

## Enhancing rabies control in Bhutan

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### Introduction

Rabies is a fatal and neglected zoonotic disease that causes an estimated 55,000 human deaths every year globally. In Bhutan, rabies is mainly reported in southern parts of the country along the border with India (1).

Domestic dogs are the main source of and vector for rabies transmission to livestock and people. In Bhutan, the large numbers of free-roaming dogs freely cohabit with people. This poses a high risk of dog bite, and the number of requests for anti-rabies vaccination by people who have been bitten is increasing (2,3).

To manage the dog population and implement rabies control, a Capture-Neuter-Vaccinate-Release (CNVR) program has been conducted in collaboration with Humane Society International. However, the effectiveness of this program has not been assessed.

This study aimed to conduct a population survey to provide information about: the population size of free-roaming dogs; the proportion of owned dogs that are free-roaming; and vaccination coverage in the country. These data will help to inform policy on enhancing rabies control programs in Bhutan.



Figure 1. Marking a stray dog with coloured paint.

### Objectives

- Estimate the size of the free-roaming dog population and the CNVR coverage, by means of mark-resight methods in Gelephu and Phuentsholing, South Bhutan.
- Understand the demography of owned dog population in Gelephu and Phuentsholing.
- Map the activity range of free-roaming dogs on the India-Bhutan border using radio-telemetry techniques.
- Understand the epidemiology of dog-bite incidents and the uptake of anti-rabies vaccine by people in Bhutan.



Figure 2. A free-roaming dog that has been marked with colour paint spray for dog population survey.

### Methods

The size of the free roaming dog population was estimated using a Mark-Resight framework (4). A sample of  $n_1$  individuals was sighted and marked with colour paint spray on the first day during a transect walk (Fig. 1 & 2). On the following day, a second sample of  $n_2$  individuals was sighted and examined for colour marks along the same transect. The size of the population was estimated from the number of marked individuals that were re-sighted on the second day. If  $N$  is the total population size, and  $m$  marked individuals are re-sighted, then the Lincoln-Peterson Index used to estimate the population size is:

$$m/n_2 = n_1/N$$

$$N = n_1 n_2 / m$$

For comparison, the dog population abundance was also estimated using a mixed logit-normal model using Program (MARK).

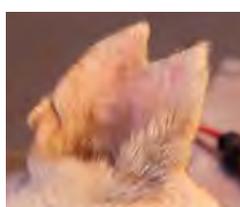


Figure 3 Surgical sterilisation of a free-roaming dog. During surgery a V-shaped ear notch is made to permanently identify that this is a sterilised and vaccinated dog.



Figure 4: Setting up the GPS dog collars.

The proportion of the dog population that was vaccinated and neutered (CNVR coverage) was estimated by counting the dogs with permanent ear-notch identification marks and dividing this by the total number of dogs observed by the counting team in a given area (Fig.3).

The demography of the owned dog population was estimated using a household questionnaire survey to address two objectives: (A) Describe the demographics of owned dogs and of dog-owning households within the study areas; (B) Determine the proportion of owned dogs that were free-roaming.

The activity patterns and home ranges of stray dogs in Gelephu town were mapped using data recovered from GPS radio collars (Fig 4 & 5). The collars (*Retrieva TM*) were fitted onto the dogs' necks, and their movement patterns were tracked through time and space for an average of 10 days via a computer programmed server and smart phone. The collars recorded the location of each dog (using XY coordinates) at 20 minute intervals, and the data were uploaded to the server every 8 hours. At the end of the study, the collars were recovered by relocating the dogs using a smart phone and the computer server. The home ranges of dogs were analysed using Home Range features in the software package Quantum GIS (Fig. 6).

The patterns of and risk factors for dog bites in humans, and trends in the uptake of anti-rabies vaccine was analysed using retrospective, hospital-based data that were retrieved from 34 health centres in the country for 2009–2012. Descriptive and regression analyses were performed to understand the patterns of human post-exposure prophylaxis (PEP) in Bhutan.

## Results

### Dog population survey

The stray dog population in the urban areas of Gelephu was estimated to be 610 (95% CI=565–657) based on evening counts and 670 (95% CI=614–727) based on morning counts, but these were not significantly different ( $D^2=0.69$ ,  $p=0.41$ ). Using a mixed, logit-normal, mark-resight model,  $640 \pm 20$  stray dogs (95% CI=605–680) were estimated to be present, which was not significantly different from the population estimates shown above that were derived using the Lincoln-Peterson method ( $D^2=0.92$ ,  $p=0.63$ ). The resighting probability was estimated to be  $0.49 \pm 0.03$  (95% CI=0.44–0.55) and  $0.46 \pm 0.03$  (95% CI=0.41–0.52) during evening and morning sampling occasions.

In Phuentsholing, the stray dog population was estimated to be 524 (95% CI=477–571) and 560 (95% CI= 505–616) using two evening counts; and 548 (95% CI=506–589) and 582 (95% CI=534–629) using two morning counts. Again, these were not significantly different ( $D^2= 3.12$ ,  $p=0.37$ ). The mixed, logit-normal, mark-resight model produced an estimate of  $544 \pm 12$  (95% CI=532–580) stray dogs present within the municipal areas, with an average resighting probability of 0.5 during secondary sampling occasions.

### Vaccination coverage

The point estimate for vaccination and sterilization coverage among stray dogs was estimated to be 55.1% (95% CI=49.7–60.4) and 66.7% (95% CI=60.8–72) in Gelephu and Phuentsholing, respectively, and these were not significantly different ( $D^2=1.1$ ,  $p=0.29$ ).

### Household surveys

Face-to-face interviews were conducted with a single member of each of 387 households (HH) [male=181, female=206] in Gelephu, and 393 HH [male=132, female=261] in Phuentsholing.

A total of 172 HHs (Gelephu: 101/387, 26%; Phuentsholing: 71/393, 18%) owned 221 dogs (Gelephu: 135, 61%; Phuentsholing: 86, 39%). There was an average of 0.35 and 0.22 dogs per interviewed HH, and 1.34 and 1.21 dogs per dog-owning HH. The human to owned-dog ratios were 13.4: 1 and 19.5:1 (based on the number of inhabitants interviewed) in Gelephu and Phuentsholing, respectively.



Figure 5. Placing a GPS radio tracking collar on a stray dog.

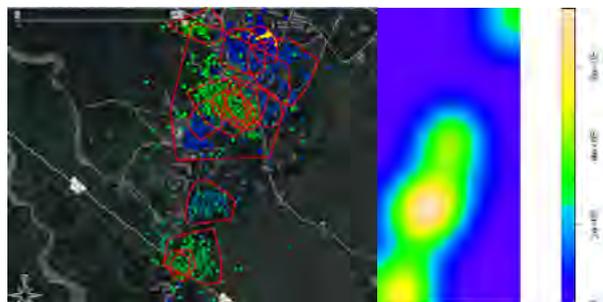


Figure 6. Maps showing the home ranges of stray dogs in Gelephu.

Dogs were primarily kept as guards and pets and were sourced either as gifts from family/friends or had been adopted from the street. The vaccination coverage of owned dogs was estimated to be above 80%. This is above the cut-off of 70%, which has been reported to be necessary to eliminate the transmission and spread of rabies among dogs.

#### Home range analysis of stray dogs in Gelephu

During the one-month study period (22 July–19 August, 2013), a total of 59 stray dogs were captured and collared in Gelephu. This generated 56,000 fixes (point location records). The size of the dogs' home ranges ranged from 0.69 to 470.29 hectares, with a median home range of 3.68 hectares. There was a high clustering pattern of fixes for individual dogs, and few instances of dogs moving across the border of their ranges were recorded (Fig. 6).

#### Epidemiology of PEP usage in Bhutan

Males (59.2%,  $n=10,924$ ) accounted for significantly more PEP events than females (41.8%  $n = 7,849$ ) across all age groups ( $p<0.001$ ), see Figure 7. The median age of patients receiving PEP was 20 years (range <1 to 93 years), and the modal age was 6 years. Children, particularly those in the 5–14 years of age group, received more rabies PEP than other age groups.

Animal bites and non-bites accounted for 80.6% ( $n= 15,163$ ) and 19.4% ( $n = 3,650$ ) of rabies PEP requests, respectively; whilst 2,286 cases (12.2%) of the PEP events had no recorded information regarding the reasons for seeking PEP. Of the total animal-bite cases, 88.67% ( $n=13,445$ ) were related to dog-bites. PEP treatment was provided throughout the year, and the number of PEP cases significantly increased between 2009 and 2012 ( $p<0.001$ ), from <1,500 to >7,000 events, respectively.

Of the 18,813 patients receiving rabies PEP, 8,067 (42.9%) patients received the standard 5-dose course intramuscular injections (Essen regimen) on days 0, 3, 7, 14 and 28, whilst 10,746 (57.1%) patients received an incomplete vaccine course (receiving between 1–4 doses). The majority of these incomplete courses were missing doses on days 14 and 28.

Multivariable analysis indicates that six variables were associated ( $p<0.001$ ) with receiving an incomplete PEP vaccine course – gender, age, type of exposure (animal bite versus non-bite), area of PEP (rabies-endemic versus rabies-free areas), season, and year. Males and adults were more likely to receive and incomplete PEP course than females and children. Patients presented to hospitals in rabies-free or non outbreak areas (interior Bhutan) were more likely to receive an incomplete course than in rabies endemic areas. Patients with animal bite injuries were more likely to receive an incomplete vaccine course than non-bite recipients, indicating a higher perceived risk of rabies infection if the exposure animal is more likely to be rabid. Similarly, compared to the autumn season, patients presenting to hospitals for PEP during spring and summer months were less likely to receive an incomplete vaccine course, whilst patients presenting during winter seasons were more likely to receive an incomplete course. In addition, compared to 2009, patients presented to hospitals for PEP during 2011 and 2012 were more likely to receive an incomplete PEP course.

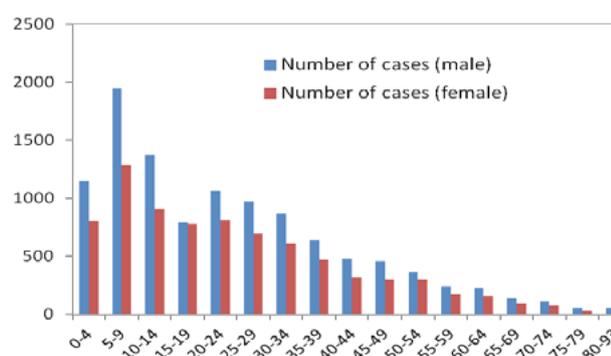


Figure 7. Uptake of rabies post-exposure prophylactic vaccination by people in Bhutan from 2009-2012, stratified by age and gender.

## Discussion

This paper describes the first study conducted in Bhutan to estimate the size of the free-roaming dog population and CNVR coverage using the mark-resight model frameworks, and to map the movement patterns of dogs using GPS radio collars.

However, the estimates derived for population size represent a standing population, i.e. the numbers of dogs present between September–October 2012, which provides baseline data for future interventions. Repeated sampling (ideally every 6 months) would provide data on the population dynamics and the impacts of the ongoing CNVR program.

The study has demonstrated that more stray dogs in-habitat the core town areas compared to the town peripheries. This may be associated with an increased human population density and the availability of food (due to poor waste management, and availability of restaurants and meat stalls) providing an ideal environment for

the strays. In addition, the territorial nature of the dogs may be other reasons for higher density of dogs in the urban areas.

The CNVR coverage estimates also relate specifically to the standing population at the time of the survey. As the CNVR program has been conducted twice (in 2010 and again in 2011), the dog populations will have undergone some turnover due to births, deaths, immigration and emigration. Therefore, the percentage coverage calculated for the CNVR program is likely to be an underestimation of the true proportion achieved. The current estimates would, however, suggest that the current dog population is protected against rabies transmission by vaccination due to the past CNVR activities providing one-time vaccination that confers immunity for three years.

The study of GPS radio collared dogs was the first of its kind to be conducted on stray dogs in Bhutan. The preliminary results (data analysis is ongoing) suggest a high clustering pattern within dogs with few instances of movement across the home range borders. The geographic availability of food within the study area may have restricted the movement of dogs in Gelephu.

The increased uptake of rabies PEP for children, and the decreasing trend with increasing age, as well as the higher uptake by males compared to females, is in agreement with previously published studies (2,3). It has been suggested that animal bites in children are more likely to be reported to hospitals for wound treatment and possible vaccination due to parental concern. The number of PEP events was also found to have increased substantially over the years, resulting in significant costs to the public health sectors. The increase in requests for PEP may be associated with an increased incidence of dog bites, increased numbers of rabies outbreaks, and increased awareness of the importance of presenting animal bites to hospitals for medical advice.

Several factors were also found to be associated with receiving an incomplete PEP course, which indicates that public education about preventing exposure to dog bites and the necessity of completing a PEP course if exposed is required.

### Lessons learned

**Communication and collaboration:** This CIP has strengthened communication and collaboration between animal and public health professionals and also enhanced networks between and within their organisations.

**Capacity building:** The participants learned new techniques in the field of epidemiology to undertake science based research, and also sensitized the concept of One Health approach towards prevention and control of zoonotic diseases.

**Policy and decision making:** The studies outcomes were used for prioritising certain aspects of the rabies control program such as the implementation of mass dog vaccination program.

### Recommendations

- The HSI sterilisation and vaccination clinics should carry out dog population surveys prior to and after implementing a program, in order to evaluate the success of the program.
- The vaccination coverage (CNVR) in both the study areas was below the threshold (below 70%) requirement for elimination of rabies in dogs. We recommend annual mass vaccination of dogs against rabies in the south Bhutan to create immune buffer (*cordon sanitaria*).
- This study provides important information for policy discussions about rabies exposure and PEP for people in Bhutan. A thorough assessment of each individual case based on the WHO guidelines would reduce unnecessary PEP (and therefore costs). The cost-effective intradermal PEP should be introduced in Bhutan, which would reduce the cost by about 70%, compared to conventional intramuscular regimens.
- The home range study using GPS radio collar should be conducted in all seasons to understand the seasonality of movement pattern for disease transmission and spread.

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