FURUNCULOSIS IN SNOW TROUT (SCHIZOTHORACINAE) IN KASHMIR: FIRST REPORT

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ABSTRACT

Incidences of furunculosis were reported in *Schizothorax* spp. (*Schizothorax niger*, *S. esocinus*, *S. curvifrons* and *S. labiatus*) in Wular Lake, Kashmir, from 2003 to 2005. The disease was reported during summer and winter months, but the percentage of infection was maximum during winter. Mortality rate ranged from 8 to 15%. Artificial challenge of *Schizothorax* spp. with *Aeromonas salmonicida* produced symptoms pertinent to furunculosis. The incidence of disease was the highest (13.87%) in December, and lowest (0.40%) in May and October. *S. esocinus* exhibited the maximum (44.48%) percentage of infection, while as *S. labiatus* exhibited the minimum (14.28%) throughout the study period. Haematological investigations revealed devastating changes in various blood parameters. Chemotherapeutic tests revealed complete recovery of the disease using 20 ppm oxytetracycline and 30 ppm streptomycin.

Keywords: Furunculosis, *Aeromonas salmonicida*, Schizothoracinae, treatment

INTRODUCTION

Furunculosis is the designation presently recognized (Ghittino, 1968) for an epidemic bacteriosis of fishes, the pathogen of which is *Aeromonas salmonicida*. Furunculosis has spread to almost all countries (Snieszko, 1973). Furunculosis and its pathogens were originally described by Emmerich and Weibel (1890, 1894) in a Bavarian fish farm. Thereafter, in a matter of few years, it was encountered in several European countries. It was reported in West North America, England and Ireland. It seems more probable that it could have been diagnosed and bacteriologically ascertained in these countries subsequent to the first descriptions by Emmerich and Weibel (1890, 1894), although it had occurred earlier in these countries. In this respect, the exposition by Heuschmann-Brunner (1974) may also be referred to. McCarthy (1975) assumes a possible link

between the introduction of the shasta rainbow trout (*Salmo gairdnerii*) into Europe in 1880 and the first record of furunculosis in Germany in 1890. Andrew *et al.* (2003) reported the sporadic incidences of furunculosis in snow trout in the fish farms of Europe.

The typical symptoms of furunculosis are boils and ulcers, which are found isolated or in groups, chiefly in the dorsal region. These ulcers are tinged with blood. The bigger ones contain sticky, dark reddish pus. Ulcers may be absent, and in such an event, autopsy shows intestinal inflammation, principally in the pyloric and rectal regions. The swim bladder is hyperaemic; small spots and haemorrhages are found in the liver. Similar haemorrhages are encountered at times on the inner side of the opercula, in the eyes and on the fins. In the event of a bacteraemia, these symptoms would be usually lacking and the blood is filled with bacteria.

The causative agent of furunculosis is *A. salmonicida* (Emmerich and Weibel; 1890, 1894). It measures 0.8 x 0.5 μm, and is non-motile, non-flagellated and gram-negative. Agar colonies at 20°C become dark and even black in colour within 2-3 days due to the formation of melanin in the presence of oxygen. Gelatin slab cultures give a very characteristic liquefaction in the form of a funnel within 2-3 days, accompanied by abundant sediment formation. *A. salmonicida* grows at an optimum temperature of 20-30°C and dies at 37°C. It is ubiquitous and may be found in water and mud, often in very large numbers.

Traditionally, *A. salmonicida* was thought to have a predilection for salmonids. Over the years, however, the apparent host range of the pathogen has steadily expanded. Thus, infections are known to occur among representatives of several major families of Osteichthyes including Cyprinidae, Serranidae and Anoplopomatidae, in addition to Salmonidae and Agnatha. Non-salmonids which have been documented as suffering from the disease of *A. salmonicida* etiology include minnow and goldfish, carp (Bootsma *et al.*, 1977), perch (Bucke, 1978) bream, roach, dace, chub, tench, pike bullheads (McCarthy, 1975), and snow trout (Andrew *et al.*, 2003).

Therapy, prophylaxis and hygiene as in all other infectious diseases, and removal and destruction of dead and gravely infected fish are suggested. These should preferably be either burned or buried. Fish which are suspected of having furunculosis are isolated in special tanks, taking care to ensure that these are fed with an independent supply of water, which should be cold and clean. Sulfonamides (sulfamerazine, sulfaguanidine, sulfadiazine, sulfamethazine, sulfisoxazole, etc.) given orally with the food at the rate of 10 g for 45 kg of fish per day are useful for therapy (Snieszko and Hoffman, 1963). Among the antibiotics, chloramphenicol and oxytetracycline are reported to be the best with a dosage of 2.5-3.5 g per 45 kg of fish per day (Snieszko and Hoffman, 1963). Leaman (1965) reported excellent results in preventing furunculosis in returning Atlantic salmon by giving 10 mg chloramphenicol per 0.45 kg fish intra peritonially.

OBSERVATIONS

Occurrence

Furunculosis appeared in the winter season, *i.e.,* at the end of October. Its severity increased with the decrease in temperature reaching the peak during intense cold in the month of January. It declined with the increase in temperature in March and by the end of April, it vanished completely. Maximum number of incidences of furunculosis was recorded during winter months. It appears that low temperature is conducive for the spread of furunculosis. Mohan and Shanker (1995) contended that lowering of water temperature may lower the defense competence of fish, thereby, predisposing it to diseases. Salman *et al.* (2004) also support this view.

Symptoms

Fishes show lethargic behaviour, slight exophthalmia, blood-shot fins, bloody discharge from vent and multiple hemorrhages. Necrosis in the skin and underlying musculature is prominent. The disease is characterized by open ulcers on the back and sides of the body; the bigger ulcers contain sticky, dark reddish pus. Punctiform bleeding and petechiae appear in the viscera, especially the liver, and also on the skin, gills, musculature and the bases of fins. The intestine is very red and its blood vessels are enlarged. In the kidneys, besides bleeding, degenerative changes and necrosis of the haematopoietic connective tissue are found. In some cases, haemorrhagic liver may be observed. Ascites is observed in the abdominal cavity. Anus is prolapsed with marked reddening.

Percentage of Infection

During the first year of study, the minimum percentage of infection (1.22) was recorded in the month of May 2004 and the maximum (13.87) in the month of December 2003. During the second year of study, the minimum percentage of

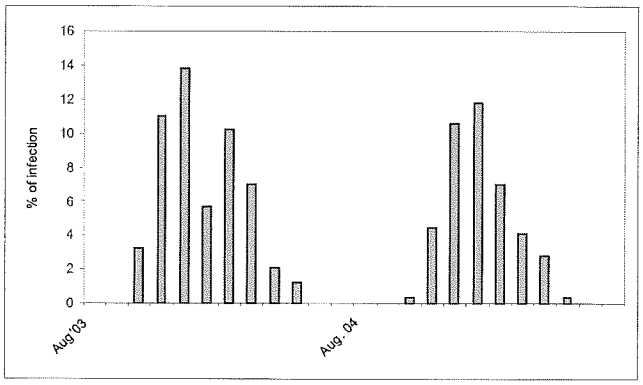

Fig. 1: Furunculosis in Schizothorax spp. from 2003 to 2005

infection (0.40) was recorded in the months of October 2004 and May 2005, while the maximum (11.83) was in the month of January 2005 (Fig. 1). During the whole

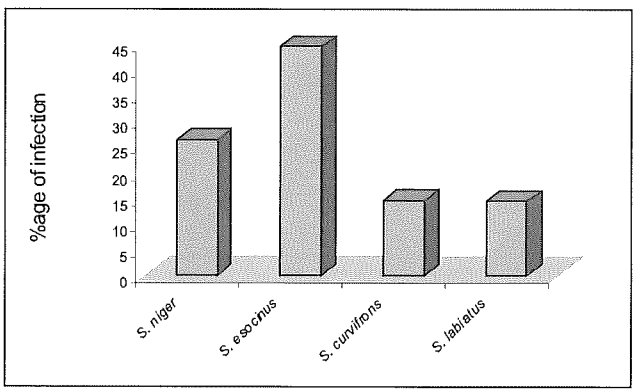

Fig. 2: Furunculosis in four species of Schizothorax in Kashmir valley

study period, the minimum percentage of infection (14.28) was recorded in *Schizothorax labiatus*, while the maximum (44.48) in *S. esocinus* (Fig. 2).

Isolation

A. salmonicida was found chiefly associated with open ulcers and viscera, especially the liver, of fishes suffering from furunculosis. Morphologically, the isolate was a gram-negative, non-motile, non-flagellated bacterium of approximately 0.8 x 0.5 µm. The bacterium was isolated on trypticase soy agar (TSA) and a selective medium, Coomassie brilliant blue (CBB). On the non-selective medium, the bacterium developed colonies surrounded by a dark brown water-soluble pigment after incubation at 20-25°C for 3-4 days. On the selective medium, the bacteria developed as dark blue colonies. Agar colonies at 20°C became dark and even black in colour within 2-3 days, due to the formation of melanin in the presence of oxygen.

Experimental infection trials with *A. salmonicida*

Table 1 exhibits the results of statistical analysis of data for pathogenicity of *A. salmonicida* against fingerlings of *S. niger*. When four different concentrations of *A. salmonicida* suspension, ranging from 2.40 x 10^3 to 9.50 x 10^3 cells per millilitre, were inoculated to *S. niger*, these caused 30 to 85% mortality within 96 hours after injection. The LD_{50} value obtained was 3.85 x 10^3 cells per millilitre. The statistical analysis such as regression equation, Chi-square, log LD_{50} ± standard deviation (SD) and fiducial limits (FL) of the data were recorded as y = -3.329 + 2.295 x, 1.925, 3.4986 ± 0.0203, 2.58 x 10^3 and 5.60 x 10^3 cells per millilitre, respectively. The Chi-square value was found to be significant at $P < 0.5$.

Table 1: Results of the experimental infection produced in *Schizothorax niger* with *Aeromonas salmonicida* isolated from furunculosis affected fishes

S. no.	Concentration (cells/ml)	Symptoms observed	Mortality (%)	Regression equation (y=a+bx)	Chi-square $\{X^2(n-2)\}$	LD_{50} (cells/ml)	Log LD_{50}±SD	95% FL (cells/ml)
1.	2.40 x 10^3	-	30	y = -3.329+	1.925*	3.85 x 10^3	3.4986±	LL: 2.58 x 10^3
2.	3.60 x 10^3	Haemorrhages	45	2.295x			0.0203	UL: 5.60 x 10^3
3.	6.10 x 10^3	Haemorrhages	70					
4.	9.50 x 10^3	Lesions	85					
5.	Control	-	0					

FL: Fiducial limit; LL: Lower limit; UL: Upper limit
*Value is significant ($P < 0.5$).

Table 2: Results of the experimental infection produced in *S. esocinus* with *A. salmonicida* isolated from furunculosis affected fishes

S. no.	Concentration (cells/ml)	Symptoms observed	Mortality (%)	Regression equation (y=a+bx)	Chi-square $\{X^2(n-2)\}$	LD_{50} (cells/ml)	Log LD_{50}±SD	95% FL (cells/ml)
1.	2.40 x 10^3	-	30	y = 4.295+	2.401*	4.90 x 10^3	3.6592±	LL: 3.82 x 10^3
2.	3.80 x 10^3	Haemorrhages	45	2.725x			0.058	UL: 5.80 x 10^3
3.	6.90 x 10^3	Ascites	65					
4.	8.90 x 10^3	Lesions	85					
5.	Control	-	5					

FL: Fiducial limit; LL: Lower limit; UL: Upper limit
*Value is significant ($P < 0.5$).

Table 2 exhibits the results of statistical analysis of data for pathogenicity of *A. salmonicida* against fingerlings of *S. esocinus*. When four different concentrations of *A. salmonicida* suspension, ranging from 2.40 x 10^3 to 8.90 x 10^3 cells per millilitre, were inoculated to *S. esocinus*, these caused 30 to 85% mortality within 96 hours after injection. The LD_{50} value obtained was 4.90 x 10^3 cells per millilitre. The statistical analysis such as regression equation, Chi-square, log LD_{50} ± SD and FL of the data were recorded as y = 4.295 + 2.725 x, 2.401, 3.6592 ± 0.058, 3.82 x 10^3 and 5.80 x 10^3 cells per millilitre, respectively. The Chi-square value was found to be significant at $P < 0.5$.

Haematology

In healthy specimens of *S. niger*, the blood parameters were: haemoglobin 8.32 ± 1.4 mg%, erythrocytes 1.325 ± 1.8 m, leukocytes 73 ± 3.9 x 10^3 t, haematocrit value 27.33 ± 10.2 mg% and blood sugar 57.16 ± 1.79 mg%. Histochemical parameters showed the value of total albumin as 2.775 ± 0.14 mg%, albumin 0.89 ± 0.01 mg% and globulin 2.12 ± 0.05 mg%. Uric acid content was 1.68 ± 0.03 mg%, total cholesterol showed a value of 289.4 ± 1.37 mg%; bilirubin was 1.84 ± 0.03 mg%, while glucose was 87.0 ± 1.83 mg%. PCV and MCV showed values of 53.11 ± 1.34 mg% and 224.83 ± 1.14 μm^3, respectively. MCH and MCHC were observed to be 65.91 ± 1.89 Pg and 33 ± 2.5 g%, respectively.

In diseased specimens of *S. niger* suffering from furunculosis, haemoglobin showed a value of 3.15 ± 2.21 mg%. The total numbers of erythrocytes and leukocytes were 0.52 ± 1.8 m and 103.2 ± 2.8 x 10^3 t respectively. Haematocrit value was 18.22 ± 1.63 mg%, while blood sugar was 33.16 ± 2.97 mg%. Out of histochemical parameters, total albumin was 0.97 ± 0.13 mg% while albumin was 0.15 ± 0.01 mg%. Globulin was 1.0 ± 0.001 mg% and uric acid was 2.89 ± 0.02

Table 3: Haematological parameters of healthy and diseased *S. niger* suffering from furunculosis

S. no.	Parameter	Unit	Normal	Diseased	Significance
1.	Haemoglobin	mg/100 ml	8.320 ± 1.4	3.15 ± 2.21	$P < 0.05$
2.	Erythrocytes	Million	1.325 ± 1.8	0.52 ± 1.80	-
3.	Haematocrit value	%	27.33 ± 10.2	18.22 ± 1.63	-
4.	Blood sugar	mg/100 ml	57.16 ± 1.79	33.16 ± 2.97	-
5.	Total albumin	mg/100 ml	2.775 ± 0.14	0.97 ± 0.13	-
6.	Albumin	mg/100 ml	0.89 ± 0.01	0.15 ± 0.01	-
7.	Globulin	mg/100 ml	2.12 ± 0.05	1.0 ± 0.001	-
8.	Uric acid	mg/100 ml	1.68 ± 0.03	2.89 ± 0.02	-
9.	Total cholesterol	mg/100 ml	289.4 ± 1.37	65.64 ± 1.52	-
10.	Bilirubin	mg/100 ml	1.84 ± 0.03	1.93 ±0.02	-
11.	Glucose	mg/100 ml	87.0 ± 1.83	42.3 ± 0.84	-
12.	PCV	mg/100 ml	53.11 ± 1.34	102 ± 0.01	-
13.	MCV	μm^3	224.83 ± 1.14	438.2 ± 0.84	-
14.	MCH	Pg	65.91 ± 1.89	60.57 ± 0.04	-
15.	MCHC	g%	33.00 ± 2.50	17.28 ± 0.05	-
16.	Leucocytes	Thousand	73 x 10^3 ± 3.9	103.2 x 10^3 ± 2.8	-

mg%. Total cholesterol was 65.64 ± 1.52 mg%. Bilirubin was 1.93 ± 0.02 mg%, while glucose was 42.3 ± 0.84 mg%. PCV had a value of 102 ± 0.01 mg%, MCV was 438.2 ± 0.84 μm^3, MCH and MCHC were 60.57 ± 0.04 Pg and 17.28 ± 0.05 g%, respectively (Table 3).

Therapeutic Measures

The drug and chemical treatment as a therapeutic measure against furunculosis in *Schizothorax* spp. revealed promising results. Potassium permanganate was used in two concentrations, *viz.,* 5 and 10 ppm, to observe the recovery of specimens naturally suffering from and artificially induced with furunculosis. In the case of naturally suffering fishes, it was observed that 40% mortality took place after eight days, while 30% was observed after 15 days, using 5 ppm concentration of potassium permanganate. By using 10 ppm concentration of potassium permanganate, it was observed that 20% mortality took place after eight days and 10% after 15 days. Complete healing was observed in fishes treated with 10 ppm concentration within five days while recovery started after ten days of treatment with 5 ppm concentration (Table 4).

In the case of artificially induced fishes, it was observed that 20% mortality took place after eight days and 30% after 15 days using 5 ppm concentration of potassium permanganate, while 20% mortality was observed after eight days

Table 4: Effect of drugs/chemicals on *S. niger* suffering from furunculosis

S. no.	Drug/chemical used	Concentration (ppm)	Fish used (no.)	Mortality (%) in lapsed days		
				1 to 8	9 to 15	16 to 25
1.	Potassium permanganate	5	09	-	20	30
		10	09		20	20
2.	Malachite green	5	09	-	40	30
		10	09		20	10
3.	Methylene blue	5	09	-	40	30
		10	09		20	-
4.	Chloramphenicol	10	09	-	30	20
		15	09		30	20
		20	09		20	-
5.	Oxytetracycline	10	09	-	30	20
		15	09		20	10
		20	09		10	-
6.	Ciprofloxacin	10	09	-	40	30
		20	09		20	10
		30	09		-	-
7.	Tetracycline	10	09	-	30	20
		20	09		40	20
		30	09		30	20
8.	Streptomycin	10	09	-	30	10
		20	09		20	10
		30	09		10	-
9.	Control	-	09	40	20	40

and 20% mortality after 15 days using 10 ppm concentration. The healing started after five days in fishes treated with 10 ppm and after 15 days in fishes treated with 5 ppm of potassium permanganate (Table 5).

Malachite green was used in two concentrations, *viz.*, 5 and 10 ppm, for 25 days. The water along with the drug was changed after every five days during the experiment. Healing started within five days in fishes treated with 10 ppm and 10 days in fishes treated with 5 ppm of malachite green. It was observed that in fishes naturally suffering from furunculosis, 40% mortality took place after eight days and 30% after 15 days using 5 ppm concentration of malachite green. A total of 20% mortality took place after eight days of treatment at 10 ppm of malachite green. However, no mortality was observed after 15 days of treatment of 10 ppm concentration of malachite green. Complete healing of fishes was observed after 10 days in fishes treated with 5 ppm concentration and five days in case of 10 ppm treated fishes (Table 4).

In the case of artificially induced furunculosis, it was observed that 40% mortality was after eight days and 30% after 15 days in fishes treated with 5 ppm of malachite green. A total of 20% mortality after eight days and 10% mortality after 15 days was observed in fishes treated with 10 ppm concentration of malachite green. The healing started after ten days in fishes treated with 10 ppm and after 15 days in fishes treated with 15 ppm of malachite green (Table 5).

Methylene blue was used in two concentrations, *viz.*, 5 and 10 ppm, for 25 days. The water along with the drug was changed after every five days during the experiment. Healing started within five

Table 5: Effect of drugs/chemicals on artificially induced furunculosis in *S. niger*

S. no.	Drug/chemical used	Concentration (ppm)	Fish used (no.)	Mortality (%) in lapsed days		
				1 to 8	9 to 15	16 to 25
1.	Potassium permanganate	5	09	-	20	10
		10	09		10	10
2.	Malachite green	5	09	-	40	30
		10	09		30	10
3.	Methylene blue	5	09	-	30	20
		10	09		30	10
4.	Chloramphenicol	15	09	-	30	20
		20	09		20	10
5.	Oxytetracycline	15	09	-	20	10
		20	09		10	-
6.	Ciprofloxacin	20	09	-	40	30
		30	09		30	20
7.	Tetracycline	20	09	-	40	30
		30	09		20	10
8.	Streptomycin	20	09	-	30	20
		30	09		10	-
9.	Control	-	09	50	30	20

days in fishes treated with 10 ppm and 10 days in fishes treated with 5 ppm of methylene blue. It was observed that in fishes naturally suffering from furunculosis, 40% mortality took place after eight days and 30% after 15 days using 5 ppm concentration of methylene blue. A total of 20% mortality was observed after eight days of treatment of 10 ppm of methylene blue. However, no mortality was recorded after 15 days of treatment in 10 ppm concentration of methylene blue. Complete healing of fishes was observed after 15 days in fishes treated with 5 ppm concentration and eight days in case of 10 ppm treated fishes (Table 4).

In the case of artificially induced furunculosis, it was observed that 30% mortality took place after eight days and 20% after 15 days in fishes treated with 5 ppm of methylene blue. A total of 30% mortality after eight days and 10% mortality after 15 days was observed in fishes treated with 10 ppm concentration of methylene blue. The healing started after five days in fishes treated with 10 ppm and after ten days in fishes treated with 5 ppm of methylene blue (Table 5).

Chloramphenicol was used in three concentrations, *viz.,* 10, 15 and 20 ppm, for 25 days. The water along with the chemical was changed after every five days during the experiment. Healing started within five days in fishes treated with 20 ppm and 10 days in fishes treated with 15 ppm of chloramphenicol. Prolonged period of healing was observed in the group treated with 10 ppm concentration, in which healing took place after 15 days. It was observed that in fishes naturally suffering from furunculosis, 30% mortality took place after eight days and 20% after 15 days using 10 ppm concentration of chloramphenicol. A total of 30% mortality was observed after eight days and 20% after 15 days of treatment in chloramphenicol, using 15 ppm concentration. After using 20 ppm concentration of chloramphenicol, 20% mortality was observed after eight days, while no mortality took place after 15 days. Complete healing of fishes was observed after ten days in fishes treated with 10 ppm concentration and five days in the case of 20 ppm treated fishes (Table 4).

In the case of artificially induced furunculosis, it was observed that 30% mortality took place after eight days and 20% after 15 days in fishes treated with 15 ppm of chloramphenicol. A total of 20% mortality after eight days and 10% mortality after 15 days was observed in fishes treated with 20 ppm concentration of chloramphenicol. The healing started after five days in fishes treated with 20 ppm and after ten days in fishes treated with 15 ppm of chloramphenicol (Table 5).

Oxytetracycline was used in three concentrations, *viz.,* 10, 15 and 20 ppm, for 25 days. The water along with the chemical was changed after every five days during the experiment. Healing started within five days in fishes treated with 20 ppm and 10 days in fishes treated with 15 ppm of oxytetracycline. Recovery was observed after 15 days in fishes treated with 10 ppm of oxytetracycline. It was observed that in fishes naturally suffering from furunculosis, 30% mortality took place after eight days and

20% after 15 days using 10 ppm concentration of oxytetracycline. A total of 20% mortality was observed after eight days and 10% after 15 days of treatment of oxytetracycline, using 15 ppm concentration. After using 20 ppm concentration of oxytetracycline, 10% mortality was observed after eight days, while no mortality took place after 15 days. Complete healing of fishes was observed after 10 days in fishes treated with 10 ppm concentration and five days in case of 20 ppm treated fishes (Table 4).

In the case of artificially induced furunculosis, it was observed that 20% mortality took place after eight days and 10% after 15 days in fishes treated with 15 ppm of oxytetracycline. A total of 10% mortality was recorded after eight days in fish treated with 20 ppm concentration. However, no mortality was observed after 15 days in fishes treated with 20 ppm of oxytetracycline. The healing started after five days in fishes treated with 20 ppm and after ten days in fishes treated with 15 ppm of oxytetracycline (Table 5).

Ciprofloxacin was used in three concentrations, *viz.,* 10, 20 and 30 ppm, for 25 days. The water along with the chemical was changed after every five days during the experiment. Healing started within five days in fishes treated with 30 ppm and ten days in fishes treated with 20 ppm of ciprofloxacin. Prolonged period of healing was observed in the group treated with 10 ppm concentration, in which healing took place after 12 days. It was observed that in fishes naturally suffering from furunculosis, 40% mortality took place after eight days and 30% after 15 days using 10 ppm concentration of ciprofloxacin. A total of 20% mortality was observed after eight days and 10% after 15 days of treatment of ciprofloxacin, using 20 ppm concentration. After using 30 ppm concentration of ciprofloxacin, no mortality was observed after eight and 15 days. Complete healing was observed after ten days in fishes treated with 10 ppm concentration and five days in the case of 30 ppm treated fishes (Table 4).

In the case of artificially induced furunculosis, it was observed that 40% mortality took place after eight days and 30% after 15 days in fishes treated with 20 ppm of ciprofloxacin. A total of 30% mortality after eight days and 20% mortality after 15 days was observed in fishes treated with 30 ppm of ciprofloxacin. The healing started after five days in fishes treated with 30 ppm and after 12 days in fishes treated with 20 ppm ciprofloxacin (Table 5).

Tetracycline was used in three concentrations, *viz.,* 10, 20 and 30 ppm, for 25 days. The water along with the chemical was changed after every five days during the experiment. Healing started within three days in fishes treated with 30 ppm and five days in fishes treated with 20 ppm of tetracycline. It was seen that in fishes naturally suffering from furunculosis, 30% mortality occurred after eight days and 20% after 15 days using 10 ppm concentration of tetracycline. A total of 40% mortality was observed after eight days and 20% after 15 days of treatment of tetracycline, using 20 ppm concentration. After using 30 ppm concentration of tetracycline, 30% mortality was observed after eight days and 20% after 15 days. Complete healing of fishes was observed after ten days in

fishes treated with 10 ppm concentration and five days in the case of 30 ppm treated fishes (Table 4).

In the case of artificially induced furunculosis, it was observed that 40% mortality took place after eight days and 30% after 15 days in fishes treated with 20 ppm of tetracycline. A total of 20% mortality after eight days and 10% mortality after 15 days was observed in fishes treated with 30 ppm of tetracycline. The healing started after five days in fishes treated with 30 ppm and after 12 days in fishes treated with 20 ppm of tetracycline (Table 5).

Streptomycin was used in three concentrations, *viz.,* 10, 20 and 30 ppm, for 25 days. The water along with the chemical was changed after every five days during the experiment. Healing started within five days in fishes treated with 30 ppm and ten days in fishes treated with 20 ppm of streptomycin. It was observed that in fishes naturally suffering from furunculosis, 30% mortality took place after eight days and 10% after 15 days using 10 ppm concentration of streptomycin. A total of 20% mortality was observed after eight days and 10% after 15 days of treatment of streptomycin, using 20 ppm concentration. After using 30 ppm concentration of streptomycin, 10% mortality was observed after eight days, while no mortality was recorded after 15 days. Complete healing of fishes was observed after ten days in fishes treated with 10 ppm concentration and five days in the case of 30 ppm treated fishes (Table 4).

In the case of artificially induced furunculosis, it was observed that 30% mortality took place after eight days and 20% after 15 days in fishes treated with 20 ppm of streptomycin. A total of 10% mortality after eight days and no mortality after 15 days was observed in fishes treated with 30 ppm concentration of streptomycin. The healing started after five days in fishes treated with 30 ppm and after 12 days in fishes treated with 20 ppm of streptomycin (Table 5).

In the case of control, the condition of the fishes improved after ten days of transferring them into clean water. It was also observed that 20% mortality took place after ten days.

DISCUSSION

The naturally infected fishes suffering from furunculosis showed the symptoms such as open ulcers on the back and sides of the body. The boils arise from deep-lying haemorrhagic muscle necrosis which became visible initially, as clear,

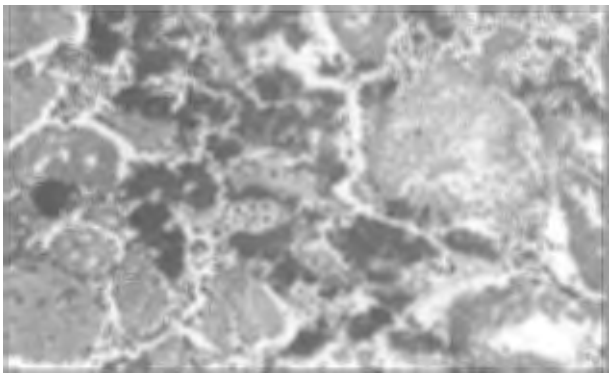

Fig. 3: Infected musculature: extensive bacterial multiplication, necrosis of musculature, haemorrhages and macrophage infiltration, and bacterial phagocytosis (HE x 100)

slightly raised, occasionally dark coloured boils, which later burst open and discharged the highly infectious contents including debris, erythrocytes and bacteria (Fig. 3-6). In some specimens,

haemorrhagic form of furunculosis was also observed which was characterized by

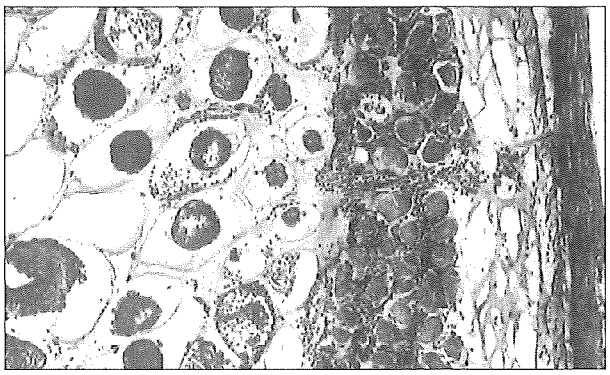

Fig. 4: Protusive lesion: bacteria extensively invade the dermis, subcutaneous adipose tissue and lateral musculature; infected tissues undergo necrosis, oedema and haemorrhages

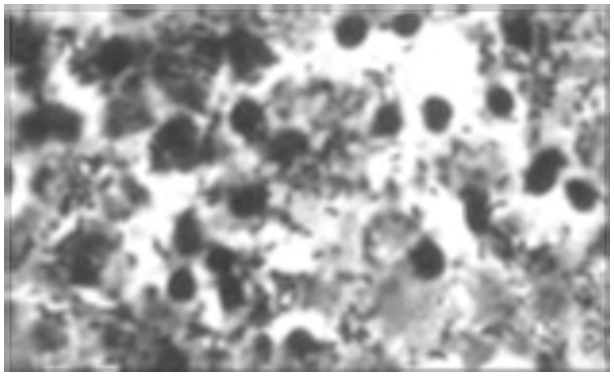

Fig. 5: View of spleen: bacterial multiplication and necrosis are extensive (GS x 800)

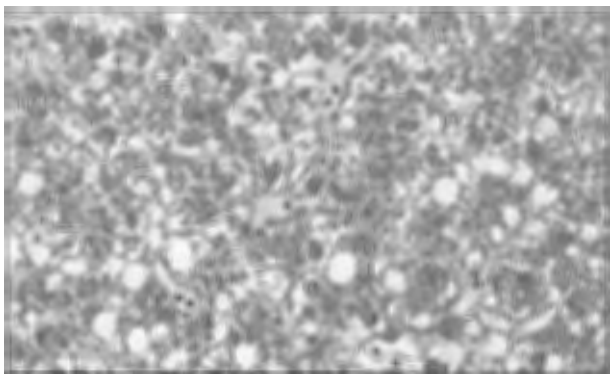

Fig. 6: Liver: all haepatocytes often undergo hyaline droplet degeneration (HE x 320)

punctiform bleeding and petechiae in the viscera, especially the liver, and also on the skin, gills, musculature, and the bases of fins. In the kidney, besides bleeding, degenerative changes and necrosis of haematopoietic connective tissue were found. In the case of intestinal furunculosis, the intestine was deep red with enlarged blood vessels. McCraw (1952), Herman (1968), Shieh and McLean (1975), Fuller *et al.* (1977), Cummings *et al.* (2000) and Andrew *et al.* (2003) also reported the same symptoms in furunculosis affected fishes.

A. salmonicida when cultured on TSA, grow as colonies surrounded by a dark-brown water-soluble pigment after incubation at 20-25°C for 3-4 days. A more recent addition to the media employed for the isolation of *A. salmonicida* is CBB, as developed originally by Udey (1982). CBB was evaluated as a differential and presumptive medium for use in the identification of *A. salmonicida* in clinical specimens (Markwardt *et al.*, 1989). The results showed that CBB is effective in differentiating *A. salmonicida* colonies from mixed bacterial population obtained from asymptomatic fish. In this case, *A. salmonicida* colonies are dark blue. *A. salmonicida* is a non-motile, non-flagellated and gram-negative bacterium. It produces a brown diffusible pigment, and forms acid and gas from glucose and other carbohydrates. Griffin *et al.* (1953) found optimal growth of *A. salmonicida* in nutrient broth at 20-22°C with maximum and minimum tolerances at 34.5° and 6.0°C, respectively.

The inoculation of *A. salmonicida* developed pathogenicity and caused symptoms of furunculosis within 48 hours of inoculation of culture filtrate. This finding conforms to the earlier ones

reporting the occurrence of lesions on head to dorsum with less mortality after inoculation of 1.2 x 10^9 cells of *A. salmonicida* into *Oncorhynchus kisutch*. It was observed that fishes injected with culture filtrates show symptoms of furunculosis such as open ulcers on the back and sides of the body as slightly raised, dark coloured boils, punctiform bleeding and petechiae in viscera, liver, skin, gills, musculature and bases of fins. The LD_{50} values of *A. salmonicida* inoculated were 3.85 x 10^3 and 4.90 x 10^3 cells per millilitre of *S. niger* and *S. esocinus,* respectively. Among the fishes tested, *S. niger* was found to be more susceptible to *A. salmonicida* than *S. esocinus*. Chi-square values were found to be significant in experimental fishes as compared to the control ones.

During an outbreak of acute furunculosis, one-year old *Salmo salar* showed marked changes in haematocrit and haemoglobin values. According to Foda (1973), the haematocrit value (%) dropped from the normal 44.5±3.5 to 18.8±2.89 in moribund fish and further to 11.0 ± 3.5 in the freshly dead fish. The haemoglobin content (g%) dropped just as drastically from 9.5 ± 0.86 to 4.3 ± 0.39 and further to 2.7 ± 0.73, respectively. These conditions were caused by the severe external and internal haemorrhages and not by the effects of toxins on erythrocytes, since no haemolysis could be observed in clear plasma in the supernatant centrifuged matter. The blood of the fish investigated was of low viscosity and had a very high erythrocyte sedimentation rate (ESR), an observation which is in agreement with result obtained by Schumacher *et al.* (1956). These authors found a significantly accelerated ESR of x14.8 mm/h (10-28 mm) in *Salvelinus fontinalis* suffering from furunculosis compared to x 5.5 mm/h (4-8 mm) in healthy specimens. Haematological investigations of diseased *S. niger* suffering from furunculosis revealed substantial decrease in haemoglobin (2.92±0.42), erythrocytes (0.49±1.72), haematocrit value (16.43±0.05), blood sugar (25.80±1.56), total albumin (1.82±0.15), albumin (0.21±1.09), globulin (0.43±0.67), total cholesterol (60.2±1.31), bilirubin (0.63±0.02) and MCH (17.77±0.05), while a substantial increase was observed in uric acid (2.68±0.01), glucose (37.8±5.25), PCV (104.4±1.21), MCV (422.8±2.12), MCHC (59.59±1.3) and leukocytes (120.3±1.1x10^3).

In vivo investigations of drugs and chemicals as therapeutic agents against furunculosis were carried out using five drugs and three chemicals. It was observed that ciprofloxacin was most effective against furunculosis, which resulted in no mortality after 15 days and 20 days of treatment of 15 ppm and 20 ppm of oxytetracycline, respectively. The treatment of 20 ppm concentration of chloramphenicol, oxytetracycline and streptomycin revealed no mortality after 20 days of application. However, tetracycline was found to be moderately effective leading to 20-30% mortality after 20 days of treatment at 30 ppm concentration. Out of three chemicals, methylene blue was observed to be most effective leading to no mortality after 20 days of treatment of 10 ppm concentration. However, 20% mortality was observed after 15 days of treatment. Potassium permanganate was ineffective

leading to 20% mortality after 15 and 20 days of treatment. However, malachite green was observed to lead to only 10% mortality after 20 days of treatment.

In the case of fishes artificially challenged with furunculosis, only oxytetracycline and streptomycin proved to be very effective leading to no mortality after 20 days of treatment at 30 ppm of the drugs. Chloramphenicol and tetracycline were moderately effective leading to 10% mortality after 20 days of treatment at a concentration of 30 ppm. Ciprofloxacin was observed to be least effective leading to 30 and 20% mortality after 15 and 20 days, respectively, of treatment at 30 ppm concentration. In the case of chemicals, more or less similar response was observed leading to only 10% mortality after 20 days of treatment at 10 ppm concentration of potassium permanganate, malachite green and methylene blue. However, out of the three chemicals, potassium permanganate was found to be most effective. The present result lends support to the work of Gutsell (1946), Barnes *et al.* (1991), Nordmo *et al.* (1994), Cipriano *et al.* (1996), Samuelsen *et al.* (1998), Ladd *et al.* (2001) and Dunaway *et al.* (2002).

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