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Rabies immune status in the stray and companion dogs in Korea

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Abstract : Rabies virus (family *Rhabdoviridae*, genus *Lyssavirus*, RV) is the causative agent of rabies in mammals. We conducted a sero-epidemiological survey for RV using sera from South Korean stray and companion dogs in the present study. A total of 533 canine serum samples were collected between February 2006 and December 2007 and were screened for rabies immunity with a neutralizing peroxidase linked assay. Both companion (49.1%) and stray (60.1%) dogs demonstrated RV seropositivity. Regional RV antibody prevalence was measured in the Jeju (87.5%), Gyeonggi (62%), Gyeongsang (59.1%), Jeonra (42%), Chungcheong (37.9%), and Gangwon (30.4%) provinces. Prevalence increased with age but did not exceed 80% in any age group. Stray and companion dogs had RV antibody prevalence values of 26.7% and 23.7%, respectively. Seroprevalence was significantly associated with age ($\chi^2 = 9.46$; $p = 0.024$) for companion dogs, although this association was not evident in stray dogs. There were no significant differences in age between stray and companion dogs and no gender differences in RV seroprevalence. Our results suggested that a widespread and reinforced vaccination program must be applied to Korean dogs.

Keywords : companion dog, rabies, sero-epidemiology, stray dog

Rabies is an important zoonotic disease that results in approximately 50,000 human deaths worldwide annually [20]. It is caused by the rabies virus (RV), which causes fatal encephalitis in all mammals, including human beings. The virus (genus *Lyssavirus* and family *Rhabdoviridae*) has a non-segmented, negative, single-stranded RNA genome which encodes five structural proteins, including nucleoprotein, phosphoprotein, matrix protein, glycoprotein, and large protein [1]. Rabies transmission primarily occurs due to a bite from an infected rabid animal [21], and the incubation period is dependent upon the bite area [5]. The first case of rabies in Korea was reported in 1907, and many rabies cases were identified nationwide until 1945 [12] due to a lack of an effective vaccine-based rabies control program. Korean dogs and livestock were vaccinated in the 1960s with an inactivated rabies vaccine created with brain tissue from rabies Pasteur strain-infected rabbits and calves. The Flury-LEP vaccine, manufactured from infected chicken fetuses, was used in 1980; this vaccine had complex manufacturing processes, questionable safety, and a restricted target animal [15]. Tissue culture-attenuated rabies virus was brought to Korea from Canada to produce higher quality vaccine (Evelyn-Rokitnicki-Alelseth; ERA strain). The virus was cloned three times by limited dilution methods using primary porcine kidney cells [10, 11]. The live vaccine (licensed in 1980) was used in both companion and domestic animals under effective vaccine control programs after safety, immunogenicity, and stability studies. This nationwide rabies control program demonstrated that rabies cases dramatically decreased to an average of 32 cases per year until 1984 [7, 12]. There were no rabies cases identified between 1985 and 1992. However, rabies cases in both domestic and wild animals have recently re-emerged around demilitarized zones in Gangwon and Gyeonggi provinces in Korea [18]. Rabies vaccines have been widely used in companion and domestic animals such as cattle, dog, cat and raccoon dog throughout Korea since that time. Vaccination of all animals throughout outbreak areas is obligatory and free of charge by the government.

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The number of pet owners has significantly increased in Korea since the 1990s, likely due to higher income. The number of elderly companion animals has also simultaneously increased. Unfortunately, pet animals have been abandoned due to various reasons, including public sanitation, zoonotic concerns, and noise concerns. To our knowledge, there have been a few reports evaluating protective RV antibodies in South Korean stray and companion animals [2]. This will allow for a better understanding of the number of immunized animals and the efficacy of RV vaccine programs. We investigated RV sero-surveillance to estimate vaccination status in the present study.

Blood samples were obtained from stray dogs from animal shelters (n = 238) and companion dogs from veterinary clinics (n = 295) to measure RV sero-surveillance between February 2006 and December 2007. The blood was taken from cephalic vein. Clotted blood samples were centrifuged at 3,000 × g for 10 min, and sera were stored at –20°C until use. Estimated age was determined according to the dental eruption and dental morphology [4]. The ERA RV strain (previously used as a vaccine strain in Canada and Korea) was used for the neutralizing peroxidase-linked assay (NPLA) [10]. Vero cells were used to propagate RV antigen in culture with α-minimum essential medium (MEM; Gibco BRL, USA) containing antibiotics (100 IU/mL penicillin, 100 μg/mL streptomycin), an antimycotic (0.25 μg/mL amphotericin B), and 10% fetal bovine serum (Gibco BRL, USA). Monolayered Vero cells were rinsed twice with PBS and inoculated with the ERA strain for propagation. Cultures were incubated in α-MEM for 7 days after adsorption at 37°C for 1 h. The harvested virus was clarified by centrifugation for 30 min at 3,000 × *g* to remove cellular debris after three freeze-thaw cycles. The propagated RV was used as the antigen in the NPLA test. The NPLA was performed in 96-well microplates [3]. Briefly, sera were inactivated for 30 min at 56°C, serially diluted (50 μL) two-fold in α-MEM (50 μL), mixed with an equal volume of 100-200 $FAID_{50}$ (fluorescent assay infectious dose) of the ERA strain, and incubated at 37°C for 1 h. Vero cells (100 μL) were added to each well at a concentration of 2×10^4 cells in α-MEM containing 10% fetal bovine serum. Microplates were incubated for 5 days at 37°C in a 5% CO_2 incubator. Plates were washed once with PBS and fixed with 80% cold acetone for 15 min. Anti-RV mAb was added to each well and incubated for 40 min at 37°C. Plates were washed three times and then stained with chromogen substrate solution 3′, 3-diaminobenzidine (Sigma, USA) and hydrogen peroxidase for 30 min at 37°C. Serum titers were recorded as the reciprocal of the highest initial dilution of sera which

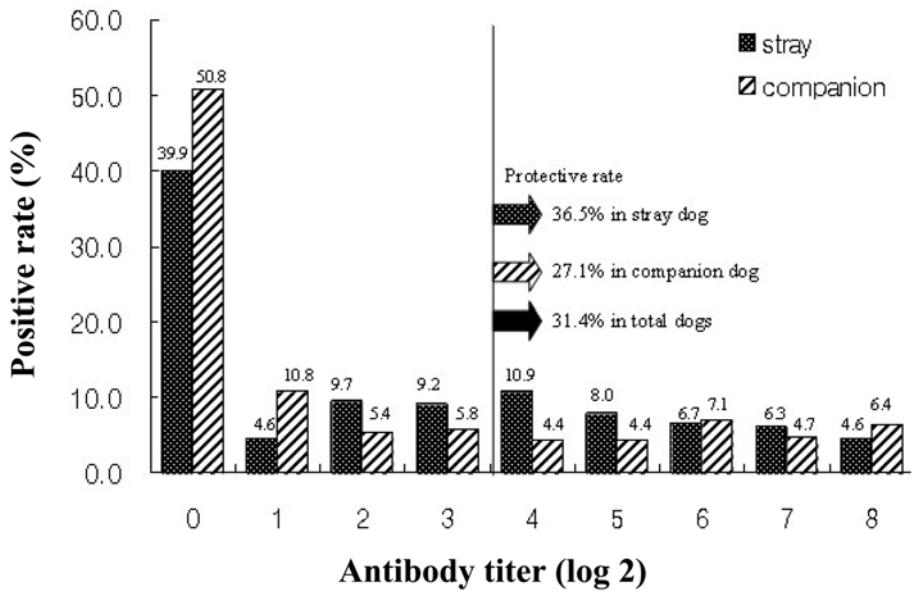

Fig. 1. Frequency distribution of antibody titers from stray and companion dogs.

Table 1. Regional distribution of rabies virus (RV) antibody-positive stray and companion dogs in Korea

Designation	Province*						Total
	GG	GW	CC	JR	GS	JJ	
Stray	96/143	2/6	13/29	7/18	21/37	4/5	143/238
(%)	(67.1)	(33.3)	(44.8)	(38.9)	(56.8)	(80.0)	(60.1)
Companion	64/115	5/17	20/58	22/51	31/51	3/3	145/295
(%)	(55.7)	(29.4)	(34.5)	(43.1)	(60.8)	(100)	(49.1)
Total	160/258	7/23	33/87	29/69	52/88	7/8	288/533
(%)	(62.0)	(30.4)	(37.9)	(42.0)	(59.1)	(87.5)	(54.0)

*GG, Gyeonggi; GW, Gangwon; CC, Chungcheong; JR, Jeonra; GS, Gyeungsang; and JJ, Jeju.

Table 2. Distribution of antibody-positive samples against RV stratified by age in Korean dogs

Designation	Age of dogs (year)				Total
	< 6 months	> 6 months-2.0	> 2.0-5.0	> 5.1	
Stray	4/15	75/120	41/73	23/30	143/238
(%)	(26.7)	(62.5)	(56.7)	(76.7)	(60.1)
Companion	14/59	30/68	79/126	22/42	145/295
(%)	(23.7)	(44.1)	(62.7)	(52.4)	(49.1)
Total	18/74	105/188	120/199	45/72	288/533
(%)	(24.3)	(55.9)	(60.3)	(62.5)	(54.0)

neutralized rabies virus replication in 100% of the wells. Serum samples demonstrating VN titers greater than 1 : 2 were considered positive and greater than 1 : 16 were considered protective to wild-virus infection [19]. Chi-square tests were used to analyze differences in seroprevalence stratified by age, gender, and geographic region. Statistical significance was defined at p-values < 0.05.

The results of RV sero-surveillance are shown in Tables 1, 2, and 3 and in Fig. 1. The average positive rate for RV seroprevalence was recorded as 54.0% among all tested dogs. The regional seroprevalence for stray dogs was 80.0% (4/5) in Jeju, 67.1% (96/143) in Gyeonggi, 56.8% (21/37) in Gyeongsang, 44.8% (13/29) in Chungcheong, 38.9% (7/18) in Jeonra, and 33.3% (2/6) in Gangwon. Seroprevalence for companion dogs was 100% (3/3) in Jeju, 60.8% (31/51) in Gyeongsang, 55.7% (64/115) in Gyeonggi, 43.1% (22/51) in Jeonra, 34.5% (20/58) in Chungcheong, and 29.4% (5/17) in Gangwon (Table 1). No significant differences were noted in regional prevalence, although the prevalence differed considerably by geographic region. RV vaccine antibody titers increased with age in the present study. However, positive rates were not greater than 80% at all ages. Further, very low antibody seroprevalence was measured in both stray (26.7%) and companion (23.7%) dogs younger than 6 months old. Seroprevalence was significantly associated with age ($\chi^2 = 9.46$; $p = 0.024$) for companion dogs but not for stray dogs. However, no age differences were identified between stray and companion dogs. Female dogs demonstrated a slightly higher seroprevalence (58.6%; 139/237) than males (50.2%; 149/297). As shown in Fig. 1, dogs with protective antibody titers greater than 1 : 16 (31.4%) and stray dogs (36.5%) both demonstrated measurably greater protective responses than companion dogs (27.1%). The most frequent VN titers in stray and companion dogs were 1 : 16 (10.9%) and 1 : 2 (10.8%) respectively.

Table 3. Distribution of positive rates against RV antibody stratified by sex

Designation	Male	Female
Stray	78/138	65/101
(%)	(56.9)	(64.4)
Companion	71/159	74/136
(%)	(44.7)	(54.4)
Total	149/297	139/237
(%)	(50.2)	(58.6)

Rabies outbreaks have been reported in most countries, including canine and bovine cases identified

in Korea [7, 8, 12]. Hyun *et al.* [13] have also reported consistent rabies outbreaks in both domestic and wild animals from 1994-2005. Rabies vaccination is recommended for prevention in all companion animals and is mandatory in all animals located near rabies epidemics. Several types of rabies vaccine have been used in domestic and companion Korean animals. Approximately 1,060,000 live rabies vaccine doses are produced annually in domestic veterinary biological companies; inactivated rabies vaccine doses (n = 538,000) and oral vaccine doses (n = 388,000) are imported to immunize domestic, pet and wild animals [9].

We estimated the nationwide rabies immune status of stray and companion dogs in the present study through NPLA testing of canine sera obtained from rescue centers and veterinary clinics. Rabies seroprevalence in stray dogs has been previously estimated from 27.7% in Japan to 62% in Thailand [6, 17]. Cho *et al.* [2] reported that 35% of dogs near the Pukhansan National park and Seoul city were seropositive against rabies. The average prevalence of antibody against rabies was 54.0% in the present study (Table 1) considering that the detected antibodies are created by vaccination. The positive rate in Korean stray dogs was greater than rates in Japan and lower than those in Thailand and higher than the rates reported in Korean dogs 2001 [2]. In addition, the positive rate in the stray dogs was greater than that of companion dogs, suggesting that some stray dogs may have been initially reared as pet animals. The serological data analysis demonstrated that the regional prevalence ranged from 30.4% to 87.5%, depending on the province. Seropositive rates in the Gangwon province were lowest, suggesting that low levels of rabies antibody seroprevalence could be correlated with rabies outbreaks in the Gangwon province and contact with raccoon dogs as carriers of rabies and domestic animals could increase the incidence of rabies. As shown in Table 2, rabies seroprevalence by age increased with increasing age, and was slightly decreased in companion dogs over 5 years old, suggesting an immune response against repeated vaccinations. Female dogs had a greater antibody prevalence than males, although this was not statistically significant (p = 0.542). Animals with antibody titers < 0.5 IU/mL or VN titers < 1 : 16 developed severe clinical signs of rabies after challenge with virulent RV. However, rabies can be prevented if protective antibody titers are present in the blood [14, 16, 21]. Only 31.4% of dogs had protective antibody titers of 1 : 16 or above in the present study, suggesting that reinforced vaccination programs should be employed to elevate immunity against rabies and to successfully eradicate canine rabies infection in Korea.

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