited per day than was expected and despite sample size calculations indicating that 350 dogs were required a total of 392 dogs were actually enrolled into the study and received a day-zero inoculation. At the day-28 visit, 339 of these dogs were available for follow up and were successfully re-sampled, 49 were lost to follow-up, and four were excluded due to operator failure (failed inoculation, sample volume was too small or the sample was misplaced). Five out of the 392 dogs (1.3%) sampled at day-zero were subsequently found to have pre-vaccination rabies titres $\geqslant$0.5 IU/ml. Two of these five dogs were lost to follow up whilst three were included in the 339 dogs re-sampled at day-28. Because these three tested seropositive at day-zero they were subsequently excluded. As a result a total of 336 dogs were included in the analyses with between 46 and 52 dogs per group.

4. Analyses

4.1. Day-28 antibody titre comparison

The primary outcome variable for this study was the day-28 post-vaccination antibody titre. The group geometric means, plus the mean and standard deviation of the log transformed titre data are shown in Table 1. The output of the ANOVA analysis, examining the explanatory effect of group on the log of titre, is presented in Table 2, whilst Fig. 1 presents a simple graphical summary. Although a downward tendency can be seen in the level of antibody production, the results indicate that groups 2–4 were not inferior to group 1 according to the pre-set criteria (i.e. the difference between the groups did not exceed 1.2 $\log_2$ titre units). Therefore for groups 2–4, titres stimulated by Nobivac® Rabies vaccine stored under elevated storage conditions are not more than 1.2 $\log_2$ titre units below titres stimulated by cold-chain stored vaccine. For groups 5–7, titres were not more than 1.2 $\log_2$ titre units below those titres stimulated by cold-chain stored vaccine but the confidence intervals did not sufficiently exclude this possibility. For this reason we were unable to conclude that groups 5–7 were equivalent to the cold-chain stored vaccine group.

Table 1
Group summary data - the number of dogs in each group and the day-28 log mean, standard deviation, and geometric mean values are shown; day-28 $\log_2$ mean and day-28 SD represent the average and standard deviation of the log (base 2) transformed data for each group.

Group	Number of dogs	Day-28 $\log_2$ mean	Day-28 SD	Day-28 Geometric mean (IU/ml)
1	50	0.8	1.9	1.8
2	46	0.9	1.7	1.8
3	52	0.6	1.5	1.6
4	46	0.2	1.4	1.2
5	47	−0.1	1.8	1.0
6	48	−0.1	1.8	0.9
7	47	0.0	2.1	1.0

Table 2
Estimates and one-sided confidence intervals for the difference of titre levels between the elevated-temperature stored vaccine groups and the cold-chain stored vaccine group as derived from ANOVA results.

	Estimate	Standard error	Lower 95% confidence limit (one-sided)
Group 2 v 1	0.05	0.35	−0.54
Group 3 v 1	−0.18	0.35	−0.75
Group 4 v 1	−0.60	0.36	−1.19
Group 5 v 1	−0.88	0.35	−1.46
Group 6 v 1	−0.96	0.35	−1.54
Group 7 v 1	−0.80	0.35	−1.39

4.2. Exploratory analysis: Seroconversion at day-28

An additional exploratory analysis was performed to examine whether storage conditions impact seroconversion. Fig. 2 summarizes the observed percentages of dogs that seroconverted. The results of the logistic regression model (Supplementary File 1) suggest that, when compared to group 1, the proportion of dogs that seroconverted was significantly lower for groups 5–7 ($p < 0.05$), whilst for groups 2–4 we were unable to demonstrate any significant effect of storage conditions on seroconversion ($p > 0.40$). As this analysis had not been specified in the design phase of the study we consider it exploratory and conclusions are not based upon the results.

4.3. Vaccine assessment

According to international regulatory criteria, a rabies vaccine can be considered satisfactory if, following immunization, the group arithmetic mean titre is $\geqslant$0.5 IU/ml and less than 10% of the animals immunized have a titre lower than 0.1 IU/ml [31]. Applying this definition (using summary data shown in Supplementary File 2), all seven groups had arithmetic mean titres >0.5 IU/ml and only group 7 had more than 10% of dogs with a titre <0.1 IU/ml. Under this definition the vaccines from groups 1–6 can be considered satisfactory at day-28 post-vaccination.

4.4. Assessment of a low-tech cooling system

The temperature logger data presented in Fig. 4 shows the range, and the number of days (count), of each midday temperature recorded by the five loggers. For loggers 1–5 the maximum midday temperature (and the number of midday temperatures that exceeded 30.0 °C) were, respectively, 29.6 °C (0), 28.4 °C (0), 36.7 °C (5), 35.7 °C (7) and 35.3 (15).

5. Discussion

This non-inferiority study has provided the first robust data that the neutralizing antibody response in dogs vaccinated with Nobivac® Rabies vaccine stored for several months at temperatures far in excess of recommended cold-chain conditions is not inferior to that of dogs vaccinated with the same vaccine stored under cold-chain conditions. Specifically the effectiveness of the vaccine at stimulating rabies antibody was not inferior to cold-chain stored vaccine when, following cold-chain storage, it was stored for up to six months at 25 °C (groups 2 and 3) or for three months at 30 °C (group 4). These findings were supported by the exploratory analysis, which suggested that the proportion of dogs that seroconverted was not significantly affected when the vaccines were stored at these same elevated temperature conditions. As the neutralizing antibody response against rabies is considered a surrogate

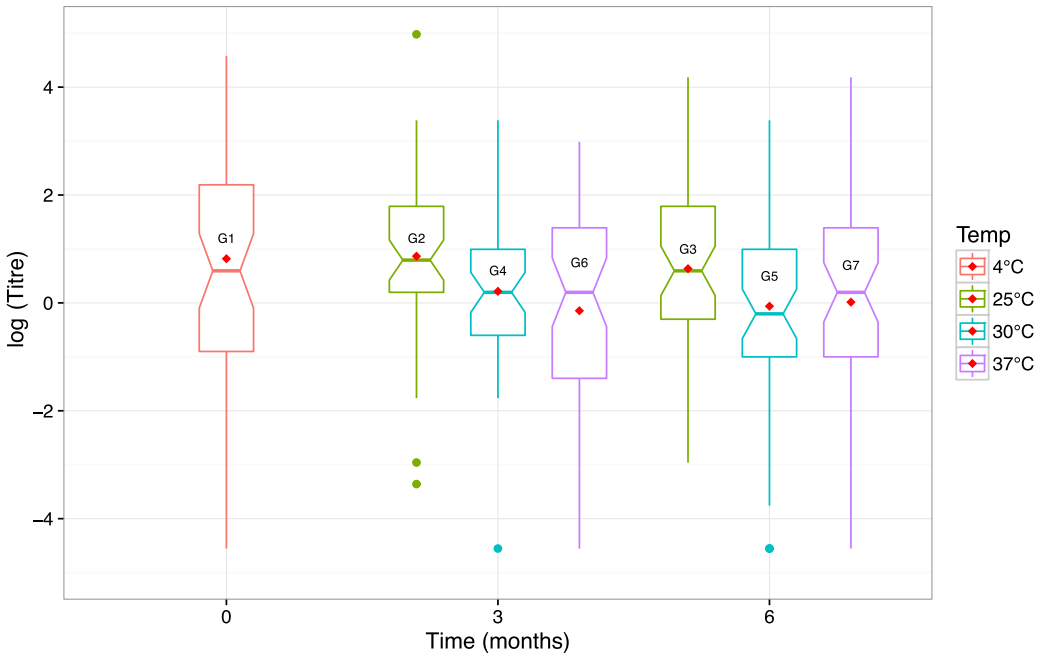

Fig. 1. A boxplot showing the range of (^{2}log) day-28 titres produced by vaccine stored at elevated temperatures for zero (cold-chain), three or six months (red diamond = ^{2}log titre mean; G1 – 7 = groups 1–7). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

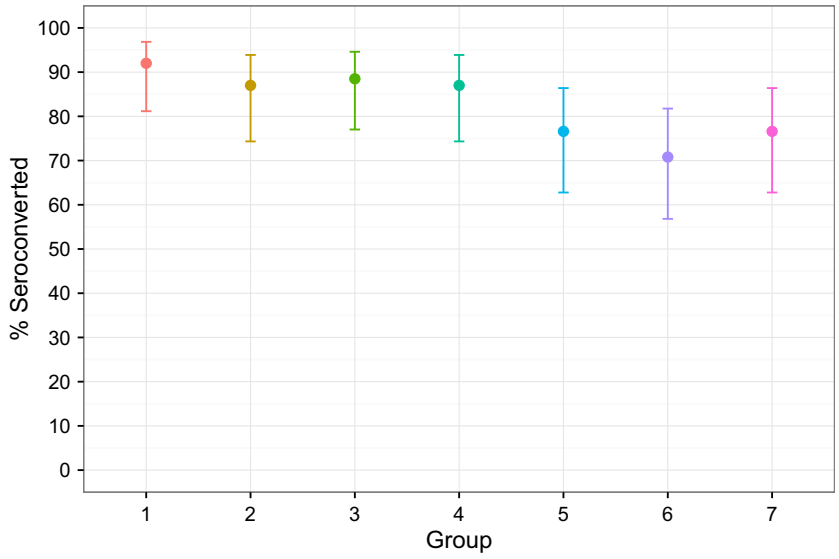

Fig. 2. The percentage of dogs within each group that seroconverted ($\geqslant$ 0.5 IU/ml) is shown. Whiskers represent the 95% confidence intervals, calculated using the Wilson method [37].

of protection [29], we consider that this vaccine, exposed to this range of temperatures, is likely to remain efficacious.

Although groups 2–4 were not found to be inferior to cold-chain stored vaccine, there was a downward tendency in the titre levels produced by vaccines stored under elevated storage conditions (Fig. 1). This tendency indicates that storage outside of the cold-chain does, as expected, impact antibody response. However the downward tendency was slight, and when applying the criteria for assessing whether a vaccine is satisfactory were used [31], vaccines stored at 30 °C for six months (group 5) and 37 °C for three months (group 6) would both be considered satisfactory. Even group 7 dogs, immunized with vaccine stored at the most extreme conditions (37 °C for six months), had an arithmetic mean titre of 2.3, considerably higher than the accepted minimum required antibody titre of 0.5 IU/ml [24].

Although we did not generate data on duration of immunity, titre following primary immunization has, for other viral infections, been shown to be a robust predictor of induction of central B cell memory and thus the response upon booster immunization [33]. As such it is possible that for dogs in groups 2–4 duration of immunity would not be significantly shorter than those vaccinated with cold-chain vaccine. Moreover, dogs in canine rabies-endemic countries are typically vaccinated during annual campaigns, irrespective of prior vaccination status, resulting in many dogs being re-vaccinated every year. Given that the label use of these vaccines recommends a booster to be given only after every three years, this

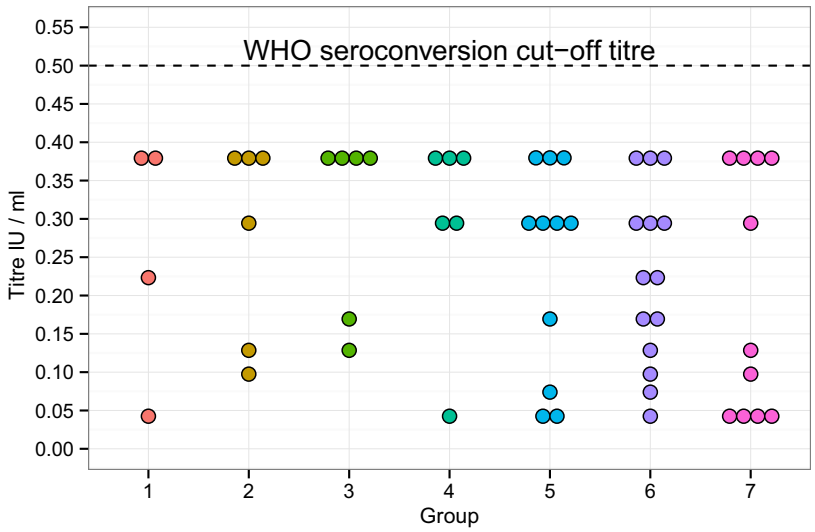

Fig. 3. A dot-plot showing the titres (IU/ml) of all the dogs in each group that did not seroconvert (dashed line indicates the minimum seroconversion titre [24]).

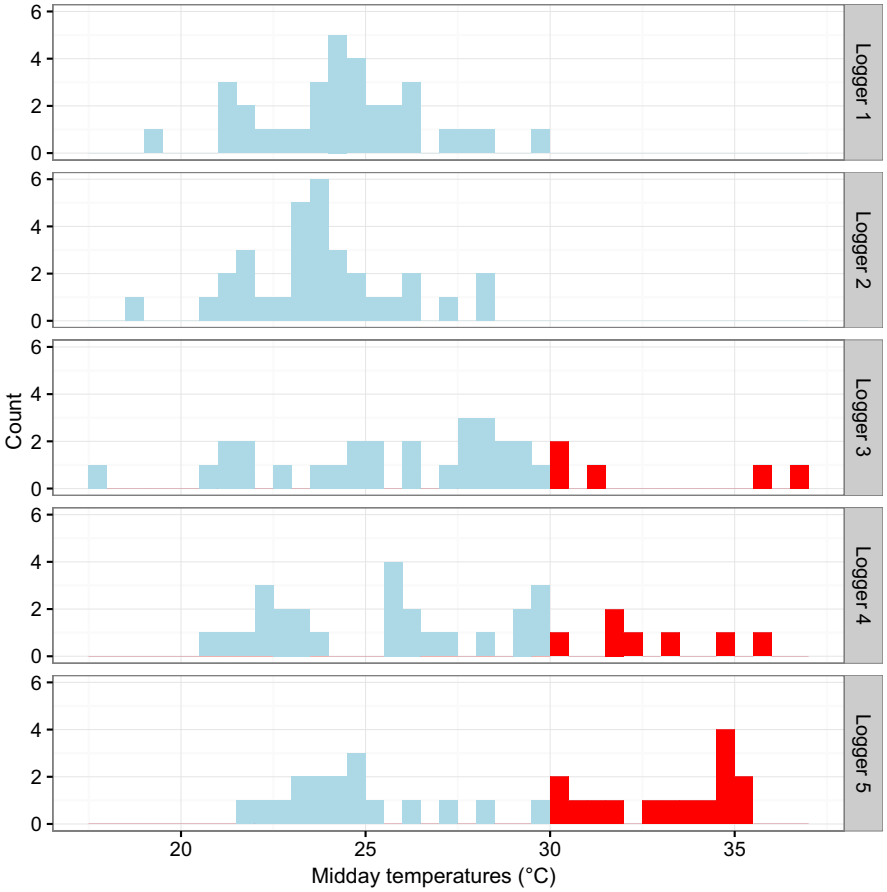

Fig. 4. A series of histograms showing the range of midday temperatures and the number of days (count) that each temperature was recorded by the five loggers (loggers 1–5). Temperatures <30 °C are coloured blue, whilst temperatures ⩾30 °C are red. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

increased frequency of dosing would further boost titres [34]. We therefore consider it unlikely that the duration of immunity of dogs immunized with vaccines stored under the conditions of groups 2–4 will be a cause for concern. Additionally, many of the 58 dogs classified as seronegative had a day-28 titre between 0.1 and the cut off of 0.5 IU/ml (Fig. 3). Some of these dogs may also have sufficient memory to be protected upon challenge and to respond with protective titres upon boosting.

The promising results from this trial raise several additional questions pertaining to the thermotolerance of Nobivac® Rabies vaccine: for example, the vaccine was stored under elevated temperature conditions for up to six months prior to use and it

would be interesting to know for how much longer this vaccine could be stored whilst retaining equivalent anti-virus antibody response. Furthermore, the raised temperature conditions that the trial vaccine was exposed to during the preparation phase were constant and it would be interesting to examine whether fluctuating temperature conditions impacts anti-virus antibody response.

What are the implications of this trial? Thermotolerance was pivotal in the eradication of small pox and rinderpest as it allowed emphasis to be placed on community involvement in both eradication campaigns [21,22]. Furthermore, it has been shown to increase the effectiveness of the delivery of a number of human and animal vaccines [20,35]. Thermotolerance could bring similar benefits to the control of canine-mediated human rabies. Specifically the availability of a thermotolerant vaccine could allow novel and cost-effective delivery models for mass dog rabies vaccination to be developed. For example, the results will give reassurance that this vaccine might be used effectively following a period of non-cold-chain storage in remote communities where access to power and cold-chain storage facilities are scarce. Further, thermotolerant vaccine could be carefully stored in remote communities for extended periods allowing dogs to be vaccinated at various times throughout the year, rather than annually when mass dog vaccination campaigns pass through. In this way puppies, born after a central-point vaccination campaign, and new dogs brought into the area, could be vaccinated in a timely manner, reducing the rate at which the inter-campaign coverage level decreases. This will be especially useful in communities where the 70% target has not been reached. Thermotolerant vaccine stored in remote areas will also provide a life-saving resource, for example in the emergency situation of an outbreak of rabies where rapid vaccination of the dog population is required to control the epidemic.

To explore the feasibility of transporting and/or storing vaccines for extended periods in remote areas where power is not available we compared the midday temperatures recorded in shady parts of buildings and within insulated vaccine storage boxes placed inside vehicles with temperatures recorded in similar boxes in which plastic water bottles, refilled daily with tap water, were placed ('low-tech' cooling system). The results suggested that the temperature inside the low-tech cooling system could be kept cooler than the ambient temperature of a building or a vehicle. Moreover, despite a maximum temperature during the study period of 36.7 °C, the temperature inside the low-tech cooling systems did not exceed 30 °C. As the tested rabies vaccine was thermotolerant when stored for three months at this temperature, this result was encouraging. This was an exploratory analysis and we only investigated one low-tech system. Further work is required to confirm whether low-tech systems are able to keep the temperature significantly lower than the ambient temperature and to explore other, potentially more effective systems, which could be made available to vaccinators working in remote areas to keep vaccines cool over extended periods of time.

In summary, this trial has shown that Nobivac® Rabies vaccine stored at elevated temperatures for extended periods of time retains its ability to stimulate a neutralizing antibody response. These preliminary findings are not an indication that the label recommendations for the storage of this vaccine should be ignored, however they will give confidence, to programs working with this vaccine to control rabies, that more flexible delivery models can be investigated, potentially involving the storage of this vaccine outside of the cold-chain for limited periods of time. These findings will contribute to the recent groundswell of momentum amongst international human and animal health agencies [4,36] that global elimination of canine-mediated human rabies is a logistically feasible, cost-effective and socially equitable goal.

Conflicts of interest

Lankester, Czupryna, Palmer, Mzimbiri and Cleaveland have no conflicts of interest.

MSD Animal Health employs Wouters, Francis, Sutton and Sonnemans.

Research and ethical clearance

The research was carried out with the approval of the Institutional Animal Care and Use Committee, Washington State University (Approval No. 04577 – 001), the Tanzanian Wildlife Research Institute (TAWIRI), the Commission for Science and Technology (COSTECH, Tanzania) and the Tanzania Food and Drug Administration (Permit Nos. 2011-213-ER-2005-141 and 2012-318-ER-2005-141).

Funding

A Washington State University intra-mural grant, kindly donated by the Autzen Endowment Fund, provided funding for the fieldwork and sample shipment. MSD Animal Health funded the vaccine preparation and laboratory analysis.

Acknowledgements

We are grateful to the Serengeti Health Initiative for their contribution to the fieldwork carried out in this trial. In addition we are grateful to the Research Committee of the College of Veterinary Medicine (Washington State University) for selecting this study for intramural funding and to the Autzen Endowment Fund which supports applied research for the benefit of the health and well-being of small animals and which provided the intra-mural funding. Finally, we are also grateful to MSD Animal Health for supplying the rabies vaccine and funding the laboratory analyses as well as for providing support and advice on the study design.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.vaccine.2016.10.015.

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