Genetic studies on the immune response in some experimental animals to DNA vaccine derived from the virulent *Pasteurella multocida*

By

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B.Sc. Agric. Sci.(Poultry production), Fac. Agric., Fayoum Univ.,2007

M.Sc. Agric. Sci. (Genetics), Fayoum University, 2014

A thesis submitted in partial fulfillment

Of

The requirement for the degree of

DOCTOR OF PHILOSOPHY

In

Agricultural Sciences

(Genetics)

Department of Genetics

Faculty of Agriculture

FAYOUM UNIVERSITY

2018

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ABSTRACT

Pasteurella multocida is an agent for many diseases of livestock. The protective effect achieved through orthodox vaccines is not ideal. The research on novel vaccines against avian *Pasteurella multocida* is imperative. In this study, ten strains of *P. multocida* isolated from chicken, cattle, buffalo and sheep had a clinical manifestation of pneumonia were identified by species specific PCR (PM-PCR) and 460 bp products were obtained. Capsular typing of *P. multocida* is useful for epidemiological evidence and has been assessed by conventional and genotyping assays. According to the results of acriflavine test, only one out of the ten strains (10%) which isolated from chicken was detected as capsular type D. The result of capsular typing by using hyaluronidase test did not detect the capsular type A for all the ten strains (0%). Moreover, nine out of the strains (90%) were unidentifiable by using the conventional typing methods. PCR was applied to confirm the capsular typing using specific primers for each type. The findings of this study showed that a uniform amplicon size corresponding to 660 bp, 850 bp and 510bp indicating that it is belong to capsular type D (one strain which isolated from chicken), Type F (one strain which isolated from cattle) and type E (eight strains which isolated from sheep, cattle, buffalo and chicken), respectively. Likewise, the data of multiplex PCR showed that one strain (10%) was detected as capsular type D and one strain (10%) was detected as capsular type F and eight out of ten bacterial strains (80%) were classified into capsular type E. Thus, Multiplex PCR for molecular typing of the capsular types of *P. multocida* can be used as a simple, sensitive, rapid, reliable technique instead of the conventional techniques for identification of the capsular types of *P. multocida.*

OmpH gene, encoding a major outer membrane protein was isolated from the ten strains and molecular typing of the PCR product

of *OmpH* was assayed using restriction fragment length polymorpahism (RFLP) technique. RFLP analysis of the 1.2 kbp*OmpH*-amplification using Mps*I* and Bgl*II*endonucleases produced different patterns for the ten strains. The PCR-RFLP of the *OmpH* gene was found to be potentially a useful method for *P. multocida* identification and therefore, for studying the epidemiology of *P. multocida* infections. The gene encoding outer membrane porin H (*OmpH*) was cloned into the expression vector pUCP24 (Novagen, Germany) and the recombinant plasmid, namely DNA vaccine (pUCP24-*OmpH*) was obtained. Four groups of rats (n=10 per group) were intraperitoneally injected with the recombinant plasmid, inactivated vaccine, control vector pUCP24 and PBS, respectively. The immune responses and protective efficacy were evaluated after immunization by serological and challenge.

A significant increase in serum antibody levels was observed in rats vaccinated with the DNA vaccine and inactivated vaccine. Furthermore, the DNA vaccine provided partial protection to the vaccinated rats; however, the protective efficacy was greater to that provided by the attenuated live vaccine.

Keywords: *Pasteurella multocida*; Capsular typing; RFLP-PCR; pUCP24-*OmpH*; DNA vaccine; Immune response and protective efficacy.