CHAPTER III

MATERIAL AND METHODS

TAXONOMY

For the taxonomy study the host was carried out regularly in each annual cycle.

The host was dissected in the mid ventral line, various organs of the viscera i.e. stomach and intestine, intestine kept separate petridish containing normal saline. These organs teased with need and observed under binocular microscope (recorded infected and non infected examined host). The worms were flattened by giving gentle pressure and fixed in 4% formalin, for 24 hours. Then they were taken out and washed in several changes of distilled water. The worms were stained with Harri’s Haematoxyline, dehydrated, cleared in xylene, mounted with D.P.X. drawings were made with the aid of camera Lucida. All the measurements are in millimeters, unless otherwise indicated.

SEASONAL VARIATIONS

The study of seasonal variation/ population dynamics was purely related with statistical application collection of cestode parasites with reference to the host calculation were based on the following formula.

1) Incidence of Infection:

It is frequency of the infection of hosts by the cestode parasites expressed in terms of percentage.

\[
\text{Incidence} = \frac{\text{No. of Infected hosts}}{\text{Total no. of hosts examined}} \times 100
\]
2) **Intensity of Infection:**

It is the quotient of the member of cestode parasites collected divided by the number of infected hosts.

\[ I = \frac{a}{b} \]

where,

- \( a \) = No. of Infected hosts.
- \( b \) = No. of cestode collected.
- \( I \) = Intensity.

3) **Density of Infection:**

It is the concentration of the cestode parasites in terms of parasites/ unit space (single host).

\[ D = \frac{b}{c} \]

Where,

- \( b \) = No. of cestode collected.
- \( c \) = No. of host examined.
- \( D \) = Density.

**Biochemical parameters:**

The hosts intestine and parasite were collected as samples for biochemical parameters from various places of Amravati district. Required quantity of these samples dried at 53-58° c for 48 hrs. Separately and then preserved powdered samples weighted for quantities estimation of protein, glycogen and lipid.
The estimation of the biochemical composition was done separately infected intestine, non-infected intestine of parasites. Percentage of component expressed for dry weight of the sample i.e. protein, glycogen and lipid.

**Protein:**

Protein was analyzed by Biuret method. Allan G. Gornall et.al (1949) the burret relation for protein estimation was one of the first Catorimetric protein assay methods developed.

**Principle:**

The number of peptide bonds is proportional to intensity of colours, colour is stabilizer for three hours. Falio phenol form intestine colours greater 10 times than Biuret. Bovine serum albumin as the standard is isolated from serum of bulk of cows.

Biuret method is so accurate because it depends only on the peptide bond and not on the particular amino acid residue but lawry and U.V. absorption method is dependent on the amino acid residue. When we have to estimate the protein or it changes the normality of acid and salt. [Biochemical methods by S. Sadasivam and A. Manickram, New age international publisher 1996].

**Reagent:**

Protein standard 10mg/ml of bovine serum albumin.

Biuret reagent: Dissolve 3gms of CuSO₄ Sulphate 5H₂O and 9gms of tartarate in 500ml of 0.2ml/lit. NaoH. Add 5mg of KI and make up to 1lit with 0.2 mg/lit NaoH.
Procedure:

Add 3ml of Biuret reagent to 2ml of protein solution mixed and warm at 31°C for 10 min. cool and read the extinction of 540 um prepare a graph of extinction against bovine serum concentration.

Glycogen:

Glycogen was analysed by the anthren method of San Seifer, Dayton S. Navie B. and Muntyw G.R. (1950).

Principle:

Anthrone reaction is the best and rapid convenient method for the determination of aldo, pentose, Hylluric acid, either or present in polysaccharides. The blue green solution an absorption maximum at 620 um although and those some Carbohydrates may give other colour. The reaction is not suitable when protein containing large amounts of the typtophan is present since red colour is obtained under these conditions. The extraction depends on the compound investigated but is constant for a particular molecule.

Reagent:

30% KOH, 2% Na₂SO₄, Absolute alcohol, Anthrone reagent (0.16 gms in 100ml of concentration H₂SO₄) and glycogen standard Glucose (5mg of glucose in 100ml of D.W.).

Procedure for glycogen:

Take 50mg of tissue, boil it for one hour on water bath Till tissue is completely dissolve cool it add 0.2 ml of 2% Na₂SO₄. Add 6ml of absolute alcohol (C₂H₅OH), Keep it for over night in Refrigerator. Centrifuge for 15min at 3000-R.P.M. Discard super
latent. Dry the residue cake for 30 min. Dilute the residue cake by adding 20 ml of D.W. add 5 ml of super latent add 09 ml D.V. add 5 ml anthrone reagent. Heat for 5 min. on water bath, cool it and read O.D. at 620 μm (Blank= 0.1+0.9 = 1 ml).

**Lipid:**

Lipids are roughly divided into simple and conjugate lipids, which serve primarily as store of oxidisable substance and those, which are parts of structural element of the cell. Fats are soluble in organic solvent and hence we shall use solvent like methanol, chloroform, and either for their estimation, Barner H. and I. Block stock (1973).

**Reagents:**

a) Chloroform methanol.

b) Vanilline (2 gms of vanilline dissolve in 200 ml of D.W. and add it to 800 ml of orthophosphoric acid (keep it for 1 month).

c) Stock-50 mg of cholesterol in 10 ml of chloroform methanol. 1 ml stock dry for two days. Add 2 ml concentration H₂SO₄ boil it on water bath, cool it for 30 min. Take 0.2 ml solution plus 5 ml of vanilline reagent wait for 30 min. Read 0.0 on calorimeter at 53 nm filter.

Chemical contamination of ascorbic acid dihydro ascorbic acid and diketoguianic acid in method of biochemical analysis vol. 1 Edited by Daniel Interscience No. PP. 115.

**Reagent:**

10% T.C.A, 2.4 Dinitrophenol Hydrazine, Hydrazine 2 gm plus Thiourea 4 gm in 100 ml 9n H₂SO₄ 85% H₂SO₄ (82.88 + 100 ml).
Procedure:

1) 100mg tissue/homogenate 5ml.
2) Add 10% T.C.A. 10ml.
3) Centrifuge at 3000 R.P.M. for 15min.
4) Take 1ml supernatant (this can be drawn from protein estimation).
5) Add 6.25 ml Hydrazine reagent.
6) Keep in boiling water bath for 20min then cool it.
7) Add ice-cold 85% H$_2$SO$_4$ 3ml drop wise with constant sterring.
8) Keep for 30 min. at room temp.
9) Read O.D. at 540 nm.

For the histopathological study infected intestine cut in to small pieces and were fixed in Bouin’s fluid, after 48hrs this tissue was cleared dehydrated and embedded in a paraffin wax. There fixed block were cut at 8-9mm,slides were stained with mallary Triple stain.

Reagent:

Alcohol grade
Lithium carbonates.
M.T.S. I: Acid fuchson- 500mg Glacial acetic acid 0.5ml distilled water 99.5ml.
M.T.S. II: Aniline blue-0.5gm orange G.200 gm oxalic acid 2.0 gm and distilled water 160ml.

Mordant solution:

Phosphotungstic acid- 1gm of distilled water 100ml.
Procedure:

Remove the wax with xylene. Then passes alcohol grade from absolute to 30% wash with distilled water (Each grade for 3-5 min.) Deep in M.T.S. for one hr. wash with D.W. pass in to mordant solution for few min stain with M.T.S. II (45 min.) wash with passes alcohol grades from 30% to 100% clearing reagent xylene. Mount in D.P.X.