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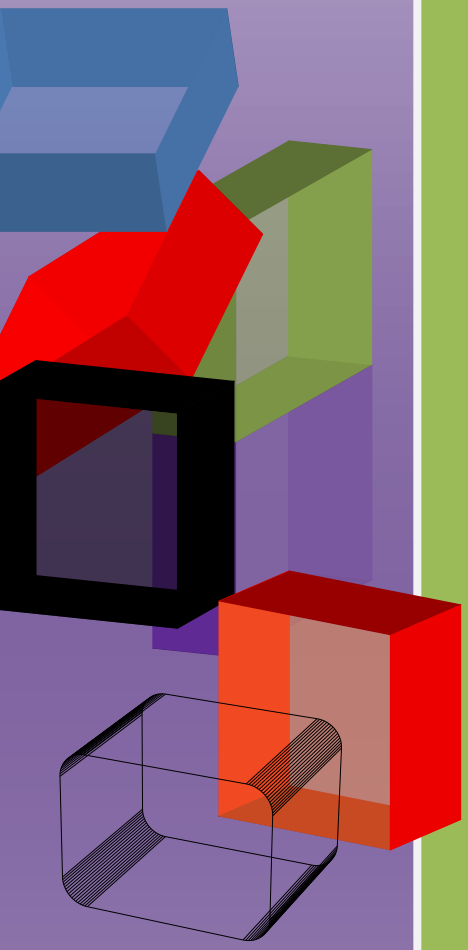

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PROTOZOAN PARASITES AND HAEMATOLOGY OF SOME FISHES IN TERAI REGION OF UTTARAKHAND

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PRESENT STATE OF KNOWLEDGE

Fishes are the earliest known vertebrates and flourishes during the Devonian period about 400 million years ago.Fish occupies an important place in an average Indian diet because of its unique qualities. It plays an important role in overcoming the protein-calorie malnutrition which is being faced by the developing countries like. India where about 60% of the population is suffering from protein deficiency. Besides, Fishes also have a rich amount of vitamins mainly A and D which are essential components of a balance diet. The inclusion of fishes in a poor man's diet is increasing day by day as now it has been established to be the cheapest source of protein for them but on The other hand, their production is hampered because of various environmental pollution hazards. Economically fishes constitute a very important group of animals and provide a rich source of food, liver oil and a number of other by- product like fish meal, fish manure is in glass etc.

The majority of infectious diseases of fish like trypanosomiasis, ichthyopthiriasis, whirling disease, hexamitiasis, microsporidiases, piroplasmosis, coccidiosis, asphyxiation, hypoglycemia, hypoglycemia etc. are caused protozoan but such findings in fishes are far from complete.Blood is very closely associated with total metabolism. Constitutes approximately7% of the total body weight and is a reliable index of physiological condition of fish. Hemoglobin percentage (Hb %), Packed cell volume (PCV) and mean corpuscular volume (MCV) also decline in infected fishes. These reports clearly confirm that total blood is an important index for studying pathogencity in fishes due to parasitic infection.

This study is based on terai region of Uttarakhand. Terai region of Uttarakhand is situated in the southern part of Himalayas. It is a part of Ganga river system. Seven reservoirs have been constructed in the terai region of kumaun hills of Uttarakhand. Some are Nanak Sager, Tumaria, Baigul, Dhaura, in the district of Udham Singh Nagar.

Unfortunately, the fishes of Terai region have been deprived of adequate studies pertaining to protozoan pathogencity though the waters have been substantially pampered by the industries and are most likely to cast their impact on the metabolic and physiological condition of the fish. It is therefore, proposed to investigate the protozoans which affect the piscine host and to observe the hematological changes incurred therein. This will facilitate on throwing light on the existing obscure problems of etiology ad the mechanism of protozoan injury to fish which will not only prove to be academically important but is likely to reveal some interesting result in applied parasitology by providing scientific basis to save valuable fish crop for nutritional and commercial purposes to meet the swelling demand of food crisis.

Histopathological lesions have been found to occur in internal organs due to protozoan parasites. A great deal of work has been done in higher vertebrates and the lesions have been found in internal organs viz. liver, spleen, kidney with hemorrhages, and severe congestion **(Shrivastava et al, 1969, Patel et al 1982)** However, similar studies on fishes are far from sufficient. Some work on this aspect has been done by **Lorn et al (1986),** who observed tissue lesions in kidney and pancreas of *Cyprinus carpio* experimentally infected with *Trypanosoma Borelli* and *Trypanosome Danilewski*.

STUDY OF AREA

During the present work some live fishes were obtained from Nanak Sagar reservoir, Tumaria reservoir, Dhaura reservoir, Baigul reservoir of tarai region of Uttaarakhand

- **Nanak sagar reservoir**
  It has been constructed on river Saryu or Deoha at Nanak matta forming Nanak Sagar and it is nearby town to Sitarganj district U.S. Nagar. Length of reservoir is $3833x10^3$ m^3. The reservoir is located at $28^o57'$ N and $79^o50'$E and area is 4662 hectare.
- **Dhaura reservoir**
  Dhaura reservoir is located near Dineshpura district U.S. Nagar constructed on river Dhaura and Katna in 1960. Length of the reservoir is 9700m and volume of it is $50.700x\ 10^3m^3$.

Irrigation potential of this reservoir is 14600 hectares, built with an estimated cost of 11.1 million Rs. The reservoir is located at 28°53' N and 79°34' E.

- **Tumaria reservoir**
  It has been constructed on river Phika and Dhela at Kashipur district U.S.Nagar in 1961. The reservoir is located at 29' 20'N and 79'0'E.
- **Local Fish Market**
  Some fishes are collected from local fish Market.

OBJECTIVES OF STUDY

Before starting or disscuss about any study there is need of some objectives, goals or aims. Without determining the objectives of any study, It is not possible to complete and gain a correct directions. So it is very necessary to understand the topic very well. There are some objectives of this study and given below :

- To find that species of protozoan parasite that are harmful for the fishes.
- To identify that fishes which are infective from protozoan parasites and collect them
- Difference between normal and parasitic infected fish species.
- Study of Haematological parameters of some fishes under normal and infected condition.

REVIEW OF LITERATURE

Disease is a major constrain in modern aquaculture system. Intensive or is indeed important to acquire knowledge sami-intensive fish culture propagates artificial stress dye to overcrowding, mal-nutrition, accumulation of metabolites, deterioration of water quality and all such factors magnify the risk of outbreak of disease. The study on fish disease in cultivable fishes in our country is limited. It is indeed important to acquire knowledge on different fish pathogens, their biology, life-cycle and pathologenecity in order to recognize fish disease.

Protozoans constitute of the major fish pathogen group amongst crustacean, bacterial and viral parasites. The protozoans are the major enemies in reaching the goal of maximum fish production as they detrimentally harm the fish altering its biochemical components and decreasing its food value., the identification of these parasites mainly come from higher vertebrates and fishes are being largely neglected in spite of profound utility and demand.

The monofloagellates, Trypanosoma are blood parasites which exist in all sorts of vertebrates fish, amphibians, reptiles, birds and mammals. They have played an interesting role in the Development of certain concepts in biology and medicine **(Chandler and Read, 1970).**

Reports on the occurrence of trypanosomes from fish blood have sprung-up over the recent years. **Danilewskyi (1885)** discovered a trypanosome from *Cyprinus carpio.* **Montel (1905)** reported *T. clariae from Clarias macrocephalus.* **Casteelani** and **Willey (1905-06**) recorded *T. saccobronchi* from *Saccobranchus fossilis* and Dutton et al. however, it was Deflein (1916) who suggested for the first time that tryoanosomes maybe pathogenic for fish.

Trypanosomes from various freshwater and hill-stream fishes have attracted attention of Joshi (1976, 1978, 1979a, 1982, 1985) who have described (H-*Clarias batrachus*) *T. vittati* (H-*Mystus vittatus*). *T. seenghali* (H-*Mystus seenghala*); *T. aori* (H-*Mystus aor*) *T. mrigali* (H-*Chirrhinus mrigala*.)

Narasimhamurti and **Saratchandra (1980)** reported two new species of trypanosomes from Clarias batrachus collected from Vishakhapatnam and Srikakulum.

Ichthyophthirius multifiliis the causative agent of "Ich" or "white spot disease" is a parasite of a wide variety of cultured and wild fishes and was first described in the late nineteenth century **(Fouquet, 1876)**

M. indirae has been described from *Cirrhinus mrigala* near Calcutta **(Kundu 1985)** and **Gupta** and **Khera (1988),** reviewed the genus Myxobolus. India fishes have drawn attention of **Haldar** and **Mukherjee (1979)** who listed the protozoan parasites from *Ophicephalus punctatus* and **Joshi (1979a)** examined 2150 freshwater teleosts recording 3.48% infectivity. **Nandi et al. (1983)** listed twenty species of trypanosomes from Indian fishes and the number was increased to thirty four by **Gupta** and **Gupta** (1988). The same workers, in 1990b, surveyed 2304 fishes from Northern India.

The pathophysiological effects of trypanosome infection on the plasma electrolytes was studied by **Dubey** and **Pandey (1985). TEC, TLC, DLC** and **Hb%** of trypanosome – infected fish (*Clarias batrachus and Channa punctatus*) have been found to show marked changes as compared to uninfected fishes (Gupta and Gupta 1985). These finding were supported by **Sharma** and **Joshi (1991)** in a hill stream fish, *Garra gotyla.*

The above review has been provided to focus the major achievements and notable contributions of researchers engaged in the fields of taxonomy, infectivity, competition of species, host range and host specificity, in vitro cultivation of trypanosome – infected hosts.

The erythrocytes of diseased fishes have been found to show a great distortion in their structure and values **(Joshi 1979c, 1985, 1989 a,b)** and **Joshi** and **Tandon (1980)** further observed haematological, changes in ten species of fresh water teleosts. **Rao et al. (1984)** made observation on Hb concentraton of parasitized hosts. The patho physiological effect of trypanosomes infection on the plasma electrolyte was studied by **Dubey** and **Pandey (1985)** TEC, TLC, DLC and Hb% of trypanosome infected fish (Clarias batrachus and Channa puntatus) have been found to show market changes as compared to uninfected fishes (**Gupta** and **Gupta 1985**). These finding were supported by **Sharma** and **Joshi (1991)** in a hill stream fish *Garra gotyla*.

Ford (1990) observed that in eels, roach rudd and tench, trypanosomes derived nutrition from the hosts erythrocyte Anemia as an outcome of trypanosome infection has been confirmed by **Gupta** and **Gupta (1990a)** in *Wallago attu* and by Islam and **Woo (1991 a)** in Carassius auratus. The latter workers (1991 b) also reported the occurrence of anorexia in the same fish. A declin in Hb% and haemotocrit values was observed by **Gupta** and **Pilacrzyk (1994)** in carps from Poland.

BOARD OUT LINE OF WORK

The present study was made on some species of fresh water fishes. Viz, Labeo rohita (cyprinidae) Chana punctatus (Ophioocephalidae), Colisa fasciatus (Anabantidae), Clarius batrachus (claridae). The fishes were identified according to the classification of fishes (Berg, 19447) as proposed by Srivastava (1980).

1. COLLECTION AND MAINTINANCE OF FISHES

The fish samples work collected from different sites Viz Nanak Sagar reservoir site 1. Dhaura reservoir site 2, Baigul reservoir Site 3, Tumaria reservoir site 4 and local fish market site 5, in summer season by the help of local fisherman by random netting for this purpose cast net was used. The fish were identified with help of meristic characters.

2. SCREENING THE HOST FOR PARASITIC PROTOZOA

A. Ectoparasitic Examination

External parts of fish viz, skin, gills, fins and scales were examined for ectoparasites. Permanent slides were made according to **kudo (1966).**

B. Endoparasitic Examination

Haematologists have adopted various methods for blood collection. For the present purpose blood was withdrawn without sacrificing the fish by two methods–

1. Running the needle deep through the median line just behind the anal fin in a dorsocranial direction approaching the vertebrae and the blood was sucked in syringe.

2. Blood samples of experimental fish were collected with the help of sterilized hypodermic syringe by puncturing the heart or from caudal in. The blood was transferred into sterilized vials having requisite amount of anti-coagulate EDTA (Ethylene diamine tetra acetic acid) at a concentration of 1 mg/5 ml of blood. To confirm the parasitic invasion there will be need of different experiments.

C. Examination of other organs

The various fish collections were also examined to detect parasites of viscera viz-liver, spleen, kidney, gall bladder, intestine and swim bladder. The examined microscopically for live parasite.

3. METHODS FOR HAEMATOLOGICAL INVESTGATION

A. Total Erythrocyte count (TEC) and Total Leukocyte count (TLC)

Total red and white cell counts were made using Numbaur's haumocytometer with improved double counting chamber. All precaution as suggested by Wintrobe (1981) were taken in using heamocytometer. Hayem's solution and truk's fluid were used as diluting fluids for TEC and TLC respectively.

$$\text{Cells/uL} = \frac{\text{No. of cells in 1 large square x Dilution factor}}{\text{Volume factor (.1)}}$$

Dilution factor = reciprocal of dilution (20)

Volume factor = (width x length x height) = .1

B. Estimation of Haemoglobin

Haemoglobin expressed as gram percent of blood was determined by the routine methods using Sahli's haemometer.

Principle – When blood is treated with .1NHCL. The blood is converts into acid haemation. The level of acid haematin is noticed by the help of Shahlis haemoglobinometer.

C. Differential Leukocyte Count (DLC)

DLC of blood were made from freshly prepared and differentially stained blood smears. The counting of cell was done by differential blood cell counter. Correlations were indicated in terms of differential haematological indices as mention below.

$$\text{Neutrophil lymphocyte index (NIC)} = \frac{\text{Neutrophil\%}}{\text{Lymphocyte\%}}$$

$$\text{Monocyte lymphocyte index (MLI)} = \frac{\text{Neutrophil\%}}{\text{Lymphocyte\%}}$$

D. Packed cell volume of red cell was estimated using Wintrobe's tub and the procedure of Wintrobe (1981) was adopted.

Procedure- Filled the Wintrobe haematocrit tube up to "0" mark with EDTA mixed blood and centrifuged, it at 3000 rpm for 60 minute or till the cell packing. Now took PCV reading from Wintrobe. In mm.and it is expressed in percentage.

E. Mean Corpuscular Haemoglobin (MCH)

The ratio of haemoglobin to red cell count which indicates the amount of haemoglobin in average corpuscles was expressed in absent terms by Wintrobe (1981).

$$\text{MCH} = \frac{\text{Hb (gm\%)}}{\text{RBC count (mil/mm}^3\text{)}}$$

F. Mean corpuscular volume (MCV)

The mean corpuscular volume (MCV) was calculated by using formula,

$$\text{MCV in cubic microns (mm3)} = \frac{\text{PCV}}{\text{Numbers of RBC}} \text{ X } 10$$

It was calculated according to Wintrobe (1981)

G. Mean Corpuscular Haemoglobin concentration (MCHC)

This index expresses the ratio of weight of heamoglobin to the volume of erythrocytes and the unit in percentage of gram per 100 ml of red cells. MCHC values were also calculated as per (Wintrobe, 1981)

$$\text{MCHC in \%} = \frac{\text{HB concentration (gm/100ml) x 100}}{\text{PVC in percentage}}$$

PERLIMINARY WORK DONE ON THE TOPIC

Some work on this synopsis been done

- Relevant literature has been done
- Sampling station has been decided.
- Field work has been started for collection of samples.
- Some species of fresh water fishes have been selected.
- Some data of annual yield from the sampling stations have been obtained.
- Haematalogical parameters and it's procedure has been selected.
- Some work has been done but no search and result is found till now.

IMPORTANCE OF STUDY

After the discussion of about any topic it is clear that every study has it's importance. This study also has importance of itself.

- This study helpful to understand about the protozoan parasites of fresh waster species.
- This study tells about the fresh water fishes of reservoirs of terai region of Uttarakhand
- This study tell about the variation in normal fish to infected fishes which infection is spreading due to protozoan parasites.
- By this study we gotted knowledge about the haematology of normal and infected fishes.

LIMITATION OF STUDY

It is not possible to study over the whole world's fishes and all over protozoan parasites. So there are some point which explain about the study area or limitation of study.

- This study focused exclusively on the Terai of Kumaun hills of Uttarakhand.
- This study focused exclusively on the some species of fresh water of Kumaun hill's reservoir.

- This study analyze only about the some infective species of protozoan parasites not involve any other parasites.
- It is based on some procedure and selected parameters of haemotology.

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