Bacterial growth and semen viability in canine semen extenders inoculated with *Brucella canis*

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*Brucella canis*, the etiological agent of canine brucellosis, was first described in 1967 by Leland Carmicheal. Since that time several serologic tests have been developed to diagnose this disease in domestic canids. The surface antigens of this bacteria make many of serologic tests highly sensitive, but lacking in specificity. In addition to this, there is a substantial lag time between the initial exposure and infection to seroconversion or positive blood culture, during which these dogs can be contagious. Due to the dilemma of accurately diagnosing canine brucellosis in a timely manner, it is difficult to determine if the dogs and semen that the practitioners and owners are handling during semen collection, processing, shipment and artificial insemination are truly disease-free in the face of negative serologic test results.

The purpose of this experiment was twofold: 1) determine if commercial semen extenders will inhibit the growth of *Brucella canis* and 2) in the event that commercial semen extenders do not prevent the growth of *Brucella canis*, determine if the addition of antibiotics to the semen extenders inhibit the growth. It was anticipated that all of the commercial extenders, with the exception of the Kenney skim milk extender would prevent the growth of *Brucella canis*. In this experiment, six commercially available extenders (Kenney skim milk extender without antibiotics (Veterinary Concepts, Spring Valley, WI), Fresh Express® (Synbiotics, Kansas City, MO), CLONE™ (CLONE Inc., Doylestown, PA), CaniPRO AI and CaniPRO Chill 5™ (Minitube of America, Verona, WI), Insemin-aid™ (Camelot Farms, College Station, TX) were examined. In experiment 1, 20 μL of a 0.5 McFarland standard *Brucella canis* suspension was added to 1 mL aliquots of each extender, a control saline, and stored at 5 °C. Brucella blood agar plates were inoculated with 100 μL (approximately 2x10^5 CFU) of each suspension at 0, 24, 48, and 120 h of chilled storage. All suspensions were plated in duplicate and incubated at 37°C for up to 72 h. None of the extenders inhibited the growth of *Brucella canis*. In experiment 2, three antibiotics (amikacin, 4 μg/mL; ampicillin, 2 μg/mL; ticarcillin, 8μg/mL) were added to each extender-*B. canis* suspension. None of the extenders with supplemental antibiotics inhibited the growth of *Brucella canis*, but all of the extenders with the exception of CLONE™ had fewer colony forming units (CFU) than those without supplemental antibiotics. With this information, semen from two stud dogs was added to the extender/*B. canis*/antibiotic mixtures at a ratio of 1:5 (semen : mixture). The addition of semen led to an increase in CFUs of *Brucella canis* in all extenders. The information gained in this study reveals that the use of commercial semen extenders with and without the addition of antibiotics does not inhibit the growth of *Brucella canis*.

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