Molecular mechanisms of *Bartonella henselae* resistance to azithromycin, pradofloxacin and enrofloxacin

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Sir,

*Bartonella henselae* are fastidious, facultative intracellular bacilli that can cause bacteremia, endocarditis, cat-scratch disease in immunocompetent patients, and bacillary angiomatosis and peliosis hepatitis in immunocompromised patients.¹² There are a limited number of studies that have addressed the antibiotic treatment of cats infected with *B. henselae*.³ Azithromycin, a macrolide compound, has seemingly become the drug of choice to treat cats and dogs for *B. henselae* infections. However, relapses after antibiotic withdrawal have been reported.⁴ Azithromycin, which is derived from erythromycin, binds to the 50S subunit of the bacterial ribosome and, thus, inhibits the translation of mRNA. Flouroquinolone antibiotics exert their antibacterial effects by inhibiting certain bacterial topoisomerase enzymes. Pradofloxacin, a third-generation fluoroquinolone, is being exclusively developed for use in veterinary medicine. Enrofloxacin is a broad-spectrum antimicrobial agent with bactericidal activity against Gram-negative and Gram-positive bacteria, mycobacteria and rickettsia. As for *B. henselae*, potential mechanisms of resistance to azithromycin, pradofloxacin and enrofloxacin are not known. Therefore, the objective of this study was to select in vitro azithromycin-resistant, pradofloxacin-resistant and enrofloxacin-resistant mutants to determine the molecular mechanism of resistance.

Six *B. henselae* isolates (BhH1, Mina Mia, Stray7, Bh94FO73, BhFO1946 and Bh95FO101) were used in this study. Four of these isolates (Mina Mia, Stray7, Bh94FO73 and BhFO1946) were derived from cats from the USA. BhH1 is the ATCC type strain obtained by blood culture from a febrile, HIV-infected patient in Houston, Texas. Isolate Bh95FO101 was from a sick pet cat from Israel.

Pradofloxacin (5 µg) and enrofloxacin (5 µg) discs were purchased from AB Biodisk (Solna, Sweden) and supplied by Bayer HealthCare, Germany. Azithromycin (15 µg) discs were purchased from VWR International, USA. The selection of antibiotic-resistant mutants was performed by serial passages of each *B. henselae* isolate on blood agar plates containing an antibiotic disc. *Bartonella* strains were considered resistant when the inhibition zone was <6 mm.

The *B. henselae* azithromycin-susceptible strains and the azithromycin-resistant mutants were screened by PCR and sequencing using primers for 23S rRNA, and L4 and L22 ribosomal proteins. Strains resistant and susceptible to pradofloxacin and enrofloxacin were screened by PCR and sequencing using primers for gyrA, gyrB, parC and parE.⁵

Six *B. henselae* azithromycin-resistant mutants were obtained after the second in vitro passage (Table 1). Compared with the parental strain, each *B. henselae* azithromycin-resistant mutant had a homogenous single nucleotide substitution at position 2058 (A2058G, *Escherichia coli* numbering) in the 23S rRNA gene. Mutations at A2058 for certain macrolides confer the highest levels of resistance.⁵ Many independent lines of evidence indicate that adenosine 2058 is the key nucleotide involved in macrolide interaction with the bacterial ribosome.⁵ An A2058G transition was the first RNA mutation shown to confer erythromycin resistance and is presently the most frequent substitution found in clinical isolates. Our in vitro results might explain relapses or treatment failures observed in vivo when using azithromycin as the sole antibiotic for treatment of *Bartonella*-related infections. We did not find any change in the L4 and L22 ribosomal proteins for the *B. henselae* azithromycin-resistant mutants. All *B. henselae* isolates became resistant to pradofloxacin and enrofloxacin after differing numbers of subculture passages; however, in contrast to azithromycin, at least five passages...

<table>
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<tr>
<th>Antibiotic</th>
<th>BhH1</th>
<th>Mina Mia</th>
<th>Stray7</th>
<th>Bh94FO73</th>
<th>BhFO1946</th>
<th>Bh95FO101</th>
</tr>
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<td>2</td>
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<tr>
<td>Pradofloxacin</td>
<td>7</td>
<td>8</td>
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<td>5</td>
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</tr>
<tr>
<td>Enrofloxacin</td>
<td>5</td>
<td>8</td>
<td>6</td>
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were necessary to develop resistance to either antibiotic (Table 1). Compared with the parental B. henselae strains, the pradofloxacin-resistant and enrofloxacin-resistant mutants had an amino acid change from serine to valine at the 83rd position (E. coli numbering), which is located in the quinolone resistance-determining region (‘QRDR’) of the DNA gyrase A protein. The Ser-83→Val mutation found in our study for pradofloxacin-resistant and enrofloxacin-resistant mutants has been reported previously by Tavío et al.⁶ in a fluoroquinolone-resistant E. coli isolate. In our study, no mutation was found in the gyrB, parC and parE genes for pradofloxacin-resistant and enrofloxacin-resistant mutants of B. henselae. These results also indicate a primary B. henselae target for quinolone antimicrobials. Because resistant mutants only showed changes in GyrA, the primary target is most likely DNA gyrase, rather than topoisomerase IV.

In conclusion, this is the first study to describe specific molecular mechanisms of azithromycin, pradofloxacin and enrofloxacin resistance for Bartonella henselae isolates obtained from cats and a human patient isolate. Our findings are clinically relevant and could explain relapses observed using azithromycin for the treatment of B. henselae infections. We believe that clinicians should be aware of these results when selecting azithromycin, pradofloxacin and enrofloxacin for the treatment of Bartonella henselae infections in veterinary patients.

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References

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Detection of pandemic B2-O25-ST131 Escherichia coli harbouring the CTX-M-9 extended-spectrum β-lactamase type in a feral urban brown rat (Rattus norvegicus)

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Sir,
Apart from the increasing prevalence of extended-spectrum β-lactamases (ESBLs) in Escherichia coli isolates from human patients and diseased livestock¹ their co-emergence in wildlife faecal samples has also been documented.² As wildlife animals are known to disseminate bacteria of human and animal health concern and may carry, in particular, ESBL-producing E. coli, it is highly important to determine how widely these bacteria have spread into rural and urban ecosystems. Recently, broad geographical dissemination of an E. coli clone (B2-O25:H4-ST131) carrying the CTX-M-15 ESBL type in a feral brown rat (Rattus norvegicus),

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