Use of Real-Time Quantitative PCR To Detect *Chlamydomphila felis* Infection

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A real-time PCR assay was developed to detect and quantify *Chlamydomphila felis* infection of cats. The assay uses a molecular beacon to specifically identify the major outer membrane protein gene, is highly reproducible, and is able to detect fewer than 10 genomic copies.

The family *Chlamydiaceae* has recently been revised on the basis of recent phylogenetic analyses of the 16S and 23S RNA genes and genetic and phenotypic data (1). In this revision, the feline strain of *Chlamydia psittaci* is given specific status in a new genus, *Chlamydomphila*. *Chlamydomphila felis* infection is common in cats and is a major cause of conjunctivitis (2, 8, 9). It can also cause fever, lethargy, lameness, reduction in weight gain, and upper respiratory symptoms (5, 7). It can be difficult to diagnose from conjunctival swabs by isolation due to the low number of organisms present and the presence of tear antibodies in chronic infection. It has been shown that conventional PCR is more sensitive than isolation in detecting this infection (3, 6). Some major problems with conventional PCR are amplicon contamination, resulting in false positives, and the inability to accurately quantify the amount of starting template in the reaction mixture. We have developed a real-time quantitative PCR assay using a molecular beacon for *C. felis* that can detect fewer than 10 genomic equivalents and has a linear dilution curve of 9 log10.

A conjunctival swab was used to obtain cells from a cat known to be infected with *C. felis*. Genomic DNA was extracted from the swab using a DNeasy tissue kit (Qiagen, Crawley, United Kingdom) according to the manufacturer’s instructions and was used as a template to generate an amplicon using primers Chl for and Chl rev designed to target the major outer membrane protein gene (Table 1) (4). The PCR mixture consisted of 25 μl of Qiagen 2X master mix (Qiagen), 0.2 μM Chl for, 0.2 μM Chl rev, 5 μl of template DNA, 120 nM *C. felis* molecular beacon (Crucemch Ltd., Glasgow, Scotland) (Table 1), and water to 20 μl. After an initial incubation at 50°C for 3 min to allow uracil DNA glycosylase (UDG) to digest any amplicon carryover and at 94°C for 2 min to inactivate the UDG, 45 cycles of 94°C for 10 s and 50°C for 30 s were carried out. Fluorescence was detected at 525 nm at each annealing step (50°C). All reactions were run in triplicate.

Figure 1 shows a plot of threshold cycle versus log10 copy number for the PCR standard in triplicate. It can be seen that the dilution is linear over a 9-log10 range with a correlation coefficient of 0.999. The estimated starting copy number was 7 × 107 molecules per PCR, and it was possible to detect as few as 7 molecules of template in two of the three reactions performed at this dilution. None of the reactions at 0.7 molecule per reaction gave a positive result.

Figure 2 shows a dilution curve for a sample isolated from a cat known to have a *C. felis* infection. Genomic DNA was diluted 10-fold and used as a template in the PCR. It can be seen that, by using a clinical sample of genomic DNA, a dilution curve that is linear over a range of 5 log10 can be produced. Using the standard curve, it is possible to accurately quantify the number of genomic copies present in the starting material. This equates to fewer than 10 genomic equivalents in the 106 dilution. At a 106 dilution no signal was seen for any of the three PCRs. However, it must be noted that incomplete lysis of the sample or the presence of inhibitors would reduce the number of genomic copies available for PCR or reduce the efficiency of amplification. Hence, there may be more organisms in the original sample than are calculated from the standard curve.

These results demonstrate for the first time the use of real-time PCR and molecular beacons to detect *C. felis* DNA isolated from a conjunctival swab. The assay is very sensitive, highly reproducible, and can be accomplished in less than 2 h. This assay is about 10 to 100 times more sensitive than our current nested PCR assay for *C. felis* and has equal specificity.
TABLE 1. PCR primers and probe used in the C. felis real-time PCR assay

<table>
<thead>
<tr>
<th>Primer or probe</th>
<th>Primer or probe sequence</th>
<th>Region of MOMP gene (nt)*</th>
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<tbody>
<tr>
<td>Chl for</td>
<td>5′-ATGCTGTACCATACATGGGG-3′</td>
<td>965–986</td>
</tr>
<tr>
<td>Chl rev</td>
<td>5′-TCTTAAAGGTTGTTCCAGG-3′</td>
<td>1071–1093</td>
</tr>
<tr>
<td>Chl molecular</td>
<td>5′-FAM-CGGCGACACTAC CGCAT TTG</td>
<td>1016–1038</td>
</tr>
<tr>
<td>beacon</td>
<td>CTCAACCGCGG-DABCYL-3′</td>
<td></td>
</tr>
</tbody>
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* From sequence with GenBank accession no. X61096. MOMP, major outer membrane protein.

The molecular beacon was labeled with a fluorescein residue at the 5′ end and a 4-(4′-dimethylaminophenylazo)benzoic acid (DABCYL) quencher at the 3′ end. The region of stem-loop formation is underlined.

The use of real-time PCR to diagnose infection and disease will allow results to be obtained more quickly than is currently possible with conventional PCR or isolation. Real-time PCR is also more sensitive than conventional PCR and allows accurate quantification if desired. The use of multiplex real-time PCR with molecular beacons can allow several infectious organisms to be identified in the same sample at the same time, yielding a quicker turnaround time and saving resources.

FIG. 1. Standard calibration curve for the C. felis real-time PCR assay. A C. felis PCR amplicon of known concentration was diluted from $7 \times 10^3$ molecules per 5 μl to 7 molecules per 5 μl, and 5 μl was used in the PCR assay. The threshold cycle was measured and plotted against the log$_{10}$ of the dilution. Each point represents the average ± standard deviation for three PCRs.

$$y = -3.578x + 41.760 \quad r^2 = 0.999$$

FIG. 2. Dilution curve of a clinical sample of C. felis DNA. Genomic DNA was isolated from a conjunctival swab taken from a cat. This was serially diluted 10-fold, and 5 μl was used in the PCR assay. The threshold cycle was measured and plotted against the log$_{10}$ of the dilution. Each point represents the average ± standard deviation for three PCRs.

$y = 3.381x + 21.992 \quad r^2 = 0.996$

REFERENCES


