

Antemortem and Postmortem Diagnosis of Rabies in Dogs from Fluorescent Antibody
Stained Corneal Impression Smears

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PURPOSE: To evaluate the corneal test for the early antemortem diagnosis of rabies in dogs.

DESCRIPTION: Recent surveys conducted at the municipal dog pound in Bangkok indicate that 3 to 5 percent of these dogs are incubating rabies as determined after euthanasia by post mortem examination of the brains utilizing the fluorescent antibody test (FAT) and mouse inoculation (MI) (See previous annual reports). This dog population will be utilized to study the ability of the corneal test (1) to identify pre-clinical cases of rabies, and to determine whether there is a reliable correlation between a positive corneal test and infectivity of the saliva. These studies have potentially important implications in the management of animals in quarantine following biting incidents.

PROGRESS: A preliminary study has been conducted utilizing dog heads submitted to the rabies diagnostic laboratory. A total of 35 heads have been examined to date. In addition to the examination of the brain, corneal impressions were prepared by repeatedly pressing a clean fluoro-slide against the cornea. After air drying, the corneal impressions were stained at room temperature with fluoresceine isothionate conjugated anti-rabies globulin of equine origin (Baltimore Biological Laboratory) for 30 minutes, washed with phosphate buffered saline pH 7.6 for 10 minutes, mounted in phosphate buffered glycerine pH 8.5, and examined under the fluorescent microscope. Normal mouse brain (20 per cent suspension) was mixed with the conjugate a short time before staining, and rhodamine counterstain was added. Non-specific fluorescence was evaluated by the examination of impressions stained with conjugate mixed with 20 percent rabid mouse brain. In some cases, impressions were stored at -70°C for up to two weeks before staining with satisfactory results. The time-consuming acetone fixation step, required in processing brain impressions for the FAT, is unnecessary in processing corneal impressions. Corneal impression slides were labelled with coded numbers so the microscopist would be unaware of the results of the brain examination prior to reading the slides. Saliva (buccal washings), parotid salivary gland tissue, and the corneas were also obtained from each head and the homogenated tissues inoculated intracranially into weanling mice for attempted rabies virus isolation.

The results of this study are summarized in Table 1. Of the 35 brains examined, 12 were positive for rabies by FAT and MI. Virus was present in the corneas of 11 (91.7 percent) of the positive dogs as determined by MI. The corneal test was positive in only 5 of the 11 corneas known to contain virus. Retrospective analysis of the histories of the dogs in the study indicates that in most cases the corneas which were positive on MI and negative on FAT came from dogs that had died or had been sacrificed more than 24 hours before the laboratory examinations were performed (although adequate refrigeration was provided). Further studies of the possibility that the sensitivity of the corneal test deteriorates with time are being undertaken.

In no case was a corneal test declared positive when the cornea was obtained from any of the 23 dogs in which the brain was negative. This indicates the high degree of specificity of the corneal test in the hands of an experienced FAT diagnostician. The morphology of the fluorescent antibody stained inclusions in the cornea are quite similar in appearance to rabies inclusions in brain, except that the inclusions are quite scanty in many impressions, and the inclusions tend to be smaller in size, and more uniform.

Laboratory analyses of saliva and salivary gland specimens are incomplete at this time. These data will be included in a future report, along with data from additional dogs to be studied.

DISCUSSION: Preliminary post mortem studies indicate that rabies virus may be demonstrated in the corneal epithelium of most dogs which die of rabies or which are sacrificed in the advanced stages of the disease. The fluorescent antibody test ("Corneal Test") was considerably less sensitive than mouse inoculation in demonstrating this virus. It is suggested that post mortem decomposition accounts, at least in part, for this decreased sensitivity. Antemortem studies in dogs are being initiated.

Table 1 Post Mortem Identification of Rabies Virus in the Brains and Corneas of Rabid Dogs

Case No.	Cornea		Brain		Time of Examination ¹
	FAT ²	MI ²	FAT	MI	
65	+	+	+	+	1
79	-	+	+	+	1
92	+	+	+	+	1
104	+	+	+	+	1
111	-	+	+	+	1
114	+	+	+	+	1
132	+	+	+	+	1
68	-	+	+	+	2
80	-	+	+	+	2
108	-	-	+	+	2
109	-	+	+	+	2
115	-	+	+	+	2

¹ Number of days between death and the time of laboratory examination

² FAT = Fluorescent antibody test; MI = Mouse inoculation test

REFERENCES:

1. Schneider, L.G.: The Cornea Test; a new method for the intravital diagnosis of rabies. *Zentralbl. Vet. Med.* 16:24-31, 1969.