Fishes are indispensable source of proteins for humans, not withstanding their importance as an object of sport fishery and pets in the case of ornamental fish. One of the most important problems facing our world today is food deficiency. The protein deficiency is one of the major global challenges facing the third world today. Fish industry also offers employment opportunities to many people as well as income at household and national levels (FAO, 1996; Srivastava, 1988). Small to large scale fish farming is on the increase as an attempt to increase fish availability to meet the ever increasing protein demand for rising human populations (Satchell, 1991; Mwangulumba, 1997). In order to maximize fish productivity farmers need to be aware of the factors that influence fish performance such as nutrition, diseases, environmental stresses and pollutants (Lebelo et al., 2001). Parasites probably cause more disease problems in fish culture than any other type of fish pathogen. If problems can be anticipated and effective measures taken, early, parasite losses may be moderated, but under adverse conditions, parasites, even normally benign ones, can result in high mortality.

*Clarias* is the genus of catfishes (*Order-Siluriformes*) of the family *Clariidae*, the air breathing catfishes. These are found in inland waters throughout much of the whole world and is one of the wide spread catfish
genera in the world. Catfish *Clarias batrachus* has come to achieve some special importance in recent years because of number of reasons. They are important part of the diet for the children and lactating mothers and also prescribed as diet for the convalescent of the patients. This fish is highly regarded for food due to its high protein (15.0%), low fat (1.0%) and high iron content (710 mg/100g tissue).

Like other physiological parameters, the blood constituents of the host are also influenced by the parasites. Fish parasites are of economic importance due to the fact that they affect the productivity of fish through mortalities, by decreasing growth rate, decreased levels of the total plasma proteins due to a fall in absorbed amino acids that are essential for protein synthesis as well as lowering the quality of the meat (*Fraser and Mays, 1986*). Haemoflagellates of the genus *Trypanosoma* are prevalent in freshwater fishes and are transmitted by leeches as vector. The parasitic leeches *P.geometra* and *H.marginata* are well known vectors of fish haemoflagellates (*Lom and Dykova, 1992; Sawyer, 1986*). They cause various diseases in the host like leucocytosis, hypoglycemia and hypocholesterolemia (*Gupta and Jairaj puri, 1983; Gupta and Gupta, 1986*), anemia, odema (*Islam and Woo, 1991*).

The thesis entitled, “Studies on the effect of Haemoflagellate Trypanosoma- *Trypanosoma batrachi, Trypanosoma maguri* on the
Histopathology and Biochemistry of the host catfish *Clarias batrachus* from Meerut District” is divisible into seven chapters. The chapters having an account of trypanosome species of host *Clarias batrachus* is illustrated with the help of 2 figures, prevalence percentage of trypanosome infection and seasonal variations in the host based on general survey throughout the year is also illustrated with the help of 2 tables and 2 figures and observation and discussion was studied in the base of host parasite relationship.

In chapter observation and discussion about the host parasite relationship study is divisible into two chapters, histopathological changes in the host and biochemical changes in the host from the effect of the trypanosome species.

Fishes were manually collected live from the different areas of Meerut district. *viz.* village Sisol, village Nangla (Kubulpur), Garh Road; village Kohalla, village Bhandora, Hastinapur Block (Mawana); village Ikri, village Kakkapur, village Durjanpur, village Daulatpur, village Baadam, village Dah, village Meharmati, village Madiyai in Saroorpur Block (Sardhana). Some fishes were also purchased from fish markets of Meerut. The fishes were transferred in oxygenated containers as soon as they were caught, then they were brought to the laboratory. They were kept alive until required in aerated glass aquaria at a controlled
temperature of 25°C( ± 5) °C and fed minced goat liver and commercial pallets.

A total of 443 host fishes were examined for the presence of haemoflagellates at regular intervals throughout 12 months period. The haemoflagellates were collected and through records of the data were maintained throughout the year. Prevalence concept as suggested by Margolis et al., (1982) was used. The prevalence percentage of trypanosomes was studied throughout the year. During the course of entire study, 88 fishes were infected out of 443. The infectivity was least during summers (May to July, temperature 25-35°C) and reached its peak value during winters (November to January, temperature 3-11°C). The observed data is shown by tables and figures. In the laboratory, fishes were examined macroscopically for the presence of leeches before transferring in the aquarium also. Before examinations for haemoflagellates weight was also recorded. The parasitic leeches P. geometra and H. marginata are well known vectors of fish haemoflagellates. The fishes were anaesthetized in 5% solution of benzocain (50 mg/l) (added in small portions until the fish was relaxed) then the fishes were killed by a blow to the head and tail was cut immediately and the blood was collected using a thin pasture pipette (heparinized) introduced through the ventral body wall or below the
pectoral fins (Lom and Dykova, 1992). Thin blood smears were made from the blood samples collected. The blood smears were allowed to air dried, fixed in absolute methanol for 5-10 minutes and stained with phosphate buffered (pH-7.2) Giemsa for 30-45 minutes and examined under 100 x objective oil immersion microscope. But if trypanosomes are present in low intensities then their detection in routine blood smears was found to be time consuming. It was detected in the following manner:-

A) Haematocrit centrifuge method (Woo, 1969).

B) Clot method (Lom and Dykova, 1992).

In Haematocrit centrifuge technique, the fish were killed by a blow to the head and the tail was cut immediately. Blood was collected directly from the severed caudal peduncle using heparinized haematocrit capillary tubes (Vitrex Microhaematocrit Tubes, 1613 NRIS, Na heparinized 80 iu/ml). Normally five capillary tubes were filled with approximately 0.06µl blood each. Four of the tubes were then sealed in one end with cement (Brand Haematocrit Sealing Compound), and centrifuged for 5 to 6 minutes in a microhaematocrit centrifuge (Hettich,Type 2075) at 12800 rpm. All smears were air dried, fixed in absolute methanol for five minutes, air dried again and stained with Giemsa (10% stain in phosphate buffered water, pH 7.2) for one hour. Blood from the last capillary tube was used to make a wet preparation. A drop of blood was administered to
a microscope slide and covered with a cover slip. The wet preparation was examined for the presence of haemoflagellates for approximately three minutes, i.e. at least 40 fields (40 x objectives, 10 x eyepiece).

In clot method, blood was allowed to clot in a centrifuge tube placed overnight in a refrigerator. The next day, the flagellates were found wriggling in the serum outside the blood clot and were concentrated by centrifugation. The trypanosomes were separated in a DEAE cellulose column (Lanham and Godfrey, 1970; Lumsden et al., 1973).

Histopathological changes in the host due to trypanosome infection was shown through histological slides of host Clarias batrachus of various parts like intestine, liver, kidney, muscles, spleen, gills. They were preserved in 10% neutral buffered formalin washed in running water and dehydrated in different grades of concentrated alcohol, cleared in xylene and embedded in paraffin. Paraffin sections of 5µ thickness were obtained and stained by haematoxylin and eosin, then covered and examined microscopically (Bancroft et al., 1990).

Fish trypanosome causes changes in somatic indices and condition factors, anemia and pale gills to general weakness, loss of escape reflex, emaciation and ascitis in infected fish (Kabata, 1985; Lom and Dykova, 1992; Laya, 1994 and Smith et al., 2004). In the liver, diffusely degenerated hepatocytes (Granular and vacuolar degeneration) was
observed in infected host as shown in figure. In the interstitial tissues of posterior kidney, wide areas of necrobiotic changes in the renal tubular epithelium with inflammatory mononuclear cells infiltrations were observed in infected host. In the spleen of infected host, swelling in the endothelium of splenic ellipsoidal capillaries, thickened trabeculea and hemorrhage was observed. The gills of infected host showed hypertrophy of epithelial lining of secondary lamellae with odema and inflammatory cells infiltrations.

In haematological biochemical analysis, haemoglobin (Hb) was measured using the standard cyanmethemoglobin method described by Baker and Silverton, (1976). Red blood cells (RBCs) and white blood cells (WBCs) were counted by Neubauer improved haematocytometer by using Hayem’s and Turk’s solutions respectively (Hesser, 1960).

In serum biochemical analysis, fresh blood samples were collected from trypanosoma infected Clarias. Serum was separated from each blood sample for the biochemical examination. Plasma total protein (TP) was determined by biuret method as described by Lawrence, (1986). Serum albumin concentration was estimated by bromocresol green as described by Gustafsson, (1976). Blood serum globulin was calculated by subtracting the concentration of albumin from that of the plasma total protein. Albumin/globulin ratio (A/G ratio) was calculated by dividing
albumin concentration over that of globulin (Coles, 1986). Cholesterol content was measured according to Tietz, (1986). Serum samples were checked for antibodies through agglutination test.

Haemoglobin content was found to be fell sharply in trypanosomal infected fish as compared to the uninfected host that is shown through table and figure. Haematocrit (Hct) or packed cell volume also decreased significantly in the infected fish due to parasitization of trypanosome. The total count of RBCs decreased gradually and is shown by table and figure. Most of the nuclei were elongated and some of them were fragmented in infected fish. The cytoplasm was vacuolated, tear drop like or completely lost leaving the nucleus either intact or swelling was observed. All the morphological abnormalities of erythrocytes found in trypanosome infected host might be due to abnormal erythropoiesis. Reduction in the volume, vacuolation of cytoplasm and fragmentation of RBCs are the indication of anemia and are discussed in detail.

The variations recorded in RBCs content were discussed that trypanosomes secrete some haemolysins capable of lysing red blood cells in infected host. White blood cells play a major role in the defence mechanism of the fish and consist of agranulocytes (lymphocytes and monocytes) and granulocytes (neutrophils, eosinophils and basophils). Differential counts of the white blood cells revealed that the percentage
of lymphocytes and monocytes was quite high in infected host as compared to healthy one. The morphological observation showed that normal lymphocytes were round in shape and nucleus surrounded by a rim of large amount of cytoplasm was observed. But in infected blood, the amount of cytoplasm was reduced and lymphocyte migrated to periphery. Nucleus and cytoplasm were vacuolated occasionally. In non-infected host monocytes were large, spherical in shape and nucleus were indented and surrounded by large amount of cytoplasm where as in trypanosome infected fish monocytes were reduced in shape and both cytoplasm and indented nucleus was vacuolated. The percentage of neutrophils was found to be lower in infected host as compared to healthy one. Neutrophils were irregular in shape as compared to normal fish. There is no change in eosinophil and basophil percentages in infected host as compared to uninfected host where as in normal fish they were rounded and normal basophil was roughly oval in shape, but in infected blood, morphological deformities were observed as is shown through tables and figures.

The plasma total protein content was decreased in the infected host. Similarly serum proteins, albumin and globulin were observed to be decreased in infected host. The serum globulin was also diminished as compared to normal or non-infected host. In A/G ratio, which is a
measurable humoral component at the non-specific defences, was found to be decreased in infected host. Similarly serum cholesterol content was observed and found to be decreased in infected host. All the parameters are shown through table and figures.
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