NEUROPATHOLOGY AND DIAGNOSIS OF RABIES IN DOMESTIC ANIMALS

By

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DECLARATION

I hereby declare that the thesis entitled **"NEUROPATHOLOGY AND DIAGNOSIS OF RABIES IN DOMESTIC ANIMALS"** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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CERTIFICATE

Certified that this thesis, entitled **"NEUROPATHOLOGY AND DIAGNOSIS OF RABIES IN DOMESTIC ANIMALS"** is a record of research work done independently by Sri.S.Silamban under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to him.

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To my brother
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Introduction

INTRODUCTION

Rabies is the most dreaded zoonotic disease. A disease of antiquity, rabies is endemic worldwide, except in a few isolated geographical areas. Each year approximately four million people in rabies endemic countries receive post exposure rabies treatment and over 30 000 people die after being bitten by rabid dogs. In India, more than 25 000 people die of rabies every year (WHO,1992).

Many species of domestic animals including dog, cat, cattle, sheep and goat can act as reservoirs and transmit the disease to human beings.

Almost all human deaths from rabies occur in the tropics, where about three-fourth of the world's population live. In over 80 countries, rabies is still prevalent in its most dangerous reservoir, the dog population. In more than 99 per cent of all human rabies cases, the virus is transmitted from dogs and over 90 per cent of people who receive rabies post-exposure treatment live in areas of canine rabies (WHO, 1992).

Because of the dangers inherent in the use of the standard anti-rabies vaccines and the cost involved, it is desirable to administer them only to persons who had a real exposure to the virus. Obviously, speedy and accurate diagnosis of rabies becomes essential.

In many underdeveloped countries, the principle means of rabies diagnosis is by clinical signs.However, the signs are varied, non-specific and difficult to differentiate from other frequently occurring diseases. Therefore in certain cases actually rabid dogs may be judged to be uninfected, resulting in danger to human beings. Hence, the diagnosis of rabies in the laboratory has an important role in meeting the threat of rabies; its verdict often decides whether or not to proceed with a long and painful course of treatment and to elaborate the measures for the control of rabies in epizootics.

Among various tests available for the diagnosis of rabies in the laboratory, tests based on the detection of Negribodies, biological testing by mouse inoculation (MI), fluorescent antibody technique (FA test) and immunoperoxidase test (IP test) are commonly utilized by many of the laboratories undertaking rabies diagnosis.

In developing countries like India, every laboratory cannot afford to be equipped with the costly sophisticated equipments. And so, there is a need for developing a rapid, reliable, economic and easy method for the diagnosis of rabies. In the light of above facts, a study was undertaken with the following objectives :

1. Survey of the prevalence of rabies in domestic animals in the state of Kerala for the last 12 year period from July 1983 to June 1995.

2.Elucidate the comparative neuropathology of the disease and evaluation of the efficacy of the common diagnostic techniques available at present.

3.Identify a simple, rapid method for the diagnosis of rabies.

Review of Literature

REVIEW OF LITERATURE

2.1. Epidemiology

2.1.1.Other Countries

Democritus was thought to have made the first recorded description of canine rabies 500 years B.C. Since then,rabies epidemic was reported from different countries of the World (Steele,1975).

During 1979, 140 cases of rabies in cats and 184 dogs were reported in USA (Diesch et al. 1982).

Presently, with the exception of Antar tica, Oceania,certain Islands such as Japan and the British Isles and few isolated geographical areas, such as Sweden, the disease is endemic in most parts of the World (King and Turner, 1993).

In Botswana, Zimbabwe 1242 confirmed cases of rabies was reported in the 10-year period between 1983 to 1992 ,with an increase from 68 in 1983 to 193 in 1992. Of this, 42.3 per cent were in cattle and 19.3 per cent in dogs (Mushi et al.1993).

In 1993, there were 9495 cases of rabies reported from USA, out of which 8889 cases were in wild and 606 in domestic animals.This was 9.9 percent higher than the reported rabies

in 1992 (8645). Interestingly, cats continued to be the domestic animal most frequently affected (Krebs et al.1994).

Between October and December 1994, 2785 cases were reported in Europe, including 2072 wild and 713 domestic animals. The total rabies cases reported in Europe in 1994 were 8819, excluding eight from rabies-free countries (Muller et al.1994).

Brochier et al.(1995) reported rabies in 43 foxes and 18 domestic animals during 1994,in Belgium.

From the rabies statistics for Malawi from 1986 to 1992, Edelsten(1995) reported an average of 186 confirmed cases of rabies every year and observed that 83 per cent of this occurred in dogs.

In 1994, from USA 8224 cases of rabies were reported in animals, and this included 93 per cent (7632) in wild and 7 per cent (592) in domestic species.Of this 592, 153 were dogs, 111 cattle, 267 cats and 61 others (Krebs et al. 1995).

Between 1980 and 1990, Orihuela and Solano (1995) reported an average of 3.3 human rabies cases annually in the state of Morelos, Mexico. Sixty per cent of this originated from the bites by domestic dogs under 3 months of age. From 1985 to 1990, 3173 animal rabies cases were recorded, out of which 75-95 per cent were canines.

Of the 2622 rabies cases reported in Europe during January to March 1995, 2076 were in wild animals and 546 in domestic animals(WHO,1995a).

WHO (1995b) reported 1432 rabies cases in Europe, for the third quarter of 1995, of which 1005 were in wild and 427 in domestic animals.

Dabrowska et al.(1996) reported 1678 rabies cases in Western Poland, including 50 dogs and 30 cattle, during the period between 1965 and 1994.

2.1.2. India.

SatyaPrakash(1970) estimated that over 15 000 deaths occurred due to hydrophobia and that over 3 million people underwent post-bite anti-rabic therapy every year.

Srinivasan et al.(1973) reported that 52.5 per cent of 394 dogs suspected for rabies were actually affected with the disease.

One hundred and fifteen cases of rabies were reported in farm animals in Madras during the period from 1973 to 1977. Of these, 91 were cattle or buffalo, 22 were goats and two were horses. Forty six per cent cases had dog bite history and 95 per cent took furious form (Parthasarathy et al.1978). The incidence of hydrophobia in man during the above period was 292 (Rao,1980),while incidence of rabies in dogs during the same period was 691(Parthasarathy,1981).

Narayan and Konar (1986) noted an upward swing in the prevalence of rabies every year in Ranchi and reported 2083 cases of post-exposure anti-rabic vaccinations during 1986.

Sekar et al.(1989) reported 771 cases of human rabies and 1701 cases of canine rabies in Madras city during a 11-year period between 1973 and 1983.

In Tamil Nadu, during 1984-1992, 2535 hydrophobia deaths in human beings were reported.Also,189 cattle,1082 dogs and 313 other animals were recorded to be rabid during the period (Basheer,1995).

Every year, substantial deaths were reported in human beings from all the States and Union Territories of India. During 1971-1981,the number of deaths per year was 561 in Maharastra, 326 in Tamil Nadu, 64 in Uttar Pradesh, 132 in West Bengal and 135 in Andhra Pradesh. In Kerala,about 22 deaths were reported in human beings every year due to rabies. During the period between 1971 and 1981, 220 deaths were reported in Kerala (Choudhuri,1995).

According to the Kerala state Disease Surveillance yearly Report, in the year 1985, 176 rabies death in animals was reported while in 1988 it was 146. During the period 1993-95, 665 animals were reported dead of rabies. It included 354 cattle, 18 buffaloes, 115 goats, 174 dogs and four other species.

2.2.Clinical signs

2.2.1. Dogs

Two types of syndromes were noted in dogs, dumb and furious form of rabies.

In the furious form, the predominant changes were excitation, unusual violence, frenzied behavior, tendency to bite and failure to obey its master. There was no deviation in appetite in the initial phase, but later it became depraved. There was drooling of saliva,change in barking tone,the mouth was wide open with dropped jaw and the tongue protruded with dropped down head. They showed inco-ordintion and muscle tremors. The dog developed dyspnoea, ascending paralysis, coma and died. The total episode lasted as long as 10 days (Appel and Carmichael,1979).

The dumb form was characterised by paralysis of the lower jaw, tongue, larynx and hind quarters. The dogs seeked solitude and appeared sluggish. They showed progressive weakness,staggered and fell. Late, they developed coma and died. The entire clinical course of the disease upto death took one to seven days (Beran,1962).

Srinivasan et al. (1973) observed the dropped jaw, excessive salivation and not responding to owner's call as common symptoms shown by 53.1 per cent of rabid dogs. These were followed by symptoms like fits, hoarse barking, biting

other dogs and animals in 25 per cent of cases. Symptoms like off feed, staggering gait and paraplegia were noticed in 18.8 per cent of cases.

Following the bite of an infected animal, signs of the disease in the dog usually became apparent within two to eight-weeks period. Incubation varied with the amount of virus transmitted, the site of inoculation and the type of wound involved. Accordingly, signs were noticed as early as 10 days after infection in the case of head wound. Conversely, it was even five months, with a sparingly infected leg wound. The course of the disease lasted for three to eight days and ended with complete paralysis and death (Bedford,1976).

In the rabid dogs, Hoon et al. (1995) observed anorexia, aggressive biting of other animals and biting of the soil, other objects and even their own tail.

2.2.2. Cats

Usually the furious form of rabies occurred in cats, but occasionally, increased affectionate disposition was also noted. The course of the disease was shorter than that for the dog, and death ensued some two to four days after the onset of the clinical signs. Initially, there was loss of appetite, but then it rapidly became vicious. Hiding from the light, continuous howling and attacking other animals and people were observed. Excessive salivation was a

relatively constant feature in the early stages, and later paralysis of the masseter muscles, pharynx and hindquarters followed by generalized paralysis and death was noted (Bedford,1976).

2.2.3. Cattle

The clinical signs in cattle varied. The early signs of infection were non-specific namely depression, poor appetite and reduction in milk yield. Furious form was characterised by restlessness, irritability, stamping of the feet and excessive salivation. They showed starring eyes and incessent bellowing. Trembling of pinna, grinding of the teeth, constant twitching of tail, constipation and straining, sexual excitement and tendency to head butt other animals and attendants were also noticed in rabid cattle. Death ensued within two to four days. The dumb form of the disease was characterised by excessive salivation, difficult swallowing and eventually, paralysis of the hindquarters, coma and death in about four to five days after the initial signs of infection(Bedford,1976).

Bellowing and excessive salivation were reported as the most common signs in field cases (Schneurrenberger et al. 1970).

Hoon et al. (1995) observed anorexia, severe salivation, bellowing and excitement in the rabid cattle.

2.2.4.Other species

The course of the disease in sheep, goats and other ruminants was similar to that in cattle. In the fox, badger, squirrel, rabbit and rat, manifestations of rabies were similar to those seen in the dog. There was characteristic loss of the natural fear usually shown towards man, and all these species had attacking tendency and bit human beings (Bedford,1976).

2.3.Gross Lesions

Perl (1975) reported that rabid animals showed little visible alterations in the central nervous system;congestion of the meningeal vessels being the only usual externally visible abnormality of the brain. A mild degree of oedema was occasionally present. Focal congestion of white matter, brainstem and spinal cord and hemorrhages in rare occasions were noted. Jubb et al. (1993a) described occasional severe hemorrhages in the spinal cords of rabid horses and cattle.

2.4.Laboratory diagnosis of rabies

2.4.1.Sellers' impression smear staining

Negri(1903) was the first to give complete description of the intracytoplasmic inclusion bodies found as an oval or oblong shaped,eosinophilic bodies of varying size,mainly being localized in the pyramidal cells of Ammon's horn, in

the Purkinje cells of the cerebellum and in the cells of the medulla and various ganglia in street rabies virus infection.

Williams(1908) used saturated alcoholic solutions of basic fuchsin and methylene blue for staining impression smears.

The diagnostic value of Negribodies was shown by Negri himself: he examined 98 clinically rabid dogs and found inclusions in all the brains except one (Babes,1912).

Sellers (1927) was the first to use mixture of one per cent alcoholic solutions of methylene blue and basic fuchsin in the proportion of 2:1 for the demonstration of Negribodies in the impression smears.

Goldwasser and Kissling (1958) reported that Negribodies in the brain smears or sections could not be demonstrated in approximately 10 per cent of naturally infected animals and that sometimes it rose to 26 per cent.

McQueen et al.(1960) reported the percentage of heads with Negribodies in confirmed cases of rabies as 93.3.

Carski et al.(1962) reported 36 of 40 rabid specimens (experimentally infected foxes and skunks) to be positive by brain impression smear examination.

Tustin and Smit(1962) reported the percentage of heads with Negribodies in confirmed cases as 66 per cent.

Sellers' method, Tanamal's method, Frothingham's method and William's modification of Vangieson's method were used to stain the impression smears by Seshadri and Chandrasekaran (1963). The Sellers' staining was the least time consuming method yielding uniformly satisfactory results. They also noted that other methods used for staining were with slight modifications, either in the choice of quantity of the dyes used or in th pH of the staining solution.

Subrahmanyam and Pathak (1971) identified Negribodies in 15 cases by Sellers' staining, out of 34 rabies positive samples. They also identified Negribodies in 16 mouse brains of 23 biologically inoculated positive samples.

Tierkel(1973) described Sellers' technique as the method which combined speed, accuracy and economy that could be employed in the laboratory diagnosis of rabies and recommended it as the most suitable stain that could be followed universally.

Derakhshan et al.(1978) detected 48 (20.6%) rabies positive cases by Sellers' impression staining.

Dinakaran(1980) demonstrated Negribodies in 12 of the 22 positive rabies bandicoots by Sellers' staining method.

Of 886 dogs examined between 1972 and 1976,77.49 per cent of cases were found positive by the presence of Negribodies in the hippocampal impression smears stained by Sellers' (Parthasarathy,1981).

Brain impression smears stained by Sellers' detected Negribodies in 76 per cent of 1400 specimens examined by Anjaria and Jhala (1985).

Kotwal and Narayan (1987) showed Negribodies by Sellers' staining technique in all the five samples examined.

Sahasrabuddhe and Sherikar(1990) reported 53 positive cases of rabies by Sellers' staining,out of 92 suspected brains examined and reported that 57.60 per cent of specimens were diagnosed by the Sellers' method.

Sureau et al. (1991) found that Negribodies were more numerous and larger when the incubation period and the clinical phase of the disease were longer;the size and number varied according to the virus strain.

2.4.2. Histopathological studies

2.4.2.1.Central Nervous System

Shortt(1935) introduced the use of iron haematoxylin for the morphological studies of Negribodies.

Gradwohl (1943) used Mann's eosine methyl blue for staining Negribodies.

Seshadri and Chandrasekaran (1963) stained histological sections of rabies positive brain using H & E, Mann's methyl eosin, method of Andral & Gentle, Schleifstein's rapid method, Lillie's modification of Stovall & Black and

Zlotnick's selective method. Mann's methyl blue eosin method was found classical for staining Negribodies. Lillie's modification of Stovall & Black's method was best for formalin fixed materials.

Lepine (1973) described histopathological diagnosis of rabies. The changes in rabies consisted of signs of meningo-encephalomyelitis, perivascular cuffing, parenchymatous infiltration, formation of encephalitic nodules (Babe's nodules), ganglion infiltration with Satellitosis and neuronophagia and the presence of rabies specific Negribodies in the different types of neurons.

Murphy et al. (1973) reported that the evidence of meningo-encephalitis was noticed only in terminal case of infection, as mild to moderate accumulation of mononuclear cells in the meninges and perivascular spaces. Inflammatory cells infiltrated into the surrounding brain with the perineuronal clustering in the places where perivascular accumulations were concentrated.

Gross and histological changes in tissues of rabies affected animals were not pathognomonic.Predominantly, a non-suppurative polioencephalomyelitis with perivascular lymphocytic infiltration was noticed. Varying degrees of neuronal degeneration and glial proliferation around damaged neuron (Babe's nodules) were also noticed (Buxton and Fraser,1977).

H & E and Mann's staining of the formalin-fixed rabid materials (Hippocampus and/or Cerebellum) showed clear evidence of encephalitis in 20.7 per cent of cases and the presence of intracytoplasmic Negribodies in 10.3 per cent of the specimens (Derakhshan et al. 1978).

Non-suppurative meningo-encephalitis,characterised by vascular cuffing with lymphocytes,plasma cells and a few neutrophils, mild focal gliosis and infrequent neuronal degeneration were noticed in experimentally infected skunks (Charlton and Casey,1979 and Charlton et al. 1987).

Dinakaran (1980) detected 10 cases of rabies out of 22 positive rabies cases in bandicoots by H & E staining method.

Nayak et al.(1982) reported that 40.9 per cent positive cases (90 of 220 rabid brains) were histologically positive for rabies with neuronal degenerations, glial reactions and perivascular cuffing and the presence of Negribodies.

Gonzalez and Stephano (1984) observed non-suppurative meningo-encephalitis in 35 out of 40 rabies cases, with intracytoplasmic inclusion bodies in 34.

Palmer et al. (1985) noticed encephalitis in 26 of 34 naturally infected rabid wild and domestic animals. Eight animals had no encephalitis lesions and two among this showed no Negribodies. They also noticed that 21 out of 26 encephalitis brain showed Negribodies atleast in one brain area examined by H & E staining.

Kotwal and Narayan (1987) demonstrated Negribodies with H & E staining in all the eight samples examined.

Meningitis to some degree, perivascular cuffs containing lymphocytes, plasma cells, macrophages and occasionally erythrocytes and number of plasma cells that increased with the duration of clinical signs were observed by Charlton (1988).

Of 187 brains from wild and domestic animals examined, Zimmer (1988) observed histological lesions of rabies in 53.

Zimmer et al. (1990) reported histopathological alterations comprising of Negribodies, inflammatory and degenerative lesions in 53 per cent of the rabies positive brains examined.

Hamir and Rupprecht (1990) and Hamir et al. (1992b) observed no histological lesions of encephalitis in a raccoon diagnosed positive by FA test.

Histopathological studies detected only 67.5 per cent of 45 rabies positive cases confirmed by FA test (Burnes et al. 1991)

Sureau et al.(1991) described H & E as the best stain for screening Negribodies in sections, followed by Mann's stain.They described rabies encephalomyelitis in detail.They found that the lesions varied with the strain of the virus, animal species, and the duration of the disease.Meningo-

encephalitic hyperemia was always prominent, but mononuclear infiltration was rarely severe, more often moderate, and sometimes absent. There were perivascular cuffs consisting of lymphocytes, with occasional participation of polymorphonuclear cells. The lymphocytic infiltration was usually moderate, and occurred in foci in the brain and meninges. The characteristic Babes nodules, consisting of glial cells, were frequently associated with these perivascular infiltrates.

Lesions of non-suppurative encephalomyelitis with marked perivascular cuffing and Negribodies were noticed in only two of the four rabies positive horses by Hamir et al. (1992a). Extensive congestion, non-suppurative encephalomyelitis with mild to moderate perivascular mononuclear cuffing, focal gliosis and Negribodies were noticed in H & E sections of rabies positive horses (Hamir et al. 1992a) and raccoons (Hamir et al. 1992b).

Inflammatory changes in rabies were usually present, but, notably, they were very mild or absent. When present, were typical of non-suppurative encephalomyelitis (Jubb et al. 1993a).

Hamir and Moser (1994) observed diffuse mononuclear perivascular cuffing and multifocal gliosis in all rabies positive cases. Negribodies were detected in 10 out of 17 cases.

Foley and Zachary (1995) observed marked perivascular and meningeal lymphocytic meningo-encephalitis and locally extensive spongiform changes of the grey matter of a rabies positive heifer. Negribodies were not detected in sections. Perivascular space dilatation was noticed.

Hamir et al. (1995b) noticed perivascular cuffing,multifocal gliosis and the presence of Negribodies within the neuronal cytoplasm in the brain of rabies positive animals.

2.4.2.2.Salivary gland

Presence of rabies virus in the saliva was detected by various authors (Nair et al. 1978 ; Cortes et al. 1979 and 1987 ; Jayakumar and Ramadass,1991).

Only limited reports are available on the histological alterations of the salivary glands in rabies infection.

Sialadenitis due to the infection of salivary glands by the virus was reported by Ninomiya,1955 ; Sikes,1962 ; Soman, 1963 and Samol,1976.

Degenerative changes of the acinal epithelial cells along with the infiltration of plasmacytes and lymphocytes were noticed by Dierks et al.(1969).

On histological examination of the parotid salivary glands of 55 rabies positive animals, Nayak et al.(1982)

found sialadenitis in 50 animals. Moderate to marked focal or diffuse mononuclear cell infiltration in the interlobular and interalveolar spaces, sometimes even replacing the glandular parenchyma was noticed. Perivascular cuffing of lymphocytes in the interalveolar spaces and the presence of Negribodies in the ganglion cells was also observed in some cases.

Hamir et al. (1992a) noticed no lesions in the acinar tissue of the salivary gland in rabies positive horses or any inflammatory or degenerative changes suggestive of viral infection in rabies positive raccoons (Hamir et al. 1992b).

In mammals, rabies virus has affinity for the salivary glands and they replicated in the acinar cells. This leads to focal lysis of acinar cells, mononuclear cell infiltration and the presence of the Negribodies in the ganglionic neurons (Jubb et al. 1993b).

2.4.2.3.Trigeminal Nerve

Frothingham (1920) studied sections of trigeminal ganglion(Gasserian) from 1100 rabies affected animals and stated that the examination of the Gasserian ganglion was a valuable adjunct in the diagnosis of rabies.They reported that the lesions were sufficiently characteristic to permit a presumptive diagnosis of rabies when Negribodies were not found in the brain. It was confirmed by Lapi et al. (1952). They noted scattered proliferations of the capsule cells, mild infiltrations of lymphocytes and plasma cells.

Charlton and Casey (1979) observed few chromatolytic neurons and mild perivascular and interstitial accumulations of mononuclear cells in the trigeminal and dorsal root ganglia of experimentally infected skunk. They also found that the severity of accumulation of inflammatory cells varied and generally correlated with the degree of neuronal degeneration which was extensive in terminal stages of the disease.

A striking proliferation of the capsular cells around infected cranial nerves and spinal ganglia, called lesions of Van Gehucht were described by Sureau et al.(1991). They reported that significant lesions were found in the dogs but less intense in rabbits and human beings.

Hamir et al. (1992a) noticed non-suppurative ganglio-neuritis in Trigeminal (Gasserian) ganglion in rabies positive horses.

Jubb et al. (1993a) described inflammatory changes in the trigeminal ganglion in rabies, being present without any inflammatory or neuronal changes in the brain.

2.4.3.Fluorescent Antibody Test (FA test)

Coons and Kaplan (1950) were the first to use Fluorescent microscopy for detecting reactions between antigen and antibody. Goldwasser and Kissling (1958) subsequently adopted it for the rabies virus.Later FA test

was adopted for the use as a diagnostic procedure for rabies (Goldwasser et al. 1959; Etchebarne et al. 1960; McQueen et al. 1960; Roger,1960).

Carski et al.(1962) on examining the specimens of experimentally infected fox and skunk brains by mouse inoculation (MI), microscopic examination for Negribodies and FA test, found that FA test and MI were the most sensitive diagnostic tests. They also showed 41 animals to have rabies virus or antigen in their salivary gland out of 46 experimentally infected foxes and skunks examined by FA test.

On examination of 989 specimens, Stone et al.(1962) found FA test as a comparable method to MI and that both had more accuracy than histological examination.

Immunofluorescence was demonstrated in 128 of 153 infected brains by FA test (Schneider,1964) and it was considered superior to conventional routine methods for its accuracy, comparative rapidity and simplicity.

Beauregard et al. (1965) employed Sellers' staining, FA test and MI for the examination of a large series of 750 domestic and wild animals submitted to their laboratory for the diagnosis of rabies; 175 specimens were diagnosed as positive. Of these, only 58 (33 per cent) were detected by conventional staining of impression smears; two animals

infected with rabies were found to be negative by MI and four by the FA test. The last two methods showed almost equal sensitivity and both were far more reliable than the conventional impression smears stained for Negribodies.

Wachendorfer (1966) reported 100 per cent correlation between MI and FA on 320 fresh specimens, but in the same study five of 34 rabid brains with advanced decomposition were negative by FA test.

A review by Jentzsch (1967) comparing the sensitivities of FA test and MI showed very high degree of correlation. Only 30 of 1537 (2 per cent) specimens , positive by MI, were negative in FA test.

Roslyakov et al.(1970) described immunofluorescence procedure as highly specific and more easier than MI procedure.

Subrahmanyam and Pathak (1971) demonstrated rabies specific immunofluorescence in 23 samples of 37 positive cases examined and found coincidence with the results of the biological test.

Dean and Abelseth (1973) perfected FA test for the detection of rabies antigen to such an extent that it was the quickest, most reliable method available and alternate to MI test for the routine diagnostic purposes.

Kaplan (1973) described direct FA test as superior to all other tests for speed and accuracy combined, which allowed diagnosis to be made within 2-4 h period.

Of 912 specimens of brain examined, Atanasiu et al. (1974) found 62 cases to be positive by FA test as against 60 by immunoperoxidase test (IP test).

Kissling (1975) reported that FA test was 98 per cent positive in acetone-fixed fresh brain tissues and found only 23 of 1468 MI-positive specimens to be negative by FA test.

Derakhshan et al. (1978) detected 104 cases by FA test out of 108 rabies positive specimens.

When the relative efficacy of the immunofluorescence and immunoperoxidase tests were tested on 500 samples, Genovese and Andral (1978) found 1.2 per cent higher accuracy with FA test than IP test. FA test detected 223 out of 230 positives detected by MI test, as against 217 detected by IP test.

Examination of 30 samples each of saliva and brain from rabid dogs revealed perfect correlation between FA test and MI (Cortes et al. 1979).

Dinakaran (1980) detected 18 cases of rabies in bandicoots by FA test.

Ramkumar and Mehrotra (1983) detected four FA positive rabies cases, which showed no Negribodies in Sellers' staining.

An evaluation of applied FA procedure by U.S.Public Health Services' 1983 with 129 participating laboratories revealed a combined efficacy of 86 per cent, when strongly positive and weakly positive slides were used along with the positive and negative controls. (Griffin,1984).

Anjaria and Jhala (1985) detected 92.5 per cent of 1400 rabies suspected specimens to be positive by direct FA test.

When 32 specimens including eight from field were examined by FA test, 31 showed rabies specific immunofluorescence (Kotwal and Narayan,1985a).

Emmons (1986) were of the opinion that the accuracy of the FA test largely depended upon the experience of the individual reading the slides. The most difficult diagnostic specimens were those with a very small amount of antigen.

The submaxillary and parotid salivary glands of 30 dogs naturally infected with rabies were examined by the direct FA test on tissue smears of the salivary glands. It was found that submaxillary salivary glands were 100 per cent positive, whereas in one case the material from the parotid glands showed negative in FA test (Cortes et al. 1987).

Kotwal and Narayan (1987) showed rabies specific immunofluorescence by direct FA test in all the 11 specimens examined.

Of 187 brains from wild and domestic animals examined, Zimmer (1988) noticed rabies specific immunofluorescence in 98.

Bourhy et al. (1989) showed that FA test was comparable with the recent techniques for the diagnosis of rabies viz. Rabies Tissue Culture Infection Test (RTCIT) and Rapid Rabies Enzyme Immuno Diagnosis (RREID).

Narayan and Kotwal (1989) demonstrated rabies immunofluorescence in all the 12 positive samples examined by FA test.

Sahasrabuddhe and Sherikar (1990) reported that the maximum number of rabies-positive specimens (60 of 92 samples) were detected by FA test. These included 53 Sellers' positive specimens. They also reported that 35 brain impression smears of mice which were MI test negative proved positive by FA test and considered it to be the most sensitive test that not only detected the live but also the inactivated antigen.

Zimmer et al.(1990) reported that FA test detected 98 per cent of the rabies positive animals.

Burnes et al. (1991) found 44 of 219 dogs to be FA positive and it had 100 per cent agreement with the biological assay.

Jayakumar and Ramadass (1991) on examining brain and submaxillary salivary glands of 56 dogs by FA test, Rapid Rabies Enzyme Immuno Diagnostic Test (RREID), indirect IP test and Counter-Immuno Electrophoresis Test (CIEP), reported that sensitivity, specificity and diagnosability of FA test were 100 per cent. The compared sensitivity and specificity of IP test and FA test were 95.3 and 99 per cent respectively.

Hamir et al.(1992a) detected four cases of rabies in horses by FA test.

WHO (1992) report described FA test as the rapid and sensitive method for diagnosing rabies infection in animals as well as human beings.

Foley and Zachary (1995) detected rabies in a heifer by FA test, which showed no Negribodies in sections.

Jayakumar et al.(1995a) reported the sensitivity of FA test as 74.5 per cent as compared to 71.25 per cent in the case of Dot ELISA, a recent technique. In another study Jayakumar et al. (1995b) reported 100 per cent specificity and sensitivity for FA test.

Hamir et al.(1996) detected 55 positive rabies cases out of 90 suspected cases examined from domestic and wild animals using FA test.

2.4.4.Immunoperoxidase test (IP test)

The introduction of enzyme-conjugated antibodies for the detection of antigens in histochemistry (Nakane and Pierce,1966) and the immunoperoxidase technique in virology (Kurstak et al. 1969, 1972 and Kurstak,1971) , prompted the application of the immunoperoxidase technique for the diagnosis of rabies.

Among the various techniques used for the diagnosis of viral diseases, the immunoenzymatic techniques, particularly the IP technique was the most adequate for the specific and rapid diagnosis of viral infections(Kurstak,1971).

IP test, which uses enzyme labelled antibodies, was introduced as an alternative technique for FA test in the diagnosis of rabies by Atanasiu (1973). Out of 912 specimens of brain examined for rabies, Atanasiu et al. (1974) found 60 to be positive by IP test as against 62 by FA test.

Though IP test detected only 217 of 230 positive cases, which was 1.2 per cent less accurate than FA test, Genovese and Andral (1978) were of the opinion that IP test was still sensitive and useful, particularly when FA test could not be carried out.

Different types of IP reactions were described for the diagnosis of rabies. Peroxidase-antiperoxidase method (PAP) (Kotwal,1984), direct IP reactions (Kotwal and Narayan,1985a) and indirect IP reactions (Kotwal and Narayan,1985b) were common among them.

Direct IP test detected 91 per cent of 1400 specimens examined with paraffin-embedded brain sections and was considered as an alternate method to replace FA test (Anjaria and Jhala,1985).

Das et al. (1985) examined brains of five rabies suspected field dogs and 45 experimentally infected animals and found all positive for rabies by IP test. They also reported that IP test was comparable with FA test and MI.

All 11 mouse brains infected with the CVS strain of rabies virus were positive by direct IP test. When 30 specimens including eight from field were examined by IP test, 28 showed IP positive reactions (Kotwal and Narayan,1985a).

Jonsson et al.(1988) applied immunoperoxidase staining to tissue sections of formalin-fixed, paraffin-embedded skunk brain for the detection of rabies antigen.

Of 187 brains from wild and domestic animals examined, Zimmer (1988) found 96 of them to show positivity to rabies by peroxidase-antiperoxidase reaction.

Narayan and Kotwal (1989) demonstrated immunoperoxidase reactions in all 12 positive cases examined by direct and indirect IP test.

Zimmer et al.(1990) demonstrated rabies antigen in 98 per cent of the rabies positive animals by peroxidase-antiperoxidase technique.

Jayakumar and Ramadass (1991) on examining 56 brain and submaxillary salivary glands of dogs by FA test, RREID test, indirect IP test and CIEP test, reported the sensitivity of indirect IP test as 92.85 per cent and specificity and diagnosability of it as 100 per cent each. The compared sensitivity and specificity of IP test and FA test were 95.3 and 99 per cent respectively.

When Avidin-Biotin Complex method (ABC) was used for a retrospective survey of rabies in equines, (Hamir et al.1992a) and for documenting the distribution of lesions and rabies antigen in tissues of raccoons (Hamir et al. 1992b), it was found that the detection of Negribodies by H & E and immunoperoxidase was even though comparable, immunoperoxidase method detected many more Negribodies than were seen in the corresponding H & E stained sections.

Sinchaisri et al. (1992) considered Avidin-Biotin Complex (ABC) as a useful method for the early diagnosis of suspected cases when conventional histopathological and

immunofluorescent antibody techniques failed to detect lesions or viral antigens.

Hamir and Moser (1994) described ABC technique to yield good results in sections unsuitable for histopathological examination, due to freezing prior to fixation. They detected 39 of 40 rabies cases with ABC technique using formalin-fixed, paraffin-embedded brain tissues. They also observed that monoclonal antibody (mAb) preparations provided more intense and discrete positive reaction in immunoperoxidase tests than polyclonal antibody.

Jayakumar et al. (1994) examined one hundred formalin fixed, paraffin embedded brain tissues from rabies positive animals and reported that both ABC and Peroxidase-Antiperoxidase procedure (PAP) gave good results and were similar to the results of FA test.

By the application of two immunoperoxidase techniques, the ABC and PAP, Last et al. (1994) identified both "viverrid" and "canid" rabies-virus antigen in formalin fixed, paraffin embedded brain tissue sections.

Hamir et al. (1995a) showed positive immunoperoxidase reactions in 55 rabid cases using formalin- fixed, paraffin-embedded brain tissues of domestic and wild animals, utilizing monoclonal antibody (mAb) 802-2 and they reported that these results were similar to those of the FA test results.

Hamir et al.(1995b) showed rabies positive reactions by ABC test on formalin-fixed, paraffin-embedded brains of a rabies positive raccoon using monoclonal antibodies, mAb 802-2.

Jayakumar et al. (1995b) reported 100 per cent specificity and sensitivity for PAP test.

Hamir et al. (1996) on examining 214 tissue sections of 90 rabies positive animals (both wild and domestic) by ABC-immunoperoxidase procedure, found that the results were identical to those of the FA test results.

Materials and Methods

MATERIALS AND METHODS

3.1. Epidemiology

Review of epidemiology of rabies in domestic animals (species-wise) in Kerala for the last 12 year (from July 1983 to June 1995) was made based on the post-mortem register maintained in the department of Pathology, College of Veterinary and Animal sciences, Mannuthy, Thrissur.

3.2.Specimens

One hundred and six cases of rabies suspected specimens brought to the department were examined during one and half year period of study. Specimens included 62 dogs, 11 cats, 16 cattle (white cattle and buffalo),five calves, seven goats,two civet cats, a bandicoot, leopard and a squirrel.

3.3.History

Information related to clinical history,symptomatology and preventive vaccination were collected from the veterinarians or the animal owners bringing the carcass for examination,through a questionnaire(Appendix 1).

3.4.Post-mortem examination

Postmortem lesions in rabies positive animals were summarised from the records maintained in the department of Pathology and lesions observed in rabies positive animals during the period of study.

3.5.Examination of brain, preparation of the slide and specimen collection

The brain was removed from the skull as per the method described by Tierkel (1973). It was examined for any congestion of cerebral vessels or exudate in the meninges. The brain was dissected longitudinally, to seperate the two hemispheres and the cerebellum and medulla were detached from the hemispheres.A longitudinal incision was made externally in the posterior third of each hemisphere , about 1.5 cm from the midline; incision was continued through the greymatter and whitematter until a narrow groove, the third ventricle was reached. The hippocampus was seen on the floor of the ventricle in the form of a glistening white, semi-cylindrical bulge, extending laterally on each side.

With a pair of scissors, small transverse sections of 2-3 mm thickness were cut (Ammons horn/hippocampus or cerebellum) and placed on a clean blotting paper, with the cut surface facing upward.

A clean microscopic slide was then touched against the cut surface of the section and pressed gently downward with enough pressure exerted to create a slight spread of the exposed surface of the tissue against the glass slide. Two impressions were made on one slide.

Eight such impression slides from each animal were prepared either from hippocampus (dog, cat and wild animals) or from cerebellum (cattle, goat).

Parts of brain (Ammons horn, cerebrum and cerebellum), submaxillary and parotid salivary glands and infra orbital branch of the trigeminal nerve were collected and fixed in 10 per cent formol-saline for histopathology and immunoperoxidase test.

3.6.Sellers' impression smear staining

3.6.1.Sellers' stain (Tierkel,1973)

Stock solution of Methylene blue

Methylene blue (C.I.No.52 015).....10 g.

Methanol (absolute acetone-free)..to make 1000 ml.

Stock solution of Basic fuchsin

Basic fuchsin (C.I.No.42 510).....5 g.

Methanol(absolute acetone-free) to make 500 ml.

The stains and chemically pure Methanol were obtained from Merck-Germany.

Working solutions

Solution 1

Methylene blue stock	2 parts
Basic fuchsin stock	1 part

Solution 2

Methylene blue stock	1 part
Basic fuchsin stock	1 part

Solution 3

Methylene blue stock	1 part
Basic fuchsin stock	2 parts

Thoroughly mixed solutions were stored in refrigerator in screw capped bottles separately, without filtration.

3.6.2.Sellers' staining of impression smears

While the impression smear was still moist, the slide was flooded with Sellers' stain, allowed to remain for a desired time, rinsed quickly in running tap water, and air-dried without blotting. The smear was screened for Negribodies under oil immersion, after locating the area/field rich in neurones in the low-power view.

a. Three slides were stained with Sellers' stain consisting of three different concentrations (2:1, 1:1 and 1:2 parts of Methylene blue and Basic fuchsin - solution 1, solution 2 and solution 3 respectively) with traditional timing (5 seconds).

b. Three slides were stained with the standard Sellers' stain (2:1 parts of Methylene blue and Basic fuchsin- solution 2) each for 2 seconds, 6 seconds and 9 seconds.

3.6.3.Wright's staining

One slide was stained by Wright's staining procedure as per the method described by Benjamin (1978).

The other slide was fixed in cold acetone at -20^{o}C for FA test.

3.7 Histopathology

Paraffin embedded tissue sections of 4-6 micron thickness (ammon's horn, cerebrum, cerebellum, submaxillary and parotid salivary glands and infra orbital branch of the trigeminal nerve) were stained with H & E.

In addition, sections of Ammon's horn and cerebellum were also stained with Sellers' method and Mann's method for the demonstration of Negribodies as per the procedure adopted by Lepine (1973).

3.8.The Fluorescent Antibody Test(FA test)

(Dean and Ablseth,1973)

Impression smears prepared from hippocampus and/or cerebellum, were airdried and fixed in acetone at -20^{o}C for 2-4 h and used for the test.

3.8.1.Preparation of the conjugate

Rabies antibody tagged with the Fluorescein Isothiocyanate (FITC) dye was obtained as a lyophilized powder from Central Research Institute, Kasauli, H.P. The material was reconstituted and thoroughly mixed with 0.5 ml of distilled water. From this suspension, 20 per cent Normal Mouse Brain suspension (NMB) and Infected Mouse Brain suspension(IMB) were separately prepared.

3.8.1.1.NMB suspension

The reconstituted conjugate was diluted 1:16 with a 20 per cent Normal mouse brain diluent. The diluent was a 10 per cent suspension of PBS(pH 7.4) prepared with the yolk of 6-7 days embryonated eggs.

3.8.1.2.IMB suspension

The reconstituted conjugate was diluted 1:16 with a 20 per cent infected mouse brain diluent. Infected mouse brain was prepared using brains from young mice inoculated intracerebrally with 1:100 to 1:1000 suspension of the CVS strain of fixed-rabies virus (obtained from the Central Research Institute, Kasauli) and harvested when moribund.

These conjugates, after centrifugation for 10 min. at 1000 g.,the supernatant was stored in glass containers as aliquots at -20 ^{o}C until used.

3.8.2.Control slides

Impression smears prepared from normal mouse brain and infected mouse brain (obtained by intracerebral inoculation of 1:100 to 1:1000 suspension of the CVS strain of fixed-rabies virus and harvested when moribund) were used as negative and positive control slides respectively.

3.8.3.FA staining

The direct FA staining as per the procedure described by Dean and Abelseth (1973) was followed.

Positive and negative control slides were also stained in a similar manner.

The examination of the slide was carried out using a Zeiss fluorescent microscope (with UG 1 exciter filter and 410 nm barrier filter) with epi-illumination and photographed on Kodak High speed Ektachrome film (400 ASA).

3.9.Immunoperoxidase test (IP test)

Conjugation and performance of direct IP test procedure described by Avrameas and Ternynck (1969) was followed with little modifications.

3.9.1.Rabies antibody - immunoperoxidase enzyme conjugation

Refined equine antirabies globulin, available as 5 ml. vials (300 IU/ml) were obtained from the Central Research Institute, Kasauli, H.P.

Horse Radish Peroxidase (HRPO) enzyme type IV obtained from Sisco Research Laboratories, Bombay was used.

1. The anti-rabic globulins were dialysed against four to five cnanges of PBS, pH 7.4-7.6 (0.9 % NaCl and 0.01 M phosphate buffer) for 24 h at 4 ^{o}C to remove the preservatives.
2. Protein estimation was done by Biuret method (Inchiosa,1964).
3. Globulins obtained was reconstituted with the same buffer to obtain 5-7 mg of globulins/ml.

4. pH was adjusted to 6.9 by addition of 0.1 M solution of potassium phosphate (K_2HPO_4).
5. For every ml of the above solution, 10-14 mg of HRPO enzyme was added, and, after its complete dissolution, 0.05 ml of one per cent solution of freshly prepared glutaraldehyde was added.
6. The mixture was shaken for 2 h at room temperature by end-over-end rotation.
7. The product thus obtained was then dialysed overnight at 4°C against physiological saline, pH 7.4.
8. The next day, the solution was centrifuged for 15 min.at 1500-2000 g.
9. The conjugate so obtained was diluted 1:10 before use.

3.9.2.Immunoperoxidase (IP) staining

1. Sections hydrated with PBS (pH 7.4).
2. Treated with 0.25 per cent trypsin for one h at 37°C in a humid chamber.
3. Washed in PBS.
4. Flooded with 0.5 per cent albumin to remove non-specific adsorption.
5. Washed in PBS.
6. Treated with 0.3 per cent Hydrogen peroxide in 100 per cent Methanol for 30 min. to destroy any endogenous peroxidase.
7. Washed thoroughly in PBS.

8. Diluted conjugate was added and allowed to react at $37^{o}C$ for 45 min.in a humid chamber.
9. Washed thoroughly in PBS.
10. Sections treated with 0.05 per cent of 3,3,di-amino benzidine (DAB) in Tris-HCl buffer (pH 7.6) in the presence of 0.1 per cent hydrogen peroxide(0.3 ml of 33 per cent solution of hydrogen peroxide for every 100 ml) for 15-20 min. at room temperature.
11. Washed thoroughly in PBS.
12. Couterstained with haematoxylin for few seconds.
13. Dehydrated in ethanol, cleared in xylene and mounted with DPX.
14. Examined for brown colored deposits under light microscopy.

Representative samples were counterchecked for the diagnosis, with the Veterinary Diagnostic Laboratory, College of Veterinary Medicine, Oregon State University, U.S.A.

Results

RESULTS

4.1.Epidemiology

Epidemiological data for the last 12 year period from July 1983 to June 1995 was collected from the post-mortem record maintained at the Department of Pathology, College of Veterinary and Animal Sciences, Mannuthy.

4.1.1.Regional distribution

Regional distribution of rabies in domestic animals in Kerala is presented in Fig.1.

There were reports of the disease from all the districts of the state.The occurrence was relatively high in Trichur (22 per cent) district, followed by Ernakulam (18 per cent), Kozhikode (14 per cent) and Palghat (10 per cent). The cases reported in the following districts were very low; Trivandrum (1.6 per cent),Pathanamthitta (1.5 per cent) and Kasargod (0.7 per cent).

4.1.2.Species distribution

The prevalence of rabies in domestic animals in the state of Kerala during the period from July 1983 to June 1995 is presented in Fig.2.

During the period from July 1983 to June 1995,1306 animals of different species were diagnosed to be positive for rabies. Canine rabies was found to be predominant (969),

followed by cattle (131), cats(114) and goats(86). Besides, two foxes, one monkey,one bandicoot, one mongoose and one civet cat were also diagnosed to have rabies during this period.

4.1.3.Year-wise distribution

The year-wise occurrence of rabies during 1983-1995 is presented in Fig.3. The occurrence of rabies in the period varied each year and was never constant. It was highest during the year 1990-91 (158 cases) and was lowest in 1993-94 (72 cases).

During the period of study, 106 rabies suspected animals that died after showing clinical symptoms of rabies were examined. It included 62 dogs, 11 cats, 16 cattle, five calves, seven goats, two civet cats and one each of bandicoot, leopard and squirrel. Twenty-two out of this [13 dogs, eight cattle(incuding one calf) and a cat] were confirmed positive for rabies.

4.2.History and clinical signs

Three of the rabies positive dogs and the cat brought for confirmatory diagnosis of rabies were stray animals.All the cattle and goats brought were of domestic type.

Details regarding stray dogs were not available and they were killed and brought for examination.

All the rabies positive dogs brought for examination were between one to three years of age, except two dogs which were five and eight months old respectively. The cat was an adult. Except one calf, all cattle were adults.

Four of the domestic dogs were reported to have died suddenly. Among the other six, five were reported sick for five to nine days before death. One died two days after showing clinical symptoms. The duration of clinical illness in cattle was between seven to nine days.

All the dogs confirmed to have rabies, had history of scratching or biting other animals or human beings. These dogs had scratched 14 persons and another 36 were reported to have bitten at different parts of the body. One stray dog had bitten eight persons, including two children, before it was killed. Twenty other animals had contact with these rabies positive dogs either by scratching or biting. The cat was furious and scratched one person before it was killed and presented for testing. Other than the accidental exposure during handling while attempting to treat them and contact with saliva, men were not exposed to any cattle.

All the dogs and the cat were males, except three dogs which were females.All the cattle were females, except two bulls.

Rabies prophylactic vaccination was not carried out for any animals, except for two dogs.

Detailed clinical manifestations were available for the domesticated dogs only.

Six of the dogs developed furious form and other four had dumb (paralytic) form of rabies.

Unusual violence, frenzied behavior, aimless wandering and tendency to bite other animals and human beings were noted in the furious form of rabies. Dullness, sluggish behavior, not responding to call, weakness and staggering were the symptoms noted in the dumb form of rabies.

Anorexia and vomiting were commonly reported in the early stages.Enteritis was reported in few cases. Profuse salivation and respiratory difficulty before death were reported in most of the cases.

Cat had a furious behavior and had a biting tendency.

Anorexia and sudden drop in the milk yield were reported in all rabies positive cattle. Incessant bellowing, and frequent urination were also noticed in many of them.

4.3.Gross lesions

The post-mortem lesions recorded in rabies positive dogs collected from the records maintained in the department of Pathology and cases presented during the present study, are summarized below.

Post-mortem lesions were predominantly seen in the

liver and lung, followed by lesions of the kidney. Involvement of the heart and gastroenteric lesions were also observed.

About 45 per cent of rabies positive animals showed lesions in the liver. The lesions varied from mild degeneration and congestion to severe necrosis of the liver parenchyma. Hemorrhages were also noticed in a few cases.

More than 50 per cent of the animals affected with rabies had pulmonary lesions. About 60 per cent of them showed congestion and edema. Presence of pneumonia at different stages was noted in 15 per cent of the total lung lesions. Twenty per cent lung lesions were characterized by complete pulmonary collapse. Consolidation of the lung, emphysema and frank hemorrhages were noted in a few cases.

About 30 per cent of rabid animals had renal damage, which varied from mild degeneration to severe nephritis.

In the digestive system, catarrhal gastroenteritis was a common post-mortem finding. More than 70 per cent of rabid animals had some lesions in the digestive system and about 40 per cent of the lesion was catarrhal gastroenteritis. Hemorrhages and ulcerative type of gastroenteritis were also noticed in about 25 per cent of cases.

Though cardiac lesions were detected in only about 15 per cent of cases, the degree of damage caused to the heart

muscle was significant. Severe hypertrophy, dilatation of the ventricle or pericarditis with the presence of fluid in the pericardium were noted in many of the cases. Endocardial haemorrhage was seen in few instances.

Parasitic infestation both internal and external was seen in about 20 per cent of rabies positive cases.Ancylostomiasis, Toxacara infection, Taeniasis and Spirocerca lupi were commonly noticed.

About 10 per cent of rabies positive animals had brain lesions. They consisted mostly of meningitis. Few of them were found to have fluid in the lateral ventricles. Hemorrhages were noted in few occasions. During the period of study, five rabies positive cases showed grossly visible changes ranging from simple congestion to areas of inflammation and oedema. No striking structural alterations could be observed in other cases. Congestion of meninges, petechiae, softening of the lateral ventricles,haemorrhage, softening of part the of cerebrum and cerebellum, dilatation of the lateral ventricles, excessive accumulation of cerebrospinal fluid in the ventricles and flattening of the gyri were observed. The cerebellar folia showed patchy dark areas of softening. Mild congestion of meninges and moderate engorgement of the gyri were noticed.

4.4.Laboratory diagnosis of rabies

Employing rapid Sellers' staining, all the rabies suspected specimens were examined for the disease. They were

also tested by histopathology, Fluorescent antibody test (FA test) and Immunoperoxidase test(IP test). Out of 106 rabies suspected specimens examined, 22 specimens [13 dogs, eight cattle(including one calf) and a cat] were detected positive for rabies.

The results of these tests are presented in Table 1.

All the four tests applied together detected 22 rabies cases. Sellers' rapid staining detected 12 specimens. All these 12 specimens were positive by histopathological staining also. Ten out of these 12 specimens showed rabies specific immunofluorescence (FA test) and the other two were negative to FA test. Another ten specimens also showed rabies specific immunofluorescence (totally 20 FA positive specimens). IP positive reactions were seen in 19 cases. Three other specimens were negative for IP reactions.

These three specimens which were negative in IP test were positive in FA test. The two specimens which were FA negative, were positive in all other three tests; Sellers' staining, histopathological staining and IP test.

The comparative efficacy of these tests in the diagnosis of rabies is presented in the Fig.4. FA test and IP test were more reliable in detecting rabies, which showed positivity in 20 and 19 cases respectively, as against 12 each detected by Sellers' staining and histopathology.

Table 1

Analysis of the Sellers' staining, histopathology, FA test and IP test in the diagnosis of rabies

Speci. No.	Species	Sellers' Staining	Histopa-thology	FA test	IP test
1.	Cattle	Positive	Positive	Positive	Positive
2.	Cattle	Negative	Negative	Positive	Positive
3.	Dog	Negative	Negative	Positive	Positive
4.	Dog	Negative	Negative	Positive	Positive
5.	Dog	Negative	Negative	Positive	Positive
6.	Dog	Positive	Positive	Positive	Positive
7.	Dog	Positive	Positive	Negative	Positive
8.	Cattle	Positive	Positive	Positive	Positive
9.	Dog	Positive	Positive	Positive	Positive
10.	Cattle	Positive	Positive	Positive	Positive
11.	Dog	Negative	Negative	Positive	Negative
12.	Dog	Negative	Negative	Positive	Negative
13.	Cattle	Negative	Negative	Positive	Positive
14.	Cat	Negative	Negative	Positive	Negative
15.	Cattle	Positive	Positive	Positive	Positive
16.	Dog	Positive	Positive	Negative	Positive
17.	Dog	Negative	Negative	Positive	Positive
18.	Dog	Positive	Positive	Positive	Positive
19.	Cattle (Calf)	Positive	Positive	Positive	Positive
20.	Dog	Positive	Positive	Positive	Positive
21.	Dog	Negative	Negative	Positive	Positive
22.	Cattle	Positive	Positive	Positive	Positive

4.4.1.Impression smear staining

Examination of the impression smears by Sellers' staining revealed evidence of Negribodies in 12 out of the 22 positive rabies cases(55 per cent).

The results of the examination of impression smears stained by different concentrations and timings of Sellers' stain are given in the Table 2.

Impression smears stained with 2 : 1 concentrations of Methylene blue and basic fuchsin yielded good results when stained for five seconds. With this concentration and timing, Negribodies appeared distinctly as magenta red bodies within the cytoplasm. Nerve cell and interstitial tissue stained pale blue and pink respectively. Erythrocytes were stained copper red. Negribodies were clear and prominent in the Purkinje cells in the cerebellum of cattle (Fig.5) and in the neurons of the hippocampus in dogs and cat (Fig.6).

Negribodies appeared heterogenous. They varied greatly in size, shape and number.The size of the Negribody ranged from 8-10 μm to 20 μm.Their average size was generally between 10 μm to 15 μm. Round, oval, elongated and sometimes spheroid shapes were noted. The number ranged from one to four. Usually they were one to two and most commonly they were seen as two in a single neuron.

Table 2

Results of the staining of impression smears at different concentrations and timings of Sellers' stain

Ratio of the stain	Time (sec.)	Result of staining			
		Negribody	Erythrocytes	Nerve cell	Interstitial tissue
2:1	3sec.	Not differentiated	yellow colour	fuchsin stain	fuchsin stain
2:1	5sec. and 6sec.	appeared magenta red coloured	copper red coloured	pale blue	pink
2:1	9sec.	red coloured	light copper coloured	faint blue	pink
1:1	5sec.	light red	yellow coloured	light blue	pink
1:2	5sec.	Not differentiated	intense copper red coloured	blue	deeply pink

In few instances, Negribodies were noted just outside the neuron and still,was easily differentiated from the erythrocytes,which stained copper red.

Basophilic inner blue bodies (Innerkorperchen) within the magenta red colored Negribodies, though recognised with difficulty, were noticed as small granules.

With other concentrations or with different timings, the impression smears were poorly stained for the routine examination of Negri bodies. They stained either too lightly or too deeply and posed problem in differentiating them from the other structures.

In addition to the presence of Negribodies, increased number of glial cells, mononuclear lymphocytes and few degenerated neurons were also observed in rabies positive smears stained with Sellers' stain.

Impression smears stained with Wright's stain didn't yield consistent and satisfactory results. Negribodies could not be differentiated in the cytoplasm. Erythrocytes stained orange colour, nerve cell intense violet and interstitial tissue stained faint purple.

It was found that working solution of Sellers'stain prepared by mixing 2 : 1 parts of one per cent methylene blue and basic fuchsin improved in the staining quality when it was allowed for ripening. The working solution gave good results when stored at room temperature for 7-10 days. And it

was also found that such stain can be used for even a month when kept at refrigeration temperature after ripening.

4.4.2.Histopathological studies

Cerebrum, cerebellum, hippocampus,salivary glands and the trigeminal nerve were examined for histopathological alterations. Special stains like Sellers' and Mann's were utilized for staining Negribodies.

4.4.2.1.Central Nervous System

In H & E stained sections, Negribodies appeared as purple colored inclusion body and was clearly differentiated from the pink staining eosinophilic cytoplasm. The cells and interstitium stained dark blue and pink respectively (Fig.7). The H & E stain satisfactorily stained Negribodies and the method was easy and simple. It was also a good staining method to study the histological changes of the brain.

In Sellers' stained sections Negribodies appeared deep red or red. Cells and interstitial tissue stained blue-violet and pale pink respectively (Fig.8). Staining of the Negribodies was satisfactory and the method was simple and one step.

In Mann's stained sections,Negribodies stained vermilion red; nucleoli of neuron, violet red; cells,faint to moderate blue; erythrocytes, pink and stroma pale pink

(Fig.9). Negribodies were more numerous and striking than other two methods. Staining method required 14-24 h time and involved considerable care. Negribodies and erythrocytes couldn't be differentiated in some sections, since both of them were stained strikingly red.

In the cerebrum, the lesions consisted of neuronal degeneration, phagocytosis of the degenerating cells and perivascular collection of lymphocytes and plasma cells. Meningeal congestion, collapse of the capillaries and subsequent dilatation of the Virchow-Robin space in the substance of the molecular layer in focal areas and perineuronal cavitation were observed. There was gliosis, occasional satellitosis and focal loss of cells in the molecular layer in some cases. In some of the cases, diffuse encephalitis was evident characterised by perivascular cuffing (Fig.10). One or two Negribodies within the cytoplasm were observed in some of the neurons.

Increase in the cellularity of the granular cell layer was commonly seen in the cerebellum. Condensation and pyknosis of the Purkinje cell and occasional loss of them were noticed (Fig. 11). There was congestion of the cerebellar folia and focal vacuolation. Dilatation of the perivascular space and the presence of intracytoplasmic Negribodies in Purkinje cells were also noted (Fig. 7). In some, the nucleus of the Purkinje cell appeared pyknotic.

Hippocampus showed degeneration of the granular layer. Some of the degenerated neurons appeared as eosinophilic spheroids. Dilatation of Virchow-Robin space, perivascular cuffing, severe congestion and hemorrhages were noticed Extensive gliosis distended perineuronal space,neuronophagia and occasional satellitosis were also noticed in the hippocampus(Fig.12 and 13).

4.4.2.2.Salivary glands

Fifteen of the 22 rabies cases showed sialadenitis. The salivary glands showed congestion of vessels, distension of the acini, vacuolation of the cytoplasm of lining cells and diffuse degeneration of glandular epithelium in most of the cases (Fig.14). In few cases, frank inflammatory changes could be observed in the gland. This consisted of congestion, collapse of the acini and extensive infiltration of the lymphocytes (Fig.15). Periductular fibrosis and infiltration were also noticed in one case.

4.2.2.3.Trigeminal Nerve

Epi and perineural oedema, fascicular degeneration and inflammation were observed in the trigeminal nerve. A few of the nerve fascicles were seen extensively necrosed and infiltrated by inflammatory cells. The inflammatory cells were also found infiltrating the epineurium (Fig.16). Only two cases out of 22 rabies positive animals had such trigeminal nerve lesions.

4.4.3.Fluorescent antibody test (FA test)

FA test was done on acetone-fixed brain impression smears of all rabies suspected specimens.

FA test revealed rabies specific immunofluorescence in 20 cases (91 per cent). Immunofluorescence was noticed in 10 specimens,out of 12 specimens showing Negribodies(Sellers' staining and histopathology). Rabies positivity was established by FA test alone in three cases.

In positive cases of rabies the antigen was detected by the presence of apple-green or greenish-yellow fluorescence as dust or sand like tiny fluorescent bodies against the dark background, indicating the rabies positivity. There was sharp and intense fluorescence towards the margins of these fluorescing bodies. The size of these fluorescing bodies varied and were comparable to the size of the Negribodies (Fig.17 and 18).

The positive control smears, prepared from fixed rabies virus (CVS) injected mouse brain, showed heavily positive rabies antigen as a greenish-yellow,tiny fluorescence.

No fluorescence was detected in the negative control smears prepared from the normal mouse brain, and hence excluded the possibility of the false positive fluorescence in the FA test.

4.4.4.Immunoperoxidase test (IP test)

Out of the total 22 rabies positive cases, immunoperoxidase reactions were evident in 19 specimens (86 per cent). These three IP negative specimens were positive for rabies by FA test.

In IP positive cases, rabies antigen was detected by the presence of brown colored deposits of varying size spread throughout the microscopic field. The precipitate particles varied in size and on an average for every two to three microscopic fields, at least one big semispherical brown deposit was noted, indicating the presence of rabies antigen. These precipitates were comparable to the size of the Negribodies in the corresponding tissue sections of H & E. (Fig 19 and 20). In some cases, the IP reaction was moderately faint and made evaluation of the case difficult. In some areas, only dust-like brown deposits were seen scattered throughout the microscopic field.

Sixteen paraffin blocks from six rabies positive cases were counter-checked with the Veterinary Diagnostic Laboratory, College of Veterinary Medicine, Oregon State University, U.S.A. It included different areas of the brain from six rabies positive cases (three dogs and one each of a cat, cattle and calf).

The specimens were examined for rabies by the laboratory employing immunohistochemical staining(IHC) using

anti-rabies monoclonal antibodies, mAb-802-2. The results of the laboratory test is presented in the Table 3.

Five out of six cases were positive for IHC. Only nine out of 12 paraffin sections from different areas of the brain of these rabies cases were positive for IHC. The Other areas of the brain from the rabid animals were negative for IHC. The sections from the sixth case (dog) was rabies negative in all the four areas examined.

Table 3

Results of the enzyme immunochemistry (immunoperoxidase test) done on rabies positive specimens

Ser. No.	Spec. No.	No.of sections Species(areas of brain) examined	No.of sections (areas of brain) positive
1	Dog	two	one
2	Cat	three	two
3	Dog	one	one
4	Cattle	two	two
5	Calf	four	four
6	Dog	four	none

Fig.1 REGIONAL DISTRIBUTION OF RABIES IN DOMESTIC ANIMALS IN THE STATE OF KERALA, JULY 1983 - JUNE 1995

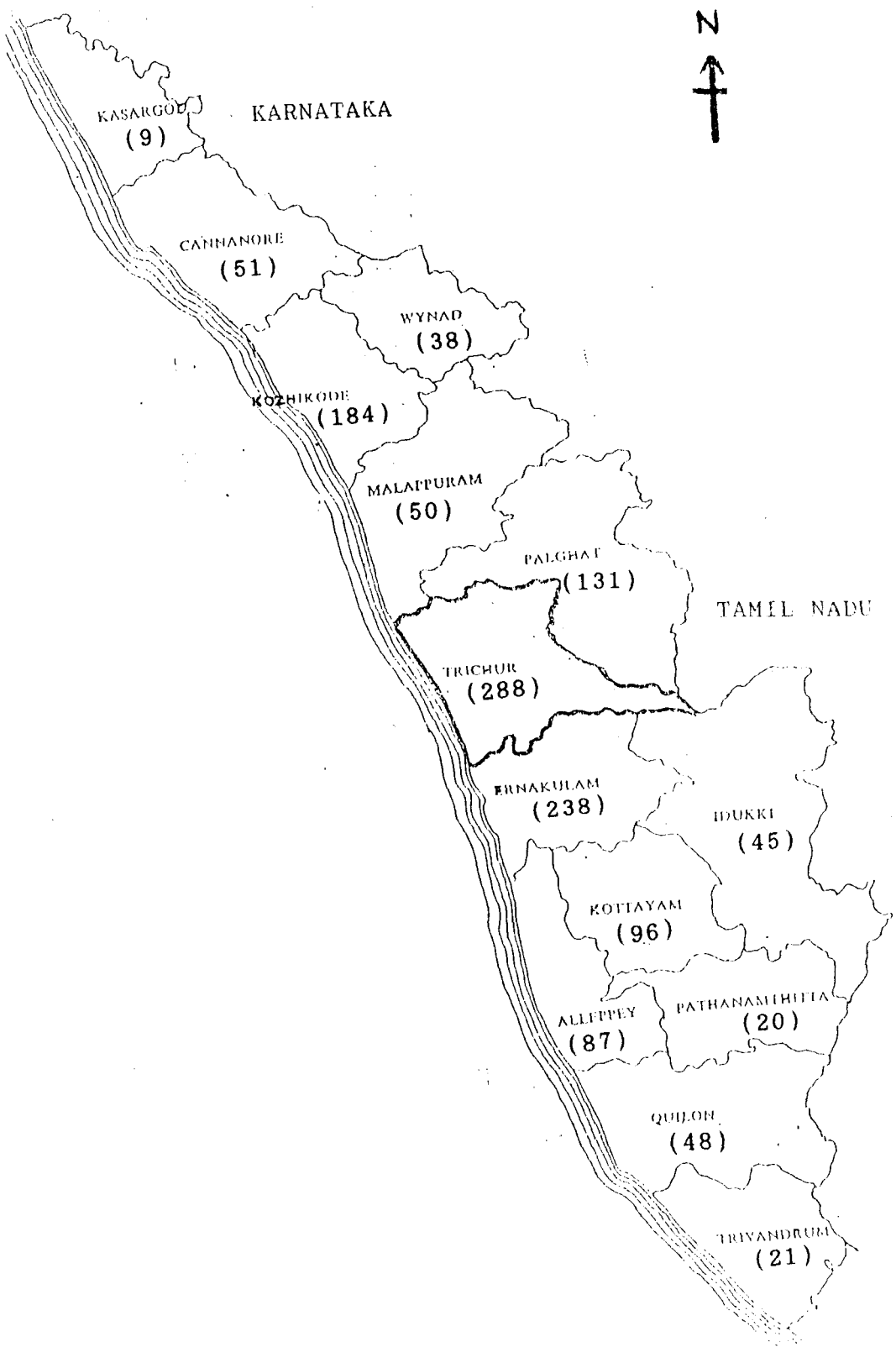
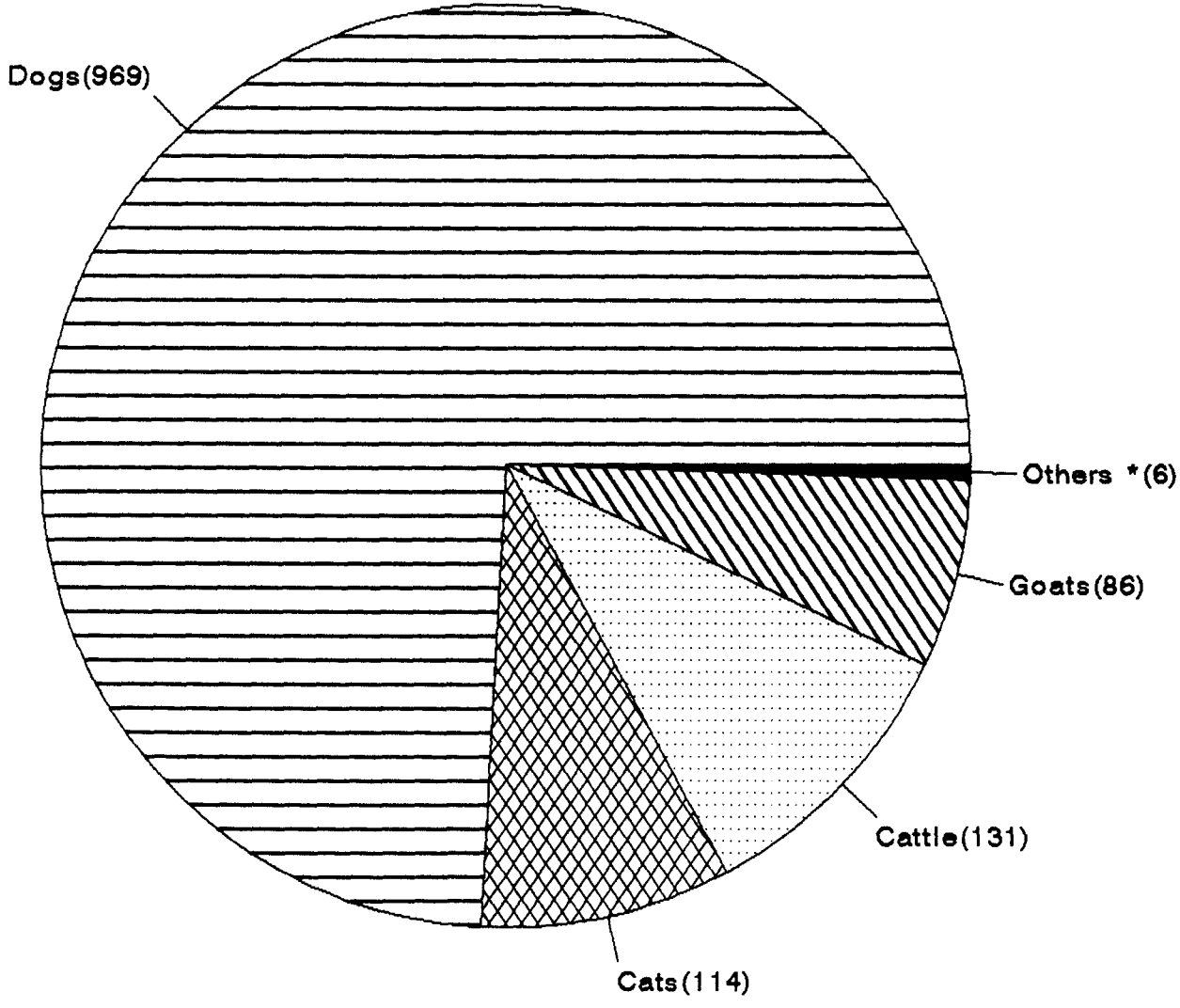


Fig.2 NUMBER OF CONFIRMED RABIES CASES IN THE STATE OF KERALA, JULY 1983 - JUNE 1995



* Includes two foxes, one monkey, one mongoose, one civet cat and one bandicoot

Fig.3 PREVALENCE OF RABIES IN DOMESTIC ANIMALS
IN THE STATE OF KERALA, JULY 1983 - JUNE 1995

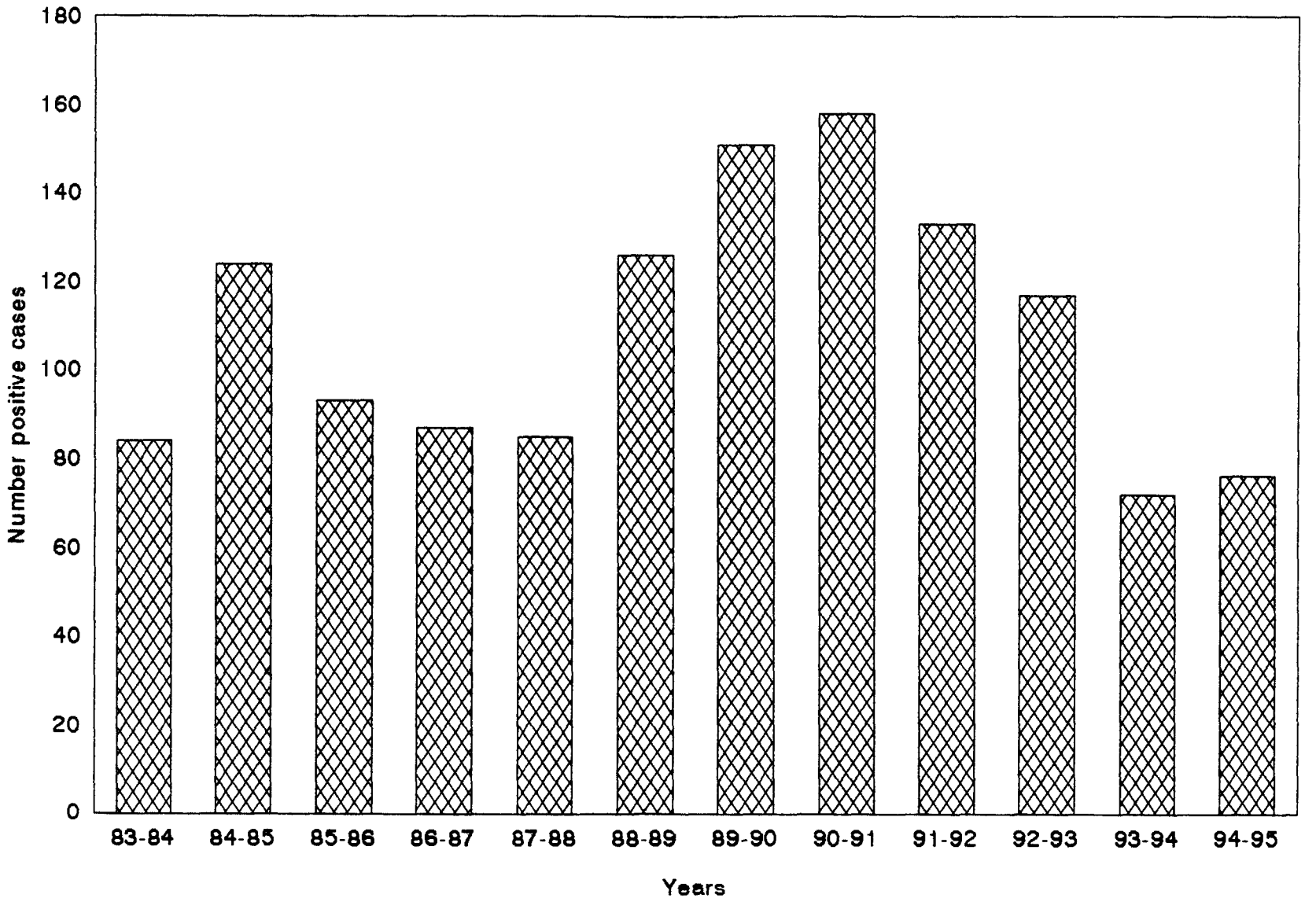
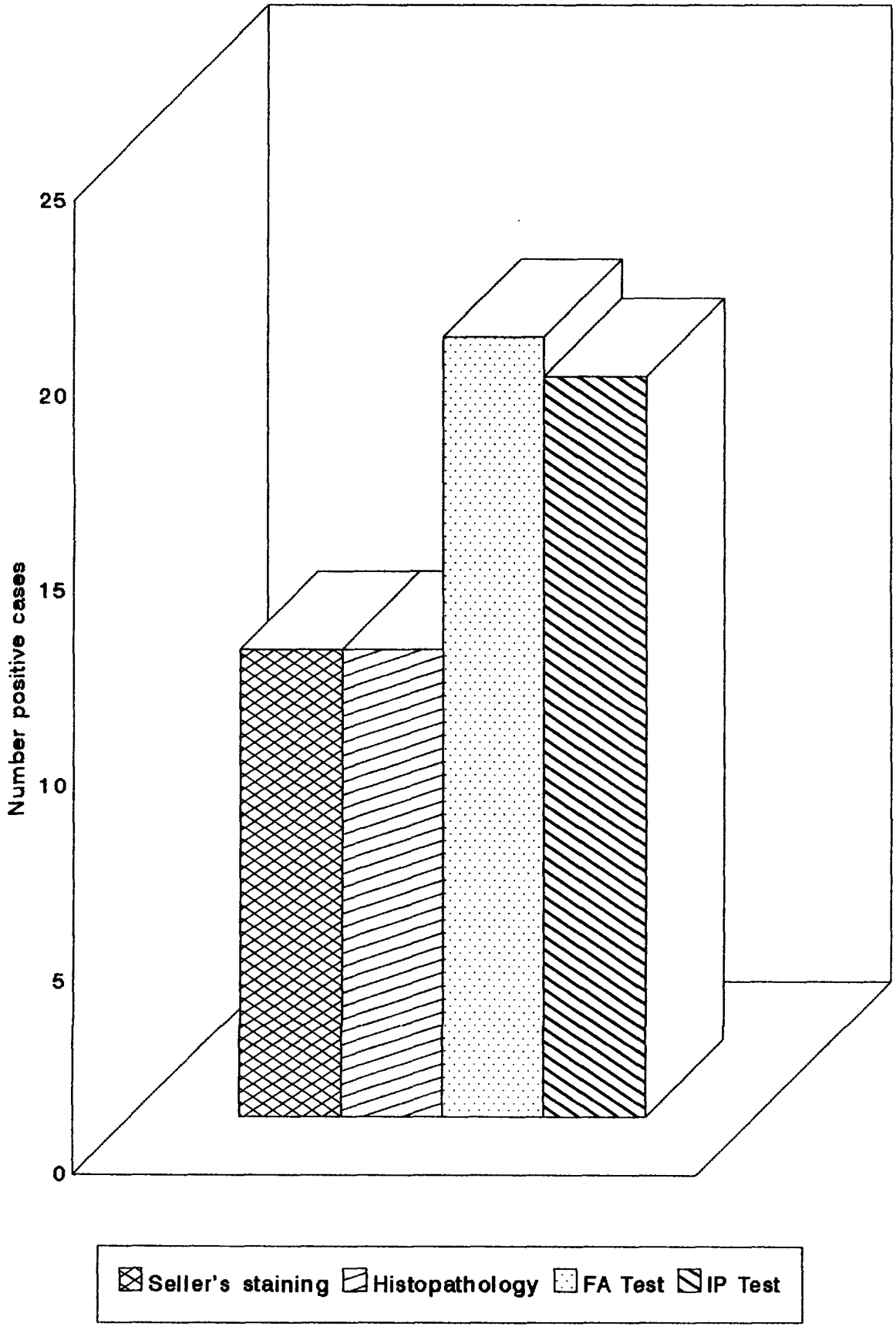


Fig.4 RELATIVE EFFICACY OF DIFFERENT DIAGNOSTIC TESTS FOR RABIES



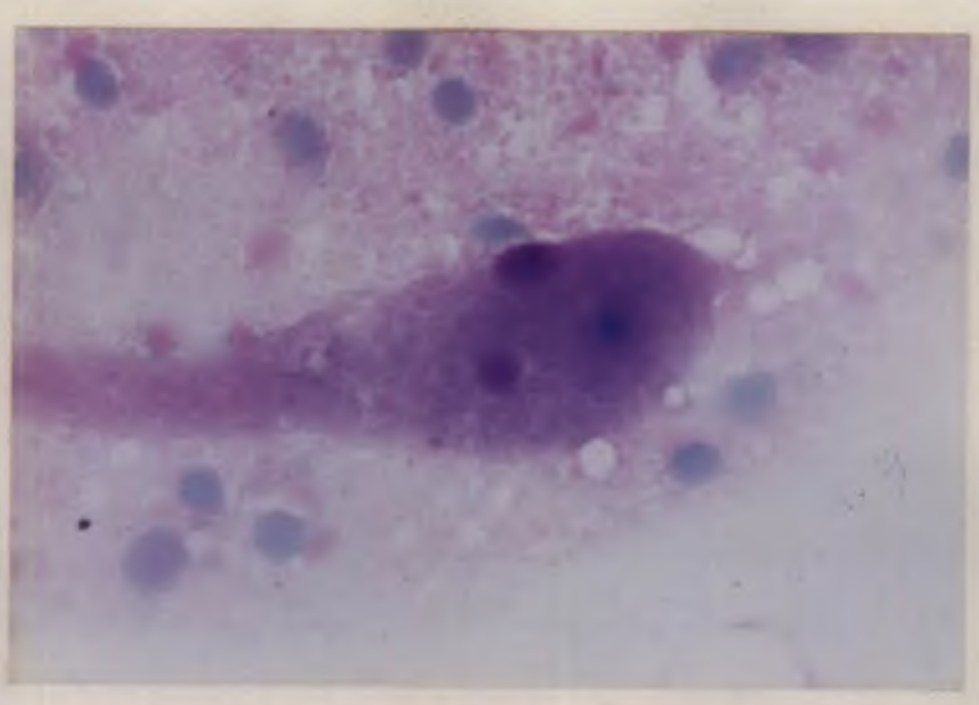


Fig.5. Impression smear from cerebellum of a cattle. Magenta red coloured Negribodies in the Purkinje cell. Sellers' x 1000

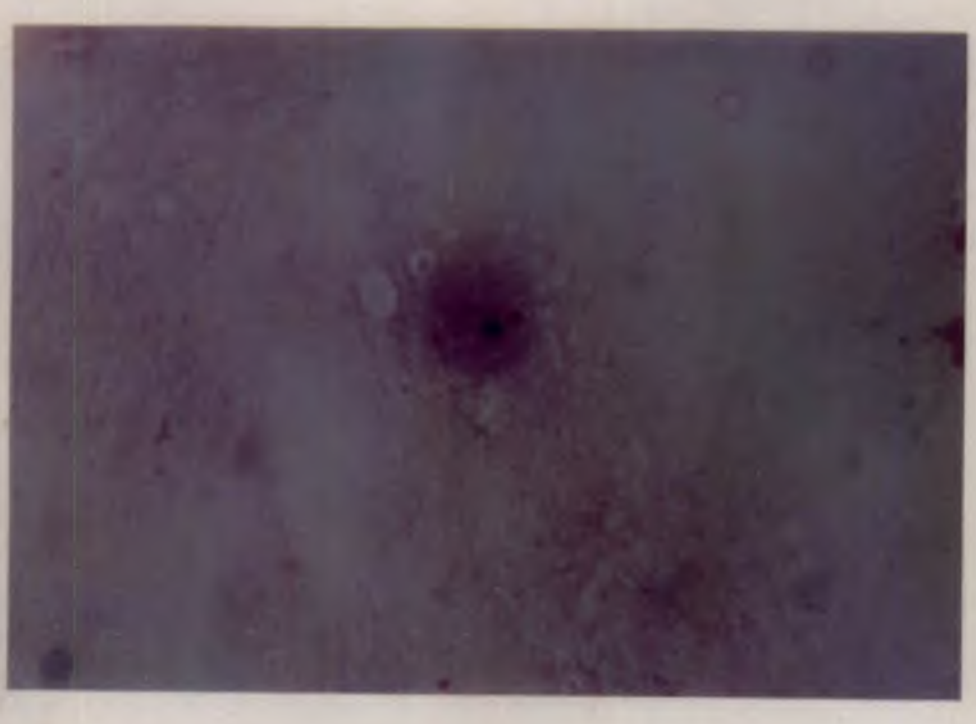


Fig.6. Impression smear from hippocampus of a dog. Magenta red coloured Negribody in the neuron. Sellers' x 1000

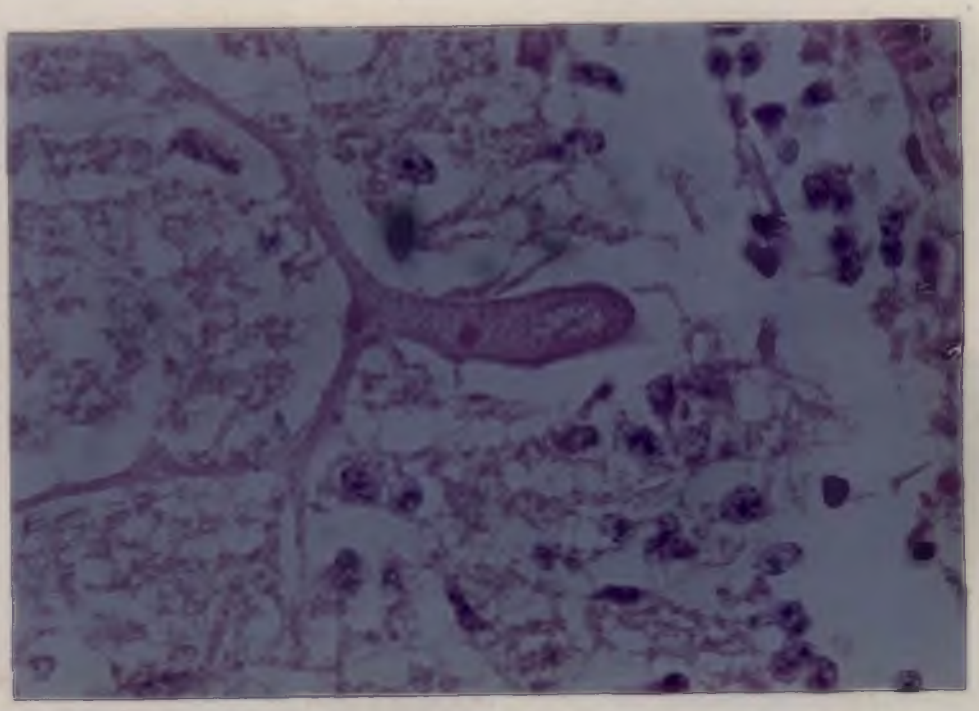


Fig.7. Cerebellum of a cow. Purple coloured Negribodies in the Purkinje cell. H & E x 1000

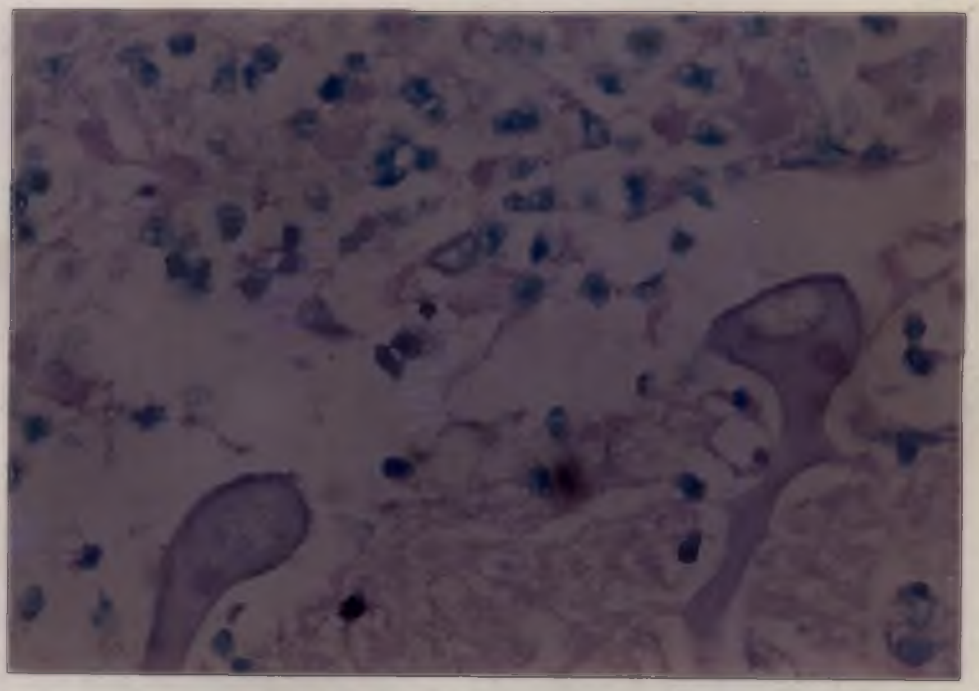


Fig.8. Cerebellum of a calf. Red coloured Negribodies in the Purkinje cell. Sellers' x 1000

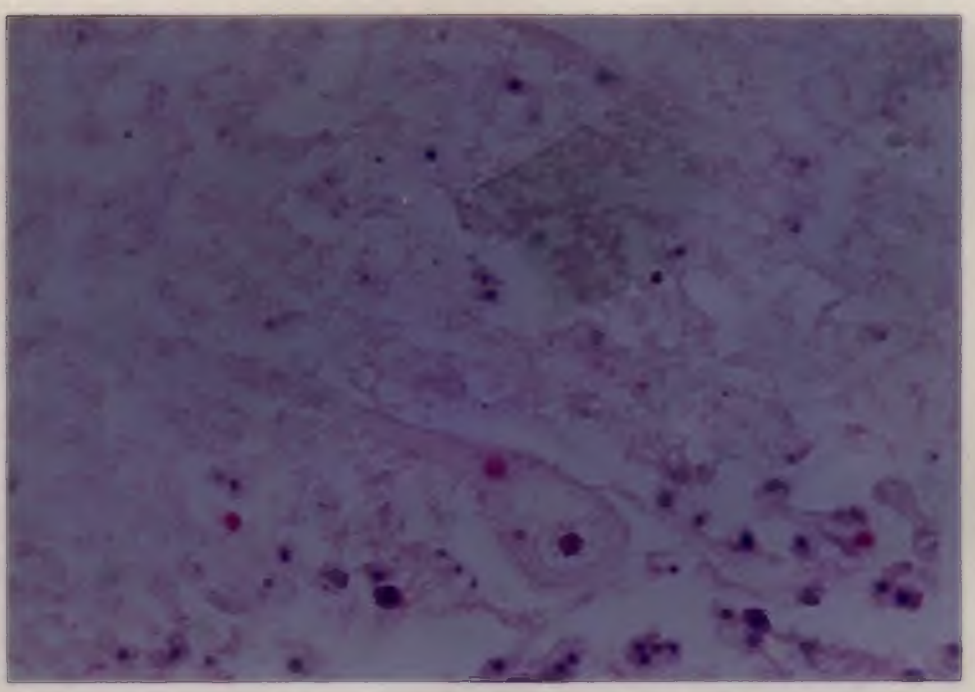


Fig.9. Cerebellum of a calf. Vermilion red coloured Negribodies in the Purkinje cell. Mann's x 1000

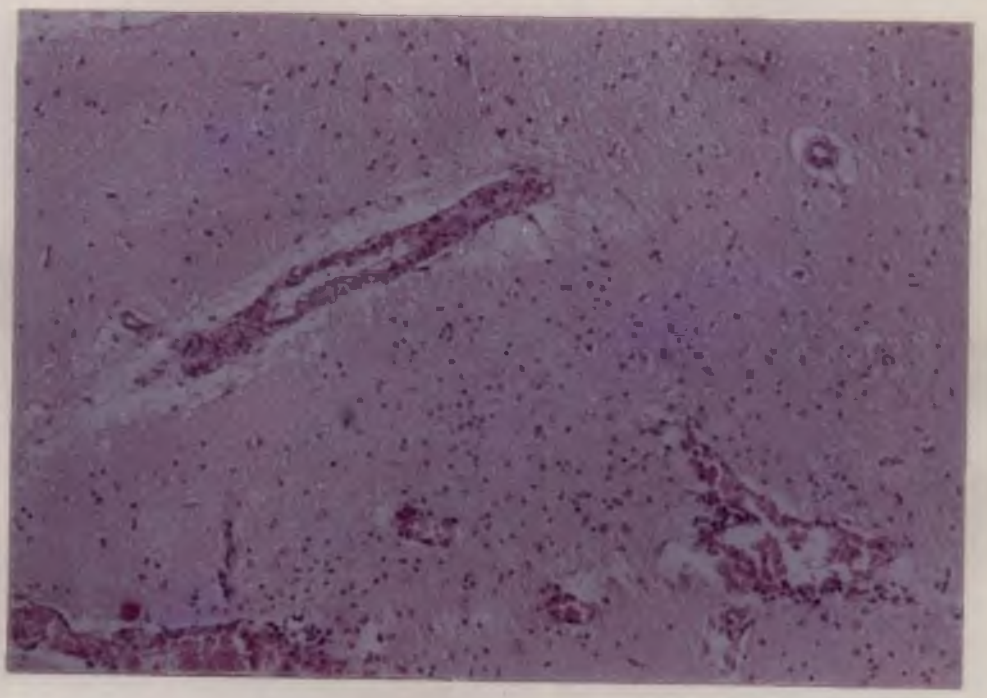


Fig.10. Cerebrum of a dog. Congestion, dilatation of Virchow-Robin space and perivascular cuffing of lymphocytes. H & E x 160

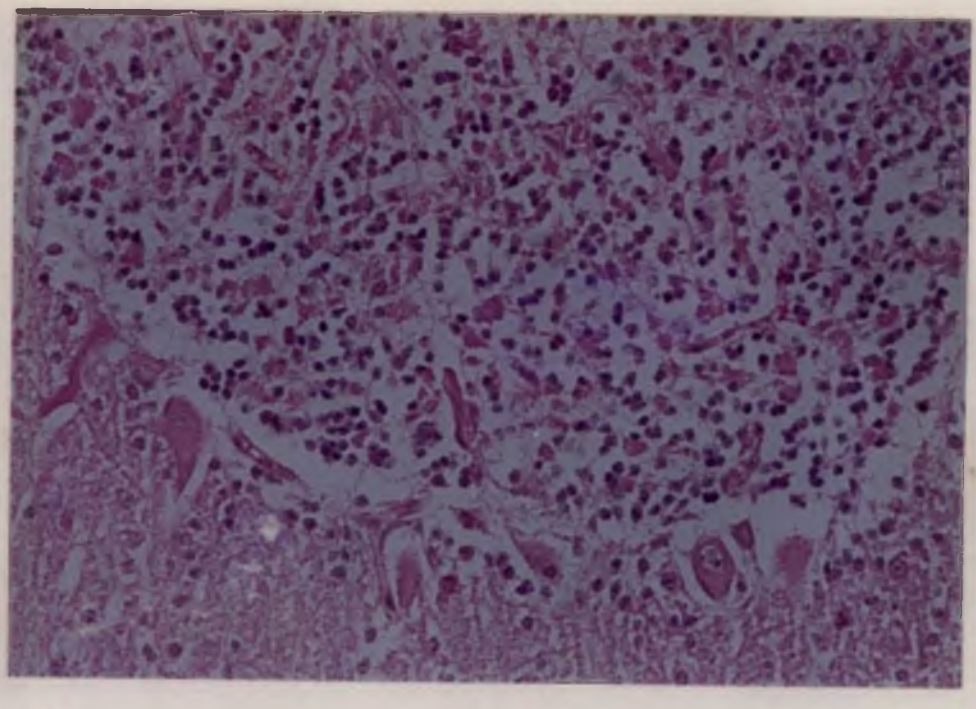


Fig.11. Cerebellum of a calf. Increased cellularity of the granular cell layer, condensation and pyknosis of the nucleus and occasional loss of the Purkinje cells. H & E x 400

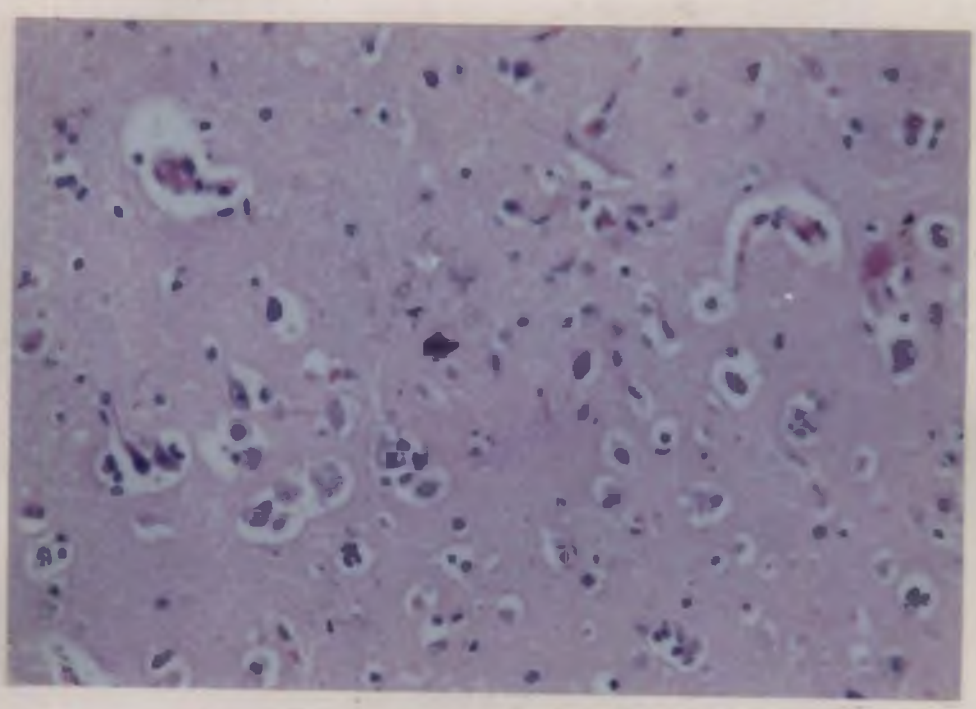


Fig.12. Hippocampus of a dog. Degeneration of the neurons, distended perineuronal space and occasional satellitosis. H & E x 400

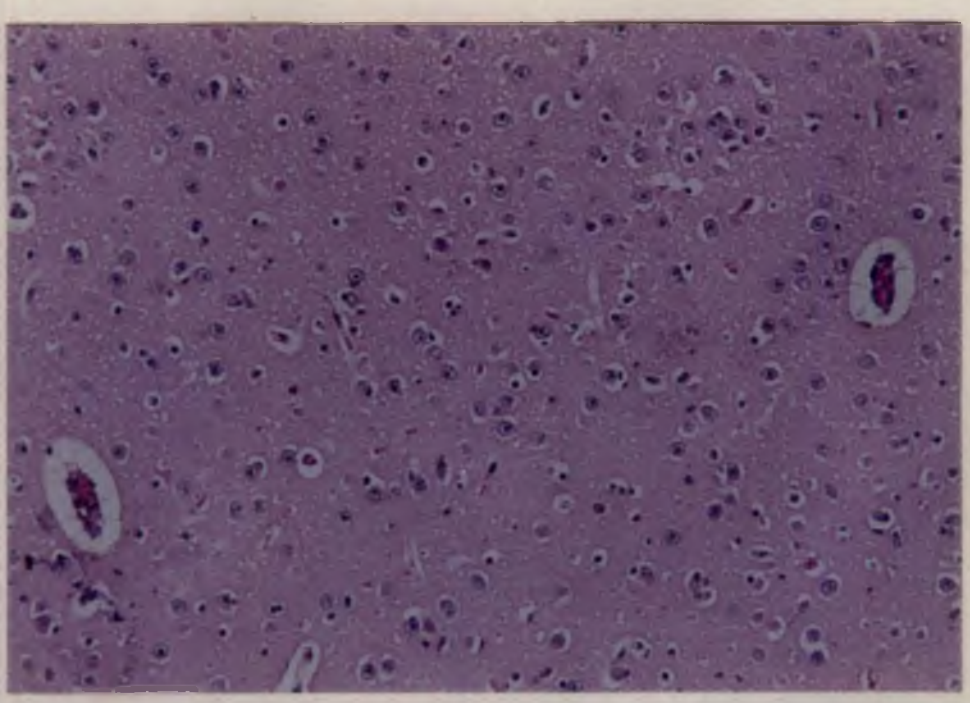


Fig.13. Hippocampus of a dog. Extensive gliosis. H & E x 400

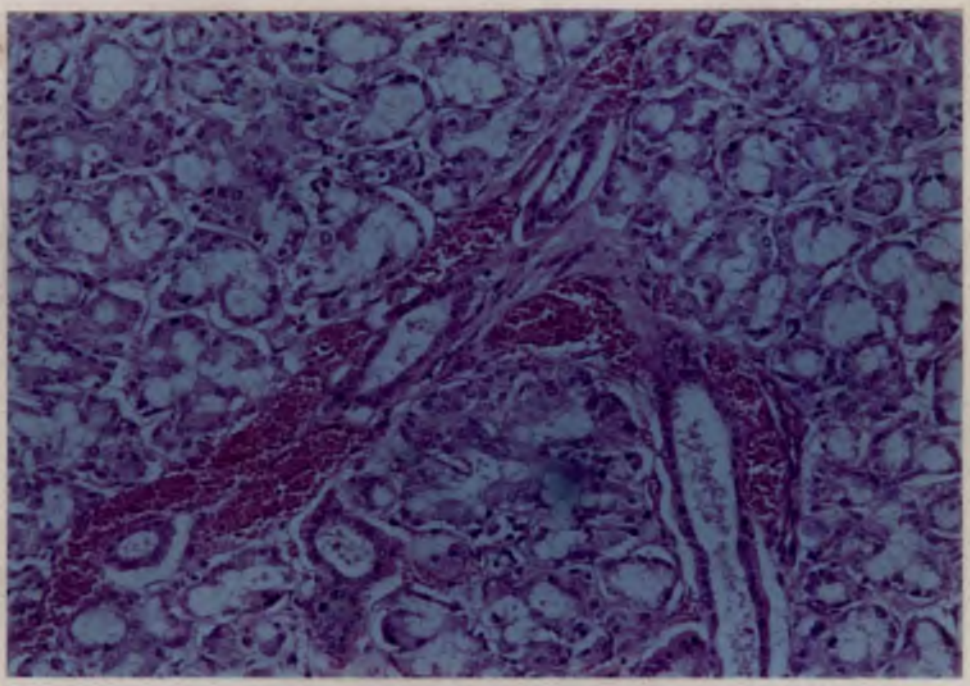


Fig.14. Submaxillary salivary gland of a dog. Congestion of the vessels, distention of the cytoplasm of the lining cells and degeneration of the gladular epithelium. H & E x 250

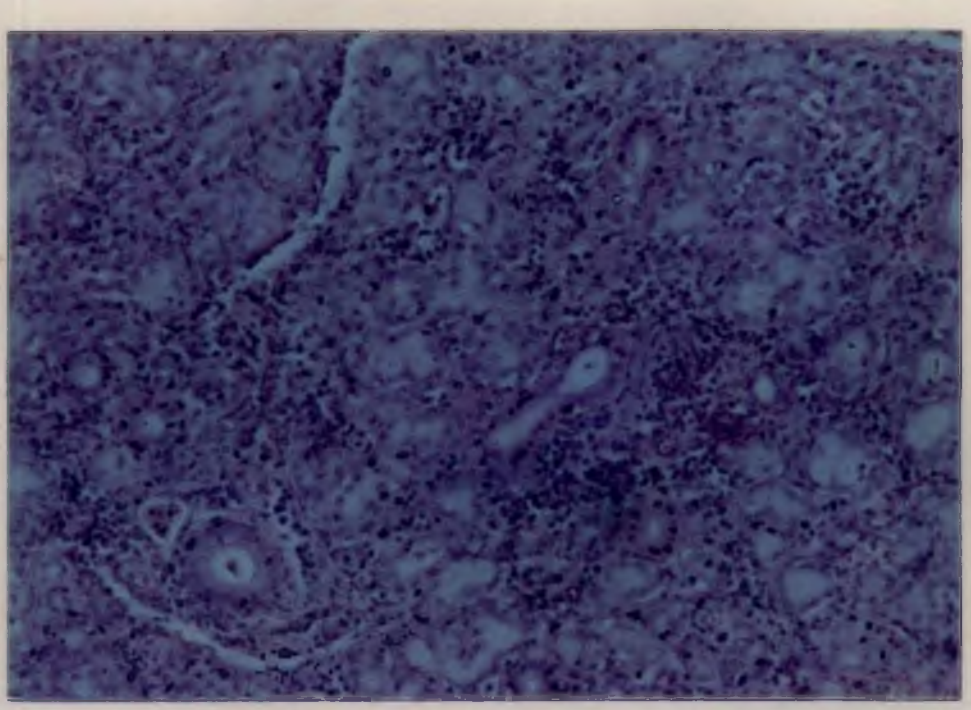


Fig.15. Submaxillary salivary gland of a dog. Sialadenitis with extensive infiltration of lymphocytes. H & E x 250

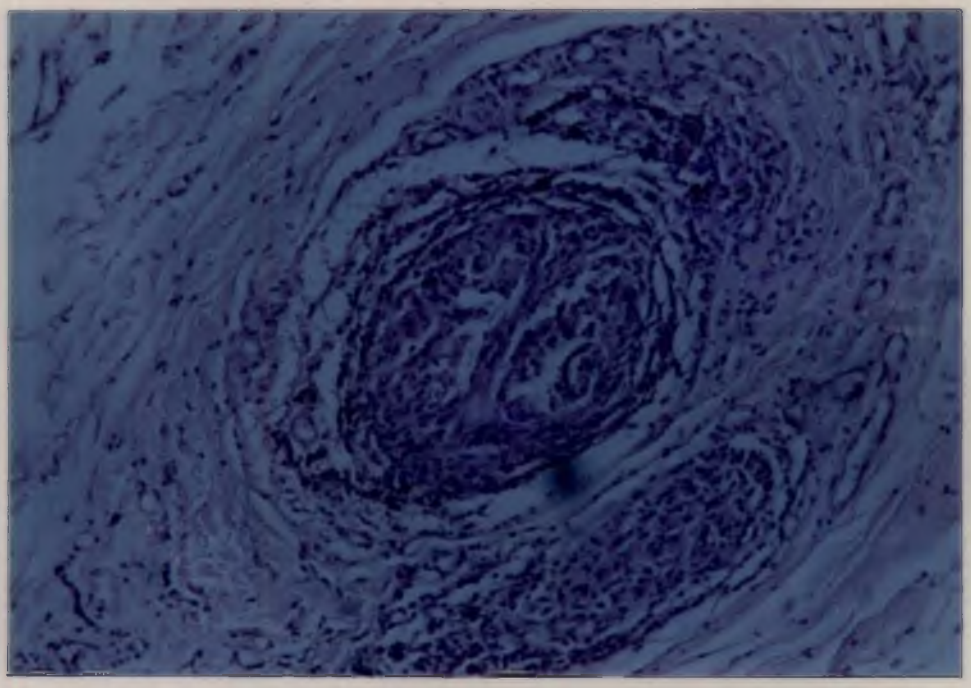


Fig.16. Trigeminal Nerve of a calf. Neuritis characterised by necrosis and infiltration of the inflammatory cells.
H & E x 160

Fig.17. Rabies specific immunofluorescence in the impression smear of a dog. Direct fluorescent staining x 400

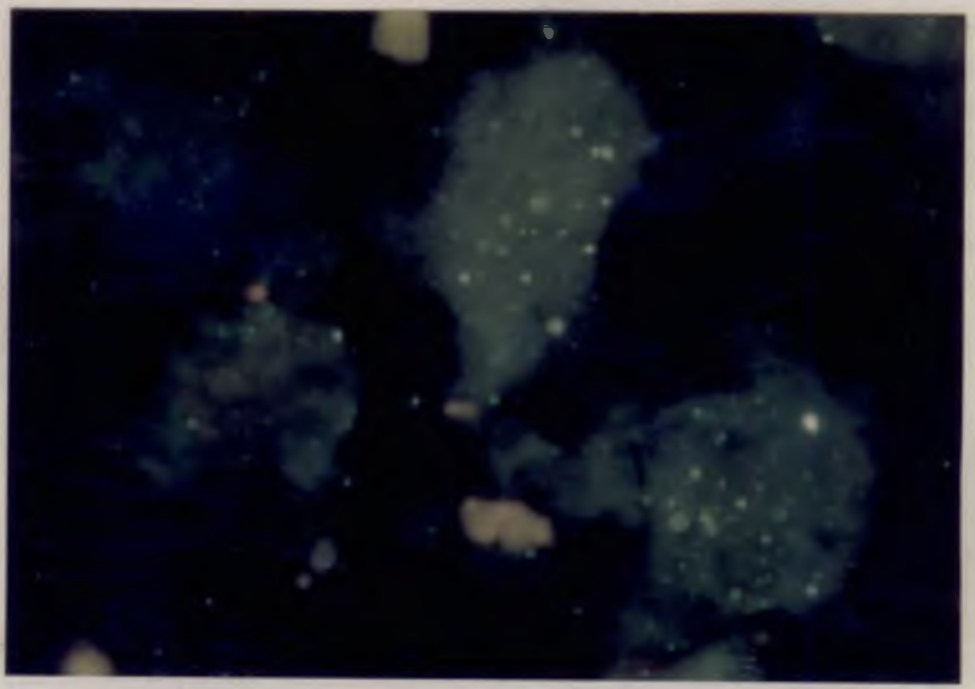


Fig.18. Rabies specific immunofluorescence in the impression smear of a calf. Direct fluorescent staining x 400

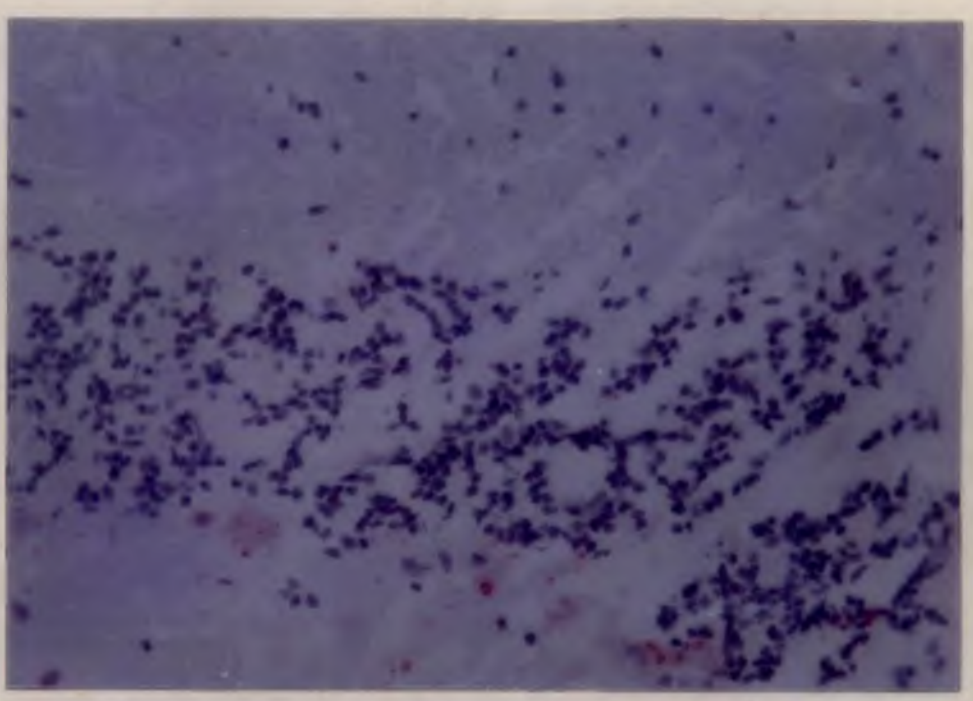


Fig.19. Immunoperoxidase reaction in a rabies positive dog. Brown coloured precipitates in the Purkinje cells of the cerebellum.
Direct immunoperoxidase staining x 400

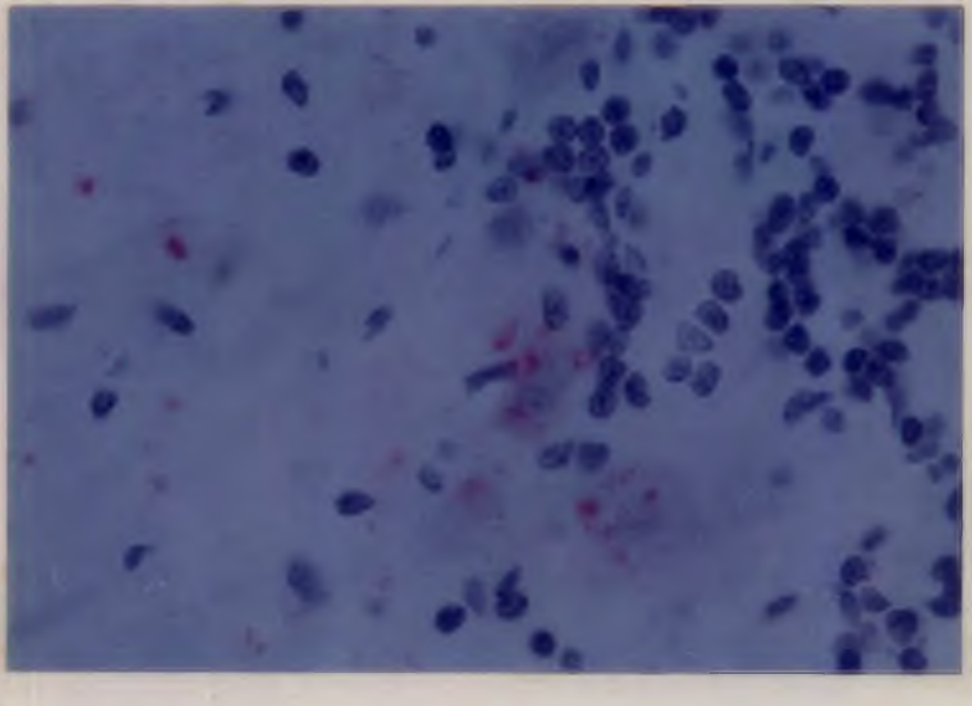


Fig.20. Immunoperoxidase reaction in a rabies positive cattle. Brown coloured precipitates in the Purkinje cells of the cerebellum.
Direct immunoperoxidase staining x 1000

Discussion

DISCUSSION

A detailed investigation on the epidemiology, clinical signs, pathologic lesions and diagnosis of rabies was undertaken. In the study, special emphasis was given to compare the results of diagnostic techniques used for rabies, namely cytological, FA and IP test.

5.1.Epidemiology

Surveillance of rabies is the basis of any programme for rabies control. This is essentialy required for planning, organizing and implementing the rabies control programmes. Prevalence of rabies in the state of Kerala based on the records available for the last 12 year period from July 1983 to June 1995 was assessed in the study. This has helped to understand the trend in the epidemiological features of the disease in the state in various domestic animals.

5.1.1.Regional distribution

The available data collected indicated that the disease is prevalent in all parts of the state, with more cases being recorded in the central zone of the state comprising the districts of Trichur, Ernakulam, Kozhikode and Palghat.

The reason for this high rate of prevalence of rabies in these districts may be attributed to the high density of domestic dog population. As per the 1987 livestock census,

the dog population in these four districts form approximately one third of the total dog population in Kerala(1.3 million). There are also reports of wolves and jackals invading the urban limits and attacking flocks of domestic livestock and dogs, especially in these districts which revealed high incidence of rabies. Moreover, places located in these districts are nearer to and easily accessible to this institution and therefore people can easily bring the rabies suspected carcasses for confirmatory diagnosis of rabies. This is the only centre in the state, where rabies diagnosis is undertaken. Trivandrum, the state capital shows less cases of rabies, in spite of having a more dense dog population.However, the data presented do not reflect the real status of the prevalence, since Trivandrum is far off from Trichur and hence animals may not be brought to the laboratory at Trichur for diagnosis. Moreover, in city like Trivandrum people are more educated and more conscious about vaccinations againt rabies. This reason might be true because, about one sixth of the total dog population in Kerala is in Trivandrum (Livestock census, 1987). Rabies in many other parts of the state is diagnosed clinically and not brought for confirmation.

5.1.2.Species distribution

Both of the epidemiological types of rabies prevailed in Kerala, the urban type involving domestic dogs and

sylvatic type, with the existence of rabies in wild species. However, the urban rabies, where transmission takes place through dogs are predominant and rabies in wild species is comparatively less significant.

According to WHO (1992) urban rabies, affecting stray and feral dogs and cats, is by far the most dangerous to man, accounting for an estimated 99 per cent of all recorded human cases and for 90 per cent of all human post-exposure treatments. In India, dogs are presumed to be the main transmitter of rabies (95 per cent),and it was estimated that there is one dog for every 10 people and the dog population is around 80 million(Chakrabarti,1993).

In Kerala, 74 per cent of the rabies positive cases during the last 12 year period were reported in dogs. In India, large population of stray dogs act as reservoirs for rabies. In the state of Kerala also, there is significant number of stray dog population, which may contribute largely to the transmission of rabies.

Though the dog is the main transmitter of rabies, the role of wild animals as a reservoir of rabies cannot be ignored. Wild species as a source of rabies in India, has been recorded by Veeraraghavan (1956). Maintenance of rabies infection in wild animals is related to their habitat, population density and annual reproductive capacity. Highly neurotropic virus rapidly kills these wild species

(Turner, 1990) and hence the prevalence of rabies in sparsely populated wild species in the state is of less concern. Even though, only meagre number of rabies cases were recorded in wild species, there might be more number of wild animals affected by rabies, but not presented for examination. Thus, rabies in wildlife is likely to be passed unrecorded, with attention being centered in domestic or semi-domestic animals like dogs, which act as a bridge between the human population and wild-life pools of infection. The disease may spread rapidly through wild population and contribute to the epizootics of rabies in domestic animals.

Prevalence of rabies in domestic animals can be, to some extent, related to the existence of the disease in wildlife. Cattle are particularly vulnerable victims for rabid carnivores. Frequently, reports from Malabar region indicate that wolves and jackals cross the forest limits, attacking the livestock and dogs. However, herbivores in general have little significance in rabies epidemiology and human rabies transmitted by rabid bovines are rare (Dierks, 1981 ; Turner, 1990). Rabies in domestic livestock of India has been reported to occur in cattle, buffalo, horse, sheep and pigs (Parthasarathy et al. 1978). Even though relatively less cases were reported from domestic livestock during the last 12 years (17 per cent, including cattle, buffalo and goat), exposure to human beings had often been

reported through attempts to examine or treat the sick animals.

Domestic cats are usually infected by a "spill-over" from other domestic or wild animals. According to World Survey of Rabies XXIII, between 1986 and 1987, 5.3 per cent of human exposure to rabies was due to cats as against 87.5 per cent by dogs, whereas reports from USA indicates cats to be the domestic animal most frequently affected (Krebs et al. 1994). During the last 12 year period, nine per cent of rabies recorded were from cats alone and hence, the role of cats in transmitting the disease to human population is significant. During the period 1993-95, rabies in cats were not reported by the Kerala state Disease surveillance Report (1993,1994 and 1995) and this may not be due to absence of the disease in the species but due to unreporting. This is revealed by the record of rabies in 114 cats during the last 12 years.

5.1.3.Year-wise distribution

From the epidemiological data it is evident that the prevalence of rabies was not static in different years of the period, which may be due to variable rabies epidemic during that period. The reason for the low prevalence of rabies in the year 1993-94 may be attributed to the preventive measures taken against the disease by the department of Animal husbandry in the recent past.

Epidemiology of rabies in animals has not been recorded and monitored properly in India. In most states, the death due to rabies in animals is confirmed only in veterinary colleges and government institutions. In Kerala, even though farmers and veterinarians are well informed and aware that the rabies diagnosis is being undertaken in the institution, frequently, rabies suspected specimens are not brought for confirmation. This is evident from the fact that the rabies was confirmed from the specimens brought from all the 14 districts of the state but recorded cases were less from the regions far away and less accessible to the institution. Actually the prevalence of rabies would be much more in the state as all cases are not recorded or are brought for confimatory diagnosis of rabies.

It cannot be disregarded that cases of rabies have to be recorded systematically, by which only the epidemiology can be defined sharply.

5.2.History and clinical signs

Rabies was diagnosed both in domestic and stray dogs. This indicates the presence and possibility of transmission within them in the urban areas. The disease was diagnosed in domestic cattle and goats also. This might be due to the transmission of the disease from the dogs which are usually allowed to move with the domestic livestock in the fields. Stray dogs, though killed and brought for examination,

showed Negribodies for rabies. This might be due to the development of complete clinical illness, before it was killed; development of such clinical illness would be the reason for them to attack and bite other animals and human beings.

With the exception of two, all the dogs were adults, one to three years old. According to Tierkel (1975) young dogs were more susceptible to rabies infection than older dogs. Usually they were let free in the streets and the possibility of spread of rabies virus among them might be more because they often fight each other in the streets. Rabies was not recorded in older cattle, this might be because they were slaughtered at young age.

The duration of illness in dogs were between five to nine days and in cattle it was between seven to nine days. The clinical episode appeared within the durations reported by previous authors for rabies (Beran, 1962 ; Bedford, 1976; Appel and Carmichael, 1979).

Except for two dogs, preventive vaccination was not taken for other animals. This indicates the paucity of awareness among the farmers and pet owners and lack of communication about vaccinations.

In the present study six of the dogs had signs of furious form and four had signs of dumb (paralytic) form. Though the clinical signs shown by dogs affected with rabies

varied widely (Bedford, 1976), the rabies positive animals showed typical signs of rabies in many cases, before death. Still, there are possibilities of missing the early signs of rabies in many instances by the owners and these are appreciated only in the later stages of the disease. However, signs of dropped jaw, ascending paralysis, coma etc. described for rabies (Chakrabarti, 1993) were not appreciated clinically.

Cat showed furious form and attacking tendency, which were described as clinical signs of rabies in that species(Bedford, 1976).

Cattle showed symptoms of anorexia, drop in milk yield, bellowing, salivation and excitement and were described as signs of bovine rabies (Hoon et al. 1995).

The clinical symptoms were mainly related to the behavioural changes. Behavior is a complex neural function governed by the limbic system and the furious and aggressive behavior may be associated with the pronounced localization of virus in the limbic system. It may be that aggression in rabies is related to the presence of virus in the midbrain raphe nuclei and the medial hypothalamus, since these are inhibitory centers of aggressive behavior (Charlton, 1988).The distribution of the virus in the brain has the bearing whether rabies develops in the dumb form or furious form. The former is characterised by neuronal destruction, micro-glial proliferation and perivascular cuffing mainly in

the spinal cord and brain stem, whereas in the latter, the inflammatory reaction, vascular changes and inclusion bodies are more widespread and includes thalamus, hypothalamus, cerebellum and cerebral cortex (Chopra et al. 1980). This indicates that the clinical diagnosis is non- specific and unreliable. All the animals showing nervous signs should be considered for the differential diagnosis of rabies. WHO (1992) report also emphasised the necessity of such differential diagnosis in rabies suspected animals.

5.3.Gross lesions

It was seen from the published literature that detailed and systematic gross pathological studies were not made on rabid animals.

Predominant lesions were observed in the liver, lung, kidney and heart, in addition to the gastroenteric system.

The lesions in the liver included mild degeneration, severe necrosis, congestion and haemorrhage. Degenerative changes and congestion may be due to circulatory changes as a part of central venous congestion occurring due to pulmonary collapse and subsequent hypoxia or anoxia. Rupture of blood vessels leading to haemorrhage may be due to struggling. Kidney lesions may also be due to same sequence of events.

Pulmonary lesions were conspicuous in rabid animals. Paralysis of the respiratory muscles and asphyxia were the

cause of death in rabid animals. This leads to the pulmonary lesions like pulmonary collapse, pneumonia or congestion and edema.

Gastroenteric lesions noticed in rabid animals might be correlated to the habit of indiscriminate eating of animate and inanimate objects by the rabid dogs.

Hypertrophy and dilatation of the heart in rabid animals might be a sequelae to the pulmonary collapse. Haemorrhage and hemopericardium might be a part of circulatory disturbances due to pulmonary lesions.

Parasitic infestations were casual observations and might not have much relevance with lesions in rabid animals.

About 10 per cent of rabies affected animals showed lesions of non-suppurative encephalitis. During the study period, only five cases of rabies showed lesions of congestion, inflammation or edema. This indicates that gross lesions in the brain of rabid animals are not pathognomonic. This is in accordance with Perl (1975), who reported only little grossly visible alterations in the CNS of rabid animals.

5.4.Laboratory diagnosis of rabies

Clinical diagnosis of rabies was difficult because the symptoms were not constant and typical ; only by laboratory diagnosis it could be confirmed.

Even though, many advanced techniques are available, histological examination with classical staining techniques even now remain as the important methods for the diagnosis of rabies. Besides this, newly introduced immunofluorescence and immunoperoxidase methods were recommended and widely used for the diagnosis of rabies(Sureau et al. 1991).

All the rabies suspected specimens were subjected to these four diagnostic tests viz. Sellers' staining, histopathological studies, FA test and IP test.

All these four tests applied together detected 22 rabies cases. Out of the 22 positive animals, 12 cases were detected by demonstration of Negribodies by Sellers' staining and later by histopathological examination. Immunofluorescence was noticed in 10 out of these 12 specimens. A total of 20 specimens out of 22 gave positive results with FA technique, including the 10 cases, which showed Negribodies. IP positive reactions were seen in 19 out of 22 cases.

These three specimens which were negative in IP, were positive in FA test. The two specimens which were FA negative, were positive in all other three tests ; Sellers' staining, histopathological staining and IP test.

An evaluation of the ccmparative efficacy of these tests in the diagnosis of rabies indicated that FA test and

IP test could detect more number of cases than compared to Sellers' staining and histopathology.

5.4.1.Impression smear staining

Out of 22 confirmed cases of rabies, Sellers' rapid staining detected 12 cases positive (55 per cent). Failure to detect positive cases of rabies in 45 per cent of cases by Sellers' staining may be due to the death of the animal before the Negribodies could form within the cytoplasm of neuron. Sureau et al.(1991) reported that Negribodies were numerous and larger when the incubation period and the clinical phase of the disease were longer.In the present study, the number of Negribodies detected were frequently two and hence the incubation period in the other cases might be of shorter duration for the development of Negribodies.

Several investigations showed that the frequency of occurrence of inclusion bodies in the brain of rabid animals varied from 10 per cent to 93 per cent (Kissling, 1975; Atanasiu, 1975; Perl, 1975; Derekhshan et al. 1978). However, a quick and correct diagnosis could be made with greater reliability when specimens were fresh and impression smears were made properly. Goldwasser and Kissling, 1958; Etchebarne et al. 1960; Tustin and Smit, 1962; Subrahmanyam and Pathak, 1971 ; Anjaria and Jhala, 1985; Palmer et al. 1985 and Sahasrabuddhe and Sherikar, 1990 reported that Negribodies were detected in 50 to 70 per

cent of cases in such circumstances.During the present study,55 per cent were diagnosed on the basis of this test.

Rabies carcasses were brought from different places to the laboratory, showing advanced autolytic changes. This might be also a factor for the absence of Negribodies in the impression smears. Errors in smear preparation, defective staining procedure and improper microscopical examination also might be the possible factors for false-negative diagnosis.

In the impression smear staining procedure, the rabies virus antigens are not specifically identified by an immunological method, but merely by an acidophilic staining reaction (Gardner and McQuillin, 1980). This might be the reason for the lower sensitivity of the Sellers' method compared to FA and IP test. The reason for the failure of Wright's stain to demonstrate Negribodies satisfactorily might be also the same.

Presence of increased number of mononuclear lymphocytes, glial cells and degenerated neurons in the rabies positive impression smears might be as a result of the inflammatory reaction occuring in rabies. This could be a supplementary criteria for considering the rabies positivity in the impression smears.

Touch impression smears stained with classical Sellers' stain consisting of 2 : 1 parts of one per cent alcoholic

solutions of Methylene blue and basic fuchsin gave good results when stained for five seconds. Negribodies appeared magenta red with pale blue background staining and copper red colored erythrocytes. Staining of impression smears with other concentrations and timings of Sellers' stain didn't give convincing results for the detection and differentiation of Negribodies.

Staining is an adsorption exchange process. The adsorption exchange between the cells in the impression smear and the Sellers' stain might be complete within five seconds, during which time it gives the optimum staining. When stained for less time or more time, this adsorption exchange process might be in imbalance, reducing the staining perfection. This is in agreement with the study by Seshadri and Chandrasekaran (1963).

Working solution of Sellers' stain was usually prepared fresh by mixing the stock solutions whenever necessary. In the present study, Sellers' working solution kept at room temperature for seven to ten days period gave better results than the one prepared fresh. Atanasiu (1975) noted the improvement in quality of staining upon ageing. Mixing of these dyes may be a contributory factor for quick ripening as observed in the present study. The above modification of the Sellers' staining technique is recommended for rapid diagnosis of rabies.

Sellers' stain even though the simplest and quickest method, failed to detect Negribodies in 10 out of 22 specimens. It might be that in 55 per cent of rabies positive animals Negribodies were developed and Sellers' stain detected only these specimens showing Negribodies. Therefore, Sellers' staining of impression smears could be highly reliable and accurate for the diagnosis of rabies where Negribodies had developed, and for rapid and preliminary on the spot diagnosis, this can be recommended. It supports the findings of Tierkel(1973) who described Sellers' method as the suitable procedure that can be followed universally.

WHO (1972) reported that 20 out of 64 countries in which rabies is endemic, diagnosis was based solely on light-microscopical criteria, adopting the method of detection of Negribodies.

5.4.2.Histopathological studies

Out of 22 rabies positive cases 12 showed Negribodies in the neurons of brain sections. All Sellers' positive samples revealed Negribodies in sections also. So when the diagnosis was made by Sellers' technique, histopathological examination has less relevance.

In addition to H & E, special stains like Mann's stain and Sellers' stain were employed for Negribodies.

Sellers' stain for histopathology was simple, easy and stained Negribodies satisfactorily. Mann's methyl blue eosin method was considered a classical method and gave excellent results for the demonstration of Negribodies. But it stained erythrocytes and Negribodies bright red, required 14-24 h for completing the staining procedure and some dexterity for full success.

The H & E staining was the simplest method of staining sections and gave satisfactory results for both Negribodies and other histopathological alterations. In rabies positive cases, where Negribodies were not developed, characteristic histopathological change in the brain helped for the diagnosis. This is in agreement with Lepine (1973), who considered H & E as the good method for staining rabies suspected sections, followed by Mann's stain. In the present study, H & E was found satisfactory for routine histopathological confirmation of rabies.

5.4.2.1.Central Nervous System

In this study 16 out of 22 cases (73 per cent) had lesions of non-suppurative policencephalomyelitis. Neuronal changes varied from mild degeneration to necrosis, particularly in the hippocampus and cerebrum. Other changes in histological sections, gliosis, satellitosis, neuronophagia and perivascular cuffing with mononuclear cells, predominantly lymphocytes, were seen in rabies

positive cases. This was in accordance with the changes described by Lepine, 1973.

Encephalitis and presence of inclusion bodies in histological sections were reported to vary widely between 20 per cent to 67 per cent (Derekhshan et al.1978; Nayak et al. 1982; Zimmer et al. 1990; Burnes et al.1991). According to Sureau et al.(1991), histopathological procedure was a relatively slow method which still remains as the basic technique for diagnosis of rabies. Encephalitis negative rabies positive cases can occur and hence it is recommended that all rabies suspected, Negribody negative (or encephalitis negative) specimens should be subjected to other techniques for confirmation of diagnosis.

5.4.2.2.Salivary glands

When submaxillary and parotid salivary glands were examined for histological changes of rabies positive cases, 15 out of 22 rabies cases showed salivary gland lesions. Both submaxillary and parotid salivary glands were affected.

Since the virus replicates in the salivary gland acini (Jubb et al. 1993) and is secreted in the saliva (Nair et al.1978; Cortes et al.1979 and 1987; Jayakumar and Ramadass, 1991), it might cause considerable damage to the cells of the salivary gland (Dierks et al.1969). The histological alterations when recognised might indicate the possibility of rabies virus secretion, indicating the risk of the

infection being transmitted to exposed persons. Hence examination of histological changes in the salivary gland becomes significant and would be valuable in situations where tests for rabies diagnosis are not available.

Nayak et al.(1982) found 50 out of 55 total rabies positive cases to show lesions in the salivary gland. In the present study 15 out of 22 cases had sialadenitis. This indicates the possibility of rabies virus causing inflammatory reaction in the salivay gland.

There was no evidence for the presence of rabies virus inclusions. The absence of inclusion bodies in the salivary gland epithelium might be due to the lack of suitable biochemical environment in these cells, unlike in the neurons of the nervous system.

5.4.2.3.Trigeminal nerve

In the present study, inflammation of the trigeminal nerve in rabies positive cases was noted.

The trigeminal nerve is the largest of cranial nerves (Vth), the ophthalmic, maxillary and mandibular branches of which supply to the muscles of the face and mastication. Wright (1953) reported the possibility of virus travelling through the nerve fascicules (in the tissue spaces in the epineurium, perineurium or between the nerve fibers). Neuritis of the trigeminal nerve indicates that the virus might be passing through the nerve fasciculus. The damage

to the trigeminal nerve supplying to the muscles of the head and face might be a contributory factor for the clinical signs in rabid animals.

5.4.3.Fluorescent antibody test(FA test)

In the present study, FA test detected 20 of 22 total rabies positive cases (91 per cent). It included 10 cases which showed Negribodies in Sellers' staining and histopathological sections. FA test failed to detect rabies in two cases which were positive for Negribodies. In three cases rabies positivity was decided based only on FA test. These three cases were negative for Negribodies and IP test.

FA test detected maximum number of rabies positive specimens in this study. and the results are in accordance with Derakhshan et al.1978; Anjaria and Jhala,1985; Sahasrabuddhe and Sherikar, 1990; Zimmer et al. 1990; Jayakumar et al.1995a and Hamir et al.1996, who showed FA test to give maximum sensitive results in the specimens without deterioration.

The failure of FA test to detect rabies antigen in two cases may be due to inadequate sampling. On repeated occasions Dean et al. (1963) found the brain to be positive for rabies when smears prepared from the hippocampus, cerebellum or the cerebral cortex were negative. In the present study, impression smears were prepared from the

hippocampus or cerebellum and that might be the reason for missing rabies antigen in these two cases. Examination of more number of smears from different parts of the brain might reduce the possibility of not detecting rabies antigen by FA test.

It was notable that these two cases were positive for Negribodies in Sellers' staining and histopathology. Therefore the failure of FA the test in these two cases could be due to the defect in processing of the slide, staining procedure or screening, during which the antigen loss or reading error might have occurred. The presence of very small amount of antigen in the smear might also be a reason for this failure.

FA test, though detected maximum number of cases, required fresh specimens, fluorescent microscope and dexterity in doing the test.

5.4.4.Immunoperoxidase test(IP test)

In the present study, IP test detected 19 out of 22 positive specimens(86 per cent). In the other three specimens it was negative.

The failure of IP test to detect rabies in two cases might be due to the processing methods undertaken for IP test by routine chemical processing methods (viz. fixation, dehydration, clearing etc). This indicated that

the antigen is more fragile when treated by the IP test. It was supported by the results of Atanasiu et al.1974 and Jayakumar et al.1994.

Hamir and Moser (1994) were of the opinion that the chances of detection of a rabies positive case by IP test were more when multiple areas of the brain (cerebrum, cerebellum and brainstem) of a suspected animal were examined. As per Chopra et al.(1980) the inflammatory changes in the dumb (paralytic) form was mainly seen in the spinal cord and brainstem, whereas in the furious form, they were more widespread in the thalamus, hypothalamus, cerebellum and cerebral cortex. Hence, the examination of multiple areas of the brain could exclude the possibility of false-negative diagnosis. In the present study, sections of the hippocampus, cerebrum and cerebellum were routinely examined by IP test for rabies. It would be preferable to include parts of the cerebrum (anterior, middle and posterior), cerebellum, hippocampus and brainstem to minimize the possibility of error in IP test.

The brown precipitations in the IP reactions were comparable to the size of the Negribodies in the corresponding tissue sections stained by H & E. This is in accordance with the observations of Hamir et al. (1992a and 1992b) and this might be because of the specific immunologic binding of the rabies antibody and antigen, that could detect even small amount of antigens present.

The IP reaction was moderately faint and made evaluation of the case sometimes difficult. The use of polyclonal antibodies for enzyme conjugation may be the reason for this faint reaction as against the use of monoclonal antibodies. Hamir and Moser (1994) when they used both monoclonal and polyclonal antibodies to examine 83 positive tissue sections, found both of them corresponding except in three cases, where polyclonal antibodies showed faint reaction. Monoclonal antibody preparation gave more intense and discrete positive reaction. In the present study, equine rabies globulins obtained from the Central Reserch Institute, Kasauli was utilized. The use of monoclonal antibodies specifically raised against rabies nucleocapsid might improve the test quality. The quality of the dye, Di-amino-benzidine (DAB) might also be a factor for this faint reaction and good quality DAB can be recommended for better IP reactions.

Sixteen paraffin blocks of different areas of the brain from six rabies positive animals were sent to the Veterinary Diagnostic Laboratory, College of Veterinary Medicine, Oregon State University, USA for counter-checking the results of rabies diagnosis. It included samples from three dogs and one each from a cat, cattle and calf. On examination by immunohistochemical staining using antirabies monoclonal antibodies, nine blocks showed positive reactions and confirmed rabies in five cases. All the four

blocks from the sixth case were negative for rabies. The results of the immunohistochemical test for rabies done by the Veterinary Diagnostic Laboratory, College of Veterinary Medicine, Oregon state University,USA showed that multiple areas of the brain should be examined for the detection of rabies.

From the study, it was seen that Sellers' stain was simple and satisfactory for the rapid diagnosis of rabies. It can useful to detect all cases of rabies in which Negribodies had developed. Histopathology, is relatively slow method for the diagnosis of rabies. It could be useful for screening histological alterations of the rabies affected brain. FA and IP test were equally good in detecting rabies. FA test required costly microscope and dexterity in performing the test. IP test, though required only light microscope, it was not sufficiently rapid for making decisions in rabies suspected cases and could be considered best for the retrospective study where formalin-fixed, paraffin embedded tissues are available and these could be utilized for any length of period.

Summary

SUMMARY

A detailed investigation of the epidemiology, clinical signs, pathologic lesions and diagnosis of rabies was undertaken. Special emphasis was given to compare the results of diagnostic techniques used for rabies.

One thousand three hundred and six cases of rabies were reported in animals during the 12 year period from July 1983 to June 1995 in the state of Kerala. This constituted 969 dogs, 131 cattle, 114 cats and 86 goats. Six were in wild animals like the fox(two), monkey(one), bandicoot(one), mongoose(one) and civet cat(one). The disease was reported from all 14 districts of the state with more cases in Trichur (22 per cent) followed by Ernakulam (18 per cent), Kozhikode (14 per cent) and Palghat (10 per cent). The cases reported from Trivandrum (1.6 per cent), Pathanamthitta (1.5 per cent) and Kasargod (0.7 per cent) were very low.The prevalence of rabies was not static in different years of the period and was high during the year 1990-91 (158 cases) and low during 1993-94 (72 cases).

Rabies in dogs were often reported at the age of one to three years. It was seen mostly in male dogs. The clinical illness in dogs were 5-9 days where as in cattle it was 7-9 days. Both furious and dumb form (paralytic) of rabies were

noted in dogs. Cats showed furious form. Anorexia, reduction in the milk yield and incessant bellowing were the common symptoms observed in rabid cattle.

Gross lesions were not pathognomonic for rabies. Lesions were predominantly seen in the liver, lung, kidney and heart, in addition to the gastroenteric system. Gross lesions in the brain were seen in about 10 per cent of rabies cases. They mostly consisted of meningitis. Congestion of meningeal vessels and edema were the other lesions noticed.

One hundred and six rabies suspected animals (62 dogs, 11 cats, 16 cattle, five calves, seven goats, two civet cats and one each of bandicoot, leopard and squirrel) were examined for the confirmatory diagnosis of rabies by employing Sellers' staining, histopathological staining, FA test and IP test and 22 of them were confirmed positive for rabies (13 dogs, eight cattle and a cat).

Sellers' staining and histopathological staining showed Negribodies in 12 cases. Fluorescent antibody test (FA test) and Immunoperoxidase test (IP test) detected rabies in 20 and 19 cases respectively. The FA test and IP test were demonstrated to be very effective and equally good.

Negribodies were demonstrated clearly when the brain impression smears were stained for five seconds with Sellers' stain containing 2 : 1 parts of one per cent

alcoholic solutions of methylene blue and basic fuchsin. The quality of the stain was improved when working solution of the Sellers' stain was kept at room temperature for 7-10 days. This was identified to be the simple test to be employed in the field laboratories.

Non-suppurative encephalomyelitis characterised by perivascular cuffing, gliosis and occasional satellitosis were noticed in histological sections of the brain. The salivary gland sections showed sialadenitis characterised by extensive infiltration of lymphocytes. Perineural edema, fascicular degeneration and neuritis were observed in the trigeminal nerve.

Sixteen paraffin blocks from different areas of the brain from six positive specimens were counter checked with the Veterinary Diagnostic Laboratory, College of Veterinary Medicine, Oregon State University, USA. On examination by immunohistochemical staining using antirabies monoclonal antibodies, nine blocks showed positive reactions and confirmed rabies. Examination of multiple areas of the brain is suggested to avoid the false-negative diagnosis.

References

REFERENCES

Anjaria,J.M. and Jhala,C.I. (1985).Immunoperoxidase reaction in diagnosis of rabies. Int. J.Zoon. **12** (4):267-275.

Appel,M.J. and Carmichael,L.E. (1979). In: Canine Medicine. Vol.I. 4th Edn. Ed. Catcott,E.J. Amer. Vet. Pub. Inc. p.36.

Atanasiu,P. (1973). The immunoperoxidase reaction for demonstration of rabies virus. In: Laboratory Techniques in Rabies. 3rd Edn. Eds. Kaplan, M.M. and Koprowski,H. WHO Monograph Series No.23. WHO, Geneva, pp. 358-360.

Atanasiu,P.(1975). cited by Baer (1975).

Atanasiu,P., Perrin,P., Favre,S., Chevallier,G. and Tsiang,H. (1974). Immunofluorescence and immuno-peroxidase in the diagnosis of rabies. In: Viral Immunodiagnosis. Eds. Kurstak,E. and Morisset.R. Academic Press,London. pp.141-155.

Avrameas,S. and Ternynck,T. (1969). cited by Atanasiu,P. (1973).

Babes,V. (1912). cited by Baer (1975).

Baer,G.M. (1975). In: The Natural History of Rabies. Vol.I. Academic Press, London.

Basheer,A.M. (1995). Control of Rabies in India. In:Summer Institute on recent advances on the epidemiology and diagnosis of Zoonotic diseases. Department of Preventive Medicine, Madras Veterinary College, Madras. pp.34-37.

*Beauregard,M., Boulanger,P. and Webster,W.A. (1965). The use of fluorescent antibody staining in the diagnosis of rabies. Can. J. Com. Med. **29** : 141.

Bedford,P.G.C. (1976). Diagnosis of rabies in animals. Vet. Rec. **99** : 160-162.

Benjamin,M.M. (1978). In: Outline of Veterinary Clinical Pathology. 3rd Edn. Iowa State University Press, Ames, Iowa. pp. 25-37.

Beran,G.W. (1962). J. Phil. Anim. Indus. **25** : 69. cited by : Chakrabarti (1993).

Bourhy,H.,Rollin,P.E.,Vincent,J. and Sureau, P. (1989). Comparative evaluation of the fluorescent-antibody test, virus isolation from tissue culture and enzyme immuno-diagnosis for rapid laboratory diagnosis of rabies. J. Clin. Microbiol. **27** (3) : 519-523. Vet. Bull.(1989) **59** (8) :Abstr. 4614.

Brochier,B., Costy,F., Coninck,V.DE., Hallet,L., Bourhy, H., Peharpre,D., Mosselmans,F., Beyer,R., Lecomte,L., Mullier,P., Bauduin,B. and Pastoret,P.P. (1995). Annales de Medecine Veterinaire **139** (4) : 263-266, 269-273. Vet. Bull. (1996) **66** (1) : Abstr.114.

Burnes, M.J., Paz,M.A., Solares,C.A., Mendez,V.G., Hernandez,R.A., Valenzuela,A.A. and Ginez,Z.V. (1991). Comparative techniques for the diagnosis of rabies and its incidence in Tamaulipas, Mexico in 1987. Revista Latinoamericana de Microbiologia **33** (2/3) : 209-217. Vet. Bull. (1992) **62** (11) : Abstr.6786.

Buxton,A. and Fraser,G. (1977). Rabies. In: Animal Microbiology. Vol.II. Rickettsias and Viruses. Blackwell Scientific Publications, London. pp. 553-567.

Carski,T.R., Wilsnack,R.E. and Sikes,R.K. (1962). Pathogenesis of Rabies in wildlife. II. Fluorescent antibody studies. Am. J. Vet. Res. **23** (96):1048-1051.

Chakrabarti,A. (1993). In: A textbook of Preventive Veterinary Medicine. 1st Edn. Kalyani Publishers, New Delhi. pp.166-189.

Charlton,K.M. (1984). Rabies : Spongiform lesions in the brain. Acta Neuropathologica **63** (3) : 198-202. Vet. Bull. (1984) **54** (8) : Abstr.4970.

*Charlton,K.M. (1988). The pathogenesis of rabies. In: Rabies. Eds. Campbell,J.B. and Charlton.K.M. Kluwer Academic, Boston. pp. 101-150.

Charlton,K.M., and Casey,G.A. (1979). Experimental Rabies in skunks and foxes. Immunofluorescence, Light and Electron microscopic studies. Lab. Invest. **41** (1): 36-44.

Charlton,K.M., Casey,G.A., Webster.W.A. and Bundza,A. (1987). Experimental Rabies in skunks and foxes. Pathogenesis of the spongiform lesions. Lab. Invest.57 (6) : 634-645.

*Chopra,J.S. Banerjee,A.K. Murphy,J.M. and Pal,S.R. (1980). Paralytic rabies : a clinico-pathological study. Brain **103** : 789-802.

Choudhuri,P.C. (1995). Epidemiology of rabies in India. In: Summer Institute on recent advances on the epidemiology and diagnosis of zoonotic diseases. Department of Preventive Medicine, Madras Veterinary College, Madras. pp. 26-27.

Coons,A.H. and Kaplan,M.H. (1950). Localization of antigen in tissue cells. II. Improvement in a method for the detection of antigen by means of fluorescent antibody. J. Exp. Med. **91** : 1

Cortes,V.DE A.,Ito,F.H., Vasconcellos,S.A.and Cortes,J.DE A. (1987). Demonstration of rabies antigens in the submaxillary and parotid salivary glands of naturally infected dogs. Use of the fluorescent antibody (FA) technique on smears of semi-ground salivary gland tissue. Revista de Faculdade de Medicina Veterinaria e Zootecnia da Universidade de Sao Paulo **24** (2) : 181-186. Vet. Bull. (1989) **59** (5) : Abstr. 2175.

Cortes,V.DE A., Paim,G.V. and Oliveira,M.C.G.DE. (1979). Diagnosis of rabies in dogs. I. Comparison of saliva and brain samples. Revista de Saude Publica, Brazil. **13** (4) : 353-356. Vet.Bull.(1980) **50** (9) : Abstr.5626.

Dabrowska,A., Sadowski,A. and Dobkiewicz,Z. (1996). Occurrence of rabies in the Opole region (Poland).Medycyana Weterynaryjna **52** (1) : 35-37. Vet. Bull. (1996) **66** (6) : Abstr.3619.

Das,S.K., Sarkar,P. and Sen,G.P. (1985). Evaluation of immunoperoxidase test in routine diagnosis of rabies in animals. Indian J. Anim. Sci. **55** (12) : 979-982.

Dean,D.J. and Abelseth,M.K. (1973). The fluorescent antibody test. In: Laboratory Techniques in Rabies. 3rd Edn. Eds. Kaplan,M.M. and Koprowski,H. WHO Monograph Series No.23. WHO, Geneva, 1973. pp.73-80.

*Dean,D.J., Evans.W.M. and McClure,R.C. (1963). Pathogenesis of rabies. Bull. Wld Hlth Org. **29** : 803-811.

Derakhshan,I., Bahmanyar,M., Noorsalehi,S., Fayaz,A., Mohammad, M. and Ahouraii,P. (1978). Light-microscopical Diagnosis of rabies. A Reappraisal. Lancet **II** (8059) : 302-303.

Dierks,R.E. (1969). Am. J.Pathol. **54** : 251. cited by : Chakrabarti (1993).

Dierks,R.E. (1981). In: Diseases of cattle in the tropics. Eds. Ristic,M, McIntyre,I. and Nijhoff Pub. p.107. cited by Chakrabarti (1993).

Dierks,R.E., Murphy,F.A. and Harrison,A.K. (1969) Extraneural rabies virus infection. Virus development in fox salivary gland. Amer. J. Path. **54** : 251-273.

Diesch,S.L., Hendricks,S.L. and Currier,R.W. (1982). The role of cats in human rabies exposures. J. Am. Vet. Med. Ass. **181** (12) : 1510-1512.

Dinakaran,A.M. (1980). Survey of incidence of rabies in Bandicoots in Madras city by FAT. M. V. Sc.,thesis submitted to TamilNadu Agricultural University. TamilNadu.

Edelsten,R.M. (1995). Epidemiology and control of rabies in Malawi. Trop. Anim. Hlth Prod. **27** (3) : 155-163.

Emmons,R. (1986). cited by : Trimarchi and Debbie (1991).

Etchebarne,M., Bernal,P.G. and Leyton,G.R. (1960). Purification of rabies antibodies in horse serum and diagnostic importance of the fluorescent antibody technique. J. Immunol. **84** : 6-9.

*Frothingham,L. (1920). Something about glanders and rabies. Cornell Vet. **10** : 163-175.

Foley,G.L.and Zachary,J.F. (1995). Rabies-induced spongiform change and encephalitis in a heifer. Vet. Pathol. **32** (3) : 309-311.

Gardner,P.S. and McQuillin,J. (1980). In: Rapid virus diagnosis. Application of immunofluorescence. 2nd Edn. Butterworths, London. pp. 174-184.

Genovese,M.A. and Andral,L. (1978). Comparison of two diagnostic tests of rabies: immunofluorescence and immunoperoxidase. Recueil de Medecine Veterinaire **154** (7/8) : 667-671. Vet. Bull. (1979) **49** (5) : Abstr.2471.

Goldwasser,R.A. and Kissling,R.E. (1958). Fluorescent antibody staining of street and fixed rabies virus antigens.Proc. Soc. Exptl. Biol. Medi. **98** : 219-223.

Goldwasser,R.A., Kissling,R.E. Carski,T.R. and Hosty,T.S. (1959). Fluorescent antibody staining of rabies virus antigens in the salivary glands of rabid animals.Bull.WHO. 20 : 579.

Gonzalez,C. and Stephano,H.A. (1984). Histopathology of central nervous system in rabid dogs. Veterinaria, Mex. 15 (1) : 39-52.

Gradwohl,R.B.H. (1943). Clinical laboratory methods and diagnosis. Vol. I. 3rd Edn. Henry Kimpton, London.

Griffin,C.W. (1984). Performance evaluation critique. Fluorescent rabies antibody test 1983-1984, U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control, Atlanta, GA. cited by : Trimarchi and Debbie (1991).

Hamir,A.N. and Moser,G. (1994). Immunoperoxidase test for rabies : utility as a diagnostic test. J. Vet. Diagn. Invest. 6 : 148-152.

Hamir,A.N., Moser,G. Fu,Z.F., Dietzschold,B. and Rupprecht,C.E. (1995a).Immunohistochemical test for rabies : identification of a diagnostically superior monclonal antibody. Vet. Rec. 136 : 295-296.

Hamir,A.N., Moser,G. and Rupprecht,C.E. (1992a). A five year (1985-1989) retrospective study of equine neurological diseases with special reference to rabies. J. Comp.Path. 106 : 411-421.

Hamir,A.N., Moser,G. and Rupprecht,C.E. (1992b). Morphologic and immunoperoxidase study of neurologic lesions in naturally acquird rabies of raccoons. J. Vet. Diagn.Invest. 4 : 369-373.

Hamir,A.N., Moser,G. and Rupprecht,C.E. (1995b). Immunohistochemical test for rabies : utility of monoclonal antibody. Vet. Pathol. 32 (5) : 574.

Hamir,A.N., Moser,G. Wampler,t., Hattel,A., Dietzschold,B. and Rupprecht,C.E.(1996). Use of a single-nucleocapsid monoclonal antibody to detect rabies antigen in formalin-fixed, paraffin-embedded tissues. Vet. Rec.138 : 114-115.

Hamir,A.N. and Rupprecht,C.E. (1990). Absence of rabies encephalitis in a raccoon with concurrent rabies and canine distemper infections. Cornell Vet. 80 : 197-201.

Hoon,J.K. Kyung,E.H., Joo,H.S. Chan,Y.B. and Dhunk,Y.Y. (1995). Recent occurrence of rabies if Korea Republic (1993-1994). R. D. A. J. Agric. Sci. Vet. 37 (1) : 508-518. Vet. Bull. (1996) 66 (4) : Absrt.2243.

Inchiosa,M.A. (1964). Direct biuret determination of total protein in tissue homogenates. J. Lab. Med. 63 : 319-324.

Jayakumar,R., Nachimuthu,K. and Padmanaban,V.D. (1994). A comparison between Avidin-Biotin Peroxidase Complex (ABC) and unlabelled antibody (PAP) procedures in rabies diagnosis. Indian Vet. J. 71 (9) : 866-869.

Jayakumar,R., Nachimuthu,K. and Padmanaban,V.D. (1995a). A Dot Enzyme Linked Immunosorbent Assay (DOT ELISA) : Comparison with standard fluorescent antibody test (FAT) for the diagnosis of rabies in animals. Comp. Immun. Microbiol. Infect. Dis. 18 (4) : 269-273.

Jayakumar,R., Nachimuthu,K. and Padmanaban,V.D. (1995b). Comparison of immunofluorescence with peroxidase-antiperoxidase technique in the diagnosis of rabies. Indian J. Anim. Sci. 65 (1) : 33-34.

Jayakumar,R. and Ramadass,P. (1991). Evaluation of diagnostic tests for rabies in dogs. Indian Vet. J. 68 (8) : 765-768.

Jentzsch,K.D. (1967). The degree of reliabilityp and sensitivity threshold of the fluorescence-serological demonstration of the rabies virus in the brain. Zantralbl. Bakteriol. Parositenk. Infektionskr. Hyg.202 : 307. cited by : Trimarchi and Debbie (1991).

Jonsson,L., Kelly,R. and Book,M.L. (1988). Demonstration of rabies infected tissues. Svensk Veterinartidning 40 (1) : 9-10. Vet. Bull. (1988) 58 (5) : Abstr.2497.

Jubb,K.V.F., Kennedy,P.C. and Palmer,N. (1993a). Inflammation of the central nervous system. In : Pathology of domestic animals. Vol. I. 4th Edn. Academic Press, London.

Jubb,K.V.F., Kennedy,P.C. and Palmer,N. (1993b). The alimentary system. In : Pathology of domestic animals. Vol.II. 4th Edn. Academic Press, London.

Kaplan,M.M. (1973). An assessment of laboratory techniques in the diagnosis and prevention of rabies and in rabies research. In : Laboratory Techniques in Rabies. 3rd Edn. Eds. Kaplan, M.M. and Koprowski, H. WHO Monograph Series No.23. WHO, Geneva, pp. 19-37.

King,A.A. and Turner,G.S. (1993). Rabies : A review. J. Comp. Path. **108** : 1-39.

Kissling,R.E. (1975). The fluorescent antibody test in rabies. The Natural History of rabies. Vol.I. Ed. Baer,G.M. Academic Press, London.

Kotwal,S. (1984). M.V.Sc., thesis submitted to Birsa Agricultural University, Ranchi.

Kotwal,S. and Narayan,K.G. (1985a). Direct immunoperoxidase test in the diagnosis of rabies-an alternative to fluorescent antibody test. Int. J. Zoon. **12** (1) : 80-85. Vet. Bull. (1985) **55** (12) : Abstr.7719.

Kotwal,S. and Narayan,K.G. (1985b). Indian J. Pub. Hlth **31** : 94. cited by : Narayan and Kotwal (1989).

Kotwal,S. and Narayan,K.G. (1987). Comparative evaluation of ELISA, FAT and immunoperoxidase tests in the diagnosis of rabies. Indian J. Anim. Sci. **57** (2) : 65-71.

Krebs,J.W., Strine,T.W., Smith,J.S., Rupprecht,C.E. and Childs,J.E. (1994). Rabies surveillance in the United States during 1993. J. Am. Vet. Med. Ass. **205** (2) : 1695-1709.

Krebs,J.W., Strine,T.W., Smith,J.S., Rupprecht,C.E. and Childs,J.E. (1995). Rabies surveillance in the United States during 1994. J. Am. Vet. Med. Ass. **207** (12) : 1562-1575.

*Kurstak,E. (1971). In : Methods in virology. Vol.V. Eds. Maramorosch,K. and Koprowski,H. Academic Press, London. pp.423-444.

*Kurstak,E., Corte,J.R. and Bellonik,S. (1969). C. R. Acad. Sci. Paris, **268** : 2309. cited by Atanasiu et al.(1974).

*Kurstak,E., Lanzon,M., Kurstak,C. and Morriset,R. (1972). C. R. Acad. Sci. Paris, **274** : 3145. cited by Atanasiu et al.(1974).

Lapi,A.C., Davis,C.L. and Anderson,A.W. (1952). The gasserian ganglion in animals died of rabies. J. Am . Vet. Med. Ass. **120** : 379-384.

Last,R.D.,Jardine,J.E., Smit,M.M.E. and Vander,L.J.J.(1994). Application of immunoperoxidase techniques to formalin-fixed brain tissue for the diagnosis of rabies in Southern Africa. Onderstepoort J. Vet. Res. **61** (2): 183-187.

Lepine,P. (1973). Histopathological diagnosis. In: Laboratory Techniques in Rabies. 3rd Edn. Eds. Kaplan,M.M. and Koprowski,H. WHO Monograph Series No.23. WHO, Geneva, pp. 56-71.

*McQueen,J.L. (1960). Rabies diagnosis. Special application of fluorescent antibody techniques. Proc. 63rd Meet. U. S. Live stk Sanit. Ass. San Francisco, 1959. pp. 356-363. Vet. Bull. (1960) **30** (11) : Abstr.3569.

*McQueen,J.L., Lewis,A.L. and Schneider,N.J. (1960). Rabies diagnosis by Fluorescent antibody. I.Its evaluation in a Public Health Laboratory. Am. J. Pub. Hlth **50** :1743.

Muller.W.W. and Cox,J.H. (1994). Summary of rabies in Europe. Rabies Bull. Europe. **19** (3) : 3-4.

Murphy,F.A., Harrison,A.K., Winn,W.C. and Bauer,S.P. (1973). Comparative pathogenesis of rabies and rabies-like viruses. Infection of the central nervous system and centrifugal spread of virus to peripheral tissues. Lab. Invest. **29** (1) : 1-16.

Mushi,E.Z., Diteko,T. and Nthoyiwa,L. (1993). Epidemiology of rabies in Botswana 1983-1992. Zimbabwe Vet. J. **24** (4) : 159,161-163.

Nair,S., Dighe,P.Y. and Nanavati,A.N.D. (1978). Role of bandicoots in rabies transmission. Indian J. Med. Res. **67** (3) : 347-358.

*Nakane,P.K. and Pierce,G.B. (1966). J. Histochem. Cytochem. **14** : 929. cited by Atanasiu et al.(1974).

Narayan,K.G. and Konar,M, (1986).Int. J. Zoon. **13** : 63. cited by Narayan and Kotwal (1989).

Narayan,K.G. and Kotwal,S. (1989). Laboratory diagnosis of rabies in animals in Ranchi (1983-1986). Indian Vet. J.**66** (9) : 797-800.

Nayak,B.C., Rao,A.T., Parhi,N.K. and Das,B.C. (1982). Sialadenitis as an aid to routine laboratory diagnosis of rabies. Indian J. Vet. Path. **6** : 39-41.

Negri,A. (1903). Cited in : Baer (1975). pp.373-400.

Ninomiya,A.S. (1955). Gunma J. Med. Sci. Japan. **4** : 117. cited by : Nayak et al.(1982).

Orihuela,T.A. and Solano,V.J. (1995). Rabies in the state of Morelos, Mexico. Trop. Anim. Hlth Prod. 27 (3) : 164-166.

Palmer,D.J., Ossent,P., Suter,M.M. and Ferrari,E. (1985). Demonstration of rabies viral antigen in paraffin tissue sections : Comparison of the immunofluorescence technique with the unlabeled antibody enzyme method. Am. J. Vet. Res. **46** (1) : 283-286.

Parthasarathy,G. (1981). Rabies in dogs with a comparative note on the incidence of its occurrence in farm animals and man in Madras city. Indian Vet. J. **58** (4): 335-336.

Parthasarathy,G., Rajan,T.S.S. and Krishnamoorthy,R. (1978). Rabies in farm animals. Indian Vet. J. **55** (9) : 744-745.

Perl,D.P. (1975). The pathology of rabies in the central nervous system. In: The Natural History of Rabies. Vol.II. Ed. Baer,G.M. Academic Press, London.pp.235-272.

RamKumar and Mehrotra,M.L. (1983). Evaluation of various laboratory techniques used for rabies diagnosis. Indian J. Vet. Path. **7** : 82-84.

Rao,A.V.N. (1980). cited by : Parthasarathy,G. (1981).

*Roger,E.W. (1960). J. Am. Vet. Med. Ass. **137** : 319. cited by Seshadri and Chandrasekaran (1963).

Roslyakov, A.A., Korobchenko, M.I., Shvanskii, V.B. and Steblyuk,T.D. (1970). Some special features in diagnosing rabies in animals. Veterinariya, Moscow (7): 100-101. Vet. Bull. (1971) **41** (3) : Abstr.1145.

Sahasrabuddhe,M,G. and Sherikar,A.A. (1990). Comparison of rapid rabies enzyme immunodiagnosis (RREID) technique with routinely used tests. Indian J. Anim. Sci. **60** (11) : 1271-1273.

Samol,S. (1976). The role of submaxillary salivary glands in the laboratory diagnosis of rabies. Bulletin de l'Office International des Epizooties (1976) **86**: 259-265. Vet. Bull. (1978) **48** (5) :Abstr. 2908.

SatyaPrakash (1970). Paper presented at the First WHO Regional seminar on Veterinary Public Health. 8-18 April 1970, Delhi.

*Schneider,L.G. (1964). Experiences with fluorescent antibody in routine laboratory diagnosis of rabies. II. Efficacy of the technique. Tierarztl. Umsch. **19** : 502-504 & 507-509. Vet. Bull. (1964) 35 : Abstr.142.

*Schneurrenberger,B.R. et al. (1970). J. Am. Vet. Med. Ass.156 : 1455. cited by : Chakrabarti (1993).

Sekar,M., Rao,A.V.N. and Raghavan,N. (1989). Seasonal occurrence of rabies in man and animals. Cheiron 18 (1) : 5-9.

*Sellers,T.F. (1927). A new method for staining Negribodies of rabies. Am. J. Pub. Hlth. 17 : 1080-1081.

Seshadri.S.J. and Chandrasekaran,K.P. (1963). Studies on diagnosis of Negribodies. Indian Vet. J. 40 (4) : 194-205.

*Shortt,H.E. (1935). Indian J. Med. Res. 23 : 407. cited by: Seshadri and Chandrasekaran(1963).

Sikes,R.K. (1962). Pathogenesis of rabies in wildlife. I. Comparative effect of varying doses of rabies virus inoculated into foxes and shunks. Am. J. Vet. Res. 236 : 1041-1047.

Sinchaisri,T.A., Negata,T., Yoshikawa,Y., Kai,C. and Yamanouchi,K. (1992). Immunohistochemical and histopathological study of experimental rabies infection in mice. J. Vet. Med. Sci. 54 (3) : 409-416.

Soman,W.W. (1963). Transmission of rabies by the bite of an apparently healthy dog. Indian Vet. J. 40 (8) : 519-521.

Srinivasan,R., Victor,D.A. and Natarajan,N. (1973). Epidemilogical studies on the incidence of rabies in dogs in Madras city. Cheiron 2 (1) : 44-48.

Steele,J.H. (1975). History of Rabies. In: The natural History of Rabies. Vol. I. Ed. Baer,G.M.Academic press, London. pp. 1-29.

Stone,W.S., Goodrich,W.O. and Dean,D.J. (1962). The fluorescent antibody test as a diagnostic aid in rabies. Ann. Rep. New York St. Dept. Hlth, Div. Lab. Res. 1961 p.61. Vet. Bull. (1963) 33 (6) :Abstr. 1956.

Subrahmanyam,B. and Pathak,R.C. (1971). Diagnosis of rabies in dogs - Comparative study on microscopic, biological and fluorescent antibody tests for diagnosis. Indian Vet. J. 48 (2) : 123-127.

Sureau,P., Ravisse,P. and Rollin,P.E. (1991). Rabies diagnosis by animal inoculation, identification of Negribodies, or ELISA. In: The Natural History of Rabies. 2nd Edn. Ed. Baer,G.M. Academic Press, London. pp. 203-217.

Tierkel,E.S. (1973).Rapid Microscopic examination for Negribodies and Preparation of Specimens for Biological test. In: Laboratory Techniques in Rabies. 3rd Edn. Eds. Kaplan,M.M. and Koprowski,H. WHO Monograph Series No.23. WHO, Geneva, pp. 41-55.

Tierkel,E.S. (1975). Canine rabies. In : The Natural History of Rabies. Vol.I. 1st Edn. Academic Press, London. pp.123-137.

Trimarchi,C.V. and Debbie,J.G. (1991). The fluorescent antibody in rabies. In: The Natural History of Rabies. 2nd Edn. Ed. Baer,G.M. Academic Press, London. pp. 219-233.

Turner (1990). Rhabdovirus and rabies. In: Topley and Wilson's Principles of Bacteriology, virology and Immunity. 8th Edn. Eds. Collier,L.H. and Timbury, M.C. Arnold, London. pp.8-20.

*Tustin,R.C. and Smit,J.D. (1962). Rabies in South Africa. An analysis of histological examinations. J. S. Afr. Vet. Med. Ass. **33** : 295-310.

*Veeraraghavan,N. (1956). Ann. Report of Director for 1954, Pasteur Inst.of Southern India, Coonoor.

Wachendorfer,G. (1966). Fluorescein-labelled antibodies in the diagnosis of viral diseases, using rabies as a model. Dtsch. Tieraerztl. Wochenschr. **73** : 446. cited by : Trimarchi and Debbie (1991).

WHO (1975). WHO Report No.231. WHO, Geneva.

WHO (1992). WHO Expert Committee on Rabies : Eigth report.WHO technical report series : 824. WHO, Geneva.

WHO (1995a). Rabies in Europe. Ist quarter : January-March 1995. Rabies Bull. Europe. **19** (1) : 3-8.

WHO (1995b). Summary of rabies in Europe. Rabies Bull. Europe. **19** (3) : 3-8.

*Williams,A.W. (1908). Am. J. pub. Hyg. **18** : 1. cited in : D'Souza,B., Rao,J.R., Victor,D.A. and Khader,T.G.A. (1968). A preliminary investigation on the role of Bandicoota malabarica in the transmission of rabies among dogs in the city of Madras. Indian Vet. J. **45** : 633.

Wright,G. (1953). Proc. Roy. Soc. Med. **46** : 319-330. cited by Baer (1975).

Zimmer,K. (1988). Rabies diagnosis in formalin fixed paraffin sections by means of the peroxidase-antiperoxidase technique. A comparison with the routine investigation techniques - immunofluorescence, mouse inoculation and cell culture. Thesis, Justus-Leibig- Universitat-Giessen.German Federal Republic Vet. Bull. (1989) **59** (2) :Abstr. 848.

Zimmer,K., Wiegand,D., Manz,D., Frost,J.W., Reinacher,M. and Frese,K. (1990). Evaluation of five different methods for routine diagnosis of rabies.J. Vet. Med. Ser. B. **37** (5): 392-400. Vet. Bull.(1990) **60** (10) :Abstr. 6850.

* originals not consulted

NEUROPATHOLOGY AND DIAGNOSIS OF RABIES IN DOMESTIC ANIMALS

By

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ABSTRACT OF A THESIS
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requirement for the degree

MASTER OF VETERINARY SCIENCE
Faculty of Veterinary and Animal Sciences
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ABSTRACT

An investigation was made to study the epidemiology, clinical signs, pathologic lesions and diagnosis of rabies in domestic animals in the state of Kerala. Epidemiology of rabies for the last 12 year period from July 1983 to June 1995 was assessed from the post-mortem records maintained at the department of Pathology. Regional distribution, Prevalence in each species and year-wise occurrence were studied. Canine rabies was found preponderant. Rabies was recorded in wild animals also.

Detailed clinical signs and gross pathological changes in the rabies positive cases were studied.

One hundred and six rabies suspected carcasses of different species of animals were examined for the disease employing Sellers' impression smear staining, histopathology, fluorescent antibody test (FA test) and immunoperoxidase test (IP test). Twenty-two of them were confirmed positive for rabies.

Negribodies were demonstrated with Sellers' staining and histopathological staining. Sellers' staining was identified to be the simple and rapid staining method that could be employed in the field laboratories. In addition to H & E, Mann's stain and Sellers' stain were applied for

staining histological sections. H & E was found to be satisfactory for routine staining.

Histological changes in the brain (cerebrum, cerebellum and hippocampus), salivary gland (parotid and submaxillary) and trigeminal nerve of rabies positive animals were studied.

The efficacy of the diagnostic techniques used for rabies diagnosis was compared. FA test confirmed maximum number of rabies positive cases.

The results of the rabies diagnosis was cross-checked by Veterinary Diagnostic Laboratory, College of Veterinary Medicine, Oregon State University, USA.

Appendix

APPENDIX 1

Request for conducting post-mortem examination of rabies suspected carcass

(Mark **x** in appropriate columns)

Name and address
of the owner:

District:

Phone No.:

Details of the animal

Species : Dog / cat / cattle / goat / others(specify)

Breed:

Sex : Male / Female

Age:

Colour:

1. Carcass brought as whole body / head alone
2. Domesticated / stray
3. Killed / died suddenly / sick for the past .....days
4. Date and time of death:
5. Whether preserved properly in ice immediately after death :yes / no
6. Has it been bitten by any other rabid or rabies suspected animal Give details:
7. Has it been vaccinated against rabies. Give details:
8. Contact with other animals or human beings(in Nos.)

   scratched ....persons,....animals
   bitten .....persons,....animals

9. Clinical symptoms

profuse salivation	change in the voice
vomiting / diarrhea	off feed
not responding to call	difficulty in respiration
aimless wandering/biting tendency	paralysis
frequent urination	any other symptoms

10. Any other information :

Signature

Date

To

Professor and Head,
Dept. of Pathology,
College of Veterinary & Animal Sciences,
Mannuthy.