CHAPTER I

INTRODUCTION

Leptospirosis, an infectious disease commonly seen in tropical areas, is caused by a spirochaete of the Genus *Leptospira* species *interrogans*. The incidence of the disease increases during the period between the end of the rainy season and early winter, owing to the high rainfall and stagnation. Water is then easily contaminated by the urine from infected animals, after which it flows into the rivers, canals, and contaminates animal feedstuffs on farms. Humans may become an accidental host, as a result of exposure to these contaminated environments. In the past, people in occupations associated with water or sewage were particularly at risk from leptospirosis as they often worked in rat infested conditions or in water polluted with leptospira-infected urine. The major occupational risks today are among farm workers (1) and the people who walk in contaminated stagnant water.

There are about 12 serovars of infectious leptospires reported in Thailand. According to the report of Bangkok Leptospirosis Centre, Faculty of Tropical Medicine, Mahidol University in 1978-1985, the predominant infecting serovars were *L. interrogans* serovar bataviae in Bangkok, and serovars autumnalis, bataviae, icterohaemorrhagiae and wolffi in rural areas respectively (personal communication).

Clinical manifestations vary from mild to severe (2). These includes fever, myalgia, muscle tenderness, headache, injected conjunctiva, hepatomegaly, renal failure and jaundice, etc. In Thailand, human leptospirosis has first been reported in 1943 by Yunibandha (3).

Leptospirosis may remain undiagnosed since the symptoms are relatively non-specific. It may be confused with other infectious disease such as malaria, typhoid fever, viral hepatitis and haemorrhagic fever, especially in the early stages of infections (4). Therefore, laboratory tests are frequently needed in order to confirm the diagnosis. This may be established either by bacteriological cultivation (5) or by demonstration of a rise in specific antibody titer. Since culturing requires a long incubation time (about 2-4 weeks), the immunological tests for leptospiral antibody are most important. The microscopic agglutination (MA) test (6) is the most widely employed as the standard reference test because of its high specificity and sensitivity. However, it requires multiple serovars of live leptospira, and hence the maintenance of a large number of stock cultures to provide antigens.

Many sensitive, rapid and easily performed immunological tests have been developed for the determination of specific leptospiral antibody They are the macroscopic agglutination test (slide test) (7, 8), complement fixation (CF) test (9, 10), haemolysin (HL) test (11), indirect immunofluorescent (IF) test (12), indirect haemagglutination (IHA) test (13), enzyme-linked

immunosorbent assay (ELISA) (14), IgM specific dot ELISA (15). Although the specific leptospiral antibody was used for diagnosis, it was not sensitive enough in early infection, as the antibody response can be detected only after 7-10 days of leptospiremia (5, 16). However, the antibody response may be negative in fatal cases (17).

In 1982, Adler *et al* (18) compared the sensitivity of several immunological technique for detecting *L. interrogans* serovar hardjo. The best sensitivity test was obtained by ELISA and radioimmunoassay, which used 10^5 and 10^4 to 10^5 leptospires per ml respectively. However, the clinical specimen was not tested to detect leptospiral antigen and only one serovar was used. In a pilot study, we have studied the detection of leptospiral antigen by double sandwich ELISA technique using a rabbit anti-*L. interrogans* serovar bataviae as a probe. It was found that $5x10^6$ cells per ml of homologous sonic extracted *L.interrogans* serovar bataviae antigen could be detected, while $1x10^8$ cells per ml of heterologous antigen of *L. biflexa* serovar patoc and *L. interrogans* serovar autumnalis could not. The possible reason may be that there was competition of whole cell antigen in raising the specific antibody. This data showed that a rabbit anti-wholecell antibody against serovar bataviae was only useful for detection of homologous antigen. Furthermore, antigen could not be detected in patient's sera by this ELISA technique, in spite of demonstrating leptospires by culture in sera taken on the same day and the convalescent sera shown to be infected with serovar.bataviae.

Recently, examination of antigen or antibody characteristics has been facilitated by the introduction of the immunoblotting technique by Towbin *et al*. (19). The immunoblotting takes advantage of the high resolution achieved using SDS-PAGE (20, 21) and identifying the immobilized proteins on nitrocellulose membranes by immunochemical reaction. Many investigators have used this technique for studying on the antigenic components or immune response of various organisms such as *Treponema* (22), *Legionella* (23), *Campyrobacter* (24) and *Leptospira* (25, 26, 27, 28, 29).

Thus immunoblotting techniques are important tools for analysis of antigenicity and immune responses to many organisms. We decided to study the difference of antigen in both non-pathogenic and pathogenic leptospira which are responsible for leptospirosis in Thailand by this immunoblotting technique. So we looked for a common specific pathogenic leptospiral antigen which was useful for the characterization of pathogenic leptospira, and for the preparation of specific antibody or monoclonal antibody and using this antigen as a probe to detect speicfic leptospiral antibody.