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Estimation of *Rickettsia rickettsii* copy number in the blood of patients 1 with Rocky Mountain spotted fever suggests cyclic diurnal trends in bacteremia

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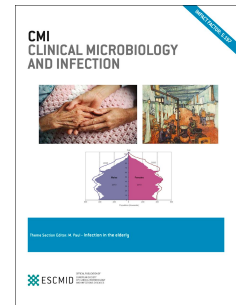
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1 Estimation of *Rickettsia rickettsii* copy number in the blood of patients with Rocky

2 Mountain spotted fever suggests cyclic diurnal trends in bacteremia

3 Sir,

4 Infection with *Rickettsia rickettsii*, an obligate intracellular gram-negative bacterium, causes
5 Rocky Mountain spotted fever (RMSF), one of the most lethal of all known infectious diseases.
6 Because *R. rickettsii* infects predominantly fixed endothelial cells of small and medium-sized
7 blood vessels, there are few quantitative assessments of bacteremia for RMSF and limited data
8 suggest that low numbers of *R. rickettsii* circulate in the peripheral blood of ill patients [1]. This
9 observation was described by early investigators who recognized that *R. rickettsii* bacteria could
10 scarcely be found in stained peripheral blood smears of patients, even those with severe disease
11 [2]. Herein we use TaqMan real-time PCR to evaluate levels of rickettsial DNA in a series of
12 clinical samples obtained from patients with RMSF. Whole blood (n=23), serum (n=6), and
13 plasma (n=1) specimens were collected from 13 US patients with RMSF from 2010-2015 and
14 submitted to the Rickettsial Zoonoses Branch Reference Diagnostic Laboratory at CDC
15 (Supplemental Table). Individual specimens were accompanied by clinical information that
16 varied in completeness. Each sample was positive for PanR8 *Rickettsia* spp., and RRI6 *R.*
17 *rickettsii*-specific targets [3], with controls performing as expected (Supplemental Figure).

18 RMSF specimens were quantified with PanR8 in duplicate (undiluted or 1:10), using ten-fold
19 dilutions of *R. rickettsii* DNA from 0.1 fg to 10.0 pg (1 fg = 0.8282 copies). Samples from
20 patients with fatal (n=20) and nonfatal (n=10) outcomes ranged from $1.41 \times 10^3 \pm 2.78 \times 10^2$ to
21 $2.05 \times 10^6 \pm 1.89 \times 10^3$ copies /mL (median = 1.63×10^5), and $8.40 \times 10^1 \pm 4.19 \times 10^1$ to $3.95 \times$
22 $10^5 \pm 3.33 \times 10^4$ copies /mL (median = 2.82×10^3), respectively (Supplemental Table). Copy
23 numbers of *R. rickettsii* DNA were significantly greater in patients with fatal outcomes ($p < 0.05$,

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