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Prevalence of autoantibodies that bind to kidney tissues in cats and association risk with antibodies to feline viral rhinotracheitis, calicivirus, and panleukopenia

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ABSTRACT

Background: The feline viral rhinotracheitis, calicivirus, and panleukopenia (FVRCP) vaccine, prepared from viruses grown in the Crandell-Rees feline kidney cell line, can induce antibodies to cross-react with feline kidney tissues.
Objectives: This study surveyed the prevalence of autoantibodies to feline kidney tissues and their association with the frequency of FVRCP vaccination.
Methods: Serum samples and kidneys were collected from 156 live and 26 cadaveric cats. Antibodies that bind to kidney tissues and antibodies to the FVRCP antigen were determined by enzyme-linked immunosorbent assay (ELISA), and kidney-bound antibody patterns were investigated by examining immunofluorescence. Proteins recognized by antibodies were identified by Western blot analysis.
Results: The prevalences of autoantibodies that bind to kidney tissues in cats were 41% and 13% by ELISA and immunofluorescence, respectively. Kidney-bound antibodies were observed at interstitial cells, apical border, and cytoplasm of proximal and distal tubules; the antibodies were bound to proteins with molecular weights of 40, 47, 38, and 20 kDa. There was no direct link between vaccination and anti-kidney antibodies, but positive antibodies to kidney tissues were significantly associated with the anti-FVRCP antibody. The odds ratio or association in finding the autoantibody in cats with the antibody to FVRCP was 2.8 times higher than that in cats without the antibody to FVRCP.
Conclusions: These preliminary results demonstrate an association between anti-FVRCP and anti-cat kidney tissues. However, an increase in the risk of inducing kidney-bound antibodies by repeat vaccinations could not be shown directly. It will be interesting to expand the sample size and follow-up on whether these autoantibodies can lead to kidney function impairment.

Keywords: Kidney diseases; autoantibodies; vaccines; immunofluorescence; feline

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Conflict of Interest

The authors declare no conflicts of interest.

Author Contributions

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INTRODUCTION

Kidney disease can be divided into acute kidney injury and chronic kidney disease (CKD), both of which can lead to the same result: kidney failure [1,2]. CKD is one of the most common causes of illness and death in elderly domestic cats [3,4].

In an attempt to elucidate the risk factors associated with CKD, a longitudinal questionnaire and follow-up investigation revealed that frequent vaccinations and the severity of dental disease were factors associated with the development of CKD [5]. There are several reports that the frequency of vaccinations is associated with autoimmune diseases in animals and humans [6-10]. Vaccines, especially viral vaccines, do not only contain antigens of the relevant organism, but also contain other ingredients, such as adjuvants, preservative materials, and tissue or cell culture proteins that are used when growing organisms [11-13]. Tissue culture components that contaminate vaccines can induce immune responses and may cross-react with host tissue antigens, known as molecular mimicry [14-17]. Proteins from the Crandell-Rees feline renal cell line (CRFK), which was derived from feline kidney tissues, are used by some companies to grow feline herpesvirus 1 (FHV-1), feline calicivirus (FCV), and feline panleukopenia virus (FPV) for use in feline viral vaccines such as feline viral rhinotracheitis, calicivirus, and panleukopenia (FVRCP) vaccine. Remnant proteins from the CRFK cell line have been shown to induce antibodies to kidney tissue lysates in an experimental model [18]. Therefore, frequent or over-vaccination by FVRCP might be considered a risk for producing antibodies that bind to kidney tissues after vaccination.

The FVRCP vaccine is a core vaccine that is used to immunize cats annually. However, viral vaccines usually induce a long-lasting immune response [19,20]. The American Association of Feline Practitioners first recommended triennial rather than annual FVRCP revaccination in 1998 [21].

This study's primary aim was to estimate the prevalence of antibodies to proteins extracted from kidney tissues in unvaccinated cats and cats known to have been administered FVRCP vaccines. The secondary aim was an attempt to determine the localization of kidney-bound antibodies.

MATERIALS AND METHODS

Sample collection

A total of 156 serum samples were collected from 69 unvaccinated and 87 FVRCP-vaccinated cats aged between 4 months and 16 years. The FVRCP-vaccinated cats received at least one complete vaccination protocol, and blood sera were collected at least one month after vaccination. The sampled cats were from a local shelter or were owned cats that came to the Small Animal Hospital at Chiang Mai University, Thailand. Serum creatinine and blood urea nitrogen (BUN) concentrations and the number of FVRCP vaccinations in each cat were recorded. The normal standard value of serum creatinine and BUN is referenced from Duncan & Prasses's Veterinary Laboratory Medicine Clinical Pathology [22]. All serum samples were aliquoted and kept at −20°C until tested. Demographic data and vaccination history of unvaccinated and FVRCP-vaccinated cats are shown in **Tables 1** and **2**, respectively.

A total of 26 paraffin-embedded cat kidneys from cats that had died from different causes were collected from the Veterinary Diagnostic Laboratory, Chiang Mai University. The

Table 1. Demographic and laboratory data of unvaccinated cats

Cat	History data			Laboratory data (mg/dL)		Cat	History data			Laboratory data (mg/dL)		Cat	History data			Laboratory data (mg/dL)	
	Age	Sex	Breed	Cr	BUN		Age	Sex	Breed	Cr	BUN		Age	Sex	Breed	Cr	BUN
U01	2 yr	F	Mix	1.18	17.10	U24	1 yr	F	DSH	2.88	57.40	U47	2 yr	F	DSH	1.26	19.10
U02	3 yr	M	DSH	1.29	29.50	U25	1 yr	F	DSH	2.24	38.10	U48	4 yr	M	DSH	1.05	34.90
U03	4 mo	M	Mix	1.12	33.00	U26	6 mo	M	DSH	1.90	38.90	U49	7 mo	M	DSH	1.26	21.60
U04[a]	7 yr	F	DSH	2.50	51.00	U27	3 yr	M	Mix	1.35	27.30	U50	8 mo	F	DSH	1.23	19.80
U05[a]	15 yr	F	DSH	3.86	166.90	U28	1 yr	F	DSH	1.18	12.40	U51	2 yr	M	DSH	1.34	24.40
U06[c]	6 mo	F	PS	1.20	77.50	U29[a]	7 yr	M	DSH	3.29	73.00	U52	8 mo	M	DSH	1.11	22.20
U07	4 mo	M	DSH	1.06	21.10	U30	4 mo	M	PS	0.83	22.20	U53	1 yr	F	DSH	1.34	26.50
U08	1 yr	M	DSH	1.11	23.30	U31	2 yr	M	DSH	2.28	30.90	U54	1 yr	F	DSH	0.83	23.10
U09	3 yr	M	Mix	1.37	20.60	U32	3 yr	M	DSH	1.75	26.30	U55	1 yr	F	DSH	1.35	25.20
U10	2 yr	M	DSH	2.28	25.70	U33[c]	6 mo	F	DSH	5.92	101.00	U56	5 yr	F	DSH	1.42	56.20
U11	6 mo	M	DSH	1.44	27.80	U34[c]	8 mo	M	DSH	0.96	19.70	U57	7 mo	F	DSH	1.24	20.90
U12	2 yr	F	DSH	1.27	28.80	U35[c]	7 mo	M	DSH	1.01	25.50	U58	1 yr	F	DSH	1.17	18.00
U13	5 mo	F	DSH	1.18	17.00	U36[c]	6 mo	M	DSH	1.06	10.10	U59	1 yr	F	DSH	1.28	14.70
U14	5 mo	F	DSH	0.99	22.80	U37	8 mo	F	DSH	1.31	17.10	U60	1 yr	M	DSH	1.20	20.30
U15	6 mo	M	DSH	1.32	28.30	U38	9 yr	F	DSH	0.96	21.40	U61	1 yr	F	DSH	1.22	20.30
U16	5 mo	M	DSH	1.13	18.80	U39	1 yr	M	DSH	0.84	30.50	U62	2 yr	M	DSH	1.49	13.90
U17	5 mo	F	DSH	0.91	19.00	U40	1 yr	F	DSH	1.87	40.20	U63	2 yr	M	DSH	1.59	16.70
U18	8 mo	M	DSH	0.82	22.40	U41	6 mo	F	DSH	1.18	18.90	U64	2 yr	F	DSH	1.62	24.10
U19	2 yr	M	DSH	2.85	42.50	U42	6 mo	F	DSH	0.67	24.40	U65	3 yr	F	DSH	1.45	21.40
U20	3 yr	M	DSH	1.95	34.70	U43	6 mo	F	DSH	0.79	25.60	U66	1 yr	M	DSH	1.24	14.00
U21	2 yr	F	DSH	2.52	43.20	U44	1 yr	M	DSH	1.25	15.60	U67	1 yr	M	DSH	1.40	25.80
U22	2 yr	F	DSH	1.58	34.40	U45	3 yr	M	DSH	1.22	28.20	U68	1 yr	M	DSH	1.27	21.00
U23	1 yr	F	DSH	1.81	38.10	U46	8 mo	F	DSH	1.02	20.40	U69[b]	1 yr	F	DSH	2.70	15.00

Note: There were 69 unvaccinated cats.
Cr, creatinine; BUN, blood urea nitrogen; DSH, Domestic Shorthair; PS, Persian; Mix, mixed breed; SF, Scottish Fold; CHI, Chinchilla.
[a]Chronic kidney disease; [b]Acute kidney disease; and [c]Feline parvovirus.

kidneys from a healthy cat that had died in a traffic accident were collected and divided into two parts; 1) kept at −20°C for antigen preparations, and 2) fixed in 10% formalin and stored at room temperature (RT) for 16–18 h before tissue processing. This cat had no previous FVRCP vaccination history, and its kidneys were PCR tested as FPV-negative.

Protocols applied in the animal experiments were approved by the Institutional Animal Care and Use Committee, Faculty of Veterinary Medicine, Chiang Mai University (approval No. R25/2559).

Antigen preparations

Proteins from the healthy cat kidney extract were obtained and purified using the Qproteome mammalian protein preparation kit (Qiagen, USA) according to the manufacturer's instructions, as previously reported [23]. The CRFK cell line was obtained from Dr. Kakanang Piyarungsri of the Department of Companion Animal and Wildlife Clinic, Chiang Mai University, Thailand (purchased from ATCC [CCL-94], LOT 60980362, passage No. 189). The cell line was cultured in Dulbecco's modified Eagle's medium supplement with 10% fetal calf serum in a 5% CO_2 incubator, as described in a previous study [23]. The CRFK protein was prepared by using the Qproteome mammalian protein preparation kit, as described in the procedure for cat kidney extract. Both kidney and CRFK protein extracts were aliquoted and kept at −20°C.

The modified-live attenuated FVRCP vaccine (Felocell CVR, Zoetis, USA) was inactivated by ultraviolet inactivation before use as an antigen, as previously described [24].

Table 2. Demographic and laboratory data of FVRCP-vaccinated cats

Cat	History data					Laboratory data (mg/dL)		Cat	History data					Laboratory data (mg/dL)	
	Age	Sex	Breed	Vac.	Last vac.	Cr	BUN		Age	Sex	Breed	Vac.	Last vac.	Cr	BUN
V01	3 yr	M	DSH	2	13 mo	1.37	26.30	V23[a]	12 yr	M	CHI	4	12 mo	7.90	178.00
V02	3 yr	M	DSH	2	5 mo	1.97	34.10	V24	4 yr	F	DSH	1	12 mo	1.70	26.70
V03	1 yr	M	DSH	2	2 mo	2.19	30.90	V25	11 yr	M	DSH	1	24 mo	1.34	32.60
V04	1 yr	F	DSH	2	2 mo	1.69	23.50	V26	2 yr	M	DSH	1	12 mo	1.67	20.90
V05	1 yr	F	DSH	2	2 mo	2.55	32.80	V27	5 yr	M	DSH	2	12 mo	1.85	26.10
V06	8 yr	M	DSH	6	2 mo	1.82	23.90	V28	7 yr	M	DSH	1	8 mo	1.55	23.70
V07	9 yr	M	DSH	9	2 mo	2.01	28.80	V29	8 mo	F	DSH	1	3 mo	1.35	28.60
V08	6 yr	M	ASH	4	7 mo	1.47	28.30	V30	9 mo	F	DSH	1	3 mo	1.17	22.70
V09	6 yr	F	ASH	4	7 mo	2.21	47.50	V31	1 yr	F	DSH	1	3 mo	1.43	27.90
V10	11 yr	M	DSH	2	7 mo	1.20	36.10	V32	11 mo	M	DSH	1	8 mo	1.71	24.60
V11	5 mo	F	ASH	2	1 mo	1.01	17.20	V33	4 yr	F	MIX	2	16 mo	1.50	23.10
V12	5 mo	F	ASH	2	1 mo	1.05	17.90	V34	3 yr	M	PS	3	2 mo	1.42	26.00
V13	3 yr	F	ASH	3	13 mo	0.85	19.90	V35	2 yr	M	PS	1	2 mo	1.62	25.80
V14	3 yr	F	ASH	3	3 mo	1.03	25.40	V36	2 yr	M	DSH	1	2 mo	2.18	27.40
V15	5 yr	F	DSH	4	13 mo	1.22	24.50	V37	2 yr	F	DSH	1	2 mo	1.92	29.70
V16	5 yr	F	ASH	5	3 mo	1.01	24.70	V38	2 yr	M	DSH	1	2 mo	2.15	28.10
V17	5 yr	F	ASH	5	3 mo	1.23	30.00	V39	2 yr	F	DSH	1	2 mo	2.08	28.80
V18	4 yr	F	ASH	4	3 mo	1.00	28.00	V40	2 yr	F	DSH	1	2 mo	1.76	32.00
V19	3 yr	F	ASH	3	3 mo	1.25	30.40	V41	3 yr	F	DSH	1	4 mo	1.86	24.40
V20[a]	3 yr	M	DSH	4	5 mo	1.76	21.20	V42	3 yr	M	DSH	2	4 mo	1.82	29.60
V21[a]	16 yr	F	DSH	15	1 mo	4.00	74.60	V43	3 yr	M	PS	3	3 mo	1.73	24.20
V22[a]	3 yr	M	PS	1	24 mo	1.25	16.70	V44	2 yr	M	DSH	2	4 mo	1.90	26.80
V45	1 yr	M	DSH	1	4 mo	2.13	24.60	V67	5 yr	F	DSH	6	2 mo	1.64	28.10
V46	8 mo	M	SF	1	4 mo	1.24	22.20	V68	5 yr	F	DSH	6	2 mo	1.48	22.50
V47	4 yr	F	DSH	2	4 mo	2.34	26.80	V69	4 yr	M	DSH	4	4 mo	1.24	13.50
V48	5 yr	F	DSH	4	12 mo	2.10	25.70	V70	4 yr	M	DSH	4	3 mo	1.43	25.60
V49	5 yr	M	DSH	4	13 mo	1.83	28.90	V71	4 yr	F	DSH	4	9 mo	1.26	27.50
V50	5 yr	F	DSH	4	14 mo	1.80	20.40	V72[a]	2 yr	M	DSH	2	1 mo	5.83	116.90
V51	2 yr	F	DSH	2	15 mo	2.21	27.10	V73[a]	6 yr	M	DSH	1	1 mo	1.88	36.00
V52[a]	1 yr	M	DSH	1	5 mo	10.77	220.00	V74	2 yr	F	MIX	1	20 mo	0.90	18.70
V53	8 yr	M	MIX	8	5 mo	2.18	23.70	V75	4 yr	M	DSH	3	12 mo	1.23	32.10
V54	5 yr	F	DSH	5	5 mo	1.54	31.40	V76	9 yr	F	DSH	6	12 mo	0.93	22.80
V55	7 yr	M	DSH	7	5 mo	1.78	31.80	V77	1 yr	M	DSH	1	12 mo	1.92	29.20
V56	7 yr	M	DSH	7	5 mo	1.95	30.20	V78	7 yr	M	MIX	1	12 mo	1.84	36.50
V57	5 yr	M	DSH	5	5 mo	1.89	20.10	V79[a,c,d]	5 yr	M	DSH	4	5 mo	3.67	142.70
V58	4 yr	M	DSH	4	5 mo	1.81	25.40	V80	1 yr	M	PS	1	7 mo	1.50	20.20
V59	5 yr	F	DSH	1	5 mo	2.13	34.10	V81[a]	11 yr	F	DSH	11	12 mo	4.99	89.70
V60	1 yr	M	DSH	1	5 mo	1.59	28.70	V82	5 yr	M	DSH	6	12 mo	1.26	19.20
V61	6 mo	M	DSH	1	5 mo	1.33	28.50	V83	8 mo	M	DSH	1	1 mo	1.44	27.80
V62	6 yr	M	DSH	2	1 mo	1.80	50.20	V84[d]	3 yr	M	DSH	3	12 mo	1.38	23.70
V63[a]	4 yr	M	PS	5	1 mo	2.63	32.80	V85	4 yr	F	DSH	3	5 mo	1.34	25.10
V64[b]	2 yr	M	DSH	1	2 mo	9.80	129.30	V86[a]	10 yr	F	DSH	10	8 mo	10.00	101.00
V65	6 yr	M	MIX	6	12 mo	1.83	18.50	V87[a]	7 yr	M	DSH	7	6 mo	2.83	33.80
V66[c]	5 yr	M	DSH	3	2 mo	2.13	30.10								

Note: There were 87 FVRCP-vaccinated cats.
FVRCP, feline viral rhinotracheitis, calicivirus, and panleukopenia; DSH, Domestic Shorthair; ASH, American Shorthair; PS, Persian; Mix, mixed breed; SF, Scottish Fold; CHI, Chinchilla; Vac., number of vaccinations; Last vac., time since the last vaccination.
[a]Chronic kidney disease; [b]Acute kidney disease; [c]Feline immunodeficiency virus; and [d]Feline leukemia virus.

Detecting antibody to kidney extract and FVRCP antigen by enzyme-linked immunosorbent assay (ELISA)

Detection of antibodies to healthy cat kidney extract and FVRCP antigen was performed by ELISA, as previously described [18], with slight modification. Briefly, kidney extract (30 mg/mL) was diluted with carbonate coating buffer (pH 9.2) to 1:500, and coated on 96-well microtiter plates overnight at 4°C. The nonspecific reaction was blocked with 3% bovine serum albumin (BSA; Bio Basic, USA). Serum samples were diluted to 1:5,000, added to

the plates, and the assay run in duplicate. For detection of anti-FVRCP, the FVRCP antigen dilution used was 1:10 and serum was 1:500, and the procedure was the same as described for the kidney extract. Thereafter, horseradish peroxidase (HRP)-conjugated goat anti-cat immunoglobulin G (IgG) secondary antibody (KPL, USA) at a dilution of 1:80,000 was added to each well. Finally, SureBlue TMB Peroxidase Substrate (KPL) was added, and the enzymatic reaction stopped by adding 1N of H_2SO_4. The plate was washed five times after every step with phosphate-buffered saline/0.5% Tween-20, except for the last step (stop reaction step). Color intensity was measured at 450 nm absorbance by using a microplate reader (Synergy H4 Hybrid Reader, Biotek, USA).

Kidney-bound antibody detection by immunofluorescence assay

Determination of antibodies bound to kidney tissues in the 26 cats that died from multiple causes was performed using kidney sections; each section contained both cortex and medulla regions. A healthy kidney section was used as a control, and it was determined to be negative for autoantibody or immune complexes by staining with negative cat serum and/or goat anti-cat fluorescein isothiocyanate (FITC)-conjugated IgG. The immunofluorescence protocol used for elephant tissue was adapted for the cats in this study [25]. Briefly, the tissues were fixed with 10% formalin solution, and the formalin-fixed, paraffin-embedded (FFPE) cat kidney tissues were cut into 4 μm thick slices. FFPE slides were deparaffinized and rehydrated. BSA (1%) in Tris-buffered saline with 0.25% Tween (TBST) was added to the slides for 30 min at RT. Cadaveric cat kidneys were stained in order to examine autoantibody binding in the kidney. The goat anti-cat IgG conjugated FITC (KPL) at a dilution of 1:200 was dropped onto the slide, and the slide incubated for 1 h at RT. Nuclei were counterstained with 2-(4-amidinophenyl)-6-indolecarbamidine dihydrochloride (DAPI; Sigma Aldrich, USA). The slides were observed under a fluorescence microscope (Zeiss, Germany). Cadaveric cat kidneys, positive for kidney-bound antibodies by direct immunofluorescence, were examined histologically by applying hematoxylin and eosin (H&E) stain [26].

The detection of antibodies to kidney tissues in cat sera was performed by following the same procedure. The sections were cut from healthy kidneys, with each section containing cortex and medulla regions. Serum samples were stained on this kidney section. Cat serum was diluted to 1:100 with 1% BSA in TBST, dropped onto slides. The steps after serum sample staining were performed as described for the cadaveric kidneys.

Western blot analysis of antibody recognition of kidney proteins

Proteins from the healthy cat kidney extract or the CRFK cell line were run on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and immunoblotting was carried out, as previously described [25], with a slight modification. Briefly, 25 μg of cat kidney proteins or 100 μg of CRFK cell line protein were loaded into wells of 12.5% gel and run for 90 min at 100 V. The SDS-PAGE gel was then blotted to a polyvinylidene difluoride membrane (Thermo Scientific, USA). The 5% BSA in TBST was added to the membrane to block nonspecific binding. Diluted cat sera (dilution of 1:200 for kidney extract and 1:100 for CRFK protein) were added to the membrane blotted with kidney and CRFK cell line proteins. The HRP-conjugated goat anti-cat IgG secondary antibody (dilution of 1:1,000 for kidney extract and 1:800 for CRFK protein) was then added to the membrane. After washing, the 3,3′-diaminobenzidine tetrahydrochloride (DAB; Thermo Scientific) substrate was added to the membrane for a 30 sec exposure and the protein band appeared. The molecular weight (M.W.) of protein bands was determined by using image analysis software (GeneTools, USA).

Statistical analysis

Sample size estimation, a minimal sample size of at least 80 cats was calculated by performing power analysis based on the assumption of a finite population proportion expected to be 50% of vaccinated cats that had autoantibodies in the serum with 95% confidence interval and 10% error [27].

The statistical analyses used in this study included the following: *t*-test for determining the *p* value; odds ratio (OR) for the association between cats with and without antibody to FVRCP antigen and anti-kidney autoantibody; and Spearman's rank correlation and Pearson's χ^2 test for the correlation assessment. The sensitivity and specificity of the ELISA developed in this study were calculated by examining the receiver operating characteristic curve (ROC curve). Statistical significance was designated at a *p* value of < 0.05.

RESULTS

Profile of anti-FVRCP antibody levels in cat sera

All FVRCP-vaccinated cats were tested firstly for antibody to FVRCP antigen. Unvaccinated cats were tested together as negative sera in order to find a cut-off value. Determination of the cut-off value according to sensitivity and specificity is shown in **Table 3**. An optical density (O.D.) cut-off value of > 0.186 was calculated by analyzing the ROC curve; the chosen cut-off value had 50% sensitivity and 60% specificity. The O.D. cut-off value had a relatively high specificity, which was considered a priority in order to reduce false-positive results. The profiles of the antibodies against the FVRCP antigen in unvaccinated and vaccinated cats are shown in **Fig. 1**. Thirteen percent (9/69) and 66% (57/87) of unvaccinated and FVRCP-vaccinated cats, respectively, were positive for the anti-FVRCP antibody. Nearly half of the vaccinated cats did not show anti-FVRCP antibody presence.

Table 3. Determination of the cut-off value for detecting anti-FVRCP antibody

No.	Cut-off value	Sensitivity	Specificity	Anti-FVRCP (ELISA+)	
				Un-vac.	FVRCP Vac.
1	≥ 0.155	70%	47%	25% (17/69)	78% (68/87)
2	≥ 0.176	60%	55%	15% (10/69)	70% (61/87)
3	≥ 0.186	50%	60%	13% (9/69)	64% (56/87)

FVRCP, feline viral rhinotracheitis, calicivirus, and panleukopenia; ELISA, enzyme-linked immunosorbent assay; Un-vac., unvaccinated cat; FVRCP vac., FVRCP-vaccinated cat.

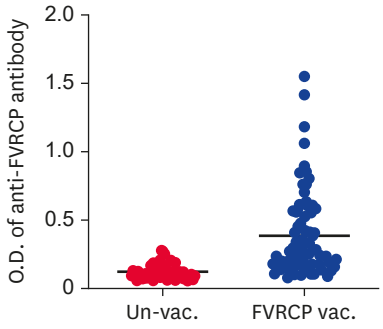

Fig. 1. Profile of anti-FVRCP antibodies in unvaccinated and FVRCP-vaccinated cats. Antibodies to the FVRCP antigen were determined by ELISA. The O.D. of anti-FVRCP antibodies is shown, and each dot represents an individual cat. The average O.D. (mean ± SD) of antibody levels in the unvaccinated and FVRCP-vaccinated cats (O.D. of 0.122 ± 0.051 and 0.381 ± 0.299, respectively) are shown as solid lines.
FVRCP, feline viral rhinotracheitis, calicivirus, and panleukopenia; ELISA, enzyme-linked immunosorbent assay; O.D., optical density; Un-vac., unvaccinated cats; FVRCP vac., FVRCP-vaccinated cats.

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Table 4. Determination of the cut-off value for detecting antibodies that bind to kidney tissues

No.	Cut-off value	Sensitivity	Specificity	Antibodies that bind to kidney tissues (ELISA+)	
				Un-vac.	FVRCP vac.
1	≥ 0.349	70%	64%	32% (22/69)	47% (41/87)
2	≥ 0.363	60%	68%	26% (18/69)	43% (37/87)
3	≥ 0.367	50%	71%	20% (14/69)	41% (36/87)

FVRCP, feline viral rhinotracheitis, calicivirus, and panleukopenia; ELISA, enzyme-linked immunosorbent assay; Un-vac., unvaccinated cat; FVRCP vac., FVRCP-vaccinated cat.

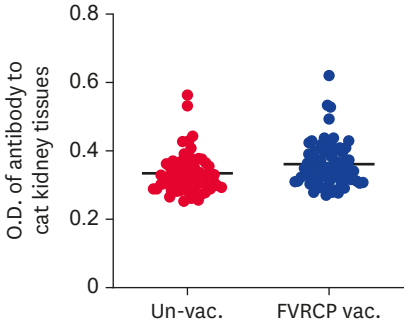

Fig. 2. Profile of antibodies that bind to kidney tissues in unvaccinated and FVRCP-vaccinated cats. Antibodies that bind to kidney tissues were determined by ELISA. The O.D. of antibody that bind to kidney tissues is shown, and each dot represents an individual cat. The average O.D. (mean ± SD) of antibody levels in the unvaccinated and FVRCP-vaccinated cats (O.D. of 0.334 ± 0.058 and 0.361 ± 0.061, respectively) are shown as solid lines. FVRCP, feline viral rhinotracheitis, calicivirus, and panleukopenia; ELISA, enzyme-linked immunosorbent assay; O.D., optical density; Un-vac., unvaccinated cats; FVRCP vac., FVRCP-vaccinated cats.

Profile of antibodies that bind to kidney tissues in cat sera

Unvaccinated and vaccinated sera were evaluated for antibodies to healthy cat kidney extracts in the optimized ELISA. Determination of the cut-off value according to sensitivity and specificity levels is shown in **Table 4**. The cut-off O.D. value of cats with antibodies that bind to kidney tissues, calculated by ROC curve analysis, was ≥ 0.349, which had 70% sensitivity and 64% specificity. The chosen O.D. cut-off value had a relatively high sensitivity, which was considered a priority in order to screen for antibodies that bind to kidney tissues. The profiles of antibodies that had bound to kidney tissues in unvaccinated and vaccinated cats are shown in **Fig. 2**. Antibodies that reacted against healthy cat kidney extracts were detected in 32% (22/69) and 47% (41/87) of the unvaccinated and FVRCP-vaccinated cats, respectively. Although FVRCP-vaccinated cats typically had higher positive antibody levels (*i.e.*, higher ELISA O.D.) bound to cat kidney tissues than that of non-vaccinated cats, there was no significant difference in the antibody bound to cat kidney tissue results between unvaccinated and FVRCP-vaccinated cats ($p = 0.055$; test of proportion statistic).

Kidney-bound antibody detection by direct immunofluorescence

Twenty-six paraffin-embedded cat kidneys were screened for antibodies that bind to kidney tissues. Regarding their clinical diseases, cats died from viral infection (n = 15), kidney disease (n = 2), disseminated intravascular coagulation (DIC; n = 2), endotoxemia (n = 2), viral and 2nd bacterial infection (n = 1), heatstroke (n = 1), feline hypertrophic cardiomyopathy (FHC; n = 1), septicemia (n = 1), and chronic bronchopneumonia (n = 1). Cat histories and diagnoses from the paraffin-embedded cat kidney samples are shown in **Table 5**.

Antibodies that bind at the apical border of the kidney tubule and interstitial cells were detected in 15% (4/26) of the cats tested. The profiles of the 4 cats with positive kidney-bound antibodies were as follows: kidney-bound antibodies were present at the apical border of the kidney tubules in cat 1 (**Fig. 3A**), while those in cats 2, 3, and 4 were present at the interstitial cells (**Fig. 3B-D**).

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Table 5. Cat history and diagnosis derived from the paraffin-embedded cat kidney samples

Cat No.	Age	Sex	Breed	History	Vac.	Necropsy reports	
						Microscopic findings[a]	Diagnosis[b]
1	nd	nd	nd	SD	nd	Severe acute diffuse hydropic degeneration in the tubules	Viral and 2nd bacterial infection
2	nd	nd	PS	Seizure	nd	Severe chronic multifocal non-suppurative interstitial nephritis, multifocal necrosis, and multifocal tubular degeneration	Viral infection
3	2 yr	M	DSH	Anemia	nd	Multifocal inflammatory cell aggregation and diffused tubular necrosis	FeLV
4	3 yr	M	PS	nd	nd	Severe subacute focally extensive necrotic and non-suppurative nephritis	DIC
5	7 mo	nd	DSH	SD	nd	Autolysis	Endotoxemia
6	2 mo	M	DSH	Depress	nd	Hemorrhage, tubular degeneration	Endotoxemia
7	6 yr	F	DSH	Seizure	nd	nd	Heatstroke
8	7 yr	M	PS	HL paresis	nd	Severe diffuse acute tubular degeneration (swelling)	KD
9	1 mo	F	PS	Diarrhea	nd	Infiltration of mononuclear in the glomerulus	FPV
10	2 yr	nd	DSH	nd	nd	Severe diffuse tubular degeneration (vacuolization)	Viral infection
11	nd	nd	nd	nd	nd	Severe acute diffuse tubular degeneration	Viral infection
12	1 mo	nd	PS	Anxious	nd	Hyperemia, diffuse tubular degeneration	FPV
13	2 mo	M	PS	Depress	nd	Diffuse tubular swelling	FPV
14	3 yr	F	PS	Seizure	nd	Multifocal hemorrhage in the tubular areas	Viral infection
15	12 yr	M	DSH	SD	nd	Severe subacute multifocal granulomatous interstitial nephritis	FIP
16	12 yr	F	DSH	SD	nd	Moderate subacute multifocal granulomatous nephritis	FIP
17	9 mo	F	ASH	nd	nd	Moderate subacute multifocal granulomatous glomerulonephritis	FIP
18	nd	F	ASH	Abortion	nd	Autolysis	DIC
19	nd	nd	PS	nd	nd	Severe chronic focal extensive granulomatous interstitial nephritis	FIP
20	nd	F	PS	Ascites	nd	Severe subacute multifocal necrotic glomerulonephritis	FIP
21	1 yr	M	PS	SD	nd	Moderate subacute multifocal necrotic glomerulonephritis, diffuse tubular necrosis	Viral infection
22	1.5 yr	M	Bengal	SD	nd	Severe subacute multifocal necrotic nephritis, diffuse tubular necrosis	FHC
23	2 mo	M	DSH	nd	nd	Severe subacute multifocal necrotic glomerulonephritis	Septicemia
24	10 mo	M	DSH	SD	nd	Nephrosis, severe diffuse tubular necrosis and degeneration	KD
25	4 yr	M	PS	nd	nd	Severe chronic multifocal pyogranulomatous interstitial nephritis	FIP
26	6 yr	M	DSH	nd	nd	Severe tubular nephritis, diffuse tubular necrosis, severe subacute to chronic multifocal necrotic glomerulonephritis	Chronic bronchopneumonia
27	5 mo	M	DSH	TA	-	No remarkable lesions	Healthy[c]

nd = no data, DSH, Domestic Shorthair; ASH, American Shorthair; PS, Persian; SD, sudden death; HL, hind limb; FeLV, feline leukemia virus; DIC, disseminated intravascular coagulation; KD, kidney disease; FPV, feline panleukopenia virus; FIP, feline infectious peritonitis; FHC, feline hypertrophic cardiomyopathy.
[a]Microscopic findings presented only for kidney samples. Lesions in other organs are not presented in this table. [b]Diagnosis was based on gross and histopathological examination. [c]Healthy cat was used as a control for kidney tissue staining and antigen preparation.

H&E-stained kidney sections showed severe acute diffuse hydropic degeneration in the tubules in cat 1 (**Fig. 3E**). H&E-stained kidney sections showed severe chronic multifocal non-suppurative interstitial nephritis, multifocal necrosis, and multifocal tubular degeneration in cat 2 (**Fig. 3F**). H&E-stained kidney sections showed multifocal inflammatory cell aggregation and diffused tubular necrosis in cat 3 (**Fig. 3G**), and H&E-stained kidney sections showed severe subacute focally extensive necrotic and non-suppurative nephritis in cat 4 (**Fig. 3H**). It was noted that the positive fluorescence signals were located in the same area of inflammatory and degenerative tissues of cadaveric cats. These cats were diagnosed pathologically as having viral infection and had unknown vaccination histories when they died.

Serum antibodies against kidney tissues detected by indirect immunofluorescence

When sera from the 69 unvaccinated and 87 FVRCP-vaccinated cats were applied to the healthy cat kidney tissue sections, antibodies that bound to the kidney tissue were detected in 15% (10 cats) and 12% (10 cats), respectively (**Fig. 4**). However, there was no statistical difference in the positive autoantibody results between the two groups. Two patterns of kidney-bound antibodies were observed at the cytoplasm and apical brush border of the proximal and distal tubules. Each cat presented individual profiles, with some showing kidney-bound antibodies in the cytoplasm, while others showed them in the apical border of the kidney tubules.

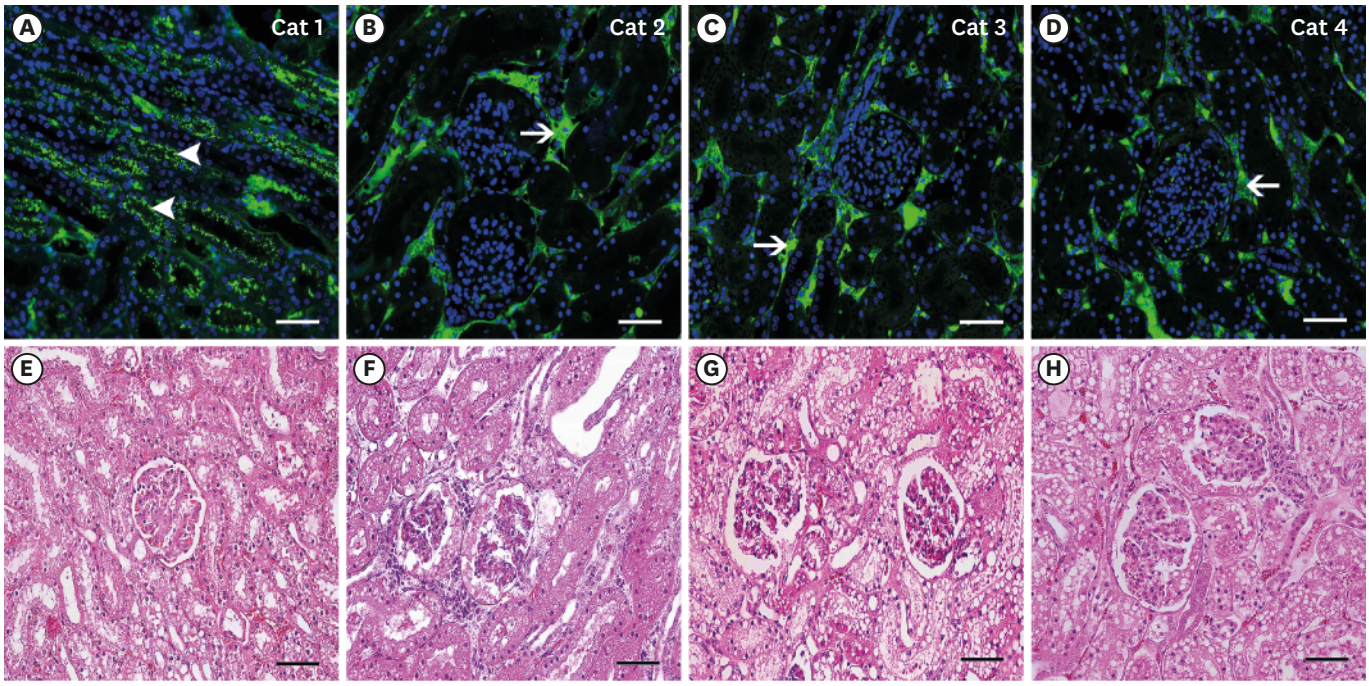

Fig. 3. Immunofluorescence and H&E staining of kidney tissue from cat cadavers. Cat kidney tissue sections were stained with FITC-conjugated goat anti-cat IgG (A-D), and nuclei were counterstained with DAPI (blue color). Results from four cats are shown with each one presented as cat 1, 2, 3, or 4. The arrowheads pinpoint the presence of positive green fluorescence at the apical border of the distal tubule, and the arrows indicate interstitial cells. H&E staining is shown in (E-H) (scale bars = 100 μm).
H&E, hematoxylin and eosin; FITC, fluorescein isothiocyanate; IgG, immunoglobulin G; DAPI, 2-(4-amidinophenyl)-6-indolecarbamidine dihydrochloride.

Recognition of kidney and CRFK cell line proteins by cat sera

In a previous study using similar Western immunoblots, the proteins recognized most frequently had apparent molecular masses of 47, 40, and 38 kDa. According to Whittemore et al.'s study [28], band appearances, including those with M.W. of 47, 40, and 38 kDa, were considered.

Twenty cats with positive indirect immunofluorescence results were analyzed to provide protein recognition. All of the cats showed a similar pattern, but additional proteins were recognized in 7 cats (**Fig. 5**). These additional proteins had M.W. of 47, 40, 38, and 20 kDa. When using the CRFK cell line proteins as an antigen, most cats showed similar band patterns, but 8/20 cats showed additional bands. These additional cat antibodies were bound to proteins at M.W. of 47, 38, and 20 kDa. It was noted that no antibody to a 40 kDa protein was detected; considered to be a result of using kidney protein. Two unvaccinated cats showed a band at M.W. 38 for both the CRFK and kidney proteins. These unvaccinated cats were then tested by a commercial VacciCheckR kit and were FVRCP positive.

Profile of antibodies that bind to kidney tissues in cats with positive and negative antibodies to FVRCP antigen

As only 66% of all FVRCP-vaccinated cats produced antibody responses after vaccination, the relationship of antibodies that bind to kidney tissues was compared with the anti-FVRCP antibody instead of comparing between the unvaccinated and FVRCP-vaccinated cats. The profiles of antibodies that bind to kidney tissues in cats with positive and negative anti-FVRCP antibodies are shown in **Fig. 6**. Vaccinated cats with an antibody to the FVRCP antigen should have a greater chance of having an antibody to CRFK cell protein contamination during vaccine preparation. There was a difference in the prevalence of antibodies that bind

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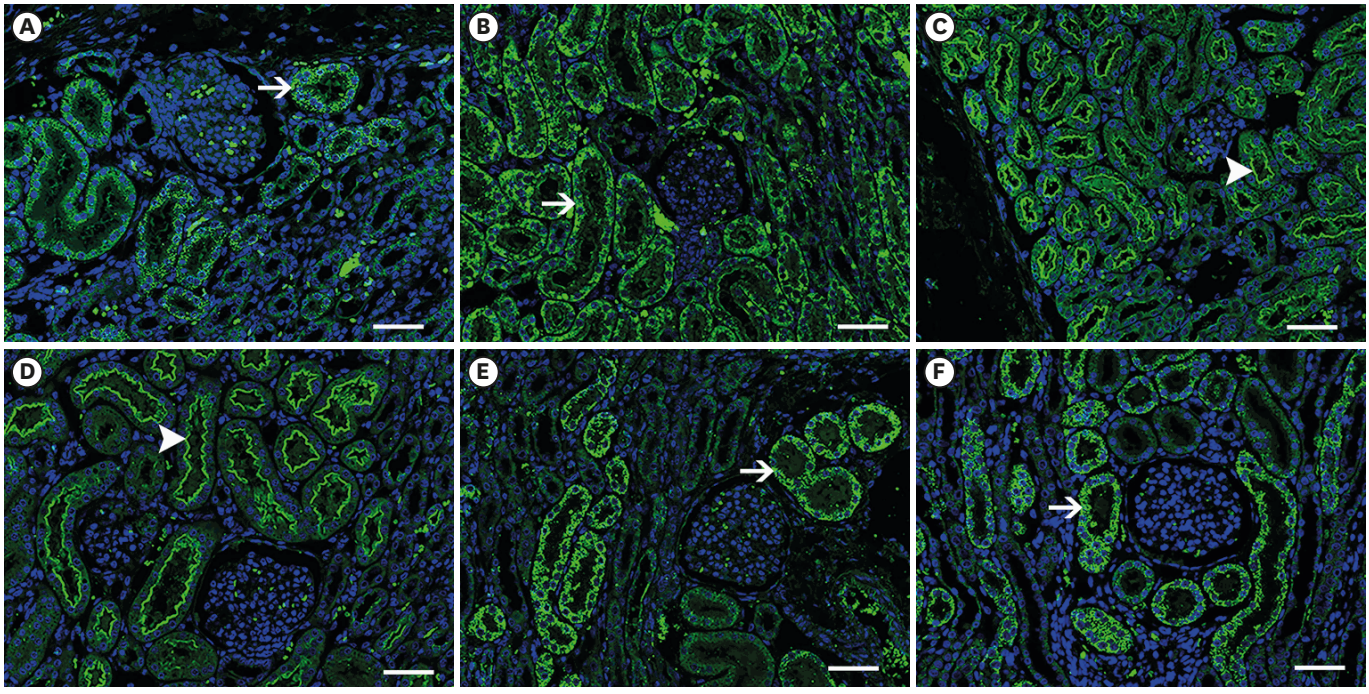

Fig. 4. Immunofluorescence pattern of cat kidney-bound antibodies. Normal cat kidney tissue sections were stained with sera from unvaccinated (A-C) and FVRCP-vaccinated cats (D-F). Nuclei were counterstained with DAPI (blue color). Arrows indicate the cytoplasm of the kidney tubule and arrowheads indicate the apical border of the kidney tubule (scale bars = 100 μm).
FVRCP, feline viral rhinotracheitis, calicivirus, and panleukopenia; DAPI, 2-(4-amidinophenyl)-6-indolecarbamidine dihydrochloride.

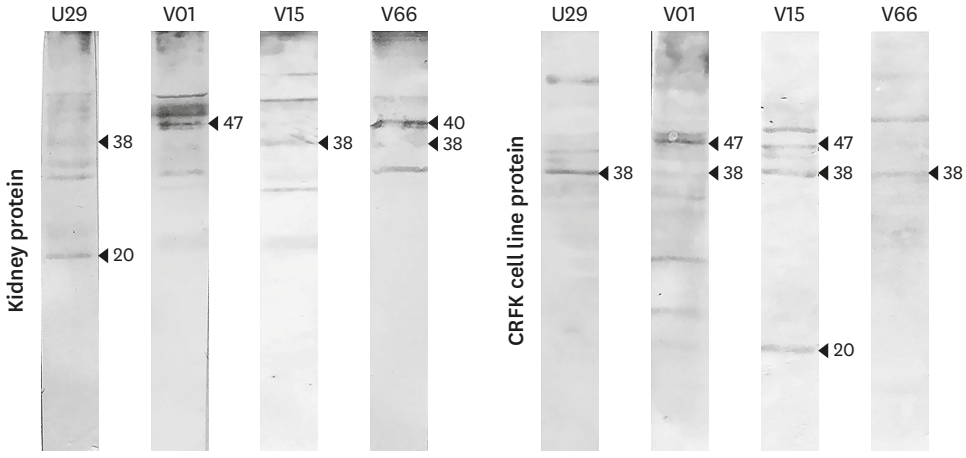

Fig. 5. Western blot analysis of cat kidney and CRFK cell line proteins recognized by antibodies that bind to kidney tissues. Four cats are presented, and each cat's code (U29, V01, V15, and V66) is indicated at the top. Closed arrowheads indicate molecular mass (kDa) on the right.
CRFK, Crandell-Rees feline renal cell line; U code, unvaccinated cats; V code, FVRCP-vaccinated cats.

to kidney tissues found in cats with and without antibodies to the FVRCP antigen. Among 156 cats, 66 and 90 cats were positive and negative for antibody to the FVRCP antigen, respectively. Fifty-five percent (36/66) of cats with positive antibodies to FVRCP antigen had antibodies that bind to kidney tissues. However, 30% (27/90) of cats with negative antibodies to the FVRCP antigen had antibodies that bind to kidney tissues. Based on χ^2 test results, positive antibodies that bind to kidney tissues were associated significantly with the anti-FVRCP antibody ($p = 0.002$).

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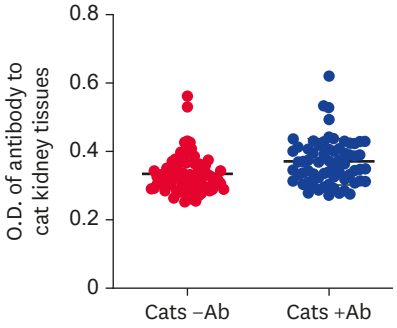

Fig. 6. Profiles of antibodies that bind to kidney tissues in cats with positive and negative antibody responses to FVRCP antigen. The levels of antibodies that bind to kidney tissues are presented as an O.D. value. Each dot represents an individual cat. The mean ± SD of antibodies that bind to kidney tissues in cats with positive and negative antibody to FVRCP antigen (O.D. of 0.371 ± 0.067 and 0.333 ± 0.052, respectively) are shown as solid lines.
FVRCP, feline viral rhinotracheitis, calicivirus, and panleukopenia; O.D., optical density; +Ab, positive antibody; −Ab, negative antibody.

The association of anti-cat kidney tissues in cats with positive and negative antibodies to FVRCP antigen was compared by determining the OR. The results showed that cats with positive antibodies to FVRCP antigen had 2.8 times more risk of having antibodies that bind to kidney tissues (OR, 2.8; 95% confidence interval, 1.37–5.73).

Correlation between creatinine and BUN levels and antibodies that bind to kidney tissues

The mean ± SD of the creatinine concentrations in cats with and without antibodies that bind to healthy cat kidney extracts were 2.04 ± 1.62 mg/dL and 1.76 ± 1.41 mg/dL, respectively, whereas the mean ± SD of the BUN concentrations were 34.78 ± 26.79 mg/dL and 33.06 ± 31.54 mg/dL, respectively (**Fig. 7**). Eight of 156 (5%) cats with positive antibodies that bind to kidney tissues had creatinine and BUN levels above the normal range (creatinine 0.9–2.2 mg/dL, BUN 19–34 mg/dL) [22], which is considered a criterion indicating kidney disease. No correlation was found above the normal range between the creatinine and BUN levels and positive antibodies that bind to kidney tissues (Spearman's rho = 0.048).

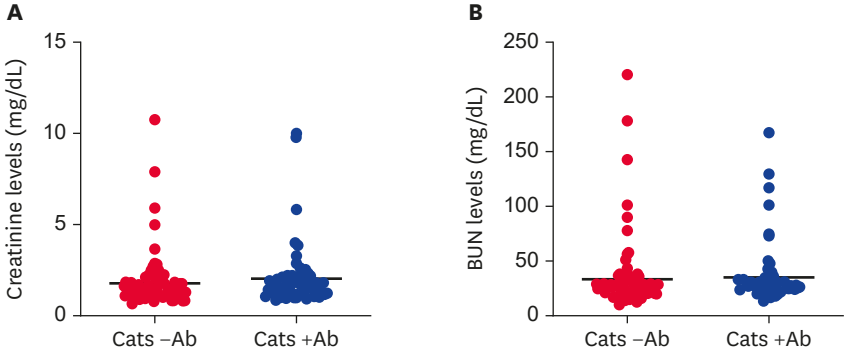

Fig. 7. The levels of creatinine and BUN in cats with and without antibodies that bind to kidney tissues. The antibodies that bind to kidney tissues and their correlation to (A) creatinine and (B) BUN levels are shown. Each dot represents individual cats. The mean ± SD level of creatinine in cats with negative and positive antibodies that bind to kidney tissues are presented as solid lines.
BUN, blood urea nitrogen; +Ab, positive antibody; −Ab, negative antibody.

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Table 6. Associations among cat history and serum antibodies that react to healthy kidney tissues

No.	Cat code	Vac.	Last vac.	Cat history	Cr level (mg/dL)	BUN level (mg/dL)	Ab to FVRCP	Ab to kidney	IF pattern
1	U15	-	-	N	1.32	28.3	–ve	+ve	+ve (C)
2	U19	-	-	[a]	2.85	42.5	+ve	+ve	+ve (C)
3	U20	-	-	[a]	1.95	34.7	–ve	+ve	+ve (C)
4	U21	-	-	[a]	2.52	43.2	–ve	+ve	+ve (C)
5	U22	-	-	[a]	1.58	34.4	+ve	+ve	+ve (A)
6	U23	-	-	N	1.81	38.1	–ve	+ve	+ve (C)
7	U25	-	-	N	2.24	38.1	–ve	+ve	+ve (C)
8	U29	-	-	CKD	3.29	73	+ve	–ve	+ve (C)
9	U36	-	-	FPV[b]	1.06	10.1	–ve	–ve	+ve (C)
10	U67	-	-	N	1.4	25.8	–ve	–ve	+ve (A,C)
11	V01	2	13 mo	N	1.37	26.3	+ve	–ve	+ve (C)
12	V15	4	13 mo	N	1.22	24.5	+ve	+ve	+ve (C)
13	V34	3	2 mo	N	1.42	26	+ve	+ve	+ve (A)
14	V41	1	4 mo	N	1.86	24.4	–ve	+ve	+ve (A)
15	V45	1	4 mo	N	2.13	24.6	+ve	+ve	+ve (C)
16	V47	2	4 mo	N	2.34	26.8	+ve	+ve	+ve (C)
17	V54	5	5 mo	N	1.54	31.4	–ve	–ve	+ve (C)
18	V66	3	2 mo	FIV[b]	2.13	30.1	+ve	+ve	+ve (C)
19	V71	4	9 mo	N	1.26	27.5	+ve	+ve	+ve (C)
20	V80	1	7 mo	N	1.5	20.2	–ve	–ve	+ve (A)

-, none; +ve, positive; –ve, negative; U code, unvaccinated cats; V code, FVRCP-vaccinated cats; Vac., number of vaccinations; Last vac., time since the last vaccination; N, normal cats; CKD, chronic kidney disease; FPV, feline parvovirus; FIV, feline immunodeficiency virus; Cr, creatinine; BUN, blood urea nitrogen; Ab, antibody; FVRCP, feline viral rhinotracheitis, calicivirus, and panleukopenia; IF pattern, immunofluorescence pattern; A, apical border of the kidney tubule; C, cytoplasm of the kidney tubule.
[a]Upper respiratory clinical symptom; [b]Laboratory diagnosis using Bionote FPV Ag and Witness FeLV-FIV test kits.

Associations among cat histories and serum antibodies that react to healthy kidney tissues

Twenty cats with serum antibodies bound to the healthy cat kidney sections in the direct immunofluorescence assay were examined. Clinical history and laboratory findings, as well as serum antibodies against healthy cat kidney lysates, were recorded (**Table 6**). Fourteen cats were diagnosed as CKD; only one of them had positive kidney-bound antibodies detected by direct immunofluorescence, whereas 4 cats had antibodies that bind to kidney tissues detected in serum by ELISA. It was interesting that a FVRCP-vaccinated cat (V47) was positive for kidney-bound antibodies, a high antibody response to FVRCP antigen, a high level of antibodies that bind to kidney tissues, and a creatinine level slightly above the normal range. However, it is unknown whether the autoantibody found in this cat was pathogenic; a follow-up study will be performed.

In this study, 6 of the 20 cats with positive kidney-bound antibodies based on indirect immunofluorescence included one with FPV, one with a feline immunodeficiency virus (FIV) infection, and 4 with no available laboratory disease confirmation but had upper respiratory clinical signs.

DISCUSSION

Based on an increase in reports about the frequency of vaccinations and its association with autoimmune disease, it is reasonable to speculate that frequent FVRCP vaccinations in cats might induce antibodies that bind to kidney tissues, leading to kidney diseases. The prevalence of finding antibodies that bind to kidney tissues and the associated risk with FVRCP vaccination were investigated in this study, in which detection of antibodies that bind to kidney tissues was laboratory-confirmed, indicating a higher risk of antibodies that bind to kidney tissues in cats with positive antibodies to FVRCP antigen. There was no statistically

significant difference in finding antibodies to cat kidney tissues between unvaccinated and vaccinated cats. However, positive antibodies that bind to kidney tissues were significantly associated with the anti-FVRCP antibody. The odds ratio results showed that the association of finding antibodies that bind to kidney tissues in cats with antibody to FVRCP antigen was 2.8 times higher than in those without it. In addition, 7 of 10 FVRCP-vaccinated cats, which were shown to have kidney-bound antibodies by indirect immunofluorescence analysis, had multiple vaccinations (2–5 times). A previously mentioned study showed that although parenteral administration of multiple FVRCP vaccines or CRFK cell lysate in cats induced antibodies to kidney tissue lysates, no kidney disease had been observed during the 56-week study period [18]. However, it was shown that after 3 cats were immunized with CRFK cell line lysate, one exhibited lymphocytic-plasmacytic interstitial nephritis after receiving 13 inoculations in 2 years [29].

When considering the risk of FVRCP vaccination, there was no statistically significant difference in finding antibodies to cat kidney tissues between unvaccinated and vaccinated cats. Several factors affect vaccination responses such as the health of animals, vaccine company, and time of sample collection since the last vaccination. As the purpose of this study was to screen for anti-kidney proteins (feline CRFK cell line) that are contaminants in FVRCP vaccine preparation, the finding of anti-FVRCP in vaccinated cats would confirm that the vaccination was successful; therefore, indicating a greater chance of finding anti-kidney protein contamination. As only 64% of the FVRCP-vaccinated cats showed antibodies to the FVRCP antigen in this study, it was considered that time since the last vaccination or the vaccination protocol could affect both anti-FVRCP titer and anti-kidney antibody titer. However, there was no clear correlation between the positive anti-FVRCP result and time since the last vaccination in this study. This result was inconsistent with those in other studies in which the factor time since the last vaccination was associated with the presence of pre-vaccination when using ELISA antibody titers; however, there was no association with viral neutralizing antibodies [30,31].

A limitation in the detection of the antibodies to FVRCP antigen in this study was related to sensitivity and specificity. As the commercial gold standard test of ELISA had not been used widely or was not available, an in-house ELISA was developed, and an internal quality control system was applied. The positive and negative serum controls were found by interviewing pet owners about their cat's history. Large samples were collected in order to calculate the cut-off value more precisely. It was considered that the FVRCP antigen preparation contains FHV-1, FCV, and FPV, and many cats are exposed naturally to these viruses, leading to positive antibody results against FVRCP protein extracts in cats, even if they were not vaccinated. This was a limitation in the interpretation of this study of a cat population in which natural exposures could not be avoided. Thirteen percent of unvaccinated cats were positive to anti-FVRCP antibodies with very low titer or ELISA O.D. values that were little above the cut-off value. They were examined by using a commercial test kit for antibodies to FVRCP, and almost all of them (6/7 or 86%) showed positive results for both the in-house ELISA and the commercial test kit. Therefore, these unvaccinated cats might have been exposed to natural infections. In other studies, surveillance results for antibodies to FCV and FHV-1 ranged from 7%–23% in unvaccinated cats or cat populations [30,32]. In addition to natural exposures, maternally derived anti-FVRCP antibodies can produce significant titers in young kittens; they displayed antibody titers against FPV at 8 and 12 weeks of age and up to 20 weeks of age in some kittens [33]. However, two unvaccinated kittens (aged 5 months) were found to have negative anti-FVRCP and anti-cat kidney tissues in this study.

The kidney-bound antibody found in cats in this study showed a similar profile to anti-mitochondrial antibody results in human autoimmune hepatitis, in which the autoantibody stained the cytoplasm of the kidney tubule and showed increased intensity at the distal tubular cells [34]. In addition, some cats showed a profile of antibody binding at the apical border of the proximal convoluted tubule, similar to that in human patients with immune complex tubule-interstitial nephritis [35]. It was considered that, currently, CRFK cells appear phenotypically similar to fibroblasts rather than tubular epithelial cells. This was interesting, as antibodies bound to the proximal and distal tubules of cat kidneys in this study were inconsistent with the current characterization of CRFK cells as a fibroblast phenotype. Neoplastic transformation commonly occurs, and the CRFK cell line utilized for viral vaccine preparation might be in a passage related to epithelial-to-mesenchymal transition [36].

Four of the 26 embedded cat kidney tissues of cadaveric cats exhibited kidney-bound antibodies at the apical border of the kidney tubules and interstitial cells. The diagnoses were based on clinical symptoms and histopathological results showing altered cell structures, cytopathic effects, and a particular type of cell infiltration and inflammation based on clinical and histological diagnosis standards [37]. These cats were morphologically suspected to be infected and died due to viral infection. Several infections have been shown to associate with autoimmune diseases [38,39]. Viral infection associated with an autoantibody to kidney tissue has been reported in FIV infection [40]. Similar to that previous report, in our study, one cat with kidney-bound antibodies had an FIV infection.

Antibodies that bind to kidney tissues in cats were shown to recognize kidney proteins with M.W. of 47, 40, 38, and 20 kDa. Protein bands with M.W. of 47 and 40 kDa have been identified as alpha-enolase and annexin A2, respectively [28]. These 2 proteins were found only in FVRCP-vaccinated cats with kidney-bound antibodies in cytoplasm, not on the apical surface of the kidney tubule (as detected by immunofluorescence assays). The 38 kDa band is reportedly a macrophage capping protein, also known as Cap G, distributed primarily in cytosol, although it may have a nuclear distribution in some tissues [41,42]. Based on database searches, the 20 kDa protein in this study did not match any previously reported cat kidney protein. It was noticed that some unvaccinated cats had antibodies to the 38 kDa band. This might be due to natural exposure to the virus; in those cats, the antibodies to FVRCP results were positive when using a commercial test kit but not the in-house ELISA. A causative role of viral infection in renal disease has been reported in FIV in cats and HIV in humans [43-45]. Thus, antibodies responding to the FPV, FHV-1, or FCV viruses, which might have cross-reactivity or pathogenesis involvement of the kidney, are of interest and should be investigated further. Our study could not show directly that a higher frequency of FVRCP vaccination was associated with detecting antibodies bound to kidney tissues. However, the presence of antibodies to FVRCP was associated with a greater chance of having positive antibodies to kidney tissues. Nevertheless, repeat vaccination should be considered carefully, as some cats with positive kidney-bound antibodies appeared to have histories of more frequent FVRCP vaccination in this study. FVRCP-vaccinated cats can exhibit antibodies from two sources: virus- or CRFK cell protein-induced autoantibodies to kidney tissues. Based on the results presented in several studies, it appears that there might be no need to administer FVRCP vaccines more frequently than every three years after the 1-year booster vaccine, and the duration of vaccine-based immunity is possibly much longer [31]. Serological test results for antibodies against FPV, FCV, and FHV-1 can be used as an aid in determining the need for a vaccine [46].

In conclusion, this study could not show directly that the frequency of FVRCP vaccination was associated with finding antibodies to kidney tissues. However, having an anti-FVRCP antibody was associated with a greater chance of the presence of a positive antibody to kidney tissues. FVRCP-vaccinated cats can have antibodies derived from either virus or CRFK cell protein-induced autoantibodies. Thus, a feline vaccination schedule should be considered carefully, as over-vaccination might increase the risk of inducing the production of antibodies that bind to kidney tissues. Post-vaccination serology should be used as a guide in deciding the need for repeated vaccination in order to avoid adverse reactions and prevent the risk of vaccine-induced autoimmune disease.

REFERENCES

1. Polzin DJ. Chronic kidney disease in small animals. Vet Clin North Am Small Anim Pract. 2011;41(1):15-30.
PUBMED | CROSSREF
2. Lee YJ, Chan JP, Hsu WL, Lin KW, Chang CC. Prognostic factors and a prognostic index for cats with acute kidney injury. J Vet Intern Med. 2012;26(3):500-505.
PUBMED | CROSSREF
3. Lulich J, Osborne C, O'Brien T, Polzin D. Feline renal failure: questions, answers, questions. Compend Contin Educ Vet. 1992;14:127-153.
4. Ross SJ, Polzin DJ, Osborne CA. *Consultations in feline internal medicine*. 5th ed. Saint Louis: W.B. Saunders; 2006, Chapter 42, Clinical progression of early chronic renal failure and implications for management; 389-398.
5. Finch NC, Syme HM, Elliott J. Risk factors for development of chronic kidney disease in cats. J Vet Intern Med. 2016;30(2):602-610.
PUBMED | CROSSREF
6. Kohn B, Garner M, Lübke S, Schmidt MF, Bennett D, Brunnberg L. Polyarthritis following vaccination in four dogs. Vet Comp Orthop Traumatol. 2003;16(1):6.
CROSSREF
7. McAnulty JF, Rudd RG. Thrombocytopenia associated with vaccination of a dog with a modified-live paramyxovirus vaccine. J Am Vet Med Assoc 1985.186(11):1217-1219.
PUBMED
8. Duval D, Giger U. Vaccine-associated immune-mediated hemolytic anemia in the dog. J Vet Intern Med. 1996;10(5):290-295.
PUBMED | CROSSREF
9. Hogenesch H, Azcona-Olivera J, Scott-Moncrieff C, Snyder PW, Glickman LT. Vaccine-induced autoimmunity in the dog. Adv Vet Med. 1999;41:733-747.
PUBMED | CROSSREF
10. Scott-Moncrieff JC, Azcona-Olivera J, Glickman NW, Glickman LT, HogenEsch H. Evaluation of antithyroglobulin antibodies after routine vaccination in pet and research dogs. J Am Vet Med Assoc. 2002;221(4):515-521.
PUBMED | CROSSREF
11. Hegde NR. Cell culture-based influenza vaccines: a necessary and indispensable investment for the future. Hum Vaccin Immunother. 2015;11(5):1223-1234.
PUBMED | CROSSREF
12. Offit PA, Jew RK. Addressing parents' concerns: do vaccines contain harmful preservatives, adjuvants, additives, or residuals? Pediatrics. 2003;112(6 Pt 1):1394-1397.
PUBMED | CROSSREF
13. HogenEsch H. Mechanisms of stimulation of the immune response by aluminum adjuvants. Vaccine. 2002;20 Suppl 3:34-39.
PUBMED | CROSSREF
14. Segal Y, Shoenfeld Y. Vaccine-induced autoimmunity: the role of molecular mimicry and immune crossreaction. Cell Mol Immunol. 2018;15(6):586-594.
PUBMED | CROSSREF
15. Waisbren BA Sr. Acquired autoimmunity after viral vaccination is caused by molecular mimicry and antigen complimentarity in the presence of an immunologic adjuvant and specific HLA patterns. Med Hypotheses. 2008;70(2):346-348.
PUBMED | CROSSREF

16. Hemachudha T, Griffin DE, Chen WW, Johnson RT. Immunologic studies of rabies vaccination-induced Guillain-Barré syndrome. Neurology. 1988;38(3):375-378.
PUBMED | CROSSREF

17. Hemachudha T, Griffin DE, Giffels JJ, Johnson RT, Moser AB, Phanuphak P. Myelin basic protein as an encephalitogen in encephalomyelitis and polyneuritis following rabies vaccination. N Engl J Med. 1987;316(7):369-374.
PUBMED | CROSSREF

18. Lappin MR, Jensen WA, Jensen TD, Basaraba RJ, Brown CA, Radecki SV, et al. Investigation of the induction of antibodies against Crandell-Rees feline kidney cell lysates and feline renal cell lysates after parenteral administration of vaccines against feline viral rhinotracheitis, calicivirus, and panleukopenia in cats. Am J Vet Res. 2005;66(3):506-511.
PUBMED | CROSSREF

19. Schultz RD, Thiel B, Mukhtar E, Sharp P, Larson LJ. Age and long-term protective immunity in dogs and cats. J Comp Pathol. 2010;142 Suppl 1:S102-S108.
PUBMED | CROSSREF

20. Jensen WA, Totten JS, Lappin MR, Schultz RD. Use of serologic tests to predict resistance to Canine distemper virus-induced disease in vaccinated dogs. J Vet Diagn Invest. 2015;27(5):576-580.
PUBMED | CROSSREF

21. Elston T, Rodan H, Flemming D, Ford RB, Hustead DR, Richards JR, et al. 1998 report of the American Association of Feline Practitioners and Academy of Feline Medicine Advisory Panel on Feline Vaccines. J Am Vet Med Assoc. 1998;212(2):227-241.
PUBMED

22. Latimer KS, Duncan JR. *Duncan & Prasse's veterinary laboratory medicine: clinical pathology*. 5th ed. Chichester (UK): Wiley-Blackwell; 2011, Chapter 13, Generating and interpreting test results: test validity, quality control, reference values, and basic epidemiology; 374-375.

23. Songaksorn N, Petsophonsakul W, Pringproa K, Lampang KN, Sthitmatee N, Sriphawattana N, et al. Production of polyclonal antibody against kidney antigens: a model for studying autoantibody in feline chronic kidney diseases. J Vet Sci. 2019;20(6):e73.
PUBMED | CROSSREF

24. De Chiara G, Marcocci ME, Civitelli L, Argnani R, Piacentini R, Ripoli C, et al. APP processing induced by herpes simplex virus type 1 (HSV-1) yields several APP fragments in human and rat neuronal cells. PLoS One. 2010;5(11):e13989.
PUBMED | CROSSREF

25. Kochagul V, Srivorakul S, Boonsri K, Somgird C, Sthitmatee N, Thitaram C, et al. Production of antibody against elephant endotheliotropic herpesvirus (EEHV) unveils tissue tropisms and routes of viral transmission in EEHV-infected Asian elephants. Sci Rep. 2018;8(1):4675.
PUBMED | CROSSREF

26. Pringproa K, Madarame H, Sritun J, Bumpenpol P, Pedsri P, Somgird C, et al. Histopathological and immunohistochemical characterization of spontaneous uterine leiomyomas in two captive Asian elephants. Thai J Vet Med. 2015;45(2):289-294.

27. Enderlein G. Daniel, Wayne W.: Biostatistics — A Foundations for Analysis in the Health Sciences. Wiley & Sons, New York—Chichester—Brisbane—Toronto—Singapore, 6th ed. 1995, 780 S., £58.—, ISBN 0–471–58852-0 (cloth). Biom J. 1995;37(6):744.
CROSSREF

28. Whittemore JC, Hawley JR, Jensen WA, Lappin MR. Antibodies against Crandell Rees feline kidney (CRFK) cell line antigens, alpha-enolase, and annexin A2 in vaccinated and CRFK hyperinoculated cats. J Vet Intern Med. 2010;24(2):306-313.
PUBMED | CROSSREF

29. Lappin MR, Basaraba RJ, Jensen WA. Interstitial nephritis in cats inoculated with Crandell Rees feline kidney cell lysates. J Feline Med Surg. 2006;8(5):353-356.
PUBMED | CROSSREF

30. Bergmann M, Speck S, Rieger A, Truyen U, Hartmann K. Antibody response to feline calicivirus vaccination in healthy adult cats. Viruses. 2019;11(8):702.
PUBMED | CROSSREF

31. Mouzin DE, Lorenzen MJ, Haworth JD, King VL. Duration of serologic response to three viral antigens in cats. J Am Vet Med Assoc. 2004;224(1):61-66.
PUBMED | CROSSREF

32. Henzel A, Brum MCS, Lovato LT, Weiblen R. Serological survey of feline calicivirus and felid herpesvirus in Rio Grande do Sul, Brazil. Acta Sci Vet. 2013;41:1153.

33. Jakel V, Cussler K, Hanschmann KM, Truyen U, König M, Kamphuis E, et al. Vaccination against Feline Panleukopenia: implications from a field study in kittens. BMC Vet Res. 2012;8(1):62-62.
PUBMED | CROSSREF

34. Liberal R, Mieli-Vergani G, Vergani D. Clinical significance of autoantibodies in autoimmune hepatitis. J Autoimmun. 2013;46:17-24.
PUBMED | CROSSREF

35. Rosales IA, Collins AB, do Carmo PA, Tolkoff-Rubin N, Smith RN, Colvin RB. Immune complex tubulointerstitial nephritis due to autoantibodies to the proximal tubule brush border. J Am Soc Nephrol. 2016;27(2):380-384.
PUBMED | CROSSREF

36. Lawson JS, Syme HM, Wheeler-Jones CPD, Elliott J. Characterisation of Crandell-Rees Feline Kidney (CRFK) cells as mesenchymal in phenotype. Res Vet Sci. 2019;127:99-102.
PUBMED | CROSSREF

37. Srivorakul S, Guntawang T, Kochagul V, Photichai K, Sittisak T, Janyamethakul T, et al. Possible roles of monocytes/macrophages in response to elephant endotheliotropic herpesvirus (EEHV) infections in Asian elephants (Elephas maximus). PLoS One. 2019;14(9):e0222158.
PUBMED | CROSSREF

38. Wucherpfennig KW. Mechanisms for the induction of autoimmunity by infectious agents. J Clin Invest. 2001;108(8):1097-1104.
PUBMED | CROSSREF

39. Kivity S, Agmon-Levin N, Blank M, Shoenfeld Y. Infections and autoimmunity--friends or foes? Trends Immunol. 2009;30(8):409-414.
PUBMED | CROSSREF

40. Grant CK, Fink EA, Sundstrom M, Torbett BE, Elder JH. Improved health and survival of FIV-infected cats is associated with the presence of autoantibodies to the primary receptor, CD134. Proc Natl Acad Sci U S A. 2009;106(47):19980-19985.
PUBMED | CROSSREF

41. Dabiri GA, Young CL, Rosenbloom J, Southwick FS. Molecular cloning of human macrophage capping protein cDNA. A unique member of the gelsolin/villin family expressed primarily in macrophages. J Biol Chem. 1992;267(23):16545-16552.
PUBMED | CROSSREF

42. Prendergast GC, Ziff EB. Mbh 1: a novel gelsolin/severin-related protein which binds actin in vitro and exhibits nuclear localization *in vivo*. EMBO J. 1991;10(4):757-766.
PUBMED | CROSSREF

43. Baxter KJ, Levy JK, Edinboro CH, Vaden SL, Tompkins MB. Renal disease in cats infected with feline immunodeficiency virus. J Vet Intern Med. 2012;26(2):238-243.
PUBMED | CROSSREF

44. Poli A, Tozon N, Guidi G, Pistello M. Renal alterations in feline immunodeficiency virus (FIV)-infected cats: a natural model of lentivirus-induced renal disease changes. Viruses. 2012;4(9):1372-1389.
PUBMED | CROSSREF

45. Kimmel PL. The nephropathies of HIV infection: pathogenesis and treatment. Curr Opin Nephrol Hypertens. 2000;9(2):117-122.
PUBMED | CROSSREF

46. Lappin MR, Andrews J, Simpson D, Jensen WA. Use of serologic tests to predict resistance to feline herpesvirus 1, feline calicivirus, and feline parvovirus infection in cats. J Am Vet Med Assoc. 2002;220(1):38-42.
PUBMED | CROSSREF