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Feline calicivirus strain differentiation using monoclonal antibody analysis in an enzyme-linked immuno-flow-assay

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Abstract

Six monoclonal antibodies raised against feline calicivirus (FCV) strain F9 were used in an enzyme-linked immuno-flow-assay (ELIFA) to analyse 55 isolates of FCV. Forty seven field isolates were obtained from cats with acute oral/respiratory disease, chronic oral lesions, and from cats showing vaccine reactions, i.e. clinical signs of FCV infection shortly after vaccination. Eight reference strains including F9 and three vaccine strains based on F9 were also examined. All of the strains of F9, derived from various sources, reacted with all six of the monoclonal antibodies, whereas some of the field isolates did not react with any. In general, the field isolates showed a spectrum of reactivities and selected isolates could be distinguished. However, there were no clear cut differences between the clinical groups. Overall, the oral/respiratory group showed less reactivity with the monoclonals, suggesting they were less related to F9. Although the other groups appeared to be more closely related to F9, none of the isolates tested reacted with all six monoclonal antibodies.

Keywords: Feline calicivirus; Cat; Monoclonal antibodies; Serology; Typing; Respiratory disease

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1. Introduction

Feline calicivirus (FCV), a member of the family Caliciviridae, is an important cause of acute oral and respiratory disease in cats (Gaskell and Dawson, 1994). FCV can also cause an acute febrile lameness syndrome (Pedersen et al., 1983; Bennett et al., 1989; Dawson et al., 1994), and the virus has also been found in association with chronic oral lesions in cats (Thompson et al., 1984; Knowles et al., 1989; Tenorio et al., 1991). FCV vaccines have been available for some time and are reasonably effective at protecting against disease, but not infection. Most available vaccines are modified live products administered subcutaneously. Vaccine reactions, i.e., clinical signs of FCV infection occurring shortly after vaccination, have been reported (Dawson et al., 1993a). Virus has been isolated from such cases but differentiation between vaccine and field viruses is difficult.

Many strains of FCV have been isolated which vary slightly both in terms of pathogenicity and antigenicity (Povey and Hale, 1974; Kahn et al., 1975; Dawson et al., 1993b). By conventional cross virus neutralisation (VN) tests some differences between isolates can be demonstrated, but isolates are reasonably closely related and constitute one serotype (Povey, 1974; Kalunda et al., 1975). Monoclonal antibody analysis has also been applied to FCV isolates, and has shown both antigenic variation, and also the presence of at least seven neutralising epitopes (Tohya et al., 1990 and Tohya et al., 1991). However this work did not take into account the clinical origin of the isolates tested.

In recent work, using conventional virus neutralisation tests, we have found some differences between FCV isolates according to their clinical origin (Dawson et al., 1993b). The aim of this study was to determine whether monoclonal antibodies would also reveal such differences and perhaps aid in future typing systems including the differentiation between vaccine and field viruses.

2. Materials and methods

2.1. FCV isolates

Fifty five isolates were analysed. These comprised 11 isolates obtained from cats with acute oral/respiratory disease, 12 isolates from cats with chronic oral lesions, 12 isolates from cats with vaccine reactions associated with the use of vaccine A, and 12 from cats with vaccine reactions associated with the use of vaccine B (see Dawson et al., 1993b for a full description of the criteria used to segregate the isolates into groups). Vaccine virus A (strain F9), vaccine virus B (F9-like strain), vaccine virus N (strain F9) and 5 reference viruses were also included (Table 1). Field viruses isolated from oro-pharyngeal swabs were used after two to three cell culture passages in feline embryo-derived (FE) cells (Dawson et al., 1993b). Vaccine virus supplied by the manufacturers were passaged two to three times in FE cells before use, and reference strains were at unknown high passage.
2.2. Monoclonal and polyclonal antibodies

Six mouse monoclonal antibodies 4G12, 6E8, 4E11, 4C11, 4E7 and IG9, prepared against FCV strain F9 derived from a commercial vaccine (vaccine N) (Carter, 1989), were used. All six of the monoclonal antibodies were found to react specifically to FCV-F9 infected cells by indirect immunofluorescence and more specifically to the major capsid protein of FCV strain F9 by immunoprecipitation (unpublished data). However, only three (4E7, 4E11 and IG9) of the six monoclonal antibodies reacted with strain F9 in sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and western blotting (Dawson, 1991). This suggests that monoclonal antibodies 4E7, 4E11 and IG9 may bind to linear epitopes in the major capsid protein, whereas the binding of the others may be conformationally dependent, as has been suggested by Shin et al. (1993). Dawson (1991) has also demonstrated that only three of these monoclonal antibodies (IG9, 4E7, and 6E8) possess the ability to neutralise vaccine N virus in vitro. All monoclonal antibodies were isotypes IgG2b except IG9 which was IgG1.

Polycional antiserum to FCV strain LS015 was produced in specific-pathogen-free cats (Dawson et al., 1993b).

2.3. Virus stocks

Viruses were grown in feline embryo-derived cells, harvested by freeze–thawing three times, and clarified by low-speed centrifugation. Clarified preparations were then mixed with an equal volume of phosphate-buffered saline (PBS)/0.01% (v/v) Tween 20, passed through 220-nm filters, aliquoted and stored at −20°C until use. A single batch of each antigen was used for all tests.

2.4. Monoclonal antibody analysis of FCV isolates

A 96-well format enzyme-linked immuno-flow-assay (ELIFA) was used (Pierce and Warriner, UK). Briefly, monoclonal antibodies were immobilised on nitrocellulose

| Isolate | Origin | Reference |
|---------|--------|-----------|
| F9 | Field isolate | Bittle et al., 1960 |
| Vacc. N | Commercial vaccine | Carter, 1989 |
| Vacc. A | Commercial vaccine | Dawson et al., 1993a and Dawson et al., 1993b |
| Vacc. B | Commercial vaccine | Dawson et al., 1993a and Dawson et al., 1993b |
| A4 | Field isolate | Povey et al., 1973 |
| G1 | Field isolate | Omerod and Jarrett, 1978 |
| 68/40 | Field isolate | Povey, 1970 |
| 69/1112 | Field isolate | Povey, 1970 |

a High passage laboratory strain F9.

b Modified live vaccines based on F9 strain.

c Modified live vaccine based on an F9-like strain.
membranes in the 96-well format, and used to capture the various FCV isolate antigens, whilst unbound material, including unreactive FCV antigens, passed through. Positive binding of FCV isolates to the various monoclonal antibodies was detected using the polyclonal anti-FCV serum, followed by monoclonal anti-cat IgG-biotin (Sigma) and ExtrAvidin-peroxidase conjugate (Sigma) and tetramethylbenzidine (TMB) (10 μg/ml in 0.1 M acetate buffer pH 6.0/0.005% hydrogen peroxide) chromogenic substrate. After passing through the membrane to produce the colour reaction, the substrate was collected in a 96-well ELISA plate and 15 μl of 2 M sulphuric acid added to each well. The optical density (OD) of each well was then determined at 450 nm.

Reagent concentration, volumes and flow rates were optimised according to the manufacturer’s instructions (Table 2). Each 96-well test contained reference positive (Vaccine N derived strain F9) and negative (uninfected cell culture) antigen controls. Each isolate was also tested against both the test FCV monoclonal antibody and an irrelevant (anti-feline coronavirus) monoclonal antibody. Each test was carried out in triplicate wells, and repeated on at least two further occasions.

A result was considered positive if the mean OD of the triplicate test wells produced by the relevant anti-FCV monoclonal antibody was at least 0.2 of an OD greater than that of the irrelevant monoclonal antibody. If the OD of any of the three triplicate wells of an isolate test fell outside the mean for the three by more than 10% then that test result was rejected and the test repeated. If the ELIFA test for any sample gave a negative result against all of the monoclonal antibodies used, this sample was further analysed by SDS-PAGE and western blotting with polyclonal sera to confirm the

Table 2
Detailed ELIFA protocol

| Step | Reagent                          | Volume (μl) | Dilution                  | Flow rate (μl/min) |
|------|----------------------------------|-------------|---------------------------|--------------------|
| 1    | PBS a wash                       | 400         | NA d                      | 50                 |
| 2    | Monoclonal antibody              | 100         | 1:1000 in PBS a           | 10                 |
| 3    | Bovine serum albumen             | 200         | 3% in PBS a               | 50                 |
| 4    | FCV isolate                      | 100         | 1:1 in PBS-T b            | 10                 |
| 5    | Bovine serum albumen             | 200         | 3% in PBS a               | 50                 |
| 6    | Feline anti-FCV serum            | 80          | 1:1000 in PBS-T b         | 20                 |
| 7    | PBS-T b                          | 200         | NA d                      | 50                 |
| 8    | Monoclonal anti-cat-biotin conjugate | 80        | 1:1000 in PBS-T b         | 20                 |
| 9    | PBS-T b                          | 200         | NA d                      | 50                 |
| 10   | ExtrAvidin-peroxidase conjugate  | 80          | 1:1000 in PBS-T b         | 20                 |
| 11   | PBS-T b                          | 200         | NA d                      | 50                 |
| 12   | PBS-T b                          | 400         | NA d                      | 50                 |
| 13   | Acetate buffer c                 | 50          | NA d                      | 50                 |
| 14   | Chromogen(3',3',5',5'-tetramethylbenzidine-
|      | dihydrochloride)                 | 200         | 0.1 mg/ml in acetate buffer c | 20                 |

a Phosphate-buffered saline.
b Phosphate-buffered saline/0.05% Tween-20.
c 0.1 M sodium acetate buffer pH 6.0.
d Not applicable.
| Clinical                     | Monoclonal antibodies (positive/total) (%) | No reaction with any monoclonal antibody |
|------------------------------|--------------------------------------------|-----------------------------------------|
|                              | 4G12 | 6E8 | 4E11 | 4C11 | 4E7 | 1G9 |                              |
| Acute oral/respiratory disease | 4/11 (36.4) | 4/11 (36.4) | 3/11 (27.3) | 2/11 (18.2) | 1/11 (9.1) | 0/11 (0) | 7/11 (63.6) |
| Chronic oral lesions         | 8/12 (66.7) | 8/12 (66.7) | 7/12 (58.3) | 4/11 (36.4) | 4/12 (33.3) | 1/12 (8.3) | 2/12 (16.7) |
| Vaccine A reactions          | 10/12 (83.3) | 8/12 (66.7) | 4/12 (33.3) | ND b         | 2/12 (16.7) | 4/12 (33.3) | 1/12 (8.3) |
| Vaccine B reactions          | 8/12 (66.7) | 10/12 (83.3) | 8/12 (66.7) | 9/12 (75)   | 1/12 (8.3) | 0/12 (0)   | 0/12 (0)   |
| Total cross reactivity a     | 30/47 (63.8) | 30/47 (63.8) | 22/47 (46.8) | 15/34 (44.1) | 8/47 (17)   | 5/47 (10.6) | 10/47 (21.3) |

a The total reactivity of each monoclonal antibody for all isolates.

b Monoclonal antibody not used with this group.
Table 4
Individual monoclonal antibody binding patterns for 55 FCV isolates

| Clinical group | Isolate | Monoclonal antibody | Reactivity |
|----------------|---------|---------------------|------------|
|                |         | 4G12  | 6E8 | 4E11 | 4C11 | 4E7 | 1G9 |
| Resp a         | JOK28   | +     | +   | +   | +    | +   | -   | 5/6 |
| Resp a         | JOK15   | +     | +   | +   | +    | -   | -   | 4/6 |
| Resp a         | JOK20   | +     | +   | +   | -    | -   | -   | 3/6 |
| Resp a         | JOK195  | +     | +   | -   | -    | -   | -   | 2/6 |
| Resp a         | JOK36   | -     | -   | -   | -    | -   | -   | 0/6 |
| Resp a         | JOK54   | -     | -   | -   | -    | -   | -   | 0/6 |
| Resp a         | JOK92   | -     | -   | -   | -    | -   | -   | 0/6 |
| Resp a         | JOK86   | -     | -   | -   | -    | -   | -   | 0/6 |
| Resp a         | JOK34   | -     | -   | -   | -    | -   | -   | 0/6 |
| Resp a         | JOK1    | -     | -   | -   | -    | -   | -   | 0/6 |
| Resp a         | JOK216  | -     | -   | -   | -    | -   | -   | 0/6 |
| CO b           | LS002   | +     | +   | +   | +    | +   | -   | 5/6 |
| CO b           | LS004   | +     | -   | +   | +    | +   | -   | 4/6 |
| CO b           | LS013   | +     | +   | +   | -    | +   | -   | 4/6 |
| CO b           | PS166   | +     | +   | +   | +    | -   | -   | 4/6 |
| CO b           | PS055   | +     | +   | +   | -    | +   | -   | 4/6 |
| CO b           | F297    | +     | +   | +   | +    | -   | -   | 4/6 |
| CO b           | F252    | +     | +   | +   | ND f | -   | -   | 3/5 |
| CO b           | LS003   | -     | +   | -   | -    | +   | -   | 2/6 |
| CO b           | LS015   | +     | +   | -   | -    | -   | -   | 2/6 |
| CO b           | LS031   | -     | -   | -   | -    | -   | -   | 0/6 |
| CO b           | PS064   | -     | -   | -   | -    | -   | -   | 0/6 |
| CO b           | G378    | -     | -   | -   | -    | -   | -   | 0/6 |
| VA c           | G83     | +     | +   | +   | ND f | +   | +   | 5/5 |
| VA c           | G84     | +     | +   | +   | ND f | +   | -   | 4/5 |
| VA c           | F213    | +     | +   | +   | ND f | -   | +   | 4/5 |
| VA c           | F227    | +     | -   | -   | ND f | -   | +   | 3/5 |
| VA c           | F211    | +     | +   | -   | ND f | -   | +   | 3/5 |
| VA c           | G85     | -     | +   | +   | ND f | -   | -   | 2/5 |
| VA c           | F474    | +     | +   | -   | ND f | -   | -   | 2/5 |
| VA c           | G320    | +     | +   | -   | ND f | -   | -   | 2/5 |
| VA c           | G297    | +     | -   | -   | ND f | -   | -   | 2/5 |
| VA c           | F208    | +     | -   | -   | ND f | -   | -   | 1/5 |
| VA c           | G323    | +     | -   | -   | ND f | -   | -   | 1/5 |
| VA c           | F433    | -     | -   | -   | ND f | -   | -   | 0/5 |
| VB d           | G243    | +     | +   | +   | +    | +   | -   | 5/6 |
| VB d           | E314    | +     | +   | +   | +    | +   | -   | 4/6 |
| VB d           | F418    | +     | +   | +   | +    | -   | -   | 4/6 |
| VB d           | G303    | +     | +   | +   | +    | -   | -   | 4/6 |
| VB d           | E250    | +     | +   | +   | +    | -   | -   | 3/6 |
| VB d           | E272    | +     | +   | -   | +    | -   | -   | 3/6 |
| VB d           | G244    | -     | +   | +   | +    | -   | -   | 3/6 |
| VB d           | E249    | +     | -   | -   | +    | -   | -   | 3/6 |
| VB d           | F6/8    | +     | -   | -   | +    | -   | -   | 2/6 |
| VB d           | G156    | -     | +   | +   | -    | -   | -   | 2/6 |
| VB d           | G332    | -     | +   | +   | -    | -   | -   | 2/6 |
| VB d           | F16     | -     | -   | -   | +    | -   | -   | 1/6 |
**Table 4 (continued)**

| Clinical group | Isolate | Monoclonal antibody | Reactivity |
|----------------|---------|---------------------|------------|
|                |         | 4G12 | 6E8 | 4E11 | 4C11 | 4E7 | 1G9 |
| Ref *           | F9      | +   | +   | +   | +   | +   | +   | 6/6 |
| Ref *           | Vacc. N | +   | +   | +   | +   | +   | +   | 6/6 |
| Ref *           | Vacc. A | +   | +   | +   | +   | +   | +   | 6/6 |
| Ref *           | Vacc. B | +   | +   | +   | +   | +   | +   | 6/6 |
| Ref *           | A4      | +   | +   | +   | -   | -   | -   | 3/6 |
| Ref *           | G1      | +   | +   | +   | -   | -   | -   | 3/6 |
| Ref *           | 68/40   | +   | -   | +   | -   | -   | -   | 2/6 |
| Ref *           | 69/1112 | +   | -   | -   | -   | -   | -   | 1/6 |

a Resp = isolates obtained from cats with acute oral/respiratory disease.
b CO = isolates from cats with chronic oral lesions.
c VA = isolates from cats with vaccine reactions associated with vaccine A.
d VB = isolates from cats with vaccine reactions associated with vaccine B.
e Ref = reference isolates.
f ND = not done.

presence of FCV-specific antigens in that sample. Using these criteria, repeatability was 100%. The mean of 10 tests ± 1 standard deviation results for the reference positive control (vaccine N derived F9 strain) against monoclonal antibodies 4E7, 6E8, 4G12, 1G9, 4E11, and 4C11 were 0.27 ± 0.05, 0.66 ± 0.04, 0.66 ± 0.03, 0.54 ± 0.08, 0.67 ± 0.04, and 0.65 ± 0.06, respectively.

### 3. Results

All of the monoclonal antibodies reacted with the F9 vaccine strain they were raised against. They also reacted with our reference strain of F9 and both of the other vaccine strains which were also based on F9 (Table 4). However, none of the remaining reference or clinical isolates reacted with all of the monoclonals tested with the possible exception of isolate G 83 (vaccine A reaction group) (Table 4). In general, there were varying patterns of reactivity, with monoclonals 4G12 and 6E8 being the most cross reactive amongst the clinical isolates tested (63.8% of the total clinical isolates tested) and 1G9 the least (10.6%) (Tables 3 and 4). Ten of the 47 clinical isolates (21.3%) tested did not react with any of the monoclonal antibodies used (Tables 3 and 4).

A spectrum of monoclonal antibody binding patterns was observed both within and between the clinical groups, and selected isolates could be distinguished from each other (Table 4). However, there were no consistent differences in the overall binding patterns between any of the clinical groups. The oral/respiratory disease group contained a higher proportion (63.6%) of isolates which did not react with any of the monoclonal antibodies (Table 3). This reflected a lower reactivity of the acute oral/respiratory disease isolates overall compared to the other clinical groups (Table 4). In contrast, all of the isolates from vaccine B reactions reacted with at least one of the monoclonals.
4. Discussion

The purpose of this study was to determine if monoclonal antibodies could be used to type isolates of feline calicivirus and enable separation of isolates according to their clinical origin. Conventional polyclonal VN analyses have shown that feline caliciviruses are closely related and constitute one serotype, although a spectrum of antigenic differences is seen within this (Povey, 1974; Kalunda et al., 1975). Similar findings have been demonstrated in other studies using monoclonal antibodies, although direct comparisons between studies are not possible since a range of different isolates and antibodies were used (Tohya et al., 1990 and Tohya et al., 1991). Little work has been done, however, in attempting to correlate antigenic differences with the clinical origin of the isolate.

In previous work we have shown differences between the four clinical groups tested here using selected polyclonal antisera in VN tests (Dawson et al., 1993b). Thus we attempted to use the more focused approach of monoclonal antibody analysis to show these differences more clearly. However, although this study was able to distinguish between selected strains, no clear cut differences between the clinical groups were seen. This would suggest that the epitopes analysed by these monoclonal antibodies were not indicators of FCV clinical origin, and it might be possible that monoclonal antibodies derived either from other epitopes within F9 or from other FCV strains would have enabled better separation. Also, comparison between polyclonal VN analysis of FCV isolates and monoclonal antibody binding patterns may not necessarily yield similar results. Tohya et al. (1990) have shown that there are at least seven neutralising epitopes present on the FCV major capsid protein. Complex interplay between these epitopes, that may not be represented by the monoclonal antibodies used in this study, may have produced the separation seen by Dawson et al. (1993b).

Although the monoclonal antibody binding patterns were not distinctive enough to group isolates, a higher proportion of isolates from cats with acute oral/respiratory disease than those from other groups did not react with any of the monoclonal antibodies used, suggesting that they were less related to F9. However, numbers of isolates in each group were relatively small, and the groups themselves may well overlap.

The origin of isolates obtained from the apparent vaccine reactions is unclear. In the previous study using VN tests, isolates from vaccine B reactions appeared similar to their parent vaccine B, and it was suggested that these isolates may have originated from the vaccine (Dawson et al., 1993a). In contrast, vaccine A reaction isolates looked different to their parent vaccine and appeared more likely to be field viruses. However, in the present study, using monoclonal antibodies, such differences between the groups were not seen, and, except possibly for isolate G83, none of the isolates appeared to be identical to their parent vaccines.

There are several possible reasons for this. Firstly, these apparent vaccine reaction isolates may in fact be field viruses and may not have originated from the vaccines. Alternatively, they may be derived from vaccine virus but have altered on passage through the cat. However, limited work with serial re-isolates made on days 1 and 6 from experimentally infected cats has shown no loss of reactivity to our panel of monoclonal antibodies on short-term passage through the cat (unpublished data). Greater
selection pressures may however operate in the field. It is also possible that virus may alter in cell culture during re-isolation and passage. However the F9 strains used in this study appeared to be stable in vitro, in that they were of different cell culture passage histories yet still retained the ability to bind all of the monoclonal antibodies.

Two of the monoclonal antibodies (IG9 and 4E7) used in this study have previously been mapped to a 37 amino acid sequence in a variable region of the centre of the capsid protein of F9 (Milton et al., 1992). In the present study, these two monoclonal antibodies did not react similarly with all of the isolates, supporting the suggestion of Milton et al., 1992 that they bind to different epitopes within this sequence.

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