Chapter 1

Introduction

Rabies is one of the commonest causes of central nervous system (CNS) infection in human in the topic, where dogs are inadequately immunized. In Thailand,300 human cases were reported per annum, whereas more than 100,000 required postexposure prophylaxis (1).

Rabies is caused by a neurotropic single - stranded RNA virus of bullet - shaped structure. The core is a helix of nucleoprotein which is surrounded by lipid envelope thus rending them easy to be disrupted by treatment with organic solvents or surface active agents (2).

Virus replicates in the cytoplasm of neuron as well as other non-neuron cells in the infected animals. Initially, rabies virus multiplies in muscle cells at the site of inoculation and then spreads via the axoplasm of the peripheral nerve to the CNS. Dissemination rapidly occurs throughout the spinal cord and brain, then centrifugally spread via autonomic nerves to the peripheral organs which include salivary glands, the most important one for further transmission of the disease. Rabies is noncytopathic virus. Disease signs in humans and animals are cause by disrupted homeostatic mechanism resulting from immunopathologic as well as viral multiplication processes and neurotransmitter dysfunction.

Rabies is usually transmitted by the introduction of virus-laden saliva into a bite or scratch wound inflicted by a rabid animal. However, transmission can occur via other nonbite routes. There is no specific treatment for rabies once signs of illness develops but intensive supportive care might prolong course of illness. Treatment is effective only when the virus is still at the bite area (3,4). A recommended prophylactic regimen in all persons exposed to rabid animals is a combination of local wound treatment, vaccination and antiserum in seriously exposed cases (5,6). Major problem in initiating postexposure prophylaxis in dog rabies endemic area is due to the fact that rabid dogs may excrete virus in the saliva long before disease signs develop (7). The appearance of the biting animals thus is not a reliable indicator. Further, dog carrier with intermittent excretion of rabies virus into saliva exists (8). However, the exact number of these carriers is still not known in Thailand. At present, canine that have bitten people are, if caught, quarantined and observed for abnormal behavior. If an illness compatible with rabies develop, they will then be sacrified and brain will be removed for examination of rabies antigen. Following this protocol, at least 2 human cases at Queen Saovabha Memorial Institute (QSMI),Bangkok, were reported to have virus during 1987 - 1988 due to delay in treatment. Postmortem diagnosis of rabies is usually made by the followings :-

- Detection of Negri body by Sellers' stain (SS).
- Detection of rabies antigen by fluorescent antibody test (FAT).
- Virus isolation by mouse inoculation (MIT).

The limited sensitivity of Negri bodies by Sellers' stain and the inherent delay in animal inoculation gave FAT to become the preferred diagnostic technique. Since 1967 FAT has been recommended by WHO as the definite diagnostic test for rabies. FAT can usually be done within a few hours with a sensitivity of approximately 98% on brain impression smear (9). The remaining of 2% were dose in which the virus titers was low (below a level of approximately $10^{2.5}$ mouse intracerebral LD50/0.03 ml) and no virus could be found in the salivary gland. This finding indicates that rabies virus has to multiply to a critical level in the CNS before centrifugal spread to salivary gland occurrs. However, during the past 2 years (1986-1987) at the rabies diagnostic unit of QSMI,19 from 2253 and 11 from 2191 of animal brain specimens examined FAT, which yield negative result from six areas of the brain each, were reported to contain virus on MIT (10). Therefore, a new rapid and specific test of high sensitivity that helps establishing the diagnosis in observed animals as well as specimens obtained from postmortem examination is needed.

The purpose of this study is to develope a dot - immunoblot technique for detection of rabies antigen in the salivary gland suspension as well as in the saliva of the suspected animal either postmortemly or during the observation period. The method is modified from the dot - immunoblot described by Lakeman(11). Since rabies is transmitted by the bites of an animal harboring virus in the saliva, the presence of rabies antigen in the saliva and/or salivary gland suspensions would indicated the need for

postexposure prophylaxis if the bite is significant. Further, time that virus can be excreted in the saliva before disease signs develop (in dog) is also investigated.