

Preliminary *in vitro* and *in vivo* findings of hyperbaric oxygen treatment in experimental *Bb* infection

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In these studies, we evaluated repeated HBOT for its ability to kill *Bb* *in vitro*, and *in vivo*, in a murine model of Lyme disease. Several North American tick-derived and recently obtained patient isolates were studied separately in our assay systems. To test for *in vitro* susceptibility, one-half to one million *Bb* were cultured in a small volume (0.1 - 0.2 ml) of BSK media using small snap-cap test tubes. With the caps removed, these cultures were then exposed, for one hour (twice daily for 2 consecutive days), to pure, filtered oxygen pressurized to 2-3 times normal atmospheric conditions. This was achieved using a specially constructed, miniaturized cylindrical chamber (length = 12 inches; diameter = 8 inches), equipped to accept any pressurized gas mixture through its portal opening. After the final HBOT, all cultures received an additional 0.5 ml of BSK media (making the final volume now 0.6 - 0.7 ml), and their caps were snapped shut. Matching control cultures received no HBOT. All cultures were incubated at 33°C for 2-3 days and were examined microscopically for live *Bb*.

Our results showed that 14 of 17 strains of *Bb* had their growth inhibited by 33-94%, while there was little or no inhibition of 3 *Bb* strains. For the *in vivo* studies, separate groups of C3H or CD1 mice were infected intradermally with 100,000 *Bb*. Two to 4 weeks later, one group of infected mice received two, 1.0-1.5 hour HBO exposures, for two consecutive or alternating days. The treated mice were sacrificed one day after the last treatment, and extract cultures of their urinary bladders were prepared in BSK media. It was found that no *Bb* grew out of 80% of these extract cultures, whereas live *Bb* organisms were recoverable from 90% of extract cultures prepared from matched, infected control mice not treated with HBO. These data suggest that HBOT may be considered as a clinically useful form of adjunct therapy in the treatment of Lyme disease.

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