

Survival of *Mycobacterium avium* subspecies *paratuberculosis* in Biofilms on Livestock Watering Trough Materials

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Introduction

Mycobacterium avium subspecies *paratuberculosis* (*M. paratuberculosis*) is the causative agent of Johne's disease, a chronic enteric infection that affects ruminants. Despite the ubiquitous occurrence of *Mycobacterium* spp in nature, and the fact that Johne's disease has been reported worldwide, little research has been done to assess its survival in agricultural environments. The goal of this study was to evaluate the ability of *M. paratuberculosis* to become established and persist in biofilms on coupons composed of common livestock watering trough materials.

Materials and Methods

M. paratuberculosis was inoculated into triplicate containers with 32 L of trough water and either concrete, plastic, galvanized or stainless steel coupons. One set of samples was treated with chlorine (5.7 ppm final concentration) every 7 to 28 days and the other set was left untreated. The concentration of *M. paratuberculosis* was determined by targeting the IS900 sequence in DNA extracted from samples by using quantitative, real-time PCR.

Results

The average concentration of *M. paratuberculosis* at the beginning of the experiment was $4.6 \pm 2.8 \times 10^4$

cells/mL water or $1.6 \pm 1.3 \times 10^5$ cells/cm² coupon. *M. paratuberculosis* could still be detected on coupons of all materials after 149 days of incubation. The concentration of *M. paratuberculosis* was lowest on stainless steel ($9.3 \pm 6.2 \times 10^1$ cells/cm²) and highest on concrete ($1.9 \pm 1.1 \times 10^4$ cells/cm²) coupons. Chlorine had the greatest affect on biofilms present on concrete. (Cell concentrations were 90% lower for chlorine-treated than for non-chlorine treated biofilms.) Biofilms on galvanized and stainless steel coupons were lower in chlorine-treated samples taken between days 35 and 70 (between 60% and 99% lower), but these differences disappeared thereafter. Chlorine addition had no affect on biofilms on plastic coupons

Significance

These results suggest that *M. paratuberculosis* survives well (over 149 days) in biofilms present on different trough materials. Trough material composition influenced the survival of *M. paratuberculosis* with the lowest survival exhibited on stainless steel, followed by plastic, galvanized steel and concrete.