

## Serologic Detection of Antibodies Against Rabies Virus in Dogs from Animal Shelters in Seoul, South Korea

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**Abstract:** Outbreaks of rabies in South Korea are no longer limited to the demilitarized zone or non-residential areas. Vaccination is a key factor for preventing virus transmission. The present study was conducted to measure the level of antirabies antibody in dogs from animal shelters in Seoul, South Korea. A total of 1,750 sera samples (999 collected in 2011 and 751 collected in 2012) from dogs kept at abandoned animal shelters in Seoul were tested using a commercial ELISA kit. The overall rate of antirabies antibody prevalence was 24.0% (420/1,750) in 2011 to 2012. The rate of antirabies antibody prevalence was 25.6% (256/999) in 2011 and 21.8% (164/751) in 2012. When analyzing the data according to age, the prevalence rates for young ( $\leq 1$  year), adult ( $>1$  and  $\leq 5$  years) and older ( $>5$  years) dogs were 12.7, 26.4 and 30.8%, respectively in 2011. These rates were 4.5, 17.7 and 30.4%, respectively in 2012. These results demonstrate that prevalence of the rabies virus increased with age ( $p < 0.005$ ). The prevalence rates between male and female dogs were 25.9 and 25.3%, respectively in 2011 and 22.3 and 21.2%, respectively in 2012. When the data were evaluated according to region, the prevalence rates of dogs from the North and South of the Han River were 27.2 and 24.4%, respectively in 2011 and 21.9 and 21.8%, respectively in 2012. The prevalence rates were similar throughout the year with Spring and fall rates of 26.0 and 25.3%, respectively in 2011 and 22.0 and 21.7%, respectively in 2012. Since, an immunological barrier that prevents from spreading of rabies virus is considered to be  $>70\%$  of dogs vaccinated, findings from the present study indicate that the prevalence of antirabies antibodies from shelter dogs in Seoul is still not sufficient for preventing transmission of the rabies virus. Thus, more intensive vaccination campaigns and programs for reducing the number of stray dogs are prerequisites for the prevention and control of rabies.

**Key words:** Antirabies antibody, ELISA, seroprevalence, shelter dog, vaccination, Seoul

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### INTRODUCTION

Rabies is a fatal viral disease of warm-blooded animals caused by the rabies virus that can be transmitted through the bite of animals infected by the virus (Kang *et al.*, 2007). The rabies virus is neurotropic and infects the central nervous system resulting in encephalopathy and ultimately death (Timoney *et al.*, 1988). It is a single stranded RNA virus belonging to the genus *Lyssavirus* of the family *Rhabdoviridae* (Pringle, 1991; Wunner *et al.*, 1995; Kang *et al.*, 2007). Rabies remains an important zoonotic disease found in both the developed and developing world (Gongal and Wright, 2011). Each year, cases of rabies are reported in  $>150$  countries and cause the death of 55,000 people, especially in Asia and Africa (WHO, 2013). About 40% of

individuals who are bitten by animals suspected to be rabid are children  $<15$  years of age (WHO, 2013). Almost all patients who develop clinical features die from the disease.

Several diagnostic methods have been used to study the structure, histopathology, molecular typing and virulence of rabies virus. These techniques include the Fluorescent Antibody Test (FAT), electron microscopy, histologic examination, immunohistochemistry, reverse transcription PCR and isolation from cell cultures (Anthony and Werner, 1994; Dean *et al.*, 1996; Warner *et al.*, 1997; Loza-Rubio *et al.*, 2005). Some assays require specialized laboratory equipment with expertise. More recently, ELISAs with high sensitivity and specificity have become available (Servat *et al.*, 2007; Singh *et al.*, 2011).

The first official outbreak of rabies among animals in Korea was reported in 1907 (Kim *et al.*, 2006; Oh *et al.*, 2012). The virus was subsequently reported every year with various prevalence rates until 1984. From 1985 to 1992, no rabies outbreaks occurred in the country (Kim *et al.*, 2006; Oh *et al.*, 2012). In 1993 however, a rabies-infected dog was identified in Cholwon city of Gangwon Province located in the Northern part of South Korea adjacent to the wild terrestrial animals preserved demilitarized zone (Kang *et al.*, 2007). Since then, 7-61 rabid animals in the country have been reported every year. The majority of rabies cases reported annually, since 1996 have occurred in dogs that transmit the virus to other animals and humans (Joo *et al.*, 2011).

Raccoon dogs are the only wildlife species in South Korea in which has been reported and these animals serve as a principal rabies vector in Gangwon Province (Shin *et al.*, 2004). Recent studies showed that stray dogs which come into contact with rabid raccoon dogs can acquire and transmit the rabies virus (Joo *et al.*, 2011; Oh *et al.*, 2012).

Over the recent past decades, outbreaks of rabies have gradually spread from the demilitarized zone to adjacent Southern Provinces of South Korea. Thus, a national rabies control program has been established for domestic and wild animals using a rabies bait vaccine. It has been estimated that vaccinating >70% of dogs would be sufficient to create an immunological barrier that prevents the spread of the rabies virus (Olugasa *et al.*, 2011). It is imperative to maintain a specific level of antirabies antibody in the animals to confer effective protection and prevent virus transmission. The current study was performed to assess the level of antirabies antibody in the sera of dogs from animal shelters in Seoul, South Korea and evaluate the vaccination status of stray dogs in the area.

## MATERIALS AND METHODS

**Blood samples:** A total of 1,750 blood of dogs that were kept at abandoned animal shelters located throughout Seoul were collected in 2011 and 2012. The blood samples were taken from 500 dogs in the Spring of 2011, 499 dogs in the fall of 2011, 387 dogs in the spring of 2012 and 364 dogs in the fall of 2012. Blood was collected from the cephalic or jugular vein and sera were recovered according to standard procedures and stored at -20°C until serological analysis. The serum samples were analyzed according to survey year, the age and gender of the dogs, region where the dogs resided and season when the blood samples were drawn.

Age groups were divided into young ( $\leq 1$  year), adult ( $>1$  and  $\leq 5$  years) and older ( $>5$  years) dogs. Study area of Seoul was divided into regions from North and South of the Han River which divides Seoul into North and South.

**Detection of antirabies antibody:** The concentration of antirabies antibody in the dogs was measured with a commercial Platelia™ Rabies II kit (Bio-Rad, Hercules, CA, USA) according to the manufacturer's instruction. Positive and negative control sera were provided with the kit. The optical density was read at wavelengths of 450 and 620 nm with an ELISA reader (Infinite M200, Tecan, Mannedorf, Switzerland). A titer of  $>0.5$  IU mL<sup>-1</sup> was considered positive based on the WHO criteria (Olugasa *et al.*, 2011; WHO, 2013).

**Statistical analysis:** A  $\chi^2$ -test was used to analyze differences among the variables (Version 5.04; GraphPad Software Inc., La Jolla, CA, USA). The  $p < 0.05$  were considered statistically significant.

## RESULTS

### Seroprevalence according to survey year and animal age:

A total of 1,750 sera samples (999 in 2011 and 751 in 2012) were collected from dogs kept at animal shelters located throughout Seoul and analyzed with an ELISA. The overall prevalence rate of antirabies antibody was 24.0% (420/1,750) for 2011 to 2012 (Table 1). The annual rates were 25.6% (256/999) in 2011 and 21.8% (164/751) in 2012. The prevalence rates among young ( $\leq 1$  year), adult ( $>1$  and  $\leq 5$  years) and older ( $>5$  years) dogs were 12.7% (20/157), 26.4% (142/537) and 30.8% (94/305), respectively in 2011 and 4.5% (4/88), 17.7% (58/328) and 30.4% (102/335), respectively in 2012. Seroprevalence of the antirabies antibodies in 2011 and 2012 varied significantly according to the age of the animals ( $p < 0.001$ ).

### Seroprevalence according to gender, region and season:

When assessed according to gender, the seroprevalence was 25.9% (152/588) for male dogs and 25.3% (104/411) for females in 2011 and 22.3% (92/412) for males and 21.2% (72/339) for females in 2012 (Table 2). No significant differences in seroprevalence between male and female dogs were observed ( $p > 0.05$ ).

The prevalence rates for dogs from the North and South of the Han River were 27.2% (119/438) and 24.4% (137/561), respectively in 2011 and 21.9% (75/342) and 21.8% (89/409), respectively in 2012. No significant difference in seroprevalence was observed between the two regions ( $p > 0.05$ ).

Table 1: Prevalence of antirabies antibodies in dogs from animal shelters in Seoul according to survey year and age

Age	2011		2012	
	No. of tested	No. of (%) positive*	No. of tested	No. of (%) positive**
Young (≤1 year)	157	20 (12.7)	88	4 (4.5)
Adult (>1 and ≤5 years)	537	142 (26.4)	328	58 (17.7)
Older (>5 years)	305	94 (30.8)	335	102 (30.4)
Total	999	256 (25.6)	751	164 (21.8)

\*Statistically significant difference ( $p = 1.12 \times 10^{-4}$ ); \*\*Statistically significant difference ( $p = 5.92 \times 10^{-8}$ )

Table 2: Prevalence rates of antirabies antibodies in shelter dogs according to gender, region and season

Variables	2011		2012	
	No. of tested	No. of (%) positive	No. of tested	No. of (%) positive
<b>Gender</b>				
Male	588	152 (25.9)	412	92 (22.3)
Female	411	104 (25.3)	339	72 (21.2)
<b>Region</b>				
North of the Han River	438	119 (27.2)	342	75 (21.9)
South of the Han River	561	137 (24.4)	409	89 (21.8)
<b>Season</b>				
Spring	500	130 (26.0)	387	85 (22.0)
Fall	499	126 (25.3)	364	79 (21.7)

In 2011, the prevalence rates were 26.0% (130/500) in the Spring and 25.3% (126/499) in the fall. In 2012, these rates were 22.0% (85/387) in the Spring and 21.7% (79/364) in the fall. These findings demonstrate that seroprevalence did not vary significantly according to season in either 2011 or 2012 ( $p > 0.05$ ).

**DISCUSSION**

Rabies is a fatal disease that is most commonly transmitted to humans through the bites of rabid dogs, especially in developing or underdeveloped countries (Olugasa *et al.*, 2011; WHO, 2013). Each year, this disease causes the death of about 55,000 people in >150 countries (WHO, 2013). Animal vaccination is a key factor for rabies prevention and the control of virus transmission. The WHO recommends that countries where the rabies is endemic should implement a preventive vaccination program for dogs (Singh *et al.*, 2011; WHO, 2013). For successful disease control, it is important that vaccinated animals maintain a protective level of antirabies antibodies.

In South Korea, rabies outbreaks have continuously occurred since, 2001 only in the Gyeonggi and Gangwon Provinces near the wild terrestrial animals preserved demilitarized zone (Joo *et al.*, 2011; Oh *et al.*, 2012). The occurrence of rabies has been confined to dogs and cows since, 2003. It was observed that infection in wild animals was limited to only raccoon dogs (Oh *et al.*, 2012). In 2011,

about 270,000 dogs were bred in Seoul; this represented a significant increase compared to an average of 150,000 dogs bred from 2006 to 2010. However, the number of abandoned dogs has decreased since, 2010. This might be due to changes in pet owner’s awareness and a newly revised act of animal protection in which all the dogs with >3 months must be enrolled. The decreased number of abandoned dogs is expected to suppress rabies virus transmission.

The current study was performed to detect antirabies antibody in dogs housed in abandoned animal shelters of Seoul between 2011 and 2012. A total of 1,750 sera samples (999 collected in 2011 and 751 collected in 2012) from the dogs were analyzed. The overall prevalence rate of antirabies antibody was 24.0% (420/1,750). The annual prevalence rates were similar with 25.6% (256/999) in 2011 and 21.8% (164/751) in 2012. The rates observed in this investigation were lower compared to the one reported in a earlier study (35%) on the stray dogs from South Korea in 2000 (Cho *et al.*, 2001). Furthermore, the seroprevalence observed in the present study was lower than those obtained in similar surveys (Kasempimolporn *et al.*, 2007; Ogawa *et al.*, 2009) conducted in other countries including Japan (27.7%) and Thailand (62%). Variation in the results could be due to differences in the study methods, animals or regions. While the low rates observed in the current investigation should be interpreted with caution, these findings could indicate that there is a need to reevaluate the current bait vaccine strategy against rabies, especially in stray dogs.

Seroprevalence significantly increased with age among dogs evaluated in the current study. This might be due to the fact that adult dogs move over a larger range than puppies which tend to remain in one location. This result was in agreement with findings from earlier studies conducted in other countries (Kasempimolporn *et al.*, 2007; Belsare and Gompper, 2013). No significant difference in gender was observed in the present study while another investigation showed that the prevalence rate among male dogs is higher than that for females (Belsare and Gompper, 2013). Likewise, no significant difference was found between animals kept at animal shelters located at North and South of the Han River in this study. This could be due to the fact that the study was conducted in an urban area (Seoul) that is geographically and climatically homogenous.

A principal host of the rabies virus in South Korea and other endemic countries is the raccoon dog (Joo *et al.*, 2011). This wild animal can transmit the rabies virus to stray dogs roaming in a city which in turn could transmit the virus to other domestic animals and humans

(Joo *et al.*, 2011; Oh *et al.*, 2012). Since, the first case of animal rabies in Korea was reported in 1907 (Kim *et al.*, 2006; Oh *et al.*, 2012), incidences of this disease have been continuously observed except during a period from 1985 to 1992 (Kim *et al.*, 2006). A recurrence of rabies was reported in a dog from Gangwon Province in 1993. Subsequently, annual outbreaks of rabies have been persistently reported in domestic (primarily dogs and cattle) and wild (primarily raccoon dogs) animals (Kang *et al.*, 2007). In addition, outbreaks of rabies have been gradually reported in the Southern areas of Gyeonggi and Gangwon Provinces of South Korea over recent decades (Kim *et al.*, 2006). Thus, programs to vaccinate canines against rabies using a rabies bait vaccine and remove stray dogs have been developed. Since, animal vaccination is a key element for preventing the spread of the rabies virus, efforts have been made by the Korean government to vaccinate >70% of dogs which is the epizootiological baseline for maintaining herd immunity in a community (Olugasa *et al.*, 2011). Since, the effectiveness of bait vaccines is questionable, this study was designed to assess the presence of antirabies antibody in sera from canines from animal shelters in Seoul, South Korea to ascertain the vaccination status of stray dogs.

### CONCLUSION

The overall low prevalence (24.0%) of antirabies antibody observed in the present study demonstrated that the appropriate threshold antibody level has not been attained. Since, this may be attributed to the lack of a stable rabies vaccination program, there is an urgent need to actively reexamine the current strategy for vaccinating stray dogs against rabies. In addition, periodic veterinary surveillance should be conducted to evaluate the vaccination status and prevalence of antirabies antibodies in companion and stray dogs to maintain the protective levels recommended by the WHO.

### ACKNOWLEDGEMENT

This research was supported by a fund (2011-674, 2012-349) from live stock division of Seoul Metropolitan Government Research Institute of Public Health and Environment.

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