

## **Evaluation of six decontamination procedures for isolation of *Mycobacterium avium* complex from avian feces**

### **ABSTRACT**

Culture is considered the gold standard for definitive diagnosis of mycobacterial infections. However, consensus about the most suitable culture procedure for isolation of nontuberculous mycobacteria is lacking. The study compared the recoveries of mycobacteria after decontamination of spiked and fresh avian feces with 4% sodium hydroxide (NaOH), 12% sulfuric acid (H<sub>2</sub>SO<sub>4</sub>), or 1% cetylpyridinium chloride (CPC), with and without mixture of three antibiotics, namely vancomycin (VAN, 100 µg/ml), nalidixic acid (NAL, 100 µg/ml), and amphotericin B (AMB, 100 µg/ml). The antibiotic mixture was referred to as VNA. Decontamination procedures were evaluated using two (n = 2) avian fecal samples spiked with 106, 104, and 102 CFU/ml of *Mycobacterium avium* subsp. *avium* (ATCC 15769) and fresh avian feces (n = 42). *M. avium* subsp. *avium* was detected on the culture media from spiked samples (106 and 104 CFU/ml) decontaminated with NaOH, NaOH-VNA, H<sub>2</sub>SO<sub>4</sub>, and H<sub>2</sub>SO<sub>4</sub>-VNA for 2–6 weeks. These bacteria were detected in 2–4 weeks when using CPC and CPC-VNA. *M. avium* subsp. *avium* cannot be isolated on culture media from spiked samples (102 CFU/ml) decontaminated with any decontaminating agent. Two mycobacterial isolates, namely, *Mycobacterium terrae* and *M. engbaekii*, were isolated from field samples decontaminated with NaOH and CPC-VNA. With regard to the contamination rate, the use of CPC-VNA showed lower contamination rates (5.5% and 19.0%) from spiked and field samples than those of the other methods (NaOH: 22.2% and 59.5%, NaOH-VNA: 16.7% and 21.4%, H<sub>2</sub>SO<sub>4</sub>: 11.1% and 40.5%, H<sub>2</sub>SO<sub>4</sub>-VNA: 5.5% and 21.4%, and CPC: 66.7% and 50%). In conclusion, the decontamination of fecal samples following a two-step procedure with 1% CPC and VNA can ensure high recovery rate of many mycobacteria with the lowest contamination in cultures.

**Keyword:** Mycobacterial infections; *Mycobacterium avium*; Mycobacteria; Avian feces