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Role of Recent Vaccination in Production of False-Positive Coronavirus Antibody Titers in Cats

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Statistical support was obtained for an association between recent vaccination of cats and the presence of elevated background (i.e., anti-cell culture) reactivity in certain of their sera, as detected with a kinetics-based enzyme-linked immunosorbent assay for coronavirus antibodies, implicating routine vaccination as a potential cause of false-positive antibody test results.

In feline coronaviral serology, control of nonspecific background reactivity is critical to obtain useful estimates of specific antibody responses (1). Antibodies against ruminant serum components can be found in certain feline sera that can potentially react with antigenically similar components present in cell culture preparations used for virus propagation in both the kinetics-based enzyme-linked immunosorbent assay (KELA) and the conventional immunofluorescence assay (1). The resulting elevation in background (i.e., anti-cell culture) reactivity can be misinterpreted as a coronavirus-specific response (i.e., a false-positive result) unless appropriate negative antigen controls are included for each individual serum sample evaluated (1). Elevated background reactivity can be minimized (but not always completely eliminated) in many cases by substituting gamma globulin-free newborn calf serum for fetal bovine serum in cell culture preparations, suggesting that it is the gamma globulin fraction that is most antigenic (1).

One possible explanation for the presence of antibodies against ruminant serum components is routine vaccination (4). Cell culture vaccines prepared for use in cats (as well as vaccines for many other species) frequently contain ruminant serum components that could conceivably produce elevated background reactivity in the sera of vaccinees—reactivity that might be especially strong in samples drawn soon after vaccination (1, 4). In this paper we describe retrospective and prospective studies from which statistical support for this hypothesis has been obtained.

Background reactivity was assessed by testing five replicates of each serum sample plus a panel of three to five control sera (calibrators for each run), using a "negative control antigen," i.e., cell culture preparation without coronavirus (1, 3). Elevated background reactivity for a serum sample was defined as a mean negative control antigen reaction rate slope greater than two standard deviations above the mean slope of all control sera in an individual run. In a recent 1-year survey of KELA data, 141 of 2,379 (5.9%) feline serum samples submitted to the New York State Diagnostic Laboratory for analysis of coronavirus antibody titers were found to contain such reactivity (J. E. Barlough, R. H. Jacobson, G. P. Sorresso, T. J. Lynch, and F. W. Scott, submitted for publication). Of these, 40 (28.4%) cats were identified for which complete vaccination histories could be obtained and for which exact age-, breed-, and sex-matched controls (also with complete vaccination histories) could be located among the remaining 2,238 cats. In age, sex, and breed statistics the sample population of 40 cats was generally representative of the total population of cats with high-background serum (Table 1). The slightly greater percentage of domestic shorthair cats in the sample population reflected the difficulty in locating exact matches for the less commonly represented breeds.

The possible influence of recent vaccination on the presence of elevated background reactivity was assessed by comparing the time intervals (days) between the date of the most recent vaccination and the date of serum collection for KELA testing for individual members of matched pairs (Table 2). Because of the nonnormal distribution of these intervals for both case and control groups, the difference between the two populations was evaluated statistically by the signed rank method of Wilcoxon (13). The relatively large number of pairs ($n = 40$) required calculation of the approximate normal deviate (10). The results of this analysis were highly significant ($Z = 4.58$), strongly suggesting an association between recent vaccination and elevated background reactivity. Vaccinated cats in the study had been inoculated with a wide variety of vaccine preparations, and no association of elevated background reactivity with any product of a particular manufacturer was noted.

To further test the hypothesis, owners of two catteries in which one or more cats with high-background serum had been previously identified were contacted and asked to participate in a joint study. Sera from a total of 13 cats, including domestic shorthair and Himalayan breeds ranging in age from 2 months to 5 years ($\bar{x} = 20.8$ months), were tested by KELA, and all were found to have negative control antigen reactivities within normal limits. Seven cats were then vaccinated subcutaneously with a commercial biological preparation containing modified live feline parvovirus, feline herpesvirus I, and feline calicivirus (Pitman-Moore, Inc., Washington Crossing, N.J.). In both catteries this same product had been used previously for routine vaccination. Three control cats were inoculated similarly with sterile diluent, and the remaining three were left as uninoculated controls. In both catteries, cats participating in this study were allowed to freely intermingle with each other and with the general cattery population in an attempt to determine whether any transmissible factor might be involved in some way with background reactivity elevation. Young cats that had never before been vaccinated as well as older cats with annual revaccination histories were included to study both primary and anamnestic immune responses. Approximately

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TABLE 1. Age, sex, and breed characteristics of cats with elevated background reactivity in serum[a]

Characteristics	Total population	Sample population
Total no. of cats	141	40
Mean age (months)	41.48	36.66
Sex		
Male	49 (34.7)[b]	14 (35.0)
Female	84 (59.6)	26 (65.0)
Unknown	8 (5.7)	0 (0.0)
Major breeds represented		
Domestic shorthair	65 (46.1)	24 (60.0)
Himalayan	15 (10.6)	5 (12.5)
Persian	11 (7.8)	5 (12.5)
Domestic longhair	10 (7.1)	3 (7.5)
Siamese	6 (4.3)	3 (7.5)

[a] Sera submitted to the New York State Diagnostic Laboratory between April 1982 and April 1983.
[b] Number of cats (percentage of total).

2 weeks after initiation of this study, serum samples were again obtained from all 13 cats and retested by KELA. Five of the seven vaccinated cats (but no cats from either control group) demonstrated elevated levels of background reactivity in their serum. These five included three kittens that had not been previously vaccinated. The data were analyzed after merging both control groups into a single control population to compare them to the vaccinated population. The null hypothesis was tested by calculating the normal deviate, derived from the normal approximation to the binomial (10). This analysis indicated significance at the 1% level for a two-tailed test ($Z = 2.568$).

Because KELA relies on a subtraction procedure to generate final or corrected slope values for calculation of titers (1), the possibility exists that elevated background reactivity might artificially lower titer results if it is not additive with coronavirus-specific reactivity. Some evidence to support this idea has been gathered. Several cats with relatively constant coronavirus antibody titers that have been followed serologically for more than 1.5 years have demonstrated titer declines associated with elevated background activity after vaccination. Once background reactivity has returned to normal, however, titer levels have returned to prevaccination levels (data not shown). It was thus of added importance to investigate the persistence of elevated background reactivity in postvaccination sera so that a minimum waiting period between vaccination and serotesting might be established. To obtain an initial estimate of this interval, 8 of the 40 cats in the retrospective case population (Table 2) were bled periodically after vaccination. The days postvaccination on which elevated background reactivity was first detected by KELA and on which it was first found to have dissipated were then tabulated (Table 3). These rough data indicated disappearance of elevated background reactivity between 6 and 15 weeks postvaccination.

For statistical validation of a practical interval recommendation for veterinary practitioners, all 40 case intervals in Table 2 were first converted to natural logarithmic values as an approximation to a normal distribution. By using the estimators $\bar{x}$ and s, the upper one-sided tolerance limit (95% confidence) of this sample population giving at least 99% coverage was calculated to be 1,076.4 days (2, 6). Because most vaccines for use in cats are administered at yearly intervals (9), 99% coverage at the 95% confidence level appeared to be an unrealistic expectation based on the available data. Therefore, the upper limits for a series of coverage percentiles at 2 confidence levels were calculated (Table 4, $n = 40$).

In a small number of cases in Table 2, the interval between vaccination and serotesting was extremely large (case members of pairs 14, 15, 19, 20, and 22) and thus appeared to be generally unrepresentative of this sample population as a whole. On the assumption that intervals of this magnitude are correspondingly uncommon in the general population, a second series of tolerance limits was calculated from which these five cases were omitted (Table 4, $n = 35$). Combined examination of both series indicated reasonable confidence (90 to 95%) that, on repeated sampling, at least 75 to 90% of sera containing elevated background reactivity would be

TABLE 2. Retrospective case-control study of the effect of recent vaccination on the presence of elevated background reactivity in serum[a]

Pair no.	Interval (days) between vaccination and collection of serum for KELA		X − Y	Signed rank
	Cases (X)	Controls (Y)		
1	15	56	−41	−12.5
2	8	93	−85	−22
3	15	73	−58	−18
4	52	61	−9	−3
5	9	69	−60	−19
6	34	52	−18	−6
7	13	63	−50	−15
8	34	70	−36	−10
9	29	76	−47	−14
10	13	54	−41	−12.5
11	24	110	−86	−23
12	7	235	−228	−28
13	42	22	20	7
14	207	366	−159	−26
15	210	173	37	11
16	134	119	15	5
17	36	237	−201	−27
18	9	71	−62	−20
19	300	307	−7	−2
20	207	320	−113	−24
21	11	336	−325	−33
22	340	76	264	30
23	21	25	−4	−1
24	63	530	−467	−36
25	8	390	−382	−35
26	8	92	−84	−21
27	57	172	−115	−25
28	20	1,095	−1,075	−38
29	5	352	−347	−34
30	56	335	−279	−31
31	67	297	−230	−29
32	44	2,009	−1,965	−40
33	68	79	−11	−4
34	131	620	−489	−37
35	82	139	−57	−17
36	129	156	−27	−8
37	86	119	−33	−9
38	70	16	54	16
39	12	1,228	−1,216	−39
40	15	309	−294	−32

[a] $T_{min} = 69$.

found among the population of samples drawn within 113 and 127 days of vaccination. Thus, for practical purposes, serum samples for KELA testing should be drawn no sooner than 4 months after the most recent vaccination to minimize the probability of encountering elevated background reactivity. It is also obvious from the data, however, that not all cats develop elevated background reactivity after vaccination.

Collectively, these studies suggest an association in certain cats between recent vaccination and elevated background reactivity in KELA for detection of coronavirus antibodies. This finding is of significance primarily because it implicates routine vaccination of cats as a potential cause of false-positive antibody test results unless appropriate negative antigen controls are included for each serum sample tested (1). Thus, in KELA, elevated background reactivity may actually lower antibody titers because of the subtraction procedure used to calculate corrected slopes and titers (1).

We can only speculate on the implications of these observations for other diagnostic assays in which cell culture-derived antigen preparations are used. Kraaijeveld et al. (5) have presented evidence for the tight adherence of bovine serum components from cell culture medium to gradient-purified human respiratory coronavirus 229E and murine hepatitis virus 3. These serum components were highly immunogenic and capable of producing false enzyme-linked immunosorbent assay cross-reactions between the two viruses. Johansson et al. (4) have shown that elevated levels of background fluorescence in immunofluorescence tests can be attributed in many cases to antibodies directed against bovine serum components tightly attached to cell surfaces. They speculated that bovine serum components present in cell culture-derived vaccine preparations might induce production of antibodies that could participate in this type of reaction. Parenteral hyperimmunization of a cat with a cell culture-derived transmissible gastroenteritis coronavirus preparation has been shown to produce both specific antiviral antibodies as well as non-coronavirus antibodies directed against cell culture components, using an immunofluorescence assay (7). Similarly, subcutaneous inoculation of cats with cell culture medium during coronavirus 229E experiments in our laboratory has produced markedly elevated background reactivity in their sera (J. E. Barlough, unpublished data). In KELA, this reactivity has been controlled primarily by growing cells in medium containing gamma globulin-free serum and by generating corrected slope data by using five replicates each of infected and noninfected cell culture preparations for each individual serum sample (1). Corrections analogous to this latter procedure have been reported for some conventional enzyme-linked immunosorbent assays (8, 11, 12, 14). Although variable levels of elevated background reactivity (presumably due to trace gamma globulins in gamma globulin-free calf serum and to serum components other than gamma globulins) still exist in some serum samples (5.9% of 2,379 submissions over a 1-year period), these two modifications have minimized their effect and, more importantly, have partially corrected for their presence during titer determination. The statistical data in the present report further emphasize the importance of controlling nonspecific reactivity in feline coronaviral serology.

TABLE 3. Disappearance of elevated background reactivity from sera of vaccinated cats

Cat no.	Days postvaccination	
	Elevated background reactivity first detected	Normal background reactivity re-established
1	12	66
2	22	85
3	69	117
4	12	128
5	127	188
6	65	80
7	8	95
8	16	90
Mean	41.4	106.1

TABLE 4. Tolerance limits for observation of elevated background reactivity in postvaccination serum samples

Coverage (%)	Upper one-sided tolerance limits (days postvaccination)[a]			
	95% Confidence		90% Confidence	
	$n = 40$	$n = 35$	$n = 40$	$n = 35$
99	1,076.4	466.2	906.7	400.0
95	418.6	212.1	366.0	188.3
90	254.6	140.4	227.0	126.6
75	113.4	71.7	103.8	66.2

[a] ($n = 40$), All 40 case intervals as in Table 2; ($n = 35$), same intervals with five outliers removed.

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