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Detection of Antibodies Against Melioidosis from Animal Sera in Thailand by Indirect Haemagglutination Test

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ABSTRACT

Antibody detection against melioidosis from 8,153 serum samples of cattle, sheep, goats, pigs and deers of 18 provinces in Thailand was done by using indirect haemagglutination test during the year 2005-2006 (6,576 goat sera , 1,050 pig sera, 78 cattle sera , 83 deer sera and 366 sheep sera). Serum antibodies titer at ≥ 1:160 against melioidosis were found in goats (0.33%), pigs (7.23%), cattle (2.56%), deers (7.23%) and sheep (6.83%), however, more information is needed for the conclusion of this study.

Key words: antibody detection, indirect haemagglutination test, melioidosis, animal sera, Thailand

INTRODUCTION

Melioidosis is an infectious disease of humans and animals caused by *Burkholderia pseudomallei*. It was first described under the name *Bacillus pseudomallei* by Whitmore and Kishnaswami (1912) following its isolation in Rangoon of Myanmar. Subsequent reports described the causative agent under a variety of names, including *Pseudomonas pseudomallei*. Stanton and Fletcher (1921) introduced the term "melioidosis" which was derived from the Greek name, and described a condition resembling glanders. Not only migration and transportation of animals around the world but also tourisms increase the risk for melioidosis to leave its endemic boundary and establish itself elsewhere.

B. pseudomallei is a motile, gram-negative rod, oxidase positive bacteria which grows readily on routine culture media under aerobic condition. However, *B. pseudomallei* can also multiply under anaerobic conditions in the presense of nitrate or arginine (Yabuuchi *et al*., 1992). It also survives in the absence of nutrients in distilled water for several years (Wuthiekanun *et al*., 1995a).

Melioidosis occurs in tropical countries between latitudes 20° N and 20°S, predominantly in Southeast Asia and Northern Australia (Ketterer *et al*., 1975; Thomas *et al*.,1981; Ketterer *et al*., 1986) China (Dance, 2000), Thailand (Srikitjakarn *et al*., 2002) Singapore, (Yap *et al*., 1995) and Brazil (Miralles *et al*., 2004). *B. pseudomallei* is a natural saprophyte that can be isolate from soil and muddy water in endemic areas (Nachiangmai *et al*., 1985).

The number of registered cases of infection increases with rainfall, e.g. during rainy season in the tropics when it is more likely that animals and humans come into contact with muddy water and soil particles carrying bacteria from deeper layers to the surface (Currie and Jacups, 2003).

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Infection in humans and animals is thought to occur by inoculation, ingestion or inhalation of environmental pathogens (Leelarasamee and Bovornkitti,1989). Animal-to-man transmission has rarely been documented but can result in fatalities (Yap *et al*.,1995; Mollaret, 1998; Idris *et al*., 1998; Choy *et al*., 2000).

B. pseudomallei infection is often found in pigs, goats and sheep. It occurs less in cattle, horses, dogs, rodents, birds, dolphins, tropical fishes and primates. Hamsters, guinea pigs and rabbits can also be infected by *B. pseudomallei* in the laboratory.

Incubation period of melioidosis can vary from days to months or years. However, asymptomatics with presense of abscess may occur in goats, sheep and pigs.

B. pseudomallei infection results in suppurative or caseous lesions in lymph nodes or other organs. There are varieties of clinical signs including fever, anorexia and lymphadenopathy. In pigs, it is often involved with submandibular lymph nodes in pigs. In sheep and goats, lung abscesses and pneumonia are commonly found.

Nowaday culture method, remains the gold standard and the most reliable diagnostic method for *B. pseudomallei* infection. It generally takes 4-7 days for bacterial identification. The specimen from animals for cultivation are blood, nasal swab, wound exudates, pus or tissues. Molecular techniques sush as, DNA hybridization and genetic engineering play an important role in diagnosis of this diseases. The use of PCR as daily routine diagnostic method is, however, performed well in environmental survey (Brook *et al*., 1997; Kao *et al*., 2003). PCR method is an important method in epidemiological study of the disease. For immunological method, the most common approach is antibody detection. It is simple and requires minimal laboratory equipments. A number of serological test has been developed such as bacterial agglutination, indirect haemagglutination test (IHAT), complement fixation test (CFT), Enzyme Link Immuno Serological Assay (ELISA) and immunofluorescence antibody test (IFA) (Sirisinha, 1991). However, sensitivity and specificity of these serological tests are questionable in endemic areas, as healthy individuals may show persistent IgG levels. In non-endemic areas, the tests might be useful for detection of chronic infection (O' Brien *et al*., 2004). In veterinary diagnosis, serology has always been used for detection *B. pseudomallei* of anti-*Burkholderia* antibodies in horses, goats and dairy cattle. (Thomas *et al*., 1988).

Serological study of melioidosis in livestock is aimed at providing information that would contribuite to future control of melioidosis in livestock.

MATERIALS AND METHODS

Eight thousands one hundred and fifty-three sera samples from 78 cattle, 6,576 goats, 366 sheep, 1,050 pigs and 83 deers were collected during 2005-2006 from 18 provinces in Thailand (Si Sa Ket, Kanchanaburi, Lop Buri, Ratchaburi, Chiang Rai, Nakhon Ratchasrima, Bangkok, Chachoengsao, Chonburi, Rayong, Saraburi, Nakhon Sawan, Satun, Sing Buri, Ranong, Nakorn Si Thammarat, Phuket and Songkhla). The sera were kept at 4°C for further serological diagnosis by IHAT.

Indirect haemagglutination test

The absorbed serum by serums were incubate 56°C 30 min add 3% sRBc glutaraldehyde and centrifuge at 1,500 rpm 15 min. Serums were serially diluted two-fold at 0.05 ml volume in microtiter plate starting from 1: 10 using 0.15 M PBS containing 0.5% BSA and 0.1% NaN_3 as diluent. To the test wells starting from 1:10 serum dilution (well No.1-9), 50 µl of Melioidin antigen sensitized sRbc was added in each well and the last two wells 50 µl of 0.5% suspension of unsensitized sRbc in diluent were

added, but one of these wells was added with the tested serum at the dilution of 1:5 as a control. The haemagglutination results at room temperature read 2 hours later. Positive and negative controls were routinely included. An IHA titer of ≥ 1:160 was considered evidence of exposure to *B. psuedomallei*. It was based on IHA titers studied by Mekaprateep *et al*., (1998).

RESULTS

Indirect haemagglutination test (IHAT) from cattle, sheep, goats, pigs and deers in Thailand 8,153 sera (goats 6,576 sera, pigs 1,050 sera, cattle 78 sera, deers 83 sera and sheep 366 sera). The result of detection antibody against melioidosis occur in goats 0.33%**,** pigs 7.23%**,** cattle 2.56%, deers 7.23% and sheep 6.83%**.** (Table 1)

DISCUSSION

Melioidosis is considered as an emerging disease with high impact on animals and humans. In the past century, the disease spreaded from east Asia to many parts of the world. Today it can be imported to regions with inappropriate climate if a subclinical carrier capable of contaminating its surroundings survives. Sporadic acute cases may also be found in animals imported from enzootic areas. The variability of the clinical course in different animal species may lead to delayed diagnosis in non-endemic area, where the veterinarians are not familiar with melioidosis. As a result of chronic character of the disease, the possibility of animal-to-man transmission, the natural resistance of *B. pseudomallei* to many antibiotics and the tendency to become endemic, the culling of infected animals is recommended. Detection of the agent is a major challenge, as the agent has to be handled in laboratories of biosafety level 3 and test kits are not yet commercially available. Veterinarians and medical doctors should be aware of melioidosis (and glanders) not only as an agent of public interest but also in terms of a bioterrorist attack. It is noteworthy to mention their statement on the status on the control of melioidosis is still valid. No international standard for medical microbiology in diagnostics of melioidosis exists which is the drawback in reliable proceeding. No evaluated test kit neither based on the detection of species specific DNA sequences is commercially available. Efforts have to be made for closing this gap in the future.

Goats and dairy cows (Thomas *et al*., 1988; Li *et al*., 1994; Srikitjakarn *et al*., 2002) were studied by evaluating IHAT, CFT and microtiter agglutination. Thomas *et al*., (1988) demonstrated that screening by the IHAT of Alexander and confirmation by using the complement fixation test (CFT) test is sensitive and specific in caprine melioidosis (Thomas et *al*., 1988). It is noteworthy that antibodies had developed within a week after inoculation with a high infective dose, but only after a period of up to 16 days when small amounts of bacteria were

Table 1 Detection of antibody against melioidosis from 8,153 animal sera from 18 provinces of Thailand during the year 2005-2006.

Examined animals	Examined of sera	Number positive (%)
Cattle	78	2 (2.56%)
Goats	6,576	22 (0.33%)
Sheep	366	25 (6.83%)
Pigs	1,050	76 (7.23%)
Deers	83	6 (7.23%)
Total	8,153	131 (1.60%)

injected. To date, none of newly developed tests has been or is commercially available and no positive and negative control sera of the various animal species which could be infected with *B. pseudomallei* are available. Serological test is still a technique restricted to a limited number of laboratory experts worldwide. In view of international transport of animals from endemic to non-endemic areas, the development of a sensitive and specific serological test is unavoidable in order to prevent the spread of *B. pseudomallei* infection in the future.

As a result, pigs and sheep were found to be the highest incidence of melioidosis. For eradication control program, valuable methods need to be used. The present test for detection of antibody against melioidosis is IHAT, which is only screening test. Thus, confirmation of the test should be considered.

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