The Thai Journal of Veterinary Medicine

Volume 27
Issue 4 *December, 1997*

Article 1

12-1-1997

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Abstract

A review was carried out on 2,689 brains from animals that were found to be positive for rabies virus by a fluorescent antibody test (FAT) and mouse inoculation test (MIT). Samples were taken from the brain stem, cerebellum, cerebrum and hippocampus of each animal. FAT sensitivity was found to be 99.59, 99.29, 98.88 and 98.74 percent for each of the different sampling sites. It was concluded that the brain stem is the best site for routine rabies FAT diagnosis.

Key words: rabies, fluorescent antibody test, sensitivity

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Introduction

The Queen Saovabha Memorial Institute (QSMI) has served as the principal rabies postexposure treatment center for the Bangkok Metropolitan region and neighboring provinces since 1913. Laboratory diagnosis of animal and human specimens suspected of carrying the rabies virus have been carried out by fluorescent antibody microscopy (FAT) since 1981. Each sample found negative by FAT is routinely tested by mouse inoculation (MIT). The diagnosis of rabies by FAT or MIT has been reported to be 99.4% and 98.3% accurate, respectively (Lennette *et al.*, 1965). Although false negative FAT's are uncommon when performed in an experienced laboratory, rabies in humans is almost invariably fatal and physicians, in endemic regions, are reluctant to rely on a negative FAT alone, before starting postexposure treatment (Wilde *et al.*, 1991). The World Health Organization (WHO) recommended that bilateral impressions from the hippocampus (Ammon's horn) and the brain stem be used for increased sensitivity (WHO, 1992). Previous work at this institute found that increasing the number of samples from the hippocampus from 1 to 3 improved the sensitivity rate from 99.02% to 99.83% (Tepsumethanon *et al.*, unpublished).

The aim of this study was to examine the sensitivity of FAT on samples taken from the brain stem, cerebellum, cerebrum and hippocampus of mammals suspected of suffering from rabies.

Materials and methods

The number of brains examined at QSMI for rabies diagnosis was 5,768. eighty six % were dogs, 11% cats and 3% humans, domestic and wild mammals. Decomposed samples were not used. Four sites of brain tissue were examined: brain stem, cerebellum, cerebrum and hippocampus. Impressions were obtained bilaterally (two on each slide) from each site, yielding a total of 4 slides with 2 impressions each for microscopy from each brain submitted.

Commercial conjugate* was used. The method for preparation and staining followed the instructions on the package insert. Microscopy was performed by one of the authors (VT, BL, CM) using a fluorescent microscope** and 400 X magnification. Approximately 5 minutes were spent in a dark room reading each individual slide.

All negative and doubtful cases were tested by mouse inoculation as recommended by WHO (Koprowski *et al.*, 1996).

Result

Of the total 5,768 brains tested, 3,079 were negative by FAT and MIT, 2,683 were positive by FAT, 6 were negative by FAT and positive by MIT (Table I). There was complete correlation between the FAT positive results from all 4 sites in 2,648 brains (98.70%). Incomplete correlation was noted in 35 of the brains studied (1.30%) (Table I). The highest FAT sensitivity was seen in samples taken from the brain stem (99.59%) and the lowest in those from the hippocampus (98.74%) (Table II). When we combined the FAT results from all the 4 sample sites examined, there were 6 false negative cases among FAT negative brains that were later identified as positive by the MIT (99.78% sensitivity) (Table II).

Discussion

Our study showed that when we sampled the brain stem, cerebellum, cerebrum and hippocampus by FAT, there were few false negative diagnoses of rabies (Table II). Whether these results justify the discontinuation of the expensive and time consuming MIT has to be decided individually by each center, on the basis of their "in house" experience. Rabies is a fatal disease and medical staff making management decisions are reluctant to accept anything

* BBL Anti-Rabies Globulin, Fluorescein Labeled.

** Olympus Model BH-2

other than near 100% sensitivity of the tests used in the decision-making process. The clinical staff at our animal bite facility were not willing to accept the very few false negative FAT results (6 among 5,768 brains examined) and insisted on the MIT, while commencing postexposure treatment on all subjects in WHO categories II and III (WHO, 1992) who were suspected of rabies exposure, untill a negative MIT became available 4 weeks later. Approximately 1 percent of the patients presented to our clinic during this study accordingly received expensive postexposure rabies vaccination and immune globulin therapy, that later was found not to have been necessary.

Table I. Results of rabies diagnosis from 5,768 brains.

Negative by FAT and MIT	3,079	brains
Positive by FAT	2,683	brains
a) Complete correlation from all 4 sample sites	2,648	brains
b) Incomplete correlation from 4 sample sites	35	brains
Negative by FAT and positive by MIT	6	brains

Table II. False negative and sensitivity of FAT from brain stem, cerebellum, cerebrum and hippocampus in 2,689 rabid brains.

	False negative FAT (brains)	Sensitivity (%)
Brain stem	11	99.59
Cerebellum	19	99.29
Cerebrum	30	98.88
Hippocampus	34	98.74
Combined 4 sample sites	6	99.78

Acknowledgement

The authors thank the Thai Red Cross Society (under Royal Sponsorship) for technical assistance.

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ความไวของการตรวจวินิจฉัยโรคพิษสุนัขบ้า ด้วยวิธีฟลูออเรสเซนต์ แอนตี้บอดี้ จากสมองส่วน Brain stem, Cerebellum, Cerebrum และ Hippocampus

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บทคัดย่อ

ทำการศึกษาสมองสัตว์จำนวน 2,689 ตัวอย่างที่ตรวจพบเป็นโรคพิษสุนัขบ้า ด้วยวิธีฟลูออเรสเซนต์ แอนตี้บอดี้ และวิธีฉีดเข้าหนูทดลอง พบว่า ความไวของการตรวจวินิจฉัยโรคพิษสุนัขบ้าด้วยวิธีฟลูออเรสเซนต์ แอนตี้บอดี้จากสมองแต่ละส่วน คือ brain stem, cerebellum, cerebrum และ hippocampus เท่ากับ 99.59%, 99.29%, 98.88% และ 98.74% ตามลำดับ สรุปได้ว่า brain stem เป็นสมองส่วนที่ดีที่สุดที่ใช้ในการวินิจฉัยโรคพิษสุนัขบ้าด้วยวิธีฟลูออเรสเซนต์ แอนตี้บอดี้

คำสำคัญ : โรคพิษสุนัขบ้า, ฟลูออเรสเซนต์ แอนตี้บอดี้, ความไว

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