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ANNUAL PROGRESS REPORT OF RESEARCH SCHEME

1. Project title : Incidence, aetiology and Pathology of tumours of the Ethmoid in domestic animals.

2. Sanction No : F.1.(4)74-ASR(iv), dated 17-8-76 of the Assistant Director General.

3. Report period : 1-1-80 to 31-12-80

4. Date of start : 22-2-78

5. Date of termination : 21-2-81

6. a. Name of Institute : College of Veterinary & Animal Sciences, Kerala Agricultural University.

   b. Division : Department of Pathology

   c. Location of work : Mannuthy

7. Technical Personnels employed:

Sl.No.	Name	Designation	From	To
1.	Dr.A.Rajan., M.V.Sc., Ph.D.	Professor of Pathology (Project Officer)	3-6-78	
2.	Dr.S.Sulochana M.V.Sc., Ph.D.	Associate Professor (Virologist)	22-2-78	
3.	Dr.Valsala C. Joseph	Research Fellow	14-1-80	13-6-80
4.	Dr.Harshakumar	-do-	25-7-80	6-9-80
5.	Smt P.C.Mary	Laboratory Technician on working arrangement	16-8-78	
6.	Sri.M.Gangadharan	working on daily wages	16-3-78	

8. Total outlay : 2,08,936/-

9. Total amount spent in previous year : 54,398.08

10. Total amount under report:

Sanctioned for the year	: *	
Pay	:	35,300.00
Allowances	:	9,800.00
DA	:	7,200.00
TA	:	1,500.00
C and OA	:	1,100.00
Recurring contingencies	:	12,000.00
		66,900.00
Spent during the year	:	
Pay and allowances	:	38,370.00
TA	:	774.90
Recurring contingencies	:	14,718.75
Total	:	53,863.65

* The University budget is for the Financial Year and not for the Calander year.

11. Objectives:

a. Assess the incidence of tumour of the ethmoid in domestic animals in Kerala.

b. Investigate the aetiology of tumours of the ethmoid.

c. Study the epidemiological and pathological features of the neoplasms and the facters influencing the spread in herds.

d. Find out whether any hereditary factors are involved in the initation of this neoplastic process.

e. In vitro culture of tumour cells and study their biological behaviour.

f. Explore suitable preventive and curative measures.

12. Approved technical programme:

a. For the year under report:

1. Nasal washings/swabs from animals in the susceptible age group in endemic farms will be subjected to detailed cytological studies.

2. Tumour cells will be cultured in different tissue culture media and attempts will be made to propogate the cell line.

3. Fresh tumour tissue to be implemented into homologus and heterologus hosts by different routes.

4. Cell free, bacteria free filtrate of the tumour tissue will be inoculated into chicken embryo and tissue culture system.

5. Electronmicroscopic studies on tumour tissue will be continued.

6. Anti-carcinogens used in medical practice will be tried on selected clinical cases of tumour.

7. Histopathological and histochemical studies on ~~medical~~ tumour tissue will be continued.

b. For the next year:

The scheme is to be terminated as per the original schedule on 21-2-1981. However, a proposal for extending the scheme for two more years has been submitted with the following technical programme.

1. Tissue culture work to establish the cell line.

2. In-depth-studies on immunotherapeutic effect of vaccination.

3. Further studies on viral aetiology- study the pathogenic potential of viral isolates.

4. Study the role of mycotoxins in the causation of the tumour.

5. Chemotherapeutic trials on tumours in goats.

6. ~~For the~~ Electronmicroscopic studies to elucidate the viral aetiology.

7. Explore the posibility of developing electronic equipments for diagnosis.

13. Progress of Research:

1. Incidence

To gauge the incidence of the tumour, Veterinarians/ officers-in-charge of Livestock farms in the state were requested to report the cases of tumours encountered by them to the Project Officer. When the reports were received detailed information on the animals were obtained. Animals bearing ethmoid tumour and located in nearby places were examined by the Project Staff and suitable cases were procured and brought to Mannuthy for detailed investigation. Besides this the heads of animals slaughtered at different slaughter houses in the state were examined for detecting tumour cases.

Number of animals examined during the year:

Cattle	:	18072
Buffaloes	:	1987
Goats		18926
Sheep		922
Pigs		94

During the year 1980,107 cases were recorded. The details of the animals have been set out in table I and II (Appendix). Out of 107 cases observed 70 animals were brought to the Department for detailed investigation. These are cases which have been reported. There may be many cases which were not reported by the Veterinary Surgeons and farmers. The incidence of the tumour is on the increase and it has also shown a tendency to appear in other species of animals also in increasing frequency.

1.1. Breed-wise incidence

The breed-wise distribution of the tumour has been shown in table III. The tumour was encountered in white cattle (86), buffaloes (3) and goats (7), and pigs (11). A tumour was also recorded in a spotted deer (Axis axis) belonging to the zoological gardens,

Trichur. The observation made indicated that there is no breed predisposition or species barrier. It is worthy to mention that there were cases of the tumour in the pig breeding farms of the University and Animal Husbandry Department. It may be pointed out that there had been no record of the tumour in pigs since the first report in 1968. During 1965-68 there were as many as 32 cases of ethmoid tumour in pigs. After 12 years of tumour free period in the herd, the incidence of the tumour was recorded. This is a significant epidemiological observation. The data collected shows a high incidence among cross-bred cattle. As the cattle population in Kerala is significantly dominated by cross-bred cattle this observation may not have any significance. It may also be pointed out that even non-descript cattle were found affected. The incidence in cattle is largely in agreement with the observation made during the last year. The data collected shows that the tumour has established itself in an endemic farm in the state. There is also evidence to show that it spreads to different species of animals.

1.2. Sex-wise incidence

The data presented in table IV shows a high incidence in females.

Table IV

Sex-wise incidence of the tumour

Species	Male	Female	Total
Cattle	7	79	86
Buffaloes	2	1	3
Goats		7	7
Pigs	3	8	11
Total	12	95	107

This observations again confirms the reports made during the last two years that the incidence is more

in females. This can be explained only as a relative phenomenon since female population is significantly higher than the males.

1.3. Age incidence:

The age wise incidence has been set out in table V. The incidence was high in animals in the age group of 6-9 years followed by above nine years, group. The

Table V

	3-6 years	6-9 years	Above 9 years	Total
Cattle	8	59	19	86
Buffaloes	-	3	-	3
Goats	7	-	-	7
Pigs	11	-	-	11
Total	26	62	19	107

tumour appears during the maximum productive period of the animal. The production stress and the immunological depression taking place as age advances might contribute to the development of the tumour. In the case of pigs they belonged to the age group of 3-4 years. These data also goes to show that this neoplasia is a disease of the aged rather than ~~in~~ the young.

1.4. Seasonal incidence:

The season-wise distribution of the incidence has been documented in table VI. The data presented in the table indicates a relatively high reporting of cases during July - August. During April and May the incidence was relatively low. The observations made during

Table VI

Jan	Feb	Mar	Apr	May	June	July	Aug	Sept	Oct	Nov	Dec.
9	11	5	15	11	14	10	9	7	3	7	6

this year are very similar to that observed during the last year. There has been more reporting of cases during April to July.

2. Symptomatology.

The symptomatology was studied in detail in 70 tumour bearing animals. Nasal discharge, was the earliest clinical symptom as reported earlier. In most of the cases this symptom was overlooked and an personal discussion with the owners the occurrence of this symptom was brought to light. Veterinary Surgeons have been informed about this feature of this disease by letters and farmers have been provided with a pamphlet on describing the clinico-pathological features of this disease. It is felt that this would greately help in spotting out the disease in the early stages. The details of the clinical symptoms observed in the animals examined during the year are shown in the table (table I appendix).

2.1. Epistaxis:

The first symptom noticed in all cases was intermittent epistaxis. This was of course not a consistent symptom. There were a few instances where there was uncontrollable bleeding. Massive blood clots were sneezed out. This symptom was commonly seen in pigs.

2.2. Respiratory difficulty:

The second step in the symptomatology was respiratory difficulty. In the initial stages the difficulty was mild. But very soon as the growth advanced there was pronounced respiratory difficulty, ultimately resulting in severe snoring. Abdominal type breathing and frothy salivation were also seen.

2.3. Exopthalmos:

Exopthalmos was a common symptom. This was unilateral or bilateral. Unilateral exopthalmos was more common than bilateral. The degree of exopthalmos varied from mild to severe. In advanced cases this was associated with blindness. In pigs exopthalmos was not a feature. Conjuntivites and keratitis often supervened.

2.4. Subcutaneous swelling on the forehead:

A swelling on the forehead due to the expansive growth of the tumour was observed in 36 cases. In the initial stages small perforation was palpable and

later it became enlarged and through this the tumour growth protruded out. The size of the growth varied from that of lemon to that of a football. The location of the swelling was on the forehead either just above or below the eye. This was consistent symptom in pigs. The deer also had a very big sized swelling on the forehead.

2.5. Pregnancy:

Out of 131 animals examined 50 animals were pregnant. This is an observation which would indicate an association between tumour development and pregnancy. During the last year also a similar finding was recorded. No doubt, pregnancy will exaggerate the clinical respiratory symptoms. The Gonodotrophic hormones associated with pregnancy may also stimulate the tumour growth. The hormonal influence on tumour growth has to be studied.

3. CLINICOPATHOLOGICAL STUDIES.

3.1. Haematological studies:

The haematological data of tumour bearing animals are shown in table VII. There was anemia characterised by low haemoglobulin level. There was no variation in the ESR. There was moderate to severe leucocytosis. In advanced cases there was neutrophilic leucocytosis. The observations made were similar to that recorded during the last year. The haemotological changes would appear to be secondary manifestations of tumour growth.

3.2. Calcium and Phosphorous:

Calcium and phosphorous levels in the serum of 15 tumour bearing animals were estimated. The data are drawn in table VIII. There was an appreciable imbalance in the calcium phosphorous ratio. The variation was observed in animals which had perforation of the frontal bone. The rarefactive changes in the bone might be responsible for this. The observations made during this year confirms the previous years finding.

Table VIII

Serum Calcium and Phosphorous level of tumour bearing animals.

Sl.No.	Serum calcium mg/dl	Serum phosphorous mg/dl
1.	6.40	5.30
2.	10.80	5.40
3.	9.50	6.80
4.	8.50	6.90
5.	10.40	7.40
6.	10.20	9.60

Sl.No.	Serum Calcium mg/dl	Serum Phosphorous mg/dl
7.	11.10	7.40
8.	7.60	5.40
9.	10.80	8.40
10.	9.20	4.30
11.	8.60	4.10
12.	10.60	5.80
13.	10.20	5.40
14.	8.70	5.30
15.	11.20	6-10

3.3. Serum protein pattern:

The serum protein pattern of 12 tumour bearing and 10 control animals were studied by electrophoretic serum fractionation. The data are presented in table IX. There was decrease in albumin percentage and reduction in albumin-globulin ratio in tumour bearing animals. This was associated with an increase in Gamma globulin and alpha-1 globulin in these animals.

Table IX

Serum fractions in tumour bearing and non-tumour bearing healthy animals.

Serum protein fraction	Tumour animals	Non-tumour bearing animals
Albumin (%)	28.642 ± 1.690	36.997 ± 2.099
Alpha- 1 globulin (%)	13.277 ± 1.651	12.383 ± 1.921
Alpha- 2 globulin (%)	8.569 ± 1.103	12.470 ± 2.473
Beta - globulin (%)	8.143 ± 1.337	11.222 ± 1.890
Gamma-globulin (%)	41.366 ± 3.273	28.077 ± 3.810
Albumin/globulin ratio	0.40	0.60

Significant increase in gamma globulin level was observed in all the tumour bearing animals. This shows that there has been an immunological stimulation in tumour bearing host. Hypoalbuminaemia observed in the present

Table VII

Sl. No.	Animal No	Stage	Arrival TC *	Arrival L *	Arrival N *	Arrival M	Arrival E	15 days TC *	15 days L *	15 days N *	15 days M	15 days E	30 days TC *	30 days L *	30 days N *	30 days M	30 days E	45 days TC *	45 days L *	45 days N *	45 days M	45 days E
1.	140	I	7.1	3.8	2.9	142	142	13.4	5.7	7.2	268	134	10.2	4.8	4.9	105	204	13.2	4.1	9.0	132	0
2.	122	I	11.5	5.7	5.3	114	229	9.6	4.6	4.8	96	96	10.6	4.8	5.6	106	-	12.2	4.3	7.6	122	0
3.	109	II	6.7	3.7	2.4	-	472	5.8	2.8	2.6	58	293										
4.	120	II	12.5	5.3	6.9	-	251	12.1	5.1	6.9	121	-	11.8	5.1	6.6	-	-					
5.	92	II	7.5	4.4	2.1	-	900	16.5	7.9	8.2	165	165	-									
6.	82	III	13.2	7.1	4.4	265	397															
7.	95	III	5.8	2.3	2.8	175	468	7.3	2.5	3.9	438	438										
8.	107	III	4.0	1.5	2.4	40	40	20.7	3.7	17.0	-	-										
9.	108	III	7.4	2.6	4.5	74	74	36.0	2.5	33.4	-	-										
10.	116	III	10.6	3.6	6.6	212	106	11.8	2.2	9.0	236	236										
11.	121	III	9.1	3.0	5.8	91	183	7.9	2.3	5.4	79	79										
12.	142	III	5.7	3.2	2.3	-	172	12.7	2.9	9.3	127	382										
13.	143	III	9.2	4.7	3.9	-	555	9.8	2.8	6.4	98	392	10.2	3.1	6.6	102	306					
14.	125	III	7.2	2.3	4.8	-	72															
15.	89	III	5.7	2.4	3.2	-	-															

* Thousand

study might be due to improved air synthesis of protein as a result of low intake of food.

3.4. Serum enzymology of tumour bearing animals

The enzyme profile of 36 tumour bearing animals were also studied. Depending on the severity of clinical symptoms the tumour bearing animals were grouped as follows.

Stage I: Unilateral exopthalmos - Mucus discharge - slight bulging of forehead.

Stage II: Mucus discharge - Moderate to severe respiratory distress - unilateral exopthalmos - Bulging of the forehead.

Stage III: Nasal discharge with or without epistaxis - ~~F~~d severe respiratory distress - bulging on the forehead.

Enzyme estimation:

Approximately 8 ml of blood was obtained in a clear test tube from the jugular vein. The blood was allowed to clot and then placed in a Refrigerator for an hour. The clot was then gently dislodged and test tubes were then centrifuged at 300 rpm for 10 minutes. The serum was then separated. The following enzymes were estimated.

1. Alkaline phosphatase
2. Acid phosphatase
3. Glutamic pyruvic transaminase
4. Glutamic oxalo-acetic transaminase
5. Lactic dehydrogenase

The enzyme kits for these enzymes were employed for the estimations (Decruz corporation, Bombay).

To make a comparative study the enzyme levels in ten healthy cattle was also estimated.

The serum enzyme levels of the ten control healthy animals and tumour bearing animals have been set out in table ~~III~~ IX to XIII and ~~IV~~.

In order to find out whether there is any statistically significant difference between the above two groups a one way classification of completely randamised block as an experimental design was applied.

Statistically no significant difference was found between the tumour bearing animals and controls regarding the activity of enzymes Alkaline phosphatase, Acid phosphatase, Gluatamic pyruvic, oxalo-acetic transaminases and lactate dehydrogenase. However, when animals were clinically staged, it was found that serum LDH activity was increased from stage II onwards reaching maximum activity in stage III. With a fall in activity in advanced case. The serum LDH activity was found to be increased in those tumour bearing animals with increased tumour mass, especially in those found in clinical stage III and advanced stage, where the tumour mass extended beyond half the nasal passage. Serum enzyme estimation was not found to be of clinical value in the early diagnosis of tumour arising from the ethmoid region. The mean serum enzyme level of animals bearing tumours in different stages of growth was calculated in order to assess the significance of enzyme level, if any. There was no significant difference in the level of enzymes depending on the stage of growth except for LDH. The serum LDH was highest in stage II. The stage III animals showed higher level of LDH when compared to the animals in stage I. There was however, increase in serum LDH activity in advanced cases of the tumour. The histopathology of the tumours were also studied and they were diagnosed. No correlation was found between the hisological type and serum enzyme levels.

Table IX

Srum enzyme levels in control animals

Sl.No.	ALP mMU/L	ACP mM/L	SGOT units/ ml	SGPT units/ ml	LDH units
1.	3.25	0.33	84.00	17.00	440
2.	4.5	0.33	79.00	16.00	530
3.	3.6	0.34	70.00	17.00	420
4.	3.6	0.33	84.00	15.00	520
5.	3.23	0.33	76.00	16.00	460
6.	3.9	0.33	72.00	16.00	540
7.	4.0	0.34	86.00	15.00	420
8.	4.25	0.34	75.00	15.00	410
9.	3.75	0.33	74.00	16.00	420.
10.	3.5	0.33	75.00	17.00	420

Table XI

Serum enzyme levels of the tumour bearing animals in Stage I.

Animal No	ALP mMu/L	ACP mM/L	SGOT units/ ml	SGPT units/ ml	LDH units
69.	3.6	0.33	68	16	420
124.	4.0	0.33	75	20	410
111.	3.6	0.33	68	16	520
132	3.0	0.33	79	22	530
134	3.75	0.34	79	16	630
136	3.5	0.33	72	18	580
139	3.5	0.34	75	20	420

Table XII

Serum enzyme levels of the affected animals in stage II.

Sl. No.	Animal No	ALP mMu/L	ACP mM/L	SGOT units / ml	SGPT units / ml	LDH units
1.	101	3.3	0.33	79	14	420
2.	100	3.9	0.33	82	17	630
3.	108	4.0	0.34	74	18	630
4.	109	3.5	0.33	76	16	530
5.	126	3.75	0.34	74	18	420
6.	128	4.0	0.33	72	15	630
7.	112	4.0	0.33	84	14	605
8.	120	3.75	0.33	72	16	600
9.	121	3.95	0.33	68	15	605
10.	122	3.60	0.34	81	18	630
11.	140	3.65	0.33	78	22	410
12.	142	4.1	0.34	76	16	580
13.	144	3.75	0.33	86	16	600
14.	145	3.95	0.34	74	15	630
15.	146	4.0	0.34	76	18	605
16.	148	3.6	0.33	79	18	660
17.	150	3.65	0.33	86	18	630
18.	151	3.5	0.33	79	16	630

Table XIII

Serum enzyme levels of the affected animals in stage III

Sl. No	Animal No	ALP mMu/L	ACP mM/L	SGOT units / ml	SGPT units / ml	LDH units
1.	103	3.6	0.33	70	16	600
2.	125	4.7	0.34	68	23	600
3.	116	3.95	0.34	88	16	630
4.	110	3.65	0.33	78	15	420
5.	123	3.65	0.33	72	15	600
6.	130	3.75	0.33	76	17	630
7.	143	3.6	0.34	82	15	420
8.	149	3.75	0.33	82	15	630

Table XIV

Mean serum enzyme levels of the affected animals as compared to the controls.

Clinical stage	Alp mMu/L	ACP mM/L	SGOT units / ml	SGPT units / ml	LDH units
Control	3.76	0.33	77.5	16.00	458
Stage I	3.57	0.33	73.71	18.29	501.43
Stage II	3.78	0.33	77.56	17.44	578.89
Stage III	3.83	0.33	77.00	16.5	566.25

3.5. Exfoliative cytological studies:

The nasal washings, deep mucosal smears and free nasal discharge smears from animals in the susceptible age group and tumour bearing animals were subjected to detailed cytological studies. The smears were stained with Papanicolaou's method, Shorr's method besides routine Haematoxylin and eosin and Giemsa's stain. In all 541 nasal smears from cattle, 61 smears from goats and 46 smears from pigs were examined. In cattle 41 positive cases were detected and in goats and pigs (7, 11) all positive cases were detected and confirmed subsequently. The cytological features of the neoplastic cells were well defined. The desquamated tumour cells were often identifiable as cell clumps. The hyperchromia, syncitial formation, cells in mitotic division, were all common consistent findings. In squmous cell carcinoma large orangeophilic cells with vide variation in cell size were evident. There was often a back ground of inflammatory cells consisting mainly of neutrophils. The cellular pattern and diagnostic criteria have been given in detail below.

Undifferentiated carcinoma:

Exfoliation: Mainly in loose clusters or singly. Majority of the cells were small and resembling more to a columnar shape.

Nucleus: Shape varied from round to oval. Nuclear cytoplasmic ratio was 1:1. Mitotic figures in different stages of development were noticed.

In most cases, nucleus was placed centrally.

Nuclear membrane: Was smooth and distinct except in one case, in which it was angulated.

Chromatin: Moderately hyperchromatic. Chromatin clumping was not much pronounced, but the distribution was abberant and in certain cases, the chromatin was marginated to the nuclear rim causing thickening of the nuclear rim.

Nucleoli: Nucleoli was distinct, and either one or two in number. In two cases, it was enlarged.

Cytoplasm: Cytoplasm was scanty in majority of the cells. In two cases, the cytoplasm was basophilic and in three cases, it was eosinophilic. In 3 cases, out of 5, there was no evidence of vacuolization. But in one case, there were vacuoles with red small round hyalinised bodies in the ~~xxxxx~~ vacuoles.

Background: The inflammatory cells varied in each case. Necrotic (dirty) background was evident in those specimens which had necrosis grossly.

Simple adenocarcinoma:

Exfoliation pattern : Exfoliated mainly in clusturés. Columnar cells were the predominant type cells.

Nucleus: Round to oval. Large number of angulated and also pyknotic cells were noticed. Nucleus was mostly placed eccentrically. Nucleus was also enlarged in size without altering ation in the nuclear - cytoplasmic ratio. In certain cases, mitotic figures were also noticed.

Chromatin: In majority of cases, it was markedly hyperchromatic. Chromatin was either granular or clumped. Clumped chromatin ~~were~~ was distributed in ~~all~~ an abberant manner.

Nucleoli: In almost all the cells except those showing pyknosis, had one nucleoli. But they were not much enlarged.

Cytoplasm: Stained slightly eosinophilic. Cytoplasm has got a vesiculated appearance and it was light in texture. Certain cells have got long tailed cytoplasm. The cytoplasmic border was not distinct unlike squamous cell carcinoma and undifferentiated carcinoma. Vacculization was noticed mainly in only one case.

Background: There was lot of neutrophilic inflammatory cells in 7 out of 9 cases.

Cystadenocarcinoma:

Exfoliated in clusters. Type cells were predominantly columnar cells. Squamous cells were also noticed in one case.

Nucleus: Round to oval without any enlargement. Nuclear membrane ~~were~~ was either thickened or angulated.

Chromatin: In one case, chromatin had formed into a clump which was markedly hyperchromatic. In the other case, chromatin was marjined towards the nuclear rim.

Nucleoli: Was distinct in the moderately hyperchromatic cells.

Cytoplasm: was stained eosinophilically. Light in texture. Cytoplasm had small round vacuoles close to the nuclear membrane.

Background: There were lot of inflammatory cells with a dirty background indicative of necrosis.

Squamous cell carcinoma:

Exfoliated mainly in sheets of 3 or 4 cells.

Nucleus: Round in shape. Not much enlarged except in 2 cases. Nucleus was placed centrally and eccentrically in cases where there was vacuolization of the cytoplasm.

Chromatin: Chromatin was clumped and markedly hyperchromatic in one case, whereas in other cases, it was moderately hyperchromatic.

Nucleoli: Nucleoli were either absent or masked by the clumped hyperchromatic nucleus.

Cytoplasm: In non-keratinizing squamous cell carcinoma cytoplasm was stained bright eosinophilic in one case, and in the other case it was slightly eosinophilic . In both cases cytoplasm was thick in texture.

In keratinizing squamous cell carcinoma in both cases, exfoliated cells stained deep eosinophilic and they were also very thick in consistency.

Cytoplasmic border was very much distinct in all the cases. Vaculoization was evident in 2 cases.

Background: Was clear except, neutrophilic background.

Differentiating features of Adenocarcinoma, Undifferenting carcinoma and Squamous cell carcinoma.

	Adeno carcinoma	Undifferentiated carcinoma	Squamous cell carcinoma
1.	Exfoliated mainly in clusters	Exfoliated simply or in loose clusters	Exfoliated mainly in sheets of 3 or 4 cells or in group of small number of cells.
2.	Nucleus enlarged with out alteration of N:C ratio. More number of pyknotic and angulated nucleus	Enlarged nucleus with N:C - 1:1 Round to oval in shape.	No nuclear enlargement
3.	Hyperchromatism varied from moderate to marked and distribution was either uniform or abberant.	Same as adeno carcinoma	Same as adeno carcinoma
4.	Nucleoli was only one in most cases and without enlargement	Nucleoli 1 or 2 in number and enlarged in majority of cases.	Either absent

5. Cytoplasm - light in texture and vesiculated.	Scanty	Dense# in structure and was deeply eosinophilic or orangeophilic.
6. Vacuolization was not frequent	Not frequent	In 2 out of 5 cases, there was vaculization.
7. Mitotic figures present	Present	Not frequent
8. Cytoplasmic border not distinct	Moderately distinct	distinct

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4. IMMUNOLOGICAL STUDIES

4.1. Response to tumour antigen:

The test was conducted to assess the immune response of the tumour bearing animals. The tumour extract was employed as antigen to explore the possibility of employing the test as a diagnostic tool. The tumour anitgen was prepared as described in the 1978 Annual Progress Report. The antigen was given i/D and the dose employed and the site selected were the same as followed earlier. The skin thickness at the ~~right~~ site of injection was measured before giving the tumour antigen and at 24 hours and 48 hours after the inoculation. The test was done in 13 tumour bearing animals and six healthy animals. The data on skin measurements on statistical analysis did not reveal any significant difference in tumour bearing animals when compared to the controls. The skin tissues collected in formal saline were processed for histopathological examination.

Histopathology of skin lesion: Healthy animals: 24 hours:

There was moderate diffuse infiltration of lymphocytes and few macrophages in the epidermis and upper part of the dermis. The cellular infiltration was more in the perivascular location. Dermal layers showed moderate

degree of oedema and focal capillary congestion. The lymphatics were moderately dilated.

Forty eight hours: Infiltration of lymphocytes and macrophages was more severe in the epidermis and dermis. Perivascular accumulation of lymphocytes and macrophages was more pronounced in focal areas. The capillaries were slightly engorged. Intermuscular oedema was evident.

Histopathology of skin lesion: Tumour bearing animals.

Twenty four hours: Intermuscular oedema and separation of muscle fibres were evident in the dermis. Moderate congestion was a common finding. There was perivascular infiltration of lymphocytes and macrophages. In certain areas there was fibrinous exudate and neutrophilic infiltration. The dermal layer was moderately infiltrated with lymphocytes and few macrophages.

Forty eight hours: The reaction was more pronounced. Perivascular infiltration of lymphocytes and macrophages was evident in the deep epidermis and superficial dermis. There was severe capillary congestion. The infiltration of lymphocytes, macrophages and neutrophils was marked in the upper part of the dermis. The infiltration extended slightly into the deeper layer of dermis also. Interstitial tissue revealed oedema and focal areas of necrosis.

The histological reaction would suggest the involvement of a hypersensitivity reaction. However, the reaction was not significant enough to be measured in quantitative terms. Consistent finding of perivascular cuffing by lymphocytes and macrophages is indicative of a sensitisation reaction. It is also worthy to note that the histological reaction was more severe in tumour bearing animals. This is an expected type of reaction since the immune system of the tumour bearing animals has already been exposed to the tumour antigens. This is an observation which would suggest the shedding of antigen from the tumour tissue. ~~as stimulation of the immune system in tumour bearing animals.~~

4.2. Reaction of the lymphnode to tumour antigen and BCG:

The tumour bearing animals were divided randomly into two groups of five animals each. In the 1st group of 5 animals tumour antigen was given i/D in the skin fold at the flank region near the prefemoral lymphnode. The unstimulated lymphnode on the right side served as control. In the second group of five animals the same experiment was carried out with BCG instead of tumour antigen. Biopsies of the stimulated and unstimulated lymphnodes were taken at regular intervals of 3 days up to 15th day. The detailed experimental design and the gross features of the lymphnodes have been given in the last years report.

Histopathology: Tumour antigen:

Three days : There was diffuse hyperplasia of the lymphoid tissue in the cortex. There was increased number of lymphoid follicles. In the subcapsular zone there was increased number of histiocytes and macrophages. Diffuse hyperplasia was also evident in the para cortical zone. The medullary cords were videned and contained numerous large and medium sized lymphocytes. The sinuses contained more number of lymphoid cells and histiocytes.

Unstimulated: No stimulatory response was observed.

Stimulated lymphnode: 6 days Pi

The cortical area was increased in size. Numerous lymphoid follicles were seen with active germinal centers. Diffuse hyperplasia of paracortical region - Histiocytic proliferation was also evident. Medullary cords were increased in size. Moderate to marked sinus histiocytosis was evident.

Unstimulated lymphnode: No stimulatory effect was noticed.

Stimulated lymphnode: 9 days Pi:

Cortical area slightly increased-lymphocytic content increased. Lymphoid follicles were few. There was diffuse hyperplasia of paracortical area. Histiocytes were predominant. Medulla showed marked histiocytosis.

Unstimulated lymphnode:

No stimulatory effect was noticed.

Stimulated lymphnode: 2 days Pi

Cortical tissue appeared normal. Normal lymphoid follicles with germinal centers were seen. There was slight lymphoid proliferation in the paracortical region. Medullary sinus histiocytosis was slight.

Unstimulated lymphnode - 12 days Pi

Nothing abnormal was noticed.

Stimulated lymphnode - 15 days Pi

Cortex appeared normal. Follicles did not reveal active germinal ~~contents~~ centre. There was slight hyperplasia of the paracortical region. Slight histiocytic reaction was observed in the paracortical region.

Unstimulated lymphnode:

No abnormality was observed.

Reaction to BCG:

There was numerous lymphoid follicles with well defined germinal ~~contents~~ centres. There was slight but diffuse paracortical hyperplasia. Medullary region contained many large lymphocytes and histiocytes.

Unstimulated lymphnode:

No lesions.

Stimulated lymphnode - 6 days Pi:

There was severe diffuse cortical hyperplasia. The follicles were hypertrophied. The paracortical zone was hyperplastic and contained many histiocytes and macrophages. Medulla contained many histiocytes.

Unstimulated lymphnode:

No lesions.

Stimulated lymphnode: 9 days Pi:

Slight to moderate hyperplasia ofcortex. Lymphoid follicles contained small and medium sized lymphocytes. Paracortical region appeared hyperplastic. Moderate histiocytic reaction in the medulla.

Unstimulated lymphnode:

No gross lesions.

Stimulated lymphnode - 12 days Pi:

Slight cortical and paracortical hyperplasia - Histiocytic reaction marked in the paracortical region. Sinus histiocytosis moderate in the medulla.

Unstimulated lymphnode:

No lesions.

Stimulated lymphnode:

Lymphocyte content slightly more in the cortical area. Follicles made up of small lymphocytes. Moderate number of histiocytes in the cortical and paracortical region.

Unstimulated lymphnode:

No lesions

The reaction in the lymphnode on stimulation with tumour antigen and BCG was evident by 3rd day. The reaction was maximum by 6th and 9th day and gradually subsided thereafter. In both instances the nodal reaction was indicative of a stimulation of cell mediated immune response. The macrophage-lymphoid system was stimulated to proliferative activity. Tumour antigen ~~evolved~~ induced a severe reaction although slightly less than that induced by BCG. BCG is a powerful non-specific immunostimulant and the reaction induced by tumour antigen was similar to that. The tumour animals are thus shown to be immunoresponsive. This would suggest that tumour cells are antigenically potent and they can induce immunological stimulation. In this instance the antigen had been drained into the lymphnode from the skin and had stimulated immunological reaction. The centrolateral lymphnodes did not reveal any stimulatory effect. This would imply that there was localisation of the antigen in the regional lymphnode. The reaction induced in the lymphnode in this study ~~minimise~~ mimieri the reseponse in the regional lymphnodes draining the tumour. The observation indicates that the draining

lymphnodes would be reactive even in early stages of the tumour growth. The tumour bearing animals, which were mostly in the early stages of tumour growth, were not immunologically deficient, the reaction was indicative of immunological comptency.

4.3. Distribution of T lymphocytes in the peripheral blood of tumour bearing animals:

In recent years reports have appeared indicating the usefulness of demonstration of alpha napthyl acetate estrase activity (ANAE) as a T cell marker in human beings. The studies were all carried out on lymphocytes seporated by Ficoll-hypaque gradiant centrifugation. Employing E rosette forming technique and ANAE activity simultaneously it was also established that ANAE positive cells are T lymphocytes. In cattle the results of classical E rosette forming technique have been variable and opinion on this has been contraversial. Therefore, a simple technique for demonstrating T lymphocytes in the peripheral blood smear of cattle was developed. Thin blood smears were made from the peripheral blood of cattle. Before air drying they were fixed in buffered methanol acetone solution at 4°c for 2 minutes. This solution was prepared by adding 10 ml of methanol, 54 ml of acetone 36 ml of citric acid phosphate buffer PH 5.4. The slides after fixation were washed with distilled water and placed in reaction mixture and incubated for 18-21 hours. The reaction mixture consisted of hexazotized pararosaniline and 10 mg alpha naphthyl acetate in o.4 ml of acetone, PH 5.8. Following 21 hour incubation the slides were washed with distilled water and counterstained with 1.1. toulidine blue for 30 minutes. The slides were then rinsed in distilled water, dehydrated in ascending grades of alcohol.

The monocytes revealed diffuse reddish brown reaction product. ANAE positive cells (T cells) revealed single well defined reddish brown nodule of the reaction product at the periphery of the cytoplasm close to the border. The neutrophils contained multiple small granular or nodular reddish brown reaction product in the cytoplasm.

This technique has clearly demonstrated the ANAE activity in monocytes, T lymphocytes and neutrophils. Employing this simple elegant technique it is now possible to demonstrate the population of T cells in the peripheral blood smear and can assess the cell mediated immune response.

Employing this technique the distribution of T cells in the peripheral blood of cattle bearing ethmoid tumour was studied. In a few animals studied in the early stages of tumour growth the proportion of T cells was found to be more. Further studies are in progress and data are being collected.

4.4. Delayed cutaneous hypersensitivity reactions:

4.4.1. Reaction to DNCB:

The standardisation of the test in cattle was done and the results were incorporated in the report for 1979. The test was conducted in six healthy non-tumour bearing animals and ten tumour bearing animals. The details of the test has been mentioned in the report for 1979.

During primary sensitisation both in tumour bearing and control animals there was slight hyperaemia and oedema with in one to two hours after application and this subsided in 5 to 6 hours. At the site of challenge the thickness of the skin and diameter of the reacting area were perceptibly increased. These changes continued up to three days in both control and tumour bearing animals and during this period slight exudate oozed out from the area in some animals. But this subsided in 4 to 5 days.

The measurements of the skin thickness and diameter of the reactive area in both tumour bearing and control animals are shown in table XV and ~~III.~~ XVI

The control animals showed significantly higher mean value in skin thickness both at 24 hours (PL 10.561) and at 48 hours (PL 8.178) than the tumour bearing animals. The diameter of the reactive area in the control animals also showed a significant higher mean value both at 24

hours (P< 1.73) and at 48 hours (P< 8.82) when compared to the reaction zone in tumour bearing animals.

Tabl XV

Skin thickness before and after sensitisation with DNCB in healthy control animals.

Sl. No.	Animal No.	Before sensitisation Diameter	Thickness 1st site	Thickness 2nd site	After sensitisation 24 hr Diameter 1st site	Diameter 2nd site	Thickness 1st site	Thickness 2nd site	48 hr Diameter	48 hr Thickness
1.	C. 35	3 cm	7 mm	7 mm	5.2	5.0	16	17	5cm	12mm
2.	C.666	3	5	5	5.0	5.5	16	17	4.8	14
3.	C.429	3	6	6	5.5	5.2	18	19	5.0	16
4.	c.552	3	4	3	4.2	4.0	11	12	4.0	11
5.	C.487	3	5	6	5.8	5.7	16	15	5.9	18
6.	C.650	3	4	4	6.5	6.6	38	40	6.5	38

Table XVI

Skin thickness before and after sensitization with DNCB in tumour animals.

Sl. No	Animal No.	Before sensitisation Diameter	Thickness 1st site	Thickness 2nd site	After sensitisation 24 hours Diameter 1st site	Diameter 2nd site	Thickness 1st site	Thickness 2nd site	48 hours Diameter	48 hours Thickness
1.	C.108	3 cm	4mm	4 mm	4.2	4.4	9	10	4.2	9
2.	C.110	3	3	3	4.4	4.5	9	9	4.4	9
3.	C.115	3	6	6	4.8	4.6	12	10	4.4	10
4.	C.116	3	3	3	3.6	3.5	6	5	3.5	5
5.	C.120	3	6	6	4.5	4.2	10	10	4.2	10
6.	C.122	3	6	6	4.2	4.0	10	10	4.0	10
7.	C.128	3	4	4	4.7	4.6	10	10	4.5	10
8.	C.130	3	6	6	4.1	3.7	10	11	3.7	8
9.	C.140	3	5	5	4.7	4.6	11	10	4.5	10
10.	C.142	3	6	6	4.8	4.6	10	9	4.7	10

Histopathology - Control animals:

Twenty four hours: There was focal and scattered infiltration of moderate numbers of lymphocytes in the epidermis and upper part of the dermis. Amidst the lymphocytes a few macrophages and neutrophils and eosinophils were also seen. The cellular infiltrate was seen in the perivascular location. Congestion of capillaries and separation of muscle fibres by oedema was also evident.

Forty eight hours: The cellular infiltration was more severe. Perivascular cuffing of cells was more pronounced. Vascular changes were less.

Tumour bearing animals:

Twenty four hours: Slight diffuse infiltration of lymphocytes and macrophages in the epidermis. Perivascular cuffing of mononuclear cells was a consistent feature.

Forty eight hours: The cellular infiltration was more diffuse but less intense. Perivascular accumulation of lymphocytes and macrophages was evident. Vascular congestion and oedema was less.

The test revealed that the immunological response was pronounced in control animals. The response of the tumour bearing animals was relatively less. This would suggest a slight reduction in the immunological comptency in tumour bearing animals. The reduction is only relative and is evidenced by the biometry of the interactive lesion. However, there was not much difference histologically. The pattern of reaction was identical.

4.4.2 Response to phytohaemagglutinin:

The details of the test and data on the biometry of the skin reaction have been presented in the last years report. The results of histopathological studies are detailed below.

Six control and 33 tumour bearing animals were used in this trial.

Control:

Twenty four hours: Slight to moderate focal infiltration or in some cases scattered mononuclear cells was noticed in the epidermis. The cells were mostly made up of lymphocytes and a few macrophages. A few lymphocytes were seen scattered in the perivascular zone. Congestion and varying degree of oedema were also observed.

Forty eight hours: The mononuclear infiltration was more diffuse and pronounced. Macrophages and lymphocytes were in equal numbers. Congestion and oedema were less severe. Perivascular cuffing of lymphocytes and macrophages was still evident.

Seventy two hours: The cellular infiltration was much less. The cells were seen scattered in all the layers of dermis and epidermis.

Tumour bearing animals:

Twenty four hours: Slight to moderate focal infiltration and in some cases scattered infiltration of lymphocytes and macrophages was evident in the epidermis and upper layer of dermis. Scattered lymphocytes were seen in the perivascular zone. Slight congestion and oedema was also evident.

Forty eight hours: The cellular infiltration was more severe and diffuse. Perivascular cuffing of cells - lymphocytes and macrophages was intense. Congestion and oedema were still evident.

Seventy two hours: The cellular infiltration was much less. A few lymphocytes were seen scattered in the epidermis and dermis.

The histological studies did not reveal any significant difference in the response of control non-tumour bearing animals and tumour bearing animals to PHA. From the observation made it can be concluded that in the tumour animals tested, there has not been any significant depression of cell mediated immune response.

4.4.3. Response to BCG:

The cell mediated immune response in tumour bearing animals was evaluated using BCG as the test antigen. The BCG was administered intradermally to 21 animals bearing tumour and six healthy non-tumour bearing animals. The details of the experiment has been incorporated in the progress report for 1979. The biometry of the indurative lesion at the site of inoculation was taken at 24, 48 and 72 hours. There was no significant difference at 5% level. The biopsy of the skin at the site of inoculation was done at 24, 48 and 72 hours. The tissues were processed by routine method and sections were stained with ahematoxylin and eosin.

Histopathology of the skin lesions:

Non-tumour bearing animals:

Twenty four hours: Histopathological examination of skin biopsy at 24 hours revealed the following structural changes. There was marked perivascular infiltration of lymphocytes and macrophages. Interstitial oedema was evident in the dermis. Capillary engorgement was a common observation in the dermal layer. Focal infiltration of lymphocytes, macrophages and few neutrophils was evident just beneath the epidermis.

Forty eight hours: Capillary congestion, perivascular infiltration of lymphocytes and macrophages were evident in the upper part of the dermis. The deeper part of the dermis revealed congestion and interstitial oedema besides infiltration of lymphocytes and few macrophages. There was intense lymphocytic infiltration in the interstitial tissue of the muscle bundles in the dermis.

Seventy two hours: Diffuse infiltration of lymphocytes, few histocytes, macrophages and neutrophils was seen in dermal layers and interstitial tissue. There was also pronounced perivascular infiltration of lymphocytes and macrophages.

Histopathology of skin lesion: Tumour bearing animals:

Twenty-four hours: There was congestion and infiltration of neutrophils, macrophages and few histiocytes in the dermis. Focal areas of perivascular infiltration of lymphocytes were also evident.

Forty-eight hours: Interstitial tissue revealed oedema, haemorrhage and scattered mononuclear cell infiltration. Small areas of necrosis were also evident in certain parts of the dermal layer. Moderate number of lymphocytes and macrophages was present in the superficial and deeper part of the dermis.

Seventy-two hours: The dermal layer showed engorgement of capillaries. Moderate to maked perivascular accumulation of lymphocytes and macrophages was evident. Interstitial oedema separated the muscle fibres in the dermis. Moderate but diffuse infiltration of lymphocytes and macrophages was observed in the upper layer of dermis, epidermis and in between muscle fibres.

The histological reaction observed was one of delayed hypersensitivity type and was identical in both control and tumour bearing animals. It showed that there had been no immunosuppression in tumour bearing animals and that BCG can be used as an effective non specific immunostimulant to elicit a hypersensitivity reaction in tumour bearing animals.

4.4.4. Response to tuberculin:

The hypersensitivity response of tumour bearing animals was evaluated using tuberculin as the recall antigen. The tuberculin was given five weeks after BCG vaccination. The test was performed in 17 tumour bearing animals and 6 healthy non-tumour bearing animals. The data have been presented in table XVII and XVIII. Statistical analysis did not reveal any significant difference in the response of tumour bearing animals when compared to the control animals at 5% level.

Histopathology of skin lesion: Control animals:

Twenty-four hours: Dermal layers revealed engorgement of capillaries and perivascular infiltration of lymphocytes and few macrophages. Diffuse infiltration of lymphocytes and few macrophages was evident in the upper part of dermis.

Forty-eight hours: There was interstitial oedema, and separation of muscle fibres in the dermis. Moderate to severe congestion was also evident in the dermis. Diffuse infiltration of lymphocytes and macrophages in the dermis was a characteristic feature. The infiltration of cells was also noticed in some places of the dermis.

Histopathology of skin: Response of tumour bearing animals to tuberculin:

Twenty-four hours: The lesion was characterised by focal areas of congestion in the upper part of dermis. Beneath the epidermis infiltration of lymphocytes intermingled with macrophages was evident. Interstitial oedema caused separation of muscle fibres. Infiltration of cells also extended to the deeper layer of dermis in focal areas.

Forty-eight hours: There was perivascular infiltration of macrophages and lymphocytes in the dermis. In some places it also extended into the deeper layers. Engorged capillaries were also evident in focal areas. There was also slight interstitial oedema.

The histological studies have revealed an hypersensitivity reaction to tuberculin. The data on skin measurements did not reveal any significant difference in the immunological response of tumour bearing and control animals indicating that there has been no suppression of immunological competance in tumour bearing animals.

4.5. Lymphoproliferative response in vitro:

The cell mediated immune response in tumour bearing animals was also evaluated making use of blast transformation percentage of peripheral lymphocytes in vitro.

with PHA(M) stimulation as an index. The test was carried out in 12 tumour bearing animals, and five healthy non-tumour bearing animals. The technique adopted has been detailed in the progress report for 1979. The data have been presented in the figure(6) The percentage of cells undergoing blast transformation in non-tumour bearing animals varied from 50 to 60. In contrast, in the four tumour bearing animals the blast transformation rate was between 40 - 50 percent. The blast transformation rate was below 40 percent in eight animals. This showed that the tumour animals were less immunocompetent. However, it may pointed out that these animals were in advanced stages of growth and immunosupressor facters may play a significant role.

Table XVII

Response to tuberculin 24 hours:

Anova Table

Source	df	SS(x)	SS(xy)	SS(y)	Residual	df	MSS	F
Treatment	1	7.932	2.292	0.662				
Error	22	22.177	29.339	94.419	55.006	21	2.647	
Total	23	30.109	31.631	95.081	61.852			2.359

Table XVIII

Response to tuberculin 48 hours

Anova Table

Source	df	SS(x)	SS(xy)	SS(y)	Residual	df	MSS	F
Treatment	1	7.932	4.250	2.276				
Error	22	22.177	26.827	121.327	88.876	21	4.232	
Total	23	30.109	31.077	123.603	91.527			0.626

4.6. Response of macrophages to the mononuclear substance Dextran sulphate:

Dextran sulphate (DS) was given (0.4 ml) subcutaneously to 7 non-tumour bearing animals and 7 tumour bearing animals on two sides of the anal fold region. After giving the injection the reaction at the site was noted from the next day onwards up to 7 days. At the site of injection after 24 hours there was slight thickening and this was appreciable on palpation of the site, on the 2nd day the thickening was slightly increased. On the third and fourth day the thickening was pronounced and was visible even on gross examination. From the 5th day onwards reaction became less perceptable and by 7th day the site appeared normal. In the tumour bearing animals the reaction followed the same pattern except that at 3rd and 4th day the thickening was not as much appreciable as that was observed in control animals. From 5th day onwards the skin at the site was normal in appearance.

Histological changes - Control animals:

24 hours Pi: Focal accumulation of both macrophages and lymphocytes in equal numbers. The cellular accumulation was more in the perivascular region. Scattered neutrophils and eosinophils were also seen.

Forty eight hours:Pi: Accumulation of macrophages and lymphocytes was more. Many macrophages contained congested dextran particles. The proportion of neutrophils was more.

Seventy-two hours:Pi:Intense macrophage infiltration - lymphocytes in moderate numbers - cytoplasm of macrophages was filled with dextran.

Ninety-six hours:Pi:Extensive cellular infiltration was diffuse. Lymphocytes were seen in few numbers. Still perivascular cuffing of cells was evident.

One hundred and twenty hours Pi: Number of infiltrating cells was few. Macrophages were seen scattered but the lymphocytes were very few.

One hundred and forty four hours Pi: The cell infiltration was much less. Few macrophages were seen scattered in the dermal layer.

One hundred and sixty eight hours Pi: Few macrophages were seen scattered in the dermal layer. Very few lymphocytes and macrophages around the blood vessels.

Tumour animals:

Twenty four hours Pi: Perivascular accumulation of lymphocytes and macrophages in the dermal layer. Few neutrophils were also seen.

Forty eight hours Pi: Cellular infiltration was more. Lymphocytes and macrophages were in equal numbers. Few scattered neutrophils.

Seventy two hours Pi: Cellular reaction more diffuse but less intense. Macrophages were more in number.

Ninety six hours Pi: Diffuse and scattered infiltration of macrophages and few lymphocytes. Cellular infiltration was poor.

One hundred and twenty hours Pi: A few scattered macrophages in the dermis. Isolated lymphocytes and macrophages in the perivascular location.

One hundred and forty four hours Pi: Very poor cellular infiltration. At places a few macrophages and lymphocytes were seen.

One hundred and sixty eight hours Pi: Very few macrophages and lymphocytes were seen scattered in the dermis.

The macrophage response was comparatively less in tumour bearing animals. The intensity of inflammatory response was less and subsided relatively early. This observation indicates a slight reduction in immunological responsiveness in tumour bearing animals and less activity of lymphocytes.

Table XIX

Cellular response to dextransulphate in sections.

Days	M	L	N	E	D.S.	M	L	N	E	DS
	Control animals					Tumour animals				
1	+	++	+	+	-	±	+	-	-	-
2	+++	++	++	±	+	++	++	+	-	-
3	++++	+++	++	±	++	+++	++	±	-	-
4	+++	++	++	-	+	++	±	-	-	±
5	++	+	±	-	-	+	±	-	-	-
6	+	±	-	-	-	±	±	-	-	-
7	±	±	-	-	-	±	-	-	-	-

Table XX

Cellular response to dextran sulphate in impression smears.

	Control animals					Tumour animals				
Days	M	L	N	E	D.S.	M	L	N	E	DS
1	+	++	+	-	±	±	+	-	-	-
2	++	++	++	±	++	+	+	+	±	±
3	+++	++	+	±	+++	++	++	±	±	++
4	++	++	+	±	++	++	+	-	-	±
5	+	±	±	±	+	±	±	-	-	±
6	±	±	-	-	±	±	±	-	-	-
7	±	±	-	-	-	±	-	-	-	-

4.7. Studies on lymphnode reaction to BCG and tumour antigen in tumour bearing and control animals.

The pathological changes induced by BCG (Bacillus Calmette Guerin) and TA (Tumour antigen) on prefemoral lymphnodes in these two groups were studied in order to assess the immune response in tumour bearing animals. The methodology has been described in detail in the last years report. The tumour antigen was always given on the left side and the node on the right side served as control. The stimulated lymphnodes always weighed slightly more than the unstimulated right lymphnode. The increase in weight of the stimulated lymphnode ranged from 50 to 550 mg. Maximum increase in weight was noticed on the 3rd day. Thereafter the weight gradually reduced. It was 350 mg at 6 days, 100 mg at 9 days and 50 mg at 12 days and 100 mg at 15 days. The data showed that the lymphnode (prefemoral) responded to the tumour antigen but the reaction was transient.

When BCG was given the weight of the stimulated lymphnode was always more than the unstimulated lymphnode. The gradual increase in weight was observed from 3rd day onwards. The increase in weight reached maximum on the 9th day (600 mg) and then there was gradual fell up to 15th day.

Nitrobluetretrazolium salt reduction by macrophages in lymphnode impression smears:

Tumour antigen:

The impression smears from both the stimulated and unstimulated lymphnodes were taken as soon as they were removed by surgical technique and NBT dye reduction test was carried out. The percentage of NBT positive cells were calculated for both stimulated and unstimulated lymphnode at each time interval. At 3 days the NBT positive cells in the stimulated lymphnode smear was 28.4% as against 21[illegible].8% in unstimulated. At 6 days the percentage of positive cells was 24.8% as compared to 15.4% unstimulated lymphnodes. At 9 days 28.2% as compared

to 17.8% in unstimulated lymphnode. At 12 days 20.4% when compared to 15.6% in unstimulated. At 15 days 17.6% when compared to 16.2% in unstimulated lymphnode.

BCG

At three days the stimulated lymphnode showed 19.2% NBT positive cells when compared to 14.8% in unstimulated node. At 6 days it was 24.6% when compared to 18.6% and at 9 days 24.4% when compared to 14% and at 12 days 23.8% when compared to 15.6% and at 15 days 21.2% when compared to 16.8% in unstimulated lymphnode. Both BCG and tumour antigen had ~~looked~~ induced identical reaction. This is proof to show that tumour extract is antigenic and has effect comparable to BCG. The stimulatory effect of TA was observed by 3 days and reached a peak in 6 days and it came down ~~absent ly~~ early where as in the case of BCG the reaction reached a peak by 6-9 days and came down slowly. This shows that TA is weekly antigenic but previous exposure of the node to circulating TA in tumour bearing animals might induce an immediate response.

The histological reaction of the harvested lymphnode at specific intervals were also studied.

In the control animals both TA and BCG induced a stimulatory response.

Tumour Antigen:

There was varying degree of cortical and paracortical hyperplasia. From 3rd day onwards large lymphocytes and macrophages were predominant. Sinus histiocytosis in the medullary sinuses was a feature. The medullary cords were thickened and consisted of many large lymphocytes. Cells in mitotic division ~~was~~ were high. Focal collections of epithelioid cells were evident by 6th day. The lymphoid follicles at the cortex was activated. They appeared increased in size and number. The germinal ~~centers~~ centres were enlarged and they contained macrophages. The response gradually subsided and by 6th day there was no indication of much activation.

BCG:

There was diffuse cortical and paracortical hyperplasia. Small and large lymphocytes were seen crowding the cortical area by the 3rd day. These follicles were hyperplastic and their number was also found to be increased. Germinal centre contained macrophages and large lymphocytes. This was more evident by 6th day. Histiocytic-macrophage reaction was discernible by the 4th day. The medullary cords were thickened. They contained lymphocytes and macrophages.

The histological picture of the contralateral lymphnode in both the groups did not reveal any evidence of stimulatory response. This would suggest that the antigen was localised in the lymphnode near the site of inoculation in the skin (Prefemoral lymphnode).

The pattern of lymphnode reaction when BCG and TA were given was almost identical. However, the reaction with TA was quick in onset while with BCG it was slightly delayed. In both instances a cell mediated immune reaction was evoked. The reaction was transient with TA but was more sustained with BCG. The study showed that tumour bearing animals are immunologically comptent and tumour antigen could be used with suitable modification for immunomodulation.

5. DIAGNOSTIC TESTS

5.1. Evaluation of agar gel diffusion and passive haemagglutination tests for the diagnosis of ethmoid tumour:

The neoplastic cells in certain instances are immunogenic for the host in which they arise as the neoplastic cells acquire certain antigens called tumour specific trahsplantation antigens. These antigens may stimulate antibody production and therefore the serum of patients are likely to contain antibody to tumour associated antigen. If it is possible to detect these antibodies in the serum it would be possible to diagnose the malignancy. Therefore, attempts were made in the laboratory to evolve a suitable diagnostic test for the early diagnosis of the cancer employing gel diffusion test and passive haemagglutination test for the detection of antibody and reverse passive haemagglutination test for the

detection of circulating antigen.

Haemagglutination test for the detection of circulating Antigen.

Three types of antigens were employed, a crude 15% suspension of freshly collected tumour tissue in Hank's balanced salt solution (Antigen A); 3 M potassium chloride extracted antigen (Antigen B) and whole serum of tumour bearing animals. Antigen B was extracted from freshly collected tumour tissue following the method of Mavlight et al. (1973) for human solid tumours. The extract was dialysed against distilled water for 1 hour and against phosphate buffered saline (PH 7.2) for 24 hours, changing the buffer every 8 hours. The dialysed solution was centrifuged at 6000 g for 3 hours, the supernatent was collected, the concentration of protein was estimated by Biuret's method and stored at -20°c after freeze drying in 2 ml quantitives till use.

Antisera to all the above antigens were raised in rabbits. Initially 1 ml of antigen mixed with an equal quantity of Freund's complete adjuvant was administered subcutaneously and 2 ml of antigen alone was given intra venously. Subsequently they received four injections each of 2 ml antigen alone at 3 day intervals beginning from the 3rd day of the first dose. After 10 days of the last injection, the antibody response was tested by agar gel precipitation test against homologus antigen. When a stisfactory response was obtained, the rabbits were blood from the heart, sera separated, inactivated at 56°c for 30 minutes and stored at -20°c in 1 ml aliqueots for later use.

Test sera:

Each of the serum samples separated from the blood of tumour bearing animals was divided into four parts. The first part was inactivated at 56°c for 30 minutes (Serum -a), second part was inactivated and adsorbed with heat inactivated normal bovine serum (serum b), third part was heat inactivated and adsorbed with 50% formalinised tanned sheep red cells (SRBC) (serum c) and the fourth part was left untreated (serum d).

Gel diffusion test:

Immunodiffusion analysis was done according to the double diffusion method of Ouchterlaney (1958) with 1% noble agar in 0.15 m sodium chloride. The serum a and b were tested separately with all the three types of antigen.

Passive haemagglutination test:

Sheep red blood cells collected in Alsevers solution were washed thrice in physiological saline and a 10% suspension was made. An equal volume of 3% formalinised saline was added to the suspension and the mixture was incubated at 37°c for 20 hours. After incubation the formalinised cells were washed thrice in normal saline and stored as a 10% suspension at 4°c till it was used for tanning. For tanning the cells, 4% formalinised and 1.10000 tannic acid solution in normal saline were mixed in equal quantities, incubated at 37°c for 30 minutes, centrifuged and washed thrice in normal saline. A 2% suspension of the tanned cells was used for antigen activity. This was done by mixing equal quantities of 2% freshly tanned cells and 1% tumour antigen (Antigen A, B) and keeping it for 1 hour at 37°c. The cells were then centrifuged gently, washed twice with normal saline and resuspended to give a concentration of 1% antigen coated cells in 2% rabbit serum in normal saline.

Serial two fold dilutions of the test sera (serum c) in RSD were made in persplex haemagglutination plates. An equal volume of 1% antigen coated SRBC was added to each of the serum containing wells, mixed well and the plates were incubated at room temperature. The following controls were also set; the tanned SREC coated with normal bovine serum and tested against rabbit antibovine serum, the antigen coated cells and RSD, and the tanned SRBC coated with normal bovine serum and RSD.

Reverse passive haemagglutination test:

The presence of circulating antigen in the test sera (serum a, and d) was tested by reverse passive haemagglutination using formalinised tanned cells coated with antisera to antigen A and B.

No precipitin lines were noticed when antigen A or B was tested against the serum from tumour as well as non-tumour bearing animals, while they produced precipitin lines with the specific antiserum. The antiserum to antigen C showed specific reaction with sera of both tumour and non-tumour bearing animals but the number and nature of lines were similar. The precipitin lines completely disappeared when the antiserum to antigen C was adsorbed with normal bovine serum. The above observations indicate that the tumour animals do not have any circulating antigen, any protein constituent or any tumour specific antibodies which could distinguish the serum from that of a non-tumour bearing animal by gel diffusion test. The failure to obtain any specific reaction to tumour antigen or their antisera when mixed with the sera of tumour bearing animals may thus be considered as an indication of lack of tumour specific antigen or antibody in circulation or their absence in circulation in sufficient concentration to produce a visible precipitin reaction.

Fifty serum samples from tumour bearing animals were tested for the presence of circulating antibodies or antigen by direct and reverse passive haemagglutination tests respectively. None of the samples tested revealed the presence of either the antibody or antigen. The results obtained during this study showed that the passive haemagglutination and gel diffusion tests are not suitable for the diagnosis of endemic ethmoid carcinoma in cattle.

5.2. Ehrlich test for the diagnosis of the carcinoma of the mucosa of the ethmoid:

Ehrlich test, a simple colorimetric test was described by Nixan (1973) to detect malignancy in man. In this plasma from patients bearing tumour was incubated with Ehrlich reagent and the colour developed was measured by colorimetric methods. Chitnis et al. (1977) employed this test in oral cancer patients and observed that accurate distinction between normal individuals and patients with progressive cancer is possible. A study was therefore

undertaken to assess the usefulness of this test in detecting malignancy. The study was conducted on 15 tumour bearing animals and eight adult healthy animals. The tumour bearing animals were divided into three groups, based on the stage of tumour growth.

Stage I: Animals having slight respiratory distress mucus or mucosangimneous discharge without any bulging of the eye.

Stage II: Animals having mucus discharge and also moderate bulging of either one of the eyes or both.

Stage III: Animals having marked respiratory distress and abdominal type of respiration, marked exopthalmos rarefaction of the frontal bone and protrusion of the tumour mass into the subcutaneous tissue of the forehead.

Six animals in stage I group, five animals in stage II group and four animals in Stage III group and eight normal animals were tested.

Two ml of plasma, 4 ml of tap water and one ml of Ehalich reagent (0.7 g of para-dimethyl amino aze benzyl dehyde, 150 ml of con. Hydrochloric acid and 100 ml of distilled water) were mixed in a test tube, shaken and incubated for seven hours at 50°c in a water bath.

After incubation the tubes were centrifuged at 2000 RPM for 15 minutes and the optical density was measured at 640 millimicrons and 500 millimicrons using a Beckman spectrophotometer.

When the reagent was added to plasma immediately there was a dense white precipitate formation. During incubation in the water bath the colour of the suspension became purple blue. The optical density has been graphically represented (Fig 1). From the figure it could be seen that highest mean optical density was seen in Stage II. But between Stage I and normal animals there was not much difference. It was also seen that in animals with slow developing cancer the optical density was low.

Animals having adenocarcinoma had high OD . Animals bearing tumour with advanced necrosis OD was found to be low.

Significant diffuse detectable in the animals in stage two groups indicate that for the dectable changes to xxx occur by this test, the cancer growth should assume significant mass. The measurements of OD were made at two wave lenghts. Scattering of values was better at 640 millimicrons. There was not much difference in the values between normal animals and animals in stage I growth by this test, it was not possible to detect early cases of this cancer. It could be used along with other tests as one of the tests to confirm malignancy.

6. TUMOUR CELL CULTURE

Two different techniques were employed to establish the tumour cell line in vitro.

6.1. Explant culture:

Pieces of tumour tissue collected immediately after slaughter were suspended in Hank's balanced salt solution containing 100 im of penicillin and 1000 µg of streptomycin. The tissue pieces were washed three times in the same solution before it was minced into small pieces of 1mm size. A portion of this minced tissue pieces were suspended in tissue culture growth medium and were seeded on to Roux flasks, 100 ml tissue culture bottles or 160mm petridishes and incubated at 37°c after gentle shaking to distribute the tissue pieces. On the 4th day the flasks were gently shaken to dissolve the larger pieces from the site of cellular attachment to the glass surface and growth. The medium was poured off and replaced with fresh growth xxxxxx medium. Cases with apparently ~~new~~ uncontaminated growth tissue culture was attempted. In three instances growth was evident. The cells had attachment and grew for 4-5 days. But an subsequent passage the cells lost their viability. Bacterial and fungal contamination was very heavy and often cultural examination failed.

6.2. Dissociated cell culture:

The other half of the minced tumour tissue was washed three times in calcium magnesium free buffer (PH 7.6) and trypsinised for 20-30 minutes with 0.25% Difco trypsin (1.250) CMF. After allowing the tissues to settle the supernatent containing dispersed cells was filtered through double layered sterile muslin cloth and centrifuged at 1000 RPM for 8-10 minutes. The cell sediment was resuspended in fresh growth medium and again washed three times. These washed cells were resuspended in fresh growth medium to get a final concentration of 5 X 105 cell/ml. Then the material was seeded on to Roux flasks, tissue culture bottles and test tubes. They were incubated at 37°c. The tumour tissue from 8 tumour bearing animals were cultured by this technique and cell growth was obtained in 2 instances. Here also the cells were viable and showed progressive growth till 6th day but subsequently the cells degenerated and did not grow.

It has not been possible to establish the cell line. Inspite of using antibodies like Gentamycin and Ampicillin there was overgrowth by contaminant microbes. The use of heavy dose of antibiotics might have interfered with cell growth. The laboured deep respiratory inhalation may cause numerous environmental microbes to invade the tumour growth.

7. Virological studies

Experimental infection studies:

Three, one month old calves were infected with one of the isolates (STV-15) to see whether it can induce tumours. The calves received 4 ml of the virus in the form of infected allantoic fluid into the sinus. All the calves did not show any noticeable symptoms. The sera from these calves were screened at intervals to see whether there was ny increase in antibody titer. They were also screened by X-ray for the development of any tumours.

None of the three calves showed any increase in antibody titer. One of them died two months following infection. On postmortem examination the ethmoid region

was found highly congested and had a small swelling which contained semisolid substance. On histological examination there was severe diffuse hyperaemia and oedema of the ethmoid mucosa. The submucosa was infiltrated with moderate number of lymphocytes and macrophages. In Focal areas there was degeneration and desquamation of epithelial lining.

The second one was destroyed one year after infection. It also had the changes in the ethmoid region, as shown by the Ist calf but the reaction was more. Histologically there was moderate congestion of blood vessels. Subcutaneous oedema was slight. A few scattered lymphocytes and macrophages were seen. In focal areas there was hyperplasia of epithelial lining and the two or three layers of cells were seen lining the mucosa. In certain other locations there was metaplasia of the lining epithelial cells into squamous cell type. The mucosa was moderately and diffusely thickened.

The observations made have shown an inflammatory - hyperplastic type of reaction. Further studies are in progress.

The third calf is still alive and is about 1 year and 9 months now. On X-ray it showed some shadow in the region of the ethmoid.

None of the serum samples from these calves had antibodies (HI) to STV - 15.

7.2. Immunization:

STV - 15 infected allantoic fluid was inactivated with 0.5% formalin and inoculated subcutaneously into 3 tumour bearing animals. Unfortunately all of them were in advanced stage of the tumour. Although one of them survived for 1 month after inoculation it also failed to produce any antibodies to the virus or any other isolates from tumour bearing animals.

8. Immunotherapy

8.1. Non-specific immunotherapy:

The response of the regional lymphnode of tumour bearing animals to BCG was evaluated by intranodal inoculation of BCG. BCG was inoculated into the left mandibular lymphnode of the tumour bearing animals. By this study it was also envisaged to assess the immunotherapeutic effect of BCG. The animals were under clinical observation if any, till death. Clinically there was no palpable enlargement of the node and regression of tumour growth was not appreciable. The cytomorphological changes in these lymphnodes were studied histologically. The animals were grouped into different categories based on the survival time after BCG inoculation.

In the first group of ten animals the lymphnodes were examined with in a period of 3 days to one month. after inoculation of BCG. The consistent lesion was diffuse lymphoid hyperplasia in the paracortical region associated with depletion of lymphoid cells in the medullary region. The medullary region revealed moderate sinus histiocytosis. In focal areas epithelioid cells with foamy abundant acidophilic cytoplasm were also seen.

Group II included those animals which survived for 1-2 months after inoculation of BCG. There were four animals in this group. Histologically the reaction in the lymphnodes was characterised by diffuse hyperplasia of lymphoid cells. In the cortical and paracortical region well defined follicles were few or absent. There was reticular hyperplasia in the follicular region. Marked sinus histiocytosis was a feature.

The third group consisted of three animals which survived for 2-3 months after inoculation of BCG. The node was palpable but there was no visible enlargement. Histologically there was focal lymphoid hyperplasia in the paracortical and medullary region. There was

severe degree of stromal reaction. Sinus histiocytosis characterised by sheets of proliferating histocytes was evident in the stroma.

In the fourth group there were three animals. These animals survived for a period of more than 3 months after BCG inoculation. Histologically the nodes revealed focal cortical and paracortical hyperplasia of lymphoid cells with an attempt at germinal centre formation. Focal areas of necrosis were also evident in the medullary region. Sinus histiocytes was moderate to marked. Stromal reaction was slight.

The consistent histological picture observed in the nodes in this study was paracortical hyperplasia and moderate sinus histiocytosis. These observations are evidences which would categorically show that there had been stimulation of thymus dependant areas in the lymph-node. Therefore, there has been stimulation of CMI response in tumour bearing animals. and this has helped to enhance the survival period.

Inoculation of BCG intralesionally:

The immunotherapeutic effect of administration of BCG by this technique was attempted in 9 animals. The details of administration etc have been furnished in the last years report.

There was enhancement of tumour growth in 3 animals. The intralesial administration of BCG did not reveal any clinical regression of tumour growth. From the literature it would appear that the tumour burden should be minimal to have a beneficial effect. It may have a desired effect only in early stages of the growth. In the present study intralesial injection of BCG was given to those animals which had perforation of the frontal bone. These are naturally advanced cases. In early stages, when there is no bone perforation, it may be difficult to administor BCG intralesionally.

Histological examination of tumour tissue on death revealed necrosis and diffuse infiltration with lymphocytes and macrophages. In some areas giant cells and plasma cells were also seen. The intensity of reaction was more in those animals which survived long. There has been immunological response in tumour tissue but since the tumour burden was heavy the treatment was not effective.

8.2. Specific immunotherapy:

The vaccine using irradicated tumour cells and Freund's complete adjuvant was administered in 15 tumour bearing animals in different stages of tumour growth. In three animals a vaccine consisting of 3 ml of tumour cell suspension + 3 ml of paraffin and+1.3 mg of BCG was given. The method of preparation and administration of vaccine staging of tumour etc. has been described in earlier reports.

The animals belonging to the Stage I showed marked clinical improvement characterised by reduction in respiratory distress and general condition. One the animal in Stage I was kept under observation for 181 days and then it was taken away by the owner. The animals has not shown any symptoms even after a year. The mean survival time in Stage I group was 151.66 ± 19.02 days. The mean survival periods in Stage II and III were 56.16 ± 16.74 and 23.66 ± 2.56 days respectively.

The data clearly indicated that vaccination is effective in early stages of growth when the tumour burden is low.

On the death of the vaccinated tumour bearing animals post-mortem examination was done and tumour tissue was examined. The tumour tissue in Stage I and II on gross examination revealed necrosis and the whole tissue appeared as crumbling mass loosely and adherent to the ethmoid region. Animals in Stage III revealed focal areas of necrosis in tumour tissue.

The tumour tissue from the vaccinated animals was subjected to detailed histopathological examination on death.

In Stage II there was severe necrosis. The tumour tissue appeared as an eosinophilic mass surrounded by stroma. Diffuse collection of lymphocytes, macrophages and histiocytes was noticed in the stromal tissue. Haemorrhagic foci were observed in focal areas.

In stage II the reaction was characterised by varying degree of necrosis and focal areas of haemorrhage. Focal and diffuse collection of lymphocytes, macrophages and giant cells were evident. Infiltration of lymphocytes and macrophages was noticed in the stromal tissue. Histiocytic reaction was evident in focal areas.

In Stage III intensity of reaction was less. Mild to moderate degree of infiltration of lymphocytes and macrophages was evident. Patchy areas of necrosis was evident.

An attempt was made to correlate the survival period of vaccinated tumour bearing animals and histological type of growth. The observation made did not indicate any relationship between the survival period and histological pattern of tumour growth.

The response to vaccination was monitored by (1) Leukocytic response (2) Enhancement of survival period and by (3) exfoliative cytological studies.

There was significant progressive increase in the leucocyte count following vaccination. This was reflected both in total leucocyte count and differential count. In the Stage I group, the leucocyte count showed a steady increase up to 45 days and by 60 days there was slight fall and again there was leucocytosis by 120 days. There was also progressive increase in the absolute lymphocyte count. But in later stages showed a decline in the count.

In stage II absolute lymphocyte count showed a sharp increase by 15th day and reached maximum by 30th day. Then there was a sharp fall by 45th day and reached below base line value by 75th day.

In stage III absolute leucocyte count increased towards the later stages but the lymphocyte count was less than the initial value.

There was lymphocytic leucocytosis following vaccination but as days passed the ~~was~~ response came down. The tumour burden was ~~much~~ high even in stages classified as Stage I. Immunotherapy can be effective only when the tumour burden is low. The immunological response indicates that vaccination can be effective in very early stages. The second factor of importance is the toxaemia resulting from extreme degree of tumour necrosis following vaccination.

The relationship between tumour burden and vaccination is again idicated by the survival period.

In unvaccinated animals (5) in stage I the survival perid on the average was 45.5± 3.5 days. In the vaccinated group of animals (4) mean survival period was 178.33 ± 2.33 days.

In the stage two groups of animals (5) the mean survival period was 29.6 ± 3.50 days for unvaccinated animals and 78.007 ± 10.88 days for animals in the vaccinated group (13). The fourteen unvaccinated animals in stage III group had a mean survival period of 15.57 ± 2.20 days and eleven vaccinated animals in the same group had a survival period of 37.545 ± 2.906 days.

8.3. <u>The response of the prescapular lymphnode in vaccinated animals</u>:

The prescapular lymphnode near the site of vaccination was collected immediately after death/ sacrifice. Reaction from twenty day up to 90 days was studied.

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The cortical area was very much increased. Follicles were normal in number but their size was increased. The centre of the follicles contained very large lymphoid cells and moderate number of macrophages. In the sub-capsular area increased number of macrophages was seen. Diffuse hyperplasia of paracortical region was a feature. Groups of epithelioid cells were seen. Medullary cords were widened. In the sinus histiocytic reaction was marked. The sinuses were dilated and contained large number of small and medium sized lymphocytes. As the days passed the cortical follicles increased in number and size. Number of macrophages became more. Many of the macrophages had vaccuolated cytoplasm. Medullary sinus histiocytosis was marked. At about ninety days the activity was relatively less and cells were mostly small lymphocytes. Only few macrophages were seen. Medullary sinus histiocytosis was also less intense.

The regional draining lymphnode as evidenced by histological reaction responded to the antigen drained into the node. There was stimulation of cortical and paracortical area and histiocytic reaction. The histological picture was one of CMI response and there was stimulatory response of lymphocytes and macrophages. The vaccine was found to be a useful immunostimulator and the host response was found to be good.

9. CHEMO THERAPY

Chemotherapy with cyclophosphamide in goats bearing ethmoid tumours:

Cyclophosphamide (Endoxan) is one of the common cytotoxic anticarcinogenic agents employed. Initially an experiment was conducted to study its effect on normal goats.

Five healthy goats were administered cyclophosphamide (CY) intravenously at the rate of 500 mg/animal. Four injections were given with an interval of three days between injections. Haemogram was studied every third day till the normal values were obtained after the test.

dosage. Data have been graphically represented (Fig 2-5). There was significant leucopaenia on the third day itself. Lowest leucocyte count was obtained on the 12th day and the value came back to normal by 21st day (Fig 3). Supression of bone marrow was indicated by drop in neutrophil count and haemoglobin level (Fig 4, 5). Haemoglobin level reached the lowest level by 12 th day and gradual raise was noticed by 15th day. The eosinophil count also came down to by 3rd day and maximum drop was noticed by 9th day. Then the count went up but there was drop in the count by 21st day (Fig 4). The effect of Cy was transient and normality was reached soon after the withdrawl of administration of the drug. The dosage schedule adopted was found to have no adverse effects.

The cyclophosphamide was employed for treating goats bearing endemic ethmoid cancer. In five tumour bearing animals in varying stages of tumour growth the therapy was attempted. A course of 7 injections (500 mg per animals per injection) were given at an interval of one week. There was progressive reduction in clinical symptoms, and regression of tumour growth. The bulging growth became shrunken. In two animals there was complete disappearance of growth. In three animals three months after discontinuation of treatment these growth recurred. But these animals were in advanced stages of growth before starting the treatment. But the drug appears to be useful in treating and curing cases in early stages of growth. Further trials are being made.

10. Pathological features:

During the year 70 animals were subjected to detailed post-mortem examination. The detailed report pertaining to each animal has been given separately. The animals examined consisted of cross-bred cattle (43), non-descript cattle (6), buffaloes (3), goats (7) and pigs (11).

10.1. Gross lesions:

Cattle: The gross features observed were similar to that reported earlier. The tumour was found to invariably arise from the ethmoid region. Complete destruction

of the nasal turbinates was a feature. The frontal sinus was most commonly involved. The maxillary and sphenopalatine sinuses etc. were involved only occassionally. The perforation of the frontal bone was observed in 17 cases. Except in two instances the tumour was found to extend forward and involve the frontal sinus. In two instances the tumour tissue invaded backwards and perforated the cranial plate and occupied the cerebrum destroyed it and occupied the lateral ventricles of the brain. There was no extension into the nasal chamber and it appeared on gross examination as a primary tumour of the brain. Besides this in 15 cases brain invasion was encountered along with filling of the frontal sinus and nasal passage. Pharngial passage was almost completely filled up with the tumour mass in 16 instances. The growth had often smooth glistening fibrous capsule. The growths were fleshy, soft or hard and contained scattered bony spicules. In some areas cystic spaces were seen containing mucinous material. Scattered foci of degeneration and necrosis were seen.

The draining lymphnodes of the head revealed congestion, oedema and enlargement. In cases of metastasis greyish white mass of growth was encountered. All the head lymphnodes were involved in 12 cases. Retropharyngeal lymphnode was the commonest lymphnode involved, followed by parotid and mandibular lymphnodes. The enlarged retropharyngeal lymphnodes often caused pressure on the pharynx.

Metastatic foci were encountered on the bronchial lymphnode and lung in two instances. The detailed autopsy findings have been given separately.

In goats the lesions were very much advanced. The growth extended through the nostrils to the outside. Exopthalmos was not significant. The respiratory difficulty was seen only in very late stages. The turbinates often showed inflammatory oedematus swellings. The tumour originated invariably from the ethmoid region. Invasion into the brain was seen in two instances.

In pigs the growth appeared as a bulging on the dished part of the frontal region. The growth was soft and arose from the ethmoid region. It invaded the brain and frontal sinus. It did not came down into the nostrils much. Exopthalmos was not a feature, but expistaxis was the consistent symptom.

10.2. Histopathological studies:

The histopathological examination of tissues collected from tumour bearing animals ~~were~~ was completed. Several blocks were prepared from the tumour tissue from each animal. The other visc~~i~~eral organs like liver, kidney, heart, spleen etc were also examined. Lymphnodes of the head were subjected to detailed exaination taking multiple blocks from each of the lymphnodes. Sections were routinely stained with Harei's haematoxylin and eosin, Van Gieson's picrofuchisin, PAS, Mallory's trichroma and PTAH.

The histopathological feature of tissues from individual animals have been given separately.

The tumours studied were classified as follows:

1. Squamous cell carcinoma
2. Adenocarcinoma
3. Undifferentiated carcinoma

Adenocarcinoma was observed in 27 animals. They were sub-classified into: Simple carcinoma (20) and paillary adenocarcinoma (7). Well differentiated acini contained mucinous secretary material. Varying degree of fibrovascular stroma was seen separating the acinar tissue. Bony tissue undergoing osteolysis was seen scattered in the tumour tissue.

Adenocarcinoma often showed squamous metaplasia. Transition from columnar to squamous cells was evident in serial sections of tumour tissue. The transitial stage in squamous cell carcinoma was observed in cases. Once differentiation occurred it was designated as squamous cell carcinoma.

Squamous cell carcinoma consisted of well defined squamous epithelial cells arranged in groups. Non-keratising squamous cell carcinoma was more common than keratinising type. The stroma was relatively less. Cells in mitotic division were seen in large numbers. The squamous epithelial cells in certain instances showdd intra epithelial hyaline formation.

Undifferentiated carcinoma consisted of anaplastic cells. The cells were arranged in sheets and stroma was slight to moderate. Degenerating osseus spicules were embedded in the tumour tissue.

The invasion into the brain tissue resulted in replacement of the brain parenchyma. Lesions were seen in the anterior pole of the cerebellum. There were no lesions on the posterior aspect of the brain. Slight gliosis and perivascular cuffing was evident in focal areas. But the reaction was relatively week. Even-though in certain instances $\frac{1}{4}$ th to 1/3 rd of the cerebrum was involved. Clinical manifestation of nervous symptom was much less. The entry into the brain was by direct invasion destroying the cribriform plate of the ethmoid.

10.3. Reaction in the Head lymphnodes:

Cytomorphological changes observed in the regional lymphnodes were grouped mainly into five categories depending on the pattern of cellular reaction in these nodes following largely the classification adopted by (Vannagell et al 1977). They were

1. Lymphocyte predominant
2. Germinal centre predeominant
3. Unstimulated
4. Lymphocyte depleted
5. Sinus histiocytosis.

Lymphocyte profominant:

This type of reaction was observed in 50% of the lymphnodes examined. The consistent feature in this group was diffuse hyperplasia of lymphoid tissue on the cortical, paracortical and medullary region. The

lymphoid follicles in the cortical area were devoid of germinal centre. The sinus was filled by proliferating lymphoid tissue. In some instances well defined follicles were evident in the cortical region. The lymphoid cells in this group were large with eosinophilic cytoplasm and loosely arranged chromatin. Reticular hyperplasia characterised by proliferating reticular cells in the follicles was also evident. The cells were large with irregular border and vesicular nucleus and faintly eosinophilic cytoplasm. There was also diffuse paracortical and focal histiocytic reaction characterised by islands of histiocytes in the medullay region.

Germinal centre predominant:

In this group the lymphnodes exhibited germinal centre formation. This type of reaction was observed in 10% cases. The area appeared as pale compared to other parts of lymphnodes. The cells in this area were enlarged and hypertrophied. The immunoblasts seen in the paracortical zone showed large centrally placed nucleus with pyrininophilic cytoplasm. Surrounding this area, large lymphocytes with notched nucleus were evident. The cytoplasm of these cells was eosinophilic.

Unstimulated:

It was characterised by few or no well defined lymphoid follicles in the cortical region. There was no hyperplasia of the cells in the paracortical region. This type of reaction occured in 25% of the lymph nodes studied. The stromal reaction was moderate. Depletion of lymphoid cells in the medullary region was evident. The blood vessels were sclerotic. The lymphatics were dilated and contained pale pink staining lymph. Twenty percent of this nodes revealed metastatic foci of the tumour. Islands of neoplastic cells were seen embedded in the lymphoid tissue. Amidst the neoplastic cells there were foci of lymphoid accumulation. These metastatic foci were surrounded by moderately thick bands of fibrovascular stroma and few lymphoid cells. Increase in trabecular tissue was observed in these cases.

Lymphocytic depletion:

In this group there was total depletion of lymphoid tissue in the cortical and medullary region. This type of reaction was observed in 15% of lymph nodes studied. The proliferating stromal tissue was seen to replace most of the lymphoid tissue. In focal areas, necrosis and hayalinisation were evident in the follicles as well as in the medullary region. Dilatation of lymphatics and moderate degree of stromal oedema were also evident. Focal areas of congestion and haemorrhage were seen scattered in the node. The proliferation of stromal tissue was more in the medullary region. The blood vessels were sclerotic and capsular thickening was evident. Sinus histiocytic reaction was sparse to minimal.

Histiocytosis:

This type of reaction was more evident in stimulated lymph nodes and least in metastatic and lumphocyte depleted nodes. The medullary region was completely or almost filled with large oval cells with abundant slightly foamy granular eosinophilic cytoplasm. They replaced the lymphoid tissue in the parenchyma in many areas. Certain instances these hypertrophic macrophages extended into the the paracortex and lymphoid follicles in the cortical region. Associated with histiocytosis macrophages were also noticed. In most cases the sinus histiocytic reaction was meagre in metastatic lymph nodes. In some instances perivascular accumulation of histiocytes was also observed. In 15 cases the metastatic foci in the regional lymphnode were evident only by histological examination. The metastatic involvement was more in the retropharyngal lymph nodes (13). The other lymph nodes involved in the decreasing order of frequency were parotid (7) and mandibular (4). Adenocarcinomas were observed in eight instances and squamous cell carcinoma (16) instances. Proliferating columnar or squamous neoplastic cells were seen in focal areas in the cortex and occassionally in the medullary region. The common location of

of the metastatic foci were in the cortex and particularly in the subcortical sinus region. Clumps of neoplastic cells were seen in the capsule of the lymph node embedded in the stroma.

In some cases the proliferating anaplastic ells replaced major portion of the lymphoid tissue and foci of tumour tissue were often seen in the lymphoid follicle. The growth spread the subcortical sinus region and extended towards the medullary. In some areas the neoplastic cells were intimately dispresed with lymphocytes. The lymph sinus in some cases were filled with neoplastic cells. In some cases formation of well defined cell nests with keratinisation was evident. The histiocytic reaction was minimal. Focal areas of necrosis, congestion and haemorrhage were also evident in these nodes associated with oedema and dilated lymphatics. The stromal reaction was moderate to severe. Depletion of lymphocytes was a characteristic feature.

The common types of morphological manifestation observed were lymphocyte predominant and unstimulated pattern. The increased activity in the thymus dependant area indicated an anti-tumour T cell mediated immune response. There was evidence of immunological stimulation of the draining lymphnodes in 50% of the tumour bearing animals examined. This can be considered as an indication of stimulation of the lymphnode by the tumour associated antigens and a defence against formation of secondaries in the node. Increased germinal centre activity was observed only in a few cases. The tumour bearing animals are exposed to variety of antigens other than the tumour associated antigens. Tumour necrosis inhalation of many viruses and bacteria certainly could induce a strong reaction in these nodules. High incidence of metastasis was seen in unstimulated and lymphocyte depleted nodes. Sinus histiocytosis in the draining lymphnodes of tumour bearing animals was a common feature. This is another morphological manifestation of CMI response The pattern of reaction in the draining lymphnodes provides evidence for immunological stimulation of the node by tumour antigen. The observation made strongly suggests that our attention should be directed to exploit immuno therapy as a therapeutic measure.

10.4. Pathology of the spleen in tumour bearing animals:

The spleen is an important organ associated with immunological reactions. In order to assess the pathological reactions in the spleen in tumour bearing animals a specific study was undertaken. The spleen of the tumour bearing animals after death were collected. The organs were weighed and tissue, were fixed in 10% formalin and paraffin section cut at 5 to 6 u thickness were stained with haematoxylin and eosin.

The observations on ten animals have been completed. There was reduction in size and weight of the spleen. The capsule appeared wrinkled. The white pulp was much less and the red pulp was relatively more. Histologically the connective tissue trabecular was prominant. The splenic capsules were atrophic and lymphocytes were only few. Congestion of sinusoids was seen. In certain instances haemosiderosis was noted. Around the arterial sheath and lymphoid follicles the lymphocytes were only few and they did not reveal any mitotic activity. Lymphoid follicles which were only few had washed ~~at~~ out appearance. The work has not been completed. Further studies are in progress.

10.5. Pathology of the adrenal gland in tumour bearing animals:

The adrenal glands are associated with the maintenance of internal haemostasis and in chronic disorders like the malignancy there is likely to have changes in these glands. The adrenal glands of 10 healthy animals and 15 cross-bred tumour bearing animals were subjected to detailed studies. ~~The weight of the adrenal glands and measurements of the cortex and medulla are shown on the table.~~ There was enlargement or the adrenal glands as evidenced by the weight of the glands in tumour bearing animals. The cortex was broader than the healthy animals.

There was congestion and focal areas of haemorrhage in the cortex. Zona fasciculate consistently showed ~~dx~~ diffuse hyperplasia. There were also focal areas of degeneration and fatty change and necrosis in these zone.

Glandular formation and cystic dilatation were seen in two instances. Cortico-medullary fibrosis and congestion were seen five cases.

Varying degree of capsular fibrosis was noticed in one case and this animal, which had advanced tumour growth also had multiple accessary cortical nodules which contained zona fasciculta cells. Medulla revealed congestion.

The increase in the weight of the adrenal gland is an indicature of stress response in the tumour bearing animals. Diffuse hyperplasia, degeneration, fatty change and necrosis in the cortex are again observations which could support a stress response. The general Pathological change in the adrenal indicated an adaptive response in tumour bearing animals.

11. ELECTRON MICROSCOPIC STUDIES

A consideration of the epidemiological features of this neoplasm suggested the association of some infectious agent with this constitution. In the studies made we could isolate seven haemagglutinating agents from a total of 17 tumour tissues (41%) processed for virus isolation by chick embryo. In order to identify the nature of the involvement of the virus in the causation of the tumour tissues collected from animals sacrificed were examined.

The affected animals were slaughtered and the tumour tissues were collected immediately as small pieces of 2-4 mm size and fixed in 2.5% gluteraldehyde in cacodylate buffer. They were subsequently refixed in 1% osmium tetroxide buffered with S.coludine, dehydrated through graded series of alcohol and embedded in epon. Thin sections were cut with glass knives on LKB ultratome, stained with uranyl acetate and lead citrate and examined with philips EM 301 at 60 KV.

The sections from one of the tumour tissue showed thickening and small crescent like projections of the plasma membrane at certain regions. The projections contained distinct electron dense spots which were arranged symmetri-

cally in a circular fashion. These dense spots could be the cross sections of filamentous structures, possibly the nucleocapsids. The cell surface projections showed a tendency to form distinct buds which got separated and existed as free particles (110 to 150 mm in size). The particles that got released in this manner and appeared as free particles were more or less uniform in size and had identical morphological features. They also contained electron dense spots (15 to 17 mm) as described above. However, pelomorphic particles were also seen in few sections. The outer membrane of the bud like projections and the free particles were covered with peplomer like structures.

The maturation and assembly at the plasma membrane, their release by budding, presence of nucleocapsid like structures, well defined outer rim covered by peplomer like structures and the size of the free particles suggest that they are (could be) envelpped virus possessing some similarity to members of the family Paramyxoviridae. In this connection it is interesting to note that the haemagglutinating agents isolated from the tumour cases by Sulochana *et al* (1980) were sensitive to chloroform, agglutinated chicken and some mammalian erythrocytes and were related to newcastle disease virus by haemagglutination inhibition test. The importance of these virus like particles in the causation of tumour of the mucosa of ethmoid needs further investigation.

12. SUMMARY

1. Animals were regularly screened for the incidence of the tumour. Information was gathered on the prevalence of the tumour from Veterinarians all over the state. Suspected cases were screened and 70 confirmed cases were brought to Mannuthy for detailed investigation.

2. During the year 1980, 107 cases were recorded. The tumour was encountered in cattle (86) buffaloes (3) goats (7) and pigs (11). A case was also encountered in a spotted deer.

3. The incidence was more in females. The most susceptible age group was 6 to 9 years. The incidence was more frequent during April to July. Out of 70 cases subjected to detailed investigation 16 animals were pregnant.

4. Haematological data revealed anemia and moderate to severe leucocytosis. In early stages there was lymphocytic leucocytosis and in advanced stages there was neutrophilic leucocytosis.

5. There was appreciable imbalance in the serum calcium phosphorus ratio, particularly in animals which had perforation of the frontal bone.

6. Serum proteinogram was characterised by decrease in albumin ~~globuxin~~ percentage and reduction in albumin globulin ratio. There was also increase in gammaglobulin and alpha globulin.

6. Employing different cytological and cytochemical parameters the cellular pattern and cytomorphological features of the exfoliated cells in the nasal discharge were studied. The morphological characteristics of the neoplastic cells were delineated and diagnostic criteria for different histological types of malignancies were described.

7. The serum enzyme profile of tumour bearing animal was evaluated. There was no significant variation in ALP, ACP, SGOT and SGPT levels in different stages of tumour growth. There was increase in the LDH level in advanced stages of tumour development.

8. The cutaneous response to tumour antigen in tumour bearing animals was one of allergic hypersensitivity type and the tissue hypersensitivity response was significantly more when compared to control animals.

9. The response of the lymphnode to tumour antigen and BCG was assessed and evaluated. The tumour bearing animals were immunologically competent. However, the response was quick in onset and decline with tumour antigen when compared to BCG.

10. The distribution of T lymphocytes in the peripheral blood of healthy cattle and tumour bearing animals was studied employing AHAE as a marker. The reaction pattern in different leucocytes was described and the technique was perfected. The proportion of T cells was found to be slightly more in tumour bearing animals in early stages indicating a slight enhancement in CMI response. In later stages the proportion of T lymphocytes was less. This indicated a lowering of CMI.

11. The evaluation of the delayed cutaneous hypersensitivity reaction employing DNCB indicated a slight depression of CMI response in tumour bearing animals. However, there was no evidence of depression in CMI response with PHA. The histological pattern of reaction to DNCB and PHA was not significantly different in tumour bearing and non tumour bearing control animals.

12. The CMI response in tumour bearing animals when BCG was administered I/D was not significantly different from the response in non-tumour bearing animals.

13. The lympho-proliferative response of lymphocytes was studied <u>in vitro</u> using PHA and it was clarified that tumour bearing animals are less immuno competent than non-tumour bearing animals.

14. An assessment of the macrophage function in tumour bearing animals employing dextran sulphate revealed that the macrophage response in tumour bearing animals was relatively less.

15. The lymphnode (Prefemoral lymphnode) reaction to BCG and tumour antigen was evaluated. In the tumour bearing animals the response to tumour antigen was quick in onset and disappearence, suggesting a previous exposure to tumour antigen. The response to BCG was relatively delayed in onset and disapperance. The pattern of histological reaction to BCG and tumour antigen was identical. The centralateral lymphnode did not reveal any change indicating the localisation of the antigen in the ipsilateral lymphnode.

16. The agar gel diffusion test, passive haemagglutination test and the reverse passive haemagglutination tests were not found useful in tumour diagnosis.

17. The usefulness of the Ehrlich test in diagnosing the ethmoid tumour was evaluated. The test was not found useful as a single diagnostic test but it was suggested to be used along with other tests for confirming the diagnosis.

18. Attempts to grow the tumour cells <u>in vitro</u> did not succeed.

19. The virus isolate was inoculated into experimental calves inorder to assess the pathogenic potential. There was inflammatory hyperplastic and metaplastic reaction in the ethmoid region. The observations are being continued.

20. A formalinised preparation of the virus isolated was used as a vaccine for therapy. The results are being observed.

21. Non-specific immunotherapy with BCG by different routes was attempted. These studies have indicated significant increase in the CMI response in tumour bearing animals. The results of intralesional injection of BCG was not encouraging.

22. Specific immunotherapy using Tumour cells, was found to give beneficial results as indicated by enhanced survival period and enhanced CMI response, particularly in early stages of tumour growth.

23. The tissue response in the prescapsular lymphnode following vaccination was studied. There was significant stimulation of CMI response in the lymphnode. An observation which will support the beneficial effect of vaccination.

24. The therapeutic effect of cyclophosphamide was assessed in tumour bearing goats. The treatment was found to be effective, in early stages of tumour growth.

25. Detailed pathological studies were made on 51 tumour bearing animals. The tumours encountered in the decreasing order of frequency were Adeno carcinoma (27); Squamous cell carcinoma (18) and undifferentiated carcinoma (6). The tumour was found to invariably arise from the ethmoid region. Metastatic foci were seen in the regional lymphnodes in 34 instances.

26. The pattern of reaction encountered in the draining lymphnodes of the head were classified as lymphocytic predominant, Germinal centre predominant, unstimulated, lymphocyte depleted and sinus histiocytosis.

27. Studies on the pathological changes in the adrenal gland revealed histological features of stress response.

28. Pathological changes in the spleen ~~are~~ were studied. The picture in general was one of immunological incompetency.

29. Electron microscopic studies revealed the presence of enveloped virus possessing similarity to the members of the family paramyxoviridae in the tumour tissue. There was indication of maturation and assembly of these virus particles at the plasma membrane. The importance of these virus particles in the causation of tumour is being investigated further.

30. The results that ~~xx~~ can be explited on pilot or field scale.

31. The incidence of the tumour is on the increase and has been encountered in all domestic species. For early detection of cancer, nasal discharge of animal in the susceptible age group should be subjected to exfoliative cytology.

2. The established symptomatology should be made known to Farmers and Veterinary surgeons so that early detection of the tumourcan be made.

3. Cyclophosphamide can be employed in goats as a chemotherapeutic agent.

4. Specific immunotherapy can be attempted in early stages of the cancer growth.

13. Publications

1. Effect of cyclophosphamide on peripheral blood leucocytes of goats.
   Vet. Immunol immunopathol (UP)

2. Histopathology of adrenal glands in tumour bearing animals.
   Kerala.J.vet.Sci. (UP)

3. Tumours of the mucosa of the ethmoid in pigs.
   Indian J.Cancer (UP)

4. Isolation of haemagglutinating agents from tumour of the mucosa of the ethmoid in cattle.
   Kerala J.vet.Sci (1980) VolII(2):219

5. Ehrilich test for the diagnosis of endemic ethmoid cancer in cattle.
   Indian J.Cancer (UP)

6. Virus like particles in tumours of the mucosa of the Ethmoid in cattle.
   Acta.vet.Scand (UP)

7. Tumours of the mucosa of the ethmoid in cattle.
   Aust.Adv.vet.Sci. (1980) 15

14. Contributions made by the Co-operators.

15. Signature

Professor of Pathology
(Project Officer)

Head of Department

GROSS AND HISTOPATHOLOGICAL FEATURES.

Animal No.93.

Date of entry : 28.12.79.
Date & time of death: 29.2.80 - 10 AM.
Owner: Kunjappu, Punnayoor Kulam.

Species: Bovine.
Breed : N.D
Sex : Female.
Colour : Black.
Age : 7 years.

Clinical History : Respiratory distress since one month. There was copious mucus nasal discharge. Slight exopthalmos of right eye. Animal was very weak and emaciated.

Gross lesions : The tumours originated from the ethmoid region extended down half of the nasal passage. The tumour mass was completely necrosed and yellowish in colour. Few areas of haemorrhages were observed terbinate mucosa was congested.

Histological diagnosis: Adenocarcinoma.

No.94

Date of entry : 4.1.80
Date & time of death : 8.1.80
Owner : Bhaskaran, M, Manakkal, Calicut.

Species : Bovine.
Breed : Crossbred.
Sex : Female.
Age : 9 years.
Colour : Brown with black strips.

Clinical history : The animal had severe respiratory distress since 2 weeks. Conducted tracheactomy on 7.1.80 to relieve respiratory distress, The animal had frothy nasal discharge mixed with blood since 1 month - bulging on the forehead was observed since 5 days.

Gross lesions : A hard cherry red tumour mass with bony spicules filled the nasal sinuses. The tumour mass posteriorly penetrated cribriform plate and attached to the meninges. The left side of the frontal bone was rarefied and lemon sized mass was present just beneath the skin. The head lymphnodes were moderately oedematous.

Histological diagnosis: Squamous cell carcinoma.

No.95

Date of Entry : 5-1-80
Date & time of death: 21-1-80
Owner : St. Joseph Seminary, Alwaye.

Species : Bovine.
Breed : Crossbred Jersey.
Sex : Female
Age : 7 years.
Colour : Brown

Clinical history : Exophthalmos left eye with ulceration - Forehead bulging was observed since 2 weeks - Tennis ball size. Respiratory distress since 2 months. The nasal discharge copious and mucous in nature.

Gross lesion : A soft fleshy yellowish mass arising from the ethmoidal region, which extended into the frontal and maxillary sinus and ¾ of the nasal passage. The frontal bone was completely rarefied and appeared as tennis ball size covered with skin. Focal areas revealed necrosis. Retropharyngeal lymph node was enlarged and metastatic foci were noticed which appeared as whitish mass embedded in the parenchyma.

Histological diagnosis: Adenocarcinoma.

No.96

Date of entry : 16.1.80
Date and time of death : 19.1.80.
Owner : Shobhanan, Anthicadu.

Species : Bovine.
Breed : Crossbred Jersey.
Sex : Female.
Colour : Brown.
Age : 7 years

Clinical history : The animal having respiratory distress since 2 months. Exophthalmos of the left eye Mucous nasal discharge. Tracheotomy was performed to relieve respiratory distress on 26.1.80.

Gross lesions : A dark red mass arising from the ethmoid region was noticed, which extended into the ½ of the nasal passage and left retrobulbar region. The growth penetrated the cranial cavity and entered into the brain and passed the cerebrum, Parotid, mandibular & retropharyngeal lymph nodes were enlarged and had the size of tennis ball cut surface revealed yellowish white and hard to touch. Metastatic foci were observed in lungs and appeared as whitish hard nodule on the surface and distributed focally. Mediastinal and bronchial lymph nodes moderately enlarged and contained metastatic foci.

Histological diagnosis: Adenocarcinoma.

No.97

Date of entry : 18.1.80
Date & time of death: 21.1.80
Owner: N.Parameswaran Nambeesan, Calicut.

Species : Bovine.
Breed : Crossbred
Colour : Black.
Sex : Female.
Age : 7 years.

Clinical History : Exopthalmos of the left eye - sero sanguinous nasal discharge - severe respiratory distress.

Gross lesions : A large irregular mass of growth involved the frontal sinus and extended into pharynx and lower third of the nasal passage destroying the turbinates. Invaded into the left retrobulbar region. The growth contained numerous bony spicules.

Histological diagnosis: Adenocarcinoma.

No. 98

Date of entry: 29-1-80

Date &time of death: 8.3.80.

Owner: A.K. APpu,
Maruthaka,
Chavakadu.

Species : Bovine.
Breed : ND
Sex : Female.
Colour : Black
Age : 6½ years.

Clinical history : Severe respiratory distress since a fortnight. Exophthalmos of the left eye Bloodish nasal discharge - performation of the frontal bone - about 3-4 cm diameter.

Gross lesions : An irregular white fleshy mass arising from the ethmoidal region which extended posteriorly penetrating the cranial cavity and extended into the cerebral hemispheres. It also extended into the lateral ventricles of the brain anteriorly it extended ½ of the nasal cavity. Laterally it extended left retrobulbar region, Retropharyngeal and mandibular lymphnodes showed metastatic foci. Parotid lymph nodes severely congested and moderately oedematous.

Histological diagnosis: Undifferentiated carcinoma.

No.99

Date of Entry : 8.2.80.

Date and time of death : 10.2.80.

Owner: University livestock Farm, Thiruvazhamkunnu.

Species : Bovine
Breed - Crossbred Jersey.
Sex : Female.
Colour. : Red.
Age : 7 years.

Clinical history : Slight respiratory distress have noticed since 3 weeks. It has a tendency to press the head on objects . Intermittant eppistaxis.

Gross lesions : The tumour was found to arise from the ethmoid mucosa. Posteriorly it extended into the cranial cavity and was seen attached to the meninges. It also extended into the cerebral hemispheres and then to lateral ventricles. Metastatic foci were noticed in the head lymph nodes and enlarged to the size of a lemon.

Histological diagnosis: Adenocarcinoma.

No.100

Date of entry : 12.2.80.

Date and time of death : 24.3.80.

Owner: Holycross Convent, Kottiyam, Quilon.

Species : Bovine.

Breed : Crossbred Jersey

Sex : Female.

Colour : Reddish with black strips

Age : 8 yrs.

Clinical history : Severe respiratory distress since a fortnight. Copious amount of mucous dischharge with blood spots were observed. Exophthalmos of the left eye and terminates Keratitis, opacity and ulceration.

Gross lesions : A large irregular soft mass of growth arising from the ethmoid and extending into the maxillary sinus and left orbital cavity. Focal areas showed brown patchy areas of necrosis. The growth also adherent to turbinate scrolls. Mandibular and retropharyngeal lymphnodes moderately enlarged and oedematous.

Histological diagnosis: Adenocarcinoma.

No.191

Date of entry : 13.2.1980.

Date & time of death: 30.3.80.

Owner: Rajamma, Police Quarters, Vaikom.

Species : Bovine.

Breed : Crossbred jersey.

Sex : Female.

Colour : Brown.

Age : 6 years. (Heifer)

Clinical History : Severe respiratory distress since one week. Blood tinged mucous discharge from both nostrils exophthalmos of the left eye. There was bulging on the forehead and has the size of a coconut.

Gross lesions : Soft brownish grey mass of growth filled the nasal chambers and invaded left retro-bulbar r gion. It also extended into maxillary sinus and frontal sinus. Perforation of fronta. bone about 15 cm diameter. Growth bulged out into the subcutaneous tissue. The head lymphnodes were moderately enlarged and oedematous.

Histological diagnosis: Squamous cell carcinoma.

No. 102

Date of entry : 15.2.80
Date & time of death : 15.2.80
Owner : Viswambharan, Vaikom.

Species : Bovine
Breed : ND
Sex : Female
Colour : Reddish Brown
Age : 8 years.

Clinical history : Severe respiratory distress and mucous discharge from both the nostrils. Exophthalmos of the right eye.

Gross lesions : Fleshy irregular growth arising from the ethmoid region extended downwards into the pharynx and lateraly right retrobulbar region. It also invaded ¾ of the nasal passage. The growt was soft and focal areas showed haemorrhage. The growth also invaded into cranial cavity and presse the brain(cerebrum)

Histological diagnosis: Squamous cell carcinoma.

No.103.

Date of entry : 15.2.80
Date and time of death : 1.3.80
Owner: Pannu, K. Udayanopuram, Vaikom.

Species : Caprine.
Breed : N.D
Sex : Female
Colour : White.
Age : 5 years.

Clinical history : Respiratory distress since one month. The animal was under cyclo phosphamide treatment. Slight exopthalmos of the right eye-mouth breathing Mucosanguinous discharge.

Gross lesions : A soft polypoid growth arising from the ethmoid mucosa was noticed. The mucosa of turbinates were highly congested and oedematous. The right retrobulbar region contained myxomatous and vicid material.

Histological diagnosis: Adenocarcinoma.

No.104

Date of entry : 19.2.80.
Date & time of death : 31.3.80
Owner: Baby Edathwa.

Species : Brown
Breed : H.FCB.
Sex : Female
Colour : Black.
Age : 3 years.

Clinical history : Intermittant nasal discharge mixed with blood was observed since 1½ months. Pressing the forehead on walls since 10 days - exophthalmos of the right eye - bulging of the forehead was observed since 10 days. Opsithotonus were noticed two days back. Animal was 8 month pregnant and aborted on 21.2.80.

Gross lesions : A large encapsulated necrotised mass filled the ethmoid & frontal sinuses. There was a swelling on the forehead, size of a large coconut, covered by subcutaneous tissue and skin. The frontal bone was rarefied. The growth laterally extended into right retro-bulbar region. Posteriorly growth invaded cranial cavity and adhered to the meninges. The head lymphnodes were slightly oedematous.

Histological diagnosis: Squamous carcinoma

No. 105

Date of Entry : 22.2.80
Date & time of death : 22.2.80
Owner : Pigbreeding Farm, Mannuthy.

Species : Porcine
Breed : York Shire.
Colour : White
Sex : Male.
Age : 3½ years.

Clinical history : Intermittant epistaxis since one month. Moderate to severe respiratory difficulty. Coconut size swelling on the forehead.

Gross lesions : An irregular fleshy pedenculated mass originating from the ethmoid region was noticed. It anteriorly extended ¼th of nasal passage and invaded into the frontal sinuses and rarefied the frontal bone, appeared as a mass covering skin and subcutaneous tissue. Head lymphnodes slightly enlarged and oedimatous.

Histological diagnosis: Adeno carcinoma.

No. 107

Date of entry : 15.3.80
Date & time of death : 5.4.80
Owner: Mrs. Ersila Job,
Thyvellikattu House,
Shertali.

Species : Bovine.
Breed : Swiss Brown cross
Sex : Female
Colour : Brown.
Age : 4 years.

Clinical history : Respiratory distress since 2 months exophthalmos of the right eye since a fortnight. Mucosanguinous nasal discharge.

Gross lesions : A white fleshy and gritty mass originating from the ethmoid mucosa which extended anteriorly ¾th nasal passage. In some areas the growth was necrotic and contained cystic spaces. Lateraly the growth extended right retrobulbar region and rarefied the frontal bone and bulged as mass size of a lemon. Posteriorly it encroached the cranial cavity and adhesion with brain.

Histological diagnosis: Undifferentiated carcinoma.

No. 108

Date of entry : 17.3.80
Date & time of death : 20.4.80.
Owner : C.I.Antony,
Chiramal House,
Chalakudi.

Species : Bovine.
Breed : Jersey Cross.
Colour : Brown.
Sex : Female.
Age : 5 years.

Clinical history : Severe respiratory difficulty since one month. Abdominal type of breathing noticed in later period. Exophthalmos of the right eye. In later stages the animal was in recumbant state. Mucosanquinous discharge.

Gross lesions : A dark red mass arising from the ethmoid region, which extended anteriorly to ¾th of the nasal passage. Many foci of suppuration in the fleshy growth. Head lymphnodes moderately hyperaemic.

Histological diagnosis: Adenocarcinoma.

No. 109

Date of entry : 26.3.80
Date & time of death : 17.4.80
Owner: ICDP, Calicut

Species : Bovine.
Breed : Crossbred Jersey.
Colour : Brown.
Sex : Male.
Age : 9 years.

Clinical history : Severe respiratory difficulty since 1½ month. Snoring and abdominal breathing in later stage. Exopthalmos of left eye was noticed in very late stage. Nasal Mucous discharge.

Gross lesions : White firm and fleshy growth arising from the ethmoidal region which extended into the nasal passage and frontal sinus. It laterally extended to the left retrobulbar region pushing the eye ball. Foci of haemorrhage were noticed in the growth.

Histological diagnosis: Adenocarcinoma.

No. 110

Date of entry : 28.3.80.

Date & time of death : 12.7.80

Owner: K. Sreedharan, Purappanagadi.

Species : Bovine.
Breed : ND
Colour : Dark brown.
Sex : Female.
Age : 9 years.

Clinical history : Mild respiratory difficulty since one month. Mucous discharge in little amount from both nostrils. Exopthalmos of the right eye.

Gross lesions : A growth arising from the ethmoid region was noticed. The growth was fleshy and firm, and contained a few bony spicules in the tumour mass. It filled up the frontal, maxillary and sphenoid sinuses and anteriorly extended ¾th of nasal cavity. Foci of necrosis were seen, throughout the growth. The growth posteriorly invaded cranial cavity by destroying ethmoid bone and was seen attached to the meninges. It laterally extended into the right retrobulbar region and pushed the eyeball.

Histological diagnosis: Squamous cell carcinoma.

No.111.

Date of entry : 29.3.80.

Date & time of death : 25.5.80

Owner : S.K. Balan, Puthur, Kaparamba.

Species : Bovine
Seed : Kangayam.
Colour : Brown with strips.
Sex : Male.
Age : 12 years.

Clinical history : Respiratory distress since 2 weeks. Copious mucous discharge from both nostrils. Slight bulging of left eye.

Gross lesions. : A hard dark brown mass of growth contained scattered bony spicules - extended into ¾th of the nasal passage, pharynx and left retrobulbar region. Head lymphnodes slightly hyperaemic.

Histological diagnosis : Adeno carcinoma.

No. 112.

Date of entry : 5.4.80
Date & time of death : 16.6.80.
Owner : A.K. Krishnan, Triprayar.

Species : Bovine.
Breed : Crossbred.
Colour : Brownish black.
Sex : Female
Age : 8 years.

Clinical history : Respiratory distress since 2 months. Bilateral nasal bleeding observed- Six months pregnant- In later stages copious amount of mucous discharge with blood. There was protrusion of the right eye and bulging of the forehead.

Gross lesions : A dark brown growth arising from the ethmoid region, filling the maxillary sphenoid and palatine sinuses and extended down upto 2/3rd of the nasal passage. The growth revealed extreme necrosis and focal haemorrhagic area. The growth also laterally invaded to both retrobulbar region. Ethmoid bone was completely destroyed and the growth extended posteriorl to the cranial cavity and was seen pressing on the cerebrum and meninges.

Histological diagnosis : Adenocarcinoma.

No. 113.

Date of entry : 12.4.80.
Date & time of death : 12.4.80
Owner : E.K. Karthiyani, Kaloor.

Species : Bovine.
Breed : ND
Colour : Brown.
Sex : Female.
Age : 7 years.

Clinical history : The animal had severe respiratory distress since 2 weeks. Since four days the animal was recumbent position.

Gross lesions : A white fleshy and firm growth originating from the ethmoid region extended into the nasal passage and paranasal sinuses. Posteriorly the growth penetrated through ethmoid bone and formed adhesion with the meninges. Retropharyngeal lymphnodes were very much enlarged and revealed metastatic foci.

Histological diagnosis: Squamous cell carcinoma.

No. 114

Date of entry : 13.4.80.
Date & time of death : 14.4.80
Owner : University Pig Breeding Farm, Mannuthy.

Species : Porcine.
Breed : Large White Yorkshire.
Colour : White
Sex : Female
Age : 3½ years.

Clinical history : The animal had intermittant nasal discharge and epistaxis since a month. Severe respiratory distress since a fortnight.

Gross lesions : A fleshy growth originating from the ethmoid region. Perforated the cribriform plate and invaded the cerebral poles. The anterior pole was completely invaded by the tumour tissue. Adhesion developed between meninges and brain. Retropharyngeal lymphnode revealed metastatic foci.

Histological diagnosis: Adenocarcinoma.

No. 115

Date of entry : 22.4.80.
Date and time of death: 11.5.80
Owner: Mohamed Ali, Blangad, Chavakad.

Species : Bovine
Breed : Crossbred.
Colour : Brown.
Sex : Female
Age : 7 years.

Clinical history. : Severe respiratory distress since 2½ months. Bulging on the forehead since 1 month. Occassional epistaxis from both nostrils. Right eye bulged out.

Gross lesions : An irregular greyish white growth extending from the ethmoid region. Filled ¾th of the nasal passage. The growth was firm and contained bony spicules. The growth revealed focal areas of necrosis and suppuration. The

ethmo turbinates were completely destroyed. Frontal bone was rarefied and tumour mass was lying just beneath skin & subcutaneous tissue.

Histological diagnosis: Adenocarcinoma.

No. 116

Date of entry : 16.4.80
Date & time of death : 11.5.80.
Owner : Achuthan, P. Mullasseri.

Species : Bovine.
Breed : N.D.
Sex : Female.
Colour : Brown.
Age : 7 years.

Clinical history. : Respiratory distress since 2 months. exopthalmos of right eye - occassional epistaxis. Slight bulging of the forehead.

Gross lesions : An irregular fleshy dark brown growth arising from the ethmoid region. Extended anteriorly down into the nasal passage and filled the paranasal sinuses. The frontal bone was rarefied and the growth was covered by skin and subcutaneous tissue only. The growth also invaded the cranial cavity and was pressing on the brain and meninges.

Histological diagnosis: Adenocarcinoma.

No.117

Date of entry : 7.5.80
Date & time of death : 14.5.80
Owner : Pothen Philipose, Kadvil, Ramamangalam.

Species : Bovine.
Breed : Crossbred Jersey.
Colour : Light Brown.
Sex : Female.
Age : 6 years.

Clinical history : Copious mucous discharge from both nostrils since 2½ months. It had also occassional nasal bleeding. Since the past 15 days there was bulging of the left eye ball, ~~since a fortnight~~.

Gross lesions : A white soft fleshy mass arising from the ethmoid region and laterally extended to the left retrobulbar region. It posteriorly extended into the cranial cavity and attached to the meninges. Focal areas of haemorrhage and necrosis were noticed in the tumour mass.

Histological diagnosis: Adenocarcinoma.

No. 118

Date of entry : 8.5.80
Date & time of death : --
Owner : Ismaliseth, Engandiyoor.

Species : Bovine.
Breed : H.F Jersey cross.
Colour : Black with white spots.
Sex : Female.
Age : 2½ years.

Clinical history : Exopthalmos of both eyes since one week. Mucous discharge from both nostrils. Slight respiratory difficulty. The animal has taken back by the owner on 18.7.80.

No. 119

Date of entry : 13.5.80
Date & time of death : 7.6.80.
Owner : V.P. Kunjunny, Vallathuparambil, Pattambi.

Species : Caprine
Breed : N.D
Colour : White
Sex : Female.
Age : 4 years.

Clinical history : The animal had severe respiratory distress since 1½ months. There was mouth breathing - exophthalmos of the left eye. It was under cyclophosphamide therapy (200 mg I/v weekly).

Gross lesions : An irregular growth, fleshy in consistency originated from the ethmoid region and filled the maxillary sinus and anteriorly extended upto ¾th of the nasal passage. Turbinate scrolls were completely destroyed. The tumour tissue posteriorly invaded the brain and laterally left orbital cavity.

Histological diagnosis: Adenocarcinoma.

No. 120

Date of entry : 21.5.80.
Date and time of death: 1.7.80
Owner : K.Ramankutty, Alwaye.

Species : Bovine.
Breed : N.D
Colour : Brown.
Sex : Female.
Age : 8 years.

Clinical history : It had severe respiratory distress since 2 months. Exophthalmos of the left eye. The animal was in full term pregnancy.

Gross lesions : An irregular fleshy greyish white growth arising from the ethmoid region and filled the paranasal sinuses. The growth laterally invaded left retrobulbar region. Anteriorly it extended ½ of the nasal passage. Periphery of growth revealed focal areas of necrosis.

Histological diagnosis: Adenocarcinoma.

No. 121

Date of entry : 28.5.80
Date & time of death : 29.6.80.
Owner : K.S. Madhavan, Vadakkupurath Veedu, Shertali.

Species : Bovine.
Breed : N.D
Colour : Black
Sex : Female
Age : 4 years.

Clinical history : The animal had respiratory distress since 2 months. Blood tinged mucous discharge from both nostrils. The animal was 6 months pregnant. Exophthalmos of the right eye.

Gross lesions : A fleshy dense growth arising from the ethmoid region and filled the paranasal sinuses. Laterally it extended into the right retrobulbar region. Focal areas of haemorrhage were observed on the growth and periphery of the growth showed necrosis. The growth destroyed cribriform plate and invaded into the brain. The right parotid, mandibular and retropharyngeal lymphnodes were enlarged to the size of a lemon and ~~extreme~~ metastatic foci were noticed.

Histological diagnosis: Squamous cell carcinoma.

No. 122.

Date of entry : 30.5.80
Date & time of death : 19.6.80.
Owner : Karappan, Mattathil Veedu, Mala.

Species : Bovine
Breed : Crossbred Jersey
Colour : Brown.
Sex : Female.
Age : 10 years.

Clinical history : Severe respiratory distress since 1½ months. Epistaxis was also noticed. Occasional mucous nasal discharge - Exophthalmos of the left eye.

Gross lesions : A reddish hard growth arising from the ethmoid bony spicules. It extended into

the left retrobulbar region and paranasal sinuses and completely destroyed ethmo-turbinates. The tumour mass also invaded cranial cavity and pressed the cerebrum. Focal areas of haemorrhage and necrosis of tumour growth.

Histological diagnosis: Undifferentiated carcinoma.

No. 123

Date of entry : 3.6.80

Date & time of death : 12.6.80

Owner : Sarada,
Kuriyakott House,
Potot.

Species : Bovine.

Breed : Crossbred

Colour : Browth with white spot.

Sex : Female.

Age : 6 years.

Clinical history : Respiratory difficulty was noticed since 2 months. Intermittent dpistaxis. Animal was 6 month pregnant.

Gross lesions : A red hard growth arising from the ethmoid mucosa and filled the paranasal sinuses. Extreme haemorrhage and bony spicules were seen embedded in the growth. It invaded the cranial cavity and adhered to the brain and compressed the cerebrum. Haemorrhages in the meninges.

Histological diagnosis: Squamous cell carcinoma.

No. 124

Date of birth : 11.6.80

Date & time of death :

Owner : P.C. Varghese,
Palathil,
Edthwa.

Species : Bovine.

Breed : Swiss Brown

Colour : Brownish black

Sex : Female

Age : 3 years.

Clinical history : Severe respiratory distress since one month. Mucous discharge from both nostrils. The animal was pregnant.

Gross lesions : A hard fleshy growth arising from the ethmoid region filled the paranasal sinuses. It extended 3/4th of nasal passage and pharynx. The head lymphnodes moderately congested & oedematous.

Histological diagnosis: Undifferentiated carcinoma.

No.125

Date of entry : 13.6.80
Date & time of death : 25.6.80
Owner : K. Thressamma, Udayanapuram.

Species : Bovine.
Breed : ND
Colour : Brown.
Sex : Female.
Age : 7 years.

Clinical history : The animal had severe respiratory distress since 3 months. Mucous nasal discharge with traces of blood. A lemon sized swelling over the forehead region was observed since 2 weeks.

Gross lesions : The tumour growth was involving the ethmoid region and extended to the para nasal sinuses. The growth revealed focal areas of haemorrhage, necrosis and bony spicules. The growth was moderately firm and hard. Metastatic foci on the retropharyngeal lymphnode.

Histological diagnosis: Adenocarcinoma.

No. 126

Date of entry : 17.6.80
Date & time of death : 17.6.80
Owner : Pig Breeding Farm, KAU, Mannuthy.

Species : Porcine.
Breed : Yorkshire.
Colour : White
Sex : Female.
Age : 2 years 2 months.

Clinical history : Intermittent Epistaxis since 4 months. It also exhibited severe respiratory distress.

Gross lesions : A small growth arising from the ethmoid bone and extended into the frontal sinuses growth revealed haemorrhages. The nasal mucous membrane oedematous and congested.

Histological diagnosis: Undifferentiated carcinoma.

No. 127

Date of entry : 17.6.80
Date & time of death : 19.8.80
Owner : Capt. P. Nair, Moothedath.

Species : Bovine
Breed : H.F.
Colour : Black with white strips.
Sex : Female.
Age : 8½ years.

Clinical history : Severe respiratory distress, exophthalmos of the right eye. Animal was 8 months pregnant.

Gross lesions : An irregular reddish tumour arising from the ethmoid region which extended to the middle of the nasal passage. Numerous bony spicules were embedded in the growth. The growth also extended to the pharyngeal passage and right retrobulbar region. Deeper part of the growth revealed necrosis. It invaded and entered the cranial cavity and formed adhesion with meninges.

Histological diagnosis: Squamous carcinoma.

No.128

Date of entry : 21.6.80
Date & time of death : 11.7.80
Owner: K. Ayyappan, Kanyath Veedu, Engandiyoor.

Species : Bovine
Breed : N.D
Colour : Black
Sex : Female.
Age : 8 years.

Clinical history : Respiratory distress since 1½ months. Exophthalmos of the right eye. Animal was pregnant 8 months. Mucous discharge from both nostrils.

Gross lesions : An irregular growth originating from the ethmoid region. Dark brown in colour and extended 3/4th of the nasal cavity and down to the pharyngeal passage. The growth was necrotic in the deeper portion. It also invaded right orbital fossa and posteriorly it extended to the cranial cavity and formed adhesion with meninges.

Histological diagnosis: Undifferentiated carcinoma.

No. 129

Date of entry : 22.6.80
Date & time of death : 22.6.80
Owner : Gopi, N. Nadhawakattil, Malaparamba, Calicut.

Species : Bovine
Breed : N.D
Colour : Brown.
Sex : Female.
Age : 6 years.

Clinical history : Exophthalmos of both eyes. Frontal bulging since 15 days. Sero sanguinous nasal discharge.

Gross lesions : A whitish, firm and encapsulated growth arising from the ethmoid region extended into the maxillary and frontal sinus and 1/3rd of the nasal cavity. The growth encroached the cranial cavity destroying cribriform plate and formed adhesion with

: the meninges. Frontal bone undergone osteolysis and tumour growth bulged as a mass covering skin and subcutaneous tissue.

Histological diagnosis: Squamous cell carcinoma.

No. 130

Date of entry : 24.6.80
Date & time of death : 9.7.80

Owner: K.S. Gopalakrishnan,
Asok Vihar,
Tripunithura.

Species : Bovine.
Breed : Crossbred
Sex : Female
Colour : Light brown.
Age : 7 years.

Clinical history : Severe respiratory distress since 1½ months - Mucosanguinous nasal discharge - exophthalmos of the right eye since 1 month - Frontal bulging.

Gross lesion : A growth arising from the ethmoid region which extended into the maxillary, frontal and sphenoid sinuses and anteriorly it come upto the external nares. It also extended down into the pharyngeal passage. The growth was irregular, soft and fleshy and the periphery showed necrosis. It also invaded the cranial cavity and compressed the brain tissue.

Histological diagnosis : Undifferentiated carcinoma.

No.131.

Date of entry : 28.6.80.
Date and time of death : 24.7.80

Owner : V.Krishnankutty Menon,
Kizhakee Veetil,
Nayakkanal,
Trichur.

Species : Caprine.
Breed : ND
Colour : White
Sex : Female.
Age : 8 years.

Clinical history : Severe respiratory distress since a fortnight - mucopurluent nasal discharge - pressing the head on objects - A lemon sized swelling on the forehead.

Gross lesion : A greyish white fleshy irregular mass filled the nasal cavity and anteriorly extended upto half of the nasal passage. The left side frontal bone rarefied and bulged as a mass covered by skin and subcutaneous tissue. A polyp 4-6 cm. was seen in the anterior part of the tumour. The mandibular lymphnodes were hyperaemic and oedematous.

Histological diagnosis : Adenocarcinoma.

No. 132

Date of entry : 4.7.80
Date & time of death : 30.12.80
Owner: K. Krishna Das,
Elayedathu House,
Calicut-16.

Species : Bovine.
Breed : ND
Colour : Black.
Sex : Female.
Age : 6 years.

Clinical history : Moderate respiratory distress since 1 month. Mucous discharge and occasional epistaxis. The animal was pregnant.

Gross lesion. : A small growth arising from the ethmoid region and filled the paranasal sinuses. The growth revealed focal area of haemorrhage and necrosis.

Histological diagnosis: Adenocarcinoma.

No. 133.

Date of entry : 5.7.80
Date & time of death : 9.7.80
Owner : Gynaecologist,
Polyclinic,
Kunnamkulam.

Species : Porcine.
Breed : Yorkshire.
Colour : White.
Sex : Female.
Age : 2 years 6 months.

Clinical history : Intermittent epistaxis since one month and severe respiratory distress.

Gross lesions : A fleshy greyish coloured grape sized growth originating from the ethmoid region and extended half of the nasal cavity. Focal areas of cystic dilatation. Destruction of cribriform plate and adhesion to the meninges Mandibular lymphnode moderately enlarged & oedematous - Had cirrhosis of liver.

Histological diagnosis: Squamous cell carcinoma.

No. 134.

Date of entry : 14.7.80
Date & time of death : 12.8.80
Owner : C. Gopalakrishnan,
Chalayil Veedu,
Kanjanny.

Species : Bovine.
Breed : Crossbred
Colour : Brown.
Sex : Female.
Age : 6 years.

Clinical history : Severe respiratory distress since 2 months - exophthalmos of the left eye - mucous nasal discharge.

Gross lesions : A cherry red growth originating from the ethmoid region and extended anteriorly 5-7 cm to the nasal passage. The growth was rough and gritty to touch and contained bony spicules. Both retropharyngeal lymph nodes enlarged to the size of a lemon and metastatic foci were present. The growth revealed focal areas of necrosis. Metastatic foci were present in mandibular and parotid lymphnodes.

Histological diagnosis: Adenocarcinoma.

No. 135.

Date of entry : 23.7.80.
Date & time of death : 28.7.80.
Owner : P. Nanu, Perumundassery, Aroor.

Species : Bovine.
Breed : ND
Colour : Brown
Sex : Female.
Age : 5 years.

Clinical history : Severe respiratory distress - sero-sanguinous nasal discharge. Animal is in recumbent position since the last 4 days.

Gross lesions : A large fleshy growth attached to the ethmoid region and contained many cystic dilatation. Focal areas of necrosis and suppuration were observed on the growth.

Histological diagnosis: Squamous cell carcinoma.

No. 136.

Date of entry : 24.7.80
Date & time of death : 4.9.80.
Owner : K. Karunakaran, Kunjippally House, Thottada.

Species : Bovine.
Breed : Crossbred Jersey.
Colour : Brown.
Sex : Female.
Age : 4 years.

Clinical history : Snoring since two weeks back. Intermittent epistaxis - exophthalmos of the left eye. Severe respiratory distress.

Gross lesions : Large moderately hard encapsulated growth filling the frontal sinus and extending into the maxillary sinus and projecting into pharynx. There was complete destruction of turbinates and growth come down to the lower third of the nasal cavity. The growth was greyish white and showed focal areas of necrosis.

: The growth had perforated the cranial bone and had caused adhesion with the cerebrum. It also invaded and filled left retrobulbar region.

Histological diagnosis: Squamous cell carcinoma.

No.137.

Date of entry : 30.7.80.
Date & time of death : 30.7.80
Owner : University Pig Farm, Mannuthy.

Species : Porcine.
Breed : Yorkshire.
Colour : White
Sex : Female.
Age : 3 years.

Clinical history : Intermittent epistaxis, respiratory distress snoring.

Gross lesions : Soft fleshy like growth originating from ethmoid region, extended 1/3rd of nasal passage and occluded the nasal cavity. In some areas systic dilatation were observed. It invaded the cranial cavity and formed adhesion with meninges.

Histological diagnosis: Adenocarcinoma.

No.138

Date of entry : 1.8.80
Date & time of death : 6.8.80
Owner : Gynaecologist, Kunnamkulam.

Species : Porcine,
Breed : Yorkshire.
Colour : White
Sex : Female.
Age : 3½ years.

Clinical history : Intermittent epistaxis since one month. Severe respiratory distress.

Gross lesions : A fleshy greyish coloured growth originating from the ethmoid mucosa and 3-4 cm anteriorly. Focal areas showed cystic like spaces. Rarefaction of ethmoid bone. Suppuration wasnoticed in focal areas in deep layers of tumour growth. Retropharyngeal lymph node moderately enlarged and on section diffuse whitish foci were noticed. Cirrhosis of the liver.

Histological diagnosis: Adenocarcinoma.

No.139

Date of entry : 5.8.80
Date & time of death : 8.8.80
Owner: K. Sankarapillai, Kottarathil, Ambalapuzha.

Species : Bovine.
Breed : Crossbred Jersey
Colour : Greyish Brown.
Sex : Female.
Age : 12 years.

Clinical History : Respiratory distress and intermittent nasal discharge since three month. Exophthalmos of the right eye since a month. Slight frontal bulging also noticed.

Gross lesion : A large fleshy, moderately necrotic mass of growth almost filling the frontal sinus and extending into lower-down. The growth extended into the maxillary and palatine sinuses perforated slightly by into the cranial cavity. It also filled right retrobulbar region. Head lymphnodes moderately enlarged, pale and oedematous.

Histological diagnosis: Adenocarcinoma.

No. 140

Date of entry : 7.8.80
Date & time of death : 16.11.80.
Owner : University Livestock Farm, Thiruvazhamkunnu.

Species : Bovine.
Breed : Crossbred Jersey.
Colour : Brown.
Sex : Female

Clinical history : Severe respiratory distress since 1 month. Mucopurulent discharge from both nostrisl. Exophthalmos of the right eye. Bulging of the forehead.

Gross lesions : A X hard cherry red growth arising from the ethmoid region and filled almost all paranasal sinuses and right orbital fossae. It perforated the frontal bone and covered by skin and subcutaneous tissue. Head lymphnodes were moderately enlarged, hyperamic and oedematous.

Histological diagnosis: Adenocarcinoma.

No.141

Date of entry : 17.8.80
Date & time of death : 25.8.80
Owner : C.M. Abraham, Kakkode.

Species : Bovine.
Breed : Crossbred Jersey
Colour : Red
Sex : Female.
Age : 8 years.

Clinical history : Respiratory distress since 2 months - mucopurluent nasal discharge intermittent epistaxis.

Gross lesions : An irregular fleshy whitish growth arising from ethmoid region and filled ethmoid sinus, destroying ethmoturbinates. It also extended upto 2/3 of the nasal

: cavity and pharyngeal region. The growth revealed focal areas of necrosis.

Histological diagnosis: Adenocarcinoma.

No.142

Date of Entry :12.8.80
Date & time of death : 17.9.80.
Owner : Rudran Namboodiri, Aloor.

Species : Bovine.
Breed : Crossbred Jersey
Sex : Female.
Colour : Brown.
Age : 8 years.

Clinical History : Respiratory distress since 2 months. Exophthalmos left eye. 2 month pregnant.

Gross lesions : An irregular reddish growth arising from the ethmoid region and filled paranasal sinuses and invaded into retrobulbar region. It also destroyed the ethmoturbinate bones and invaded cranial cavity. Focal areas necrosis were observed on the growth.

Histological diagnosis: Adenocarcinoma.

No.143

Date of Entry : 18.8.80
Date & time of death : 17.9.80
Owner : K. Sreekumaran, Kozhupulli House, Wadakkanchery.

Species : Bovine.
Breed : Crossbred Jersey.
Colour : Red.
Sex : Female.
Age : 8 years.

Clinical history : Exophthalmos of both eyes - moderate respiratory distress. Mucopuruluent nasal discharge - forehead bulging.

Gross lesions : An irregular soft growth arising from the ethmoid region and filled 2/3rd of nasal passage. The growth revealed necrosis and bony spicules. It also invaded the frontal and maxillary sinuses and there was erosin of frontal bone. Retropharyngeal and mandibular lymph nodes were enlarged and revealed metostatic foci. The growth penetrated the cranial cavity and formed adhesion with the meninges.

Histological diagnosis: Squamous cell carcinoma.

No. 144

Date of entry : 27.8-80
Date & time of death : 6.11.80
Owner : Regional Artificial Insemination Centre, Alwaye.

Species : Bovine.
Breed : Jersey.
Colour : Brown.
Sex : Male.
Age : 8 years.

Clinical History. : Respiratory distress since 5 months. Mucous nasal discharge since three months. Exophthalmos of right eye since 2 months.

Gross lesions. : A large fleshy dark brown growth arising from the ethmoid region and extending upto 2/3rd into the nasal passage. The turbinate scrolls have been destroyed by tumour growth. The tumour involves more to the right side than the left. It invaded the frontal sinus partly extended into pharynx. Posteriorly it pierced the lamina cribrora. and for-med adhesion with the meninges and cerebrum. Masses of clot in the tumour tissue and above cerebrum. The head lymphnodes moderately enlarged.

Histological diagnosis: Adenocarcinoma.

No. 145

Date of entry : 31.8.80
Date & time of death : 18.9.80
Owner : N.K. Katharunny, Chavakkad.

Species : Bovine.
Breed : Crossbred Jersey
Colour : Light brown.
Sex : Female.
Age : 7 years.

Clinical history : Severe respiratory distress since 2 months. Bulging of the forehead mucous nasal discharge. Intermittent epistaxis.

Gross lesions : A cherry red irregular growth arising from the ethmoid region. The growth is hard in consistency and contained numerous bony spicules. It anteriorly extended upto½ of the nasal passage and filled the paranasal sinuses. It pierced cranial cavity and formed adhesion with meninges.

Histological diagnosis: Adenocarcinoma.

No. 146

Date of entry	: 3.9.80	Species	: Bovine.
Date & Time of death	: 24.9.80	Breed	: H.F Cross.
Owner : Raman, Kallingal House, Iyyal.		Colour	: Black with strips.
		Sex	: Female.
		Age	: 4 ½ years.

Clinical History : Intermittent nasal discharge since 3 weeks. Respiratory distress and mouth breeding. Later epistaxis was noticed.

Gross lesions : A large fleshy mass of growth involving the ethmoid region and extending into frontal and maxillary sinuses. Many parts of the growth revealed necrotic foci and bony spicules. Retropharyngeal lymphnodes were enlarged and revealed metastatic foci. It pierced the cranial cavity and formed adhesion with the meninges and cerebrum.

Histological diagnosis: Adeno Carcinoma.

Table I

Details of animals bearing tumours of the mucosa of the ethmoid

Sl. No.	Animal No.	Exopthalmos			Age	Nasal discharge		Frontal bone perforation	Pregnancy	Type of tumour
		Right	Left	Bilateral		Mucus	Blood			
1.	93	-	-	-	7 years	+	-	-	-	A.C.
2.	94	-	-	-	9 years	-	+	+	-	S.C.C.
3.	95	-	+	-	7 years	+	-	+	-	A.C.
4.	96	-	+	-	7 years	+	-	-	-	U.C.
5.	98	-	+	-	6½ years	-	+	+	-	
6.	99	-	-	-	7 years	-	+	-	-	A.C.
7.	100	-	+	-	8 years	+	-	-	-	A.C.
8.	101	-	+	-	6 years	+	+	-	-	S.C.C.
9.	102	+	-	-	8 years	+	-	-	-	S.C.C.
10.	103	+	-	-	5 years	+	+	-	-	A.C.
11.	104	+	-	-	3 years	-	+	+	+	S.C.C.
12.	105 (Porcine)	-	-	-	3½ years	-	+	+	-	A.C.
13.	107	+	-	-	4 years	+	+	-	-	U.C.
14.	108	+	-	-	5 years	+	+	-	-	A.C.
15.	109	-	+	-	9 years	+	-	-	-	A.C.
16.	110	+	-	-	9 years	+	-	-	-	S.C.C.
17.	111	-	+	-	12 years	+	-	-	-	A.C
18.	112	+	-	-	8 years	+	+	+	+	A.C.
19.	113	-	-	-	7 years	+	-	-	-	S.C.C.

Sl. No.	Animal No	Exopthalmos			Age	Nasal discharge		Frontal bone perforation	Pregnancy	Type of tumour
		Right	Left	Bilateral		Mucus	Blood			
20.	114	-	-	-	3½ years	+	+	-	-	A.C.
21.	115	+	-	-	7 years	+	+	+	-	A.C.
22.	116	+	-	-	7 years	-	+	+	-	A.C.
23.	117	-	+	-	6 years	+	-	+	-	A.C.
24.	118	-	-	+	2½ years	+	-	-	-	
25.	119	-	+	-	4 years	+	-	-	-	A.C.
26.	120	-	+	-	8 years	+	+	-	+	A.C.
27.	121	+	-	-	4 years	+	+	-	+	S.C.C.
28.	122	-	+	-	10 years	+	+	-	-	U.C.
29.	123	-	-	-	6 years	+	+	-	+	S.C.C.
30.	124	-	-	-	3 years	+	+	-	+	U.C.
31.	125	-	-	-	7 years	+	+	+	-	A.C.
32.	126	-	-	-	3½ years	+	+	-	-	U.C.
33.	127	+	-	-	8½ years	+	-	-	+	S.C.C.
34.	128	+	-	-	8 years	+	-	-	-	U.C.
35.	129	+	+	+	6 years	+	+	-	-	S.C.C.
36.	130	+	-	-	7 years	+	+	+	-	U.C.
37.	131	-	-	-	8 years	+	-	+	-	A.C.
38.	132	-	-	-	6 years	+	+	-	+	A.C.
39.	133	-	-	-	2½years	-	+	-	-	S.C.C.
40.	134	-	+	-	6 years	+	-	-	-	A.C.
41.	135	-	-	-	5 years	-	+	-	-	S.C.C.

Sl. No.	Animal No.	Exopthalmos			Age	Nasal discharge		Frontal bone perforation	Pregnancy	Type of tumour
		Right	Left	Bilateral		Mucus	Blood			
42.	136	-	+	-	4 years	-	+	-	-	S.C.C.
43.	137	-	-	-	3 years	+	+	-	-	A.C.
44.	138	-	-	-	3½years	+	+	-	-	S.C.C.
45.	139	+	-	-	12 years	-	+	+	-	A.C.
46.	140	+	-	-	8 years	+	-	+	-	A.C.
47.	141	-	-	-	8 years	+	+	-	-	S.C.C.
48.	142	-	+	-	8 years	+	-	-	+	A.C.
49.	143	+	+	+	8 years	+	-	-	-	S.C.C.
50.	144	+	-	-	8 years	+	-	-	-	A.C.
51.	145	-	-	-	7 years	+	+	+	-	A.C.
52.	146	-	-	-	4½years	+	-	-	-	A.C.
53.	147	-	+	-	5 years	+	+	-	+	
54.	148	-	+	-	8 years	+	-	-	-	
55.	149	+	-	-	7 years	+	-	-	+	
56.	150	-	+	-	9 years	+	+	-	-	
57.	151	+	-	-	4½years	+	-	+	-	
58.	152	-	+	-	4½years	+	-	+	+	
59.	153	-	-	-	3 years	-	+	+	-	
60.	154	-	+	-	3 years	+	-	+	-	
61.	155	-	+	-	5 years	+	-	-	-	
62.	156	-	+	-	9 years	+	+	-	+	

Sl. No.	Animal No	Exopthalmos			Age	Nasal discharge		Frontal bone Perforation	Pregnancy	Type of Tumour
		Right	Left	Bilateral		Mucus	Blood			
63.	157	+	-	-	5 years	+	+	+	-	
64.	158	+	-	-	6 years	+	+	-	-	
65.	159	+	+	-	6 years	+	-	-	-	
66.	160	+	-	-	8 years	+	+	-	+	
67.	161	-	-	-	3 years	+	+	-	-	
68.	162	+	-	-	8 years	-	-	-	+	
69.	163	-	+	-	7 years	+	-	-	+	

: 1 :

Showing the Optical Density at 500 mμ at Stage I, Stage II, Stage III and Normal.

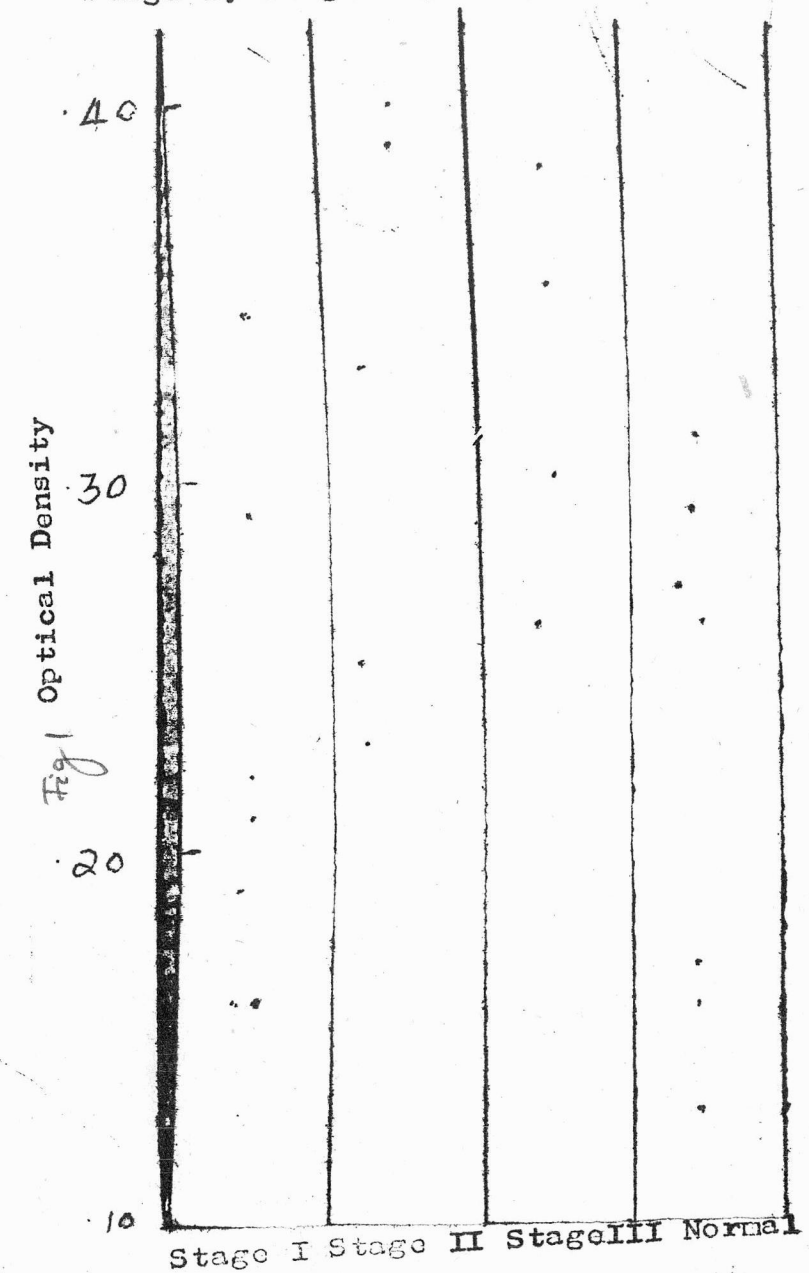


Fig 1

Showing the Optical Density at 640 mμ at Stage I, Stage II, Stage III and Normal.

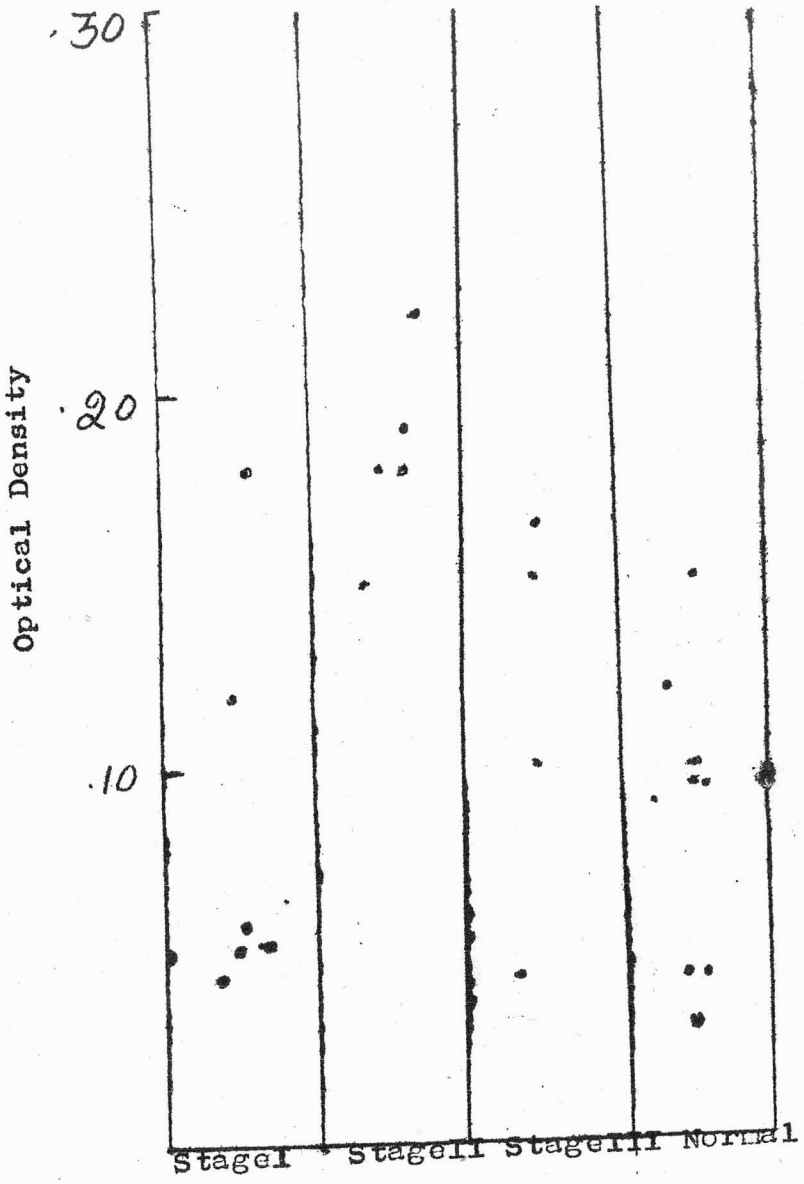


Fig 2 Changes in absolute number of Eosinophils in goats following administration of cyclophosphamide

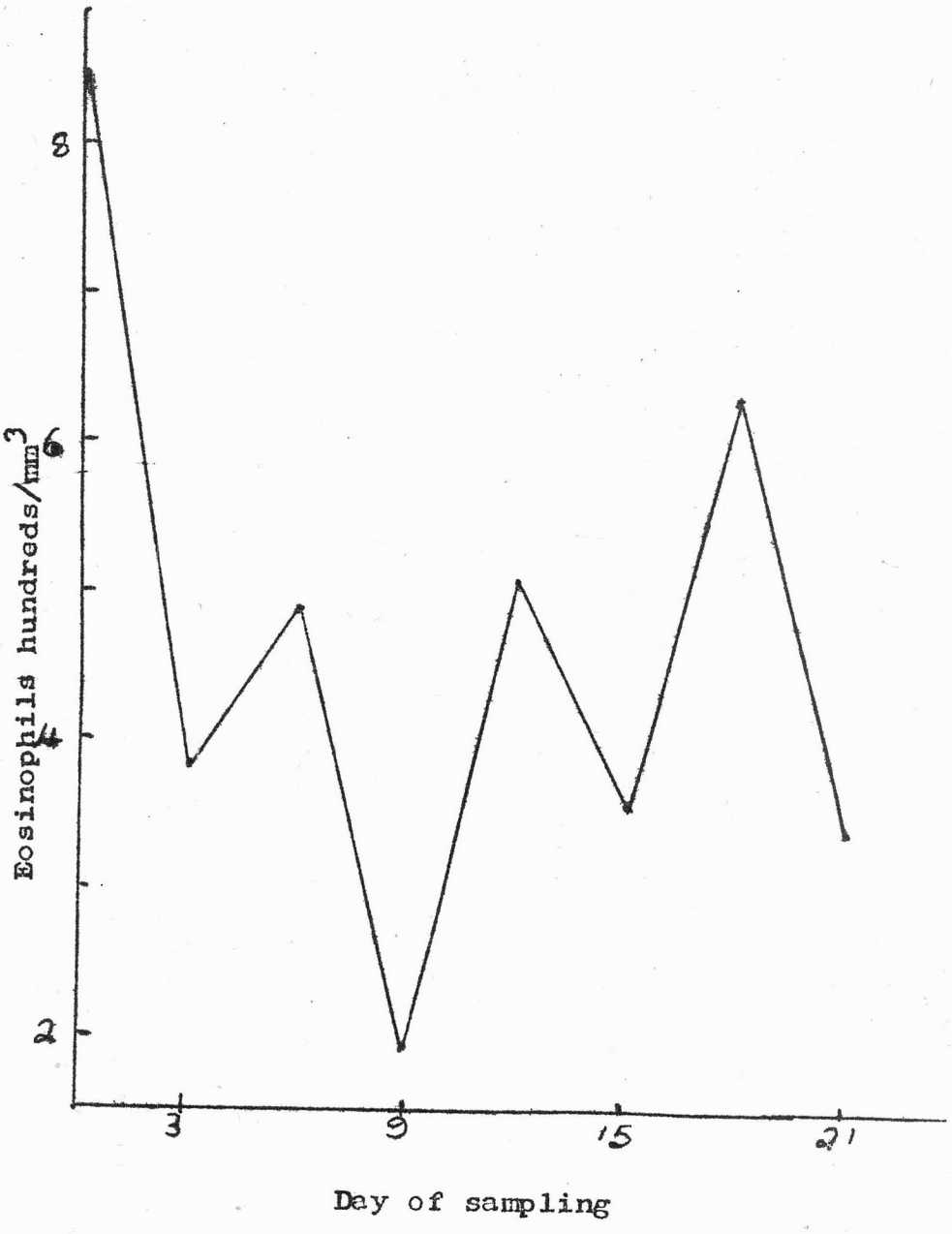


Fig 3 Changes in number of leucocytes in goats following administration of cyclophosphamide.

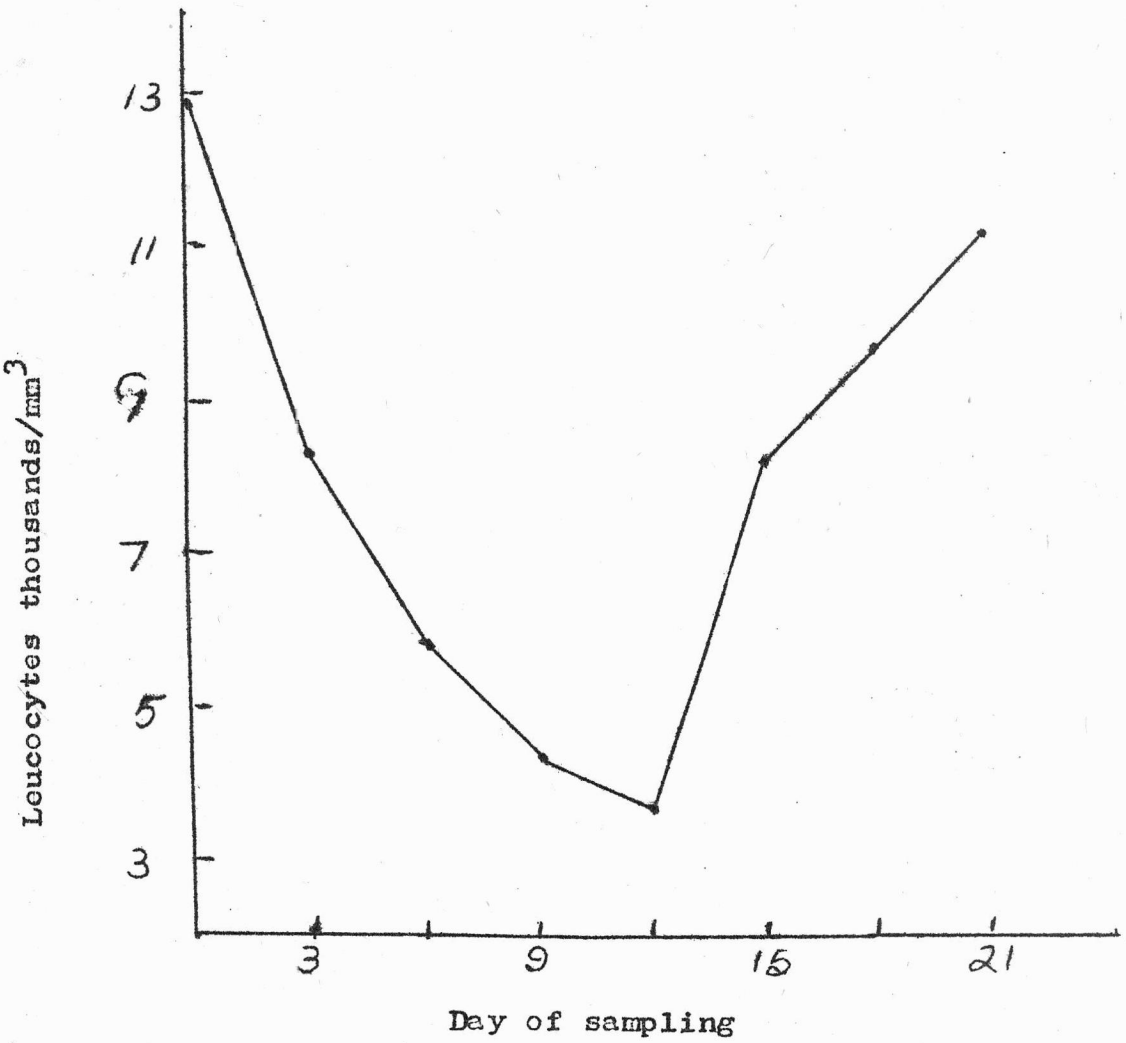


Fig 4 Changes in amount of Haemoglobin in goats following administration of cyclophosphamide

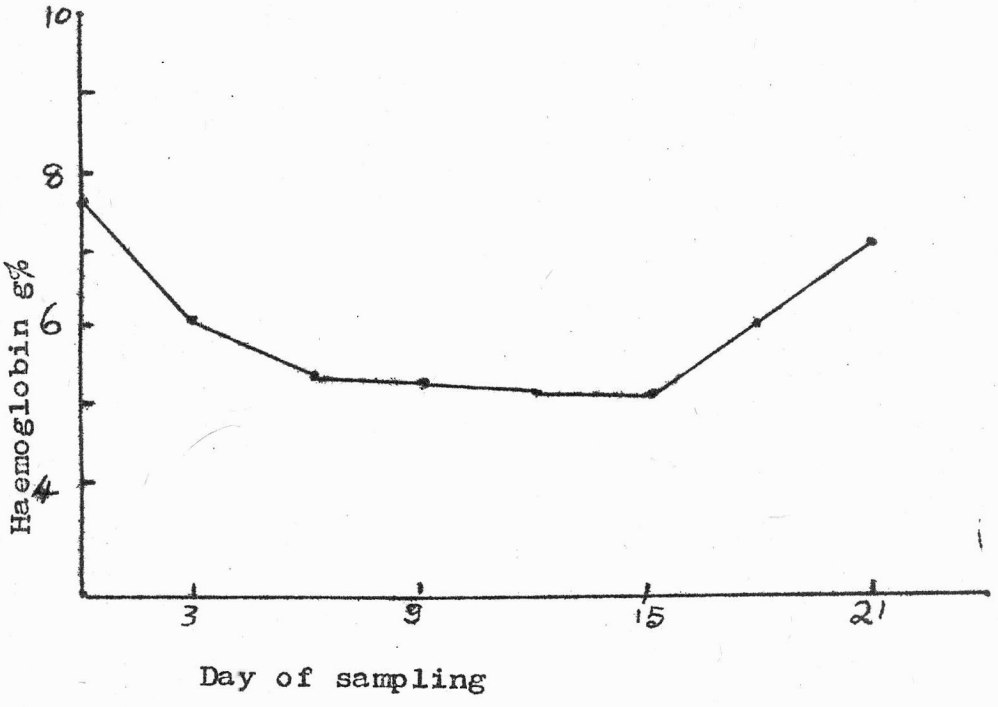


Fig 5 Changes in Absolute Lymphocytic and Neutrophilic counts in Goats following administra ion of Cyclophosphamide.

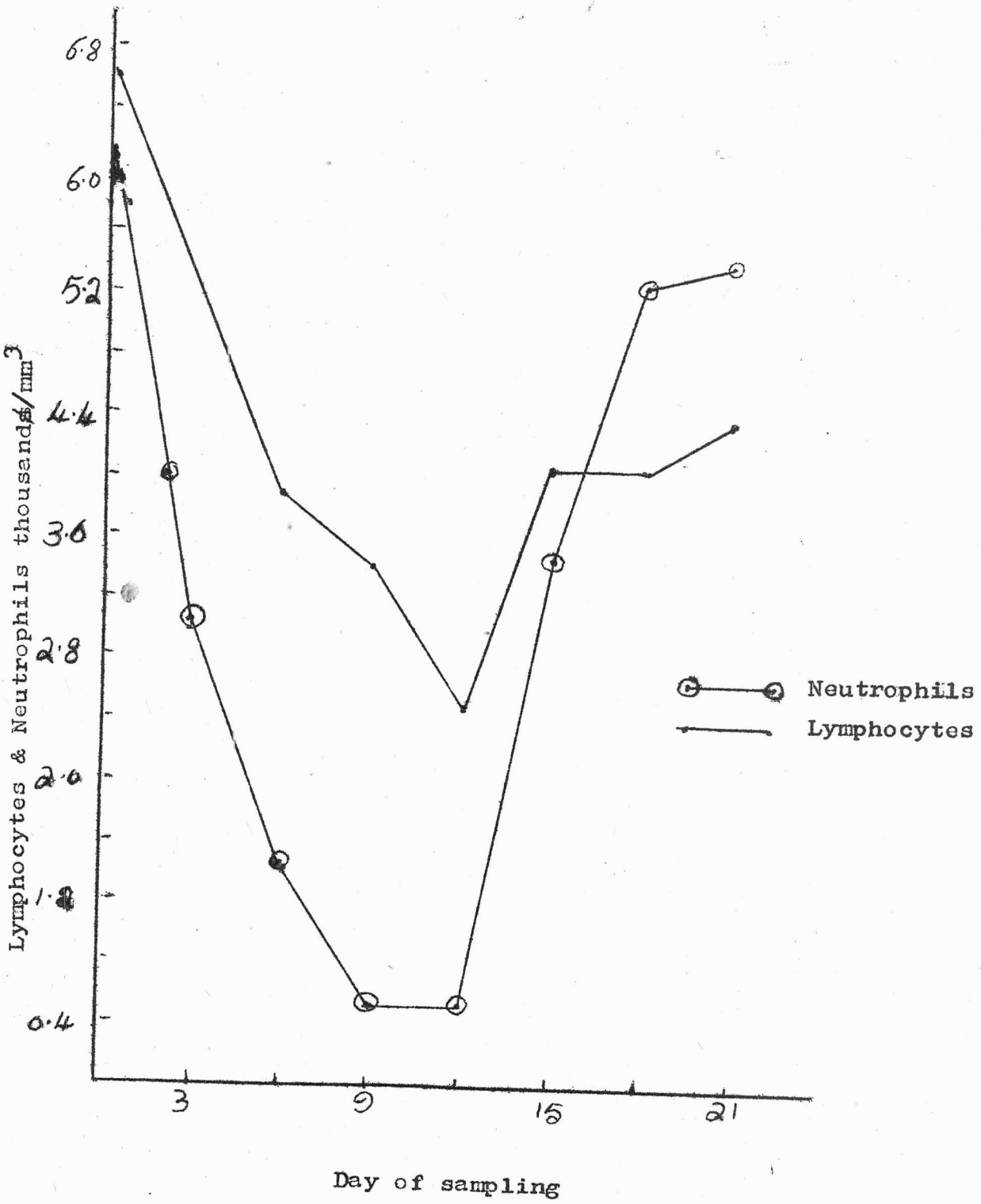
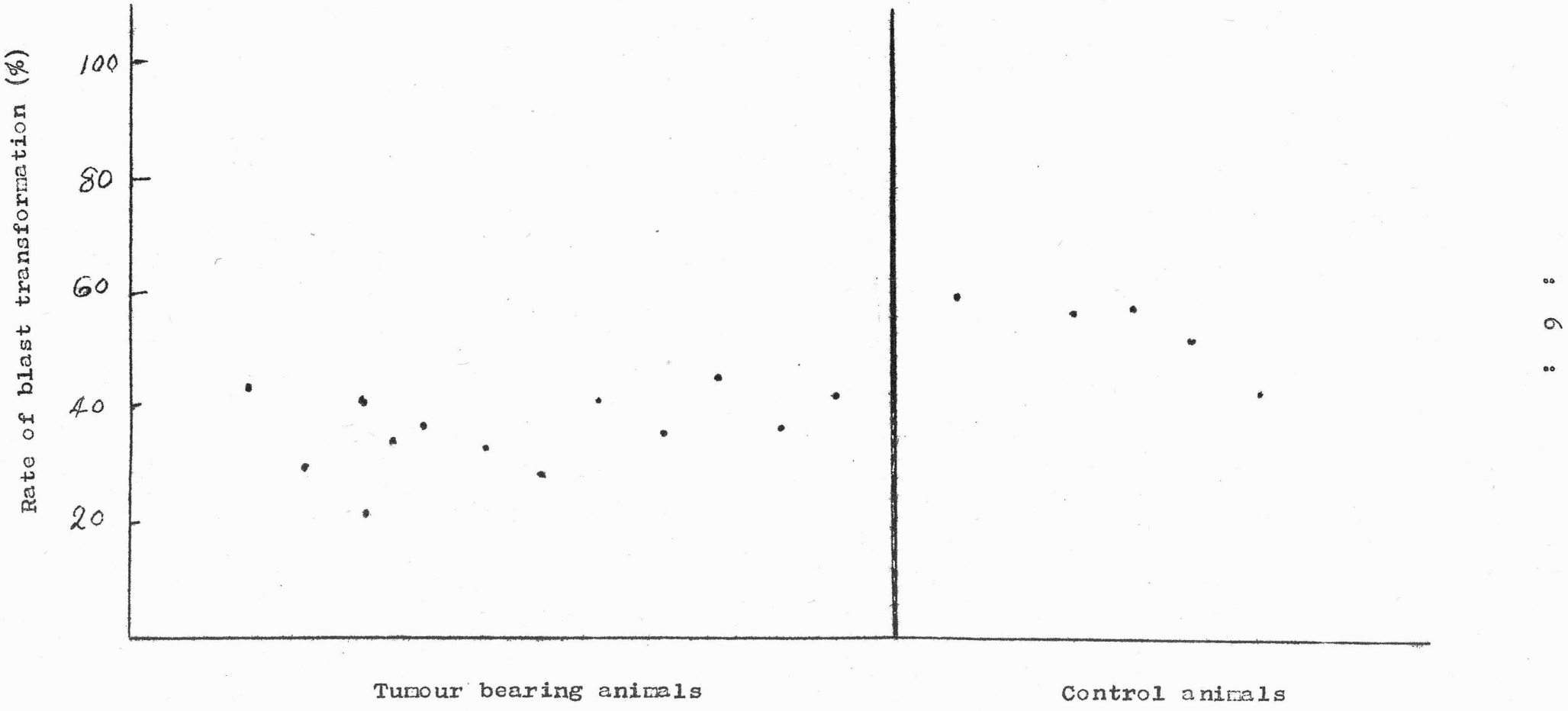


Fig 6 Scattergram of Blast transformation of peripheral blood lymphocytes induced by PHA-M in tmour bearing and Control animals.



APPENDIX

Details of animals bearing eithmoid tumour

(Subjected to detailed investigation)

Sl. No.	Date	Source	Sex	Breed	Age in years	Date of death	Remarks.
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
1.	4-1-1980	Calicut	Female	CBJ	7	8-1-80	
2.	5-1-1980	Alwaye	,,	CBJ	8	21-1-80	Left eye bulged
3.	16-1-'80	Anthicad	,,	CBJ	9	19-1-80	
4.	18-1-1980	Panimbra	,,	CBJ	8	21-1-80	
5.	29-1-1980	Chavakkadu	,,	ND	12	8-3-80	Left eye pretraded
6.	8-2-1980	Thiruvazhamkunnu	,,	CBJ		10-2-80	
7.	18-2-1980	Quilon		CBJ	9	24-3-80	Left eye
8.	13-2-1980	Vaikom	Heifer	CBJ	9	30-3-80	
9.	15-2-1980	Vaikom	Female	CBJ	8	16-2-80	Right eye bulged
10.	15-2-1980	Udayanapuram	,,	Goat	5	1-3-'80	
11.	19-2-1980	Edathwam	,,	CBJ	2½	31-3-80	
12.	20-2-1980	Pattikkad	,,	CBJ	6	5-6-'80	
13.	22-2-1980	Mannuthy	,,	CBJ	6	22-2-80	Frontal bulging
14.	15-3-1980	Shertalai	,, Heifer	Brown Cross	24		Right eye bulged Frontal Frontal bulging.
15.	17-3-1980	Chalakudy	Female	Jersey cross	5	20-4-80	Right eye elight bulging Respiratory distress. S nasal discharge. Calved months back.

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
16.	26-3-1980	Calicut	Bull	Jersey Cross.	9	17-4-80	Snoring, left eye bulged.
17.	28-3-1980	Parappanangadi	Female	CBJ	9	12-7-80	Right eye bulged
18.	29-3-1980	Kaiparambu	Bullock	ND	12	25-5-80	
19.	5--4-1980	Triprayar	Cow	CBJ		16-6-80	
20.	12-4-1980	Kulavoor				12-4-80	
21.	13-4-1980	Mannuthy	Pig	LWJ		13-4-80	
22.	16-4-1980	Chunkakkad	Cow	CBJ	8	15-8-80	
23.	22-4-1980	Mullasseri	Female	CBJ	9	11-5-80	
24.	7-5-1980	Ramamangalam	Cow	CBJ	7	14-5-80	Left eye bulged out.Respirato distress.
25.	8--5-1980	Engandiyoor	Cow	ND	6		
26.	13-5-1980	Kondoorkara	Goat	CB	3½	7-6-80	It had respiratory difficulty. Nasal discharge.
27.	21-5-1980	Chalakkudy	Cow	CBJ	8	1-7-80	
28.	28-5-1980	Shertallai	Veehoor	Veehoor breed	8	27-6-80	Respiratory difficulty with mu genious discharge.No eye bulgi
29.	30-5-1980	Mala	Cow	CBJ	10	19-6-80	Slight asymetry of the eyes.Abd nal type respiration. Holdin head upwards. Epistaxis.
30.	3-6-1980	Pattor	Cow	ND	8	12-6-80	Ails since 2 months. Mucosangu discharge. Respiration difficult
31.	11-6-1980	Edathwa	Female	Cross	3		Snoring sound since 2 months. Animal taking food and water, condition fair. 9 months preg On pressing, lower part of th right side of the nasal passa dull.Delivered on 4.7.80 is v week.calf did on 7.7.80

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
32.	14-6-1980	Udayanapuram	Female	ND	7	25-6-80	Calved 1 week back The animal respiratory difficulty and epi axis since 1 month. No bulgin eye ball. Mucosangninous nasa discharge. Animal poor in con tion.
33.	17-6-1980	Mannuthy	Female	LWY	3	17-6-80	
34.	17-6-1980	Ernakulam	CBJ	H.F.	8½	20-8-80	Slight frontal perforation.
35.	21-6-1980	Engandiyur	C x B	C x B	8	11-7-80	
36.	22-6-1980	Malaparamba	Female	C x B	6	22-6-80	Both eyes protruded. Frontal b ing. Advanced stage, Calved 6 months back.
37.	24-6-1980	Tripunithura	Cow	CBJ	7	9-7-80	Left side of the face is bulge out. Mucosangninous nasal dis rge. Nasal discharge and resp tiony difficulty noticed since months.
38.	28-6-1980	Trichur	CB	Goat	7	24-7-80	
39.	4-7-1980	Calicut	Female	Cow	6	30-12-80	5 months pregnant. Epistaxis occasionally since 2 months Na discharge. Respiratory diffic Delivered and the calf died on 28-12-1980.
40.	5-7-1980	Kunnamkulam	Female	Pig LWY	4	9-7-80	
41.	14-7-1980	Kanjany	Female	c X B	7	12-8-80	
42.	23-7-1980	Aroor	Female	CBJ	5	26-7-80	Snoring noticed 3 months back, Calved 2 months back. Respira difficulty with mucosangninou nasal discharge. Animal is in cumbent position for the last 4 years

43.	24-7-1980	Tottada	Female	C x B	4	4-9-80	Snoring started from 2-7-80. Epistaxis Noticed.
44.	30-7-1980	Mannuthy	Male	LWY	3½	30-7-80	
45.	1-8-1980	Kunnamkulam	Female	Pig	3 2 months 25 days	6-8-80	
46.	5-8-1980	Ambalapuzha	Female	C x B	12	8-8-80	Calved 4 months back.Respiratory d culty started 1 month back. Right bulged out. Slight frontal bulgin also noticed.
47.	7-8-1980	Thiruvazhamkunnu	Female	CBJ	8	16-11-80	
48.	17-8-1980	Makkada	Female	CBJ	6	25-8-80	
49.	12-8-1980	Thazhekkad	Female	C x B	9	16-9-80	
50.	18-8-1980	Wadakkenchery	Female	C x B	8	17-9-80	Calved 10 months back. Both eyes moderately protruded. Respiratory d tress and nasal discharge noticed 1 month.
51.	27-8-1980	Alwaye	Bull	Jersey	8 11 days	6-11-80	Respiratory distress noticed sinc months Exopthalmos of the right ey
52.	31-8-1980	Trichur		CBJ	6	18-9-80	
53.	3-9-1980	Eyyal	Female	H.F.	4½	24-9-80	Respiratory distress calved 3 mont back.
54.	17-9-1980	Nenmara	Female	CBJ	5	24-9-80	
55.	19-9-1980	Poruthur	Female	C x BJ	8	14-10-80	
56.	24-9-1980	Alwaye	Female	C x BS	7	14-10-80	Respiratory distress.Nasal dischar Exopthalmos of the right eye.
57.	27-9-1980	Thiruvazhamkunnu	Female	CBS	8	21-10-80	Calved three times

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
57.	27-9-1980	[illegible]hiruvazhamkunnu	Female	CBS	8	21-10-80	Calved three times
58.	30-9-1980	[illegible]annuthy	Female		4	9-10-80	
59.	30-9-1980	[illegible]annuthy	Female		4	9-10-80	
60.	7-10-1980	Kunnamkulam	Boar	Pig	4	10-10-80	
61.	25-10-'80	Calicut	Heifer		4		Returned to the owner
62.	4-11-1980	Calicut	Female	CBJ	5	9-11-80	Left eye protruded, Dry animal, respiratory.
63.	4-11-1980	Calicut	Female	ND	9	20-11-80	Sudden bulging out of the left eye 1 week back. Muco-sangninous nasal discharge, calved 4 times.
64.	6-11-1980	Vaikom	Female	CBJ	5	17-11-80	Respiratory difficulty noticed sinc 1 week.
65.	15-11-1980	Calicut	Female	C x B	6	7-12-80	Calved two days back. Epistaxisis noticed since 1 month. Respiratory difficulty no eye bulging oral brea
66.	1-12-1980	Calicut	Female	CBJ	8	5-12-80	
67.	10-12-1980	Trichur	Female	CBJ	6	28-12-80	
68.	11-12-1980	Kunnamkulam	Female	LWY	3½	1-12-80	
69.	18-12-1980	Vyttila	Female	CBJ	6		Calved 4 times. Sever respiratory distress. Slight assymet of the lef eye.
70.	20-12-1980	Angamali	Female	C x B	8		Epistaxis noticed since 1½ months. Severe respiratory difficulty.

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