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Short communication

Strain differentiating real-time PCR for *Mycoplasma gallisepticum* live vaccine evaluation studies

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Abstract

Mycoplasma gallisepticum causes respiratory disease and production losses in poultry. Vaccination of poultry with *M. gallisepticum* live vaccines is an approach to reduce susceptibility to infection and to prevent the economic losses. The development and evaluation of live vaccines usually requires the involvement of several vaccine and challenge strains in the same experimental setup. Our goal was to develop a tool to allow the differentiation between a set of known *M. gallisepticum* strains in a quantitative manner. We developed 5 real-time PCR assays that absolutely differentiated between one of the five commercial and laboratory vaccine strains: F, ts-11, 6/85, K5831, K5054, and the challenge strain R_{low} when tested on *in vitro* cultures. The assay K5831 vs. R_{low} was also tested on specimens from live birds that were vaccinated with K5831 and challenged with R_{low}, and successfully differentiated between the vaccine and the challenge strains in a quantitative manner. This preliminary *in vivo* application of the method also shed light on possible protection mechanisms for the *M. gallisepticum* K5831 vaccine strain.

Keywords: *Mycoplasma gallisepticum*; Live MG vaccine; MG strain; Real-time PCR; Dual-labeled probe

1. Introduction

Mycoplasma gallisepticum (MG) is an infectious respiratory pathogen of chickens and turkeys. It is the most pathogenic and economically significant mycoplasma pathogen of poultry. Economic losses from condemnation or downgrading of carcasses, reduced feed and egg production efficiency, and increased medication costs are factors that make this one of the costliest disease problems confronting

Abbreviations: AFLP, amplified fragment length polymorphism; CCU, color changing units; C_T, threshold cycle; HI, hemagglutination inhibition; HSD, honestly significant difference; MG, *Mycoplasma gallisepticum*; PCR, polymerase chain reaction; PBS, phosphate buffer saline; SPA, serum plate agglutination; RAPD, random amplified polymorphic DNA.

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commercial poultry production worldwide (Ley, 2003).

Prevention and control programs based on strict biosecurity, surveillance (serology, culture, and molecular identification), and eradication of infected breeder flocks are preferable. Nevertheless, the rapid expansion of poultry production in restricted geographical areas and the consequent recurring MG outbreaks necessitated the implementation of additional measurements.

Vaccination with bacterins has been shown to reduce, but not eliminate, colonization by MG following challenge. Generally, it is felt that bacterins are of minimal value in long-term control of infection on commercial layer multiple-age production sites (Ley, 2003). Live MG vaccines appear to be more effective, and therefore more popular, than bacterins (Kleven, 1997; Whithear, 1996). An important characteristic of MG live vaccines is their ability to increase resistance to wild-type strain infection, and to displace wild-type strains with the vaccine strain on multiple-age production sites (Levisohn and Kleven, 2000; Turner and Kleven, 1998). Currently there are 3 commercially licensed vaccines, containing living cultures of either F (Adler et al., 1960), 6/85 (Evans and Hafez, 1992) or ts-11 (Whithear et al., 1990) strains of MG.

Live vaccine development and evaluation require studies that involve two or more MG strains in the same experimental setup. Protection study formats can include only one vaccine strain and one challenge strain (Papazisi et al., 2002), or a few vaccine strains in different experimental groups challenged by the same virulent strain (Ferguson et al., 2004). Displacement studies, to evaluate the capability of vaccine strains to displace a virulent strain, utilized several vaccine strains and a challenge strain (Kleven et al., 1998). The study of the immune mechanisms by which the MG vaccines confer protection from challenge also requires the involvement of at least two strains (Javed et al., 2005). In all the different MG live vaccine evaluation study formats, the involved strains could not be well differentiated and analyzed separately from one another once they were introduced into the experimental system. This lack of ability to differentiate between the participating strains limits the level of control and the amount of information that could be gained from MG vaccine evaluation studies. Our objectives were to develop a research tool to allow the qualitative and quantitative differentiation between MG strains utilized in vaccine evaluation studies and to improve the reliability and efficiency of these studies.

Real-time PCR is a convenient one step procedure and has an inherent quantitative nature. A real-time PCR with a dual-labeled probe (Taqman) appears advantageous for microorganisms strain differentiation due to the superior sensitivity and improved specificity endowed by 3 hybridizing oligonucleotides (two primers and a probe). In this study, we present the concept of quantitative strain differentiating real-time PCR for the differentiation between MG strains in live vaccine evaluation studies. The developed assays could strictly differentiate between *in vitro* cultures of the vaccine strains and the R_{low} challenge strain. The implementation of this novel tool in an *in vivo* MG vaccine protection study revealed an interesting insight into the quantitative relationships between the vaccine and the challenge strains in the chicken trachea and to a putative protection mechanism induced by the vaccine strain.

2. Materials and methods

2.1. MG strains and target gene sequences

Five MG live vaccine strains and one challenge strain were used in this study. The three MG commercial vaccine strains: F (Schering-Plough, Summit NJ), ts-11 (Merial Select, Gainesville, GA) and 6/85 (Intervet America, Millsboro, DE) were used directly from sealed containers supplied by the manufacturer. The MG K5831 isolate from our laboratory depository was isolated in 2005 from chickens challenged with MG K2101. K2101 was isolated in 1984 from a commercial layer flock with drops in egg production. This isolate was used as a live autogenous vaccine on the same farm with satisfactory results (Dr. S.H. Kleven, unpublished). The MG K5054 isolate from our laboratory depository was obtained from sinus exudate of commercial turkey breeders in 2001 (Ferguson et al., 2003). Further characterization of this isolate demonstrated its prophylactic properties (Ferguson et al., 2004). The MG R_{low} strain from our laboratory depository served as the challenge strain. R_{low} strain is a virulent MG strain, which has been previously described