Patterns of Rabies Occurrence in Bhutan between 1996 and 2009

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Impacts

- This study evaluated rabies incidence and seasonal trends in domestic animals in Bhutan from 1996 to 2009.
- Rabies cases in animals were reported throughout the year with a higher incidence during spring and summer months. The number of reported cases was stable from 1996 to 2005 but increased in 2006 and 2008.
- Fifty-nine of the 205 sub-districts reported rabies between 1996 and 2009, with a higher incidence in southern Bhutan. There was a positive temporal correlation between the number of cases reported in dogs and other domestic animals.

Summary

This study was conducted to evaluate incidence and seasonal trends of rabies in dogs and other domestic animals in Bhutan from 1996 to 2009. Time series analysis approach was used to determine the seasonal trend and temporal association between species-specific rabies cases in animals. A total of 814 rabies cases were reported during the 14-year period, of which cattle and domestic dogs accounted for 55% (447/814) and 39% (317/814) of the cases, respectively. The remaining 6% of the cases (50/814) were reported in horses (2%), cats (2%), pigs (1%) and goats (1%). Rabies cases were reported throughout the year with more reports during spring and summer months. The annual patterns of cases were stable from 1996 to 2005, but the incidence increased during 2006 and 2008. Fifty-nine of the 205 sub-districts reported rabies in animals from 1996 to 2009 with increased incidences in the four districts in southern Bhutan, an area located close to the border towns of India. A significant (P < 0.05) positive cross-correlation was observed between the number of cases in

dogs and other domestic animals at time lags (months) 1–3 with the highest correlation (r = 0.94, P < 0.05) observed at time lag 0 (same month) indicating that the peak in rabies incidences occur in the same month when both dogs and other domestic animal cases are reported. Regression analysis predicted rabies in other domestic animal when there are reports of rabies in dogs during the previous months. This study provides useful information about the epidemiology of rabies that can be used to plan a rabies control programme in Bhutan.

Keywords: Rabies; domestic animals; surveillance; time series analysis; Bhutan

Introduction

Rabies is an acute viral disease that affects the central nervous system, causing encephalitis and ultimately death in all warm-blooded animals including humans. Rabies is caused by a negative-stranded RNA virus within the *Lyssavirus* genus of the *Rhabdoviridae* family and is transmitted by the bite of rabid animals via saliva rich in the virus (Kaplin et al., 1986). Rabies occurs in two epidemiologic forms: urban rabies, with domestic dogs as the main reservoir and transmitter; and sylvatic rabies, with wildlife as the reservoir and transmitter of the disease (DeMattos et al., 1996). Urban rabies is endemic in most parts of the developing countries of Asia and Africa, and in Latin American and Caribbean countries, whilst North America and Europe have wildlife rabies (Finnegan et al., 2002; Belotto et al., 2005; Bourhy et al., 2005; Knobel et al., 2005). Urban dog rabies has been largely eliminated in Europe, North America and some Asian countries (Japan, Malaysia, Taiwan, Singapore) by massive dog vaccination, stray dog reduction programmes and enforcement of dog regulations (Finnegan et al., 2002; Belotto et al., 2005; Wilde et al., 2007; Takahashi-Omoe et al., 2008). Worldwide, rabies causes an estimated 55 000 human deaths annually, mostly in Asia and Africa because of endemic dog rabies and lack of healthcare and control measures (Knobel et al., 2005; Wilde et al., 2007).

Bhutan is a small Himalayan kingdom, located in South Asia between China and India, where rabies is highly endemic in both countries (Sudarshan et al., 2007; Si et al., 2008; Wu et al., 2009). Rabies was prevalent in most parts of Bhutan until the early 1990s but has been controlled by restrictive elimination of dogs (Owoyele, 1992; Tenzin et al., 2010). Currently the disease is endemic in the southern districts of Bhutan along the border with India (Rinzin et al., 2006; Tenzin et al., 2010). Frequent outbreaks of rabies are occurring in these endemic areas affecting mostly domestic animals such as cattle and dogs (Kuensel, 2010a). Recently, outbreaks have been reported in some previously free areas in the interior as well as in southern Bhutan, indicating re-emergence of rabies in the country (Tenzin et al., 2010). Sporadic human deaths from rabies are also reported in the south rabies endemic districts of Bhutan. For instance, eight human deaths (mostly children) were reported between 2006 and June 2010, accounting for about 1.2 deaths per 100 000 population (Kuensel, 2009, 2010b; MoH, 2010). The people who are exposed to dog bites and presumed rabid animals are provided post-exposure prophylaxis free-of-charge in the hospitals, but some individuals may have failed to receive prompt treatment after exposure, resulting in death. Mass vaccination and sterilization of dogs are conducted annually in Bhutan but the coverage has been low (<20%) due to inadequate resources and rapid increase of the dog population (n = >50 000),

especially free-roaming dogs (NCAH, 2007). The translocation and trans-border movement of free-roaming dogs could also explain the persistency and high incidences of rabies in southern Bhutan (Kuensel, 2010a). However, in an effort to control the free-roaming dog population and rabies in Bhutan, a nationwide dog vaccination and sterilization programme is under implementation in collaboration with an international organization – Human Society International (MoA, 2009). Rabies is a notifiable disease in Bhutan and surveillance data for animal rabies are stored in the Veterinary Information System (VIS) database. However, no detailed analyses have been conducted on this surveillance data to describe epidemiology of rabies in Bhutan. To combat rabies successfully, a clear understanding of the disease epidemiology is required. In this study we analysed rabies surveillance data (1996 to 2009) to identify spatio-temporal patterns of reported rabies in animals and to evaluate the association between reported rabies cases in dogs and other domestic animals in Bhutan.

Materials and Methods

Data source

The data for this study were extracted from the national rabies surveillance database – Veterinary Information System (VIS) – maintained at the National Centre for Animal Health in Bhutan. The data contained both clinical and laboratory confirmed reported cases of rabies in animals. Brain tissue samples are collected from the field, preserved in 50% glycerine saline and sent to the laboratory for confirmatory diagnosis using only fluorescent antibody test (FAT) (Dean et al., 1996), due to constraints within the existing surveillance system. Note that for logistical reasons, field samples from all cases are usually not collected and submitted to the laboratory, particularly when at least one sample from the same herd or locality has positive laboratory confirmation. The remainders of the cases are then diagnosed by the veterinary officials based on the epidemiological investigation, history of dog bite and clinical signs consistent with rabies and subsequent death of animals (Tenzin et al., 2010). Rabies is not a difficult disease to diagnose (clinically) in endemic countries.

Data analysis

Descriptive and temporal analysis

Descriptive statistical analysis was performed to examine the frequencies and animal pattern of occurrences. The 14 years time series data were aggregated into monthly and yearly number of cases and time series plots were created to visualize possible trends and seasonality. The seasonal distribution was assessed by summing the frequency of cases into Bhutan's four seasons: (1) spring (March to May), (2) summer (June to August), (3) autumn (September to November) and (4) winter (December to February). The expected numbers of cases were calculated under a null hypothesis that rabies incidence was independent of season in Bhutan. Observed and expected numbers were compared by chi-squared test to evaluate the association between season and rabies incidence (Kim et al., 2006).

Spatial analysis

A Geographic Information System (ArcGIS 9.3; ESRI, Redlands, CA, USA) was used to visualize the distribution of rabies in Bhutan. Case reports in any species of animals (cattle, dogs, cats, horses, pigs and goats) were aggregated at the sub-district level and thematic choropleth maps were produced demonstrating 2-yearly (1996 to 2009) distribution of rabies in Bhutan. Since rabies is mainly reported from the four southern districts (Sarpang, Chhukha, Samdrup Jongkhar and Samtse) of Bhutan, the frequencies of cases in each district were summed by year to examine the annual trend of cases in these sub-districts.

Cross-correlation analysis

The numbers of cases in dogs and other domestic animals were aggregated into monthly series of cases (n = 168 months) to evaluate the relationship between reported cases in dogs and other domestic animals by cross-correlation functions (Diggle, 1990). The cross correlation coefficients were estimated for up to 6 month lag windows (sufficient to cover the average incubation period of rabies) (Courtin et al., 2000). The approximate standard errors for the cross-correlation coefficients were calculated on the assumption that the series are not cross-correlated and that one of the series is white noise (Hartnack et al., 2009). The analysis was performed using the Applied Statistical Time Series Analysis software program (ASTSA) version 1.0 (Shumway, 1988).

Autoregression analysis

Since the times series of reported rabies cases in dogs and other domestic animals was positively cross-correlated, we performed autoregression to determine if a value in the time series of cases of rabies in dogs at time t could be predicted by cases at a previous time (*t–1, t–2...t–p*) (Wheelwright and Marridakis, 1980; Shumway, 1988; Allard, 1998).

An autoregressive model is of the form: $X_t = \delta + \varphi_1 X_{i-1} + X_{i-2} ..., +X_{i-p} +w_t$, where X_t is the time series, δ is the constant or intercept, φ_1 is the autoregressive model coefficient, X_{i-1} is the previous observation at time *(t–1)* and *w(t)* is the random error.

The best-fitting autoregressive model describing the number of rabies cases in dogs reported each month was chosen based on the goodness-of-fit criterion (Akaike's corrected information criteria, AICc). The selected model residuals were checked by examining the autocorrelation function (ACF) and partial autocorrelation function (PACF) plots for evidence of stationary (lack of trend or patterns) and for constant variance, independence and randomness (Shumway, 1988). A *t*-statistic was used to test the significance of estimated model coefficients.

Similarly, the best-fitting predictive model of cases in other domestic animals was fitted by a multiple regression model using reported cases in other domestic animals as the dependent variable and reported cases in dogs at some previous time lag *(t–1, t–2,…, t–p)* as the predictor variables. Akaike's corrected information criteria statistics and the residual test (as described above) were used to select the best-fitting model. A *t*-statistic was used to test the significance of estimated model coefficients (Shumway, 1988). The analysis was performed in ASTSA version 1.0.

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Results

Descriptive analysis

From 1 January 1996 to 31 December 2009, a total of 814 rabies cases in dogs and other domestic animals were reported in Bhutan. The cases were most commonly reported in cattle (55%, 447/814) and dogs (39%, 317/ 814), with only a few cases in other species of animals (horses 17/814; cats 14/814; pigs 13/814; and goats 6/81 (Table 1). A total of 332 brain tissue samples were tested in the laboratory, of which 234 (70%) were confirmed positive by the FAT (Table 1). However, the majority (71%) of the reported cases were diagnosed based on clinical signs.

Table 1. Total number of reported rabies cases and the percentage of laboratory confirmed cases in different species of animals (1 January 1996 – 31 December 2009) in Bhutan.

Species	Total number of reported cases	Percentage of cases in different animal species	Number of laboratory confirmed cases	Percentage of laboratory confirmed cases	Number of cases diagnosed based on clinical signs
Cattle	447	54.91	170	38.03	277
Dogs	317	38.94	45	14.20	272
Pigs	13	1.60	9	69.23	4
Cats	14	1.72	6	42.86	8
Goats	6	0.74	3	50.00	3
Horses	17	2.09	1	5.88	16
Total	814	100	234	28.75	580

Temporal pattern

Rabies cases in animals in Bhutan were reported throughout the year, with greater numbers reported during spring and summer months (Figure 1). The reported cases were above expected values during spring and summer months and below expected values in autumn months ($\chi^2 = 113.89$; $P < 0.001$) (Table 2).
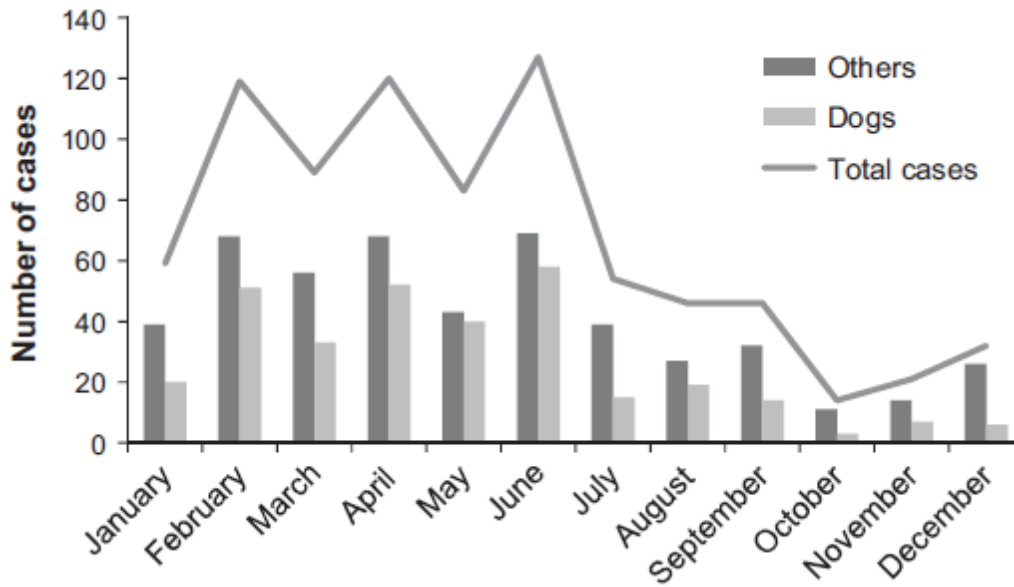
Figure 1. Monthly distribution of reported rabies cases in animals in Bhutan between 1 January 1996 and 31 December 2009

Table 2. Seasonal distribution of reported rabies cases in animals in Bhutan (1 January 1996 – 31 December 2009)

Season (month)	Observed cases	Chi-square
Spring (March–May)	292	38.48
Summer (June–August)	232	3.99
Autumn (September–November)	83	71.35
Winter (December–February)	207	0.06
Total	**814**	**113.89***

* $\chi^2 = 113.89$; df $= 3$; p<0.001

The annual trend of rabies cases reported in dogs and other domestic animals is illustrated in Figure 2. The number of reported cases was almost stable from 1996 to 2005 but increased in 2006 and 2008 (P < 0.001).
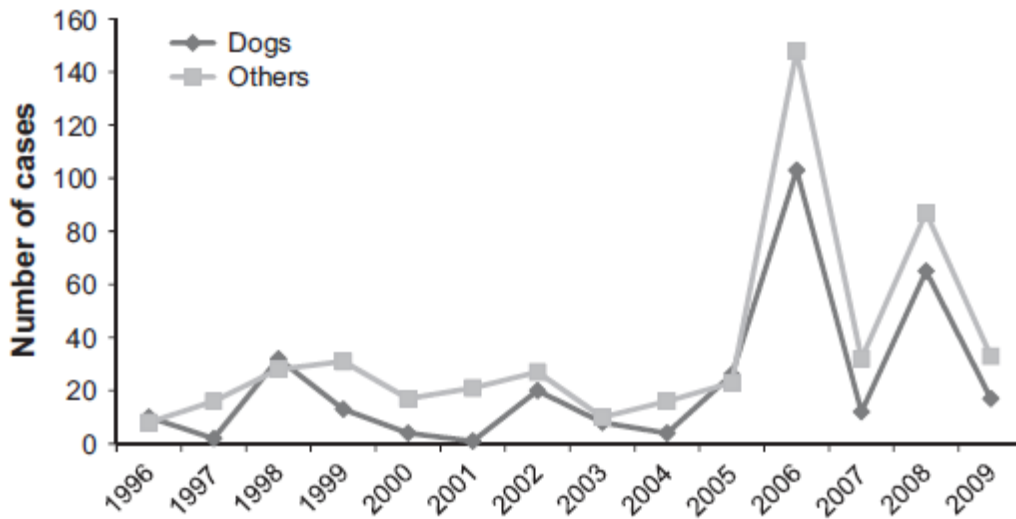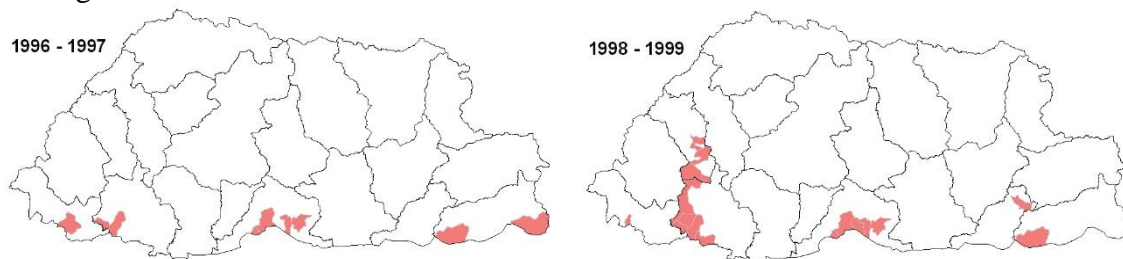
Figure 2. Annual trend of reported rabies cases in animals in Bhutan between 1 January 1996 and 31 December 2009

Spatial pattern

Figure 3 shows the spatial distribution of reported rabies cases in different sub-districts in Bhutan. Of the 205 subdistricts, 59 sub-districts (29%) reported rabies in animals from 1 January 1996 to 31 December 2009. Some subdistricts reported rabies for all 14 years while others reported once and never reported again. The sub-districts that did not report subsequent outbreaks are located away from the border areas (see Figure 3). In general, rabies outbreaks were commonly reported in the four districts (Sarpang, Samdrup Jongkhar, Chhukha and Samtse) of southern Bhutan that share a border with India (Figures 3 and 4). Sarpang (36%) and Chhukha (36%) reported the highest number of cases followed by Samdrup Jongkhar (16%) and Samtse (13%) districts. The annual pattern of rabies cases in the four sub-districts between 1996 and 2009 is illustrated in Figure 4. On visual examination of the time series plots (Figure 4), Samdrup Jongkhar district reported a stable number of cases during 2003 to 2009, whereas the other three districts (Sarpang, Chhukha and Samtse) reported slightly increasing numbers of cases during this period. Chhukha district reported the highest number of cases in 2008.
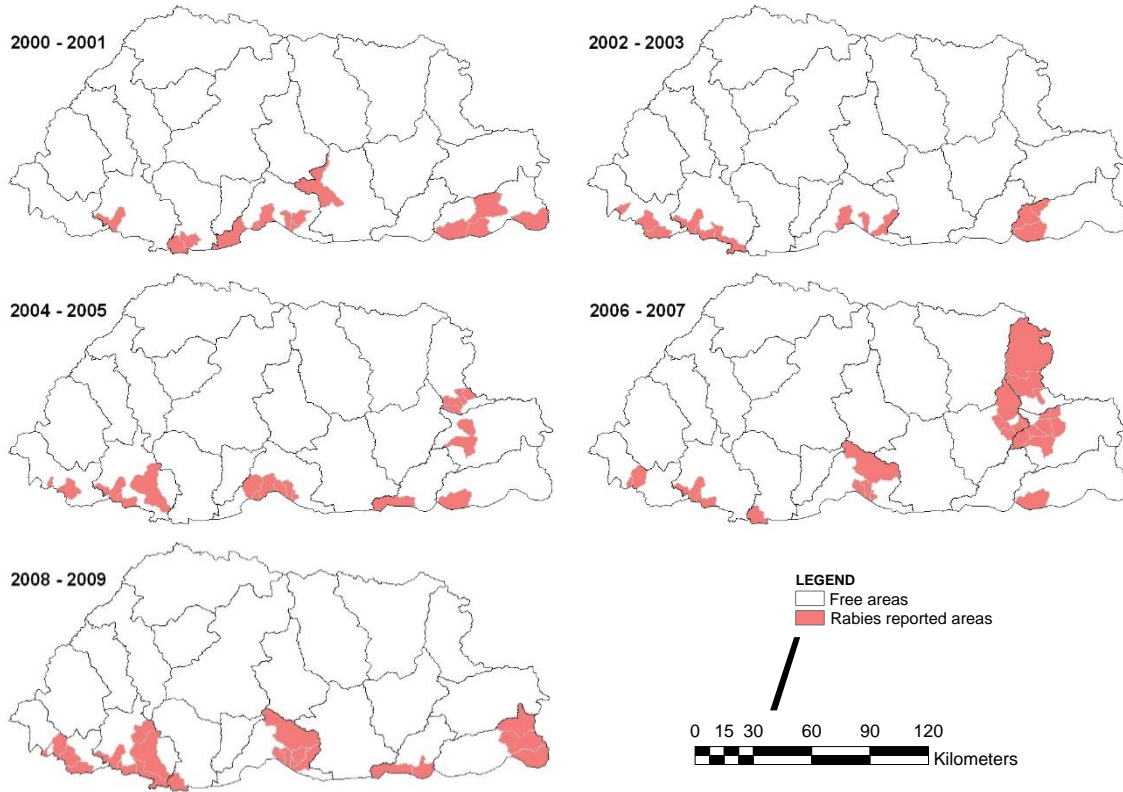
Figure 3. Geographical distribution of reported rabies cases in animals in different sub-districts of Bhutan between 1 January 1996 and 31 December 2009
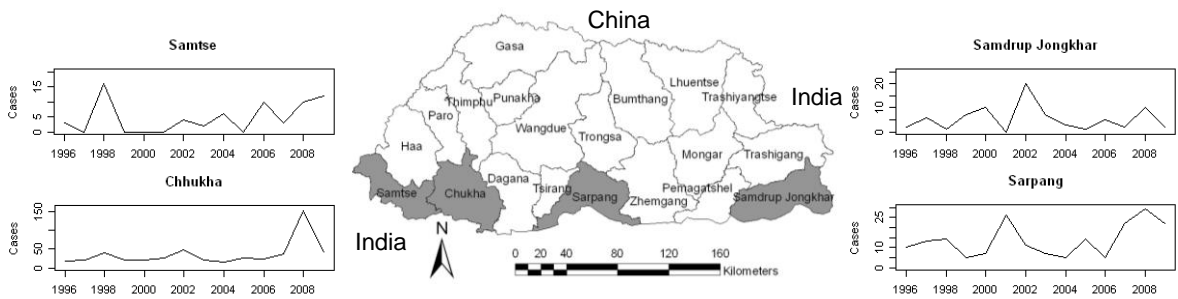

Figure 4. District map of Bhutan showing the four southern districts (shaded) that are highly endemic to rabies and their annual trend of reported rabies cases in domestic animals between 1 January 1996 and 31 December 2009.

Cross-correlation analysis

A significant (P<0.05) positive cross-correlation was observed between the number of cases in dogs and other animals at lags of 1–3 months with the highest cross-correlation (r = 0.94, P<0.001) at lag 0 (Table 3). This indicates that the peak in rabies incidences occur in the same month when both dogs and other domestic animals cases are reported. It also suggests that the report of rabies in dogs tends to precede the report of cases in other domestic animals.

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Table 3. Cross-correlation between cases of rabies in dogs and other domestic animals reported between 1 January 1996 and 31 December 2009 in Bhutan, lagged by 0–6 months. 95% significance is r > 0.1508 and is in bold

Lag in months	CCF (r)
0	**0.935**
1	**0.542**
2	**0.417**
3	**0.215**
4	0.140
5	0.080
6	0.023

95% significance is r>0.1508 and is in bold, cross correlation function (CCF)

Autoregression analysis

The best-fitting (AICc = 5.045 R^2 = 0.363) autoregressive model of dog rabies cases included rabies cases reported in dogs during the previous *1–2* months indicating that an increase of one dog case at month *t–1* and *t–2* predicted an increase of 0.52 cases and 0.21 cases in dogs at month *t* (Table 4). The fitted autoregressive model was:

Dog rabies = 2.04 + 0.52dog[t–1] + 0.21dog[t–2] + w[t]

Table 4. Best fitting (AICc = 5.045 R^2 = 0.363) autoregressive model of dog cases of rabies reported between 1 January 1996 and 31 December 2009 in Bhutan

Variable	**Lag (months)**	*b*	**S.E**	*t*	*P−value*
Constant	–	2.038	0.685	2.973	0.003
dog cases	[–1]	0.523	0.077	6.734	0.000
dog cases	[–2]	0.209	0.086	2.428	0.015

The ACF and PACF of the residuals showed autocorrelation (r > 0.15) at lag 27 months. The residuals appeared to have constant variance and were independent without any fluctuation and outliers.

The final best-fitting (AICc = 3.919, R^2 = 0.31) regression model to predict the cases of rabies in other domestic animals was found to be the report of rabies in dogs during the previous 1 and 2 months indicating that an increase of one dog case at month *t–1* and *t–2* predicted an increase of 0.250 cases and 0.086 cases in other domestic animals at month t (Table 5). The fitted model was:
Rabies in other domestic animals = 1.325 + 0.25 dog [t–1] + 0.086 dog [t–2] + w$_{(t)}$.

Table 5. Best fitting (AICs = 3.919, R^2 = 0.31) regression model of other domestic animals cases of rabies reported between 1 January 1996 and 31 December 2009 in Bhutan

Variable	Lag (months)	*b*	S.E	*t*	*P-value*
Constant	–	1.325	0.382	3.466	0.000
dog cases	[–1]	0.250	0.044	5.688	0.000
dog cases	[–2]	0.086	0.044	1.957	0.050

The ACF and PACF of the residuals showed autocorrelation (r > 0.15) at lag 28 months. The residuals had constant variance and were independent without any fluctuation and outliers.

Discussion

This study describes the temporal and spatial distribution of reported animal rabies in Bhutan. The data showed that most of the reported cases are in dogs and cattle but the number of cases in cattle exceeded that in dogs and other animals. This could be due to either under reporting of cases of rabies in free-roaming dogs or a single rabid dog might have infected many cattle during outbreaks. In Bhutan, cattle are grazed in open fields and come into contact with free-roaming dogs, increasing the risk of dog bites. Cases in free-roaming dogs are more likely to have been underreported than cases in cattle due to difficulties in tracing the cases in dogs. Moreover, rabid dogs (especially strays) are difficult to trace due to trans-border movement in the south border towns. Cases in livestock would have been captured by the reporting system, perhaps because of the greater economic value of cattle: farmers often report the illness of cattle to veterinary officials for treatment or investigation.

Rabies cases were reported throughout the year with more cases reported during spring and summer months (Figure 1 and Table 2). This finding is in agreement with a previous report in Bhutan in which more rabies outbreaks were reported during February to June compared to other months (Rinzin et al., 2006). Increased incidence of rabies during spring and summer months may be associated with the breeding season of dogs. It has been reported that the dog-breeding season is associated with increased contact rates between dogs, leading to frequent fights and increases the risk of virus transmission (Malaga et al., 1979; Ezeokoli and Umoh, 1987; Mitmoonpitak et al., 1998; Panichabhongse, 2001). Malaga et al., (1979) have also suggested that the seasonality of canine rabies could be due to changes in the age structure of the susceptible dog population following the breeding season, when large numbers of puppies enter the population and are present in the streets, increasing the risk of rabies virus transmission. Study in Thailand revealed that 14% of rabid dogs were <3 months old and 42% were <6 months old suggesting that young dogs were a risk factor for rabies transmission (Mitmoonpitak et al., 1998). Nevertheless, the seasonality of rabies in wildlife (including foxes, skunks and raccoons) elsewhere has clearly been shown to follow a marked seasonal pattern due to their highly seasonal breeding pattern and strong seasonal territorial instincts (Pool and Hacker, 1982; Gremillion-Smith and Woolf, 1988; Wandeler and Bingham, 2000; Harnos et al., 2006; Zienius et al., 2007). Further studies may be necessary to substantiate the seasonality of rabies in Bhutan by establishing a proper surveillance and reporting system for rabies in free-roaming dogs. This would provide information for better planning of vaccination programmes in dogs. Currently in Bhutan, vaccination of free-roaming dog is usually carried out in conjunction with a sterilization programme during the cooler months of the year to avoid double catching of the dogs and post-operative

complications during the hot summer months. In Thailand, the mass dog vaccination campaign is generally scheduled prior to the dog breeding season (Mitmoonpitak et al., 1998).

There was no significant change in the reported number of rabies cases from 1996 to 2005, but peaks in 2006 and 2008 were observed. A series of rabies outbreaks that occurred in the east, southeast and southwest of Bhutan in recent years might be associated with these increases (Tenzin et al., 2010). Although the number of reported cases remained almost constant over the study period, the frequencies of outbreaks (data not shown) and the area of spread have increased (see Figure 3). For instance, places or sub-districts that were previously free of rabies have reported outbreaks recently (see Figure 4) (Tenzin et al., 2010). This increased incidence may be due to higher free-roaming dog population densities and the absence of a sustained vaccination programme (low vaccination coverage: <20%) resulting in the maintenance of rabies endemicity in the border areas of Bhutan, as observed elsewhere (Beran and Frith, 1988; Cleaveland and Dye, 1995; Kitala et al., 2002). It may also be due to translocation and trans-border movement of infected free-roaming dogs in southern Bhutan. Further studies are necessary to understand in detail the transmission dynamics of rabies in southern Bhutan.

We also observed a significant temporal correlation between reported cases in dogs and other domestic animals, wherein the report of cases in dogs predicts cases in other domestic animals. The relationship is biologically plausible since dogs are the reservoir and vector of rabies virus transmission to other livestock species in canine rabies endemic countries. Unlike in other countries (Courtin et al., 2000; Milius et al., 2004; Vos et al., 2009) no other intervening species (such as wildlife) have been found to be involved in the epidemiology of rabies in domestic animals, including in dogs in Bhutan (Tenzin et al., 2010). To date, no wildlife rabies cases have been reported or confirmed in Bhutan, and dogs have been the source of spillover infections of cattle, other domestic animals and sporadic infections in humans. Furthermore, the association of rabies in dogs in the previous months with the observation of rabies incidences in other domestic animals suggests the need for enhancing rabies surveillance in the event of rabies occurrence in dogs. Similar results were obtained in a previous investigation in Namibia, in which black-backed jackals predicted rabies in dogs and domestic ruminants (Courtin et al., 2000). The association between cases in dogs and other domestic animals in Bhutan could be substantiated by conducting monoclonal antibody testing and genetic typing of the virus. Such techniques would reveal the source and transmission dynamics of rabies virus infections between reservoir and dead end hosts (such as domestic livestock).

There are some limitations in this study. The data that we used are surveillance data collected by different units and submitted to the VIS database. Although there has been no dramatic change in the surveillance system for rabies over the years, some minor differences in the reporting system from the different rabies outbreak areas may have occurred. For instance, more detailed investigation and follow-up of cases are expected to have been carried out during the period of major outbreaks than during that of the sporadic occurrence of cases. This may be also one of the reasons for observing in our analysis a strong correlation between reported rabies cases in dogs and other domestic animals at lag 0 (same month).

Some underreporting of cases could have also occurred due to lack of awareness among farmers, or due to the remote location of some outbreaks. However, we assume that underreporting remained relatively constant during the study period.

It is also important to consider proper sampling and rabies laboratory diagnostic procedures being followed in Bhutan. Brain tissue samples are collected in the field, preserved in 50% glycerine saline and submitted to the laboratory for confirmatory diagnosis using the FAT (Dean et al., 1996). Because of the generally long distances from the field to the laboratory, the final confirmatory diagnosis takes some time (weeks) and it is likely that the diagnostic test is affected by the quality of the sample (McElhinney et al., 2008; Fooks et al., 2009). In some instances, the samples would be unfit for testing. All these limitations can result in poor test performance. However, rabies is not a difficult disease to diagnose (clinically) in endemic countries. Although the FAT is a standard test for rabies diagnosis, other rapid diagnostic field test kits would be useful in a country such as Bhutan so that a quick decision can be made in the field regarding implementation of control activities and also to advice people who have been potentially exposed to the disease about post-exposure treatment.

In summary, our analysis shows a stable trend in rabies reported in Bhutan from 1996 to 2005, with increased number of cases and area of spread from 2006 to 2009. There was also significant seasonal variation – increased incidences during spring and summer months. A significant correlation between rabies in dogs and other domestic animals was also demonstrated, suggesting the need to improve surveillance in the event of rabies outbreak in dogs. We recommend that the surveillance system should be improved, especially in free-roaming dog populations. Investigation of the existence of any rabies reservoirs in wildlife species should be undertaken. Because of the limited distribution of rabies in southern Bhutan, successful control and elimination of rabies is achievable by strict enforcement of dog vaccination rules and the dog population management programme.

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