Epidemiologic Trends of Rabies in Domestic Animals in Southern Thailand, 1994–2008

Anyarat Thiptara, Edward R. Atwill,* Wandee Kongkaew, and Bruno B. Chomel

Department of Population Health and Reproduction, School of Veterinary Medicine, University of California, Davis, California;
Epidemiology Section, Veterinary Research and Development Center (Southern Region), Thungsong, Nakhon-si-thammarat, Thailand

Abstract. Rabies and associated risk factors in dogs, cats and cattle ($n = 3,454$) in southern Thailand during 1994–2008 were evaluated by using a mixed-effect logistic regression model. Overall prevalence was 48%. In dogs, odds of being rabid were 1.7 times higher in unvaccinated dogs than in vaccinated dogs and two times higher in dogs with bite history than in dogs with no known bite history. Similarly, aggressive dogs were more likely to be rabid than non-aggressive dogs. In cattle, aggression, pharyngeal paralysis, hyperactivity, and depression were clinical signs associated with being rabid. Annual fluctuations of the species-specific prevalence of rabies is suggestive of a positive correlation between canine and either feline ($r = 0.60$, $P = 0.05$) or bovine rabies ($r = 0.78$, $P = 0.004$). Insufficient vaccination coverage led to maintenance of rabies, which could be easily controlled by increased vaccine coverage and public education.

INTRODUCTION

Rabies is an acute viral encephalomyelitis caused by a lyssavirus in the family Rhabdovirus that affects wild and domestic mammals. Worldwide, human deaths caused by this disease are estimated to reach 55,000 cases annually.¹ In North America and Europe, where dog rabies has been eliminated, rabies is still maintained in wildlife. With an estimated mean human-to-dog ratio of 7.4 in urban areas and 14.3 in rural areas in Asia, approximately 2.5 billion persons are at risk for rabies exposure in this region.² In Asia, Thailand had been a country highly endemic for rabies with more than 200 human deaths per year in the 1990s. The incidence of human rabies has considerably decreased over the past decade; only eight deaths were recorded in 2008 in part because of mass rabies vaccination campaigns in dog populations and a major increase in rabies post-exposure prophylaxis of humans exposed to potentially rabid animals.^{3,4}

Transmission of rabies is usually through the bite of a rabid animal, which introduces the virus present in its saliva into the bite wound. Rabid animals of all species commonly exhibit typical signs of central nervous system disturbance. Dogs may have either the furious or the paralytic (dumb) form of rabies. Major clinical signs in dogs are aggression, biting, laryngeal paralysis, mastication muscle paralysis, roaming, abnormal barking, and excessive salivation.⁵ Clinical signs in cats include a change in behavior (a tendency to hide), irritation, abnormal vocalization, photophobia, fractious aggression, muscle tremors, incoordination, and paralysis. Mandibular and laryngeal paralyzes commonly observed in dogs is rare in cats.⁶ Major clinical signs in cattle included excessive salivation, behavioral changes, vocalization (bellowing), aggression, hyperesthesia and/or hyperexcitability, and pharyngeal paresis/paralysis.^{7,8}

In Thailand, dogs are the main reservoir of rabies and play an important role in the rabies transmission cycle in humans and animals.^{5,9,10} Cats, other domestic and farm animals, and humans are accidental hosts of the rabies virus.¹¹ Rabies vaccination campaigns in dogs and cats are conducted on a yearly basis, yet rabies is still endemic in its dog reservoir.¹² This finding is likely the result of insufficient vaccination coverage to

break the rabies transmission cycle among stray and owned dogs.¹³ The use of rabies vaccines via parenteral injection requires a capture or restraint process, which may be difficult, especially in stray dogs, and may be one cause of vaccination failure. Lack of reliable data on dog population and their ecology and limited surveillance of rabies infection in dogs and humans also hamper control and prevention.^{3,14}

Clinical signs and selected demographic characteristics have been used as epidemiologic tools for rabies diagnosis in animals and humans, especially in areas where necropsy and reliable laboratory diagnoses are not possible. Clinical criteria of rabid dogs were evaluated retrospectively and this technique had a value for *intra vitam* (during life) diagnosis of rabies in dogs with a sensitivity of 90% and a specificity of 96%.¹⁵ However, relatively few studies have been published on the risk factors associated with rabies, especially in veterinary research. We hypothesize that some clinical and demographic characteristics of animals are indicative of rabies occurrence. The objective of this retrospective study was to identify clinical and demographic characteristics associated with rabies in dogs, cats, and, cattle in southern Thailand during 1994–2008.

MATERIALS AND METHODS

Study population. The study population consisted of 3,454 animals submitted to the Southern region of Veterinary Research and Development Center (SVRDC) from 14 provinces in southern Thailand for rabies diagnosis during 1994–2008. The SVRDC is the major rabies diagnostic center located in Nakhon-Si-Thammarat Province where approximately 10 animals are submitted and tested for rabies every month. Most of the rabies-suspected samples were from dogs.

Data collection. Information on demographic characteristics, including owner's address, submission date, species, breed (exotic or local), sex, age, ownership status (owned or stray), biting history, vaccination, death status (euthanasia or death), and clinical characteristics, such as biting, attacking, aggressiveness, swallowing difficulty, excessive salivation, slack jaw, flaccid tongue, abnormal posture, abnormal vocalization, roaming, depression, paralysis, and other abnormal clinical signs, were obtained at the time the animal submitted via interviews conducted by veterinary assistants with the animal's owners or persons who brought animals to the SVRDC on the submission date and the rabies submission form. Animals alive at the time of submission were examined by a staff veterinarian. All data

*Address correspondence to Edward R. Atwill, Department of Population Health and Reproduction, 2009 Haring Hall, School of Veterinary Medicine, University of California, 1 Shields Avenue, Davis, CA 95616. E-mail: ratwill@ucdavis.edu

obtained on the submission form were entered into a database program (Microsoft Access; Microsoft Corp., Redmond, WA), and the suspected samples were sent to the virology section for rabies diagnosis. Data were transferred into a spreadsheet software program (Microsoft Excel 2007; Microsoft Corp.) for analysis.

Data coding. Owner addresses were used to categorize the location of animals into 14 provinces. Season was classified into three groups using submission dates, i.e., hot and dry (February 16–May 15), hot and wet (May 16–October 15), and cold dry (October 16–February 15).¹⁶ Animals were categorized as local if its breed was native; otherwise, it was coded as exotic. Species were categorized into four groups: dog, cat, cattle, and others. Age was recorded either in months for dogs or years for cats and cattle. Animals were then categorized into groups depending on age distribution in each species: six groups based on age in months and three groups based on age in years. Ownership status was classified as owned if the animal had at least one referral household; otherwise, an animal was classified as stray.¹⁴ Bite history referred to those animals that had been bitten or that had bitten another animal or person. Rabies vaccination status differentiated those animals that had been vaccinated against rabies within less than a year from those that were either not vaccinated or vaccinated more than a year ago. Death status was categorized as euthanasia for those animals that were euthanized for rabies diagnosis; otherwise, they were reported as dead. Clinical characteristics were coded yes if an animal showed at least one sign in each clinical group definition (see below).

Clinical characteristic definition. Clinical characteristics were categorized into seven groups by using clinical history data from the submission form as follows. 1) Behavioral changes were defined as any change from normal behavior as noted by the owner, e.g., irritability, nervousness, etc. 2) Aggression was defined as pursuit of or attack on another animal, person or any moving object. For dogs, aggression referred to animals seen chewing the wire of their cages, swallowing foreign objects, breaking their teeth, or attempting to bite without provocation. Vicious biting and scratching were also noted aggressive behavior in cats. 3) Pharyngeal paralysis was defined as paralysis of the throat and masseter muscles, profuse salivation, inability to swallow, abnormal vocalization, flaccid tongue, or slack lower jaw. Dogs usually showed signs similar to those seen with a foreign body obstruction in the oral cavity. Bellowing, yawning, and drooling saliva were common signs reported in cattle. 4) Generalized paralysis was defined as muscle tremors, incoordination, reluctance to walk or stand, stiffness, abnormal posture, or recumbency. 5) Sudden anorexia was defined as sudden loss of appetite. 6) Depression was defined as decreased interest to an environmental stimulus, drowsiness, or lethargy. 7) Hyperactivity/hyperexcitability were defined as increased awareness to sound or light, pupil dilatation, increased sensitivity to touching or other sensory stimuli, or running without apparent reason.

Rabies diagnosis. Healthy brain tissue from suspected animals were examined by the direct fluorescent antibody (DFA) technique.¹⁷ Briefly, after brain dissection, the cerebellum, hippocampus, and brain stem, including pons and medulla, were obtained. A cross-section of each brain sample was made, and the cut surface of the cross-section was impressed onto two separate locations on prepared slides. For many small animals (e.g., dog-sized or smaller animals),

a complete cross-section was placed in a 15-mm diameter well. The rest of the samples were kept in plastic containers separately for virus isolation.

Test slides were fixed in acetone for 15 minutes and dried at room temperature. Each anti-rabies conjugate, fluorescein isothiocyanate-labeled monoclonal (Chemicon catalogue no. 5100; Millipore Ltd., Livingston, United Kingdom) and polyclonal anti-rabies antibody (Thai Red Cross, Bangkok, Thailand), was added to fixed samples and incubated for 30 minutes at 37°C in a high-humidity chamber. Slides were rinsed twice each using 0.01 M phosphate-buffered saline, pH 7.2–7.4, for 5-minute intervals, and then briefly air-dried before mounting. Slides were mounted by dropping a small volume of glycerol (0.05 M Tris-buffered medium with 20% glycerol, pH 8.0–9.0) and covered with a cover slip. Slides were read by using a fluorescent microscope at a magnification $\geq 100\times$ within 2 hours of mounting. A sample was considered positive for rabies when any round or oval shape of bright apple-green fluorescence was present in each area of the brain stained with anti-rabies fluorescein isothiocyanate-labeled conjugate. Positive and negative control slides were prepared, fixed, and stained in the same manner and at the same time as the test slides to control for the effect of test reagents on test performance. The DFA test not performed on autolyzed brain tissue.

Virus isolation was performed by using the mouse inoculation test whenever the DFA test yielded a negative result.¹⁸ Briefly, a 10% suspension of brain was prepared in phosphate-buffered saline containing broad-spectrum antibiotics. A 0.01-mL aliquot of the brain suspension was injected into 3–5 suckling mice (5 days of age) by the intracranial route. Inoculated mice were kept in a separate room for 30 days and were observed daily for clinical signs such as circling, seizure, and death. Impression smears of brain tissue from mice showing signs within 30 days were prepared and subjected to the DFA test. Mice used for rabies inoculation were kept in a specific isolated ward and procedures of intracerebral inoculation were performed according to the guidelines of our animal use and care committee.

Statistical analyses. The unit of analysis was the individual animal. The dependent variable was the rabies DFA test result (positive or negative). A mixed-effect logistic regression model (Intercooled Stata 9 for Windows; Stata Corp LP, College Station, TX) was used to identify risk factors for rabies in dogs, cats, and cattle. The model-building methods for logistic regression have been described.¹⁹ Briefly, independent variables were selected by using univariate logistic regression analysis. Any variable for which univariate analysis had a P value < 0.25 was a candidate for the multivariate logistic regression model along with all variables of known biological plausibility. Province of origin was tested for random effect to determine whether it should be retained in the model based on the likelihood ratio test (P value < 0.05). Age was checked for the linearity assumption in logit scale to determine whether it could be used as a continuous variable. Once these variables had been identified, we began with a model containing the variable with lowest P value, then each additional variable was entered into the model to test for its significance with α based on the likelihood ratio statistic set at 0.05. This process of deleting, refitting, and verifying continued until all significant variables were included in the model and those excluded were biologically implausible and/or not significant. Lastly, all

non-significant variables that had not been selected for the original multivariate model were re-entered in the final model to confirm their non-significance.

Interactions among the variables in the model were tested based on the likelihood ratio test. Only biologically plausible interaction terms were included in the model. In addition, the correlation coefficient (r) and the coefficient of determination (r^2) were used to evaluate the relationship between the annual prevalence of rabies in dogs compared with cat or cattle hosts (SAS system 9.1 for Windows, SAS Institute Inc, Cary, NC).

RESULTS

A total of 3,454 rabies-suspicious cases (2,833 dogs, 331 cats, 189 cattle, and 101 other animals), were submitted from 14 provinces of southern Thailand during 1994–2008. The distribution of the submitted cases is shown in Figure 1. Almost half (48%, 1,661 of 3,454) of the animals submitted for rabies testing were confirmed to be rabid. Of these positive cases, 98% were confirmed by using the DFA test and 2% by the mouse inoculation test. The prevalence of positive animals was 73%

(138 of 189) for cattle, 51% (1,454 of 2,833) for dogs, and 16% for cats (52 of 331).

The percentages of rabies-positive cases by year for each species are shown in Figure 2. The rabies trends in all species reached their lowest prevalence in 1997, 2003, and 2008, and peaked in 1994 and 2000. In cattle, the rabies trend slightly increased in 2004, peaked in 2007, then sharply decreased in 2008. In dogs, the rabies trend increased to a peak in 2004, remained stable until 2006, then decreased in 2007 and 2008. In cats, the rabies trend was stable at < 10% during 2004–2007.

The geographic distribution of animal rabies cases is shown in Figure 3. Pattalung Province had the highest percentage of animal rabies (69%), followed by Nakhon-Si-Thammarat Province (52%), and Songkhla and Satun Provinces (50% each). For dogs, Pattalung Province had the highest percentage of rabies (69%). In cats, the highest percentage of rabies was 43% in Songkhla Province. In cattle, the highest percentage of rabies was in Suratthani Province (100%).

Descriptive statistics for each potential risk factor in each species are listed in Tables 1 and 2. Only 1,614 dog cases (57%), 321 cat cases (97%) and 184 cattle cases (97%) were included in the final analysis because of missing data. The province of

FIGURE 1. Distribution of rabies suspected cases submitted in southern Thailand during 1994–2008.

FIGURE 2. Temporal descriptive statistics for rabies in southern Thailand during 1994–2008.

origin was statistically significant ($P = 0.033$) for random effect in dogs, and the proportion of total variance contributed by the province variance component (ρ) was approximately 5%. In contrast, the province of origin was not significant for cats and cattle (Table 3).

The annual fluctuations of the species-specific prevalence of rabies is suggestive of a positive correlation between dog and either cat or cattle rabies. The correlation coefficient (r) was 0.60 ($P = 0.05$) between the annual prevalence of dog and cat rabies and 0.78 ($P = 0.004$) between the annual prevalence of dog and cattle rabies. The coefficient of determination (r^2) was 0.36 for dog and cat rabies and 0.62 for dog and cattle rabies. Overall, most of the cattle rabies cases could be explained by the association with dog rabies cases. However, such an association was weaker for cat rabies cases.

Among the various dog demographic factors, age, rabies vaccination status, and bite history were significantly associated with a positive test result for rabies virus. In contrast, sex, breed, owner status, death status, and season were not significantly associated with a positive test result for rabies virus. Vaccinated dogs had one and a half times lower odds of being rabid than unvaccinated dogs ($P = 0.002$). Dogs with a bite history (biting another animal or a human or being bitten by another dog) had twice the odds of having a positive test result for rabies virus than dogs without a bite history ($P < 0.001$). The odds of having a positive test result for rabies virus also varied with age.

FIGURE 3. Distribution of all positive rabies cases (A), dog rabies (B), cat rabies (C), and cattle rabies (D) in southern Thailand during 1994–2008.

TABLE 1

Rabies-positive results for dogs, cats, and cattle suspected of having rabies in southern Thailand during 1994–2008 stratified by demographic characteristics

Covariate	Dogs		Cats		Cattle	
	No.	% Positive	No.	% Positive	No.	% Positive
Sex						
Female	720	54.2	99	15.2	110	78.2
Male	1,594	48.5	131	13.7	61	67.2
Not specified	519	56.1	101	18.8	18	61.1
Breed						
Exotic	152	28.9	1	0	24	62.5
Local	2,283	52.6	260	13.1	149	74.5
Not specified	398	52.8	70	25.7	16	75.0
Owner status						
Owned	1,924	44.6	243	16.0	189	73.0
Stray	778	69.3	73	15.1	0	0
Not specified	131	42.7	15	13.3	0	0
Vaccination status						
Yes	643	37.0	57	7.0	0	0
No	1,485	51.7	219	17.4	189	73.0
Not specified	705	63.5	55	18.2	0	0
Bite history						
Yes	1,979	57.5	173	18.5	10	80.0
No	666	35.3	142	12.7	163	71.8
Not specified	188	43.1	16	12.5	16	81.3
Death status						
Euthanasia	1,562	55.5	94	24.5	36	94.4
Dead	1,188	45.9	227	11.9	143	66.4
Not specified	83	50.6	10	20.0	10	90.0
Season						
Hot and dry	824	49.3	85	11.8	49	71.4
Wet	1,047	52.0	132	16.7	82	73.2
Cold and dry	962	52.4	114	17.5	58	74.1
Age, months						
0–3.0	533	49.0	–	–	–	–
3.1–6.0	347	53.6	–	–	–	–
6.1–12.0	358	57.5	–	–	–	–
12.1–24.0	352	48.3	–	–	–	–
24.1–36.0	198	38.9	–	–	–	–
> 36.0	249	29.3	–	–	–	–
Not specified	796	60.2	–	–	–	–
Age, years						
0–1	–	–	128	7.8	46	67.4
1.1–2	–	–	41	17.1	32	71.9
> 2	–	–	57	17.5	86	79.1
Not specified	–	–	105	23.8	25	64.0

TABLE 2

Rabies-positive results for dogs, cats, and cattle suspected of having rabies in southern Thailand during 1994–2008 stratified by clinical characteristics

Covariate	Dogs		Cats		Cattle	
	No.	% Positive	No.	% Positive	No.	% Positive
Behavioral change						
Yes	628	14.8	76	10.5	4	25.0
No	2,045	63.5	235	17.4	182	74.2
Not specified	160	38.8	20	15.0	3	66.7
Aggression						
Yes	944	89.2	36	47.2	84	98.8
No	1,728	31.8	275	11.6	101	51.5
Not specified	161	39.1	20	15.0	4	75.0
Pharyngeal paralysis						
Yes	225	72.4	19	31.6	56	80.4
No	2,447	50.2	296	14.5	129	69.8
Not specified	161	39.1	20	15.0	4	75.0
Body paralysis						
Yes	97	62.9	13	23.1	9	77.8
No	2,575	51.7	298	15.4	176	72.7
Not specified	161	39.1	20	15.0	4	75.0
Anorexia						
Yes	164	56.1	27	56.1	9	66.7
No	2,508	51.8	284	15.9	176	73.3
Not specified	161	39.1	20	15.0	4	75.0
Depression						
Yes	774	45.0	134	14.2	29	51.7
No	1,898	55.0	177	16.9	156	76.9
Not specified	161	39.1	20	15.0	4	75.0
Hyperactivity						
Yes	211	88.2	13	38.5	50	90.0
No	2,461	51.0	298	14.8	134	66.4
Not specified	161	35.4	20	15.0	5	80.0

Compared with older dogs (> 36 months of age), the odds of having a positive test result for rabies virus was 1.9 times higher in dogs < 3 months of age, 3.3 times higher in dogs 6.1–12 months of age, and 2.5 times higher in dogs 24.1–36 months of age ($P < 0.05$ for all comparisons). For clinical history before submission, only depression was excluded from the final model because of its non-significance (Table 3). Dogs showing overall behavioral changes or aggression, pharyngeal paralysis, body paralysis, anorexia, or hyperactivity/hyperexcitability had 3.4 ($P < 0.001$), 10.9 ($P < 0.001$), 2.2 ($P < 0.001$), 1.8 ($P < 0.05$), 1.7 ($P < 0.05$) and 3.1 ($P < 0.001$) times, respectively, higher odds of having a positive test result for rabies virus than dogs without these clinical signs.

For cattle, all cases were reported as unvaccinated owned cattle. Therefore, we excluded rabies vaccination status and owner status from the analysis. Although most of the demographic factors showed statistically significant associations with occurrence of rabies in the univariate analysis, only four clinical signs (aggression, pharyngeal paralysis, hyperactivity/hyperexcitability, and depression) were associated with higher

odds of having a positive test result for rabies virus (Table 4). Interestingly, the odds of being rabid among aggressive cattle was approximately 400 times higher than the odds of being rabid among non-aggressive cattle ($P < 0.001$). Cattle showing depression or pharyngeal paralysis or hyperactivity/hyperexcitability had 4.7 ($P < 0.05$), 7.3 ($P < 0.005$), and 19.7 ($P < 0.001$) times, respectively, higher odds of being rabid than cattle without these signs.

Only univariate associations were significant for rabid cats. Displaying aggression or being hyperactive/hyperexcitable was associated with higher odds of rabies ($P < 0.001$) (Table 5).

DISCUSSION

The human population of southern Thailand has increased during the past decade from 8.2 million to more than 8.8 million.²⁰ Consequently, an increase in the dog population has also been recorded. Most of these dogs are not domiciled and live either partially or fully outside the home, facilitating the conditions favoring endemic dog rabies.¹⁴ Interestingly, although rabies vaccination can prevent dogs from rabies infection, some vaccinated dogs were shown to be infected with rabies virus. There are several reasons why rabies can develop in vaccinated dogs. First, dogs could already be infected with rabies virus before being vaccinated. Second, dogs may have been reported as vaccinated, but were in fact not vaccinated, because we did not have their vaccination certificates to confirm their status. Third, dogs could have been vaccinated just once and their immunity had waned. Fourth, dogs may have been

TABLE 3

Logistic regression model for demographic and clinical characteristics associated with 1,614 dogs in southern Thailand during 1994–2008*

Covariate	β	SE	OR	95% CI	P
Constant	-3.3	0.3	—	—	< 0.001
Vaccination status					
No	0	—	1	—	—
Yes	-0.5	0.2	0.6	0.5–0.8	0.002
Bite status					
No	0	—	1	—	—
Yes	0.8	0.1	2.2	1.6–2.9	< 0.001
Age, months					
0–3.0	0.6	0.3	1.9	1.0–3.5	0.046
3.1–6.0	0.7	0.3	2.0	1.2–3.4	0.008
6.1–12.0	1.2	0.3	3.3	1.9–5.5	< 0.001
12.1–24.0	1.2	0.3	3.3	1.9–5.8	< 0.001
24.1–36.0	0.9	0.3	2.5	1.52–4.1	< 0.001
> 36.0	0	—	1	—	—
Behavioral changes					
No	0	—	1	—	—
Yes	1.2	0.2	3.4	2.4–5.0	< 0.001
Aggression					
No	0	—	1	—	—
Yes	2.4	0.2	10.9	7.7–15.4	< 0.001
Pharyngeal paralysis					
No	0	—	1	—	—
Yes	0.8	0.2	2.2	1.4–3.3	< 0.001
Body paralysis					
No	0	—	1	—	—
Yes	0.6	0.3	1.8	1.1–3.2	0.037
Anorexia					
No	0	—	1	—	—
Yes	0.5	0.2	1.7	1.1–2.5	0.017
Hyperactivity					
No	0	—	1	—	—
Yes	1.1	0.3	3.1	1.7–5.5	< 0.001

*OR = odds ratio; CI = confidence interval. Province random effect $\sigma = 0.4$, 95% CI (0.2–1.1), $p = 0.05$.

vaccinated before three months of age, and maternal residual antibodies may have interfered with their own immune response. Dogs less than one year of age usually need to receive two doses of vaccine for rabies immunity to develop to the protective level. In this study, because approximately 48% of vaccinated dogs in which rabies developed were less than one year of age, they might be vaccinated only one time during their life and they still were at high risk for rabies.

The present study suggests that three demographic characteristics, rabies vaccination status, bite status, and age, may function as predictive risk factors for dog rabies in southern

TABLE 4

Logistic regression model for demographic and clinical characteristics associated with 184 cattle in southern Thailand during 1994–2008*

Covariate	β	SE	OR	95% CI	P
Constant	-1.9	0.5	—	—	< 0.001
Aggression					
No	0	—	1	—	—
Yes	5.9	1.1	373.0	40.0–3475.5	< 0.001
Pharyngeal paralysis					
No	0	—	1	—	—
Yes	1.2	0.6	7.3	2.1–25.2	0.002
Hyperactivity					
No	0	—	1	—	—
Yes	3.0	0.7	19.7	5.1–76.8	< 0.001
Depression					
No	0	—	1	—	—
Yes	1.5	0.7	4.7	1.3–7.1	0.02

*OR = odds ratio; CI = confidence interval.

TABLE 5

Logistic regression model (univariate analysis) for demographic and clinical characteristics associated with 321 cats in southern Thailand during 1994 and 2008*

Covariate	β	SE	OR	95% CI	P
Death status					
Constant	-1.1	0.2	—	—	< 0.001
Dead	-0.9	0.3	1.9	1.7–2.7	0.006
Euthanized	0	—	1	—	—
Aggression					
Constant	-2.0	0.2	—	—	< 0.001
Yes	1.9	0.4	6.8	3.2–14.4	< 0.001
No	0	—	1	—	—
Hyperactivity					
Constant	-1.8	0.2	—	—	< 0.001
Yes	1.3	0.6	3.6	1.1–11.5	0.03
No	0	—	1	—	—

*OR = odds ratio; CI = confidence interval.

Thailand. As reported, rabies vaccination is a protective factor and important for rabies control.^{21,22} In our study, we demonstrated that the odds of being diagnosed rabid was 1.7 times higher for unvaccinated than vaccinated dogs. This finding supports the assertion that inadequate vaccination of dogs is a contributing reason for the ongoing epizootic of dog rabies in southern Thailand. Dogs less than one year of age, especially those 6.1–12 months of age, had the highest odds of development of rabies. These results are similar to data reported from Bangkok, Thailand, where dogs 1–3 months of age had the greatest odds of having rabies than other age groups. This age cohort was also less likely to be vaccinated.²³ Interestingly, in a study of dog ecology and rabies in Mexicali, Mexico, dogs 3–11 months of age had a three times higher risk of being non-vaccinated than dogs ≥ 1 year of age.²⁴

Previous studies showed that owned dogs in Bangkok, Thailand²¹ and Sao Paulo, Brazil²⁵ were more likely to have rabies than stray dogs. However, in this study, the odds of rabies were not significantly different between these two groups of dogs. One possible explanation is that many owners feed their dogs but do not vaccinate them because we reported that only 20% of stray dogs and 32% of owned dogs submitted for rabies testing were vaccinated in southern Thailand. In addition, owned dogs are often not restrained and are allowed to roam freely in developing countries.^{25,26} Therefore, owned dogs can be equally at risk for contracting rabies compared with stray dogs. A previous study reported that the sex ratio of rabid dogs in Thailand was 60% male and 32% female.²¹ Similarly in Mexico, 69% of the rabid animals during an epizootic were males.²⁷ However, sex was not a significant risk factor for dog rabies in the present study.

A previous study in Thailand showed that rabid dogs displayed at least 2 of 17 clinical signs (slack jaw, abnormal bark, dry-flaccid tongue, licking its own urine, abnormal licking of water, regurgitation, altered behavior, biting and eating abnormal objects, aggression, biting with no provocation, running without apparent reason, stiffness upon running or walking, restlessness, bites during quarantine, appearing sleepy, ataxia, and frequent demonstration of the dog sitting position) during their last week of life.¹⁵ Five additional criteria (age > 1 month, sick < 10 days, gradual onset of illness, symptoms and signs progressing in the last 3–5 days, and no circling or unavailable information for these five criteria) were more likely to be observed in rabid dogs in retrospective and prospective studies.

A previous study in Brazil reported that aggressive behavior was the most frequent sign (77%), followed by limb paralysis (42%), change in voice (30%), and jaw paralysis with excessive salivation (26%).²⁵ In our retrospective study, we identified six of seven clinical characteristics (behavioral changes, aggression, pharyngeal paralysis, body paralysis, anorexia, and hyperactivity) commonly associated with rabies. Among these clinical signs, aggression was the most significant variable because aggressive dogs were 11 times more likely to be rabid than non-aggressive dogs. Therefore, most dog rabies cases in southern Thailand were the furious form.

All cattle cases submitted to SVRDC during the study were reported as unvaccinated owned cattle. Thus, vaccination status and ownership were not useful as predictable rabies variables. Cattle are only accidental hosts for rabies usually after the bite of a rabid dog or animal.¹¹ In this study, 80% of cattle with a bite history were positive for rabies virus. However, 72% of cattle without a reported bite history also had positive rabies results. Therefore, bite status was not associated with rabies in our final model. It should be noted that most cattle rabies cases occurred in this region owned by small-holding farmers who raised their animals in public fields or rice fields after harvest. It is unlikely that they would know whether their cattle had been bitten by a rabid dog. Therefore, they are less likely to report a bite history. The temporal descriptive statistics show similar trends between dog and cattle rabies. Phylogenetic analysis from a previous study confirmed that the sequences obtained from non-dog sources (humans, cats, and other mammals) were similar to those obtained from rabid dogs.³ Therefore, it is reasonable to speculate that rabies virus could have been transmitted to cattle from rabid dogs, which cause virus spillover to humans, domestic animals, and wildlife.

A previous prospective study reported that major clinical signs of rabies in experimentally infected cattle included excessive salivation (100%), behavioral change (100%), muzzle tremors (80%), vocalization and bellowing (70%), aggression, hyperaesthesia and/or hyperexcitability (70%), and pharyngeal paresis/paralysis (60%).⁷ Only four clinical variables including aggression, pharyngeal paralysis, hyperactivity/hyperexcitability, and depression were significantly associated with cattle rabies in this study. Among these variables, aggression was the one most strongly associated with rabies, as reported for dogs. This result is consistent with results of Hudson and others⁷ because aggression was one of the clinical signs of the furious form of rabies documented in 70% of experimentally rabies-infected cattle.

Surprisingly, although cats had lower rabies vaccination coverage than dogs, rabies prevalence in dogs was higher than in cats. An explanation for lower occurrence of rabies in cats can be related to nature of rabies in Thailand, where the dog is the main reservoir^{5,9,10} and there is less contact between dogs and cats. In countries where dog rabies is endemic, cats do not constitute a natural reservoir and cases in cats are mainly a result of spillover, with limited to no risk of intra-species transmission.¹¹ It fully explained by the lower rate of rabies in cats despite lower vaccination coverage. Conversely, in countries such as the United States in which rabies is mainly endemic in wildlife and dogs are regularly vaccinated against rabies, the number of rabies cases is always much higher in cats than in dogs.²⁸ In addition, because cats live mainly indoors, they have a lower risk of contacting rabid dogs. In contrast, because dogs

are more likely to live outdoors and most can roam free during the day, they are more often in contact with rabid dogs.^{14,25,26}

Although multivariate analysis could not be used to interpret the association between the risk factors for rabies in cats, three variables showed significant associations by univariate analysis. The univariate model suggests that cat rabies was most often of the furious form because aggression and hyperactivity were highly associated with the odds of cats being rabid.

This study, although comprehensive in scope, had several limitations. The epidemiologic features of rabies in domestic animals included only those animals in which rabies was diagnosed by the SVRDC. Thus, any inference from this study could be reasonably used only for animals in similar regions. Owner interviews tend to have recall bias if the owners or senders did not recognize abnormal change in their animals. An additional limitation was that our study excluded incomplete records, which were high for dogs ($n = 1,219$). As a result, 43% of the suspected dogs and 3% of suspected cats and cattle were excluded from this study. However, we believe that the large sample size for the dog population ($n = 1,614$) should be adequate for the model and subsequent conclusions, and likely representative of the situation in the whole population. Because bite status, especially in dogs, is the combination between animals that had been bitten or which had bitten any other animal or person, we could not clearly identify which type of bite had the strongest association with rabies. As a result, we will need to improve our submission form to record those events separately. Consistent with the bite mode of transmission for the rabies virus, dogs with a bite history were more likely to show positive test results for rabies. We have also established that season is not a significant risk factor for dog rabies because rabies can occur year round within this region, in keeping with results of a similar report.²⁹

Under the assumption that rabies in cats and cattle were primarily the result of interactions with rabid dogs (spillover from rabid dogs), a significant correlation coefficient (r) was measured between the annual prevalence of canine and feline rabies and canine and bovine rabies and for coefficient of determination (r^2).

Vaccination is still the most effective preventive measure to control rabies, especially in its dog reservoir and other animals at risk. In Thailand, because dogs are the main reservoir of this disease, mass rabies vaccination is needed to break the transmission cycle among dogs and other domestic animals. The World Health Organization recommends that 70% of a dog population should be vaccinated to eliminate or prevent outbreaks of rabies.³⁰ In some areas, despite an estimated rabies vaccination coverage equal to or above this threshold, cases of rabies were still recorded. This finding strongly suggests an underestimation of the true dog population and the need for more accurate means of evaluating dog populations and dog rabies vaccination coverage. For instance, the dog:human ratio can be used as an alternative method to estimate the dog population.¹⁴ More effective means of rabies vaccine administration to stray and aggressive dogs via oral route may also increase the level of vaccination coverage. In addition, combining spay or neutering program or chemical sterilization with rabies vaccination campaigns should be promoted to reduce rabies transmission. Moreover, public education regarding rabies control and dog management still needs to be emphasized to decrease animal contact to interrupt rabies transmission

among domestic animals. In highly endemic areas, where cattle rabies cases were often observed, rabies vaccination in those animals might reduce the number of cases.

Received September 23, 2010. Accepted for publication April 4, 2011.

Acknowledgments: This study partially fulfills the Master of Preventive Veterinary Medicine thesis requirement for Dr. Anyarat Thiptara. We thank Dr. Chongmas Antarasena for consultations on this project; the Veterinary Research and Development Center (Southern region) of Thailand, especially colleagues in the epidemiology section, for their collaboration and help in obtaining data; all staff in the virology section for rabies detection; and Dr. Sharon Hunt Gerardo for reviewing this manuscript.

Financial support: This study supported by the Royal Thai government and the Master of Preventive Veterinary Medicine program at the University of California at Davis, School of Veterinary Medicine.

Authors' addresses: Anyarat Thiptara, MPVM Program, School of Veterinary Medicine, University of California, 1 Shields Avenue, Davis, CA, and Epidemiology Section, Veterinary Research and Development Center (Southern Region), Teewang, Thungsong, Nakhon-si-thammarat 80000, Thailand, and Department of Population Health and Reproduction, School of Veterinary Medicine, University of California, Davis, CA, E-mails: thiptara9@yahoo.com, thiptara9@dld.go.th, and athiptara@ucdavis.edu. Edward R. Atwill, Department of Population Health and Reproduction, School of Veterinary Medicine, University of California, Davis, CA, E-mail: ratwill@ucdavis.edu. Wandee Kongkaew, PhD Program, Royal (Dick) School of Veterinary Studies, The University of Edinburgh, Edinburgh, United Kingdom and Epidemiology Section, Veterinary Research and Development Center (Southern Region), Teewang, Thungsong, Nakhon-si-thammarat 80000 Thailand, E-mails: w.kongkaew@sms.ed.ac.uk and k_wand18@yahoo.com. Bruno B. Chomel, Department of Population Health and Reproduction, School of Veterinary Medicine, University of California, Davis, CA, E-mail: bbchomel@ucdavis.edu.

REFERENCES

- World Health Organization, 2009. *Human and Animal Rabies*. Available at: <http://www.who.int/rabies/en>. Accessed September 8, 2009.
- Knobel DL, Cleaveland S, Coleman PG, Fèvre EM, Meltzer MI, Miranda ME, 2005. Re-evaluating the burden of rabies in Africa and Asia. *Bull World Health Organ* 83: 360–368.
- Denduagboripant J, Wacharapluesadee S, Lumlertdacha B, Ruankaew N, Hoonsuwan W, Puanghat A, Hemachudha T, 2005. Transmission dynamics of rabies virus in Thailand: implications for disease control. *BMC Infect Dis* 5: 52.
- Division of Epidemiology, 2008. *Summary of Notifiable Diseases. Annual Epidemiology Surveillance Report*. Available at: http://203.157.15.4/Annual/Annual%202551/Part2_51/Annual_MenuPart2_51.html. Accessed September 8, 2009.
- Bowen-Davies J, Lowings P, 2000. Current perspectives on rabies 2. Review of classical rabies and its control. *In practice* 22: 170–175.
- Ott RL, 1983. Systemic viral disease: neurologic virus infection, rabies. Pratt PW, ed. *Feline Medicine*. First edition. Santa Barbara, CA: American Veterinary Publication Inc, 101–106.
- Hudson LC, Weinstock D, Jordan T, Bold-Fletcher NO, 1996. Clinical features of experimentally induced rabies in cattle and sheep. *Zentralbl Veterinarmed B* 43: 85–95.
- Ikede BO, 1999. Diseases caused by viruses and *Chlamydia*-II. Radostists OM, Gay CC, Blood DC, Hinchcliff KW, eds. *Veterinary Medicine*. Ninth edition. New York: W.B. Saunders, 1201–1208.
- Wilde H, 2005. Fox rabies in India. *Clin Infect Dis* 40: 614–615.
- Kasempimolporn S, Sichanasai B, Saengseesom W, Puempumpanich S, Sitprijia V, 2008. Stray dogs in Bangkok, Thailand: rabies virus infection and rabies antibody prevalence. *Dev Biol (Basel)* 131: 137–143.
- Wilde H, Chutivongse S, Tepsuethanon W, Choomkasien P, Polsuwan C, Lumbertdacha B, 1991. Rabies in Thailand: 1990. *Rev Infect Dis* 13: 644–652.
- Bureau of Disease Control and Veterinary Services, 2009. *Rabies Control and Prevention Plan*. Available at: <http://www.dld.go.th/dcontrol/02Planning/Planning52/Planning52.htm>. Accessed September 15, 2009.
- Kasempimolporn S, Jitapunkul S, Sitprijia V, 2008. Moving towards the elimination of rabies in Thailand. *J Med Assoc Thai* 91: 433–437.
- Kongkaew W, Coleman P, Pfeiffer DU, Antarasena C, Thiptara A, 2004. Vaccination coverage and epidemiological parameters of the owned-dog population in Thungsong District, Thailand. *Prev Vet Med* 65: 105–115.
- Tepsuethanon V, Wilde H, Meslin FX, 2005. Six criteria for rabies diagnosis in living dogs. *J Med Assoc Thai* 88: 419–422.
- Thai Meteorological Department, 2009. Available at: <http://www.tmd.go.th/info/info.php?FileID=23>. Accessed August 13, 2009.
- Centers for Disease Control and Prevention, 2003. *Protocol for Postmortem Diagnosis of Rabies in Animals by Direct Fluorescent Antibody Testing: A Minimum Standard for Rabies Diagnosis in the United States*. Available at: http://www.cdc.gov/ncidod/dvrd/rabies/Professional/publication/DFA_Diagnosis/DFA. Accessed August 13, 2009.
- Markson LM, Upcott DH, Hebert CN, 1971. A biological test for rabies using suckling mice. *Trop Anim Health Prod* 3: 89–92.
- Hosmer DW, Lemeshow S, 2000. Model-building strategies and methods for logistic regression. *Applied Logistic Regression*. Second edition. New York: John Wiley and Son Inc., 91–143.
- Department of Provincial Administration, 2009. *Annual Report of Population Density*. Available at: http://www.dopa.go.th/stat/y_stat.html. Accessed September 17, 2009.
- Mitmoonpitak C, Wilde H, Tepsuethanon V, 1997. Current status of animal rabies in Thailand. *J Vet Med Sci* 59: 457–460.
- Chomel B, Chappuis G, Bullon F, Cardenas E, de Beublain TD, Lombard M, Giambro E, 1988. Mass vaccination campaign against rabies: are dogs correctly protected? The Peruvian experience. *Rev Infect Dis* 10: 697–702.
- Mitmoonpitak C, Tepsuethanon V, 2002. Dog rabies in Bangkok. *J Med Assoc Thai* 85: 71–76.
- Flores-Ibarra M, Estrella-Valenzuela G, 2004. Canine ecology and socioeconomic factors associated with dogs unvaccinated against rabies in a Mexican city across the US-Mexico border. *Prev Vet Med* 62: 79–87.
- da Silva LH, Bissoto CE, Delbem AC, Ferrari CI, Perri SH, Nunes CM, 2004. Canine rabies epidemiology in Araçatuba and neighborhood, northwestern São Paulo State-Brazil. *Rev Soc Bras Med Trop* 37: 139–142.
- Ratsitorahina M, Rasambainarivo JH, Raharimanana S, Rakotonandrasana H, Andriamiarisoa MP, Rakalomanana FA, Richard V, 2007. Dog ecology and demography in Antananarivo. *BMC Vet Res* 5: 1–7.
- Eng TR, Fishbein DB, Talamante HE, Hall DB, Chavez GF, Dobbins JG, Muro FJ, Bustos JL, de los Angeles Ricard Y, Munguia A, 1993. Urban epizootic of rabies in Mexico: epidemiology and impact of animal bite injuries. *Bull World Health Organ* 71: 615–624.
- Blanton JD, Palmer D, Rupprecht CE, 2010. Rabies surveillance in the United States during 2009. *J Am Vet Med Assoc* 237: 646–657.
- Meesomboon V, 1985. Epidemiology of rabies in Thailand. Kuwert E, Merieux C, Koprowski H, Bogel K, eds. *Rabies in the Tropics*. Berlin and Heidelberg: Springer-Verlag, 721–725.
- Coleman PG, Dye C, 1996. Immunization coverage required to prevent outbreaks of dog rabies. *Vaccine* 14: 185–186.