CHAPTER VI
SUMMARY

PART I. EPIDEMIOLOGY
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I. EPIDEMIOLOGY

The present work was undertaken to study the carrier state among stray dogs, and also to study the presence of antibodies in human population where rabies is endemic. Along with this study, serology of pet dog population was undertaken to find out the danger of exposure to rabies in pet dog population as well as pet dog owners.

226 brains, 226 salivary glands, 224 sera samples and 178 cerebrospinal fluid samples obtained from stray dogs were examined for the presence of rabies virus antigen in brains and salivary glands and rabies antibodies in sera and CSF of stray dogs by mice inoculation and complement fixation and gel diffusion techniques respectively.

Rabies virus antigen could not be isolated from the brains and salivary glands examined. 12 sera (5.36 per cent) out of 224 were found to contain rabies antibodies at various concentrations, suggesting that rabies antibodies are found in stray dog population in endemic area. CSF samples did not reveal presence of rabies antibody. This is expected since rise in CSF antibody is found either in prolonged clinical illness or recovery.

A stray dog whose bite to another dog had resulted in disease was caught and kept under observation for 302 days.
During the period of observation saliva was monitored for presence of virus and serum was examined for the presence of rabies antibody periodically. At no time during the period of observation, saliva showed the presence of rabies virus or serum showed the presence of rabies antibody. The dog died due to acute gastroentritis after 302 days and therefore, further work could not be carried out. Literature mentions such carrier state in dogs, where the dogs are observed over a period of three to five years.

214 sera samples obtained from pet dogs brought for prophylactic antirabies vaccine were examined over a 15 month period. 17 pups and seven young dogs which were brought for the first vaccination did not show presence of rabies antibody in sera indicating no previous exposure to virus or vaccine nor circulating maternal antibody which was an expected observation. All the 190 dogs which were vaccinated once or more before were having a detectable presence of rabies antibody in their sera. This indicates that provided the vaccination schedule in case of pet dogs is adhered to, the danger of rabies to pet dogs and through them to pet owners and pet handlers does not exist. The 56 samples of CSF collected from large dogs wherever it was possible to collect, did not reveal presence of rabies antibody and this is expected result as stated above.

572 human sera samples were collected from individuals reported for post bite antirabies treatment at Civil Hospital. This was considered a population at risk. The desired and
relevant information was collected from them. The incidence of bite in males was higher (78.32 per cent) than females (21.68 per cent). The highest incidence of bite was in age group of three to 12 years (33.11 per cent) followed by next group of 13 to 24 years (26.22 per cent), whereas the age group of 25 to 45 years was very close to previous group (25.7 per cent). In the age group of zero to two years the bite rate was 1.4 per cent and in persons above 65 years the bite rate was 1.05 per cent. In 46-65 years age group 7.52 per cent bite rate was noted.

This indicates that high risk groups are school going children, working people and persons engaged in outdoor activity.

Of the 572 human sera examined only 11 showed presence of rabies antibody (1.92 per cent). This indicates that the population had a previous exposure to rabies antigen, which is quite possible in rabies endemic area, and the results show correlation with such study in stray dogs population of same area.

The monthwise distribution of dog bites show that highest bites were reported in month of May followed by March, October, February and June. In July and August bites were not reported. This seasonal variations in bite cases have also been found by several authors.

The factors responsible for causing bites are visiting unknown locality (130, 22.73 per cent), teasing the dog (114, 19.93 per cent) going out for work (99, 17.31 per cent),
going to school (76, 13.29 per cent) cycle accidents (58, 10.14 per cent) while playing (40, 7.0 per cent), handling pups (26, 4.54 per cent) and pet owners, handlers etc. (29, 5.1 per cent). Looking to the way of life people live, the factors responsible for causing bites correlate well. The bite rate is higher in males than females in all cases as expected.

The dog bites were classified in class I, class II and class III bites. 482 (34.26 per cent) were class I bites and rest 90 (15.74 per cent) were class II bites. Class III bites were not found, nor reported.

Leg bites (65.73 per cent) were most frequent followed by bite on hands (28.5 per cent) accounting for about 94.2 per cent bites on extremities. The next in order were bites on abdomen, back, head and face. The bite on extremities are most frequent.

In a city having a population of about 2.22 million, only 572 persons reported for ART at Civil Hospital. This number appears small, and these seems to be under reporting of dog bite cases.

Out of 572 persons reported for ART only two completed full course of 14 inoculations and 507 (88.65 per cent) took seven to 13 inoculations, 63 persons (11 per cent) took less than seven inoculations. This indicates that people do not come forward for full course and there is a very high defaulter rate. However, in cases of class I bite, seven inoculations are considered sufficient, and thus such persons might have discontinued the therapy.
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PART-II. DIAGNOSTIC METHODS
II. DIAGNOSTIC METHODS:

The presently available diagnostic methods for histologic staining of Negribodies in brain and detection of rabies antigen in rabies suspected material by direct FAT and direct IP were studied on impression smears for giving rapid results and on paraffin sections. The PCA reaction in guinea pigs as described by Mathew and Rao (1973) was also studied to find out its applicability in diagnosis of rabies.

The results obtained by staining impression smears prepared from rabies positive brains revealed that with Sellers stain Negribodies were detected in 76.28 per cent specimens, whereas direct FAT and direct IP detected rabies antigen in 92.5 and 91.36 per cent of brains respectively. This indicates that for giving rapid diagnosis Sellers' stain may be of value but negative results does not rule out positive result. Direct FAT and direct IP were found sensitive, and it can be said that at least three tests should be used for diagnosis of rabies which should invariably be confirmed by mouse inoculation test.

Paraffin sections were stained by Massiagnani Malferrari method (Eosin-phosphotungstic -EPA - method), direct FAT and direct IP. All the three methods were found equally specific and sensitive. Direct FAT is possible only when fluorescent microscope is available. Its disadvantage is its impermanancy. Nonspecific reactions are also seen. These disadvantages can largely be avoided by employing direct immunoperoxidase technique since the method does not require
a special kind of microscope, it can keep for a longer time and it can be studied under electron microscope if desired. This test can also replace direct FAT in examining impression smears where fluorescent microscope is not available. Horse radish peroxidase from SIGMA, USA type IV RZ 3.0 was found most suitable.

Passive cutaneous anaphylaxis reaction in guinea pigs was studied for diagnosis of rabies. Horse, guinea pig, rabbit and dog antirabies sera were used for testing five street virus as well as two CVS antigens. Three guinea pigs were used for each test. A good positive reaction showing a blueing of 12 mm in diameter was observed when rabies antigens were tested with rabbit and dog antirabies sera. Horse and guinea pig antirabies sera did not give satisfactory results.

Guinea pig, dog and rabbit antirabies sera were tested at different serial two fold dilutions with rabies antigens. It was seen that guinea pig antirabies serum gave unsatisfactory results but good reactions showing blueing upto 8 mm diameter were observed upto 1:2 dilution and in case of rabbit sera all antigens reacted with blueing upto 8 mm diameter at 1:4 dilution. Therefore, it is observed that rabbit antirabies serum gave better results as compared to dog serum. As low as $10^{-2.15}$ mouse LD$_{50}$ gave good reaction with rabbit and dog antirabies sera. Normal tissue antigens did not reveal any significant blueing.

Thus PCA reaction in guinea pigs seems promising for use in diagnosis of rabies.