수의학석사학위논문

Validation of a commercial ELISA kit for the detection of rabies antibodies in dogs and cats for border qurantine in South Korea

우리나라에서 수출입 검역 시 개와 고양이의 광견병항체검사를 위한 효소결합면역분석법의 적합성

2013 년 11 월

서울대학교 대학원

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지도교수 박 봉 균

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위 원 장 김 재 홍 (인)
부 위 원 장 박 봉 균 (인)
위 원 유 한 상 (인)

Abstract

Validation of a commercial ELISA kit for the detection of rabies antibodies in dogs and cats for border qurantine in South Korea

Lim Jungeun

Department of Veterinary Medicine
Veterinary Microbiology
The Graduate School
Seoul National University

Rabies is a zoonotic disease which is reported as sporadic outbreak in South Korea. Although nation-wide vaccination program have been applied, stronger control management is required because of serial outbreaks in the rural areas surrounding the Seoul metropolis. Recently, several countries are requiring rabies neutralizing antibody test reports in the quarantine of dog and cat. The requirement has been applied in South Korea since December 1, 2012.

Korean Government has adopted an OIE acknowledged standardized evaluation method, the Fluorescent Antibody Virus Neutralization Test (FAVNT). Recently, the need for a new test is rising due to some problems of the test to be used. As one of the tests, a commercial Enzyme-linked immunosorbent assay test (Platelia Rabies II kit, Bio-Rad) was evaluated by comparing with the standard method using 1,163 quarantine and 20 experimental serum samples. The specificity and sensitivity of ELISA test compared to FAVNT was 90.2% and 95.0% for dogs, 100.0% and 95.8% for cats and the *k*-values were 0.58 and 0.46 respectively. A further designed 20 experimental

German Shepherds was evaluated for the FAVNT and ELISA tests on 0, 90 and 180 days post vaccination. On the 90 and 180 days post vaccination, three and seven dogs vaccinated with the inactivated vaccine were over 0.5 International Units (IU)/㎖, which was considered as the threshold of seroconversion to a rabies antigen, in FAVNT. However none of dogs was seropositive in ELISA test. In the group vaccinated with the attenuated vaccine, all of dogs had a titer of over 0.5 IU/㎖ for both methods except one dog in FAVNT. In quarantine tests, an inactivated vaccine is required to vaccinate because of the possibility of rabies virus infection. These results suggested that a commercial ELISA test still has limitation to apply quarantine inspection as a FAVNT replacement method in Korea situation despite of OIE recommendation.

Keywords : rabies, FAVN, ELISA, quarantine
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INTRODUCTION

Rabies is a zoonotic disease that causes severe damage to the central nervous system and without treatment, it has a 100% mortality rate (OIE, 2008). In South Korea, the first rabies case was reported in a dog in 1907, and a number of rabies cases had been reported in several provinces of South Korea by 1945. As a result of the application of intensive vaccination programs that use inactivated or live attenuated rabies vaccines, rabies cases have steadily decreased and no reports were made for eight years from 1985 to 1992(Lee et al., 2009; Yang et al., 2011). However, this disease recurred in the Gangwon Province in 1993, and a number of cases have been continuously reported since that time. The disease is not reported in South Korea except the several provinces near the DMZ anymore; however, cases were recently reported near Seoul (Yang et al., 2011; Kim et al., 2006). This emphasizes the need to apply strictly controlled vaccine programs and regulations for international trade of susceptible domestic carnivores. On December 1 2012, South Korea acted its quarantine laws with new regulations for importing companion animals, specified that they must be tested for sufficient levels of rabies-neutralizing antibodies. An antibody titer of 0.5 IU/ml has been determined as the threshold of seroconversion to a rabies vaccination (Hooper et al., 1998; Oh. et al., 2012). At present, the Rapid Fluorescent Focus Inhibition Test (RFFIT) and the Fluorescent Antibody Virus Neutralization Test (FAVNT) are the international prescription test methods (OIE, 2008), though those methods take several days and require highly trained technicians as well as special laboratory facilities. For these reasons, various tests have been developed to replace those methods (Sagne et al., 2006; Welc et al., 2009; Knoop et al., 2010). The Enzyme-Linked Immunosorbent Assay (ELISA) test has several advantages: It takes less time and requires neither

highly trained technician nor specialized laboratory containment.

The purpose of this study was to determine the validation of a commercial ELISA kit that was adopted by the OIE Register, as replacement method (OIE, 2013). The performance of the ELISA kit was compared to that of the gold standard FAVNT in two different groups of serum samples: 1,163 serum samples for quarantine with a variant history and designed serum samples.

MATERIALS AND METHODS

1. Test samples

1.1 Quarantine samples

A total of 1,163 (893 dogs and 270 cats) quarantine serum samples which were included in the study have various collection history. Each sample had a different background in terms of regional origin, vaccination, sex and age. These samples were submitted to the Seoul branch of the Animal, Plant and Fisheries Quarantine and Inspection Agency (QIA) for export quarantine tests from 2009 to 2012. The origin, vaccination, sex, age, breed and year of sample collection were recorded for each animal, when as available as possible. Samples with insufficient information for analysis were excluded. All tested sera were inactivated by heating to 56℃ for 30 minutes and stored at −70℃.

Both dog and cat sera mainly came from either Seoul (54.2% of dog sera, and 31.5% of cat sera) or the Gyeonggi Province (25.7% of dogs and 15.2% of cats) (Fig.1). Each serum sample was generally collected two months after vaccination. One to three months after vaccination, 80% of the dog and 78% of the cat subjects were inquired (Table1).

Vaccines used for this study were originated from several different foreign manufacturers. As shown in Table 2, most of them came from five particular manufacturers, all of which were inactivated vaccines. The first and second amongst these companies provided the majority of vaccine(60.0% of the dogs and 57.7% of the cats, respectively) and the rest of the companies provided the vaccines for the remainder.

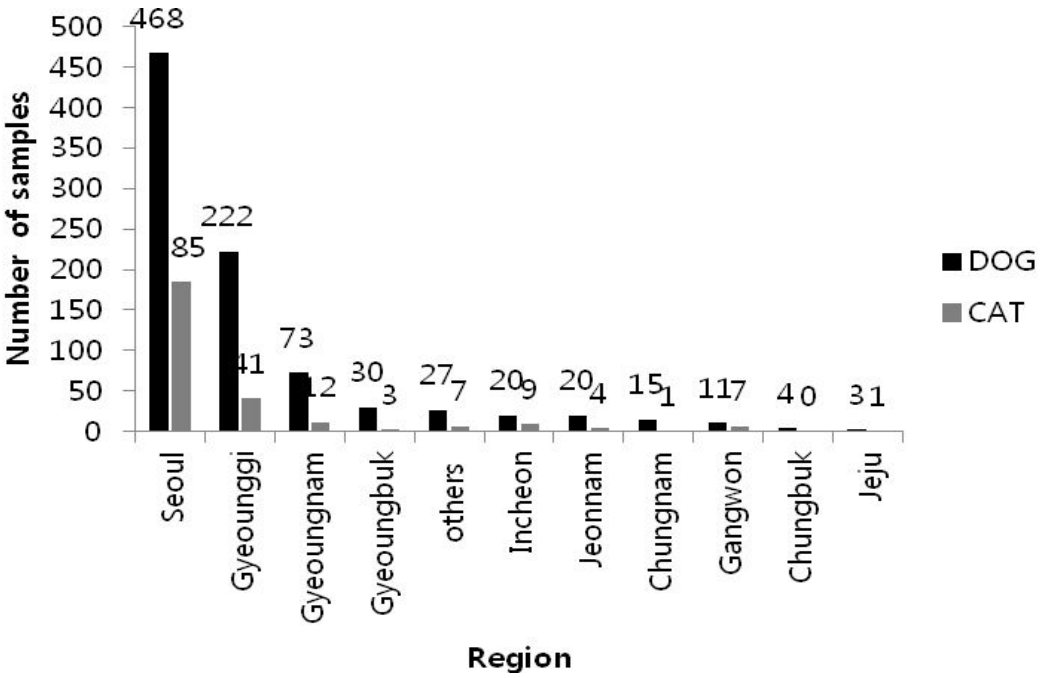

Fig 1 Originated regions of the quarantine samples. A total of 1,163 serum samples were tested for rabies under quarantine. Both dog and cat sera mainly came from Seoul and Gyeonggi province. “Others.” refers to the remaining provinces and metropolitan cities not shown in this figure.

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Table 1 FAVNT results of sera of dogs and cats sera by vaccine injection interval.

	Dog			Cat		
Month[+]	**Number of seropositive**	**Number of seronegative**	**Total**	**Number of seropositive**	**Number of seronegative**	**Total**
1	150	5	155	43	0	43
2	481	20	501	150	2	152
3	53	6	59	15	0	15
4	50	6	56	11	0	11
5	26	1	27	14	2	16
6	48	2	50	9	0	9
7	14	0	14	7	0	7
8	27	1	28	5	0	5
9	0	0	0	6	0	6
>9	3	0	3	5	1	6
Total	852	41	893	265	5	270

[+] When submitted postvaccination

Table 2 Number of test samples according to manufacturer of injected vaccine and number of seronegative samples for FAVNT and ELISA.

Manufacturer	Brand Name	No. of sera tested	No. of <0.5 IU/㎖ (FAVNT)	No. of <0.5 EU/㎖ (ELISA)
Virbac	Rabigen Mono	337	18(5.3)	35(10.4)
Schering-Plough Animal Health	Rabdomun	332	3(0.9)	6(1.8)
Pfizer	Defensor3	253	12((4.7)	25(9.9)
Intervet	Nobivac Rabies	121	9(7.4)	22(18.2)
Merial	Rabisin-R	112	4(3.6)	7(6.3)
Ect.		8	-	1(0.13)
Total		1163	46(3.9)	96(8.3)

( ) the percentage of samples with <0.5 IU/㎖ among total sera tested.

1.2 Experimental samples

To exclude environmental factors, 20 German Shepherd dogs, all of the same age, were arranged. None of the dogs in this experiment had ever been vaccinated against rabies until they were four years old and all were raised within the same environment as military dogs. All subjects were neutered and the range of their weights at the time of vaccination was 7-12kg. The subjects were divided into two groups of ten, then one 10-12kg group had an attenuated vaccine(Rabigen Mono,Virbac, France), and the other 6.6-7kg group had an inactivated vaccine(Dogibac, Daesung, Korea).

1.3 Control sera

Each test also included a reference serum: WHO, 30 IU in an ampoule, the dilution of serum contained 0.5 IU/㎖ antibodies used for the titration, and the control titration of the reference positive anti-rabies dog serum (OIE reference laboratory for Rabies, Nancy). Naïve dog serum as a negative control was provided from QIA .

2. Fluorescent antibody virus neutralization test (FAVNT)

The presence of rabies-neutralizing antibodies was determined by the FAVN test to be a standard reference serological method. A positive control was used for the OIE serum of dog origin adjusted to 0.5 IU/㎖ (WHO, 1992). Each serum sample was distributed in four consecutive wells, and then serially diluted to 1:3. The rabies challenge virus (CVS-11, ATCC VR 959) containing around $TCID_{50}$ was then added to each well. After 60 minutes of incubation at 37℃ in a humidified incubator with 5% CO_2, BHK-21 cells 50㎕of 4×10^5 cells/㎖ suspension were added to each well and the microplates were

incubated for 48 hours. They were stained by adding 100㎕ of *fluorescein isothiocyanate (FITC) anti-rabies monoclonal globulin* (Millipore, USA) to each well. The titres of serum samples were expressed in International Units per milliliter (IU/㎖) and compared to results of the positive standard. The threshold of positivity used was 0.5 IU/㎖(Hooper et al., 1998).

3. Enzyme-linked immunosorbent assay (ELISA)

The *Platelia Rabies II kit* (Bio-Rad, France) is an indirect immune-enzymatic assay that detects rabies virus antiglycoprotein antibodies. For all procedures follow the manufacturer's instructions were followed. Each serum sample was diluted to 1:100 and 100㎕ of this dilution was distributed to the microplates along with the positive, negative and quantification standard controls. The microplates were incubated for 1 hour at 37℃. After three washings were performed, 100㎕ of the conjugate (protein A labeled with peroxidase) was added to each well. The microplates were then incubated for 1 hour at 37℃. After the second washing, 100㎕ of TMB chromogen solution was added to each well. The microplates were incubated in the dark for 30 minutes at room temperature. The enzymatic reaction was then stopped by adding a solution of 1 N H_2SO_4. The microplates were measured bichromatically at 450 and 620 nm. An interpretation of the titer in ELISA was done, using the method provided by the manufacturer. The results were expressed in equivalent units (EU) /㎖, which correlate with IU/ ㎖ (Stantić et al., 2006; Welch et al., 2009). A value greater than or equal to 0.5 EU/ ㎖ represented an antibody level from 0.125, 0.5 EU/㎖ and over 4 EU/㎖. The threshold of positivity was 0.5 EU/㎖, which was the same as for the FAVNT.

4. Statistical analysis

The quarantine serum samples were grouped by region of origin, age, sex and vaccine type of manufacture. Statistical analyses were performed using the chi-square (χ^2) test or Fisher's exact test and variance analysis for one factor (Cliquet et al., 2003). Statistical analyses were performed using the SAS (statistical analysis system) program(Landies et al., 1997). The animals' ages were evaluated as a categorical variable with groups consisting of dogs and cats under 1 year, 1-5 years, 5-10 years, and over 10 years of age (Yang et al., 2010). To validate the Bio-Rad Platelia Rabies II ELISA, the obtained results were compared to those of the FAVNT as a gold standard and relative sensitivity, and specificity were also calculated. When the two methods (ELISA and FAVNT) were compared, the *kappa* value was also calculated to evaluate the agreement between them (Sagne et al., 2006).

RESULTS

1. Quarantine samples

1.1. Neutralizing antibody titer values

Large individual variations were observed in the measurement of rabies-neutralizing antibodies from 0.0 IU/㎖ to >377.9 IU/㎖, in both dogs and cats. Fig. 2 shows the results obtained, and the numbers of animals presenting antibody titers lower than the threshold value of 0.5 IU/㎖ (seronegative). Of the total 893 dog and 270 cat sera that were analyzed in FAVNT, 41(4.6%) and 5(1.9%) samples respectively showed an antibody titer lower than 0.5 IU/㎖. When using ELISA, 80(9.0%) dog and 16(5.9%) cat samples were seronegative.

1.2. Relative sensitivity, specificity and accuracy

A total of 893 dog sera and 270 cat sera were analyzed to validate *Bio-Rad Platelia Rabies II ELISA* compared to FAVNT (Table 3). When the dog sera were tested, the specificity, sensitivity and accuracy of ELISA when compared to FAVNT were 90.2%, 95.0% and 94.7%, respectively. The *kappa* value was 0.58, which indicated a moderate agreement between the two methods. In the tested cat sera, the specificity, sensitivity and accuracy of ELISA when compared to FAVNT were 100%, 95.8% and 95.9%, respectively, with a 0.46 *kappa* value. This indicated poor consistency between FAVNT and ELISA

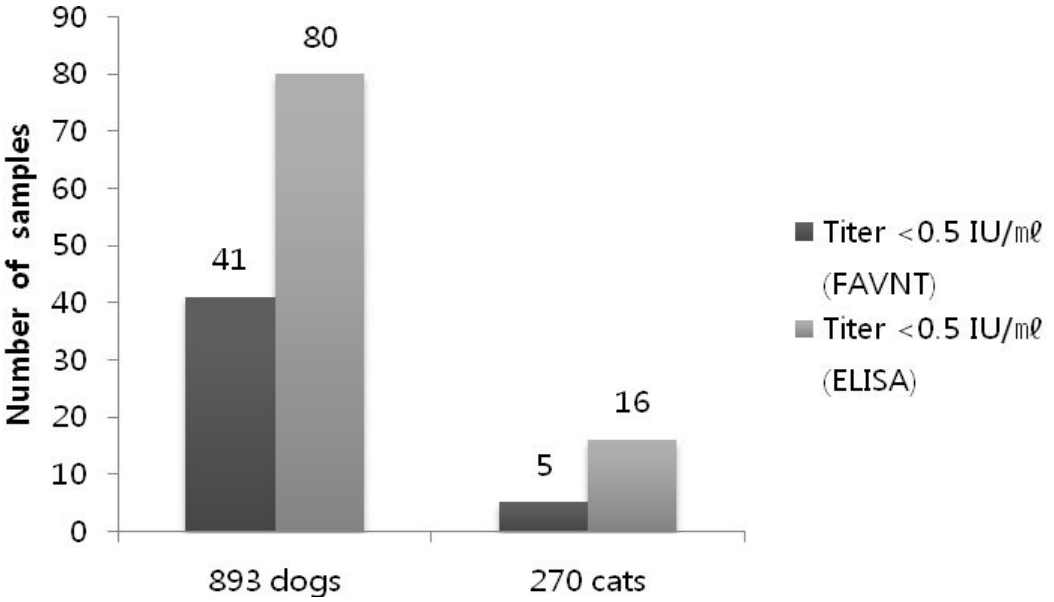

Fig 2 Numbers of animals presenting with an antibody titer of < 0.5 IU/㎖ (seronegative).

Table 3 Specificity and sensitivity of commercial ELISA kit compared with FAVNT

Dog					Cat				
		FAVNT					FAVN		
		P *	N	total			P *	N	total
ELISA	P *	809	4	813	ELISA	P *	254	0	254
	N	43	37	80		N	11	5	16
	total	852	41	893		total	265	5	270

* Seropositive value: ≥0.5 IU/㎖(FAVNT), ≥0.5 EU/㎖(ELISA)
Seronegative value: <0.5 IU/㎖(FAVNT), <0.5 EU/㎖(ELISA)

1.3. Statistical analysis

The age of the 893 tested dogs ranged from 3 months to 16 years. Table 4 shows the data in detail. The age of the 270 tested cats ranged between three months and 12 years. The “One-five years” groups for both dogs and cats included the most animals, 48.9% and 58.5% respectively, and the “over 10 years” groups included the least. The statistical analyses revealed that while dogs showed significant seronegative number according to age ($\chi 2 = 23.92$, $p<0.0001$), but cats had no correlation ($\chi^2 = 1.5153$, $p=0.6787$).

In this study, 448 female and 445 male dogs and 135 female and 135 male cats were tested. According to the FAVNT, 41 dogs were below 0.5 IU/㎖, of which 20 were male (4.5%) and 21 were female (4.7%). For the cats, five were below 0.5 IU/㎖, of which two were male (1.5%) and three were female (2.2%). In the ELISA test 80 dogs were seronegative. Of those dogs, 41 were male (9.2%) and 39 were female (8.7%). For the cats, five of the males (3.7%) and 11 of the females (8.1%) were below the standard (Table 5). Neither dogs nor cats had a significant correlation of seronegativity to sex (dog: $\chi^2 = 0.0190$, $p=0.8903$; cat: $\chi^2 = 0.2038$, $p=0.6517$).

In total, 1,163 dogs and cats were vaccinated with vaccines from several different foreign manufacturers. Five inactivated rabies vaccines manufactured by foreign companies are distributed in Korea (Yang et al., 2013). Considering that Fisher’s exact test showed a p-value of = 0.0042, which is lower than the significance probability, the seronegative number statistically is significantly related to vaccine type.

Table 4 Number of sera with rabies-neutralizing antibody titer <0.5 IU/㎖ by age of dog and cat under quarantine

		< 1	**1-5**	**6-10**	**≥11**	**Total**
Dog	**FAVNT**	26(9.7)	13(3.0)	2(1.1)	0	41(4.6)
	ELISA	44(16.4)	26(5.9)	7(3.8)	3(75.0)	80(9.0)
	Total	268	437	184	4	893
Cat	**FAVNT**	1(1.9)	4(2.5)	0	0	5(1.9)
	ELISA	6(11.3)	8(5.1)	2(4.3)	0	16(5.9)
	Total	53	158	46	13	270

( ) the percentage of samples < 0.5 IU/㎖ on total sera tested of each groups.

Table 5 Number of sera with rabies-neutralizing antibody titer <0.5 IU/㎖ by sex of dog and cat under quarantine

	FAVNT			ELISA		
	Male	**Female**	**Total**	**Male**	**Female**	**Total**
Dog	20(4.5)	21(4.7)	41(4.6)	41(9.2)	39(8.7)	80(9.0)
Cat	2(1.5)	3(2.2)	5(1.9)	5(3.7)	11(8.1)	16(5.9)

( ) Percentage of samples < 0.5 IU/㎖ of total sera tested.

2. Experimental samples

To compare the effect of the two methods, FAVNT and ELISA test, on rabies antibody titration in military working dogs in similar conditions to one another. None of the dogs in this experiment had been vaccinated against rabies until they were four years old, and they were all neutered German Shepherds. The mean residual antibody titers were 0.0 IU/㎖ in FAVNT and <0.125 EU/㎖ in ELISA (Table 6) before vaccination, respectively. However, individual results varied significantly according to the types of vaccine: On day 90 and 180, three and seven dogs, respectively, that were vaccinated with the inactivated vaccine had a titer over 0.5 IU/㎖. However, none of the dogs was over 0.5 IU/㎖ according to ELISA. In the group of dogs inoculated with the attenuated vaccine, all of them had a titer over 0.5 IU/㎖ regardless of methods, except one dog on day 90 tested by FAVNT.

Table 6 Comparison of FAVNT and ELISA test in German Shepherds

		FAVNT			ELISA		
	sample	**0**	**90**	**180**	**0**	**90**	**180**
Inactivated vaccine	1	0.10	0.07	0.17	< 0.125	< 0.125	< 0.125
	2	< 0.07	0.22	**0.5**	< 0.125	< 0.125	< 0.125
	3	< 0.07	**0.5**	**0.5**	< 0.125	0.318	< 0.125
	4	< 0.07	0.17	**2.62**	< 0.125	< 0.125	0.386
	5	< 0.07	**0.5**	**0.5**	< 0.125	0.295	0.192
	6	0.07	0.13	**0.66**	< 0.125	0.133	< 0.125
	7	0.10	**0.5**	**1.51**	< 0.125	0.186	0.208
	8	0.10	0.29	**0.87**	< 0.125	0.182	0.196
	9	< 0.07	0.10	0.17	< 0.125	< 0.125	< 0.125
	10	< 0.07	< 0.07	< 0.07	< 0.125	0.162	< 0.125
Attenuated vaccine	11	< 0.07	**18.15**	**13.77**	< 0.125	**> 4**	**2.681**
	12	< 0.07	< 0.07	<0.07	< 0.125	**3.917**	**1.114**
	13	< 0.07	**2.62**	**10.45**	< 0.125	**1.141**	**2.897**
	14	< 0.07	**6.01**	**10.45**	< 0.125	**3.552**	**2.085**
	15	< 0.07	**3.46**	**10.45**	< 0.125	**1.458**	**3.874**
	16	< 0.07	**7.92**	**10.45**	< 0.125	**> 4**	**> 4**
	17	< 0.07	**1.51**	**1.51**	< 0.125	**2.533**	**1.096**
	18	< 0.07	**10.45**	**2.62**	< 0.125	**3.727**	**1.683**
	19	< 0.07	**4.56**	**10.45**	< 0.125	**> 4**	**3.23**
	20	< 0.07	**7.92**	**13.77**	< 0.125	**3.796**	**> 4**
	Ref. serum	0.5	0.5	0.5	0.510	0.510	0.448

[+] Inactivated vaccine: Rabigen Mono(Virbac, France)
Attenuated vaccine: Dogibac(Daesung, Korea)

DISCUSSION

The rabies quarantine on exported and imported companion animals has been reinforced in accordance with the new Companion Animal Import Act since December 1. 2012. Although previously dogs and cats with a rabies vaccination certificate did not need quarantine inspection, now all animals must have an inspection certificate acknowledging the presence of neutralizing antibodies after a rabies vaccine injection. In addition, the EU, Taiwan, and other countries free of rabies outbreaks require rabies-neutralizing antibody test certificates for all animals passing the national border. Currently Korea is favoring the FAVNT out of the standard methods verified by the OIE (FAVNT and RFFIT) (OIE, 2013). Since this method utilizes cells, it is time-consuming and expensive, and the test results vary according to the skill of the tester. Thus, this study has validated the commercial ELISA kit, which is deemed appropriate by the OIE (Wasniewski. et al., 2014) for quarantine inspection in Korea.

It has been accepted that the detection of rabies neutralizing antibody is a key factor for evaluation the efficacy of vaccination in animal (Johnson et al., 2010; Moore et al., 2005). The minimum measurable antibody titer considered to represent a level of immunity in humans is 0.5 IU/㎖ of rabies antibodies, which correlates with the ability to protect against rabies infection (WHO, 2005). The same measure is used in dogs and cats to confirm a satisfactory response to vaccination (Oh et al., 2012; OIE, 2008), as neutralizing antibodies are considered a key component of an adaptive immune response against the rabies virus (Hooper et al., 1998). Here, <0.5 IU/㎖ was recorded as seronegative, and ≥0.5 IU/㎖ as seropositive.

When comparing the test results of FAVNT and ELISA with 1,163 samples, FANVT

showed 41 dogs (4.6%), and 5 cats (1.9%) as seronegative, while ELISA showed 80(9.0%) and 16(5.9%) respectively (Fig. 2). When the sera were grouped by vaccine manufacturer, the percentage of seronegative samples was similar in each vaccine and ELISA's seronegative ratio increased approximately two-fold when compared to FAVNT regardless of vaccine manufacturer (Table 2). There was the same result from grouping of age. This result represented that ELISA test had a higher sensitivity than FAVNT. Several studies on the FAVNT sensitivity have demonstrated that a threshold of 0.24 IU/㎖ should be adopted instead of 0.5 IU/㎖ (Cliquet et al., 1998; Hammami et al., 1999).

To be compared the test results of FAVNT and ELISA when targeting 1,163 samples (Table 3), for dogs, the sensitivity of ELISA compared to FAVNT was 95.0%, the specificity was 90.2%, and the accuracy was 94.7%. For cats, the sensitivity was 95.85%, the specificity was 100% and the accuracy was 95.9%. The Cohen's *kappa* values were 0.59 and 0.46. Other published reports suggest that ELISA method shows a lower sensitivity and a stronger correlation for specificity when compared to FAVNT (Cliquet et al.,2004), but in this test, the cats had a high sensitivity while that of specificity was low. Most discrepancies observed involved sera with a titer close to the threshold, 0.5 IU/㎖. When analyzing the raw data, the titers that had either an extremely high or low titer of antibodies were equal in both tests, but if it was around 0.5 IU/㎖, the results became different. The FAVNT depended greatly on the techniques of the researchers, and sensitivity, specificity, and accuracy worsened in consideration of two wells of error range. The fact that antibodies detected by ELISA had a lower titre than those obtained by the neutralization test has been reported previously (Knoop et al.,

2010; Stantic-Pavlinic et al., 2006). Furthermore, given the *kappa* values (dog: k=0.58, cat: k=0.46), ELISA methods cannot be recommend as an alternative to FAVNT as a quarantine inspection method for cats.

For the statistical results, dogs showed a correlation between seronegative subject number and age. Moreover, the Chi-square test was not affected by gender for either cats or dogs; hormones had no influence. In a study which they mentioned the effects of neutralizing antibody titers in vaccinated dogs and cats, Mansfield et al. (2004) reported that sex had no effect on dogs (Mansfield et al., 2004).

Analysis of the 1,163 samples showed that the FAVNT and ELISA methods were not affected by sex, but were affected by products of vaccine and age. Thus, samples with the same in which such external factors could be eliminated were compared. Twenty 4-year-old German Shepherds were vaccinated. Both the inactivated vaccine and attenuated vaccine that were used are mainly distributed in the domestic market. Before inoculation, the level of antibodies was checked, and collected the bloods drew the sera at both 90 days and 180 days after the vaccination to conduct FAVNT and ELISA. Both methods showed that all 20 samples were seronegative. After 90 days, FAVNT showed that three dogs that were inoculated with inactivated vaccines had a titer of ≥ 0.5 IU/㎖. However, no dogs were over 0.5 IU/㎖ according to ELISA. After 180 days, 7 out of the 10 dogs injected with the inactivated vaccine had exceeded the threshold but there was still no dog over 0.5 IU/㎖ according to ELISA. All subjects that were injected with attenuated vaccines surpassed 0.5 IU/㎖ after 90 days for both methods. For the FAVNT only one dog that had its titer of attenuated vaccine below the threshold.

In general, the antibodies of the rabies vaccine are considered as properly formulated

four weeks after the injection regardless of vaccine type (Mansfield et al.,2004). Therefore, it was expected that all of a dog's titer of neutralizing antibodies after 90 days will exceed 0.5 IU/㎖ regardless of vaccine type. Even if it was to be considered that the inactivated vaccine antibody was formed slowly compared to the attenuated vaccine, its antibody development was not appropriately done (Berndtsson et al.,2011; WHO, 2011). In particular, all dogs that were four years old, the interruption of maternal antibodies can be excluded at that age (Bernardi et al., 2000).

For quarantine tests, the contagious risk of rabies virus requires the authorities to utilize inactivated vaccine. However, the inactivated vaccine used in quarantine tests showed a different result between FAVNT and ELISA. The low sensitivity of this ELISA kit compared to FAVNT has also been reported previously (Knoop et al., 2010), with sensitivities equal to 69.8% and 75.8% for dog and cat samples, respectively. The ELISA kit's plate is coated with a purified glycoprotein G and high purification of the glycoprotein G can negatively affect sensitivity (Servat et al., 2007). Other possible thing was the underestimation of titres obtained from the ELISA kit was probably due to poor sample storage conditions involving possible degradation of samples. It was the only affected the Bio-Rad ELISA kit results reported by Wasniewski and Cliquet (2008). However, the experimental samples were tested only several months after sample collecting . Therefore, the results obtained in the current evaluation preclude any possible degradation of samples due to storage conditions. The last thing was an inactivated vaccine injection dose and an immne response. A single dose does not provided lifelong immunity and booster doses need to be administrated at specific intervals. Animals must be at least three months old when they are first vaccinated and it has been shown that animals less than one year old have a slightly incresed risk of

having a poor antibody titer (Mansfield et al., 2004). The possible reasons in resulting a poor ELISA results appear the potency of inactivaed vaccine and dog's age.

Comparing FAVNT and ELISA for the 1,163 quarantine samples, the commercial ELISA kit inspection monitoring method would be suitable for usage considering the sensitivity, especially favorable for dogs.

The international movement of pets increased a lot recently and a quarantine inspection is considered more important. Therefore rabies serological test methods should be able to discriminate protected from insufficient animals. Despite the need to replace the current prescribed methods with other methods as ELISA test, it should be carefully examined to adopt any new methods. Although the OIE has recommended the commercial ELISA kit as prescribed tests for evaluating vaccine responses in dogs and cats, it cannot sufficiently replace FAVNT.

REFERENCES

Bernardi, F. and Ito, F.H. 2000. Maternal antibody passively transferred interferes with rabies vaccination in hamsters. Comp.Immune.Microbio.Infec.Dis. Jul;23(3):185-95.

Berndtsson, L.T., Nyman, A.J., Rivera, E. and Klingeborn, B. 2011. Factors associated with the success of rabies vaccination of dogs in Sweden.Acta.Vet.Scand. 53:22.

Kim, C.H., Lee,C.G., Yoon, H.C., Nam, H.M., Park, C.K., Lee, J.C., Kang, M.I. and Wee, S.H. 2006. Rabies, an emerging disease in Korea. J.Vet. Med B, 53(3), 111-115

Cliquet, F., Aubert, M. and Sagne, L., 1998. Development of a fluorescent antibody virus neutralisation test (FAVN test) for the quantitation of rabies-neutralising antibody. J. Immunol. Methods 212, 79–87.

Cliquet, F., McElhinney, L.M., Servat, A., Boucher, J.M., Lowings, J.P., Goddard, T., Mansfield, K.L. and Fooks, A.R. 2004. Development of a qualitative indirect ELISA for the measurement of ra-bies virus-specific antibodies from vaccinated dogs and cats. J. Virol. Methods. 117(1):1-8

Cliquet, F., Verdier, Y., Sagne, L. and Aubert, M. 2003. Neutralising antibody titration in

25,000 sera of dogs and cats vaccinated against rabies in France, in the framework of the new regulations that offer an alternative to quarantine. Rev.Sci.Tech.OIE. 22(3):857-866.

Degado, S. and Cármenes, P. 1997. Immune response following a vaccination campaign against rabies in dogs from northwestern Spain. Prev.Vet.Med., 31:257-261.

Hammami, S., Schumacher, C., Cliquet, F., Tlatli, A., Aubert, A. and Aubert, M., 1999. Vaccination of Tunisian dogs with the lyophilised SAG2 oral rabies vaccine incorporated into the DBL2 dog bait. Vet. Res. 30, 607–613.

Hooper, D.C., Morimoto, K., Bette, M., Weihe E., Koprowski H. and Dietzschold B. 1998. Collaboration of antibody and inflammation in clearance of rabies virus from the central nervous system. J. Virol., 72: 3711–3719.

Johnson, N., Cunningham, A.F. and Fooks, A.R., 2010. The immune response to rabies virus infection and vaccination. Vaccine 28, 3896–3901.

Joo, Y.S., Lee, J.H., Lee, K.K., Bang, H.A., and Lee, W.C. 2011. Retrospective study of extensive vaccination programs for canine rabies control and public health in Korea. Jpn. J. Infect. Dis., 64, 513-515.

Knoop, E.V., Freuling, C.M., Kliemt, J., Selhorst, T., Conraths, F.J. and Muller, T. 2010. Evaluation of a commercial rabies ELISA as a replacement for serum

neutralization assays as part of the pet travel scheme and oral vaccination campaigns of foxes. erl Munch Tierarztl Wochenschr. 2010 Jul-Aug;123(7-8):278-85.

Mansfield, K. L. 2004. Serological response of dogs and cats to rabies vaccination. Vet.Rec. 154:423-426.

Lee,K.K. 2009. Outbreaks and control of animal rabies in Korea. Infect.Chemother. 42(1):1-5.

Landies, J.R. and Koch, G.G., 1977. The measurement of abserver agreement for categorical data, Biometrics 33(1);159-174

Moore, S.M., Ricke, T.A., Davis, R.D. and Briggs, D.J., 2005. The influence of homologous vs. heterologous challenge virus strains on the serological test results of rabies virus neutralizing assays. Biologicals 33, 269–276.

Oh, Y.N., Lee, S.H., Yang, D.K. and Song, J.Y. 2012. Risk of rabies by importing animals to South Korea. Kor. J. Vet. Publ. Hlth. 36(1):50-54.

OIE, 2008. Rabies, Chapter 2.1.13. in Manual of dianostic tests and vacccines for terrestrial animals, vol I. 6th Ed.

OIE, 2013. Rabies, Chapter 2.1.13. in Manual of dianostic tests and vacccines for

terrestrial animals, vol I 7th Ed.

Sagne, L. and Cliquet,F. 2006. Collaborative study to evaluate a new ELISA test to monitor the effectiveness of rabies vaccination in domestic carnivores. Virus. Res., 120(1-2):17-27.

Servat, A., Feyssaguet, M., Blanchard, I., Morize, J.L., Schereffer, J.L., Boue, F. and Cliquet,F. 2007. A quantitative indirect ELISA to monitor the effectiveness of rabies vaccination in domestic and wild carnivores. J. Immunol. Methods 318, 1−10.

Shimazaki, Y., Inoue, S., Takahashi, C., Gamoh, K., Etoh, M., Kamiyama, T. and Makie H. 2003. Immune response to Japanese rabies vaccine in domestic dogs. J.Vet. Med.B.(50):95-98.

Stantić-Pavlinić1, M., Hostnik, P., Levičnik-Stezinar, S. and Zaletel-Kragelj, L. 2006. Vaccination against rabies and protective antibodies - comparison of ELISA and fluorescent antibody virus neutralization (FAVN) assays. Vet.Arc.76(4):281-289.

Wasniewski, M., Labbe, A., Tribout, L., Rieder, J., Labadie, A., Schereffer, J.L. and Cliquet, F. 2014. Evaluation of a rabies ELISA as an alternative method to seroneutralisation tests in the context of international trade of domestic carnivores. J Virol Methods. Jan;195:211-20

Welch, R.J., Anderson, B.L. and Litwin, C.M. 2009. An evaluation of two commercially available ELISAs and one in-house reference laboratory ELISA for the determination of human anti-rabies virus antibodies. J Med Microbiol, 58(Pt 6):806-810.

WHO(World Health Organization), 1992. Expert committee on rabies. WHO(World Health Organization), 8th report:84-88.

WHO(World Health Organization), 2011. Immunological basis for immunization series. Module 17: Rabies

WHO(World Health Organization), 2005. WHO Expert Consultation on Rabies. World Health Organization, Geneva

Yang, D.K., Kim, S.Y., Oh, Y.I., Lee, J.A., Cho, S.D., Lee, K.W. and Song, J.Y. 2011. Epidemiological Characteristics of Rabies in South Korea from January 2004 to March 2011. J. Bacteriol.Virol.41(3):165-171.

Yang, D.K., Kim, H.H., Lee, K.W. and Song, J.Y. 2013. The present and future of rabies vaccine in animals. Clin Exp Vaccine Res 2:19-25

Yang, D.K., Yoon, S.S., Lee, K.K., Byun, J.W., Bae, Y.C., Oh, Y.I. and Song, J.Y. 2010. Rabies immune status in the stray and companion dogs in Korea. Korean.J. Vet.Res. 50(2):133-137.

국문초록

수출입 검역 시 개와 고양이의
광견병항체검사를 위한 효소결합면역분석법의 적합성

서울대학교 대학원

수의학과 수의미생물학 전공

임 정 은

(지도교수 : 박 봉 균)

국내에서 광견병은 산발적으로 발생하는 인수공통질병이다. 국가백신프로그램 정책 이후에도 최근 서울 인근에서 발생보고가 연이어 이루어지는 등, 보다 강력한 관리가 요구되고 있다. 최근 몇몇 국가에서 개와 고양이의 검역과정에서 광견병중화항체역가가 일정수치 이상 형성되었다는 검사결과 증명서를 요구하고 있으며 2012년 12월 1일 이후 우리나라에서도 개나 고양이가 입국할 시 광견병중화항체역가 검사결과서를 휴대해야 한다.

현재 국내에서 사용하는 광견병중화항체 검사법은 Fluorescent Antibody Virus Neutralization Test (FAVNT)로 OIE에서 표준 진단방법으로 인정하는 방법이다. 그러나 이 방법은 여러 불편한 점으로 인해 다른 대안방법의 필요성이 요구되어 왔다. 이에 시판 Enzyme-linked immunosorbent assay kit(Platelia Rabies II kit)를 검역검사에 사용 할 수 있을 지 그 적합성을

1,163두의 수입 및 수출 검역 혈청 샘플과 20두의 실험군 혈청샘플을 사용하여 판단하였다. FAVNT에 대한 ELISA의 특이도 및 민감도는 개에서는 90.2%와 95.0%, 고양이에서는 100.0%와 95.8%였다. k 값은 각각 0.58과 0.46으로 나타났다. 실험군은 독일 셰퍼트 20마리에 생독백신과 사독백신을 각각 접종한 후 접종당일, 90, 180일의 혈청을 FAVNT 와 ELISA로 결과를 비교해 보았다. 그 결과 사독백신을 접종한 그룹에서는 FAVNT로 검사하였을 때 접종 후 90, 180일 째 각각 3두, 7두가 0.5IU/㎖ 이상이었다 그러나 ELISA 방법에서는 0.5IU/㎖을 넘은 개체가 없었다. 생독백신을 접종한 그룹에서는 FAVNT에서 1두를 제외하고 두 실험법에서 모든 개체가 0.5IU/㎖를 넘었다. 검역검사에서는 광견병 감염의 우려 때문에 사독백신의 사용을 요구하고 있다. 따라서 ELISA kit(*Platelia Rabies II kit,* Bio-Rad)는 OIE에서 동물의 나라간 이동 시 사용을 추천하고 있지만, 아직 우리나라에서는 FAVNT 대체 방법으로 사용하기 어렵다고 판단되었다.

주요어 : 광견병, 형광항체중화시험법, 효소결합면역분석법, 검역

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