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Short Communication

Evaluation of PMS–PCR technology for detection of *Mycobacterium avium* subsp. *paratuberculosis* directly fromty bovine fecal specimens

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ABSTRACT

Mycobacterium avium subsp. *paratuberculosis* (MAP) causes paratuberculosis, or Johne's disease, in animals. Diagnosis of MAP infection is challenging because of the pathogen's fastidious in vitro growth requirements and low-level intermittent shedding in feces during the preclinical phase of the infection. Detection of these "low-shedders" is important for effective control of paratuberculosis as these animals serve as sources of infection for susceptible calves. Magnetic separation technology, used in combination with culture or molecular methods for the isolation and detection of pathogenic bacteria, enhances the analytical sensitivity and specificity of detection methods. The aim of the present study was to evaluate peptide-mediated magnetic separation (PMS) capture technology coupled with IS*900* PCR using the Roche real-time PCR system (PMS–PCR), in comparison with fecal culture using BACTEC-MGIT 960 system, for detection of MAP in bovine fecal samples. Among the 351 fecal samples 74.9% (263/351) were PMS–PCR positive while only 12.3% (43/351) were MGIT culture-positive ($p = 0.0001$). All 43 MGIT culture-positive samples were also positive by PMS–PCR. Mean PMS–PCR crossing-point (Cp) values for the 13 fecal samples with the highest number of MAP, based on time to detection, (26.3) were significantly lower than for the 17 fecal samples with <100 MAP per 2 g feces (30.06) ($p < 0.05$). PMS–PCR technology provided results in a shorter time and yielded a higher number of positive results than MGIT culture. Earlier and faster detection of animals shedding MAP by PMS–PCR should significantly strengthen control efforts for MAP-infected cattle herds by helping to limit infection transmission at earlier stages of the infection.

1. Introduction

Mycobacterium avium subsp. *paratuberculosis* (MAP) is one of the most fastidious members of the *Mycobacterium* genus. It is the causal agent of Johne's disease (also known as paratuberculosis), an untreatable disease characterized by granulomatous enteritis, diarrhea, loss of body weight and death. Occurring worldwide, this infectious disease primarily affects domestic and wild ruminants, being responsible for significant economic losses to livestock production worldwide (Sweeney, 2011; Lombard, 2011). MAP also may impact public health, since the organism has been consistently found in people with Crohn's disease, suggesting that this agent is zoonotic (Chiodini et al., 2012).

MAP infections are largely subclinical and characterized by a prolonged incubation period, roughly 5 years in

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dairy cattle. Diagnosis of MAP infection is challenging because of the pathogen's fastidious in vitro growth requirements and low-level intermittent shedding in feces during the preclinical phase of the infection. Detection of these "low-shedders" is important for effective control of paratuberculosis as these animals serve as sources of infection for susceptible calves (Khare et al., 2004).

Although PCR-based diagnostics are considered very sensitive, in practice the presence of PCR-inhibitors in clinical samples such as bovine feces forces PCR procedures to start with relatively small aliquots of feces and to dilute samples prior to DNA extraction. Thus, because of the low number of MAP in clinical samples and the impediments posed by PCR inhibitors, it is vital to find ways to selectively concentrate MAP from bovine fecal samples before performing nucleic amplification assays to detect this pathogen.

Magnetic separation (MS) has become a routine method in food and veterinary microbiology laboratories and is commonly used in combination with culture or molecular methods for the isolation and detection of pathogenic bacteria, enhancing the analytical sensitivity and specificity of detection methods (Grant et al., 1998, 2000; Foddai et al., 2010; Stewart et al., 2013). Foddai et al. (2010) reported a novel peptide-mediated magnetic separation technique (PMS) for capture of MAP from milk consisting of a 50:50 mixture of MyOne Tosylactivated Dynabeads coated with biotinylated MAP-specific peptides aMp3 or aMptD. They demonstrated that this technology provided a more rapid and sensitive method of detecting and enumerating viable MAP organisms in milk. Using alternative ligands, this group recently extended their application of MS-based detection methods to *Mycobacterium bovis* (Stewart et al., 2013). The aim of the present study was to evaluate PMS capture technology when coupled with IS*900* PCR (PMS–PCR) in comparison with fecal culture using the MGIT system for detection of MAP in bovine fecal samples.

2. Materials and methods

2.1. Study population and sample collection

The study was conducted in cattle herds in the De Los Ríos region of southern Chile between January and February 2013. Herd sizes ranged from 150 to 820 adult cattle: 10 herds of Holstein-Friesian dairy cattle and one herd of Herefords. The livestock management for most herds was extensive, characterized by cattle grazing throughout the year, with only a few herds where cattle were housed during the winter months. A targeted sampling strategy was used to maximize the likelihood of testing MAP-infected cattle by selecting herds with a history of clinical paratuberculosis. A total of 351 fecal samples were collected from adult cattle in the 11 study herds. Fecal samples (5–10 g) were collected from rectum using palpation sleeves. Samples were refrigerated until processing in the Biochemistry and Microbiology Department, Faculty of Sciences, Universidad Austral de Chile, Valdivia.

2.2. MAP culture

Feces (2 g) was processed and inoculated into MGIT ParaTB Medium™ (Becton Dickinson, Sparks, MD, USA) containing supplement and antibiotics according to the manufacturer's protocol. Each MGIT ParaTB™ medium tube contained 7 ml of modified Middlebrook 7H9 broth base with mycobactin J, 500 μL of egg yolk suspension (Becton Dickinson, Sparks, MD) and 100 μL of VAN cocktail (vancomycin, nalidixic acid, and amphotericin, Sigma–Aldrich) and a fluorescent oxygen indicator embedded in silicon at the bottom of the tube. The final concentrations of antibiotics were: 10 μg/mL vancomycin, 40 μg/mL amphotericin B, and 60 μg/mL nalidixic acid. All MGIT ParaTB Medium™ tubes were incubated at 37 °C for 49 days in a BACTEC-MGIT 960 instrument (Becton Dickinson, Sparks, MD, USA). Tubes not signaling positive by that time were considered negative for MAP and no further testing was done. Signal-positive cultures were subjected to DNA extraction and IS*900* PCR as previously described for MAP confirmation (Salgado et al., 2013). Great care, including dedicated facilities and equipment, was taken to avoid cross contamination of samples and all assays included positive and negative controls. All signal-positive tubes were verified as having MAP by IS*900* PCR. The time to detection (TTD) for all IS*900* PCR-confirmed cultures, reported by the MGIT 960 instrument, was converted to an estimated number of inoculated MAP by a standard curve created by measuring TTD values for serial dilutions of single cells suspensions of MAP (Fig. 1) (Salgado et al., 2013).

2.3. PMS–PCR for MAP detection

MAP was selectively concentrated from 2 g fecal samples using PMS methods. Briefly, paramagnetic beads (Tosylactivated Dynabeads, Invitrogen Life Technologies, Grand Island, NY) were coated with either one of two

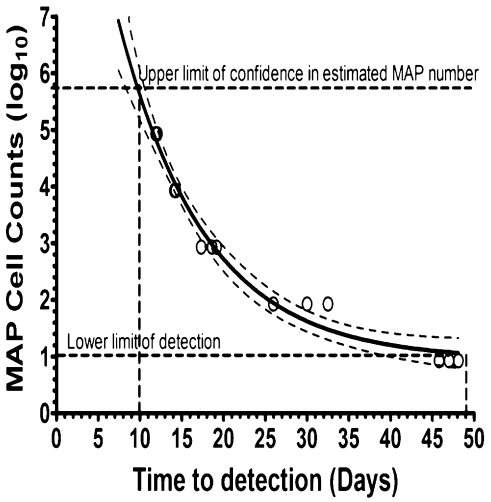

Fig. 1. Standard curve for conversion of MGIT time to detection (TTD) to numbers of MAP inoculated into the tube. Dashed line represents 95% confidence interval.