CHAPTER I

INTRODUCTION

Pasteurella multocida, the gram negative bacteria is the cause of major disease in domestic animals and avian species (1). The strains of capsular type B or E are the cause of hemorrhagic septicemia in cattle, characterized by an acute systemic disease with high morbidity and mortality. A similar disease in avian species including ducks, chickens and turkeys is known as fowl cholera (2). This acute systemic disease can result in quick death for the individual and rapid spread of the disease throughout the flock. Most avian isolates of *P. multocida* belong to capsular type A (3). Prevention and control of disease depend on good sanitation, management factors, therapeutic and vaccination. Outbreaks are usually associated with stress, and the rate of exposure and risk of infection are increased. Although a small percentage of animals have naturally acquired immunity to infection, it is necessary in most animals to induce protective immunity by vaccination. Since Pasteur (1880) developed the first fowl cholera vaccine, many other live attenuated or inactivated vaccine and bacterins products have been produced in an effort to control disease (2).

Various preparations of killed vaccine (bacterin) have been used including phenol-killed vaccine (4), oil-adjuvant (5,6), tissue-propagated bacterins (7) and turkey embryonated egg bacterins (8). Vaccination of poultry species with these

bacterins often resulted in protection against homologous serotype challenge (9) and ineffective immunity in the field, eventhough it demonstrated satisfactory results under controlled condition (10,11,12).

Many attempts in production of a live attenuated vaccine that consisted of nonpathogenic bacteria were performed and resulted in broad range protection. One of the live avirulent vaccines widely used in United States of America was the Clemson University (CU) strain (13), which had been effective in preventing fowl cholera in turkey (9,14,15,16). Several live avirulent vaccines (17,18,19), live avirulent temperature sensitive mutant strains (20,21,22) and live avirulent streptomycin dependent mutant strains (23,24,25) have been developed and found to be protective vaccines against infections. The live vaccine has advantage over the bacterin in its simplicity of application and cross protection against the infection of different serotype strains (13,26), but its serious disadvantage is that the vaccination sometimes results in systemic infection (27) and high mortality.

A reasonable approach to develop an improved vaccine against fowl cholera was to determine the immunogenic antigens of *P. multocida* (28). Antigenic substance responsible for protective immunity in fowl cholera have been investigated and several antigens with protective immunogenicity have been obtained by various extraction methods (29-40).

Antigen extract by potassium thiocyanate (KSCN) was found to be immunogenic against heterologous challenge in chicken (29), and against homologous challenge in cattle (30), mice (31) and rabbits (32,33). Capsular extract from 2.5%

sodium chloride solution provided 80-100% protection in turkey (34). Recently, outer membrane protein (OMP) has been studied as potential immunogen. Vaccination with OMP protected turkeys (35) and rabbits (36) against homologous challenge. More specifically a monoclonal antibody (MAb) against 37.5 kDa of OMP protected both mice and rabbits against challenge (37). Purified LPS was antigenic, it seem to be a major immunogen in birds (38). In addition, Tsuji and Matsumoto 1988 (39) suggested that a LPS protein complex was essential for induction of immunity against infection in turkeys. Recently, Wejiwardana 1990 (40) found that a bactericidal MAb against LPS completely protected mice against homologous challenge.

The objectives of this research :

1. Comparison of the antigenic profiles of potassium thiocyanate(KSCN) antigen extract, capsule, outer membrane protein (OMP) and lipopolysaccharide (LPS) of *P. multocida* mutant strains and parental strain by SDS-PAGE and Western blot.

2. Determination of the immunity and antibody titer of rabbit immune sera against various types of *P. multocida* antigens.