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Analytical verification of a multiplex PCR for identification of *Bordetella bronchiseptica* and *Pasteurella multocida* from swine

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Abstract

Bordetella bronchiseptica and *Pasteurella multocida* are etiologic agents of progressive atrophic rhinitis (PAR) and bronchopneumonia in swine. Only dermonecrotic toxin-producing strains of *P. multocida* play a role in atrophic rhinitis while both toxigenic and nontoxigenic strains have been associated with pneumonia. Monitoring and investigation of outbreaks involving these bacteria require sensitive and accurate identification and reliable determination of the toxigenic status of *P. multocida* isolates. In the present study, we report the development, optimization, and performance characteristics of a multiplex PCR assay for simultaneous amplification of up to three different targets, one common to all *P. multocida* strains, one found only in toxigenic *P. multocida* strains, and one common to *B. bronchiseptica* strains. Based on analysis of 94 *P. multocida* isolates (31 toxigenic) and 126 *B. bronchiseptica* isolates assay sensitivity is 100% for all amplicons. Evaluation of 22 isolates of other bacterial genera and species commonly found in the swine respiratory tract demonstrated a specificity of 100% for all gene targets. The limit of detection for simultaneous amplification of all targets is 1–10 pg of DNA per target, corresponding to a few hundred genomes or less. Amplicon mobility in agarose gels and sequence analysis indicate the amplicons are highly stable. The data presented establish this multiplex PCR as a reliable method for identification of *B. bronchiseptica* and both toxigenic and nontoxigenic *P. multocida* that may greatly simplify investigations of swine PAR and bronchopneumonia.
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Keywords: *Bordetella bronchiseptica*; *Pasteurella multocida*; PCR; Atrophic rhinitis

1. Introduction

Bordetella bronchiseptica and *Pasteurella multocida* are etiologic agents of pneumonia and progressive atrophic rhinitis (PAR) in swine (de Jong, 2006). Monitoring and investigation of outbreaks requires sensitive and accurate identification of these bacteria.

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Determination of the toxigenic status of *P. multocida* is of importance for diagnosis of PAR, since only strains that produce a dermonecrotic toxin, PMT, play a role in that disease (de Jong, 2006).

Detection of *B. bronchiseptica* and toxigenic/nontoxigenic *P. multocida* is typically based on isolation and biochemical testing of suspect colonies cultured from swabs or biopsies, an approach which is time-consuming, cumbersome, and suffers from poor sensitivity. Probe-based methods, although highly specific and sensitive (Kamps et al., 1990a; Register et al., 1995, 1998), are not readily incorporated into the workload of a diagnostic laboratory. In contrast, PCR is increasingly being implemented in diagnostic settings and often offers superior performance. Numerous investigators have described PCRs for detection of toxigenic *P. multocida* from swine (Hunt et al., 2000; Donnio et al., 1999) or for detection of all strains without regard to toxigenic status (Hunt et al., 2000; Miflin and Blackall, 2001; Liu et al., 2004). One *B. bronchiseptica*-specific PCR has been described (Hozbor et al., 1999), but it was not evaluated for veterinary applications. Additional analysis is required before this assay can be recommended for use in investigations of swine atrophic rhinitis and bronchopneumonia. A single reliable method for identification of *B. bronchiseptica* and both toxigenic and nontoxigenic *P. multocida* would greatly simplify investigations of swine PAR and bronchopneumonia. The goals of the present study were to design and optimize a multiplex PCR for this purpose and to establish its performance characteristics.

2. Materials and methods

2.1. Bacterial isolates

P. multocida strains 4533 (toxigenic) and 1059 (nontoxigenic) were used as controls (Rimler and Brogden, 1986). Ninety-four additional *P. multocida* isolates from pigs (Table 1) were obtained from the National Animal Disease Center collection. These strains originated from diverse geographic locations and over a broad span of time (approximately 8% between 1958 and 1979, 48% during the 1980s, and 44% between 1998 and 2002). Identification was based on standard biochemical testing and colony morphology. The capsular type of 62 of the isolates was previously determined for other studies, using either a passive hemagglutination test and type-specific sera (Rimler and Brogden, 1986) or a multiplex PCR typing assay (Townsend et al., 2001; G. Nordholm, unpublished data); 29% are type A and 71% are type D. A colony-blot assay using a PMT-specific monoclonal antibody was used to classify *P. multocida* strains as either toxigenic or nontoxigenic (Magyar and Rimler, 1991). Isolates were grown at 37 °C for 24 h on Dextrose Starch Agar.

B. bronchiseptica strain KM22 (Brockmeier et al., 2000) was used as a control. One hundred twenty-six additional *B. bronchiseptica* swine isolates, from a variety of locations worldwide, were evaluated (Table 1). All were originally identified based on culture characteristics and standard biochemical testing. Those originating within the United States were from the National Animal Disease Center

Table 1
Bacterial isolates included in this study

Isolate	Geographic origin	*n*
B. bronchiseptica	United States, Canada	62
	Continental Europe	47
	Ireland, United Kingdom	15
	Australia	2
P. multocida		
Nontoxigenic	United States, Canada	53
	South America	2
	United Kingdom	1
	Norway	3
	Sweden	2
	Malaysia	2
Toxigenic	United States, Canada	18
	Norway	7
	Sweden	5
	Singapore	1
Mycoplasma hyopneumoniae	United States	6
Mycoplasma hyorhinis	United States	4
Mycoplasma flocculare	United States	4
Haemophilus parasuis	United States	3
Actinobacillus suis	United States	1
Salmonella cholerasuis	United States	1
Arcanobacterium pyogenes	United States	1
Actinobacillus pleuropnuemoniae	United States	1
Streptococcus suis	United States	1

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