

THE DIAGNOSIS OF CANINE RABIES INFECTION USING THE  
"ANTIBODY CAPTURE" SOLID PHASE ELISA METHOD  
(ACELISA)

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OBJECTIVES :

1. To determine the onset of detectable IgM in the CSF and sera of dogs with acute rabies infection.
2. To determine whether the rabies virus is shed into the CSF sera during an acute phase of illness.
3. To develop a simple, rapid, reliable technique for the diagnosis of canine rabies infection that does not require long quarantine or killing of the dog for testing.
4. To compare the ACELISA technique with other established methods for the diagnosis of acute rabies infection.

BACKGROUND : Rabies is endemic in Thailand. In a recent report of a ten year period, the yearly range was 237-322 cases in man and 781-3286 cases in dog (1). With such a high incidence of disease, a reliable, rapid method of early diagnosis of infection is important. Many laboratory techniques have been developed for the diagnosis of rabies. The Seller's stain for Negri bodies, the Fluorescent Antibody technique (2), the radio immunoassay (3) and detection of IgM after vaccination by immunoperoxidase method have all been used for the diagnosis of rabies infection in man and animals. Each of the tests have required either considerable time or sophisticated equipment to obtain the result. The detection of low level IgM in human serum has been reported at the 3rd and 4th day post exposure to rabies antigen (5).

The antibody capture solid phase enzyme linked immunosorbent assay (ACELISA) has as one of its unique properties gross specific immunoglobulins functionally concentrated onto the solid from the liquid so that very low concentration can be detected (6). We propose to use this method to detect the acute rabies infection in the dog by measuring the level of IgM in CSF as well as in serum. This method may provide a means for early diagnosis of the canine rabies infection.

RESULTS : Paired serum and CSF samples were collected from 38 dogs quarantined for rabies observation at the Thai Red Cross Society. Mouse inoculation (MI) and fluorescent antibody (FA) testing was done on each dog that died (Table 1). The CSF was tested by mouse inoculation and FA and found to be positive in 8/22 dogs that had rabies confirmed by either the FA or MI methods. No dogs with negative FA and MI tests had detectable virus in the CSF (Table 2). Table 3 shows that for comparison of IgM titers the dogs tentatively can be divided into two groups based upon FA and MI test results. Dogs that died in quarantine and were MI and FA positive were considered rabid. Dogs with negative MI and FA results were considered non rabid although 5 of 16 died in quarantine. One died of canine distemper; the cause of death in the other 4 dogs was not determined. All serum and CSF will be tested for rabies IgM antibody titer first by RIA to determine if significant illness in IgM levels can be detected in rabid and non rabid dogs. If significant differences in non-rabid and rabid dogs can be detected by RIA, samples will then be tested using an ELISA method to measure IgM.

#### REFERENCES :

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5. Burke, D.S., Nisalak, A. : Detection of Japanese encephalitis virus IgM Antibodies in serum by Antibody Capture radioimmunoassay. (In press) 1981.

Table 1. Rabies diagnosis in quarantined dogs by mouse inoculation and fluorescent antibody tests.

	Mouse Inoculation	Fluorescent Antibody	Total
Positive	22	22 <sup>a</sup>	22
Negative	16	16	16
Total	38	38	38

<sup>a</sup> Four were negative on first test but positive when retested after a positive MI test result.

Table 2. Rabies virus in CSF of dogs quarantined for rabies.

	<u>Fluorescent antibody and Mouse inoculation</u>		
	Positive	Negative	Total
Rabies virus in CSF	8	0	8
Rabies virus not in CSF	14	16	30
Total	22	16	38

Table 3. Mortality in dogs quarantined for rabies observations.

	<u>Fluorescent antibody and Mouse inoculation</u>		
	Positive	Negative	Total
Survived	0	11	11
Died	22(2.4) <sup>a</sup>	5(3.4) <sup>a</sup>	27
Total	22	16	38

<sup>a</sup> Mean days of survival in quarantine.