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Prevalence of rabies virus infection and rabies antibody in stray dogs: A survey in Bangkok, Thailand

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Abstract

To investigate the rabies antigen and antibody prevalences among stray dogs in Bangkok, Thailand, we took both a saliva and serum sample from each of 3314 stray dogs captured once each between December 2003 and June 2004. One 2-year-old female was antigen positive in the latex-agglutination test and confirmed by reverse transcription-polymerase chain reaction. The overall antibody seroprevalence from the enzyme-linked immunosorbent assay that we used was 62% (95% CI: 54, 70%). Antibody seroprevalence was greater for dogs captured within central Bangkok (86% of 1208 dogs captured) than in the dogs captured in the outskirts of the greater metropolitan area (49% of 2106 dogs captured). If our samples of stray dogs are representative, then the seroprevalence achieved from previous vaccination campaigns is too low to protect the dog and human populations.

Keywords: Rabies virus; Rabies antibodies; Stray dog population; Seroprevalence; Thailand

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1. Introduction

In Thailand, stray dogs were the animals most frequently reported as rabid. Cases of rabies in domestic animals other than dogs have been traceable or believed to have been at the origin of exposure to rabid dogs. Dog bite-related rabies cases in human have accounted for 14 (70%) of the 20 deaths reported in 2005, of which 3 cases occurred in the Bangkok metropolis (Chuxnum and Choomkasien, 2006). Although the number of human rabies deaths is decreasing, an increasing number of patients receiving post-exposure prophylaxis (PEP) have been observed during the past decade. Over 400,000 persons received PEP in 2005, which almost quadrupled the number in 1991 (Ministry of Public Health Annual Report, 2005). An enormous amount of funding is spent on PEP by the Thai public and private sectors amounting to at least US$ 10,000,000 per year.

The reported incidence of dog bites is highest in the central part of the country, especially in Bangkok. In a sampling survey conducted by the Bureau of National Statistics and the Bangkok Metropolitan Administration (BMA) in 1999, it was estimated that ~630,000 dogs inhabit Bangkok. Moreover, approximately 110,000 (17%) of them were considered to be ownerless. The latest survey conducted in 2002 indicated that dog populations have continued to increase and are unlikely to level off in the near future. There are no reliable data indicating their relative population densities or distribution pattern in various areas throughout the city. In addition, it is difficult to conduct an accurate survey because the ownership status of these dogs might be unstable. Most stray dogs result from people raising dogs and later abandoning them. There is as yet no effective measure for controlling stray dogs in Bangkok. A costly intervention, such as shelters for stray dogs, has been implemented over the last 10 years. However, the financial burden made this intervention unsustainable and finally ineffective. Very little has been done so far to effectively control the disease in dogs. Preliminary assessment revealed that <20% of the estimated dog population was sterilized and vaccinated (Hemachudha, 2005). We investigated the current prevalence of rabies in the stray dog population of Bangkok using rabies virus antigen and antibody determinations.

2. Materials and methods

2.1. Clinical specimens

A total of 3314 dogs were seen for surgical sterilization at the Veterinary Public Health Division, Health Department, BMA during December 2003–June 2004. All of the dogs were captured from streets and public places of 50 districts in Bangkok as part of a campaign for sterilization and vaccination against rabies. General data about the dogs, such as estimated age, sex, ownership status, and location of capture were recorded by veterinary specialists. Examining teeth was the way that the approximate age of the dog was determined (The Humane Society of the United States, 1996). Any dog selected for this study was not on the premises of the owner or not confined or under control by leash or other recognized control method. Paired specimens of saliva and blood were taken from each dog. Sample collection was done with the dog under deep barbiturate anesthesia.

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Saliva was collected with a sponge swab. Procedures for collection and storage were the same as described previously (Kasempimolporn et al., 2000). A 5 ml sample of whole blood was drawn from each dog. The separated serum was stored frozen at −20 °C until analyzed. After being neutered and vaccinated, all dogs were brought back and released at the same location where they were initially captured.

2.2. Detection of rabies virus in saliva

The latex-agglutination test (LAT) was performed according to the method previously described (Kasempimolporn et al., 2000). Rabies virus antigen in dog saliva is detected by agglutination on a glass slide using latex particles coated with gamma globulin. The test results were confirmed by reverse-transcriptase polymerase chain reaction (RT-PCR) with nested primers for rabies virus antigen. Total RNA was extracted from saliva specimens using TRIzol® (GIBCO-BRL, Gaithersburg, MD). RT-PCR of 2 μg of extracted RNA was performed in a one-step process using the AccessQuick® kit (Promega, Madison, WI). Subsequently, the primary amplified product was subjected to a second-round PCR. All amplifications were carried out on a thermocycler (MWG-Biotech, Germany), using a denaturation temperature of 94 °C, an annealing temperature of 60 °C, and an extension temperature of 72 °C. Two sets of primers were used to amplify 1473 base-pair (5′-GTAACACCCCTACAATGGATGC-3′, at position 57–78, and 5′-CAAAGATCTTGCT-CATGTTTGG-3′, at position 1508–1529) and 524 base-pair (5′-GACATGTCCGGAA-GACTGG-3′, at position 319–337 and 5′-GTATTGCCTCTCTAGCGGTG-3′ at position 823–842) fragments of rabies virus nucleoprotein (N) gene, for the first- and second-round PCR, respectively. The second PCR products were electrophoresed in 1.2% agarose gels and stained by ethidium bromide.

2.3. Determination of rabies antibody in serum

The technique used was an enzyme-linked immunosorbent assay (ELISA). The wells of polystyrene plates were coated overnight at 4 °C with 1:100 purified Vero-cell rabies vaccine (Aventis Pasteur, France) in 0.05 M carbonate–bicarbonate buffer, pH 9.6. Plates were then quenched with 1% BSA for 30 min at 37 °C. Serum samples diluted at 1:10 were added to the plates and incubated for 1 h at 37 °C. Horseradish peroxidase-conjugated rabbit anti-dog IgG (Sigma, St. Louis, MO), at the dilution recommended by the manufacturer, was then added and followed by orthophenylenediamine (Sigma) to develop a color reaction. Absorbance was read on an ELISA reader at 492 nm. Between all steps of the reaction, plates were washed with PBS containing 0.05% Tween. For quantitative assay, a standard curve was established with anti-rabies standard serum (Statens Serum Institute, Copenhagen, Denmark) titrated in international units per milliliter (IU/ml). The test serum was read off the standard curve. Antibody levels were expressed as equivalent units per milliliter (EU/ml) corresponding to IU/ml.

Calculations of sensitivity and specificity of the ELISA were established by testing serum from vaccinated ($n = 55$) and naïve or non-vaccinated ($n = 37$) dogs. A working group recommended that a minimum value of 0.5 IU/ml of neutralizing antibodies should be attained to demonstrate seroconversion in humans, and we used the same for the dogs

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(WHO, 1992). Choosing the status of the dogs (naïve and vaccinated) as the reference, the sensitivity and specificity were 0.85 and 0.95, respectively. An estimated true prevalence of antibodies among stray dogs was calculated using the Rogan-Gladen-estimator and the confidence interval (CI) was set at 95% (Greiner and Gardner, 2000).

3. Results

All dogs tested were strays. Of these, 1425 dogs were male (43%), 1866 dogs were female (56%) and in 23 dogs sex was not recorded (1%). Estimated ages of dogs ranged from 1 month to >8 years.

The saliva of a 2-year-old female dog was found positive by both LAT and RT-PCR, as revealed by the appearance of a 524 base-pair fragment of rabies virus N gene. Saliva samples from dogs which previously known to be positive or negative for rabies virus infection were used as controls (Fig. 1). The prevalence of rabies virus infection among stray dog investigated was 0.03% (1 of 3314 dogs).

Fig. 2 shows a map of the greater Bangkok metropolitan area. Bangkok, with total area of 1600 km^2, is divided into 50 districts. We categorized 19 districts containing mostly high-rise business buildings as ‘‘central Bangkok’’. We called the remaining densely populated districts with individual houses and crowed tenements ‘‘Bangkok’s outskirts’’. Rabies antibody seroprevalence among stray dogs in each of the 50 districts ranged from 35 to 100% (data not shown). Seroprevalence $\geq$70% was found in only 20 districts (Fig. 2).

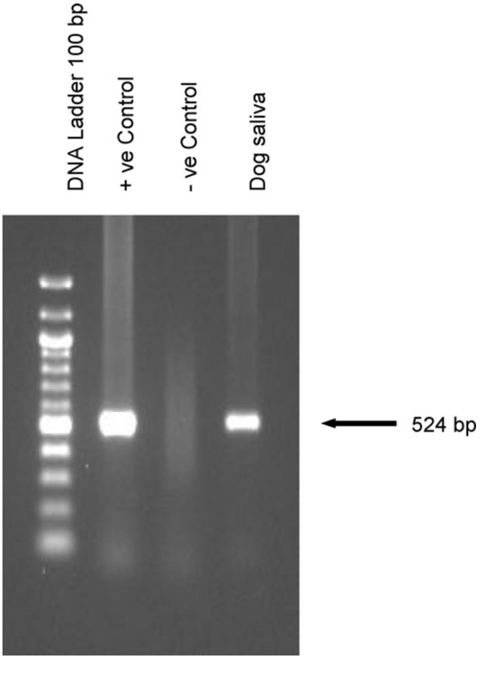

Fig. 1. Detection of rabies virus RNA in saliva specimen by RT-PCR.

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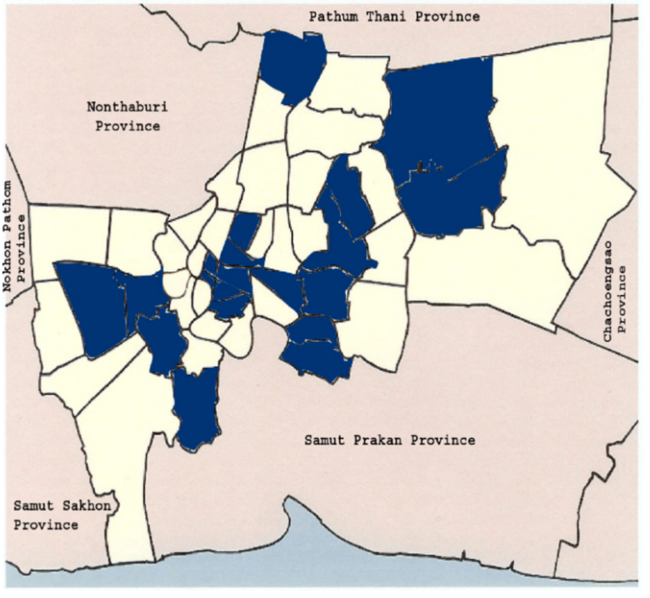

Fig. 2. Map of Bangkok depicting the 50 districts. Shaded surface areas represent the districts in which the antibody seroprevalence among stray dogs reached 70% in 2004, as recommended by WHO.

Most dogs which came from central Bangkok had specific antibodies above the WHO-recommended protective antibody level of 0.5 EU/ml, and the seroprevalence for the central area was 86% (95% CI: 76, 96%). In contrast, most dogs from the outskirts of the capital did not have protective titers and the seroprevalence in the outskirts was only 49% (95% CI: 41, 57%). The percentage of seropositivity tended to increase after the age of 4 months (Table 1). The overall seroprevalence of rabies antibodies among stray dogs in Bangkok was 62% (95% CI: 54, 70%).

Table 1
Seroprevalence of rabies antibodies among stray dogs in the central and the outskirts of Bangkok classified by estimated age, based on an ELISA cut-off level of 0.5 EU/ml

Age	Central Bangkok				Bangkok's outskirts			
	No. of positive	No. of tested	Adjusted seroprevalence		No. of positive	No. of tested	Adjusted seroprevalence	
			%	95% CI			%	95% CI
1–3 months	1	3	35	28–42	1	12	4	0–13
4–11 months	6	12	56	48–64	9	20	50	42–58
1–4 years	655	870	88	77–99	672	1628	45	38–52
5–8 years	202	275	86	76–96	218	382	65	56–74
>8 years	6	10	69	60–78	11	19	66	57–75
Not recorded	17	38	50	42–58	22	45	55	47–63
Total	887	1208	86	76–96	933	2106	49	41–57

**The WHO-recommended minimal acceptable antibody level.

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4. Discussion

Public attention and fears are most focused on dogs as the source of rabies. Dog-bite victims often experience prolonged anxiety because knowledge of rabies is widespread. Most PEP is rendered without knowledge as to whether the biting dog was indeed rabid, and dog bites represent a huge financial burden to governments that must render PEP for animal-bite recipients. The natural epidemiologic transmission of dog rabies to humans depends on the relationship of dogs to humans and on the density of the dog population. Dog-population density is usually associated with the socioeconomic values and habits of a society. Prevention of human rabies primarily depends on controlling canine rabies; this can achieved by mass vaccination and elimination of stray-dog population (WHO, 1984). However, control by elimination of strays is against religious principles for the Bangkok public.

The routine diagnosis of rabies in dogs is made on brain tissue. Ante mortem diagnosis looking for evidence of the virus in dog saliva can also be carried out (Kasempimolporn et al., 2000), but even the latest laboratory methods can be problematic. The sensitivity of all techniques during life varies with the stage of clinical illness, and serial samples should be tested because the virus is shed intermittently (Niezgoda et al., 2002). We might have found more rabid dogs, had we tested each dog repeatedly.

According to WHO recommendation, at least 70% of the dog population must be continuously vaccinated to control rabies transmission (WHO, 1984). Little is known about the vaccination status in stray dogs. Between 3 and 9% of dogs found rabid have a vaccination history. Furthermore, some dogs vaccinated with a one-dose primary injection fail to produce long-lasting neutralizing antibodies (Sage et al., 1993). The minimum level of circulating antibodies to rabies virus that is protective in dogs is not known and WHO has not defined a minimum antibody titer response in dogs. A standard antibody titer of 0.5 IU/ml is arbitrarily considered to be the minimal acceptable level in humans indicating an adequate immune response to the vaccine (WHO, 1992). Most stray dogs often receive only one rabies vaccine injection. They neither carry a collar, nor other sign of having been vaccinated at all.

The seroprevalence went up after 1 year old, indicating that the majority of dogs at these age groups had already been vaccinated at least once (Table 1). The main concern is that dogs younger than 1 year of age, especially at 1–3 months old, are clearly at higher risk of rabies (Table 1). According to the Rabies Prevention and Control in Domestic Animals Act 1992, all pet dogs (and cats) must receive rabies vaccine injection for the first time at 2–4 months of age and annual boosters after that. The age at vaccination has, nevertheless, been controversial. It is generally recommended that puppies should not be vaccinated before the age of 3 months. The reasons given are that puppies have passive antibodies of maternal origin and an immature immune system (Bunn, 1991). However, only 16% of Thai puppies had evidence of rabies antibody before primary vaccination (Kasempimolporn et al., 1996). Those data are consistent with our findings. Approximately, 15% of dog rabies cases were in puppies under 3 months of age (Ministry of Public Health Annual Report), evidence that these animals were being exposed to rabies early in life. Of the human rabies cases, 57% were bitten by puppies at this age group (Chuxnum and Choomkasien, 2006).

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An intensive citywide rabies vaccination campaign was initiated in Bangkok in June 2002, with the specific aim of eliminating dog rabies throughout the city. The Thai government has invested vast sum of money on this effort. However, the seroprevalence in most districts still has not reached 70% (Fig. 2). The overall antibody seroprevalence was 62% (95% CI: 54, 70%), varying from 35 to 100% by district. In addition, the percentage of rabies positive canine specimens from the Bangkok metropolis during this period remained high (23–29%) (Tepsumethanon and Sitprija, 2005). This indicates that the infection is still prevalent in Bangkok. Low vaccination coverage and ineffective management of stray dogs are the most likely reasons for the dog rabies elimination program's lack of success. Public-health authorities consider mass vaccination of dogs the primary tool for control of rabies. However, often it is possible to capture stray or semi-owned dogs only once for vaccination. WHO-coordinated research might bring about oral dog rabies vaccination as an adjunct to parenteral mass vaccination. This might facilitate rabies control where dogs are difficult to vaccinate by injection. However, the relatively high amount of oral vaccine virus required and low rate of seroconversion still call for consideration improvement (Bogel, 2002). Developing a mass vaccination and sterilization campaign in Bangkok city alone without considering neighboring areas is unlikely to be successful. Translocation of dogs from neighboring regions is believed to have occurred regularly and could be the source of infection. Dog vaccination levels must be sufficient to break dog-to-dog transmission cycles. Focal vaccination campaigns are to be conducted promptly whenever cases of rabies appear in any neighborhood, in band of at least 5 km wide around the contact zone. This has been part of Thailand's official rabies control programs. Vaccination campaigns at intervals >1 year were not sustainable, partly due to the short life expectancy of stray dogs and their rapid population turnover. Canine surgical sterilization was not sustainable and not effective in importantly reducing a large stray dog population. Finally, from our study, it can be said that at least 30–46% of the known number of dogs are not covered by vaccination. If this is considered in relation to the total number of dogs in Bangkok, there is a large population of unvaccinated dogs.

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