



NOTE

Internal Medicine

Anaphylaxis after rabies vaccination for dogs in Japan

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ABSTRACT. Severe adverse reactions after rabies vaccination in dogs were examined from 317 cases reported to the Ministry of Agriculture, Forestry and Fisheries (MAFF) in Japan during 15-year period from April 2004 to March 2019. We found that 109 of the 317 dogs showed anaphylaxis (0.15/100,000 vaccinated dogs), and 71 of the 109 cases of anaphylaxis resulted in death (0.10/100,000 vaccinated dogs). We measured bovine serum albumin (BSA) in four commercially available rabies vaccines and found the levels ranged from 0.1 to 16.6 µg/dose. Our survey showed that the rate of anaphylaxis to rabies vaccines in dogs is rare, although some cases of anaphylaxis resulted in death. Veterinarians should be well prepared to deal with vaccine-associated anaphylaxis.

KEY WORDS: adverse reaction, anaphylaxis, dog, rabies vaccine

J. Vet. Med. Sci.

83(8): 1202–1205, 2021

doi: 10.1292/jvms.21-0090

Received: 18 February 2021

Accepted: 31 May 2021

Advanced Epub:

9 June 2021

Rabies vaccines are widely used to protect animals, including dogs, from the disease and play an important role in public health. Although canine vaccines play an important role in the prevention of many infectious diseases in dogs, adverse reactions after vaccination have been observed [5, 18]. Allergic reactions including anaphylaxis are one example of adverse reactions after vaccination [14]. Anaphylaxis is an immediate-type reaction induced by IgE-mediated type I hypersensitivity [11]. Anaphylaxis is the most severe reaction after canine rabies vaccination, and our previous report documented that postvaccination anaphylaxis resulted in death in some dogs [16].

An epidemiological survey in Canada identified the rate for anaphylaxis to rabies vaccines as 0.343 per 10,000 cats and dogs [22]. In Japan, the law requires dogs to be vaccinated against rabies virus annually [21]. Our previous study reported that 27 dogs showed adverse reactions to rabies vaccines over a 6-year period from April 1994 through March 2000 [16]. Of the 27 cases, five died from severe allergic reactions including respiratory and/or cardiovascular clinical signs, which were considered to be anaphylaxis. Gamoh *et al.* [4] reported that 60 dogs showed adverse reactions to rabies vaccines from fiscal years 2003 to 2005. Since July 2003, manufacturers and veterinarians in Japan have been required to report all serious adverse reactions (deaths or cases that may lead to death, or diseases and disorders or cases that may lead to them that cannot be predicted from the package insert [9]) to drugs and medical devices. In April 2004, the “adverse event reporting system for veterinary drugs” to allow reporting of adverse reactions via the internet was commenced.

We previously found IgE in response to fetal calf serum (FCS) in canine sera, which obtained from dogs that had allergic reactions after vaccination against non-rabies vaccine with high levels of bovine serum albumin (BSA) [17]. Furthermore, immunoblot analysis demonstrated that a variety of FCS components, including albumin, could function as allergens in dogs that developed allergic reactions after vaccination [15]. However, there are no reports about the measurement of BSA levels in rabies

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(Supplementary material: refer to PMC <https://www.ncbi.nlm.nih.gov/pmc/journals/2350/>)

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vaccines.

In this study, we report the results of a large-scale survey of adverse reactions to canine rabies vaccines in Japan during a period of 15 years from April 2004 through March 2019. Furthermore, we measured BSA levels in rabies vaccines for dogs. Our results reveal useful information about anaphylaxis including critical details of clinical signs and death associated with canine vaccination.

Serious adverse reactions, including death, to rabies vaccines in dogs were examined from 317 cases reported to the MAFF in Japan over a period of 15 years from April of 2004 through March of 2019 [10]. The reports not only recorded standard information (breed, sex and neuter status, age, weight, and date of vaccination), but also important factors associated with adverse reactions including type of vaccine, clinical signs, and time to onset postvaccination. In the reports, the dogs were diagnosed with anaphylaxis by the veterinarians who had administered the rabies vaccines. In addition, we identified further anaphylaxis cases based on the definition of anaphylaxis as the following clinical signs: collapse, cyanosis, hypothermia, dyspnea, and/or hyperpnea observed after vaccination [13].

Four rabies vaccines for dogs are commonly used in Japan and produced by four manufacturers (A, B, C, and D). These vaccines consist of RC-EL strain of rabies viruses that have been inactivated following propagation in hamster lung HmLu-1 cell cultures and do not contain any adjuvant, excipient, or protein stabilizers (gelatin, casein, or peptone).

BSA levels in commercially available rabies vaccines for dogs in Japan were assayed using sandwich enzyme-linked immunosorbent assay (ELISA) [17] with minor modifications. A microplate (NUNC-Immuno Plate Maxisorp F96; NalgeNunc International, Rochester, NY, USA) was coated with crude extracts of anti-BSA IgG (4 µg/ml) (ITEA Inc., Tokyo, Japan) diluted with phosphate buffered saline (PBS) and was incubated at 4°C overnight. After washing, the plate was blocked with 5% rabbit serum and 0.125% Block Ace (Yukijirushi, Osaka, Japan) with PBS for 1 hr at room temperature. After washing, several concentrations of the vaccine diluted with 5% rabbit serum and 0.125% Block Ace with PBS with Tween 20 were added to the wells and the plate was incubated for 3 hr at room temperature. The plate was washed then incubated for 1 hr at room temperature with biotin-anti-BSA IgG (0.5 µg/ml) (ITEA Inc.). After washing, Streptavidin–Peroxidase Polymer, Ultrasensitive (Sigma-Aldrich, St. Louis, MO, USA) was added to the wells for 1 hr at room temperature. After washing, the colorimetric reaction was developed by adding a mixture of hydrogen peroxide and ortho-phenylenediamine dihydrochloride (Sigma Aldrich). After the enzymatic reaction was stopped by adding 4N sulfuric acid, the absorbance at 492 nm was measured with a colorimetric microplate reader (Flow Laboratories, McLean, VA, USA).

Comparison of the frequencies of serious adverse events and deaths, and anaphylaxis and deaths due to anaphylaxis by year were evaluated using the χ^2 test. In order to detect an increasing or decreasing trend in adverse event reporting, the frequency by year was evaluated using regression analysis. The proportion of anaphylaxis cases that resulted in death by year was also evaluated as the case-fatality risk of anaphylaxis. A value of $P < 0.05$ was considered significant.

Japan has been free from animal rabies since 1958 [7]. In accordance with the Rabies Prevention Law enacted since 1950, the policy of registration and vaccination of domestic dogs against rabies has been enforced by the prefectural governments under the order of Ministry of Health, Labour and Welfare (MHLW) [21]. The total number of doses of rabies vaccines administered to dogs in Japan over a 15-year period from April 2004 through March 2019 was 72,573,199 (mean 4,838,213 doses/year) (Table 1) [12]. During this time, 317 cases (0.44/100,000 vaccinated dogs) of severe adverse reactions to rabies vaccines in dogs were reported to the adverse event reporting system of the MAFF [10], of which 171 dogs (54%) died (0.24/100,000 vaccinated dogs). Based on veterinarian diagnosis and symptom estimation, 109 (34%) of the 317 dogs showed anaphylactic symptoms (0.15/100,000 vaccinated dogs), and 71 (65%) of the 109 cases of anaphylaxis resulted in death (0.10/100,000 vaccinated dogs). Of the 107 cases of anaphylaxis, 61 had anaphylaxis as a symptom in the report, 45 had veterinarians diagnosed, and 17 had our own diagnosis in this study. There were no significant differences in the frequencies of reporting serious adverse events and deaths, or reporting anaphylaxis and deaths due to anaphylaxis, by year ($P > 0.05$, χ^2 test). No significant regression for an increasing or decreasing trend was observed for any of the reporting frequencies by year (Table 2). The case-fatality risk of anaphylaxis by year slightly showed a decreasing trend, but anaphylaxis was still considered the high risk of death and the regression was not significant (Tables 1 and 2).

In this study, we found that the rate (0.44/100,000) of severe adverse reactions to rabies vaccines in dogs in Japan was lower than that (3/100,000) of severe adverse reactions to rabies vaccines in humans in the United States [3] (Table 1). We identified 109 dogs that showed anaphylaxis (0.15/100,000 vaccinated dogs) to rabies vaccines in Japan; this number was much lower than previously reported (3.43/100,000) for anaphylaxis to rabies vaccines in dogs and cats in Canada [22]. This rate is much lower than the rate (72/100,000) of anaphylaxis to non-rabies vaccines in our previous report, although the two epidemiological surveys differ in the surveyed population and the method of obtaining information [13]. These data suggest that rabies vaccination in dogs in Japan is safe and severe adverse reactions, including anaphylaxis, are rare.

Detailed information about 29 of the dogs who showed vaccine-associated anaphylaxis in 1 hr is provided in Supplementary Table 1. Anaphylaxis is distinguished from simple allergic reactions by the simultaneous involvement of several organ systems. In this study, clinical signs in Supplemental Table 1 related to anaphylaxis were seen in 19 (66%) cases in relation with the cardiovascular system (collapse 55%, hypotension 24%, bradycardia 21%, pale visible mucous membrane 10%), 11 (38%) cases with the gastrointestinal tract (vomiting 38%, diarrhea 10%), 16 (55%) cases with the respiratory tract (dyspnea 24%, tachypnea 21%, cyanosis 35%), and less frequently skin (facial edema 7%). Furthermore, astasia (28%) possibly related to anaphylactic shock was seen. For acute anaphylactic reactions in dogs reported to the United States Pharmacopeia, Veterinary Practitioners' Reporting Program, the clinical signs most often reported were in the skin (51%), usually manifesting as urticaria involving the face and ears [11]. In anaphylaxis, cutaneous signs and non-life-threatening gastrointestinal disturbance usually occur first, followed by life-threatening cardiovascular or respiratory changes and cardiac arrest. In cases with higher clinical severity, cutaneous signs may be missed [1].

Table 1. Severe adverse reactions and anaphylaxis to rabies vaccines in dogs in Japan

Year	No. of doses	Severe adverse reactions				Anaphylaxis				Case-fatality risk of anaphylaxis*
		No. of reports	No. of deaths	No. of cases per 100,000 doses	No. of death per 100,000 doses	No. of cases	No. of deaths	No. of cases per 100,000 doses	No. of death per 100,000 doses	
2004	4,801,709	22	16	0.46	0.33	14	11	0.29	0.23	0.79
2005	4,796,585	17	8	0.35	0.17	5	3	0.10	0.06	0.60
2006	4,910,047	23	13	0.47	0.26	7	5	0.14	0.10	0.71
2007	5,097,615	20	11	0.39	0.22	6	4	0.12	0.08	0.67
2008	5,091,515	22	11	0.43	0.22	5	3	0.10	0.06	0.60
2009	5,112,401	19	7	0.37	0.14	6	4	0.12	0.08	0.67
2010	4,961,401	35	17	0.71	0.34	10	8	0.20	0.16	0.80
2011	4,985,930	20	9	0.40	0.18	4	2	0.08	0.04	0.50
2012	4,914,347	18	8	0.37	0.16	8	7	0.16	0.14	0.88
2013	4,899,484	18	12	0.37	0.24	5	3	0.10	0.06	0.60
2014	4,744,364	15	11	0.32	0.23	7	6	0.15	0.13	0.86
2015	4,688,240	18	10	0.38	0.21	6	3	0.13	0.06	0.50
2016	4,608,898	19	10	0.41	0.22	6	2	0.13	0.04	0.33
2017	4,518,837	18	11	0.40	0.24	5	3	0.11	0.07	0.60
2018	4,441,826	33	17	0.74	0.38	15	7	0.34	0.16	0.47
Total	72,573,199	317	171	0.44	0.24	109	71	0.15	0.10	0.65

*Proportion of anaphylaxis cases that resulted in death.

Previously, in about 0.3–0.8 human individuals per 1,000 vaccines, contaminating neuroproteins present in nerve tissue-based rabies vaccines caused severe allergic encephalomyelitis [23]. At present, available cell culture-based vaccines are considered safe [23]. Factors known to cause vaccine reactions include the primary vaccine agent or antigen, adjuvants, preservatives, stabilizers, and residues from tissue culture used in vaccine production [2]. In human, we found that gelatin contained in some vaccines has been associated with high rates of anaphylaxis in Japan [19, 20]. In dogs, our previous study reported that canine non-rabies vaccines contained large amounts of BSA and bovine IgG and dogs with anaphylaxis to the vaccines showed FCS, gelatin, and casein-specific IgE [17]. The rabies vaccines administered to dogs in this survey do not contain gelatin, casein, or peptone, which many other canine vaccines contain. We measured BSA in two lots of the four rabies vaccines commercially available in Japan and found levels ranging from 0.1 to 16.6 µg/dose (mean value 3.5 ± 5.3 µg/dose) (Fig. 1). Compared to our previous report where BSA levels in non-rabies vaccines ranged from 61.6 to 3,678 µg/dose [15], the levels of BSA in rabies vaccines in this study are lower. This suggests that the low number of severe adverse reactions, including anaphylaxis, seen after rabies vaccination in dogs compared to the number of those of non-rabies vaccine in Japan is possibly due to the lower BSA levels and fewer additives in rabies vaccines compared to those in non-rabies vaccines.

We found that the rate of anaphylaxis to rabies vaccines in dogs in Japan is rare. Furthermore, another 19 dogs were suspected of anaphylaxis but could not be diagnosed due to lack of clinical

Table 2. Regression coefficients for reporting frequencies by year

	Regression coefficient (95% confidence intervals)
Frequencies of cases by severe adverse reactions	0.0045 (-0.0118–0.0207)
Frequencies of death by severe adverse reactions	0.0021 (-0.0070–0.0111)
Frequencies of cases by Anaphylaxis	0.0015 (-0.0082–0.0112)
Frequencies of death by Anaphylaxis	-0.0024 (-0.0095–0.0047)
Case-fatality risk of anaphylaxis	-0.0146 (-0.0331–0.0038)

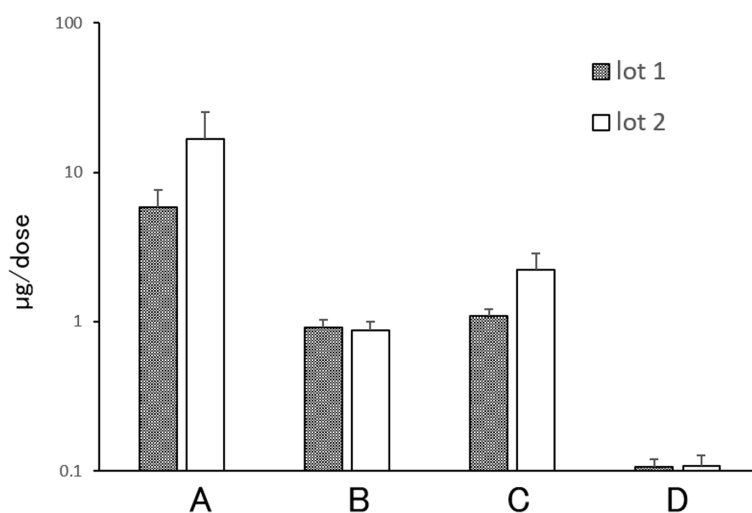


Fig. 1. Bovine serum albumin (BSA) levels in two lots of four commercially available rabies vaccines for dogs in Japan. Error bars show standard deviation.

information, although they died within 1 hr of vaccination (data not shown). In fact, more dogs may have anaphylaxis after vaccination than reported here, due to lack of clinical information. Pretreatment with antihistamines and corticosteroids is widely practiced, however there is little evidence for their effectiveness and they do not prevent a severe anaphylactic response [6, 8]. Therefore, the most important management consideration for dogs with anaphylaxis is to be alert to the clinical signs and have immediate access to treatments including fluid therapy and epinephrine. Furthermore, if a dog presents in respiratory distress, it may be necessary to secure an airway. Although rare, in the event of rabies vaccine-associated anaphylaxis in dogs, veterinarians should always be well prepared to save their life.

POTENTIAL CONFLICTS OF INTEREST. The authors have nothing to disclose.

ACKNOWLEDGMENTS. This study was supported by Ministry of Education, Culture, Sports, Science and Technology-Supported Program for the Private University Research Branding Project, 2016–2020.

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