

DETECTION OF RABIES IMMUNOGLOBULIN IN SERUM AND
CEREBROSPINAL FLUID OF QUARANTINED DOGS BY
ANTIBODY CAPTURE IMMUNOASSAY

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OBJECTIVES :

1. 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.
2. To determine whether the rabies virus is shed into the CSF sera during an acute phase of illness.

BACKGROUND : Rabies is endemic in Thailand. In a recent report of cases over a 10 year period, yearly ranges were 237-322 cases in man and 871-3286 cases in dogs. With so many annual canine cases and human exposures it is important to have an accurate reliable method for early diagnosis of canine rabies. Recently an immunoassay technique has been developed which is highly sensitive for detection of specific IgM antibodies in CSF during human viral infections of the Central Nervous system. This technique, called the "antibody capture" solid phase radioimmunoassay has the important feature that at IgM concentrations greater than 1 mg/ml, the counts per minute (CPM) bound to the solid phase are essentially independent of the absolute antibody concentration and reflect instead the proportion of IgM molecules with specific anti-viral antigen antibody.

At present, the definitive diagnosis can be made only after the dog has died. The purpose of this study was to determine the feasibility of using the antibody capture radioimmunoassay (RIA) method for detecting rabies virus IgM in serum and CSF of dogs that were in quarantine for rabies observation.

MATERIALS & METHODS : Serum and cerebrospinal fluid (CSF) samples were obtained from 37 dogs quarantined for rabies. Brain from each dead dog and CSF specimens were tested for rabies virus by mouse inoculation. Mouse deaths were confirmed by FA. Dogs were classified as rabid when the mouse inoculation was positive. Dogs that died with a clinical rabies illness but a negative mouse inoculation test were classified as unknown. All surviving dogs and a dog that exhibited a canine distemper like illness and had a negative mouse inoculation test result were classified as non-rabid. The technique for the radioimmunoassay has been previously described (2) was modified to test serum

and CSF specimens.

RESULTS : Paired serum and CSF samples from thirty-seven dogs were tested in the rabies MACRIA assay (Table 1). After entering quarantine 25 dogs died within one to five days. Twenty-four of those dogs had a rabies like illness, and in 21 dogs rabies virus infection was confirmed by mouse inoculation. Twelve of 13 dogs survived the quarantine and were released. One died of canine distemper after 2 days of quarantine. Significantly higher rabies MACRIA cpm were detected in the serum from rabid compared to nonrabid dogs. A highly significant difference was also detected between the CSF counts of rabid and non rabid dogs. Three dogs died with clinical signs of rabies but the brains of these animals were negative for rabies by mouse inoculation. Their CSF and serum IgM cpm were significantly higher than cpm from the nonrabid dogs. Serum IgG cpm were significantly higher than cpm from the nonrabid dogs.

Samples of CSF from 6 of 21 rabid dogs contained virus when tested by mouse inoculation. No mice died following injection of CSF from the nonrabid dogs. Comparing the results of serum and CSF tests in rabid dogs, CSF counts were significantly higher than serum counts (mean difference = 66.6, $p < .04$). In normal dogs, serum counts were significantly higher than CSF counts (mean difference = 64; $p < .001$). These data are consistent with the hypothesis that anti-rabies antibody is being produced concentrated in the CSF of rabid dogs.

The correlation between serum and CSF cpm in all dogs is highly significant ($r = .78$, $p < .001$), confirming that the test is measuring related variables in both serum and CSF. Sucrose density gradient separation of rabid dog serum resulted in the major count peak in the early fractions where IgM would be expected to appear. When the CSF to serum cpm ratio is compared between known rabid and non rabid groups, the ratio to rabid dogs is 1.2 whereas in nonrabid dogs it is 0.71.

FUTURE OBJECTIVES : To determine the effect of rabies vaccination on serum and CSF IgM. To determine how early in experimental infection IgM can be detected in the CSF.

REFERENCES :

1. Singhaseni, A. : Current status of rabies problems in Thailand. Japan Med. Sci. Biol. 32:367-370, 1979.
2. Burke, D.S., Nisalak, A., Ussery, M.A. : Antibody capture immunoassay detection of Japanese encephalitis virus immunoglobulin M and G antibodies in cerebrospinal fluid. J. Clin. Microbiol. 16:1034-1042, 1982.

Table 1. Mean CPM in serum and CSF of rabies quarantined dogs.

| <u>Group</u> | <u>Source</u> | <u>Number</u> | <u>Mean CPM + SE</u> | |
|--------------|---------------|---------------|----------------------|-------|
| Rabid | Serum | 21 | 358 | 27** |
| Nonrabid | Serum | 13 | 221 | 13 |
| Unknown | Serum | 3 | 511 | 156** |
| Rabid | CSF | 21 | 425 | 38** |
| Nonrabid | CSF | 13 | 157 | 7 |
| Unknown | CSF | 3 | 630 | 221** |

* Dogs that died but were negative on mouse inoculation and FA.

** Mean significantly higher than nonrabid group, $p < .001$.