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Efficient distribution of oral vaccines examined by infrared triggered camera for advancing the control of raccoon dog rabies in South Korea

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ABSTRACT. The field distribution of the oral rabies vaccine is effective in controlling the spread of rabies. The present study aimed to investigate efficient distribution locations based on the environment, contact rate, and consumption by target wildlife species in South Korea. The target species (Korean raccoon dogs, domestic dogs, and feral cats) accounted for 945 contacts (52.2%), in total 1,808 contacts. There were 863 (47.8%) contacts by non-target species. Raccoon dogs, a main reservoir of rabies in South Korea, had the highest contact rate (34.1%) among all species. The contact rate by target species was highest at riparian sites and bushy mountainous vegetation, where raccoon dogs are abundant. There was remarkable contact by raccoon dogs in mountainous areas below 150 m with bushy vegetation. Our results indicate that these locations are efficient areas for vaccine distribution, especially targeting the raccoon dog. Vaccines were continuously contacted with intervals ranging from one hour to one day. Vaccines at 94.4% of the distribution points were completely consumed within two weeks. The mean consumption rate was 95.2 ± 1.93% during the overall study period. These findings suggest that the oral rabies vaccine attracts wildlife including domestic dogs and feral cats. Our results suggest that low sections of mountainous areas with bushy vegetation and/or neighboring riparian areas are rich in target wildlife species (especially raccoon dogs) and are efficient locations for vaccine distribution to control rabies in South Korea.

KEY WORDS: oral rabies vaccine, rabies, raccoon dog, vaccine distribution, wildlife

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Rabies is an acute progressive encephalitis caused by viruses of the genus *Lyssavirus* in the family *Rhabdoviridae* [45]. Other lyssavirus such as Lagos bat virus, Duvenhage virus, European bat lyssavirus, and Australian bat lyssavirus have been mostly isolated from bats [12, 44]. Rabies is one of the oldest known infectious zoonotic diseases in the world and is mostly maintained by dogs and terrestrial mammalian carnivores [7, 38], particularly raccoons (*Procyon lotor*), skunks, foxes, and coyotes in North America, and foxes and bats in Europe [30, 32, 34, 39]. Moreover, domestic dogs are the principal reservoir of rabies virus worldwide, and raccoon dogs (*Nyctereutes procyonoides*) are vectors of the rabies virus in parts of Asia and Europe [2, 17, 36, 37, 51]. Raccoon dog is a locally common medium-sized mammal, mostly inhabiting forests and thick vegetation with a preference for riparian areas [29, 41, 50]. In addition, the population of raccoon dogs has increased due to a lack of natural predators. This has led to an extension of its habitat, increasing their virus carrying potential in South Korea [14, 27]. To prevent the spread of rabies in wildlife, oral rabies vaccine (ORV) is widely used worldwide, including in the United States, Canada, and Europe [21]. Since it was first used in Switzerland in 1978, field trials have demonstrated its effectiveness for the control of rabies [3, 4, 23, 24, 33, 40].

The first Korean rabies case was reported in a domestic dog in 1907, after which rabies rapidly became an enzootic disease spread by dogs until 1984 [13, 18, 46]. In addition, dogs have been the principal host of rabies in South Korea, as evidenced by the detection of 518 rabid dogs between 1962 and 1979 [15]. Rabies cases steadily decreased between 1976 and 1984, followed by no reports between 1985 and 1992, due to the government vaccination campaign for dogs [14, 48]. The Korean raccoon dog (*Nyctereutes procyonoides koreensis*) has become the main reservoir of rabies in South Korea since 1993 [6, 18, 27, 46], and 325 cases were reported between 1994 and 2003 [18, 27]. Since 1993 rabies reports were limited to two provinces (northern Gyeonggi

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and Gangwon) near the border of South Korea's demilitarized zone [18, 49]. Rabies has been confirmed in five animal species in South Korea since 1993, and among these, the percentages of rabid dogs, raccoon dogs, and feral cats, were 40.5%, 17.4%, and 0.9%, respectively [49]. Recently, rabies has been reported in western Seoul in 2006 and 2012, and in Hwaseong City in 2013, in the southern part of Gyeonggi province [26, 27]. This indicates that rabies is not limited to northern areas but also prevalent in the southern Han River region of South Korea [26, 49].

To control rabies in South Korea, livestock and dog vaccination is required by law under the Act on the Prevention of Contagious Animal Diseases [42]. In addition, serum analyses and the bait vaccine is distributed for wildlife use [5]. In 2001, ORV was introduced to South Korea by the Ministry of Agriculture and Forestry [25]. Since then, vaccine distribution has gradually increased, rising to 990,000 doses in 2018, and distribution has been concentrated in the northern areas of South Korea, including Gyeonggi and Gwangwon provinces, as well as Seoul [46]. Consequently, there have been no reported cases of rabies in South Korea since 2014. The seasonal mass distribution of ORV has had industrial and economic benefits by substantially decreasing rabies transmission via raccoon dogs in South Korea [5, 25, 49].

Although ORV is effective in preventing rabies originating from wildlife, research on efficient distribution methods for the rabies vaccine is insufficient for devising an informed strategy. Moreover, the seropositivity of captive raccoon dogs decreased in South Korea from 40% in 2012 to 13.7% in 2017–2018. It is a necessity to undertake an improved distribution method to deliver the vaccine targeting raccoon dogs [27, 48]. Vaccine performance in the field may be affected by a variety of factors, including the vaccine bait density and distribution pattern, habitat, vegetation, population density of the target species, and vaccine consumption by the target species [10, 28, 32]. For successful vaccination, seasonal distribution and population density are important parameters in South Korea [25, 49]. Taken together, the success of rabies vaccination depends on the development of an efficient distribution strategy with regard to diverse environmental and population factors and the contact and consumption rate of ORVs by wildlife.

Therefore, in the present study, we investigated efficient vaccination locations with regard to environmental factors and the rates of contact and consumption of ORVs by raccoon dogs, domestic dogs, and feral cats as target wildlife species in South Korea. Our findings provide a basis for the development of distribution strategies for the continuous and efficient control of rabies.

MATERIALS AND METHODS

Study period and site description

The study period and sites were chosen based on raccoon dog behavior, with data collected during early spring (March to April 2012) and late autumn (November to December 2012) to avoid periods of hibernation from December to February [16, 41]. Six sites were selected for vaccine distribution, four sites in a mountainous district, and two sites near streams (Table 1). The mountainous areas were in Bukhansan National Park (between 37° 39.262′ N, 126° 56.962′ E and 37° 39.316′ N, 127° 00.489′ E), near the northern border of Seoul city. The streams were Yangjaecheon and Tancheon (between 37° 28.054′ N, 127° 01.766′ E and 37° 29.660′ N, 127° 06.113′ E), which flow into the Han River in central Seoul (Fig. 1).

ORV distribution

A licensed recombinant ORV, Raboral V-RG® (MERIAL Corp., Lyon, France), was used; it has protective efficacy against rabies virus in the fox, raccoon, coyote, skunk, raccoon dog, and jackal [8, 11, 21]. ORVs were distributed during two seasons (spring and autumn). Each of the six sites received 1,000 doses of ORV per season, resulting in a total of 12,000 distributed vaccines across the two seasons. There were 33 distribution points designated at each site, resulting in a total of 198 points at six sites (Fig. 2). At each site, 30–40 vaccine doses were distributed at each point.

Distribution was performed by hand following the guidelines of the U.S. Animal and Plant Health Inspection Service (APHIS) [1] and the "Rabies Eradication Plan" of the National Veterinary Research and Quarantine Service (NVRQS), South Korea [25]. ORVs were distributed along the trails of Bukhansan National Park and streams near the borders of Seoul city, to prevent the transmission of the rabies virus from neighboring areas. GPS receivers (GPSmap60CS, Oregon550, and Legend Etrex; Garmin Corp., Kansas City, MO, USA) were used to verify the altitude; above sea level (ASL) and to record the coordinates of the distribution points. Satellite imagery was obtained with Google Earth (Google LLC, Mountain View, CA, USA).

Monitoring and analysis of ORV contact and consumption

In this study, contact was defined as clear contact with the bait, included sniffing, intake (or not) behavior by wildlife.

Table 1. Classification and environmental characteristics of study sites

Site No.	Location	Environment	Altitude (m)	Vegetation
1	Bukhansan National Park (Eunpyeong-gu, northwestern)	Mountainous area	70–100	Grassy
2	Bukhansan National Park (Jongno-gu, southwestern)	Mountainous area	450–500	Bushy
3	Bukhansan National Park (Seongbuk-gu, southeastern)	Mountainous area	350–400	Grassy
4	Bukhansan National Park (Gangbuk-gu, northeastern)	Mountainous area	100–150	Bushy
5	Yangjaecheon (Seocho-gu)	Riparian area	0–10	Grassy
6	Tancheon (Gangnam-gu)	Riparian area	0–10	Bushy

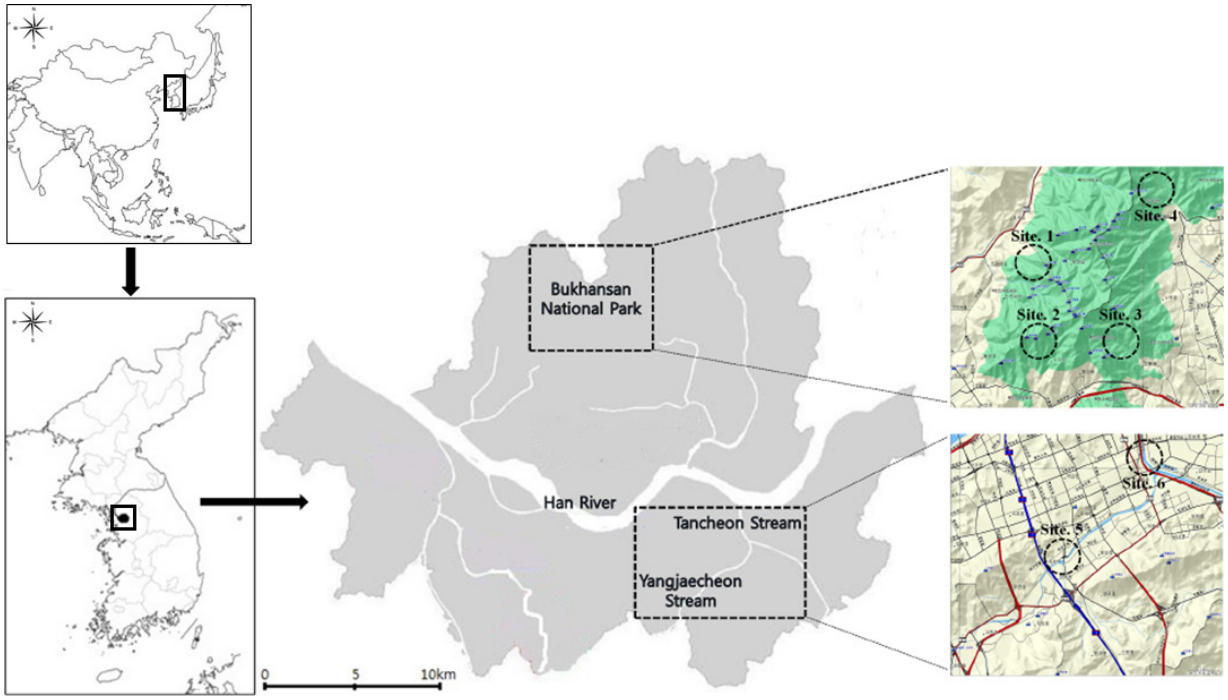

Fig. 1. Vaccination site locations at Bukhansan National Park, Yangjaecheon and Tancheon Streams, Seoul, South Korea. Site 1 (Eunpyeong), site 2 (Jongno), site 3 (Seongbuk), and site 4 (Gangbuk) are mountainous area, site 5 (Yangjaecheon) and site 6 (Tancheon) are riparian area.

Consumption was defined as the removal of the bait from the distribution point. The raccoon dog, domestic dog, and feral cat were the target species based on previously reported rabies cases in these species in South Korea [15, 25, 46, 49]. Among the three target species, the focus for the analysis was the raccoon dog because it is main reservoir of rabies in South Korea [27]. Infrared triggered cameras (Covert Extreme TLV and DLC II; DLC Trading Co., Lewisburg, KY, USA) were set up at each site to monitor contact by wildlife. Contact numbers were recorded for each site and analyzed with various factors, such as season, environment, and vegetation. Contact by the raccoon dog was analyzed by cross tabulation analysis (Pearson's χ^2 test) using SPSS ver. 22.0 (IBM Corp., Armonk, NY, USA). In all analyses a probability value less than 0.05 was considered statistically significant ($P<0.05$).

Every distribution point was checked manually once a week for 3 weeks after vaccine distribution to determine whether the vaccine had been consumed. The consumption rates were determined by collecting any remaining doses at each site 4 weeks after distribution of the vaccine, following the guidelines of NVRQS [25].

RESULTS

Analysis of vaccine contact by wildlife

A total of 11 species made contact with the baits, including the three target species: raccoon dog (*N. procyonoides*), domestic dog (*Canus lupus familiaris*), and cat (*Felis catus*). In addition, eight non-target species made contact with the bait: carrion crow (*Corvus corone*), Eurasian magpie (*Pica pica*), wild boar (*Sus scrofa*), Eurasian red squirrel (*Sciurus vulgaris*), Siberian chipmunk (*Tamias sibiricus*), Korean water deer (*Hydropotes inermis argyropus*), brown rat (*Rattus norvegicus*), and waterfowl (*Anas platyrhynchos*, *Anas poecilorhyncha*) (Fig. 3a–l).

In total, 1,808 vaccine contacts were recorded during the two distribution periods (Table 2). The total rate of contact by the target species was 52.2% (945/1,808). The eight non-target species had a total contact rate of 47.8% (863/1,808). Raccoon dogs were the primary targeted species, they had an average contact rate of 34.1% across all sites, the highest among all species (Table 2). Carrion crows and wild boars accounted for 31.7% of the total contacts and were the major non-target species.

The proportion of raccoon dog contacts was higher in the autumn than in the spring when compared with all other species ($\chi^2=552.78$; $P<0.001$). The proportion of raccoon dog contacts was higher in riparian sites than in mountainous sites when compared with all other species ($\chi^2=358.80$; $P<0.001$). The proportion of raccoon dog contacts were also higher in bushy vegetation than in grassy vegetation when compared with all other species ($\chi^2=760.26$; $P<0.001$), respectively. All individual contact numbers with respect to site are presented in Table 3.

Analysis of ORV consumption

The vaccine consumption rate was 95.2 ± 1.93% during the overall study period. Vaccines at 94.4% of the distribution points were completely consumed within two weeks (Tables 4 and 5). The vaccines were continuously contacted by animals with intervals

Fig. 2. Individual site map details of vaccine distribution points (white flag). (a) site 1 (mountainous area); (b) site 2 (mountainous area); (c) site 3 (mountainous area); (d) site 4 (mountainous area); (e) site 5 (riparian area); (f) site 6 (riparian area).

ranging from one hour to one day (Supplementary Fig. 1). There was no difference between sites with respect to contact, and repetitive contact was observed in three species (raccoon dog, carrion crow, and Eurasian red squirrel). At points where the vaccine was completely consumed, torn sachets remained; these were consumed by carnivores (Fig. 4).

DISCUSSION

ORVs have been distributed worldwide to interrupt the transmission of rabies from target animals, and eliminate the virus from vectors [20, 47]. In this regard, we investigated which locations were optimal for distributing vaccines with regard to environmental factors and the rates of ORV contact and consumption by the raccoon dog, domestic dog, and feral cat as target wildlife species in South Korea.

In this study, the population density of raccoon dogs in mountainous and riparian areas of Seoul was considered 1.0 per 1 km^2, which is the known density in neighboring Gyeonggi province in South Korea [49]. A total of 11 species came into contact with the bait (Seoul). This is a similar result to the 10–14 species reported previously in a study of ecological research project on wild animals in the rabies bait vaccine applied area in the Gyeonggi and Gangwon provinces of South Korea in 2010 [35]. In addition, both investigations revealed no noticeable difference between species according to region. This result indicates that there are no significant differences in the number of animal species making contact with ORVs in the vaccinated areas in South Korea.

Fig. 3. Species in contact with the oral rabies vaccine. (a) Korean raccoon dogs (*Nyctereutes procyonoid*); (b) domestic dog (*Canis lupus familiaris*); (c) domestic cat (*Felix catus*); (d) carrion crow (*Corvus corone*); (e) Eurasian magpie (*Pica pica*); (f) wild boars (*Sus scrofa*); (g) Eurasian red squirrel (*Sciurus vulgaris*); (h) Siberian chipmunk (*Tamias sibiricus*); (i) Korean water deer (*Hydropotes inermis argyropus*); (j) brown rat (*Rattus norvegicus*); (k) mallard duck (*Anas platyrhynchos)*; (l) spot-billed duck (*Anas poecilorhyncha*).

Fig. 4. The oral rabies vaccine was completely consumed, leaving a torn sachet (white circle and arrow).

Seasonal variation in the vaccine contact rate by raccoon dogs may be related to the hibernation period. A previous study has reported that hibernation is preceded by a period of intensive eating [41]. Table 2 shows that species contact also varied environmentally with site type. The overall contacts by target species were three times higher in mountainous areas than in riparian areas and were six times higher in bushy vegetation than in grassy vegetation. Table 3 shows that raccoon dogs made a remarkable number of contacts with vaccine at site 4, which was the only mountainous site where raccoon dogs were identified. Boyer *et al.* also reported that the rate of contact by raccoons with vaccine bait is highest under forest cover [3]. By contrast, carrion crows and wild boars accounted for 81.2% of the total contact rate at sites 2 and 3, which were above 350 m ASL in mountainous areas. These results indicate that high grounds in mountainous areas without bushy vegetation might favor the uptake of vaccines by non-target species, especially by omnivorous birds. Hence, ORV contact by the target species may be highest in bushy mountainous areas, particularly in the autumn.

Among other species, raccoon dogs made more contacts in riparian sites than in mountainous sites (Table 2). These results were consistent with the habitat characteristics of the raccoon dog, which are found mainly in forests and in thick vegetation, and prefer areas bordering lakes and streams [29, 41, 50]. Considering the overall numbers and frequency of contacts made by raccoon dogs according to sites, mountainous areas below 150 m ASL with bushy vegetation, and/or neighboring riparian areas could be the optimal candidate sites for efficient ORV distribution in South Korea for targeting raccoon dogs.

These results also correlated with the efficient ORV distribution requires target animal species that encountered the bait, and photography using infrared triggered camera is used for investigation of ORV contact by animals [4, 28, 31, 43]. Contact does not signify intake of the vaccine, it comprises all animal actions when they encountered vaccines,

Table 2. Number of vaccine contacts by season, environment, and vegetation type

Species	Season		Environment		Vegetation		Total (%)
	Spring (%)	Autumn (%)	Mountainous area (%)	Riparian areas (%)	Grassy (%)	Bushy (%)	
Raccoon dog	61 (8.7)	556 (50.2)	466 (30.8)	151 (50.8)	31 (4.7)	586 (51.4)	617 (34.1)
Feral cat	116 (16.5)	87 (7.9)	138 (9.1)	65 (21.9)	62 (9.3)	141 (12.4)	203 (11.2)
Domestic dog	91 (13)	34 (3.1)	118 (7.8)	7 (2.4)	31 (4.7)	94 (8.2)	125 (6.9)
Carrion crow	194 (27.7)	131 (11.8)	325 (21.5)	0 (0)	243 (36.4)	82 (7.2)	325 (18)
Wild boar	45 (6.4)	202 (18.2)	247 (16.4)	0 (0)	197 (29.5)	50 (4.4)	247 (13.7)
Eurasian magpie	80 (11.4)	33 (3)	95 (6.3)	18 (6.1)	32 (4.8)	81 (7.1)	113 (6.3)
Waterfowl	45 (6.4)	15 (1.4)	30 (2)	30 (10.1)	12 (1.8)	48 (4.2)	60 (3.3)
Eurasian red squirrel	42 (6)	7 (0.6)	49 (3.2)	0 (0)	20 (3)	29 (2.5)	49 (2.7)
Korean water deer	3 (0.4)	24 (2.2)	21 (1.4)	6 (2)	0 (0)	27 (2.4)	27 (1.5)
Siberian chipmunk	13 (1.9)	9 (0.8)	22 (1.5)	0 (0)	22 (3.3)	0 (0)	22 (1.2)
Brown rat	11 (1.6)	9 (0.8)	0 (0)	20 (6.7)	17 (2.5)	3 (0.2)	20 (1.1)
Total	701 (100)	1,107 (100)	1,511 (100)	297 (100)	667 (100)	1,141 (100)	1,808 (100)

Table 3. Number of vaccine contacts by various species at all sites

Species	Site 1			Site 2			Site 3			Site 4			Site 5			Site 6			Total (%)
	S[a]	A[b]	T[c]	S[a]	A[b]	T[c]	S[a]	A[b]	T[c]	S[a]	A[b]	T[c]	S[a]	A[b]	T[c]	S[a]	A[b]	T[c]	
Raccoon dog	0	0	0	0	0	0	0	0	0	9	457	466	22	9	31	30	90	120	617 (34.1)
Feral cat	30	15	45	45	9	54	0	0	0	12	27	39	17	0	17	12	36	48	203 (11.2)
Domestic dog	15	6	21	0	0	0	3	0	3	66	28	94	7	0	7	0	0	0	125 (6.9)
Carrion crow	0	3	3	47	12	59	124	116	240	23	0	23	0	0	0	0	0	0	325 (18)
Wild boar	0	3	3	18	8	26	3	191	194	24	0	24	0	0	0	0	0	0	247 (13.7)
Eurasian magpie	4	0	4	0	0	0	16	0	16	45	30	75	12	0	12	3	3	6	113 (6.3)
Waterfowl	3	0	3	12	6	18	0	0	0	3	6	9	9	0	9	18	3	21	60 (3.3)
Eurasian red squirrel	13	7	20	29	0	29	0	0	0	0	0	0	0	0	0	0	0	0	49 (2.7)
Korean water deer	0	0	0	0	0	0	0	0	0	0	21	21	0	0	0	3	3	6	27 (1.5)
Siberian chipmunk	13	9	22	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	22 (1.2)
Brown rat	0	0	0	0	0	0	0	0	0	0	0	0	11	6	17	0	3	3	20 (1.1)
Total	78	43	121	151	35	186	146	307	453	182	569	751	78	15	93	66	138	204	1,808 (100)

a) Spring, b) autumn, c) total.

Table 4. Number of distribution points at which vaccines were consumed according to season

Season of distribution	Number of points at which vaccines were completely consumed			Number of remaining points[b]	Total distribution points
	One week[a]	Two weeks[a]	Three weeks[a]		
Spring	172	14	0	12	198
Autumn	181	7	1	9	198
Total	353 (89.1%)	21 (5.3%)	1 (0.3%)	21 (5.3%)	396 (100%)

a) After distribution, b) vaccines were not completely consumed by wildlife.

including sniffing, intake (or not). Consumption was specified as the removal of the vaccine because of animal contact. Thus, infrared triggered cameras were used to investigate contact by wildlife and these contacts were taken as a proxy for consumption in this study. There were discrepancies between the ORV distribution and serological survey areas in South Korea in 2017–2018. This may have resulted in the lower seropositivity of captive raccoon dogs (13.7%) when compared to the data of 2012 (40%) [48]. Taken together, further studies are needed for better serological confirmation that take into consideration the habitat, and consumption analyses of the target animal species.

Vaccines at 94.4% of the distribution points were completely consumed within two weeks, with continuous contact (Table 4). These findings were consistent with previous results of field trials in Israel, in which up to 90% of the bait was consumed during the first night [21]. Furthermore, the time it took for the complete consumption of the vaccines was almost two weeks in the spring, whereas vaccines were consumed within days following the autumn distribution [19]. The rate of consumption across all sites and seasons was at least 91% (Table 5), which was similar or greater than previous estimates [9, 22]. Accordingly, ORV appears to be attractive to wildlife, resulting in mass consumption by both target and non-target species.

In summary, bushy vegetation in mountainous areas below 150 m ASL and/or neighboring riparian areas are rich in target

Table 5. Rate of vaccine consumption by site and season

Site	Spring (%)	Autumn (%)	Total (%)
1[a)]	98.6	95.3	96.95 ± 2.33
2[a)]	91.3	94.0	92.65 ± 1.90
3[a)]	96.2	95.1	95.65 ± 0.77
4[a)]	92.7	96.1	94.40 ± 2.40
5[b)]	95.1	96.6	95.85 ± 1.06
6[b)]	94.6	96.8	95.70 ± 1.55
Total	94.75 ± 2.57	95.65 ± 1.05	95.2 ± 1.93

a) Mountainous areas, b) riparian areas.

wildlife species (i.e., raccoon dog, domestic dog, and feral cat). These are effective locations for the distribution of vaccines to control rabies in wildlife, especially raccoon dogs, in South Korea. Continuous ORV distribution is needed for rabies control from an ecological perspective based on the habitat of the target wildlife species. Our findings suggest that efficient vaccination locations with regard to the environment, contact rate, and consumption by target species can be used to devise strategies for effective ORV distribution for the continuous and efficient control of rabies.

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