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Evaluation of methods for short-term marking of domestic dogs for rabies control

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ABSTRACT

Rabies transmitted by domestic dogs is a serious yet neglected public health threat in many underserved communities in Africa and Asia. Achieving 70% vaccination coverage in dog populations through annual mass vaccination campaigns is an effective means of controlling the disease in these communities. Evaluating the extent to which this target coverage is achieved requires either accurate pre-campaign estimates of the dog population size or accurate estimates of the coverage attained by conducting post-vaccination surveys. Short-term marking of dogs by applying visible marks may be useful to achieve these estimates, but will be affected by the performance of the marking methods. We evaluated the longevity and visibility of two readily-available livestock marking methods applied to owned, free roaming dogs.

We applied two types of marks (spray and crayon) with three different colours (red, blue and green) to each of 21 dogs and compared the time of persistence of the marks over several weeks. Two independent observers assessed the visibility and colour of the marks. Each dog was observed over 8–37 days (median: 28 days). Kaplan–Meier survival analyses and semi-parametric log-rank tests were performed separately for both observers. Spray marks remained visible significantly longer (median of 24 days for both observers) compared with crayon marks (medians of 10 and 13 days). After 10 days, 90% of spray marks were still visible, compared with only 46% of crayon marks. Visibility of marks was reduced in darker-coloured dogs. Colours of marks were frequently misclassified, and agreement between observers on the colours of the marks was low (Cohen's kappa coefficient = 0.27).

The livestock marker spray can effectively be used to mark dogs that are physically restrained, for example during vaccination campaigns. Resight surveys should be conducted within a short a time as possible after marking; however, our results suggest that loss of marks will not have a significant impact if surveys are conducted within 5–7 days after marking with the spray. Results that depend on observers' abilities to distinguish between the three colours which we evaluated may not be reliable.

1. Introduction

Domestic dogs are the primary reservoir hosts for rabies virus (RABV) across most of its distribution in Africa and Asia. Dogs are responsible for the vast majority (>90%) of human exposures and deaths due to this virus (WHO, 1999). Rabies transmitted from dogs kills an estimated 55,000 people each year in Africa and Asia, and millions of dollars are spent on post-exposure prophylaxis (PEP) to prevent the disease developing in patients bitten by suspect rabid dogs (Knobel et al., 2005). Rabies in dogs, reintroduction of rabies from bats or wildlife in dog population, and cases of the disease transmitted from dogs to people, can be controlled and in certain circumstances eliminated by the mass vaccination of dogs against the virus (Cleaveland et al., 2006; De Lucca et al., 2013; Lembo et al., 2010; Velasco-Villa et al., 2008). Using estimates of the basic reproductive rate of rabies from a number of outbreaks in dogs around the world, Hampson et al. (2009) estimate that a critical vaccination coverage of only 20–40% of a dog population is sufficient to prevent outbreaks of rabies. In circumstances when vaccination is applied through annual campaigns of relatively short duration, vaccination coverage declines in the period between campaigns as vaccinated dogs die or migrate out and susceptible dogs are born or migrate in. While studies on these demographic rates in dog populations are limited, data from northern Tanzania show that attaining 60%

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vaccination coverage during annual campaigns will be sufficient to prevent vaccination coverage falling below this critical threshold in the period between campaigns (Hampson et al., 2009). This is in line with the empirically-derived recommendation of 70% vaccination coverage in dogs to control rabies (WHO, 2013).

Evaluating the extent to which this target coverage is achieved requires either accurate pre-campaign estimates of the dog population size along with the number of dogs vaccinated during the campaign, or accurate estimates of the coverage attained by conducting post-vaccination surveys. Various methods have been used to estimate dog population sizes and vaccination coverage. Household surveys are commonly used (e.g. Acosta-Jamett et al., 2010; Kongkaew et al., 2004), but are prone to biases (Downes et al., 2013) and may be logistically difficult and costly. Other studies to estimate population sizes have applied mark-resight methods (Totton et al., 2010). While these methods may make use of natural marks or other physical characteristics of dogs (e.g. Amaral et al., 2013; Belsare and Gompper, 2013), in other cases dogs are artificially marked, often during vaccination campaigns (e.g. Gsell et al., 2012; Kayali et al., 2003). Collars may be applied to mark dogs (Gsell et al., 2012), but these can be removed by owners or dogs themselves. Paint marks may provide short-term, cost-effective alternatives sufficient for most purposes (Totton et al., 2010). The accuracy of population estimates derived through mark-recapture methods relies on a number of assumptions being met (Totton et al., 2010) including the assumption that marks are neither lost before nor overlooked during the resight survey. The degree to which this assumption is met depends on the interval between marking and survey, and will be influenced by the type of mark applied (Childs et al., 1998). The longevity and visibility of marks applied during vaccination also influences the accuracy of estimates of vaccination coverage derived from post-campaign surveys (Townsend et al., 2013). In this study, we evaluate the longevity and visibility of two readily-available livestock marking methods applied to owned, free-roaming domestic dogs.

2. Materials and methods

The study was conducted among owned, free-roaming dogs in Hluvukani, Mpumalanga Province, South Africa (S 24°39′; E 31°20′), from 7th October to the 15th November 2013 during the rainy season. Hluvukani has been the site of a health and demographic surveillance system in dogs (HDSS-Dogs) conducted since 2011 by the University of Pretoria. For this project, all of the nearly 2000 households in the designated surveillance area are visited quarterly to update data on the demographics of the owned dog population (births, deaths, migrations and rabies vaccination status). Photographs and microchip implants (Backhome Biotech, Virbac®) are used to individually identify dogs.

For the present study, 21 healthy adult (≥1 year old) dogs were selected from the HDSS-Dogs population. The selection of dogs was a convenience sample, representing dogs from households that were visited over a two-week period for quarterly updates. Owners of selected dogs were approached and informed consent was verbally obtained before dogs were enrolled in the study. A dog was included if it was easy to restrain it and if the size of its side allowed application of three marks. All selected dogs had short coats. Colour of dogs was recorded at enrolment and classified in three categories: light (white and light brown coats), fawn and dark (dark brown and black coats). At enrolment, six marks – three colours (blue, red and green) of each of two marking types (RAIDEX animal-marking spray and crayon, RAIDEX GmbH, Dettingen an der Erms, Germany) – were applied on the sides of each dog (three marks per side). The safety aspects of the RAIDEX spray (composition: ethanol) and crayon (waxes and paraffin oil) in dogs were considered the same as in livestock, and no harmful consequences have been notice after application (Identification Methods for Dogs and Cats, available from http://www.icam-coalition.org/resources). All dogs were restrained by their owners and marks were applied by the same member of the study team. Stripes of about 5 cm width were applied on the sides, from shoulder to hip. Each of the six marks (type and colour combination) was randomly assigned to one of the six positions on the sides of each dog. Following this process, all 126 marks were applied to the assigned positions on the 21 dogs.

Dogs were visited twice a week after enrolment until all marks could no longer be observed. The team member who had applied the mark restrained the dog, while two observers (A and B) independently assessed the marks from a distance of 30 m (measured on each occasion using a laser ranger finder). At the time of observation, observers were unaware of the colour or type of mark applied to each position on the dog. For each position (1–6), the observers independently recorded whether a mark was visible, and if so, its colour. Neither observer was known to have any major deficiency of their vision. Results were captured on paper forms in the field and entered into a Microsoft Excel spreadsheet. Survival analysis was performed with R (R Core Team, 2014) using the package *survival* (Therneau, 2014). A failure event was defined as the first non-observation of the mark. Kaplan–Meier survival functions were plotted and the effect of variables (type of mark, colour of mark, and coat colour) on survival time was compared by univariate analyses using the log-rank test. The correct identification of the colour of visible marks was evaluated using Chi-square tests. Correctness of observations (correct/incorrect) was compared (1) between colours for each observer and for spray and crayon separately, and (2) between observers for each combination of type and colour. Survival times of all marks were compared between both observers. Afterwards, analyses were performed independently for each of the two observers to avoid non-independence of the observations. To evaluate the influence of individual observer on the correct identification of the colour of visible marks, Cohen's kappa coefficient κ was calculated.

3. Results

Each dog was assessed during one to nine visits (median: six visits) over 8–37 days (median: 28 days). There was no significant difference in survival time of marks between observers A and B (log-rank test, $p = 1$). For each observer, the results of the univariate analyses of the survival data are shown in Table 1. Significant differences in survival time were found for the type of mark (spray vs. crayon) and the coat colour of the dog (for observer A). The colour of the mark did not significantly affect survival time. There was no difference in the survival times between colour of dogs if the analysis was separated by colour of marks ($p > 0.1$ for all comparisons).

The Kaplan–Meier survival functions for the two types of marks for observer A are shown in Fig. 1. After 10 days, 90.4% of spray marks were still visible, compared with only 46% of crayon marks. Fewer than 90% (82.5%) of crayon marks were visible up to 5 days. In the case of dark-coloured dogs, the probability of survival of spray marks up to eight days was still high (83.3%), but was only 50% for crayon marks.

For all observations when a mark was visible, the observer recorded the perceived colour of the mark. This was compared with the actual colour of the mark. Table 2 shows the results of this comparison. Within observers, red spray marks were most often correctly classified, while green crayon marks were most often incorrectly classified. There was a significant difference between observers in the classification of green spray and of blue crayon, with observer B misclassifying them more frequently than