Reduction of *Chlamydophila-felis*-associated signs by roxithromycin treatment regimen in cats showing doxycycline intolerance

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**ABSTRACT:** *Chlamydophila felis* (*C. felis*) causes chronic conjunctivitis in cats, and is frequently treated with tetracyclines. However, tetracyclines may cause gastrointestinal side effects, such as vomiting, loss of appetite, diarrhoea, and increased liver enzyme activity in some pets. We evaluated the effect of a four-week treatment regimen with roxithromycin – RXM (Rulid® Sanofi-Aventis, France) in 14 cats with conjunctivitis that tested *C. felis*-positive, and exhibited doxycycline intolerance. The treatment was given for four weeks. Assessment of clinical disease was performed on Day 0 and Day 56. Changes in severity of clinical signs were assessed on a three-step scale (increased, decreased, or no changes) every week of the therapy (Days 7, 14, 21, and 28). Additionally, conjunctival swabs were collected on Days 0 and 56 from each animal, and the *C. felis*-count was assessed through amplification of the ompA gene by real-time PCR with fluorogenic probes and normalisation to the feline DNA signal. Animals receiving RXM improved regarding the ophtalmological parameters related to manifestation of conjunctivitis, severity of ocular discharge and nasal discharge and breathing patterns (*P* ≤ 0.05). A visible improvement in relation to these parameters was already observed after two weeks of RXM administration. *C. felis*-counts decreased in nine cats that were given RXM, but in the other five *C. felis* was not eliminated. Interestingly, however, aggravation of symptoms was not observed in these five animals. Further studies are needed to fully confirm that a reduction of clinical signs and pathogen counts under conditions of natural infection can be attributed to RXM treatment, since there was no control group that received placebo or another drug in this study. The present results also indicate that in some cases 28 days of RXM administration will not be sufficient to eliminate infection.

**Keywords:** cats; chlamydophilosis; conjunctivitis; alternative therapy; roxythromycin RXM

*Chlamydophila felis* (*C. felis*) is an important pathogen in feline conjunctivitis. Infected cats are frequently treated with tetracyclines (e.g., doxycycline and oxytetracycline) (Dean et al. 2005; Stephens et al. 2009; Hartmann et al. 2010), although fluoroquinolones may also be an effective treatment (Hartmann et al. 2008). However, tetracyclines (especially doxycycline) may cause gastrointestinal side effects, such as vomiting, loss of appetite, diarrhoea, and increased liver enzyme activity in some pets. For example, a long-term study indicated that anorexia, diarrhoea, and vomiting occurred in 6% to 13% of cats treated with this antibiotic, with increased liver enzymes noted in 6% to 18% of cats (Schulz et al. 2011; Schulz et al. 2013). Furthermore, German et al. (2005) described a risk of damage resulting in oesophageal strictures after doxycycline treatment, concluding that the therapy should be accompanied by water or food supplementation. Tetracycline treatment should also be avoided in pregnant queens and growing kittens (Hartmann et al. 2008).

Roxithromycin (RXM) is a semi-synthetic macrolide antibiotic derivative of erythromycin (Koopaei et al. 2012) that shows high tissue penetration and activity against *Rickettsia* spp., *Chlamydia* spp., and...
**MATERIAL AND METHODS**

This study included 14 privately owned cats with predominant conjunctivitis that were positive for *C. felis* by PCR, based on methodology described by Marsilio et al. (2004). All cats were also tested by RT-PCR and PCR to determine the presence of feline herpesvirus 1 (FHV-1) and *Mycoplasma felis* infections, according to published protocols (Helps et al. 2003; Chalker et al. 2004). The owners gave their consent for participation and also answered a questionnaire. The main criterion for study inclusion was a negative response to doxycycline, including vomiting and/or diarrhoea, within the first three days of administration. Other frequent causes of the sudden appearance of these symptoms, such as dietary errors, foreign bodies, and hairballs, were excluded during anamnesis and clinical examination. The characteristics of the examined population are provided in Table 1. A scoring system for the evaluation of clinical signs was adapted from Hartmann et al. (2008) to enable the assessment of clinical disease (Table 2). Each sign was assessed separately and subsequently the total score was calculated summarising the score of each signs. Clinical signs were independently assessed by the same person, with a maximum score of 18. All cats were retested to confirm *C. felis* infection by RT-PCR, with a less than one-week interval between discontinuation of doxycycline treatment and sampling.

Conjunctival samples from the ventral conjunctival fornix were collected in pairs using sterile cotton-tipped swabs and prepared for RT-PCR, as described by Sykes (2005). Briefly, DNA from each swab pair was extracted directly with the QIA Amp Ultra Sens Virus kit (Qiagen, Synagen Biotech, Wroclaw, Poland). Primers specific to the *C. felis* ompA gene (forward primer, 5'-GAACTGCAAG CAACACCACT G-3' and reverse primer 5'-CCATTCGGCA TCTTGAAGAT G-3') were used together with a fluorogenic probe: 6-FAM-CGCTGCCGAC AGATCAAATT TTGCC-BHQ. The feline 28S rDNA gene served as the endogenous housekeeping gene for feline conjunctival cells, using the primers described by Helps et al. (2003) and the probe, CGGCACGCT ACTGATGATG TGTTGTTGCC GCGCG, labelled with Texas red on the 5' end and the appropriate BHQ1 on the 3' end. All reactions were performed in duplicate. Each RT-PCR-reaction was performed in a 20-μl mixture containing 10 pM of each ompA gene primer, 20 pM of each 28S rDNA gene primer, 10 pM of the FAM/BHQ, and 20 pM of the Texas red/BHQ1 probes, 1 μl of template, and 10 μl of Kappa Probe FAST qPCR Master mix (Kappa Biosystems, USA). The reaction mixture was heated to 95 °C for 3 min, followed by 45 cycles of PCR consisting of 10 s at 95 °C and 30 s at 60 °C. Fluorescence was detected at the annealing step (iQ5 Multicolor Real-Time PCR Detection System, Bio-Rad Laboratories Ltd., Poland).

DNA from the vaccine 905 *C. felis* strain was used as a control. The relative count of *C. felis* DNA was
calculated from the *C. felis* DNA signal normalised to the feline DNA signal, as described by Dean et al. (2005) and Beshir et al. (2010). The difference between the threshold cycles Ct (ΔCt) of the *C. felis* ompA gene and the Ct of the feline 28 S rDNA gene was calculated using the 2^{−ΔΔCt} method (Livak and Schmidten 2001). The change in ΔCt was calculated after a 4-week treatment (ΔΔCt), with time 0 representing the time of pre-treatment diagnostic sampling. To calculate relative *C. felis*-levels, ompA levels from cat No. 1 were arbitrarily set to 1. The percent knockdown (% KD) was calculated by subtracting the normalised ΔΔCt of the ompA gene levels at Day 0 and Day 56 and multiplying by 100.

Cats were treated with an oral suspension containing 50 mg RXM (5 mg/kg body weight) every 12 h for four weeks (from Day 0 to Day 28), followed by a four-week post-treatment period. Conjunctival swabs were collected on Days 0 and 56. Clinical exams using a scoring system were performed at the start of the study (Day 0) and at the four-week, post-treatment follow-up (Day 56). Additionally, changes in symptom severity were assessed on a three-step scale (increased, decreased, or no changes) every week of the therapy (Days 7, 14, 21, and 28).

The Wilcoxon matched pairs test (a nonparametric alternative to the *t*-test for dependent samples) was used to compare proportions and statistical analysis was performed in Statistica 10.0.0 (StatSoft).

The study was performed in accordance with the ethical principles of the Ethics Committee of the Wroclaw University of Environmental and Life Sciences.

Table 1. Characteristics of the examined cat population including clinical signs, environmental factors and potential problems with antibiotic administration, which may influence the clinical efficacy of Rulid® (Sanofi-Aventis, France)

<table>
<thead>
<tr>
<th>Cat No</th>
<th>Breed, gender, age (years)</th>
<th>Problems with antibiotic administration declared by the owners*</th>
<th>Direct contact with other cats during therapy*</th>
<th>Outside* animal</th>
<th>Duration of the disease before treatment (in months)</th>
<th>Other symptoms (accompanying conjunctivitis)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>BRI, ♂, 8</td>
<td>–</td>
<td>+</td>
<td>+/–</td>
<td>3–6</td>
<td>rubbing the eyes with paws</td>
</tr>
<tr>
<td>2</td>
<td>SPH, ♂, 2</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>6–12</td>
<td>dried scabs around eyes</td>
</tr>
<tr>
<td>3</td>
<td>MB, ♂, 2</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>6–12</td>
<td>blepharospasm</td>
</tr>
<tr>
<td>4</td>
<td>MB, ♀, 6</td>
<td>–</td>
<td>+/–</td>
<td>+</td>
<td>&gt; 12</td>
<td>dried scabs around eyes</td>
</tr>
<tr>
<td>5</td>
<td>PER, ♂, 10</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>&gt; 12</td>
<td>not reported</td>
</tr>
<tr>
<td>6</td>
<td>EXO, ♂, 8</td>
<td>+/–</td>
<td>+</td>
<td>–</td>
<td>6–12</td>
<td>coinfection with FHV-1, sequestratio corneae</td>
</tr>
<tr>
<td>7</td>
<td>SPH, ♂, 2</td>
<td>+/–</td>
<td>–</td>
<td>–</td>
<td>&gt; 12</td>
<td>dried scabs around eyes and nostrils</td>
</tr>
<tr>
<td>8</td>
<td>MB, ♀, 11</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>&gt; 12</td>
<td>dried scabs around eyes and nostrils</td>
</tr>
<tr>
<td>9</td>
<td>DRX, ♀, 7</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>&gt; 12</td>
<td>earlier coinfection with <em>P. aeruginosa</em>, dried scabs around eyes and nostrils</td>
</tr>
<tr>
<td>10</td>
<td>EXO, ♀, 3</td>
<td>+/–</td>
<td>–</td>
<td>–</td>
<td>&gt; 12</td>
<td>sequestratio corneae (L), dried scabs around eyes and nostrils</td>
</tr>
<tr>
<td>11</td>
<td>MCO, ♂, 5</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>&gt; 12</td>
<td>not reported</td>
</tr>
<tr>
<td>12</td>
<td>PER × EXO, ♀, 3</td>
<td>+/–</td>
<td>+</td>
<td>–</td>
<td>&gt; 12</td>
<td>dried scabs around eyes and nostrils, breathing problems</td>
</tr>
<tr>
<td>13</td>
<td>MB, ♀, 4</td>
<td>+/–</td>
<td>–</td>
<td>–</td>
<td>6–12</td>
<td>not reported</td>
</tr>
<tr>
<td>14</td>
<td>MB, ♀, 3</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>6–12</td>
<td>not reported</td>
</tr>
</tbody>
</table>

Breeds (according to EMS/Easy Mind System): MB = mixed breed, PER = Persian, BRI = shorthair British cat, EXO = exotic cat, DRX = devon rex, SPH = sphinx, MCO = Maine Coon

Positive answer (+); negative answer (−); ambiguous answer (+/−, sometimes)

Gender: female ♀ and male ♂

*questionnaire data
RESULTS

All 14 cats from the examined group were positive for *C. felis* DNA on day 0. FHV-1 was detected in one cat (No. 10). *Mycoplasma* spp. were not detected.

All cats receiving RXM improved significantly in all parameters (Table 2), with pre-treatment (Day 0) clinical scores of 6.71 (± 3.24) decreasing to 1.21 on Day 56 (± 1.62) (*P* < 0.001). In clinical examination, there was a reduction in the ocular discharge (scores changed from 2.71 on day 0 to 0.64 on day 56, *P* < 0.001) and conjunctivitis manifestation (2.78 on day 0 to 0.42 on Day 56, *P* < 0.001). Resolution of the conjunctivitis symptoms was observed in nine cats (64%) by Day 7 of RXM administration, and in all cats by Day 14. Moreover, an improvement in cats receiving RXM was also observed with respect to the severity of nasal discharge (*P* = 0.028) and breathing patterns (*P* = 0.043). Comparability of statistics for lung

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Table 2. Evaluation of changes in clinical symptoms between Day (0) and Day (56), after four weeks of RXN (Rulid®, Sanofi-Aventis, France) therapy. Estimation of the severity of clinical signs was performed as proposed by Hartmann et al. (2008). The maximum possible total clinical score was 18 (shaded in bold).

| Cat No. | Discharge from one or both eyes | Character of ocular discharge | Conjunctivitis | Nasal discharge | Breathing pattern | Lung sounds | Sneezing | Improvement of clinical signs illustrated as the total clinical score based on the assessment of each clinical symptom
<table>
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<tr>
<td></td>
<td>Day 0 (0)</td>
<td>Day 56 (56)</td>
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<td>Day 56 (56)</td>
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</table>

P-value (Wilcoxon matched pair test for dependent samples)  
P < 0.001  P < 0.001  P = 0.028  P = 0.043  not analysed  not analysed  P < 0.001

1° = no discharge, 1 = unilateral, 2 = bilateral;  
2° = no clinical signs, 1 = minor serous discharge, 2 = moderate serous to mucoid discharge, 3 = moderate mucopurulent discharge, 4 = severe mucopurulent discharge  
3° = no clinical signs, 1 = mild conjunctival hyperaemia, 2 = moderate conjunctival hyperaemia and mild chemosis, 3 = moderate to severe conjunctival hyperaemia and moderate chemosis, 4 = severe conjunctival hyperaemia and severe chemosis  
4° = no clinical signs, 1 = minor serous discharge, 2 = moderate serous to mucoid discharge, 3 = moderate mucopurulent discharge, 4 = severe mucopurulent discharge  
5° = normal, 1 = mild dyspnoea, 2 = moderate to severe dyspnoea; 6° = normal, 1 = mild abnormal lung sounds, 2 = moderate to severe lung sounds; 7° = absent, 1 = occasionally, 2 = frequently

*the maximum possible total pre-treatment clinical score (Day 0) = 18, (Day 56) = 18
sounds and sneezing were excluded due to small variability. According to the owners, there were no reported episodes of vomiting or diarrhoea that could be considered as side effects of RXM therapy. On Day 56, nine cats (64.2%) showed reduced \textit{C. felis} DNA signals relative to pre-treatment (1.006 to 17.1 times smaller than the initial copy number), but increased positive signals (1.2 to 128.5 times greater than the initial copy number) were seen in the other five cats (35.7%) (Figure 1).

**DISCUSSION**

The lack of standard procedures for the culture-based detection of pathogens from the Chlamydiaceae family, low recovery rates from clinical isolates, and potential heterotypic resistance to antimicrobials make diagnosis and treatment difficult. Antibiotic therapy may be inefficient against \textit{Chlamydia/Chlamydophila} infections, leading to long-term infections or perturbations in the chlamydial replication cycle (Sandoz and Rockey 2010). Treatment efficacy can also be influenced by factors such as inadequate owner compliance in administering antibiotics; contact with other cats, potentially allowing cross-contamination with \textit{Chlamydophila}; or variability in exposure to other immune challenges, if the animals are outdoors.

Treatment alternatives to doxycycline for \textit{Chlamydophila} have been extensively studied. Sturgess et al. (2001) described the efficacy of 19-day-treatment with amoxicillin and clavulanic acid in specific pathogen-free cats experimentally infected with a \textit{C. felis}-isolate. Treatment initially reduced isolate levels and improved clinical status, but recurrence occurred in 62% of cats within 40 days, requiring an additional four weeks of treatment to eradicate the infection. Enrofloxacin (5 mg/kg, for 14 days) improved the clinical signs of conjunctivitis, but an immunofluorescent antibody test on conjunctival swabs revealed that some cats were still positive for \textit{Chlamydia/Chlamydophila} spp., despite enrofloxacin levels in tears that exceeded the minimum inhibitory concentration for those pathogens (Gerhard et al. 2006). Owen et al. (2003) described the efficacy of azithromycin for the treatment of fe-line chlamydophilosis. Azithromycin (10–15 mg/kg daily for three days and then twice weekly, at the mean treatment period of 20 days) also improved the clinical signs, but \textit{C. felis} was re-isolated from the majority of examined cats. Initially, daily treatment with 10 mg/kg doxycycline for two weeks was the standard of care for \textit{C. felis} infection. However, even a three-week treatment may be insufficient to eradicate the pathogen completely, as \textit{C. felis} DNA was still detected in some animals 35 days after treatment (Dean et al. 2005). Therefore, we selected a study design with a four-week RXM treatment and a four-week post-treatment period.

To our knowledge, this is the first clinical study in which RXM was used to treat chlamydophilosis in cats with conjunctivitis and doxycycline intoler-
ance. We used a standardised clinical assessment score proposed by Hartmann et al. (2008) to allow inter-study comparison. In Hartmann and others, this score ranged from 4.5 to 4.9 on Day 0, which was lower than in our study, indicating that those animals had better initial clinical presentations. However, both studies showed similar overall improvement rates. In their study pradofloxacin therapy reduced clinical conjunctivitis during the first week of drug administration, and they observed as well as we, an improvement with respect to local effects within the conjunctiva or respiratory tract. Therefore, our four-week oral RXM regimen may be suitable for treating the clinical manifestation of chlamydial conjunctivitis in cats.

A controlled, double-blinded study, performed by Hartmann et al. (2008), involved cats receiving pradofloxacin (5 mg/kg daily for 42 days), doxycycline (5 mg/kg every 12 h) or placebo. We measured *C. felis* DNA levels with the same ompA RT-PCR primers (Dean et al. 2005) and quantification methods. In their study, of the 23 cats positive for *C. felis* on Day 0, 17% had pathogen-specific DNA at the end of the treatment. Some cats were still *C. felis*-positive on Day 42, despite being negative in the RT-PCR test during the study. We found that 35.7% of cats treated with RXM remained *C. felis*-positive on Day 56. Dean et al. (2005) concluded that 21 days of doxycycline therapy eradicated *C. felis* in 53% of pathogen-free-derived cats infected with *C. felis* (3×10^3 infectious units of field isolate). However, the differences in infectious route (inoculation onto the conjunctiva in SPF cats vs. natural infection) and timing make comparison of these studies difficult. In some cases even longer (28 days) therapy is needed to eradicate *C. felis*, according to Hartmann et al.’s studies (Hartmann et al. 2008). In naturally infected cats treated with doxycycline the initial infection level may also determine *C. felis* clearance. The variability in initial infection levels and inter-animal responses to therapy are a major study limitation. The long duration of infection (six to 12 months or longer) may have prevented complete *Chlamydia felis* clearance in the five cats that tested positive at the end of the study. Three cats that showed increased *C. felis* counts after RXM treatment also showed mucopurulent discharge during the therapy, suggesting concomitant bacterial infection. Variability in epidemiological differences, such as the risk of re-infection from contact with carriers at home or outside, could also make interpretation difficult. Three cats that did not show reduced *C. felis* counts were exposed to other cats in the house, although the infection status of these other cats is unknown. One cat was permanently living outside, which may have affected its exposure to *C. felis* from other animals. We conclude that a limited ability to isolate cats from the environment or to change owner habits makes it difficult to rule out the contribution of environmental factors to infection or re-infection rates. There are a few possible reasons for the observed improvement of clinical signs in all cats despite the increases in *C. felis* counts in some individuals. The most important ones in our opinion include, potentially additional secondary bacterial pathogens involved in conjunctivitis and sensitive to RXM and/or the possibility of *C. felis* re-infection. The incidence of these types of re-infections during conjunctivitis (under natural conditions) is not well known and should be studied in more detail. Therefore, there is no evidence regarding the replication and quantitative analysis of *C. felis* in such cases.

Outcomes of RXM therapy may also be influenced by the initial doxycycline treatment. Inhibiting the normal replication cycle of *Chlamydiae* by transient antibiotic administration can result in persistence and subsequent long-term infection. Antibiotic treatment causes the formation of aberrant reticular bodies that continue to synthesise proteins and replicate DNA but halt cell division (Sandoz and Rockey 2010). Doxycycline treatment was discontinued because of negative side effects in the animals, but such discontinuation may lead to chlamydial persistence in vivo, and increased difficulty in eradicating it in vivo (Sandoz and Rockey 2010). However, the limited extent of doxycycline administration (three days) makes it difficult to assess its contribution to the persistence of the infection. Wyrick and Knight (2004) reported the appearance of azithromycin-resistant *Chlamydia* strains in response to penicillin exposure.

In conclusion, we report a marked clinical improvement in cats infected with *C. felis* receiving a four-week dosing regimen of RXM. However, RXM treatment does not guarantee the clearance of *C. felis* from the animals, especially, if some epidemiological factors may not be eliminated. Further studies on feline populations with control groups are necessary in order to determine the whole potential of RXM in the treatment of cats with *C. felis* conjunctivitis.
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