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EMBRYOTOXICITY OF MALATHION IN DEVELOPING CHICK

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Abstract: Malathion, an organophosphorous insecticide, was tested for embryotoxic and teratogenic effects in developing chick. Different aquous concentrations of the insecticide (5.0, 10.0, 15.0, 20.0 µg/egg), were injected in yolk sac of he eggs before incubation. Embryo recoveries were made at day 7 and 14 of incubation.

At day 7, morphological, anatomical and morphometric studies revealed concentration dependent adverse effects of the insecticide. The developmental defects were significant reduction in CR length and body weight, hydroencephaly, microcephaly, microphthalmia, anophthalmia, short beak, micromelia, Amelia and ectopia cordis. Developmental anomalies in 14 days embryos included dawarfism, undistinguishable brain parts, microphthalmia, amelia, meningocoele and ectopia cordis.

The present study indicates that Malathion is potentially dangerous to avian development. This indicates that insecticides must be used with utmost care.

Key words: Malathion, insecticide, embryotoxic, teratogenic, avian development.

INTRODUCTION

Pollution is the major problem of modern age. It has increased various hazards, which are severely damaging the living conditions of almost all the organisms. Amongst many other factors causing pollution insecticides contribute a lot. The insecticides of course have increased the agro-production possibilities but environmental and health side effects of their use have rendered differences for living creatures. (Zilberman and Siebert, 1990). Worldwide productions of these insecticides continue to rise with a ten-fold increase in production between 1955 and 1985 (Rosenstock *et al.*, 1991).

Organophosphate are generally amongst the most acutely toxic of all pesticide to vertebrate animals. Using all relevant federal data on food consumption and pesticide residue on food, the environment working group concluded that 9 of 10 American children of age 6 months to 5 years ingest organophosphate insecticide in their food each day (Wiles *et al.*, 1998). Dinham (1993) revealed that there are in total 3 million acute sever cases of pesticides poisonings, of which a large proportion involves organophosphates. Organophosphate applicators had significantly more dizziness, sleepiness, headache and higher neurological symptoms scores than non-applicators (London *et al.*, 1998). Wide range of neuropsychological test, including memory, attention, problem solving and dexterity (Rosenstock *et al.*, 1991). In 1995, in Chine 15,300 pesticide poisoning cases

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were caused, including organophosphates i.e., parathion, methamidophos and diethoate (Shayang and Peipei, 1996).

Organophosphate insecticides exert their acute effect in both insects and mammals by inhibiting acetyl cholinesterase (AChE) in nervous system with subsequent accumulation of toxic level of acetylcholine (ACh), which is neurotransmitter (WHO, 1986).

Malathion is slightly toxic via the oral route, with reported oral LD_{50} values of 1000 mg/kg in the rat, and 400 mg/kg in the mouse (Gallo and Lawryk, 1991; Kidds and James, 1991). It is also slightly toxic via the dermal route, with reported dermal LD_{50} values of greater than 4000 mg/kg in rats (Gallo and Lawryk, 1991; Kidd and James, 1991). Effects of malathion are similar to those observed with other organophosphates, except that larger doses are required to produce them (Gallo and Lawryk, 1991). It has been reported that single doses of malathion may affect immune system response (Gallo and Lawryk, 1991).

Several studies have documented developmental and reproductive effects due to high doses of malathion in test animals (Gallo and Lawryk, 1991). Rats fed high doses of 240 mg/kg/day during pregnancy showed an increased rate of newborn mortality.

Above mentioned studies have indicated that organophosphate insecticides are toxic for non-target organisms and may also be embryotoxic and teratogenic. Thus the present study was planned to evaluate the embryotoxic and teratogenic potential of malathion in developing chicks.

RESULTS AND DISCUSSION

The purpose of present study was to evaluate the developmental toxic effects of an organophosphate insecticide Malathion in chick embryos. The main observations made during the present investigation revealed embryotoxicity and teratogenicity of malathion, injected in chick eggs before incubation.

The developmental defects, including increased embryo lethality, significant ($P<0.001$) reduction in CR length and body weight, hydroencephaly, microcephaly, eye defects, short beak agenesis of beak, twisted spinal cord, micromelia, Amelia and ectopia cordis were found almost in all dose groups (Fog 1 and 2) (Table 1 and 2).

During a study malathion was found to cause DNA abnormalities at all doses rested in human blood cells. Blood samples were drawn from healthy non-smoking men age 23-25. Four different concentrations (0.02, 0.2. 2,20 μg/ml) were added to blood samples. All doses showed chromosome abnormalities. A significant increase was noted for doses 2 and 20 μg/ml (Smith, 1993).

Malathion, Endosulfan, Carbafuran were tested for their toxicity in chicks. The increased level of all the pesticides in feed, with effect from 5^{th} week, showed a certain decrease in feed consumption. Feed conversion efficiency was reduced in Malathion group. When leukocytes response was studied in chick at 4^{th} to 9^{th} weeks of age after

feeding insecticides contaminated feed from the day of hatching absolute heterophilia, eosinophilia, monocytosis and lymphocytopenia were observed (Deshmukh *et al.*, 1991).

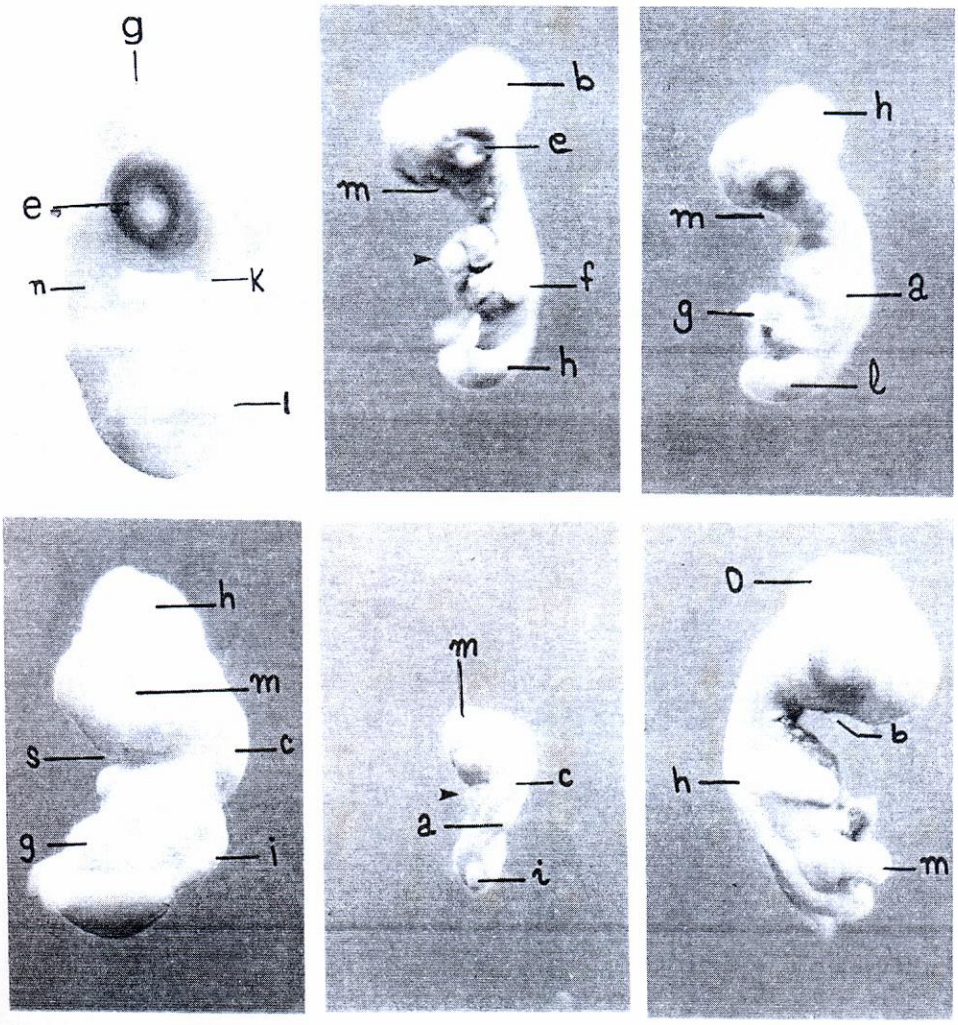

Fig 1: Macrophotographs of 7 days embryos recovered from different dose groups of Malathion. (A) control group embryo, with normal development. (B-F) embryos from 0.025, 0.25, 0.5, 1.0 and 2.0 g/egg dose group, respectively. Note: Developmental anomalies including micromelia (a), mesomelia (i), agenesis of beak (m), gastroschisis (g), hydroencephaly (h), meningomyocod (mn), anophthalmia (mo), microphthalmia (e) and ectopia cordis (arrow head).

In another study by Ooi *et al.* (1991) malathion, mathoxytryptamine and ethoxytrytophol were injected into yolk sac of chick at 4th day of incubation. Abnormalities including twisted vertebral column, abdominal hernia, exteriorizations of heart and viscera, defect of eye, beak and limb were found.

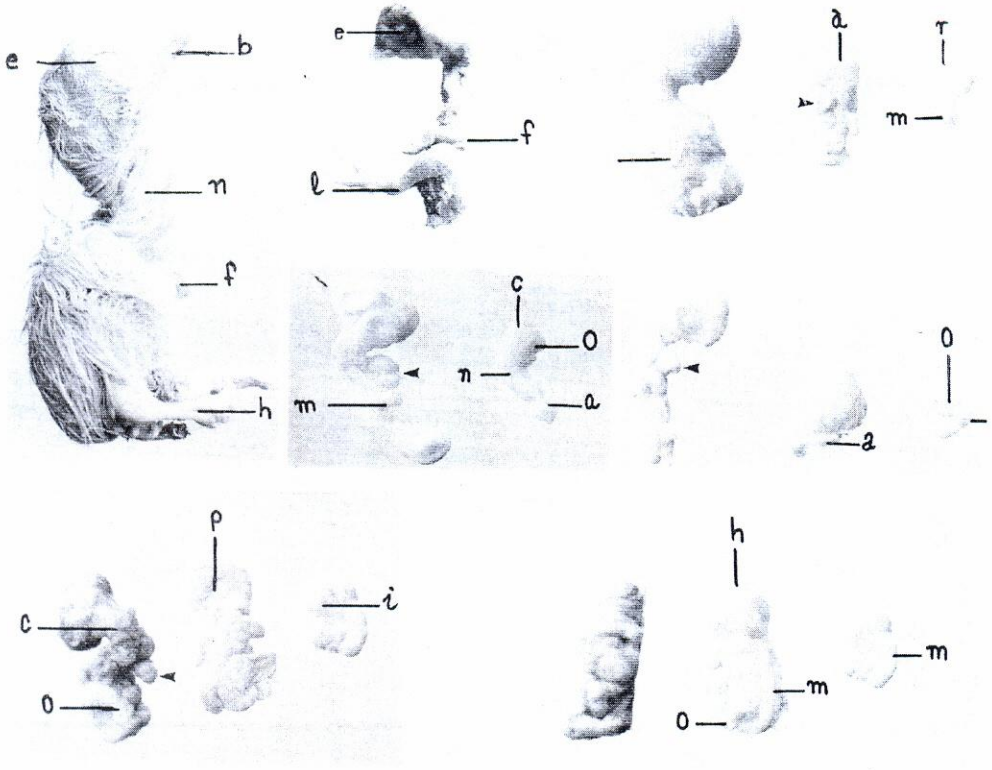

Fig 2: Macrophotographs of chick fetuses recovered, at day 14 of incubation, from different dose groups of malathion. (A) control group fetus (B-C) from 0.025 g/egg dose group; (D-G) from 0.25, 0.5, 1.0 and 2.0 g/egg dose groups, respectively. Note developmental defects including anencephaly (a), microcephaly (c), deformed neck (d) Amelia (m), micromelia (o), anophthalmia (ao), microphthalmia (e) meningomyocoel (mn) and ectopia cordis (arrow head).

Table I: Embryotoxic effects of different concentrations of Malathion in 7-day old chick embryos.

Dose (Mg/egg)	Resorbed (%)	CR Length mm ± S.D	Weight Mg ± S.D	Head	Beak	Eyes	Fore-limb	Hind-limb	Cardiac Position
0.00	0.00	19.35 ± 1.09 n = 20	600 ± 12	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)
5.00	600	16.25 ± 2.02** n = 20	320 ± 18***	Microcephaly (50) Hydroencephaly (25)	Agenesis of beak (30.00) Slightly formed (55.00)	Microphthalmia (40.00)	Phocomelia (30.00) Amelia (40.00)	Amelia (10.00)	Ectopia cordis (85)
10.00	60	14.70 ± 4.59** n = 20	310 ± 12***	Microcephaly (65) Hydroencephaly (20)	Slightly formed (15.00) agenesis of beak (50)	Samll (35.00) Microphthalmia (15.00) Monopia (20)	Micromelia (90)	Amelia (50) Micromelia (50)	Ectopia cordis (80)
15.00	70	10.41 ± 0.67*** n = 20	90 ± 2***	Microcephaly (75) Hydroencephaly (20)	Agenesis of Beak (100)	Small (75.14) Very small (42.85)	Phocomelia (20) Micromelia (60) Amelia ((20)	Micromelia (100)	Ectopia cordis (90)
20.00	70.00	10.20 ± 1.80*** n = 20	40 ± 2***	Microcephaly (75) Hydroencephaly (25)	Agenesis of Beak (100)	Anophthalmia (30.0) Microphthalmia (16.66) (70.00)	Micromelia (40.00) Amelia (60.00)	Micromelia (70.00) Amelia (30.00)	Ectopia cordis (90)

Table I **Developmental anomalies induced by different concentrations of Malathion in 14-day chick embryos, injected before incubation.**

Dose (Mg/egg)	Resorbed (%)	CR Length mm ± S.D	Body weight Mg ± S.D	Head	Beak	Eyes	Fore-limb	Hind-limb	Cardiac Position
0.00	0.00	42.75 ± 2.22 n = 20	9.55 ± 0.16	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)
5.00	40.00	18.87 ± 1.29*** n = 20	2.79 ± 0.14***	Microcephaly (65) Hydrocephaly (25)	Small Beak (25) agenesis of beak (75)	One eye is small (25) microphthalmia (75)	Short wing (25)	Micromelia (100)	Ectopia cordis (75)
10.00	40.00	18.50 ± 0.91*** n = 20	1.55 ± 1.17***	Microcephaly (75) Hydroencephaly (25)	Agenesis of beak (1000)	Microphthalmia (75)	Micromelia (75) Amelia (25)	Micromelia (100)	Ectopia cordis (80)
15.00	45.00	13.50 ± 0.82*** n = 20	0.81 ± 0.59***	Microcephaly (100)	Small (20) Agenesis of Beak (100)	Microphthalmia (80)	Amelia (40) Micromelia (600)	Micromelia (100)	Ectopia cordis (90)
20.00	60.00	10.75 ± 0.58*** n = 20	0.79 ± 0.38***	Microcephaly (100)	Agenesis of Beak (100)	Microphthalmia (80)	Amelia (40) Micromelia (60)	Micromelia (100)	Ectopia cordis (90)

Malathioni *et al.* (1997) have categorized a whole set of abnormalities encountered in chick embryos following Malathion treatment. Abnormalities such as micromelia, dwarfism, parrot beak and abnormal feathering, short neck, tibiotarsal arthrogryposis and muscular hypoplasia of the legs. Were commonly observed another set of abnormalities designated as type II Many other studies have also shown embryotoxic and teratogenic effects of different organophosphorus insecticides in chick embryos. (Greenbeeg and Laham, 1969; Gosh *et al.*, 1998; Asmatullah *et al.*, 2002; Asmatullah *et al.*, 2003).

These results and some earlier studies have indicated that Malathion is toxic to embryonic and fatal tissues and can induce teratogenicity in chick.

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TERATOLOGICAL EFFECTS OF CHLORPYRIFOS ON CHICK DEVELOPMENT

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Abstract: Chlorpyrifos, an organophosphate insecticide, was tested for embryotoxic and teratogenic effects in chick. Different aqueous concentrations of chlorpyrifos (2.0, 1.0, 0.5, 0.25 and 0.025 µg/egg) were injected in yolk sac of eggs at day 4 of incubation. Embryo recoveries were made at day 7 of incubation.

At day 7, morphological, anatomical and morphometric studies revealed concentration dependent adverse effects of the insecticide. The developmental defects were reduced in CR length, microcephaly, under-developed eyes, agenesis of beak, micromelia, Amelia, excencephaly and ectopia cordis.

The present study indicates that Chlorpyrifos is potentially dangerous to avian development at every very low doses.

Key words: Chlorpyrifos, embryotoxicity, teratogenicity, avian development.

INTRODUCTION

By controlling agricultural pests, pesticides have contributed to dramatic increase in crop-yields and in the quantity and variety of diet. Organophosphates and carbamates are however toxic to nervous system and caused many cases of acute poisoning (Blondell, 1977).

The pesticides may be rapidly absorbed through skin, lungs or gut producing acute toxic effects, e.g., headaches, dizziness, sweating, nausea, vomiting and difficulty in breathing. In severe cases of over-exposure, coma, respiratory depression and death may occur. Pesticides are responsible for birth defects, genetic mutation, damage to the immune system, reproductive system, deformations, neurological damage, mild cognitive dysfunction, lung damage, dysfunction of the endocrine system, cancer and other health effects (Schwartz *et al.*, 1990; Jeyaratnum, 1990; Arm *et al.*, 1993; Faustman *et al.*, 2000).

Each year, over 1 million people are poisoned by pesticides, with 20,000 deaths (Jeyaratnum, 1990). Distribution of toxic waste dump is associated with highest breast cancer mortality and birth defects. Approximately 250,000 U.S. children are born each

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year with birth-defects diagnosed at or shortly after birth. Birth defects are the leading cause of infant mortality (Croen *et al.*, 1997).

Women exposed to pesticides through agricultural or floricultural work, have been documented to have significantly higher risk of children born with musculoskeletal defects, limb defects, growth retardation and learning behavioral disorders (Faustman *et al.*, 2000; Schwartz *et al.*, 1990).

People are also exposed to organophosphates (e.g., diazinon, chlorpyrifos, malathion etc.) through food residues (e.g., fruits, vegetables, grains, fish and milk) and from a survey made by Wiles and Compbell (1993), it becomes clear that all the fruit, vegetables of daily use e.g., apple, banana, grapes, orange, potato, carrot etc., contain significant amount of pesticides. Malathion (75%), Chlorpyrifos (38%) and p,p, DDE (21%) were detected in at least 20% of all food samples. This is because organophosphorous insecticides are rapidly absorbed by the plants through roots or directly through leaves and fruits (Ware, 1986).

Chlorpyrifos CPF) is used to control mosquitoes and house-hold insects. In man, it has toxic effects on the central nervous system, the cardiovascular system and the respiratory system. A study investigated the toxicity of CPF on nervous system development in rat embryo using the mid-brain micromass culture system. All demonstrated toxicity in mid-brain micromass cells with IC sub (50) values below 30 ng/mL indicating a potent teratogen (Cosenza and Biodanset, 1995).

The use of chlorpyrifos to area of fish breeding causes toxicological hazards especially in view of its highest toxicity to fish. Several investigations have reported the toxicity and histopathological changes in the gills and kidneys of fish after chlorpyrifos treatment (Srivastava *et al.*, 1997).

Chlorpyrifos which is most numerous in home's atmosphere and children are its definite and ultimate target, because of their behavior, diet etc. Thus, the present study was planned to evaluate the embryotoxic and teratogenic potential of CPF in developing chicks.

MATERIALS AND METHODS

Fresh eggs (of White leghorn breed) were purchased from Veterinary Research Institute, Lahore. The eggs were divided randomly (irrespective of their size, shape and colour) into 6 groups. Of them, 5 groups were treated with different concentrations (0.025, 0.25, 0.5, 1.0 and 2.0 µg/egg). The other group was control without any treatment.

Chlorpyrifos was available in market in liquid form with trade name, Terminus-T.C.L.O. (Jia Ji Co. Ltd., Pakistan).

The eggs were cleaned with a piece of cotton soaked in alcohol. A small window was made in the shell of each egg except 'C' group eggs, with the help of a sterilized scalpel, provided shell membrane was not ruptured. The aqueous solution of Chlorpyrifos (0.1 ml) was injected into the yolk sac to eggs of respective groups with micro-applicator (glass syringe). All concentrations were applied before incubation. The eggs were incubated at standard conditions of chick egg incubation.

Embryonic recoveries were made at 7^{th} day of incubation. Embryos were fixed in Bouin's fixative for 48 hours. After that embryos were washed in 70% alcohol till they became clear and were finally preserved in 80% alcohol for morphological studies.

Morphological observations involved measurements of crown-rump length as well as gross anatomical observations. These observations included the studies of development conditions of brain, eyes, ear, heart, limbs and beak.

These organs were studied with the help of magnifying lens and with naked eye depending upon the size of the embryo. The observation data were tabulated and analyzed for comparison of development.

RESULTS AND DISCUSSION

The observations made during this study clearly showed the embryotoxic and teratogenic potential of chlorpyrifos. A significant ($P<0.001$) decrease in CR length was noted in all dose groups (Table I). In all above groups a high percentage of developmental abnormalities was studied. Developmental anomalies include agenesis of beak, microcephaly, microphthalmia, anophthalmia, micromelia and twisted spinal cord (Fig.1b-g).

Chlorpyrifos is a cholinesterase inhibiting organophosphate pesticide used extensively to treat crops and domestic animals. Cohn and Macphail (1997) determined the effects of acute and repeated CPF exposure on the acquisition and performance of response sequence. Radiometric analysis of serum cholinesterase activity showed CPF produced 90% inhibition at 3 hr and 85% inhibition at 24 hr post exposure.

In a set of experiments, Bagchi *et al.* (1995) determined the *in vitro* effects of CPF on DNA-SSB (Single Stand Break) and enhanced lactate dehydrogenase leakage (LDH) from neuroactive DC-12 cells in culture. In treatment of rats with chlorpyrifos increase of 4.3 folds was observed in hepatic lipid peroxidation and increase of 3.0 fold in

hepatic DNA-SSB, increase of 1.4 fold in brain nuclear DNA-SSB. Increase of 4.9 fold was observed in chemiluminescence, following *in vitro* incubation of the liver homogenates with chlorpyrifos and 3.1 fold in LDH leakage.

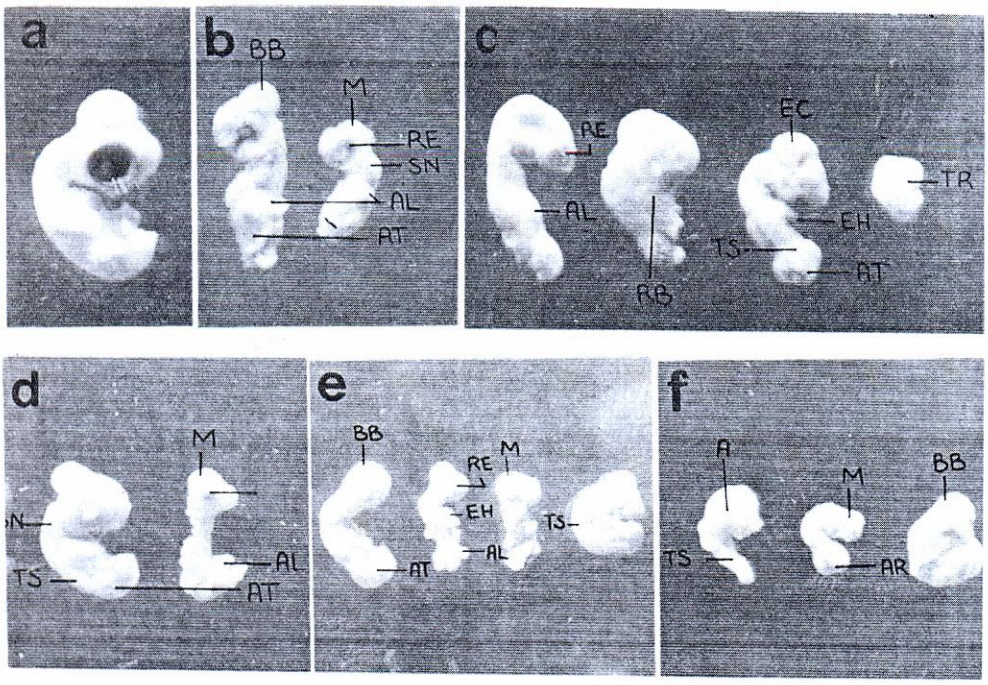

Fig. 1: Macrophotographs of embryos recovered on day 7 of incubation a) control, b) 0.025 µg/egg, c) 0.25 µg/egg, d) 0.5 µg/egg, e) 1.0 µg/egg and f) 2.0 µg/egg. Note, developmental defects caused by the insecticide in treated groups of eggs: Anophthalmia (A), micromelia (AL), abnormal rump (AR), abnormal trunk (AT), abnormal brain bulges (BB), exencephaly (EC), ectopia cordis (EH), microcephaly (M), short neck (N), resorption of limb (RB), microphthalmia (RE), twisted neck (SN), total resorption (TR) and twisted spinal cord (TS).

So, it became obvious that chlorpyrifos induces production of reactive oxygen species and oxidative tissue damage which may contribute to the toxic manifestation of programmed cell death (apoptosis) in response to many toxicants including chlorpyrifos.

Chlorpyrifos was evaluated for potential to produce developmental and reproductive toxicity in rats following oral exposure. Maternal effects noted at the two higher dose levels including decreased cholinesterase levels at 3.0 mg/kg per day and cholinergic signs (extensive salivation and tremors), decreased cholinesterase levels and decreased body weights.

Table I: Developmental anomalies induced by different concentrations of chlorpyrifos in chick embryos recovered on day 7 of incubation.

Doses (mg/egg)	CR length (mm±SD)	Beak	Eyes	Neck	Fore-limbs	Hind-limbs	Head	Spinal Cord
0.00	16.7±1.20 (20)	Normal	Normal closed	Well developed	Well developed	Well developed	Normal	Normal
0.025	12.8±4.37** (30)	Agenesis (100)	Microphthalmia (100)	Small (100)	Micromelia (100)	Micromelia (100)	Abnormal brain bulges (50) Microcephaly (50)	Twisted (100)
0.25	12.3±1.30** (30)	Agenesis (100)	Microphthalmia (100)	Small (100)	Micromelia	Micromelia	Abnormal brain bulges (100)	Twisted (100)
0.5	11.06±0.94*** (30	Agenesis (100)	Microphthalmia (100)	Small (100)	Micromelia	Amelia (50) Reduced (50)	Abnormal brain bulges (50) Microcephaly (50)	Twisted (100)
1.00	9.59±2.24** (30)	Agenesis (100)	Anophthalmia (50)	Small (100)	Micromelia	Micromelia (100)	Microcephaly (100)	Twisted (100)
2.0	7.17±1.26***	Agenesis (100)	Anophthalmia (100)	Not different-tiated (100)	Amelia (100)	Amelia (100)	Anencephaly (50) Microcephaly (50)	Twisted (100)

Significantly reduced against the controls; ** = P<0.01; *** = P<0.001.

Parental effects included decreased plasma and erythrocyte cholinesterase at 1.0 mg/kg/day and decreased plasma, erythrocyte and brain cholinesterase and histopathological alteration of the adrenal zone fasiculate at 5.0 mg/kg/day. Effects on neonatal growth and survival were observed at a maternally toxic dose level in one generation. However, this effect was not observed in the subsequent generation (Breslin *et al.*, 1996).

The mechanism of chlorpyrifos induced neurotoxicity was studied by Song *et al.* (1997) who found that chlorpyrifos evoked deficits in multiple components of the adenylcyclase cascade in brain cells, a system that mediate cholinergic as well as adrenergic signals.

Slotkin (1999) reported that the chlorpyrifos inhibits DNA synthesis *in vitro* in cultures of fetal rat neurons, additionally, cell replication is inhibited, cell acquisition is arrested and neurotoxic apoptosis is accelerated.

Developing mammals are also markedly more sensitive to acute toxicity from exposure to a variety of organophosphorous pesticides. Brain and plasma cholinesterase activity in neonatal and adult rats exposed to sublethal doses of chlorpyrifos showed that the correlation between rats was high but lower in adults (Pope and Chakraborti, 1992; Chanda and Pope, 1996; Chanda *et al.*, 1997).

Chlorpyrifos may exhibit developmental toxicity to the fetal nervous system at relatively low doses. A study by Whitney *et al.* (1995) revealed that administration of chlorpyrifos to neonatal rats at 1 day of age (approximately equivalent to human fetal exposure at 7 months of gestation) produced significant inhibition of DNA and protein synthesis throughout brain. The author interpreted these results as indicating that low doses of chlorpyrifos target the developing brain during the critical period in which cell division is occurring, effects which may produce eventual cellular, synaptic and behavioural aberrations after repeated or prolonged sub-toxic exposure.

Similarly, another recent study found that repeated exposure of pregnant rats to low doses of chlorpyrifos result in long-term neurochemical and behavioural deficits in the offspring (Slotkin, 1999).

These results and some earlier studies have indicated that chlorpyrifos is toxic to embryonic and fatal tissues and can induce teratogenicity in chick. So, it may be used under extreme necessity and great care.

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DISTRIBUTION, FOOD AND HABITAT PREFERENCES OF SMALL MAMMALS IN MACHIARA NATIONAL PARK, DISTRICT MUZAFFARABAD, AZAD KASHMIR, PAKISTAN

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Abstract: First detailed study in Machiara National Park was conducted from April 2003 to August 2003 to determine distribution, food and habitat preferences of small mammals. During this study nineteen species of small mammals, representing five orders, eleven families and eighteen genera, were recorded. Among these six species (*Vulpes vulpes, Prionailurus bengalensis, Paguma larvata, Martes flavigula, Mustela erminea and Herpestes javanicus*) were carnivores, nine (*Hystrix indica, Petaurista petaurista, Hylopetes fimbriatus, Rattus rattus, Rattus turkestanicus, Apodemus rusiges, Mus musculus, Alticola roylei and Hyperacrius wynnei*) were rodents, and two (*Lepus capensis and Ochotona roylei*) were lagomorphs. House shrew (*Suncus murinus*) and Horseshoe bat (*Rhinolophus ferrumequinum*) represented the orders insectivora and chiroptera, respectively. The other species like *Martes flavigula, Suncus murinus, Rattus turkestanicus, Apodemus rusiges* and *Alticola roylei*, were uncommon. Similarly, *Paguma larvata, Hylopetes fimbriatus, Lepus capensis* and *Ochotona roylei* were considered as vulnerable species in this area. *Vulpes vulpes, Hystrix indica, Rattus rattus, Mus musculus, Hyperacrius wynnei* and *Rhinophus ferrumequinum* were common and widely distributed species. These mammals were studied and trapped from different major habitats including villages (houses and agriculture lands), mixed temperate forests, sub alpine scrub forests and alpine meadows and their food habit was also determined in different localities. Human related activities inside the Park area, were the major threats to the small mammals of study area.

Key words: Machiara National Park, small mammals, forests.

INTRODUCTION

The State of Azad Jammu and Kashmir is located in the lower hills of Himalayas. It lies between 73°-24′E to 73°-75′E longitude and 33°-36′N to 35°-07′N latitude in the Sub-continent. The territory of the State is encircled from the north by Northern Areas, from the west by North West Frontier Province (NWFP), from the south by the Punjab and from the east by the Line of Control (Anonymous, 1996). It covers an area of 5,134 sq. miles (13,297sq.kilometers; 1,229,729 hectares). Elevation

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from sea level ranges from 902 feet in the south (Manawar) to 20750 feet in the north (Shonter top) (Anonymous, 2002).

Woods and Kilpatrick (1997) and other authors have described the biodiversity of small mammals in the mountain ranges of Pakistan. They have discussed the distribution of these animals in the Northern Areas which are similar (in climatic conditions, vegetations and topography) to the Muzaffarabad, Azad Jammu and Kashmir. They include Murree Hills, Kaghan Valley and adjacent area.

Red Fox *(Vulpes vulpes griffithi)* occurs throughout the Northern hemisphere (Ables, 1975), in Himalayas, Africa, northern and western areas of Pakistan including mountains and intermountainous valleys of Bluchistan, North West Frontier Province (NWFP) and Himalayas (Woods *et al.,* 1997; Roberts, 1997; Farooq and Ghalib, 1979), usually found in close association with human population (Roberts, 1997). Leopard Cat *(Prionailurus bengalensis)* is a globally endangered species and is distributed all over the Southern and Central Asia (Prater, 1965), some parts of Pakistan and Northern Areas including Azad Kashmir (Roberts, 1997 and Farooq and Ghalib, 1979), inhabiting Himalayan moist temperate forests (Roberts, 1997). Yellow Throated Marten *(Martes flavigula)* is found in India, Nepal, Bangladesh, Bhutan, Burma, South China, Malaysia, Taiwan, Korea and Eastern Siberia (Pocock, 1941; Prater, 1965). In Pakistan its distribution is confined to Murree Hills, Margalla Hills, Attock Distt., Hazara, Dir, Swat, Chitral districts ,Gilgit, Chilas districts and Neelum Valley of Azad Kashmir (Roberts, 1997; Woods and Kilpatrick, 1997 and Qurashi, 2000).

Masked Palm Civet *(Paguma larvata)* is distributed throughout the Himalayas to Assam Hills (Roberts, 1997). It has been reported in different forest areas of Pakistan including Murree and Neelum Valley of Azad Kashmir (Farooq and Ghalib, 1979; Woods and Kilpatrick, 1997 and Roberts, 1997), usually lives in forests and bushy lands (Roberts, 1997). Stout or Ermine *(Mustela erminea)* is found in Europe, Russia, North America, Canada (Jone et al., 1988; Baker and Rollin, 1983) Pakistan, including Northern Areas, Chitral, Kaghan, Kohistan and Azad Kashmir (Woods and Kilpatrick, 1997; Roberts, 1997). It prefers underground burrows and rocks of woodlands and open areas of alpine meadows (Wilson and Reeder, 1993). Small Indian mongoose (*Herpestes javanicus*) is distributed in South East Asia, from Pakistan to south coast of China, Malaysia, Java, Iran, Afghanistan, India, Assam, Burma and Thailand. (Nellis and Everad, 1983; Turtkovic and Krystufek 1990; Ogura *et al.*, 1998 and Roberts, 1997). Porcupine *(Hystrix indica)* is distributed throughout Southern and Central Asia, Middle East, India, South Asia (Prater, 1965; Ellerman, 1961; Gurung and Singh, 1996). In Pakistan it is found in desert areas and Himalayan moist temperate deciduous forests including Northern Areas and Azad Kashmir (Roberts 1997; Woods and Kilpatrick, 1997; Mian *et al.*, 1988 and Farooq and Ghalib, 1979).

Indian Giant Red Flying Squirrel *(Petaurista petaurista)* ranges from the eastern border regions of Afghanistan to Java including Kashmir, Taiwan, Southern China to Srilanka and in northern forest regions of Pakistan (Parker, 1990; Nowak, 1991 and Roberts, 1997). Its distribution is restricted in Murree Hills, Margala Hills, Hazara, Donga gali, Shogran, Naran and Neelum Valley of Azad Jammu and Kashmir (Roberts, 1997; Farooq and Ghalib, 1979). Small Kashmir Flying Squirrel *(Hylopetes fimbriatus)* is distributed in Afghanistan, Northern India and Pakistan

(Nowak, 1991). It is distributed in Chitral, Dir, Swat, Murree Hills, Hazara, Shogran, Kaghan, Naran valleys, Gilgit and throughout Azad Jammu and Kashmir (Farooq and Ghalib, 1979; Roberts, 1997).

Roof Rat or House Rat *(Rattus rattus)* is distributed throughout Pakistan except in the upper Northern Areas where it is replaced by Turkistan Rat *(Rattus turkestanicus)*. It is generally found in close association with human population, mainly in houses, farmhouses and their roofs (Roberts, 1997; Hassan *et al.*, 1997, 1998). Turkistan Rat *(Rattus turkestanicus)* is distributed in the higher mountainous regions of Afghanistan, Russian Turkistan, Xinjiang of China and Iran (Nowak, 1991 and Roberts, 1997). In Pakistan it is present in Murree Hills, Chitral, Dir, Swat, Kohistan, Northern Areas (Woods and Kilpatrick, 1997; Roberts, 1997 and Farooq and Ghalib, 1979).

Himalayan Wood Mouse or Field Mouse *(Apodemus rusiges)* is distributed in Southwestern Asia, Northwestern Africa and Europe (Nowak, 1991). In Pakistan it is mostly distributed in northern hilly areas including Murree Hills, Swat, Chitral, Hazara Distt. and Northern Areas (Wood and Kilpatrick, 1997; Roberts, 1997 and Farooq and Ghalib, 1979) and live in burrows and usually found in Himalayan mixed temperate coniferous forests (Parker, 1990; Nowak, 1991 and Roberts, 1997). House Mouse *(Mus musculus)* is found throughout the world (Hassan *et al.*, 1998 and Roberts, 1997), lives in houses, cultivated fields and may be found in woodlands (Nowak, 1991; Naz *et al.*, 1997).

Royle's High Mountain Vole *(Alticola roylei)* is distributed in Central Asia from Mongolia to the Western Himalayas. In Pakistan it occurs in high mountainous regions and has been reported throughout the Northern Areas (Farooq and Ghalib, 1979 and Roberts, 1997). Murree Vole *(Hyperacrius wynnei)* is one of the uniquely endemic species of Pakistan and is restricted in Murree Hills, Shogran, Kaghan and lower Swat (Roberts, 1997; Woods and Kilpatrick, 1997; Farooq and Ghalib, 1979). Murree Vole is highly fossoreal and excavates complicated food tunnels in forests and alpine pastures (Roberts, 1997).

Royle's Pika or Indian Pika *(Ochotona roylei)* ranges mainly in Nepal, Tibet, Szechuan, Western China and Northern Burma (Nowak, 1991; Orr, 1977 and Smith, 1981). In Pakistan it is reported around Gilgit, Skardu, Baltistan, Chitral, Swat, Kohistan and Naran (Farooq and Ghalib, 1979). Royle's Pika prefers rocky areas and usually nests in stone heaps (Smith, 1981). Cape Hare *(Lepus capensis)* is found in Africa, Middle East and Central Asia (Nowak, 1991), widely distributed in Pakistan, generally associated with agriculture fields, alpine meadows and tropical pine forests (Roberts, 1997).

House Shrew or Musk Shrew *(Suncus murinus)* is distributed throughout the oriental region, reported from Afghanistan, China, Iran, Saudi Arabia, Egypt, Eastern Africa, Southern Japan, Madagascar and other islands of Indian Ocean (Anonymous, 1995). In Pakistan it has been reported from Punjab, Southern Sind, NWFP and foothills of the Himalayas including Azad Kashmir (Saddiqui, 1971; Beg *et al.*, 1986; Farooq and Ghalib, 1979 and Roberts, 1997). Greater Horseshoe Bats *(Rhinolophus ferrumequinum)* are present in India, Afghanistan, Iran, Japan, Korea, Europe (Roberts, 1997) and Pakistan ((Farooq and Ghalib, 1979 and Roberts, 1997).

Study Area

The study area, Machiara National Park is situated in the foot Hills of Himalayas at about 32 km north to Muzaffarabad, on the west bank of the Neelum River, facing slopes of Ganja mountain ranges on south. It lies on latitude 34°-31N and longitude 73°-37E, covering an area of about 13537 ha (33437acres) and elevation between 1300 m and 4733 m. Villages Bheri, Machiara, Konkan and Jhing are linked to Muzaffarabad city by metalled roads via Pattika, which is about 20 km north to Muzaffarabad city. The Park has excellent scenic beauty, panoramic views, towering hills and evergreen forests.

Machiara National Park comprises, Himalayan mixed temperate forests, including Himalayan moist and dry temperate coniferous forests, Sub alpine scrub forests and Alpine pastures/meadows. Dominant plant species include *Cedrus deodara, Pinus wallichiana, Abies pindrow, Picea smithiana, Aesculus indica, Jaglans regia, Prunus padus, Vibernum foetens and V.erubeseens, Pinus roxberghii, Prunus cornuta, Betula utilus, Salix alba, Salix tetrasperma, Jumiperus communis, J. squamata, Lonicera* spp., *Rhodendron lepidotum, Polygonum amplexicaule* and *Bergenia* spp. *Caprex* spp, *Artimisia* spp. and *Medicago* spp etc.

Machiara National Park is situated at the junction of Palearctic and Oriental regions (Saharia, 1982), comprising elements of both the regions. The animal fauna includes Black Bear, Himalayan Ibex, Snow leopard, Hunting leopard, Himalayan Grey goral, Asiatic jackal, House mouse, Himalayan Wood mouse, Horseshoe bat, Leopard cat, Musk shrew (Anonymous, 1991). Murree vole (*Hyperacrius wynnei*) is the only species that is endemic to Pakistan (Roberts, 1997).

In Azad Jammu and Kashmir, so for no detailed study has been conducted on small mammals. The present work in Machiara National Park is preliminary study in Azad Kashmir and is aimed to explore the current distribution, food and habitat preference.

MATERIALS AND METHODS

The present study was conducted from April 2003 to December 2003 in order to determine the current distribution and habitat preference of small mammals in Machiara National Park. For this purpose, the study area was divided into five major localities (Table 1) on the basis of topography and climatic conditions. Each locality was further subdivided into four sub-localities i.e. villages (houses and agriculture lands), mixed temperate forests, sub alpine scrub forests and alpine meadows.

The different methods were adopted to study these animals. Most of the small mammals were nocturnal and shy, so the direct observations and physical trapping of comparatively larger animals was not possible under the available conditions. Thus the indirect evidences, such as footprints, fecal droppings, caves, burrows, beds, runways, fresh ground scratching, signs of predations and other related information were collected to locate and confirm the animal's presence. The useful information about these mammals was obtained from local residents, shepherds, hunters and game watchers of the area. Other small mammals e.g. weasels, pikas, high mountain voles, cape hares and small Indian mongooses were directly observed by using binocular (8X40) during the study

period. Most of the small mammals, like rodents were trapped by using steel snap traps (17X9.5cm) and cage traps (28X16 cm), baited with different objects in different habitats. For this purpose 30-50 traps were set in the randomly selected area of each sub-locality.

Baited traps were set and tied with unmovable objects by using rope of diameter 3mm. The traps were set in the evening and collected in the morning and vice versa. In each selected area trapping was done for two consecutive days and nights (Mian *et al.*, 1988; Knudsen, 1972). After removing the dead and live animals, photographs were taken. The morphometric measurements, geographic locality, date, elevation, soil type, stage (adult, male, female or immature) and dominant vegetations within 20X20 m were collected and identified and preserved by the method describe by Knudsen (1972). Bats were collected with the help of net and preserved in 10% formalin solution. After preservation, each specimen was tagged with a field number. The specimens were identified and classified by using photographs, literature and keys. For this purpose Roberts (1997) was followed.

RESULTS

During this study current distribution, food and habitat preferences of nineteen species of small mammals belonging to five orders, twelve families and eighteen genera were recorded (Table1), which are described below.

Red Fox *(Vulpes vulpes griffithi)* was a larger animal having grayish brown colored hair on the dorsal and somewhat rusty-orange hairs on the ventral sides. it was commonly distributed through out the National Park from elevation1500 m to 3300 m (Table 1), reported to kill poultry near Basri, Gali Khatter and Chathian, where a male Fox was observed producing characteristic voices at evening and another young male was trapped. It has also been reported from Danna, Machiara, Chathian Mohri, Koli, Punjal Gali, Konkan, Panjnand, Serli Sacha, Jing and Balgiran (Table 1). Red Fox prefers deciduous forests around villages, in close association with the human population, resting in burrows and caves and avoiding dense forests. One such burrow was observed under the bushes near Chathian. Its feces were observed containing the hairs of Royle's Pika. wing remains of beetles. It also destroys the chicks and eggs of some valuable game birds e.g. pheasants.

Leopard Cat *(Prionailurus bengalensis)* was rare and endangered, found in all localities of the study area between 2500 m and 4000 m. It was reported around Bheri, Seri, Basri, Gali Khatter, Machiara (around Mohri, Danna), Serli Sacha and Jhugian (Table 1). It prefers Himalayan moist temperate coniferous forests usually around the villages resting between rocks and holes of the trees (Table 1). It mainly preys on rodents, small birds, eggs and chicks of Monal and Koklas pheasant and reported to be hunted for its densely spotted fur.

Palm Civet *(Paguma larvata)* was present through out the study area ranging between 1500 m and 3200 m (Table 1). Feces of palm civet were observed near Bheri (1500 m), Seri (1600 m), Doba (2000 m). This animal was also reported from Chathian,

Gali Khatter, Danna (2700 m), Gahatian, Machiara, Serli Sacha and Jing areas (Table 1). It generally prefered deciduous and moist temperate coniferous forests, resting in dens and holes of trees like *Aesculus indica* and *Juglans regia*. Undigested seeds of *Prunus* spp, *Diospyrus lotus*, *Ficus palmate*, *Pyrus pashia* and *Viburnum* spp were observed in its feces.

Yellow Throated Marten *(Martes flavigula)*, was rare species, found at the elevation range of 2500 m to 4000 m (Table 1). The feces of this animal were observed at Thellan near Basri. Two individuals were seen walking on snow under the dense trees of *Pinus wallichiana* near Chathian at elevation of 2900 m during daytime and it was also reported around Machiara and Serli Sacha. The footprints were observed near Kuthiali at elevation 3500 m. (Table 1). It is usually found in the Himalayan moist temperate coniferous and sub alpine scrub forests and was observed taking rest in the dens and holes of *Aesculus Indica* and *Taxus wallichiana* (Table 1). Its diet mainly consists of small mammals (rodents), fruits, occasionally honey and was also reported to prey on Musk deer, young of Grey goral and squirrels, usually attack bigger animals in pairs.

Stout or Ermine *(Mustela erminea)* was commonly seen in different localities of the study area between the elevation of 2000 and 4500 m (Table 1). It was observed near Choki, Gali Khatter and Ganja (4100m), where five young specimens were seen and one of them trapped. It was also found around Machiara and Serli Sacha and Konkan, where an individual was observed in the stone walls along the road. It is found in deciduous, moist temperate coniferous, sub alpine scrub forests and alpine meadows and also common in villages, usually lives in burrows between rocks, rocky walls of huts and cultivated fields (Table 1). It was reported to prey on *Mus musculus, Rattus rattus, Hyperacrius wynne,* pikas, snakes, frogs, small birds and their eggs.

Small Indian Mongoose (*Herpestes javanicus*) was observed around Pattika, (1200 m), Sadka, (1300 m), Choki (1600 m), Gali Khatter (2500 m), Batdara, Madar and Konkan. (Table 1). This animal prefered lower dry areas of Machiara National Park, not above 2500m elevations and common around cultivated fields of Pattika, Madar and Batdara, observed in crevices and burrows. It consumed rodents (*Rattus rattus and Mus musculus*), Snakes, lizards, small birds, their eggs and domestic poultry.

Indian Porcupine (*Hystrix indica*) was commonly distributed in study area up to 3200 m elevation. This animal was physically seen at night, in maize crops near Gali Khatter (Table 1). This animal generally preferd to live near the cultivated fields in villages, usually avoiding dense forests. Its burrow was observed at about 2 km away from the human population, near Gali Khatter, where its fecal pellets were also observed. The animal was observed utilizing fruits of *Pranus* spp, *Lupha* spp and *Pyrus malus,* fallen on the ground and potatoes were preferably consumed. In winter it was observed eating the tubers of *Arisaema Jacquemontii,* roots of *Convolvulus arvensis*.

Himalayan Giant Red Flying Squirrel (*Petaurista petaurista albiventer*) was rare and endangered, confined to deep forest areas between 2600 m to 3800 m (Table 1). Its nests were observed near Chobsar at 2800 m and also reported around Besri and adjacent areas. An adult female was trapped from a nest near Chathian at elevation of 2600 m and a young male trapped from Gali Khatter. Inactive nests, partially eaten cones

and shoots were also observed in both villages. Indirect evidences and reports indicated its presence in the forest areas around Machiara, Serli Sacha and in locality D and E (Table 1). It prefered forests having dominent tree species of *Pinus wallichiana, Abies pindrow, Aesculus indica Picea smithiana, Cedous deodara, Juglans regia* and *Prunus cornuta* and did not penetrate into villages. Its nests were observed in the branches of *Abies pindrow, Pinus wallichiana* and *Quercus incana* near Gali Khatter. It was observed feeding on young cones and seeds, young shoots and nuts. The skin crusts of *Aesculus indica* were observed from the esophagus and stomach of a freshly killed specimen.

Small Kashmir Flying Squirrel (*Hylopetes fimbriatus*) was commonly distributed in all localities of the study area, usually preferred deciduous and Himalayan moist temperate coniferous forests, near villages at elevation range of 1500 m to 3200 m (Table 1). This species mainly inhabited the trees of *Juglans regia, Aesculus indica, Prunus cornuta* and made nests on the branches of *Pinus wallichiana* and *Pinus roxburghii*. During summer, it mostly ate the young nodes, buds, shoots and nuts of *Aesculus indica, Pinus roxburghii, Pinus wallichiana* and *Abies pindrow*. In autumn, it was noted to migrate to villages and ate the fruits, specially walnuts and frequently hunted by the local peoples. It is preyed upon by eagle, as was observed near Gali Khatter.

Roof Rat (*Rattus rattus*) was commonly found in all villages of the study area up to elevation of 3500 m. These rats were trapped from Bheri, Chathian, Gali Khatter, Machiara and Konkan (Table 1). This species usually occupied houses and cultivated fields and made burrows in roofs of houses, stores and godowns of grains and subsisted on seeds, grains and nuts etc.

Turkistan Rat (*Rattus turkestanicus*) was rare and could not be trapped except one specimen from a summerhouse in Loon Gali at 3500 m elevation. It was also reported around Jhugian (2800 m) (Table 1). It lived in the roofs of houses like *Rattus rattus* but prefered high elevation (Table 1), probably eats grains, nuts, seeds and fruits.

Himalayan Wood Mouse (*Apodemus rusiges*) was rare and unfamiliar to local people and little information was available about its distribution. Only three specimens were trapped, one from Babayan near Basri at elevation of 3600 m and other two from Lone Gali (3600 m) and Mohri near Gali Khatter. It usually prefered moist temperate coniferous forest but was also found in cultivated crops and even in houses near these forests (Table 1), making tunnels (burrows) in clay soils and ate mostly grains, seeds, roots, nuts and grasses. Pieces of grasses and maize plants were found from cheeks and esophagus of trapped specimens.

House Mouse (*Mus musculus*) was the most commonly distributed species among the small mammals, trapped form all localities of the study area (Table 1). Twenty-one specimens were trapped from Bheri, Sadka, Gali Khatter, Machiara, Konkan, and Serli Sacha. It was found extra-limitedly during the autumn season, when crops become ready for harvesting, generally found in close association with man. It usually made nest in burrows and piles of dry grasses, Maize plants and house roofs. It ate seeds, grains, walnuts and any human food that is accessible and was the greatest agricultural pests in the study area.

Royle's High Mountain Vole (*Alticola roylei*) was rare animal in the study area, present at elevations of 2800 m to 4700 m (Table 1). Two specimens were observed, one near Makkra (4300 m) and other in Kuthiali, three specimen were trapped, one from Moosa Gali (4000 m) and two from Ganja Mountains (4300 m). This species occupied the same rocky habitat as Royle's Pikas and was confined to sub alpine scrub forests and alpine meadows (Table 1). It was observed eating stems and leaves of wild herbaceous plants such as *Artimisia* spp. *Polygomum anplexicaule*, and *Thymnus serpyllum*.

Murree Vole (*Hyperacrius wynnei*) was a densely populated species in study area. It was abundantly distributed in all localities of the study area above 2600 m elevation. It was found around Ban, Chabsar, Makkara (4500m) and Banda (3900m) (Table 1). It was trapped from Mohri near Gali Khatter (2600 m) and was also present throughout Ganja Mountains range, densely populated around Kuthiali, Lambi Digi,Charal, Tahair, around Ranja. Sokar Kasi, Sar Sangarh Gali, Barhi Baik and Dana (Table 1) rarely found below 2600 m elevation. The most preferred habitat was alpine meadows, sub alpine scrub forests and agriculture fields above 2800 m elevation (Table 1), usually avoid dense forests but was found in open places. Its burrows were also present between the roots of *Vibernum* spp and *Salix alba* in sub alpine areas. It excavated extensive network of shallow tunnels (about 3 inches deep) and dump their fecal pellets in a separate tunnel that was branched from main tunnel ending at about 1.5 feet away. Food depots were also observed in some burrows. It generally did not come on the ground surface and were captured by setting traps under the ground. It usually ate the roots, stems and leaves of herbaceous plants.

Royle's Pika *(Ochotona roylei)* was common and generally associated with high altitudinal areas ranging between 3000 m to above 4800 m (Table 1). Animal was present in the mountain areas of all localities including Makkra (4300 m), Kala Jabra (3600 m) and Ban (3700 m). Four specimens were trapped; two near Banda (3700 m), one from Moosa Gali (4000 m) and other one from Ganja (4400 m). It was commonly distributed in the rocky areas of Ganja, Kuthiali, Rveri, Alia Baik and Charal (Table 1). Its nests were found in stone heaps and cavities, formed by the loose accumulations of sliding rocks. It ate a variety of wild herbaceous plants like *Artimisia* spp. *polygonum amplexicuale, Senecio chrysanthemoides, Nepeta connata* and grasses.

Cape Hare (*Lepus capensis*) was confined to the lower cultivated fields above Pattika. Cape hares were absent from other localities of the study area and was common in winter season, lived in burrows (Table 1) and subsisted on wheat plants and other grasses.

House Shrew (*Suncus murinus*) was rarely found between 1300 m and 2500 m (Table 1). Among four specimens, two were trapped from Bheri (1800 m) and other two from Gali Khatter. They were trapped from godown, shops, and houses and were also reported from agriculture fields, resting in burrows (Table 1). Animal was mainly insectivorous but was also observed eating vegetable, bread and apple pieces.

Larger Horseshoe Bat (*Rhinolophus ferrumequinum*) was commonly present in lower areas and rare above 2000 m elevation, around Bheri, Daba, Basri Chathian and Gali Khatter. One specimen was seen flying around the electric lamp in a house lawn. Two specimens were trapped from Gali Khatter at about 2300 m elevation. It was also

present in locality C up to about 3000m elevations (Table 1). One specimen was observed in an old mosque near summer residence in Gali Khatter. Pieces of moths and mosquitoes were also detected from the esophagus of freshly killed bat from Gali Khatter.

Table 1: Distribution and habitat preferences of small mammals in Machiara National Park during the year 2003.

Species name	Elevation range (meters)	Forest type and Preferred Habitat	Distribution in study sites and source of information				
			A	B	C	D	E
Vulpes vulpes griffithi	1500-3300	Deciduous and moist temperate coniferous forests. Lives in burrow and caves in rocks.	4	1	2	4	4
Prionailurus bengalensis	2500-4000	Himalayan moist temperate coniferous forests mostly between rocks or holes in trees.	4	3	4	4	4
Paguma larvata	1500-3200	Tropical deciduous and moist temperate coniferous forest. Arboreal, lives in dens in trees.	3	3	3	3	4
Martes flavigula	2500-4000	Himalayan moist temperate coniferous and sub alpine scrub forests. Lives in dens in trees.	3	2	3	4	4
Mustela erminea	2000-4500	Coniferous forests and alpine meadows, lives in holes between rocks and burrows, rocky wall of human huts and cultivated fields.	2	1	2	2	?
Herpestes javanicus	1300-2500	Forests and alpine pastures, lives between rocks	2	2	2	2	?
Suncus murinus	1500-2000	Houses, rice field. Lives in burrows.	1	1	?	?	?
Lepus capensis	1300-1500	Cultivated fields (wheat fields), lives in burrows in soil.	4	0	0	0	0
Ochotona roylei	3000-4800	Moist temperate forests, subalpine scrubes and alpine pastures. Lives in between loose rocks.	1	1	2	2	4

Petaurista petaurista	2600-3800	Himalayan moist temperate coniferous forest, in dens of trees.	3	1	3	4	4
Hylopetes fimbriatus	1500-3200	Himalayan moist temperate forest with mixture of deciduous trees.	2	2	2	3	3
Hystrix indica	1300-3200	Deciduous and coniferous forest and cultivated fields. Lives in caves and burrows between rocks and soil.	3	2	3	3	3
Rattus rattus	1300-3500	Houses, cultivated field, roofs of houses and in burrows.	1	1	1	1	1
Rattus turkestanicus	2500-3800	Houses and moist temperate forests. In burrows and roofs of houses	?	1	?	?	4
Apodemus rusiges	2700-3800	Moist temperate coniferous forests, cultivated fields and houses. Live in burrows in usually clay soil.	1	1	?	?	?
Mus musculus	1300-3500	Cultivated field and houses. Live in burrows in sandy and clay soil.	1	1	1	1	1
Alticola roylei	2800-4700	Subalpine scrubs and alpine meadows, mostly between rocks.	2	1	2	3	?
Hyperacrius wynnei	2600-4700	Cultivated field, forests, subalpine scrubs and alpine meadows in burrows in clay soil.	1	1	1	3	3
Rhinolophus ferrumequi-num	2000-3000	Deciduous and moist temperate coniferous forests. Lives in darker natural caves and old houses.	2	1	1	4	?

Key:

0: Absent
1: Trapped
2: Physically seen
3: Confirmed (by indirect evidences)
4: Reported (from local community)
?: Informations not available,

Site A: Nala Kalas, Bheri, Doba, Basri
Site B: Chathian, Gali Khatter
Site C: Gahatian, Machiara, Panjnand, Konkan.
Site D: Panjur Gali, SerliSacha, Jhing
Site E: Balgiran, Jhugian, Chakrian

Threats

Machiara National Park is endowed with a rich and diverse fauna. Unfortunately, the human population and their livestock, present in the adjoining villages, are degrading its biodiversity. Poverty, illiteracy and environmental unawareness force people to use natural resources. They and their livestock depend entirely on the park resources. The main threats to wildlife of Machiara National Park include habitat destruction through natural and human related activities. The natural factors include snow, avalanches, land sliding and thunderstorms. The human related activities cause the habitat destruction through fuel wood extraction, timber, looping, commercial logging, grazing, grass cutting, extraction of medicinal plants, encroachment, hunting and poaching. In spite of these serious problems Machiara National Park has still diverse and unique fauna making it prominent among National Parks of country.

DISCUSSION

The present study was conducted from April 2003 to August 2003 to determine distribution, food and habitat preferences of small mammalian species in Machiara National Park.

Among the animals inhabiting village areas, *Vulpes vulpes, Hylopetes flmbriatus, Hystrix indica, Rattus rattus* and *Mus musculus* are dominant. They are followed by *Herpestes javanicus, Paguma larvata*, *Rhinolophus ferrumequinum*, *Suncus murinus* and *Lepus capensis*. Similarly *Prionailurus bengalensis, Petaurista petaurista, Mertes flavigula* and *Apodemus rusiges* are the inhabitants of deep coniferous forests. *Hyperacrius wynnei*, *Ochotona roylei* and *Alticola roylei* were mainly found in sub alpine scrubs and alpine meadows.

During this study very low population of Leopard cat, Giant red flying squirrel, Turkistan rat, Wood mouse, High mountain vole and House shrew were recorded. Most of the small mammals were rodents, belonging to three families e.g. Pteromyidae, Hystricidae and Muridae. Similarly six species of carnivores were related to Canidae, Felidae, Viverridae and Mustelidae. Two families (Leporidae and Ochotonidae) were of Lagomorphs and two other (Soricidae and Rhinolophidae) belonged to order insectivora and chiroptera respectively. Among these small mammals, nine species were noted as the agricultural pests in the study area. They cause significant damage to commercially exploitable crops including Maize, Potatoes, Tomatoes and Wheat.

Red Fox *(Vulpes vulpes griffithi)* is commonly distributed in all Villages of the study area in close association with human population. This sub-species, with same habits and habitat has already been described by Roberts (1997) and Ahmed (1997) in Azad Kashmir. Similarly Leopard cat has also been reported by Roberts (1997). During the present study, the population of this cat was estimated very low and similar status has already been described by Roberts (1997). It has become endangered in this area due to frequently hunting by the locals and outsiders for its highly priced skin.

Palm civet (*Paguma larvata*) was present in the deciduous and moist temperate coniferous forests of the study area (Table 1). It has been reported by Roberts (1997) in Neelum Valley in the same habitat. Like wise Yellow Throated Marten (Martes *flavigula*) occurs in the study area up to 4000 m in coniferous and sub alpine scrub forests (Table 1). Roberts (1997) has described this species associated with *Abies pindrow* and *Pinus wallichiana* in Himalayas including Azad Kashmir. Roberts (1997) and Qurashi (2000) also described it as the predator of Musk deer in the Neelum Valley of Azad Kashmir.

Ermines (*Mustela erminea)* were found in villages, sub alpine and alpine meadows at elevation of 4500 m (Table 1). Roberts (1997) has collected this species at 4000 m elevation in Kaghan Valley and in study area they were sympatric with Alpine weasel (*Mustela altiaca).* Small Asian Mongoose (*Herpestes javanicus)* was distributed in lower cultivated areas (up to 2000 m) of the Park ((Table 1). It inhabited natural and man-made culverts and crevice in the cultivated fields and buildings. Roberts (1997) has described this species in lower Plain areas of Sind and Punjab. He has not recorded this animal in the mountainous areas of Himalayas. So, the present description of the species in the mountainous areas (study area) is a worthy of consideration. Similarly, House shrew (*Suncus murinus)* is also an inhabitant of plain area and low elevations but it has also been trapped by Roberts (1997) at 3600 m near Kaghan Valley. During present study, it was not observed above 2500 m elevations (Table 1).

Cape hare (*Lepus capensis*) was found in a small cultivated area between the elevation of 1300 m and 1500 m (Table 1). It has been described by Mirza (1998) and Roberts (1997), near Muzaffarabad and Murree Hills at 600 m and 900 m elevations respectively. Royle's Pikas (*Ochotona roylei*) were generally associated with the high elevation and rocky areas of the Machiara National Park (Table 1). Their preferred habitat was alpine and sub alpine scrubs at about 3000 m to 4800 m elevation in Machiara (Table 1). Roberts (1997) has recorded it at an elevation range of 2400 m to 3600 m. He has described it in association with *Cedrus deodara* and *Picea smithiana*. But during the present study it was noted that pikas generally avoid these forests, probably due to the presence of more predators in forests than the alpine and sub alpine areas (Table 1).

Giant red flying squirrel (*Petaurista petaurista*) was recorded at an elevation range of 2600 m to 3800 m in deep moist temperate coniferous forests of the study area (Table 1). It has also been recorded by Roberts (1997) and Mirza (1998) in Neelum Valley of Azad Kashmir at elevation of 1350 m to 3050 m. Porcupine (Hystrix *indica*) was widely distributed animal in Machiara National Park at an elevation of 1300 m to 3200 m (Table 1). Roberts (1997) has given its distribution in Blochistan, Punjab and Northern regions e.g. Hazara, Murree, Chitral and Kurram Valley up to 2750 m. Prater (1965) and Gurung and Singh (1996) have also described this animal throughout the Himalayas up to 2400 m.

Roof Rat (*Rattus rattus*) was densely populated species of the National Park. It was an agricultural pest in this area (up to 3500 m elevation) (Table 1). Roberts (1997), Hussain *et al.* (2002), Parker (1990), Nowak (1991) and Hassan *et al.* (1995) have described it as agriculture pest but were not trapped from cultivated fields during the present study and similar results were recorded by Taber *et al.* (1967). Turkistan Rat

(Rattus turkestanicus) inhabits the same habitat as Roof Rat in the study area. Its Morphology is similar (with little variations) to Roof Rat. In the study area it was trapped up to 3800 m (Table 1). In contrast, Roberts (1997) has reported it up to 3100 m. House Mouse *(Mus musculus)* was trapped from agriculture fields and village houses up to 3500 m (Table 1). Contrary to Roof Rat House Mouse and house shrew occur both indoor as well as out door (Hussain *et al.*, 1975).

High mountain vole (*Alticola roylei*) and Murree vole (*Hyperacrius wynnei*) were two other rodents present in alpine and sub alpine scrubs forests of the study area (Table 1). Roberts (1997) has recorded *Alticola* in Northern Areas up to 5300 m elevation. During present study, they were observed at 4500 m (Table 1). Like wise Murree vole has been reported by Roberts at an elevation range of 1850 m to 3050 m, but in present study they were trapped and observed up to 4600 m. Murree voles are mole like in habits and this information has already been given by Roberts (1997), Nowak (1991) and Phillips and Carleton (1969).

Afghan Vole *(Hyperacrius fertilis)* is found in Kaghan Valley but it is replaced by Murree Vole *(H. wynnei)* in Machiara National Park. Similarly Royle's Pika *(Ochotona roylei)*, High Mountain Vole *(Alticola roylei)* and Stout *(Mustela ermenea)* are absent from Murree Hills (Roberts, 1997) but they were observed in study area. Beech or Stone Marten (*Martes foina)*, Chinese Birch Mouse *(Sicista concolor)*, *Woolsly* Flying Squirrel *(Eupetaurus cinereus)*, Long Tailed or Kashmin Marmot *(Marmota caudate)*, Asiatic Pygmy Shrew *(Sorax thibetanus)* and Asiatic White-toothed Shrew *(Crocidura pullata)* are present around Murree and Kaghan Valley (Roberts, 1997) but absent in study area.

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SERUM PROTEIN PROFILE IN LUTEAL PHASE OF CYCLING WOMEN

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Abstract: Serum samples of unmarried young females (N=34) of ages ranging 19-25 years, were drawn on mid luteal phase (at 7th or 8th day before the next menstruation). Prior to sampling, it was made certain that the subjects were definitely passing through the required phase. In case of irregular cycle, the sampling was made on 21st day of the menstrual cycle. Different samples were categorized into normal regular (27-29 days), short regular (24-26 days), long regular (30-31 days) and irregular cycle. Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) based on the method of Laemmli (1970), was employed for studying the low molecular weight protein fractions in all cycling patterns. The gel photography, image storing and quantification of various protein fractions were carried out by Gene Genius Bioimaging Gel Documentation System that provided the data of molecular weights and percent areas covered by each of the fractions. The data was analysed statistically using Student 't' test and employed in finding the enhancement/reduction and/or appearance/disappearance of particular protein fractions for comparisons among normal, short, long and irregular cycles. Low molecular weight serum protein fractions were ranging between 26-11 kDa in all cycling patterns. Significant protein fractions of 14 and 13 kDa were found to be declined in short compared to normal regular cycle. Protein fractions of 17 , 15 and 13 kDa indicated a pronounced decline in irregular compared to short regular cycle. The fractions of 17 and 11 kDa indicated a significant elevation in long compared to short regular cycle. No significant protein was found in irregular compared to long regular cycle.

Key words: Gel electrophoresis, luteal phase, protein fractions

INTRODUCTION

Reproductive function, in women and female primates, follows a cyclic pattern between menarche and menopause that is termed as menstrual cycle (Randall *et al.,* 2000). The average age of menstrual bleeding in girls is 12 years although it starts as early as 10 or may be as late as 16. At menopause, when periods stop, the average age is 50, however, it can vary (Marshall, 2001).

The time course of gonadotropin secretion throughout the menstrual cycle is regulated by negative and positive feedback actions of estradiol on gonadotropin secretions. The secretions of follicle stimulating hormone (FSH) and luteinizing hormone (LH) by the pituitary gland is dependent on interplay of two major regulatory components,

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the ovary and the hypothalamus (Cone *et al.*, 2003). The length of the cycle is counted from the beginning of the menstrual period until the day before the next period commences. The average cycle lasts about 28 days but cycle can vary from 23 to 31 days (Cutler *et al.*, 1998).

Menstrual cycle is generally divided into different phases, the menstrual phase, follicular phase, ovulation phase and the luteal phase. Following ovulation, progestational or luteal phase is dominated. Normal length of the luteal phase is 10-16 days and average is 14 days. The estrogen level is elevated at mid luteal phase and decreased at the end of the menstrual cycle. The secretion of progesterone during the luteal phase is episodic and pulses are correlated with pulses of LH. LH acts as a luteotropic agent. Formation of oxytocin and vasopressin within the corpus luteum promotes luteolysis by modulating autocrine or paracrine mechanisms. Finally, LH down regulation of its own receptors may play a role in the termination of luteal phase (Marshall, 2001).

Severe reducing diets cause low levels of progesterone, slowing follicular growth, inhibiting the surge of LH and preventing ovulation (Wynn and Wynn, 1994). Exercise of sufficient rigor, particularly, when coupled by weight loss and dietary restriction is capable of producing reversible disturbances of many otherwise healthy young women (Campbell *et al.*, 2001). However, it is clear that there are many health benefits of moderate and regular exercise (Green, 1993). Menstrual cycle may be shorter regular (less than 28 days), longer regular (more than 28 days) and irregular. In physiological set up, particularly, related to reproduction, the hormonal patterns are indicative of the regulation of cycle and determine the duration of different phases. Polyacrylamide gel electrophoresis (PAGE) of serum proteins has an important role as diagnostic investigation and variations in menstrual cycle duration may be evaluated on the basis of appearance or disappearance of certain regulatory proteins in the blood. Von Wolf *et al.* (2001) analysed endometrial mRNA and protein expression of osteopentin and its receptor beta (3)-integrin throughout the menstrual cycle. Beier and Beier-Hellwig (1998) studied the proteins during menstrual cycle and identified and isolated the molecular structure of several proteins like histones, cyclophilins, transthyretin, haptoglobin and uteroglobin. Beier-Hellwig and Beier (1994) had also studied the endometrial proteins by SDS-PAGE, during luteal phase, and observed that numerous individual protein bands mainly resolved between 68 and 65 kDa. They identified several of these proteins as histones; H_2A, H_2B, H_3 and H_4. Itoh and Manaka (2001) analyzed the vaginal secretions by SDS-PAGE and reported a relatively low concentration of 67 kDa and high concentration of 56 kDa in pre and post menstrual phases. A relatively high concentration of 52 kDa protein characterized the mid cycle. By keeping in view the importance of proteins in so many physiological phenomena, it is very likely that in the present investigation, various regulatory proteins may be synchronously associated with the behaviour of the cycle.

MATERIALS AND METHODS

Serum samples of unmarried young females (N=34) age range 19-25 years were drawn at mid luteal phase (mostly on 7th and sometimes on 8th day before the onset of next menses). In case of irregular cycles the sampling was made on 21st day of menstrual cycle. Menstrual cycle was categorized into 4 types. A cycle with duration of 24-26 days was termed as short regular; of 30-31 days categorized as long regular and 27-29 days was considered as normal regular. The subject experiencing varying duration in subsequent cycles are placed in irregular category.

Polyacrylamide gels (15%) for low molecular weight protein fractions was prepared by using the method of Laemmli (1970). Protein size markers and each of the samples were loaded in separate wells and gels were electrophoresed at a current supply of 30 mA and voltage of 200 V in a cooling chamber maintained at 4°C until the tracking dye reached the lower end of the gel. Following Electrophoresing run, the gels were stained with coomassie brilliant blue up to 30 minutes and destained afterwards until the clearance of blue background. Protein fractions of different molecular weights were visible in the form of blue bands on a transparent background.

Stained gels were photographed afterwards and their images were saved on a floppy disk with Gene Genius Bio-imaging Gel Documentation system. The quantification of separated protein fractions was carried out by gene tools software that provided the data of molecular weights and percent areas covered by each of the fractions.

For the assessment of the variations in the protein profiles among different individuals in normal, short, long and irregular cycle during luteal phase, values of individual protein fractions were averaged and expressed as mean±SEM. The data was analyzed statistically using Student t-test and employed in finding the enhancement/reduction and appearance/disappearance of particular protein fractions for comparisons among different individuals.

RESULTS

An overall view of protein profile resulted in the detection of seven low molecular weight protein fractions ranging between 26-11 kDa during luteal phase of all cycling patterns in the unmarried young females (Fig. 1).

Among these, protein fractions of 26 kDa and 20 kDa were found to be elevated by 16 and 17% covering the average areas of 29.88±2.17 and 8.94±3.72, respectively, in short compared to normal regular cycle. Protein fractions of 17, 15, 14, 13 and 11 kDa were found to be declined by 60, 66, 42, 82 and 46%, respectively, with average values of 1.87±0.31, 1.71±0.46, 1.89±0.40, 0.50±0.12 and 3.41±0.83%, in short compared to normal regular cycle, respectively.

In the comparison of normal and long regular cycle, protein fractions of 26, 17 and 11 kDa did not exhibit significant variations. Protein fractions of 20, 15 and 14 were, however, declined by 17, 25 and 14% with average areas of 7.63±0.44, 3.76±1.03 and

2.79±0.32%, respectively, in long compared to normal regular cycle.

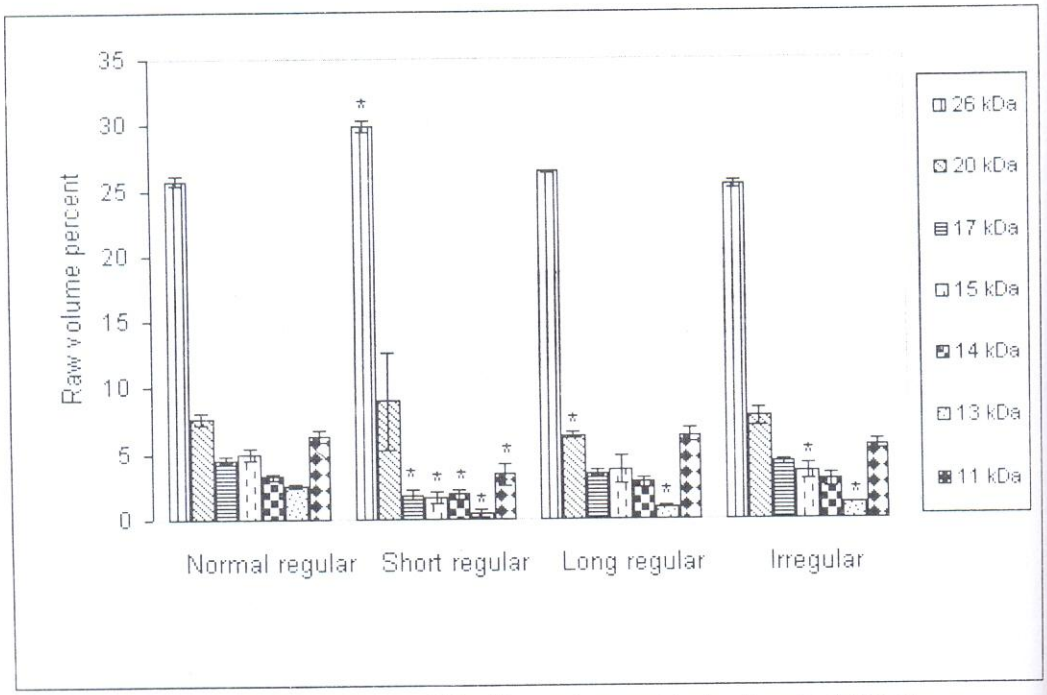

Fig. 1. Average raw volumes percent exhibited by various protein fractions in different subjects during luteal phase in normal, short, long and irregular cycles.
Values are mean±SEM * Significance at P<0.05, as compared to normal regular

Among the comparison of normal and irregular cycle, protein fractions of 17, 15, 14 and 13 kDa were declined by 23, 26, 63 and 14% with average areas of 4.33±0.24, 3.60±0.62, 297±0.54 and 19.00±0.20% in irregular compared to normal regular cycle. Protein fractions of 20, 17 and 11 kDa were almost in the same range in both cycling patterns.

In the comparison of short and long regular cycle, protein fraction of 20 kDa, exhibited a significant decline of 29%, whereas, 17, 15, 14, 13 and 11 kDa fractions were significantly elevated by 88, 119, 47, 84 and 87%, respectively, in short compared to long regular cycle.

Among the comparisons of short and irregular menstrual cycle, protein fractions of 26 and 20 kDa indicated a decline of 16 and 15% in irregular compared to short regular cycle. Other protein fractions of 17, 15, 14, 13 and 11 kDa were found to be elevated by 131, 110, 57, 133 and 63, respectively, in irregular compared to short regular cycle.

Among the comparison of long regular and irregular cycle the protein fractions of 20 and 17 kDa were elevated, respectively, by 22 and 23%, whereas, 13 kDa protein

fraction was declined by 22%, in irregular compared to long regular cycle. The rest of the protein fractions did not vary considerably, in this group comparison.

DISCUSSION

The term menstrual cycle technically refers to a series of changes that occurs in sexually mature non-pregnant females (Labb *et al.*, 2000). Complex series of hormonal interactions between thyroid, adrenal, pituitary gland, hypothalamus and ovary control menstruation (Hundscheid *et al.*, 2000). Therefore, changes in the amount or timing of hormone released by the thyroid, pituitary gland or hypothalamus may cause menstrual irregularities (Weiss, 2001). Membrane cofactor protein is found throughout the menstrual cycle and plays a role in normal reproductive function. Many insulin like growth factors binding proteins are involved in human folliculogenesis during normal and abnormal menstrual cycle (Rabahi *et al.*, 1991).

In the regulation of menstrual cycle in terms of duration of its various phases, it is evident that directly or indirectly through hormonal mediation various regulatory proteins eventually determine the behaviour of the cycle. The regulatory proteins in serum through their varying concentrations reflect their role in the function. No doubt, such responses are the main feature to know variations in luteal phase of human females. In the present study serum samples of young unmarried females were collected at luteal phase of normal, short regular, long regular and irregular cycle. The study of the protein fractions is an approach to locate these in different cycling patterns and to understand the regulatory nature of these serum proteins in four cycling patterns. The various protein fractions observed included trypsin I (26 kDa), trypsin inhibitor (20 kDa), apolipoprotein A II (17 kDa), Lysozyme (15kDa), α-lactalbumin (14 kDa), β2-microglobulin (11kDa) and an unknown fraction (13 kDa). Trypsin I (26 kDa) varied non significantly in short, long and irregular subjects compared to normal regular cycle. Trypsin inhibitor was found to be declined in long compared to normal regular cycle. It strongly indicates that this fraction's appearance in the circulation is associated with the normal behaviour of the cycle as its decrease changes cycle's duration (Bouckart *et al.*, 1986). Apolipoprotein A-II (17 kDa) was found to be declined by 66% in short compared to normal regular cycle. The same fraction was enhanced by 88% in long and 131% in irregular compared to short regular cycle. Solerte *et al.* (1996) have reported that apolipoprotein A-II remain unchanged throughout the menstrual cycle. Hence, variations in its appearance, in the present investigation, may be related to irregularity. There is variation in the level of lysozyme and albumin during the luteal phase of menstrual cycle (Schumancher *et al.*, 1977). Alpha lactalbumin (14 kDa) and many other proteins showed no significant differences in luteal phase of cycling women (Vizoro *et al.*, 1989) as, in the present study, the decrease in alpha lactalbumin in short compared to normal regular cycle suggests that decrease in duration of cycle length may be linked with the reduction of alpha lactalbumin. Lysozyme (15 kDa) was significantly enhanced by 110% in irregular compared to short regular cycle. Highly significantly expressed fraction of 13 kDa was declined by 63% in irregular

compared to normal regular cycle, whereas, an average elevation of 133% was observed in irregular subjects compared to short regular cycle indicating the role of the fraction in varying cycle duration. Beta-2 microglobulin was declined by 46% in short compared to normal regular cycle while this fraction was elevated in long compared to short regular cycle. On the other hand, Paaby *et al.* (1980) demonstrated that beta-2 microglobulin was appeared significantly during the luteal phase but not during the following phase in the serum.

Conclusively, analysis of the results of present study, analysing the pattern of serum protein fractions, indicated that some fractions alterations in luteal phase are strong indicators of their role in alteration of cycle and phase duration. The results of the present study are in agreement and also in disagreement with other reports. However, the differences, in the pattern of present study may be related to genetic and environmental differences of the populations.

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ELECTROPHORETICALLY RESOLVED SERUM PROTEIN FRACTIONS IN POSTMENOPAUSAL WOMEN

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Abstract: Blood samples of naturally postmenopausal women and healthy control cycling females were collected at different places in Lahore by the expert persons. The study concerns on the physiological adaptations after the natural menopause. Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) based on the method of Laemmli (1970) was employed for studying protein profile of postmenopausal and control subjects. Gels were photographed and their images were stored for quantification of various protein fractions by Gene Genius Bio-imaging Gel Documentation System that provided the data of molecular weights and percent raw volumes by each of the fractions. The data was analyzed statistically using Student t-test in finding the enhancement or reduction and appearance or disappearance of particular protein fractions for comparisons among the postmenopausal and the control subjects. The six low molecular weight protein fractions were detected that were ranging between 26-11 kDa. Non significant elevations were observed in 26, 20 and 11 kDa protein fractions. Highly significant elevations were observed in 17, 15 and 14 kDa protein fractions in postmenopausal subjects when compared with control subjects.

Key words: Gel electrophoresis, postmenopausal women, protein fractions

INTRODUCTION

Menopause refers to the period of climactic, which encompasses the transition from reproductive years up to and beyond the last episode of menstrual bleeding (Carr, 1998). The average age at the onset of menopause is 51. however, it can vary (Richardson *et al.*, 1987). The age is retrospectively defined after 1 year of no menses (Bromberger *et al.*, 1997). Throughout a women's reproductive life about 400 of primordial follicles grow into vesicular follicles and ovulate, while literally hundreds of thousands of ova degenerate (Guyton and Hall, 2000). Some years before the cessation of menstruation, levels of gonadotropins increase, while ovarian hormones begin to decrease (Sherman *et al.*, 1976; Chakravartis *et al.*, 1976).

Schlessinger (2001) has reported that a mutation in the newly identified gene $FOXL_2$ causes premature menopause which usually occurs at the age 30 years. A wide array of symptoms is often associated with menopause including vaginal dryness, heart palpitations, urinary tract infections, emotional changes and social dysfunction (McKinlay

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and Jeffry, 1998). Hot flushes are the most common objective symptoms of menopause (McKinlay *et al.*, 1987). These are caused by decline in estrogen blood concentrations associated with menopause (Lauritzen, 1973).

There is a close relationship between estrogen deprivation and development of osteoporosis in postmenopausal women. The susceptible women may lead to vertebral, hip and wrist fractures (Lindsay, 1978). Hormone replacement therapy (HRT) is effective in preventing the bone loss and decreasing fracture rate (Lufkin *et al.*, 1992). The principal cause of death in postmenopausal women is cardiovascular disease of which the most common form is the coronary artery disease (Henderson *et al.*, 1986).

It was reported by Ross *et al.* (1981) that estrogen replacement therapy reduces the incidence of cardiovascular disease in postmenopausal women by raising the HDL-cholesterol and lowering the LDL-cholesterol. Estrogen replacement therapy has certain risk factors like endometrial carcinoma in postmenopausal women. Therefore, the addition of progestins either cyclically or continuously reduces the risk of estrogen induced carcinoma (Carr *et al.*, 1998).

Phytoestrogens are the type of plant estrogens which serve as an alternate of hormone replacement therapy. They not only prevent the bone loss but also provide cardioprotective benefits to postmenopausal women (Polkowski, 2000).

Polyacrylamide gel electrophoresis (PAGE) of serum proteins has an important role as diagnostic investigation. Magruiness *et al.* (1993) separated the tubal epithelial proteins (TEP-1 and TEP-2) from tubal mucosa and endometria by one dimensional gel electrophoresis. These two protein bands were present throughout the ovarian cycle but were absent from the tubal mucosa obtained from postmenopausal women.

Tchernof *et al.* (2002) reported that weight loss decreased the plasma C-reactive protein levels in postmenopausal women which may mediate part of its cardioprotective effects in obese postmenopausal women. The diameter of LDL-protein was determined by gradient gel electrophoresis in postmenopausal women. The plasma concentrations of LDL-particles were increased after menopause. Lower levels of endogenous estrogen appeared to cause the size of LDL particles to be reduced (Ikenoue *et al.*, 1999).

The studies regarding the electrophoretic protein profile in response to ovarian hormones pathophysiology are meagre and non-existent. By keeping in view of the importance of proteins in so many physiological phenomena, the present study is planned to investigate the alterations in low molecular weight protein fractions in postmenopausal women sampled, in Lahore.

MATERIALS AND METHODS

Blood samples of naturally menopausal (N=18) and healthy control (N=9) cycling subjects in luteal phase of menstrual cycle were collected at different places in Lahore.

Polyacrylamide gel (14%) for low molecular weight protein fractions was prepared using the method of Laemmli (1970). Serum samples were denatured in loading

dye and diluted with distilled water to prepare the working dilutions for loading on to the gel. Each of the serum samples and low molecular weight protein markers were loaded in separate wells and gel was electrophoresed at a current supply of 30mA and voltage of 200V, in a cooling chamber maintained at 4°C until the dye reached the lower end of the gel. Following electrophoresing run the gels were stained with coomassie blue for one hour with constant agitation and destained afterwards until the clearance of blue background. Protein fractions of different molecular weights were visible in the form of blue bands on a transparent background.

Stained gels were photographed afterwards and their images were saved on a floppy disk with Gene Genius Bio-imaging Gel Documentation System. Quantification of separated protein fractions was carried out by the same system that provided the data of molecular weights and percent volumes covered by each of the fractions.

The data was analyzed statistically using Student t-test and employed in finding the enhancement or reduction and appearance or disappearance of particular protein fractions for comparison among the postmenopausal and the control subjects.

RESULTS

The electrophoretic results in the form of percent raw volumes covered by the resolved low molecular weight serum protein fractions were analyzed in comparable groups categorized as control (subjects 1-9) and postmenopausal (subjects 10-27) groups.

Control group

An overall view of protein profile resulted in the detection of six low molecular weight serum protein fractions ranging between 26-11 kDa (Figs. 1-6) during the luteal phase of normal regular menstrual cycle (subjects 1-9).

Among these, protein fraction of 26 kDa exhibited a dominant expression in all subjects with raw volume percent ranging between 22.75-44.62%.

Protein fraction of 20 kDa was next in expression. The fraction exhibited the raw volume percent ranging between 2.09-5.76%.

The protein fraction of 17 kDa indicated its considerable presence with percent raw volume ranging between 0.58-1.60%.

The protein fraction of 15 kDa was missing in subjects No. 4, 7, 8 and 9, whereas the fraction ranged between 0.19-1.27%, in rest of the subjects of control group.

The 14 kDa fraction which ranged between 0.28-0.70%, in control subjects No. 1, 2, 3 and 5 was also unexpressed in subjects No. 4, 6, 7, 8 and 9.

The last resolved fraction of 11 kDa ranged between 0.66-2.64% and was expressed in all of the subjects of the control group.

Postmenopausal group

The analysis of serum protein profile in the postmenopausal subjects (subjects 10-27) resulted in the detection of six low molecular weight fractions ranging between 26-11 kDa (Figs. 1-6).

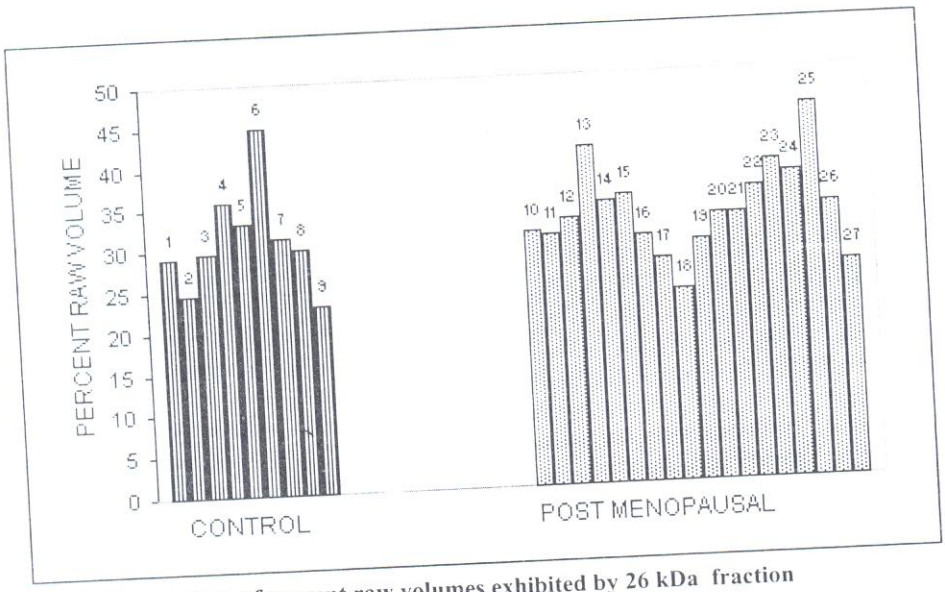

Fig. 1. Comparison of percent raw volumes exhibited by 26 kDa fraction in control (1-9) and post menopausal (10-27) women.

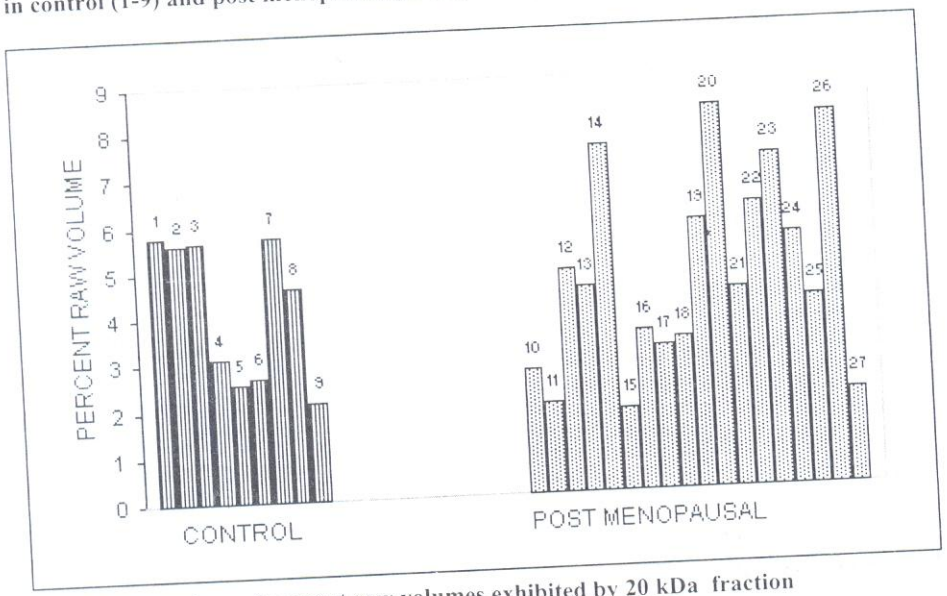

Fig. 2. Comparison of percent raw volumes exhibited by 20 kDa fraction in control (1-9) and post menopausal (10-27) women.

Among these, the dominantly expressed fraction of 26 kDa exhibited the raw volumes percent ranging between 23.36-45.54%, in various subjects of the group, indicating a slight elevation in most of the postmenopausal subjects as compared to the control group (Fig. 1).

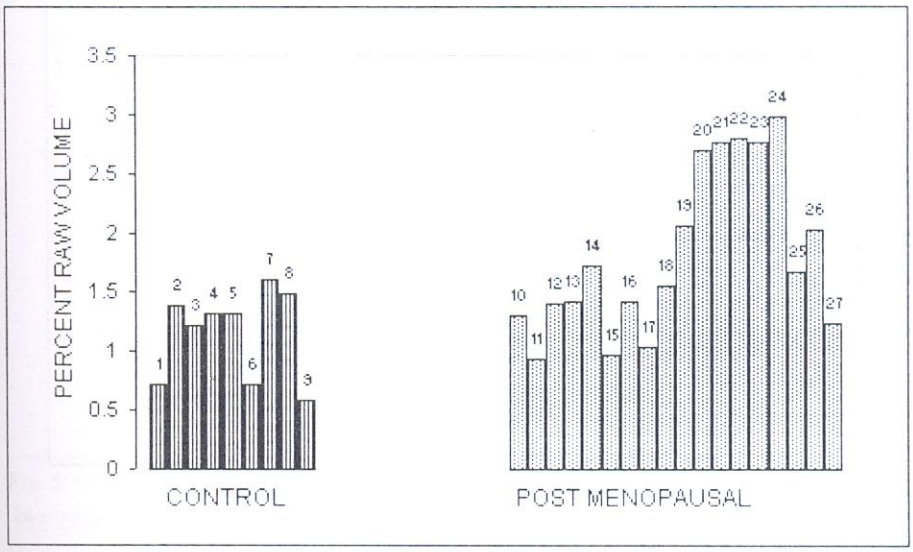

Fig. 3. Comparison of percent raw volumes exhibited by 17 kDa fraction in control (1-9) and post menopausal (10-27) women.

The fraction of 20 kDa was next in expression, ranging from 1.76-8.05%, indicating a significant elevation in postmenopausal subjects No. 14, 20, 22, 23 and 26 as compared to controls. The fraction, however, remained in the range of controls in the rest of the postmenopausal subjects (Fig. 2).

The protein fraction of 17 kDa ranged between 1.04-2.98%, indicating an elevation in postmenopausal subjects No. 14, 19, 20, 21, 22, 23, 24 and 26 as compared to controls. The fraction remained in the control range in rest of the postmenopausal subjects (Fig. 3).

The protein fraction of 15 kDa was unexpressed in postmenopausal subjects No. 11, 13, 16, 18, 19, 20, 21, 22, 24 and 26. However, it was found significantly elevated in subjects No. 12, 17, 23, 25 and 27 as compared to the controls (Fig. 4).

The fraction of 14 kDa appeared only in postmenopausal subjects No. 26 and 27. The fraction was also unexpressed in almost half of the control subjects (Fig. 5).

The last resolved fraction of 11 kDa exhibited its considerable presence in the group with percent raw volumes ranging from 1.00-2.71%. The fraction indicated a non significant elevation in post menopausal compared to control subjects (Fig. 6).

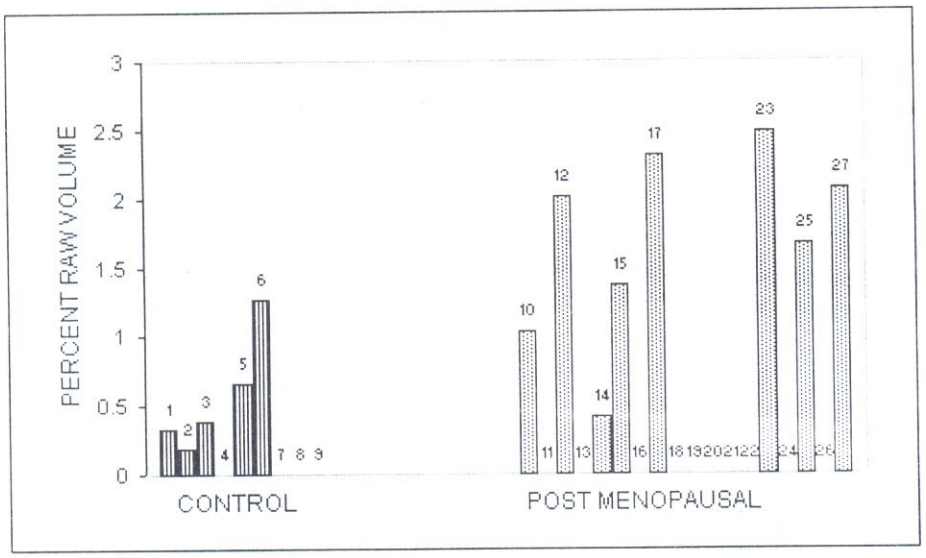

Fig. 4. Comparison of percent raw volumes exhibited by 15 kDa fraction in control (1-9) and post menopausal (10-27) women.

Average group comparison

Average raw volumes covered by 26 kDa were found to be 33.11±1.26% and 31.15±2.13, in postmenopausal and control subjects, respectively. A non significant increase of 6% was, therefore, observed in postmenopausal subjects compared with control subjects.

The 20 kDa protein fraction, with non significant elevation of 12%, exhibited average raw volumes of 4.68±4.98 and 4.19±0.524%, in postmenopausal and control subjects, respectively.

Protein fractions of 17, 15 and 14 kDa showed highly significant elevations of 58%, 198% and 331%, with average raw volumes of 1.82±0.164, 1.67±0.24, 2.15±1.74%, in postmenopausal subjects with average % raw volume of 1.15±0.125, 0.56±0.191, 0.50±0.09 in control subjects, respectively.

A non significant elevation of 7% was noticed in protein fraction of 11 kDa, with average raw volumes of 1.68±0.130 and 1.57±0.195%, in postmenopausal subjects compared with the control subjects (Fig. 7).

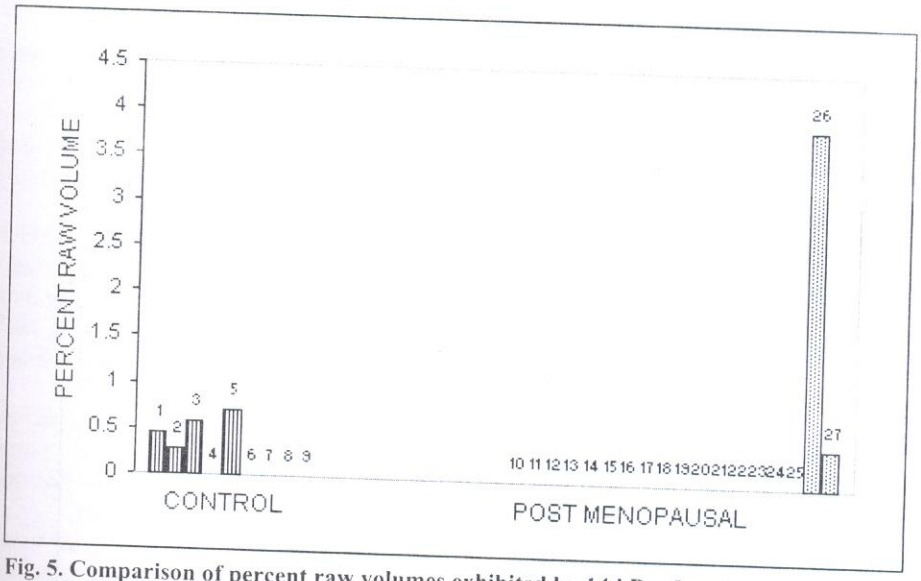

Fig. 5. Comparison of percent raw volumes exhibited by 14 kDa fraction in control (1-9) and post menopausal (10-27) women.

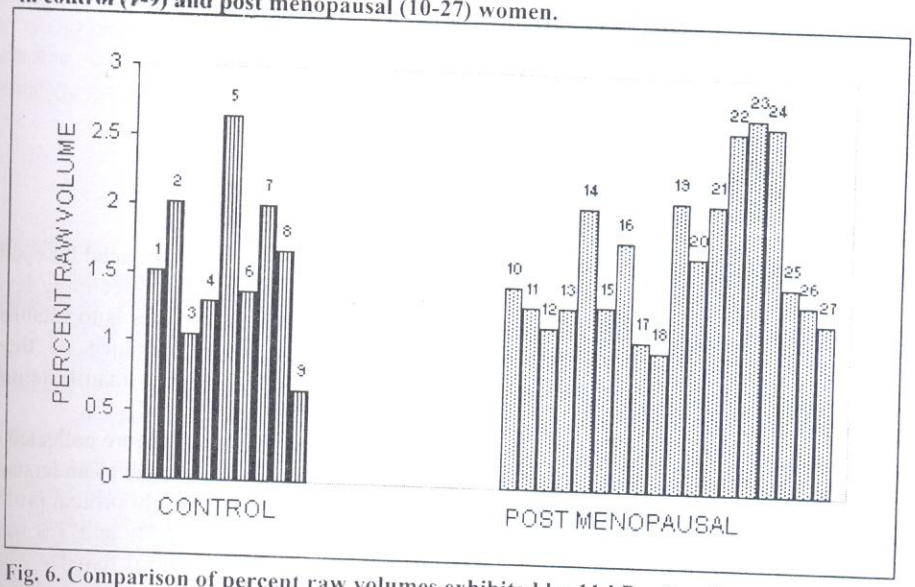

Fig. 6. Comparison of percent raw volumes exhibited by 11 kDa fraction in control (1-9) and post menopausal (10-27) women.

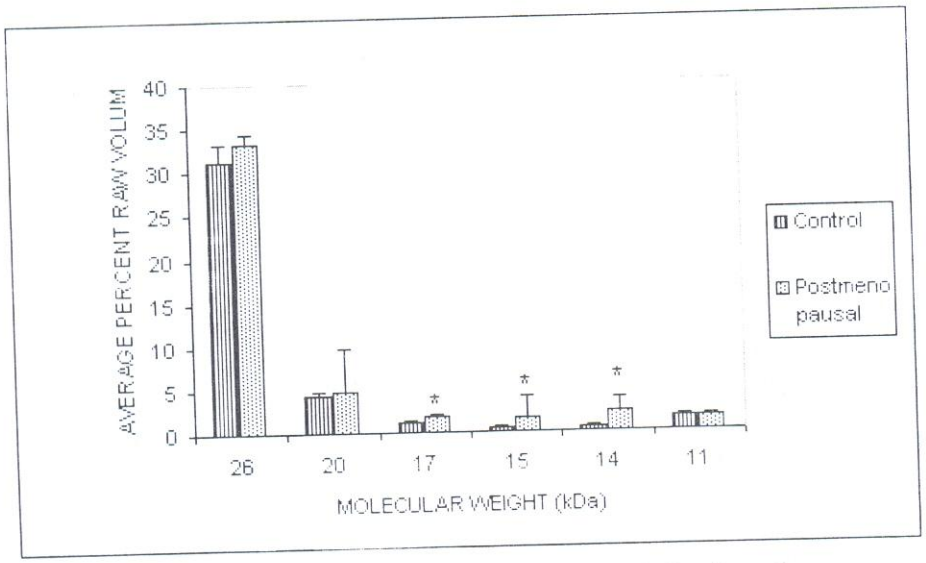

Fig. 7. Average raw volumes (%) exhibited by various protein fractions resolved by SDS-PAGE in control and post menopausal women.

Values are mean±SEM. *** Significance at P<0.05**

DISCUSSION

Menopause begins with the last episode of menstrual bleeding induced by cyclic endogenous secretion of ovarian hormones (Goldfien, 2001). It results in declining level of ovarian hormones specifically the estrogens. The role of these hormones is to establish a physiological homeostasis in cycling pattern. Therefore, disappearance of these hormones brings altered physiological state accompanied by clinical manifestations (Jonathan and Wright, 1996).

In the present study, serum samples of postmenopausal females were collected at different places and have been analyzed for protein fractions in an approach to understand their regulatory role in postmenopausal females. The low molecular weight protein profile was ranging between 26-11 kDa. Non-significant elevations of 6%, 12% and 7% were found in 26 kDa, 20 kDa and 11 kDa protein fractions in postmenopausal females when compared with controls. Similarly, highly significant elevation of 58% was observed in 17 kDa protein fraction, respectively in postmenopausal females when compared with control subjects. The 15 and 14 kDa fractions were non uniformly expressed in control and

postmenopausal subjects, however, on the average, highly significant elevations of 198 and 331% were expressed, respectively, by 15 and 14 kDa protein fractions.

Yildiri *et al.* (2002) has reported elevated levels of homocysteine in postmenopausal women but hormone replacement therapy reduces the plasma homocysteine levels thus providing cardioprotective benefits. Further investigations also revealed that production of interleukin-1 (IL-1), interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) increases in estrogen deficient oesteoporotic postmenopausal females (Heiss *et al.*, 1995).

In the present study, apolipoprotein A-II has shown highly significant elevation of 158% in postmenopausal females when compared with controls. This result reflects the significance of 17 kDa protein fraction for its greater appearance in postmenopausal state. Ikenoune *et al.* (1999) have reported that plasma levels of apolipoprotein A-II did not differ significantly between naturally and surgically induced menopausal females. Thus, absence of ovarian hormones probably affects apolipoprotein A-II.

The protein fraction of 14 kDa appeared only in two postmenopausal females. Similarly, 15 kDa protein fraction is unexpressed in half of the postmenopausal subjects. Further investigations, on these protein fractions, may contribute in understanding the molecular mechanisms in postmenopausal females.

The present study is initial or preliminary study regarding the role of low molecular weight protein fractions in menopause. It is assumed that declining levels of ovarian hormones as well as obesity and aging are responsible for alterations in these low molecular weight protein fractions. The clear picture may be seen on further investigation on menopause and its various short and long term symptoms with large population samples. The larger sample population will provide clear situation for the judgments in assessing the protein fractions as marker proteins.

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STUDIES ON THE IMMUNOPATHOLOGY AND HAEMA TOLOGY OF BROILERS EXPERIMENTALLY INFECTED WITH *ESCHERICHIA COLI*

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Abstract: This project was carried out to study immunopathological and haematological effects of experimental *E. coli* infection in broiler chicks. For this purpose one hundred (day-old) broiler chicks were randomly divided in two equal groups A and B on day-21 of the experiment. At the same day chicks of group A were inoculated with confirmed pathogenic isolate ($3x10^7$ bacilli/0.1 ml) of *E. coli* intraperitoneally while chicks of group B received sterile nutrient broth and acted as control. Result showed that haemoglobin (Hb), packed cell volume (PCV), Total erythrocyte count (TEC), antibody titre against Newcastle disease virus (NDV) and feed conversion ratio (FCR) were significantly lower while total leukocyte count (TLC) was significantly higher in treatment than control group on all the three sampling days. Moderate to marked gross and microscopic pathological changes in heart, liver and spleen were also observed in treatment group as compared to control one. Mean lymphoid organs weight/body weight ratio and post-infection mortality were higher in treatment group as compared to control one. From the foregoing study it was concluded that infection with *E. coli* resulted not only in haematological and pathological alterations but it also impaired the immune system along with high mortality in infected birds.

Key words: *Escherichia coli*, histopathology, immunopathology, haematology, broilers.

INTRODUCTION

Escherichia coli (*E. coli*) has been associated with many disease conditions of poultry. Various *E. coli* infections include colibacillosis, colisepticaemia, Hjarre's disease, coligranuloma, peritonitis, salpingitis, omphalitis and air sac disease (Gross, 1991). *Escherichia coli* infection and its association with various viral and bacterial diseases in poultry is not uncommon. Moreover, the poultry industry is constrained by high incidence of disease, high morbidity and mortality, poor quality of day-old chicks and high cost of health care (Anonymous, 1996). Colibacillosis

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is characterized by pansystemic involvement and great economic losses. It is responsible for high mortality during rearing, reduced weight gain and poor feed conversion (Anjum, 1997).

Collectively, infections caused by *E. coli* are responsible for significant economic losses to the poultry industry (Barnes *et al.* 2003). The multiplicity of disease occurring in broilers in which *E. coli* infection has been implicated represents one of the largest problems in poultry industry. Much work appears to have been done on various aspects *E. coli* infection including epidemiology, isolation and characterization, serology, drug resistance and chemotherapy. The present project was planned to study immunopathological and haematological effects of experimental infection with *E. coli* in broilers.

MATERIALS AND METHODS

Preparation of inoculum

E. coli was isolated from the birds suspected for colibacillosis. Identification and confirmation of isolated organisms was done on the basis of cultural, morphological and staining characteristics, sugar fennentation and biochemical reactions as described by Khan (2002). Pathogenicity of *E. coli* isolates was determined (Lee and Arp, 1998) and total viable count was made (Collins *et al.,* 1995).

Experimental design

A total of one hundred (day-old) broiler chicks were procured from local commercial hatchery. The birds were kept under standard managemental conditions for first 21 days of the experiment. On day-21 the chicks were randomly divided into two groups A and B having fifty chicks each. All the birds were vaccinated against Newcastle disease virus (NDV).

Group A

With the help of insulin syringe broth culture (0.5 ml/chick) of pathogenic *E. coli* ($3XI0^7$ bacilli/0.1 ml) was inoculated intraperitoneally in birds of group A on day-2l.

Group B

Chicks in this group acted as control. Sterile nutrient broth (0.5ml/chcik) was injected intraperitoneally on day-2l.

Collection of sample

The blood samples were taken from five randomly selected chicks of each group at 48, 72 and 96 hours post-infection. The blood was taken in two clean test tubes, one containing anticoagulant Ethylene diamine tetra acetate (EDTA) for hematological studies and other without anticoagulant, to separate serum. Serum samples were kept at -20°C till used for HA and HI tests. At the end of I experiment five randomly selected birds were

slaughtered and liver, heart and spleen were collected for pathological studies while bursa of Fabricius, spleen and thymus were taken and weighed.

Experimental parameters

The following experimental parameters were studied:

1. **Blood analysis**
   a) Haemoglobin determination: Haemoglobin was determined in blood by Sahli's method (Khan and Aslam, 2001).
   b) Packed cell volume (PCV): PCV percentage was estimated by using microhaematocrit method (Khan and Aslam, 2001).
   c) Erythrocyte and leukocyte count: Erythrocyte and leukocyte counts were determined by the methods described by (Khan and Aslam, 2001).
2. **Determination of antibody titre against NDV**
   Antibody titre against NDV in serum was determined by haemagglutination inhibition (HI) test as described by Thayer and Beard (1998).
3. **Pathological studies of different organs**
   a) Gross pathological examination: Liver, heart and spleen were collected at the end of experiment and were examined to record any gross pathological change.
   b) Histopathological examination: Liver, heart and spleen were processed for histopathological studies (Drury and Wallington, 1980).
4. **Lymphoid organ/body weight ratio**
   Lymphoid organs were weighed separately to determine the lymphoid organs weight/body weight ration by using following fonnula (Giambome and Closser, 1990):
   Organ-body weight ratio = weight of the organ/body weight x 1000
5. **Post-infection mortality**
   Post-infection mortality (%) in chicks of both groups was recorded.
6. **Estimation of feed conversion ratio (FCR)**
   At the end of experiment, FCR was calculated using the following formula (Singh and Panda, 1992):
   Feed Conversion Ratio = Total feed consumed/Total weight gained

Statistical analysis

Data collected were analyzed by applying unpaired t-test (Steel and Torrie, 1982).

RESULTS AND DISCUSSION

Escherichia coli is a large group of ecologically advantageous organisms and is able to grow aerobically and anaerobically. Coliform infection in birds is indicated by

depression, yellowish or greenish watery dropping, hyperthermia and gross lesions including mild to acute enteritis, perihepatitis, pericarditis, salpingitis and septicaemia.

The chicks of group A inoculated with *E. coli* became dull, depressed and took food and water very often. The chicks became dejected, had reduced weight gain and were pot bellied, The vent of these chicks was pasty with greenish feces.

Haematological alterations studies included haemoglobin (Hb) estimation, packed cell volume (PCV) and total erythrocyte (TEC) and leukocyte (TLC) counts (Table 1). There was a significant difference between these blood parameters in both treatment and control groups. Hb, PCV and TEC values were significantly lower while TLC values were significantly higher in treatment than control group on all the three sampling days. These findings are in line with the findings of Qazi (1989) who reported lowered Hb, PCV and TEC values and higher TLC values in chicks inoculated with *E. coli*.

Table 1: Mean (±S.E.) of different blood parameters of chicks of group A and B

Blood parameters	48 hrs post-infection		72 hrs post-infection		96 hrs post-infection	
	A	B	A	B	A	B
Hb estimation (gm/dl)	9.3±1.03^{a}	9.8±1.02^{b}	8.5±1.05^{c}	10.1±0.98^{b}	7.7±1.12^{d}	10.0±1.06^{b}
PCV (%)	24.7±1.07^{a}	26.4±1.10^{b}	22.7±1.05^{c}	26.9±1.10^{b}	20.3±0.92^{d}	28.4±1.09^{b}
Erythrocyte count (10^{6}/µl)	2.11±1.12^{a}	2.48±0.98^{b}	1.88±1.22^{c}	2.51±1.08^{b}	1.62±1.01^{d}	2.50±1.12^{b}
Leukocyte count (10^{3}/µl)	40.0±0.90^{a}	26.0±1.12^{b}	46.0±1.13^{c}	27.0±1.03^{b}	51.0±1.21^{d}	28.0±1.09^{b}

Mean with different superscripts across the rows are significantly different ($P<0.05$).
S.E = Standard error.

Geometric mean titres against NDV of group A and B on different sampling days are presented Table 2. On all sampling days antibody titres were significantly lower in treatment than control group. Our results are supported by the findings of Shah (2002) who reported a decreased antibody titre against NDV in chicks experimentally infected with *E. coli*.

Table 2: Geometric mean HI titre of chicks of group A and B

Group	48 hrs post-infection	72 hrs post-infection	96 hrs post-infection
A	226.0	189.3	174.6
B	368.2	352.4	371.8

The heart of treatment group was characterized by necrotic foci in cardiac muscles, thickened and inflamed pericardial sacs, discolouration of pericardial fluid and a fibrinous covering around it, There was mild to severe plasma cell infiltration. Epicardium

was edematous and epicardial sac was cloudy with light coloured exudates. The spleen of treatment group was enlarged markedly and congested. The liver from the infected birds was also enlarged and congested with green discolouration, There were multiple pale foci on liver, which were microscopically determined to be focal areas of early heterophilic, granulomatous hepatitis. Inflammatory cell infiltration, serous to fibrinous exudate, and cellular debris on serosal surfaces were present in the liver and spleen in treatment birds. Similar results in *E. coli* infection were also reported by Nakamura *et al.* (1985), Murakami *et al.* (1989), Fisher *et al.* (1998), Pourbakhsh *et al.* (1997) Anjum (1997) and Barnes *et al.* (2003).

The mean lymphoid organs (bursa of Fabricius, thymus and spleen) weights were higher while body weights were lower in treatment than control birds (Table 3). Thus mean lymphoid organs weight/body weight ratio was higher in treatment group as compared to control one, indicating a decreased confer of immunity in *E. coli* treated birds. Various workers have reported increased weight of bursa of Fabricius and thymus (Grizzle *et al.*, 1997) and spleen (Fisher *et al.*, 1998) in bacterial infections including *E. coli.* Reduced- body weight in *E. coli* infection is reported by Grizzle *et al.* (1997). Furthermore necrotic (Changlin *et al.*, 1996) and degenerative (Nakamura *et al.*, 1985) changes were also noted in bursa of Fabricius.

Table 3: Lymphoid organ weight/body weight ratio of chicks of group A and B

Group	Lymphoid organ weight/body weight ratio (Mean ± S.E)		
	Bursa of Fabricius	Spleen	Thymus
A	2.05 ± 0.04^{a}	2.29 ± 0.03^{c}	5.17 ± 0.07^{e}
B	1.83 ± 0.05^{b}	2.03 ± 0.02^{d}	3.47 ± 0.01^{f}

Means with different superscripts across the columns are significantly different ($P<0.05$).
S.E = Standard error.

Post-infection mortality percentage of group A and B is shown in Table 4. Up to day-21 no mortality was noted. In treatment group three birds died on day-2, four birds died on day-3 while five bird died on day-4 post-infection. In control group no bird died till the end of experiment. Thus total mortality in infected group was 24%. High mortality in *E. coli* infection is also reported by Leitner and Heller (1992) and Anjum (1997).

Table 4: Post-infection mortality percentage of organs of chicks of group A and B

Group	Total birds	Live birds	Dead birds	Mortality (%)
A	50	38	12	24.0
B	50	50	0	0.0

Feed conversion ratio was lower in treatment group than that of control group (Table 5). These findings are in line with the findings of Anjum (1997). It is tempting to speculate that poor FCR has resulted in reduced weight gain in bacterial infection.

Table 5: Feed conversion ratio of chicks of group A and B

Group	48 hrs post-infection	72 hrs post-infection	96 hrs post-infection
A	2.35	2.37	2.40
B	2.17	2.23	2.19

From the above discussion it can be concluded that infection with *E. coli* resulted not only in haematological and pathological alterations but it also impaired the immune system along with high mortality in infected birds.

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EFFECTS OF ACUTE AND CHRONIC EXPOSURES TO DIAZINON ON THE FECUNDITY AND EGG SIZE IN *COLISA FASCIATA*

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Abstract: The fecundity and egg size of a teleost, *Colisa fasciata* following acute and chronic exposure to different concentrations of Diazinon was investigated. Adult fish were exposed to Diazinon of concentrations 16.0 mg/l (96 R-LC_{50}), 12.0 mg/l ($LC_{37.5}$) and 6.0 mg/l ($LC_{18.75}$) for 96 hours and 2.6 mg/l (30 day-LC_{50}) and 1.3 mg/l (LC_{25}) for 30 days during the preparatory phase of the ovary. The fecundity was found to decrease by 60%, 42% and 19% in acute exposure of dose concentrations of 16.0 mg/l, 12.0 mg/l and 6.0 mg/l, respectively. In chronic exposures of doses 2.6 mg/l and 1.3 mg/l the fecundity was found to decrease by 61% and 43%, respectively. The egg size was investigated to correlate directly with the increase in dose concentrations. There was 10.6% increase in egg size in 16.0 mg/l, 6.66% in 12.0 mg/l and 3.9% in 6.0 mg/l in acute exposure, whereas in chronic exposure of Diazinon it was significant in 2.6 mg/l and non-significant in 1.3 mg/l as compared to the control.

Key words: Diazinon, environmental pollution, fecundity, egg size, *Colisa fasciata*.

INTRODUCTION

Apart from the industrial run off, the indiscriminate use of pesticides has further polluted the aquatic environment resulting in a wide range of problems that need to be resolved. These involve not only the identification of possible contaminants having harmful effects on fish and other aquatic organisms but also determination of toxic levels of contaminants and their tolerance by species of different age groups.

Since chlorinated pesticides have been proven to have many deleterious effects on non-target organisms (Renata and Arnese, 1988), there has been a shift towards a greater use of organophosphorus pesticides in agriculture sector because of their biodegradable and non-cumulative properties. But unfortunately, these pesticides too have been quite harmful to the non-target organisms (Durham and Williams, 1972; Jennings *et al.*, 1975; Harbison, 1975). Organophosphorus pesticides have been shown to cause gross morphological (Henderson and Pickering, 1958; Butler *et al.*, 1969; McCann and Jasper, 1972), histopathological (Couch, 1975; Sastry and Sharma, 1981) and biochemical (Saxena *et al.*, 1988; Asztalos *et al.*, 1988; Nemcsok and Benedeczky, 1990) effects of fish. However, the effect of acute exposure to organophosphorus insecticde on the fecundity of fish is little known. In the present study, the effects of short term exposure to

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the organophosphorus insecticide. Diazinon on the fecundity and egg size in teleost fish, *Colisa fasciata* have been investigated. This acute toxicity bioassay would provide an information in establishing the water quality criteria and to estimate a safe level for fish reproduction and fecundity.

MATERIALS AND METHODS

Female fish of almost equal length ranging from 8.3-8.8 mm were selected from the acclimated fish stock. The fish were divided into seven groups of twenty individuals each and were kept in 200 l water tanks containing water of pH 7.6; total hardness 130 mg/L as $CaCO_3$; D.O. 5.0-5.5 mg/L. Experiments for the study of acute exposures three groups were exposed to Diazinon of concentrations of 16.0 mg/L (96 h-LC_{50}), 12.00 mg/L ($LC_{37.5}$) and 6.0 mg/L ($LC_{18.75}$) for 96 h while the fourth group without any treatment served as control. Fifth and sixth groups were exposed to 2.6 mg/L (30 day-LC_{50}) and 1.3 mg/L for the study of chronic exposures. The seventh group was served as control. The LC_{50s} were calculated by Litchfield and Wilcoxon method (1949). The experiment was started in the last week of February, during the preparatory phase of the ovary. During exposure period, the fish were not fed. Dead fish were removed from the tanks daily. After exposure, continuous supply of fresh water and daily feeding were maintained till the fish became fully gravid, in the last week of May. The ovaries were then removed as a whole from each gravid fish of the exposed and control groups. After weighing the ovaries were placed in separate vials containing Gilson's fluid. After 2-3 weeks of preservation in this fluid, during which vials were periodically shaken, the ovaries were broken manually and by repeated washing, the tissue debris was removed and eggs separated from one another. The eggs were then stored in vials containing 5% formaline until they were counted and measured. For counting the eggs were whirled in one litre water and at least five sub-samples of 1/30 each were counted. The counts were averaged and multiplied by appropriate conversion factor to achieve total count. The diameter of at least 20 randomly selected eggs from each fish were measured in μm by ocular micrometer. GSI was also calculated by the following formula:

$$GSI = \frac{\text{Wt. of ovary}}{\text{Wt. of fish}} \times 100$$

The statistical significance of the data was tested using student's 't' test.

RESULTS

Acute exposure

Fecundity estimates of control and treated groups exposed to different sub-lethal concentrations of Diazinon are given in Table I. The fecundity of the control ovary was

the organophosphorus insecticide. Diazinon on the fecundity and egg size in teleost fish, *Colisa fasciata* have been investigated. This acute toxicity bioassay would provide an information in establishing the water quality criteria and to estimate a safe level for fish reproduction and fecundity.

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RESULTS

Acute exposure

Fecundity estimates of control and treated groups exposed to different sub-lethal concentrations of Diazinon are given in Table I. The fecundity of the control ovary was

2880-7320 eggs with a mean of 4654 eggs for ten fish of 8.73 ± 0.2 cm in length. In samples of fish exposed to different sub-lethal concentrations of Diazinon, fecundity decreased significantly ($P<0.001$) with the increase in dose concentration (Fig.1), particularly when treated during preparatory phase of the ovary. As compared to the control there was 19%, 42% and 60% decrease in fecundity on exposure to 6.0 mg/L, 12.0 mg/L and 16.0 mg/L of Diazinon, respectively. GSI was also found to decrease with the increase of dose concentration in lower dose groups. In the higher dose group (16 mg/L) the GSI was found to increase but the fecundity was 60% less as compared to the control (Fig.2).

Egg size was also found to be dose dependent. The diameter of the 100 control eggs measured, 10 eggs from each fish sample, ranged from 630-705 μm with the mean and SD (660 ± 19.7). In treated groups of eggs there was a significant ($P<0.001$) increase in egg size (Table I). The data collected from the treated groups of fish revealed an increase in egg size of 3.9% in 6.0 mg/L, 6.66% in 12 mg/L and 10.6% in 16 mg/L Diazinon concentration when compared with the control (Fig.3).

Chronic exposure

Fecundity estimates of chronically exposed groups of fish are also given in table. The mean egg number of fish exposed to 1.3 mg/L (30 day LC_{25}) was found to be 5659 ± 745, which was 43% less than that of control. Fecundity of fish exposed to 2.6 mg/L (30-day LC_{50}) was found to be further reduced to 1814 ± 433 which was 61% less as compared with the control.

The GSIs of both the treated groups of 1.3 mg/L and 2.6 mg/L were also found to be reduced significantly ($P<0.05$ and $P<0.001$, respectively).

As far as the egg size is concerned, the change in diameter of eggs was not significant in both the doses of acute and chronic treatment, however, in high dose group the range in diameter of eggs was almost double as compared with the control.

DISCUSSION

Fecundity studies on various species such as the European long rough dab, *Hippoglossoides platessoides* (Bagenal, 1957), Pacific herring, *Clupeaharengus pallasi* (Nagasaki, 1958), Atlantic cod, *Gadus morhua* (May, 1967) and yellow tail flounder, *Limanda ferruginea* (Pitt, 1971) have demonstrated that variations in the fecundity of these species can be adequately explained in terms of body length alone. Winters (1966) has reported that in capelin, age rather than length is the dominant factor. Previous studies (Templeman, 1948; Pitt, 1958) indicate that caplin grows rapidly up to the onset of maturity after which growth is considerably retarded and length increments become small. Hodder (1963) proposed that fish that have previously spawned have a greater fecundity than fish of the same age and size spawning for the first time. There is a clear evidence from the published data that in caplin, as the age increases the proportion of repeat spawning also increases without a significant increase in length. There are also inter- and

intra-specific differences in fecundity of fish, however, the higher or lower rate of fecundity depends upon the length, weight, GSI, age of fishes and repeated spawning (Singh *et al.*, 1982; Zirkov, 1984; Baloni, 1986) and environmental factors such as temperature or chemical stresses (Devauchelle, 1981). In present study all considerations were taken into accound in the selection of fish.

Table I: The effects of various sub-lethal doses of Diazinon on the GSI, ova number and size in *Colisa fasciata*.

Dose (mg/L)	Av. Length of fish (cm)	Av. Wt. of fish (g)	Av. Wt. of ovary (g)	GSI	Ova No.		Ova Size	
					Range	Mean ± S.D	Range	Mean ± S.D
Control	8.73± 0.2	11.19± 1.3	1.43± 0.37	12.66± 2.25	2880-7320	4654± 1112	630-717	660± 19.7
Acute treatment								
6.0	8.78± 0.20	11.168± 0.95	1.02± 0.41	9.9± 2.3	2016-5538	3763± 1230	637-714	686± 18
12.0	8.73± 0.2	11.27± 0.74	0.82± 0.30	7.31± 2.8	1160-5200	2699± 1556	660-756	704± 35
16.0	8.71± 0.22	10.89± 0.97	1.04± 0.30	9.58± 2.8	1056-2800	1857± 640	699-766	730± 25
Chronic treatment								
1.3	9.06± 0.32	10.69± 0.76	0.921± 0.30	9.109± 2.3				
2.6	9.15± 0.34	10.87± 0.77	0.643± 0.36	5.874± 3.2				

Little information is available on the possible lethal, sub-lethal and chronic effects of insecticides and other pollutants on fish fecundity (Trojnar, 1977; Barron and Adleman, 1984). However, the effects of radiation such as X-rays, metal toxicants and Dieldrin on early life stages of fishes have been investigated.

Foster *et al.* (1949) found significant decrease in fecundity of rainbow trout from parents subjected to 50 and 100 rhontgen (R) of X-rays, 3 months before spawning. The growth in weight of rainbow trout fry was impeded by X-rays of doses as low as 100 R (Kobayashi and Mogami, 1958). Till (1978) reported that ^{238}Pu (Plutonium) was uniformly distributed throughout the perivitelline fluid and yolk in the eggs of carp and fat-head minnow when exposed chronically to ^{238}Pu. Lead (Holcombe *et al.*, 1976) was shown to penetrate the chorion only slightly and hardly to accumulate in the embryos of brook trout although the exposure was at the egg stage. In experiments with Cobalt, Kunze *et al.* (1978) showed that ^{57}Co accumulated rapidly during the first 24 h, but almost all the accumulated Cobalt was reversibly bound by mucopolysaccharide in the chorion. Preferential accumulation in the chorion was also found for Cadmium (Mitchibata, 1981) and Zinc (Wedemeyer, 1968) in the embryo of the teleost, *Oryzias latipes*. Therefore, the chorion appears to 'protect' the embryo from the uptake of toxicants not by completely

preventing but by slowing down the intrusion. Aromatic hydrocarbon (Korn and Rice, 1981) and tetra-chlorobiphenyl (Guiney *et al.*, 1980) are also known to accumulate primarily in the yolk. In experiments with Dieldrin, Van Leeuwen *et al.* (1985) investigated its accumulation in the rainbow trout egg-yolk after short term exposure during egg-stage. With the resorption of yolk, these chemicals redistribute thus causing the death of juveniles of rainbow trout (Seinen *et al.*, 1981). It can thus be speculated that yolk acts as a temporary 'toxicant sink'. This is in support of the hypothesis proposed by Longwell (1977) who postulated that pollutants enter the egg with imbedded water during the process of perivitelline fluid formation. This holds true with the present study that increase in concentration of Diazinon possibly increases the accessibility of the pollutant in the egg and, therefore, the toxicity. This appears in the form of decrease in fecundity and GSI, and increase in the size of the egg.

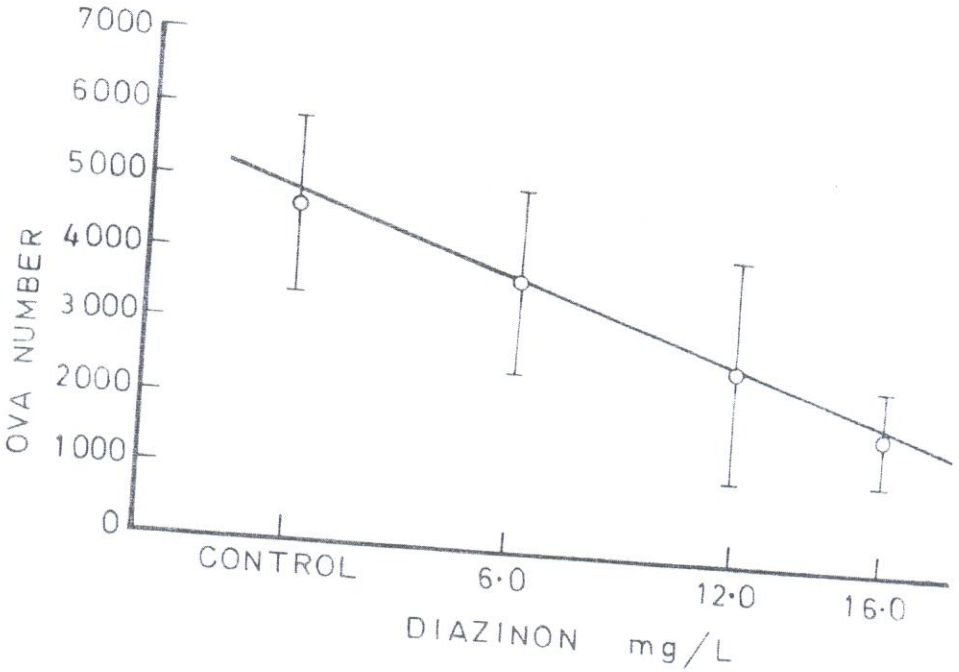

Fig. 1: Eggs (mean ± S.D) produced by *Colisa fasciata* after exposure to various concentrations of Diazinon.

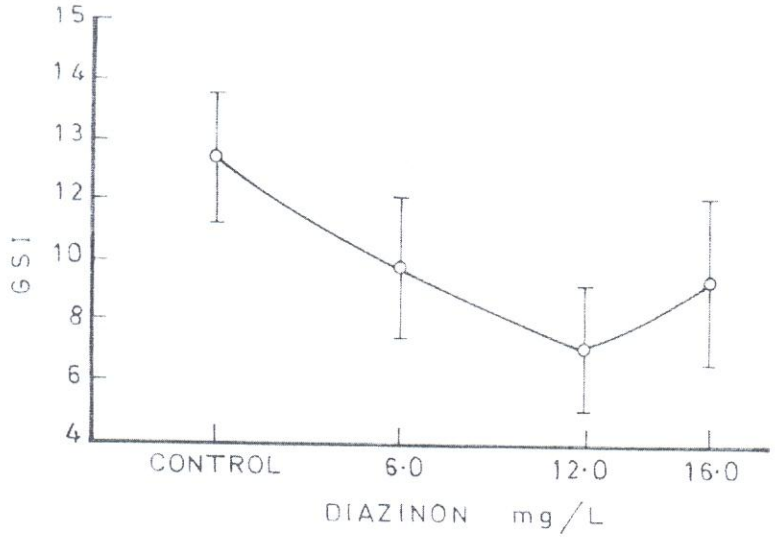

Fig. 2: **GSIs (mean ± S.D) of *Colisa fasciata* following the treatment with various concentrations of Diazinon.**

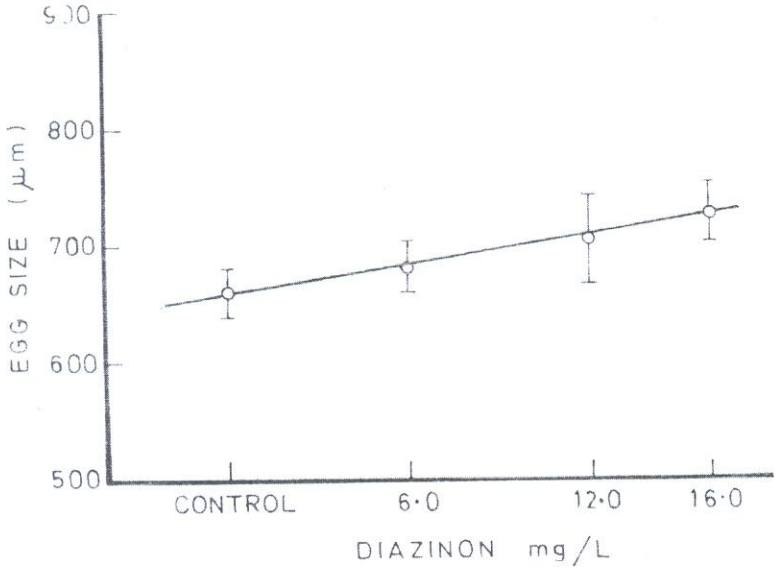

Fig. 3: **Size of the eggs (mean ± S.D) produced by *Colisa fasciata* after the treatment of various concentrations of Diazinon.**

The decreased fecundity and GSI in treated fish may be attributed to either reduced vitellogenin or other proteins, glycogen and other carbohydrates and lipids to be accumulated in the oocyte cytoplasm. Insufficient production of ganadotrophins in the Diazinon treated fish may also be one of the causes of low fecundity, since, it is well established that in teleosts, oocyte growth and maturation is accomplished by the precise production and regulation of ganotrophin and gonadal hormones.

This study thus adds new evidence to support that Diazinon has inhibitory effect on the ovarian growth and development because of its accumulative properties.

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SCANNING ELECTRON MICROSCOPY (SEM) OF EGG OF *PROTEOCEPHALUS FILICOLLIS* RUDOLPHI (CESTODA, PROTEOCEPHALIDEA)

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Abstract: Scanning electron microscopic study of eggs of *Protocephalus filicollis* has demonstrated that the outer surface of the egg has invaginations and gives an irregularly contoured appearance. The external surface of the outer envelope of the embryo has shown to have two forms of surfaces on opposite sides. This is the first study of its type on this fish cestode eggs.

Key words: *Proteocephalus filicollis*,, scanning electron microscopy (SEM) of eggs.

INTRODUCTION

The ultrastructure and the development of the egg and embryonic envelopes of proteocephalid cestode have been little studied. Formation of four main embryonic envelopes, the capsule, outer envelope, inner envelope and oncospheral membrane around the developing embryo of *Proteocephalus longicolis* is described (Swiderski and Subilia, 1978).

Proteocephalus filicollis is a host specific parasite of temperate freshwater fish three-spined stickleback, *Gastersteus aculeatus* L.. The fish is final host of the parasite. Ultrastructure of the egg and embryonic envelopes of *P. filicollis* are well described (Iqbal and Wootten, 2002), Fine structure of spermatozoon of *P. filicollis* is described by (Iqbal, 2003). Present study was aimed to understand the fine and superficial structure of the outer envelop of the egg of *P. filicollis* by histology and Scanning Electron Microscopy.

MATERIALS AND METHODS

Histology

Sampling of fish, procurement of the gravid worms is given by Iqbal and Wootten (2002). For the study of the orientation of the eggs of P. *filicollis* the gravid worm were cut into very small pieces. The pieces from the posterior of the worm were fixed in 10% buffered formalin for at least 24 hrs. The fixed material was automatically

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processed in Histokinette 2000. Sections of wax embedded worms were cut at 5-6 µm. The cut sections were spread and floated on a water bath and then placed on a clean glass slide. The glass slide was placed face down on a hot plate. Sections were stained with hamatoxylin and eosin.

Scanning electron microscopy (SEM)

Proteocephalus ,filicollis eggs were collected in water in a syringe and deposited on a Sartorius polymied filter with a pore diameter of 0.45 µm. The filter membrane with the eggs was put into a Petri dish and flooded with 1% glutaraldehyde buffered with 0.1 M sodium cacodylate and left at 4°C for one hour, after which the solution was replaced with 3% glutaraldehyde buffered with 0.1 M sodium cacodylate at 4°C, in which the specimen was kept for further two days. The eggs were than washed well in sodium cacodylate buffer and post fixed in 1 % osmium tetra oxide in 0.1M sodium cacodylate for two hours at room temperature. The specimen were dehydrated through an acetone series and then transferred to a mixture of 50% Peldri (Ted Pella Inc, Reading California) and 50% acetone in the fume cupboard for an hour. This was replaced by full strength Peldri for an hour, after which the Petri dish was placed on ice to solidify he Peldri. The peldri was sublimed off in the fume cupboard overnight. The filter was then mounted on an aluminum stub and sputter coated with gold in an Edwards 150 B spurt coater, before being examined in Philips 500 scanning electron microscope at 6 Kv.

RESULTS AND DISCUSSION

The uterus of gravid *P. filicollis* consists of a number of diverticula which contain eggs. Eggs are not tightly packed and are distributed through out the diverticula (Fig.1). When viewed by SEM the outer float membrane of *P. filicollis* egg is not swollen but is stretched as a sheath from the outer envelope (Fig.2). The external surface of the outer envelope has an irregularly contoured appearance with small invaginations and pits. In a few cases the external surface of the outer envelope showed two forms of surface sculpturing on opposite side of the embryo, one with broad invaginations and other with more wrinkled appearance (Fig.3, 4).

Scanning electron microscopy *ot P. filicollis* eggs provides little information about the membranes surrounding the oncosphere. The stretched appearance probably represents the capsule, which have obtained this shape during SEM processing. The membranous and somewhat folded structure of *P. filicollis* capsule allows it, to swell to form the float membrane when it comes into contact with water. Similarly, when the outer capsule of *Shipleya inermis* is fully formed it is folded much as a packed parachute, with a very large surface area contained in a small volume (Coil, 1977). An explanation of the invaginations or pits observed on the outer surface of the oncosphere of *P. filicollis* is somewhat problematical. However, it is reported that in cestode having the aquatic phase of their life cycle in freshwater, the egg shell is only superficially pitted (Hillhead, 1972). In the same way, it was found that in the eggs of *Shipleya inermis* the outer surface of the

outer capsule is relatively smooth but has numerous pits (Coil, 1977). The outer envelope of the oncosphere of mice tapeworm *Hymenolepis nana* has been found to have irregular contoured appearance marked by numerous pits (Fairweather and Threadgold, 1981). They also reported that much wider and deeper crests were present in the outer envelope and suggested that this may indicate that the layer is undergoing some degree of degeneration. A similar explanation may account for the surface pits on tbe eggs of *P. filicollis*. The two types of surface sculpturing on opposite sides of the egg of *P. filicollis* seen in the present study are difficult to explain and they may represent a processing artifact.

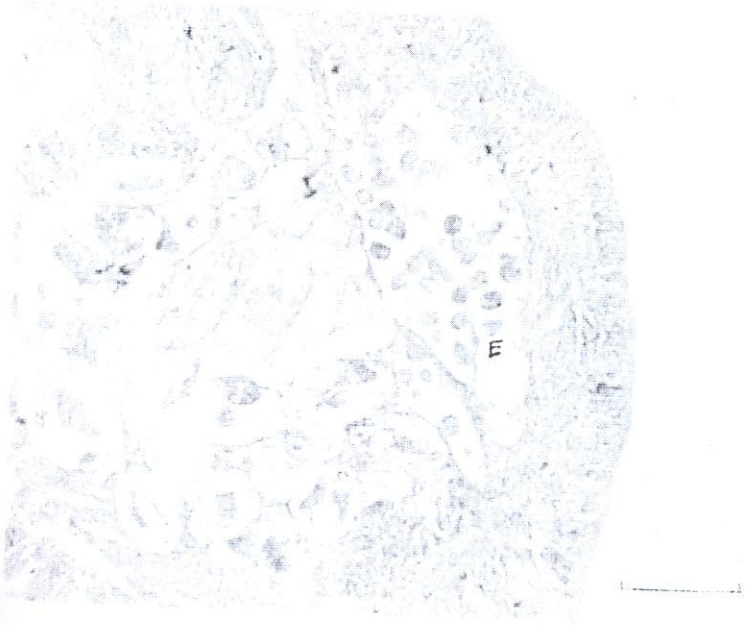

Fig.l: A transverse section of gravid proglottid of *Proteocephalus filicollis* showing eggs (E) in the uterus (H & E), Scale bar = 100 μm).

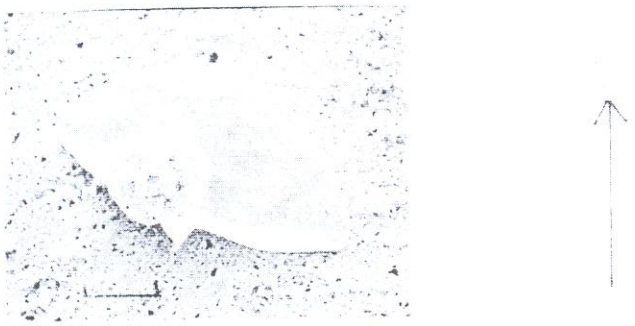

Fig. 2: SEM micrograph of egg of *Proteocephalus filicollis*. The probable capsule (C) is seen stretched as a sheet (Scale bar = 5 μm).

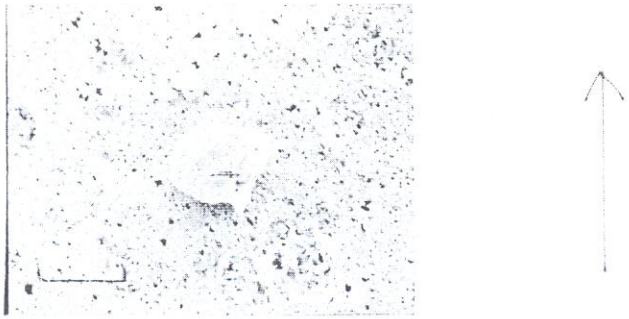

Fig. 3: SEM micrograph of egg of *Proteocephalus filicollis* showing external surface with irregularly contoured appearance (Scale bar = 7.5 μm).

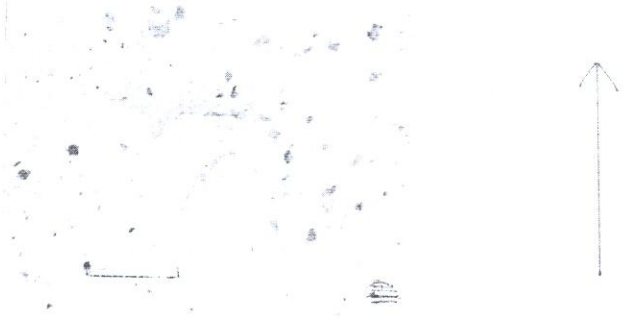

Fig. 4: SEM micrograph of egg of *Proteocephalus filicollis* showing different Sculpturing on opposite sides of the embryo (Scale bar = 5 μm).

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MORPHOMETRIC STUDIES ON *OREOCHROMIS NILOTICA* (MALE) IN RELATION TO BODY SIZE FROM ISLAMABAD, PAKISTAN

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Abstract: Twenty nine *Oreochromis nilotica* (♂) of different body sizes ranging from 7.0-25.5 cm total length and 6.1 - 328.8gm body weight were used for the analysis of morphometric variable of head length, head width, pectoral fin length, dorsal fin length, body girth, body depth, tail length and width in relation to total length and body weight of the fish to investigate allometric growth. It was observed that all these relations showed very high correlations. Slopes of the log transformed data were used to compare with an isometric slope (b = 1 or b = 0.33 or b = 3). It was found that all the parameters examined showed isometric growth except head length, dorsal fin length and tail length, which showed positive allometry in relation to total length and body weight. Growth in weight is almost proportional to the cube of its length, the values of the slope (b = 3.1) coincide with the slope of an ideal fish. Regression parameters were found to be highly significant.

Key words: *Oreochromis nilotica*, Length-Weight relationship, Condition factor; Predictive equations.

INTRODUCTION

Oreochromis nilotica is an exotic species. It was introduced in Pakistan in 1984 from Thailand. This species originated from upper Nile in Uganda. It also colonized Central and West Africa. It feeds on blue green algae and can assimilate 70-80 % of the carbon ingested. In environments having suitable temperature conditions, they are able to establish and stable populations contributing to the local fishery resources (Salam *et al.*, 1996). Keeping in view the importance of this species, it is urgently needed that the biology of this species be thoroughly studied.

A fish can change its weight without changing in length or vice versa. The relationship between weight and length for fish of a given population can be analyzed either by measuring weight and length of the same fish throughout their life or of a sample of fish taken at a particular time (Wootton, 1990, 1998).

The weight-length relationship provides an opportunity to calculate an index commonly used by fisheries biologists to compare the "condition factor" or "well being" of a fish (Bagenal and Tesch, 1978)

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Fish with a high value of "K" are heavy for its length, while fish with a low "K" value are lighter (Weatherley, 1972; Bagenal and Tesch, 1978; Weatherley and Gill, 1987; Wooton, 1990, 1998).

Several studies on length-weight relationship have been reviewed by LeCren, 1951; Sarkar, 1957; Chakrabarty and Singh, 1963; Saigal, 1964; Willis, 1988; Wootton, 1990, 1998). The present topic has received attention in Pakistan (Salam and Janjua, 1991; Naeem, *et. al.*, 1992, 2000; Salam *et. al.*, 1993, 1994; Ali *et. al.*, 2000, 2002). The present study is the first attempt in assessing length-weight, condition factor and growth allometry of an introduced exotic fish *Oreochromis nilotica* (a), is becoming important as food fish and monosex culture in farming system of Pakistan.

MATERIAL AND METHODS

Twenty-nine farmed *Oreochromis nilotica* (♂) of different body size ranging from 7.0-25.5 cm total length and 6.1-328.8 gm body weight were sampled from reservoir of Fish Seed Hatchery, Islamabad. Fish were selected at random and caught using a hand net. They were transported live to the laboratory in plastic containers. Fishes were killed, blotted dry and weighed to nearest 0.01 g on an electronic digital balance. Body length measurements were taken to nearest 0.1 cm by using Perspex measuring tray having a millimeter scale. Total length was taken as the length from tip of the snout to the tip of the caudal fin. Head length as the distance from the most anterior point on snout to the posterior edge of opercula bones, head width from broadest part of the head, pectoral fin length from dorsal base of pectoral fin, dorsal fin length between anterior and posterior end along the base of fine, body depth from dorsal and ventral surface at deepest point, body girth circumference of body at its deepest point, tail length as difference between total length and standard length and tail width as maximum width of caudal fin were measured. Condition factor was calculated using a formula $K = 100 \times W/L^3$ following the method of Weatherley and Gill (1987) and Wootton (1990, 1998).

Statistical analysis, including regression analysis and calculation of correlation was carried out by using a computer package Lotus 1-2-3 and Excel.

RESULTS

The relationship between wet body weight (W) and total length (L) is exponential having the general form $Y = aX^b$, (Fig I), or $W = aL^b$. When the data is transformed in logarithmic form (Fig.2) a linear relationship is obtained with a high correlation coefficient ($r = 0.998$; $P<0.001$), having the general form:

$$\text{Log } W = \text{Log } a + b \text{ Log } L.$$

The values of these constants and other regression parameters are given in (Table- I) .The regression coefficient "b" has a value almost equal to b =3.0.

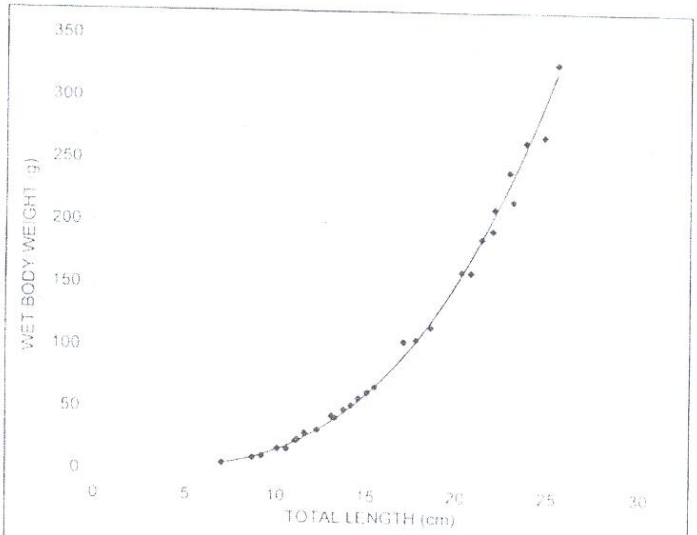

Fig. 1. Relationship between total length (cm) and wet body weight (g) in *Oreochromis nilotica (male).*

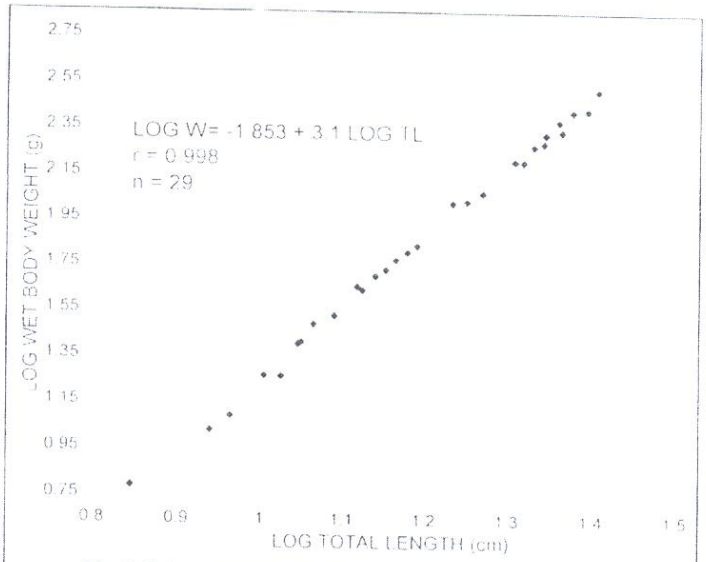

Fig. 2. Relationship between log total length (cm) and log wet body weight (g) in *Oreochromis nilotica (male).*

Table I: The regression parameters of body weight (W) on total length (TL) for *Oreochromis nilotica* (♂)

Regression equation	No. of observation (N)	Correlation coefficient (r)	Proportion of variance accounted for by the regression (r^2)	S.E. (b)	t-value when compared with b=3
Log W = -1.853 + 3.1 log TL	29	0.998***	0.996	0.0337	0.296^{NS}

*** P<0.001

Table II: The regression parameters of condition factor (K) on wet body weight (W) and total length (TL) for *Oreochromis nilotica* (♂)

Regression equation	No. of observation (N)	Correlation coefficient (r)	Proportion of variance accounted for by the regression (r^2)	S.E. (b)	t-value when compared with b=3
K = 1.818 – 0.0171 TL	29	0.506**	0.257	0.0056	3.053**
K = 1.625 + 0.0007 W	29	0.408**	0.167	0.0003	2.333*

** P<0.01; * P<0.05.

Condition factor "k" when plotted against total length and wet body weight, shows an decreasing trend with increasing length and increasing trend with increasing weight (Table-II). When the data of head length (HL), head width (HW), pectoral fin length (PFL), dorsal fin length (DFL), body depth (BD). body girth (BG), tail length (TLL) and tail width (TLW) was plotted against total length and wet body weight, these relationships were found to be highly significant, though in all these cases log transformed data generated high correlation coefficient (Table III-IV).

Table III: **The regression parameters of head length (HL), head width (HW), pectoral fin length (PFL), dorsal fin length (DFL), body depth (BD), body girth (BG), tail length (TI.L), tail width (TL.W) on total length (TL) and wet body weight (W) for *Oreochromis nilotica* (♂)**

Regression equation	No. of observation (N)	Correlation coefficient (r)	Proportion of variance accounted for by the regression (r^2)
HL = 0.224 + 0.239 TL	29	0.994***	0.988
HW = -0.086 + 0.166 TL	29	0.965***	0.932
PFL = -0.255 + 0.307 TL	29	0.962***	0.925
DFL = -0.724 + 0.511 TL	29	0.998***	0.996
BD = 0.022 + 0.375 TL	29	0.987***	0.974
BG = 0.044 + 0.750 TL	29	0.987***	0.974
TI.L = 0.236 + 0.162 TL	29	0.975***	0.952
TLW = -0.086 + 0.1662 TL	29	0.965***	0.932
HL = 2.676 + 0.013 W	29	0.962***	0.926
HW = 1.598 + 0.009 W	29	0.950***	0.904
PFL = 2.836 + 0.017 W	29	0.962***	0.926
DFL = 4.511 + 0.028 W	29	0.965***	0.932
BD = 3.868 + 0.020 W	29	0.955***	0.912
BG = 7.737 + 0.041 W	29	0.955***	0.912
TI.L = 1.875 + 0.009 W	29	0.971***	0.943
TI.W = 3.639 + 0.018 W	29	0.956***	0.915

*** P<0.001.

Table IV: **The regression parameters of head length (HL), head width (HW), pectoral fin length (PFL), dorsal fin length (DFL), body depth (BD), body girth (BG), tail length (TLL), tail wirth (TLW) on total length (TL) and wet body weight (W) for *Oreochromis nilotica* (♂)**

Regression equation	No. of observation (N)	Correlation coefficient (r)	Proportion of variance accounted for by the regression r^2	S.E. (b)	t-value when compared with b=1.00 or b=0.33
Log HL = -0.506 + 0.926 Log TL	29	0.994***	0.988	0.0191	3.874***
Log HW = -0.796 + 1.000 Log TL	29	0.963***	0.928	0.0535	$0.000^{N.S}$
Log PFL = -0.538 + 1.001 Log TL	29	0.963***	0.928	0.0575	$0.017^{N.S}$
Log DFL = -0.456 + 1.101 Log TL	29	0.998***	0.997	0.0097	10.412***
Log BD = -0.439 + 1.012 Log TL	29	0.988***	0.977	0.0292	$0.410^{N.S}$
Log BG = -0.138 + 1.012 Log TL	29	0.988***	0.977	0.0292	$0.410^{N.S}$
Log TLL = -0.591 + 0.869 Log TL	29	0.976***	0.953	0.0370	-3.600***
Log TLW = -0.359 + 0.918 Log TL	29	0.967***	0.936	0.0459	$-1.822^{N.S}$
Log HL = 0.050 + 0.297 Log W	29	0.991***	0.982	0.0077	-4.285***
Log HW = -0.194 + 0.320 Log W	29	0.958***	0.918	0.0183	$-0.552^{N.S}$
Log PFL = 0.063 + 0.321 Log W	29	0.959***	0.920	0.0181	$-0.497^{N.S}$
Log DFL = 0.204 + 0.353 Log W	29	0.996***	0.993	0.0055	4.181***
Log BD = 0.165 + 0.326 Log W	29	0.990***	0.981	0.0085	$-0.470^{N.S}$
Log BD = 0.466 + 0.326 Log W	29	0.990***	0.981	0.0085	$-0.470^{N.S}$
Log TLL = -0.068 + 0.278 Log W	29	0.971***	0.943	0.0130	-4.000***
Log TLW = 0.191 + 0.295 Log W	29	0.965***	0.933	0.0151	$-2.317^{N.S}$

*** P<0.001; N.S P<0.05

Table V: Length-weight relationship for different fish species from different localities

Fish species	Slope (b)	Reference
Labeo rohita Immature	3.01	Jhingran, 1952
Labeo rohita Ripe females	3.38	Khan, 1972
Labeo rohita Immature	3.06	Salam and Janjua, 1991
Cirrhinus mrigala	3.02	Salam and Khaliq, 1992
Labeo bata	3.17	Chatterji *et al.*, 1977
Gadusia chapra	3.06	Venkateswarlu & Banerjee, 1971
Clarias batrachus	3.33	Sinha, 1975
Oncorhynchus mykiss	2.98	Salam *et al.*, 1994
Oncorhynchus mykiss	3.12	Naeem *et al.*, 2000
Aristichthys nobilis	2.80	Salam *et al.*, 1993
Oreochromis nilotica Males & Females	2.99	Naeem *et al.*, 1992
Oreochromis nilotica Males	3.10	Present study

DISCUSSION

A review of the literature on different fish species collected from commercial as well as from natural waters shows that there is a tendency for their regression coefficient (b) in the relation $W = aL^b$ to be close to or greater than b = 3.0. Thus growth in many cases tends to be isometric (Salam *et. al.*, 1994., Wootton, 1990) since b = 3.0 for isometric growth. In the present study value of b = 3.1 which is not significantly different from b = 3.0 (the slope for an ideal symmetrical fish). Regression parameters were found to be highly significant (Table I).

Condition factor (K) shows decreasing trend with increasing length and increasing trend with increasing weight in the present study. The condition factor may vary with increasing length when average weight of fish does not increase in direct proportion to the cube of its length (Salam *et. al.*, 1994). Therefore when b =3.0 K remains constant, if however the weight increase more rapidly than cube of length, the K would increase with increase in length. When weight increases less than the cube of length, K would tend to decrease with the growth of the fish (Naeem *et. al.*, 2000).

The species under study *Oreochromis nilotica* (♂) is the ideal fish because value of slope "b" of length-weight relationship is not significantly different from b = 3.0 (the slope for an ideal symmetrical fish). This species has been well adapted in aquatic environment of Pakistan after its introduction.

During, growth changes in size bring about changes in shape and body proportions. Allometric exponents on log-log scale relating body weight to the length of body parts is b = 0.33 and body length to length of body parts b = 1.0 representing isometric growth relationship (Alexander, 1971). In this study values of "b" of various relationships as given in table-IV showed isometric growth except head length, dorsal fin length and tail length which showed positive allometry in relation to total length and body

weight. The reason for isometric growth is due to the proportionate growth of weight and length parameters, while head length, dorsal fin length and tail length which showed positive allometry in relation to total length and body weight. This is due to the fact that this species is also showing trend of faster growth and its organs/parts are also growing faster.

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REPORT ON *CERVUS SIVALENSIS* FROM THE UPPER SIWALIKS OF PAKISTAN

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Abstract: A well preserved right mandibular ramus with P_3-M_3 from Sardhok (Upper Siwaliks), Gujrat district, the Punjab province, Pakistan is being described for the first time. A comparison of the specimen under study with known material of the genus *Cervus* has shown that it is referable to the species *Cervus sivalensis*. This description gives additional information about the palaeozoogeography of cervids in the Siwaliks.

Key words: Premolars, molars, *Cervus*, Sardhok, Palaeozoogeography.

INTRODUCTION

The cervids are characterized by the presence of antlers and prominent lacrymal depressions anterior to the eyes that are occupied by the pre-orbital glands in the living animals. They appeared in the Siwalik sequence of Indo-Pakistan during Plio-Pliestocene times. Earlier studies of the Siwalik cervids based upon dentitions and antlers have recognized five to six species. Some of these species were though time-successive (Arif and Raza, 1991).

There are 6-8 species in South Asia (Indo-Pakistan subcontinent) and are mostly adapted to open woodland habitat (Roberts, 1977). The cervids show similar species diversity in the fossil record too. Their fossils are known from the Upper Siwalik sequence of the Kohat-Potwar basin and the adjoining basins of Jammu-Kashmir and the Indian-Punjab. The Siwalik cervids have been studied by Lydekker (1876, 1884), Brown (1926), Colbert (1935), Azzaroli (1954), Akhtar (1998) and Akhtar *et al.* (1999).

The cervids appeared in Oligocene with small size and lack of antlers. Early small cervids, e.g., *Eumeryx* and *Iberomeryx*, appeared in the Middle Oligocene sediments of Central Asia from where they dispersed to Europe and North America, most probably, in the early Miocene. The first appearance of cervids in South America and Africa has been reported from the Pliestocene (Romer, 1974).

The Siwalik rocks are fossiliferous throughout and thus contain an almost continuous record of mammalian evolution spanning 18 million years (Barry *et al.*, 1982). The Miocene faunal turn-over events introduced immigrants into South Asia mainly from Africa whereas the Pliocene events record mammalian faunas closely similar to contemporary ones in Northern and Western Eurasia (Barry and Flynn, 1990).

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Several species of the family Cervidae have been described mainly from the Upper Siwalik rocks of the Western Himalayas including the Siwalik Hills and adjoining ranges in India and southern Kashmir, Potwar and Trans-Indus Hill ranges of Pakistan. The earlier identifications were based on a few fragmentary specimens and their holotypes designations include maxillary fragments. The taxonomic details of Siwalik cervids given by Lydekker (1876, 1884), Brown (1926), Colbert (1935), Azzaroli (1954) and Arif and Shah (1991), have been critically reviewed and the following species are considered valid.

C. simplicidense Lydekker
C. triplidense Lydekker
C. sivalensis Lydekker
C. punjabiensis Brown
C. rewati Arif and Shah

The latest faunal turn-over events during the last Pliocene around 2.9 Ma introduced many Eurasian mammalian genera in the Siwalik faunal province including *Cervus, Equus, Elephas, Sivatherium, Sus* and *Sivachoeras*. This faunal change, termed as *Elephas Planifrons* interval zone. (Barry *et al.*, 1982).

This faunal zone is characterized by dominance of herbivore community of woodland habitat with a few adapted for riverine galley forests. The principal feature of this community is continuing into the modern South Asian wildlife assemblages.

ABBREVIATIONS

G.S.I.:	Geological Survey of India, Calcutta.
P.U.P.C.:	Punjab University Paleontological Collection, stored in the Department of Zoology, Lahore, Pakistan.
L:	Maximum preserved anteroposterior crown length of tooth.
W:	Maximum preserved crown width of tooth.
CI:	Crown shape index (W/L x 100) a ratio between width and length of crown.
mm:	Millimeter.
P_3:	Third right lower premolar.
P_4:	Fourth right lower premolar.
M_1:	First right lower molar.
M_2:	Second right lower molar.
M_3:	Third right lower molar.

SYSTEMATICS

Class	Mammalia, Linnaeus
Order	Artiodactyla Owen
Family	Cervidae Gray

Genus	*Cervus* Linnaeus
Species	*Cervus sivalensis* Lydekker

Holotype

G.S.I. No. B215, a right ramus with M_{2-3}.

Type locality

Maili, Punjab.

Horizon

Upper Siwaliks.

Diagnosis

A large cervid with relatively hypsodont molars. The skull and antlers resemble these portions in *Cervus duvaucelli*. The skull by virtue of the frontal concavity at the orbits, and the forward swell at the pedicles. The lacrymal vacuity is smaller than in *C. duvaucelli*. The browtine of the antler arise immediately above the burr, and form an obtuse angle with the beam.

Material Studied

P.U.P.C. No. 66/9: A fragment of right mandibular ramus having P_3-M_3.

Locality

Sardhok, Gujrat district, the Punjab province, Pakistan.

Horizon

Upper Siwaliks.

Description (Fig.1)

The specimen under study includes P_3-M_3 of right mandibular ramus. A portion of mandibular ramus with diastema is also well preserved with these teeth. The length of preserved mandibular ramus is 202 mm while its depth below M_2 is 62 mm. The depth of diastema is 33 mm. It is in an excellent state of preservation and at middle stage of wear. A very thin layer of cement is present on all sides of the teeth and is more prominent at the base of the crown. The teeth are narrow crowned and hypsodont except P_3 that is extremely hypsodont. The enamel is rugose and this rugosity is more prominent and evident on buccal side. The median basal pillar is prominent in M_{1-3} while absent in P_{3-4}. In M_{2-3} this is touching the summit of the crown and broken in M_1. These are also covered with thick layer of cement.

The principal conids are well preserved and prominent. In P_{3-4} the centre of the tooth is higher than that of anterior and posterior part. In M_{2-3}, the inner side of the tooth is higher than that of outer side, while in M_1 the inner conids are badly damaged at the summit of the crown. In molars the conids are broad while in premolars these are narrower. In molars the buccal conids are roughly U-shaped and broad.

The metastylid is more prominent in premolars than that of molars. In P_{3-4} it is the anterior extension of protoconid and metaconid. It is roughly V-shaped, narrow anteriorly while broad posteriorly. In M_{2-3} it is not clear because of thick layer of cement. In M_3 it is a fold like structure and also broken anteriorly. The entostylid is also prominent in premolars while not clear in M_{1-2}. In M_3 it is slightly developed.

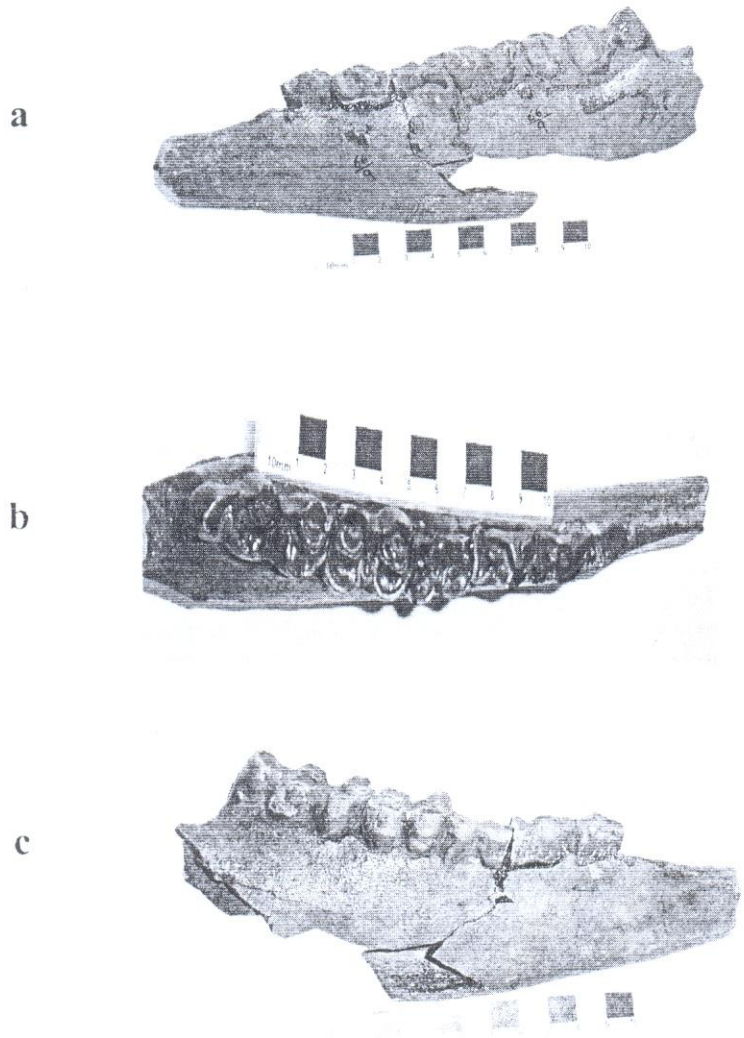

Fig. 1: *Cervus sivalensis* Lydekker, a fragment of right mandibular ramus having P_3-M_3 (P.U.P.C. No. 66/9), collected from Sardhok, Gujrat district, the Punjab province, Pakistan.
a) Lingual view, b) Occlusal view, c) Buccal view.

The anterior central cavities are absent in P_3-M_1 while in M_{2-3} these are narrow in the middle and broad anteroposteriorly. The posterior central cavity is poorly developed in P_3-M_1. These are small circular structures in P_3-M_1. In M_{2-3}, these are well developed and like anterior cavities, narrow in the middle and broad anteroposteriorly.

The median ribs are also well developed and preserved. In P_3 the posterior rib is strong than that of anterior rib while in P_4 the anterior rib is strong than that of posterior rib. In M_1 ribs are missing. In M_{2-3} anterior ribs are more strong than that of posterior ribs. The posterior ribs in M_{2-3} are slightly broken at the tips.

In M_3 the talonid is excellently preserved. It is V-shaped in its outline. It is broad in the middle while narrow anteroposteriorly. It is the postero-external extension of hypoconid and entoconid. It is also very similar to other lingual conids of molars in its general appearance.

DISCUSSION

The name *C. sivalensis* was introduced by Lydekker (1884) and then after by Pilgrim (1910), Brown (1926), Matthew (1929) and Colbert (1935). Lydekker has pointed out the resemblance of this species to *C. duvaucelli* both in the characters of the skull and of the antlers. He also mentioned that the brain case of *C. sivalensis* is large and the face is deep. The teeth of *C. sivalensis* are large and quadrate in shape. The folds are open and the enamel is rugose.

Lydekker (1880) referred the type G.S.I. No. B215 to *C. triplidense* but later Lydekker (1884) stated that this conjectural reference was incorrect, and the name *C. sivalensis* was proposed for this species to which the lower teeth belonged.

Pilgrim (1913) mentioned the name *C. sivalensis* in his list saying that it was from the Upper Siwaliks of Pinjor or Tatrot zone. Similarly Brown (1926) and Matthew (1929) mentioned this species in their account. Later, Colbert (1935) described a skull and antler under this name. According to Colbert (1935) the teeth are large and quadrate in shape. The folds are open and the enamel is rugose. The internal pillars are very small. The rugosity of the teeth was also pointed by Lydekker (1884). According to Lydekker (1884) the present teeth are also distinguished by the more rugose character of the enamel. Matthew (1929) only mentioned the name of this species in his faunal list. The material under study is well worn and maximum height in M_{2-3} is 14 mm and 13 mm, respectively. Lydekker (1883) also stated that in *C. sivalensis* the molars are very low crowned. Median basal pillars are very prominent between main cusps in M_{2-3}. M_2 is very similar to M_3 except that of talonid. In G.S.I. No. B215 the length x width of M_2 is 25 mm x 17.5 mm while in P.U.P.C. No. 66/9 it is 29 mm x 20 mm that is slightly more than that of type specimen, while the length of M_3 is exactly same as in G.S.I. No. 215. The material under study the enamel is also rugose, and this rugosity is also mentioned by Lydekker in type specimen. Similarly the crown index of M_2 under study and G.S.I. M_2 have the nearest values as 68.96 mm and 70 mm, respectively.

Table I: Comparative dental measurements (mm) of a fragment of right mandibular ramus having P_3-M_3 (P.U.P.C. No. 66/9) referred to *C. sivalensis* Lydekker.

	P.U.P.C. No. 66/9					G.S.I. No. B215				
	P_3	P_4	M_1	M_2	M_3	P_3	P_4	M_1	M_2	M_3
L	20	21.5	22	29	43	-	-	-	25	35
W	11.5	14	16	20	21	-	-	-	17.5	-
CI	57.50	65.11	72.72	68.96	48.83	-	-	-	70	-

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POLLUTION SOURCE ASSESSMENT OF RIVER WATER

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Abstract: The present paper deals with quantitative assessment of certain pollutants in wastewater originated from various industries. It draws heavily on data collected at different sampling sites of water channel receiving raw industrial effluents, which is linked with river for ultimate disposal. The characteristics of river water before mixing of industrial effluents are within the range of NEQS of Pakistan. The quality of river water, however, was deteriorated after receiving enormous amount of organic pollutants, in terms of BOD_5, from vegetable oil ghee mills and pulp and paper industrial units, in particular. Need of establishing admissible limits for different industrial effluents having specific dilution rates that might be accommodated by the river water without harming its natural biota is indicated.

Key words: River BOD, River COD, River pollution, Industrial effluents.

INTRODUCTION

Water, may be of ground or surface origin, is a basic natural resource and the use of streams or rivers as the recipient of unlimited amounts of industrial waste is definitely unacceptable. In general, the sources of river pollution are land-based and may reach the river through drains and out falls. Major chronic river pollution problems can often be attributed to the local discharge of large volume of wastes. They include materials which are potentially biodegradable such as raw sewage, food and beverage processing waste, pulp and paper mills effluents incorporated with wastes from textile, vegetable oil and dairy industries (Council of European Communities, 1976). The characterization of wastewater from various industries, however, revealed the highest concentrations of metals, halogenated hydrocarbons and a variety of settle able and suspended solids, which are found after mixing industrial effluents to the river water. These pollutants adversely affect the river ecosystem. Eventually, the effects of river pollution on human health, quality of life and fisheries in the fishing zones must fully be considered (Beavis and Rowley, 1980).

Pakistan, today is progressing towards higher stages of development and industrialization, which have brought radical changes in economic field. Consequently, various industrial units operative in the province Punjab of Pakistan are categorized as

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follows: Tanneries, Textile, Pulp and Paper, Vegetable Oil and Ghee, Chemicals, Food, Fertilizers, Sugar, Steel, Cement and Pharmaceuticals. Like most of Asian and African countries, Pakistan is an agricultural country and its economy is primarily based on the export of agricultural commodities. The water pollution is of special significance in an agriculture-oriented economy of the province Punjab, where rivers, irrigation canals network and streams are used for irrigation of cultivated land. Unexpectedly, most of the industries in Pakistan discharge their effluents directly or indirectly in the existing water bodies without proper treatments, while, polluted water significantly imbalances the ecosystem associated with agriculture productivity (Department of Environment and Welsh Office, 1971; Murrey and Norton, 1979; Shea, 1988).

This article surveys the intensity and frequency of industrial pollution and draws heavily on data collected at various disposal sites where raw effluents are mixed with drain water for ultimate discharge into river Ravi.

MATERIALS AND METHODS

Study area

The study area is situated near Sheikhupura, a historical City after the name of great Mughal Empire "Sheikhu" (Jahangir). For this work a drain namely "Barrianwala drain" was selected which was excavated in 1961under local land reclamation scheme to prevent the area from water logging and salinity.

The Barrianwala drain is the fifth important drain on the Lahore-Sheikhupura road. The existing conditions of Barrianwala drain in the study area indicate that a number of industrial units located along side the Lahore-Sheikhupura road, have been discharging their effluents into it without appropriate treatments. The drain assumes the function of a channel carrying dark brown liquid covered with a thick brown layer of scum varying from 20-30 cm, containing most of the fibrous material.The drain after receiving industrial effluents runs further 12.8 km. And then it discharges to Chichokimalia drain, which is named as Deg-nullah (II), near Mundi, where it crosses Qadirabad-Baloki link canal through siphon. It receives also the wastewater from Jaranwala sewage drain and ultimately discharges it into the river Ravi near Mouza Mubarik (Fig.1).

The Ravi is generally a clean river, maintaining a healthy aquatic environment suitable for propagation of warm water fish from its entry into Pakistan from India, up to the point near Shadbagh where it receives the first major pollution load from the city of Lahore. More than 90% of wastewater eventually finds its way into the river from the city of Lahore. The river Ravi has a wide variation in its flow with peak periods during July and August and periods between November and February, when augmentation of water is essential to meet the requirements of down stream utilization for agriculture practice. Since there was no sufficient data available indicating qualitative discharge of industrial effluents in the study area, therefore, a survey was conducted to probe the evaluation of major polluting industrial establishment.

Sampling of wastewater

Figure 1 shows the sampling sites in study area. The samples were taken from the grab wing by a plastic beaker and placed in pre-acid washed polyethylene screw bottles of 1 L capacity. The samples were stored in a refrigerator at 5°C for latter analysis.

Analytical methodology

The analysis of Chemical oxygen Demand (COD), Biochemical Oxygen Demand (BOD_5), Solids, Oils and Fats were determined according to the methods described by American Public Health Association in Standard Methods of Water and Wastewater Analysis (1985). The pH of the samples was determined by glass electrode.

RESULTS AND DISCUSSION

In the Sheikhupura-Lahore industrial estate, there are approximately 280 industrial units including paper and pulp, textile, chemicals, vegetable ghee and oil, light engineering and dairy industries. These are collectively producing more than 44000 m^3d^{-1} of wastewater that is being discharged into Barianwala drain for ultimate disposal into the river Ravi. Test results from pulp and paper industrial units indicated alkaline pH (8.6-8.7) with highest BOD_5 value of 4000-6000 mgl^{-1}, while vegetable oil and ghee mills discharged effluents contained least concentration of TSS i.e., 55-65 mgl^{-1}. The data presented in Table I also reveal that wastewaters from textile and polyester industries were of slightly acidic pH, that is, 5.4-5.5 and 6.5-6.7, respectively.

Table I: Wastewater quality

Industry	Parameters (Typical Values)			
	pH	BOD mgl^{-1}	TSS mgl^{-1}	TDS mgl^{-1}
Pulp and Paper	8.6-8.7	4000-6000	537-400	1100-1200
Vegetable oil and Ghee	8.0-8.3	350-300	55-68	300-400
Textile	5.4-5.5	200-300	200-250	700-1000
Polyester	6.5-6.7	120-130	700-900	200-300
Dairy	7.2-7.5	1300-1600	8000-10000	7200-8000

In subsequent studies, the results of sixteen sampling sites are indicated in Table II. They were located for qualitative assessment of wastewater discharged in Barianwala drain and Degh-nulla-II for ultimate disposal in river Ravi. At all sampling points the concentrations of BOD_5, TSS, pH and TDS were larger and varied probably

corresponding to the environmental conditions into which the wastewater is disposed-off in addition to their different natures from industries. From the data on characteristics of effluents mixed with water channel, it was observed that the location below Shariqpur Road Bridge gave highest BOD_5 value. The least value for these parameters were observed in the drain water before entering the industrial estate near Lahore-Sheikhupura road bridge, that is BOD_5, 90 mgl^{-1}, TSS, 170 mgl^{-1}, TDS, 200 $mgl^{-1}$and pH 7.1. A close look at the frequency of pollution at various sampling sites, however, reveals a general trend of gradual decrease in BOD. It should be noted that wastewater from pulp and paper industries contributed immensely to increase organic fraction in terms of BOD_5 in the drain water. The results of analysis at sampling point-10, near Tiba Niaz village, indicated that TSS, 2076 $mgl^{-1}$and TDS, 1502 $mgl^{-1}$are all very high near rural sites before final disposal into river Ravi. These elevated levels of the parameters may be a consequence of agriculture runoff. Further, the quality of wastewater before and after mixing with river Ravi water is also shown in Table II. Infact, the characteristics of river water prior to be contaminated with industrial pollutants are within or near to maximum limits of National Environment Quality Standard (NEQS) of Pakistan (Table III). From the data shown in this table it can be clearly seen that the river water contains higher level of BOD_5, 300 $mgl^{-1}$with slightly acidic pH (6.5) at the junction of Degh nullah and river Ravi. At this sampling site, the industrial wastewater is mixed with river water; thus, we can conclude that the estimated impact of the discharge of pollutants from industrial estate on river water quality seems to be strong. The concentration of these contents remained more or less consistent except TSS that showed tremendous increase at the distance of 9-km downstream of river Ravi. This behaviour of the parameters suggests that although the microbial picture has not been changed over a range of about 10-km downstream as evidenced by the BOD value but slight decrease in TDS is indicating the importance of establishing admissible dilution levels of different industrial effluents.

In general, during the periods of high water velocity, the wastewater could be diluted in the river water more than that during times of little water flow (Notel, 1988). Eventually, the maximum concentration of pollutants in the river water is attributed to constant wastewater discharge. However, assuming a constant discharge of pollutants, their concentration of river water should change due to varying velocity of flow, either tidal or laminar. Seasonal studies on the similar lines are needed to generate the data on a year wise pattern.

Conclusively a large quantity of untreated waste water originating from Barianwala drain and Degh nullah (Chichokimalian and Barianwala drain) are the most polluted drains with maximum BOD_5 and TDS values in the range of 500 $mgl^{-1}$to 800 $mgl^{-1}$and 200 $mgl^{-1}$to 3000 mgl^{-1}, respectively. The industrial wastewater also transports a variety of organic and inorganic pollutants, which adversely affect the ground water quality and agriculture commodities, especially when it is allowed to be spreaded on open land. Moreover, untreated industrial effluents discharged into irrigation water channels and river cause a considerable damage to phytoplankton. Therefore, issues demanding a comprehensive, integrated approach to tackle the problems of water and raw materials along with the waste minimization should have the highest priority. Obviously, cleaner

production covering both products and manufacturing process are necessary for proper waste management in the developing countries, in particular.

Table II: Quality of river Ravi receiving wastewater from Lahore-Sheikhupura Industrial Estate

Sampling Points	Sampling Location	Parameters (Typical values)			
		BOD mgl^{-1}	TSS mgl^{-1}	TDS mgl^{-1}	pH
1	Lahore-Sheikhupura road bridge	90	170	200	7.1
2	After mixing of paper mills wastewater	250	530	620	7.3
3	After mixing of Ghee mills wastewater	300	760	618	7.3
4	After mixing of paper mills wastewater	640	880	920	7.4
5	Below Shariqpur road bridge	700	900	1500	7.5
6	Near Barianwala distributary	650	800	1436	7.3
7	Chichokimalian distributary site	460	200	900	7.0
8	Lahore- Nankana road bridge	600	700	1300	7.1
9	Siphon Qadirabad-Baloki link canal	160	348	322	7.1
10	Sued wala/Tiba Niaz village	380	2076	1502	6.7
11	Moza Mubariq	312	1200	700	6.7
12	Degh nullah-Ravi junction	309	1213	813	7.3
13	Upstream of river Ravi before joining Degh nullah near bridge	15	102	186	6.8
14	Upstream, 4km from Degh nullah-Ravi junction	20	110	182	7.0
15	Upstream, 4km from Degh nullah junction	230	113	430	6.4
16	Downstream-9km from Degh nullah-Ravi junction	225	470	400	6.4

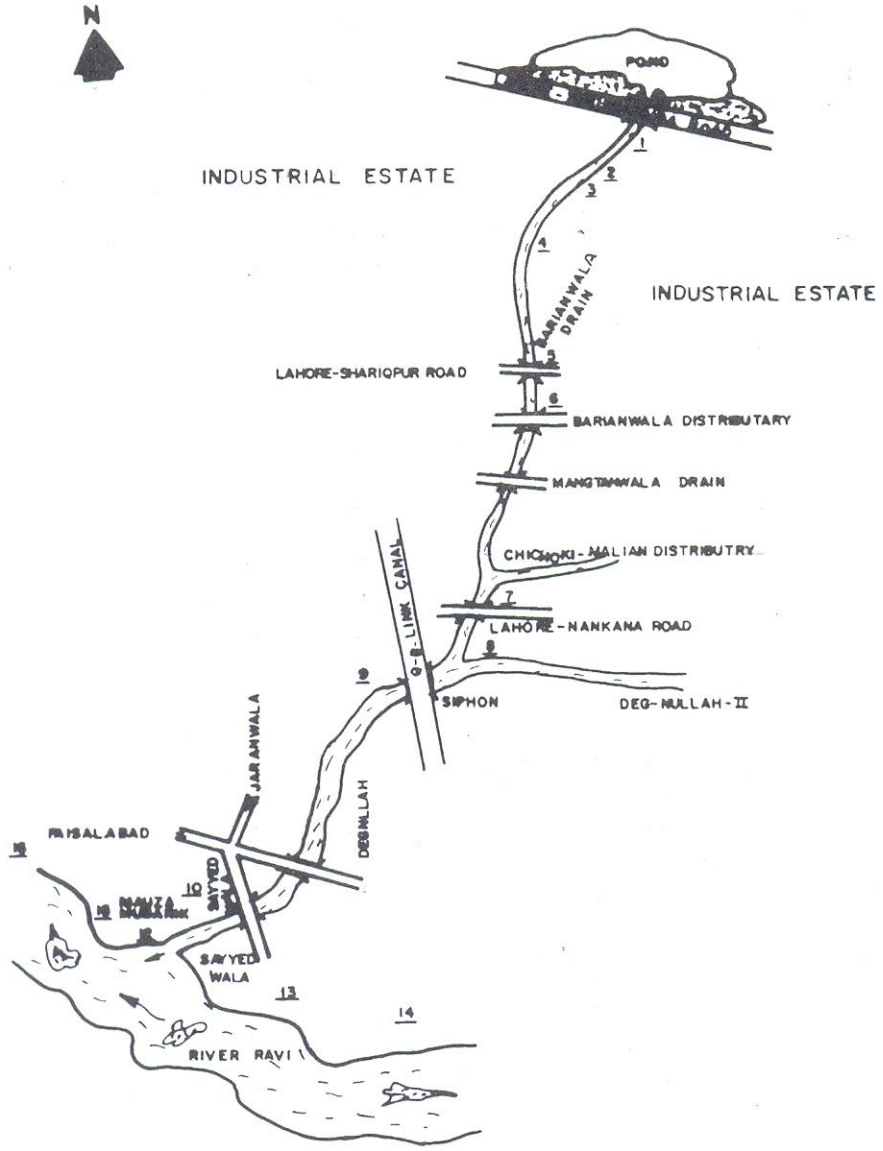

Fig. 1: Location of industrial estate as pollution source of river Ravi. Numbers indicate the sampling sites.

Table III: Proposed industry specific NEQS in Pakistan

Industry	PH	BOD_5 mgl^{-1}	COD mgl^{-1}	TSS mgl^{-1}	TDS mgl^{-1}
Pulp and paper	6.9	50	250	-	-
Dairy industry	6.9	50	250	50	-
Beverages	6.9	50	250	100	-
Fruits and Vegetable Processing	6.9	50	250	50	-
Nitrogen Fertilizers	6.9	-	-	100	-
Sugar	6.9	50	250	500	-
Dye and Dye Intermediate	6.9	100	250	100	3500
Cotton Textile Composite and processing	5.5-9.0	100	250	100	-
Tanning and Leather Finishing	6.9	80	250	200	3500
Paints	6.9	50	250	80	3500
Pesticide Formulations	6.9	30	150	80	3500

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FIRST RECORD OF *MARTES LYDEKKERI* FROM DHOK PATHAN FORMATION OF PAKISTAN

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ABSTRACT: An excellently preserved right mandibular ramus is being described from Dhok Pathan Formation (Middle Siwaliks). A comparison of the specimen under study with known material of *Martes* genus has shown that this is referable to *Martes lydekkeri*. Before this report, it was described from Chinji Formation (Lower Siwaliks) by Colbert (1933), but this addition to Siwalik carnivora indicates that the paleogeographical range of *Martes lydekkeri* is from Lower to Middle Siwaliks.

Key Words: Mandible, Dhok Pathan, Siwaliks and Martes.

INTRODUCTION

The Siwalik carnivores represent a great variety of genera and species. Bose described fossil carnivores from the Siwaliks as early as 1880. Significant contributions in this direction were, later on, made by Lydekker (1884) and Pilgrim (1932). Mammals, as group, from Siwaliks were critically observed by Matthew (1929). Pilgrim (1913) made a comparative study of the Siwaliks and European mammals. A comprehensive study of Siwalik mammals was carried out by Colbert (1935). Simpson (1945) divided the family Mustelidae into six subfamilies, but some rearrangements in different subfamilies of family Mustelidae were made by Wilson and Reeder (1992).

The genus *Martes* was discovered by Pinel (1792). It is widely distributed throughout the forested regions of Eurasia and North America. A palearctic origin is indicated and the earliest known occurrence is from Lower Miocene beds. Well adapted to their environment, they have changed very little during this long stretch of time. Never a dominant member of the fauna, the martens, small size and wandering, arboreal habits have lessened the chances of their remains being fossilized; consequently the fossil record is incomplete.

The ancestry of *M. fonia* is unknown, although it shows similarities to *M. vetus*, it occupies a different ecological niche and is not present in the European fauna until postglacial times. *M. flavigula and M. gwatkinsi*, the Asian yellow martens differ from the others by the peculiar structure of the baculum. A possible ancestor is *M. lydekkeri* from the Chinji zone of the Siwaliks and a subspecies of *M. flavigula* is known from the Middle

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Pleistocene of China. *M. gwatkinsi* survives as an isolated relict population in Southern India (Anderson, 1970).

Abbreviations

Amer.Mus:	American Museum of Natural History, New York, USA.
P.U.P.C.	Punjab University paleontological collection, stored in the Department of Zoology, Lahore, Pakistan.
L:	Maximum preserved anteroposterior crown length of tooth.
W:	Maximum preserved crown width of tooth.
CI:	Crown shape index. (W/L×100) a ratio between width and length of crown.
mm:	Millimeter.
P_2	Second right lower.premolar.
P_3:	Third right lower premolar.
P_4:	Fourth right lower premolar.
M_1:	First right lower molar.

SYSTEMATICS

Class	Mammalia Linnaeus
Subclass	Theria Haswell
Infraclass	Eutheria Gill
Super Order	Ferae Linnaeus
Order	Carnivora Bowdich
Suborder	Caniformia Wilson and Reeder
Super family	Canoidae Simpson
Family	Mustelidae Swainson
Sub family	Mustelinae Gill
Genus	*Martes* Pinel
Species	*Martes lydekkeri* Colbert

Holotype

Amer. Mus. No. 19407, a fragmentary mandible with right and left M_1

Type Locality

Chinji, Salt Range, Chakwal district, the Punjab province, Pakistan.

Horizon

Lower Siwaliks.

Diagnosis

Equal to *Martes Martes* in size. Lower carnassials with a high trigonid and a low basined talonid. Metaconid distinct and well developed (Colbert, 1933).

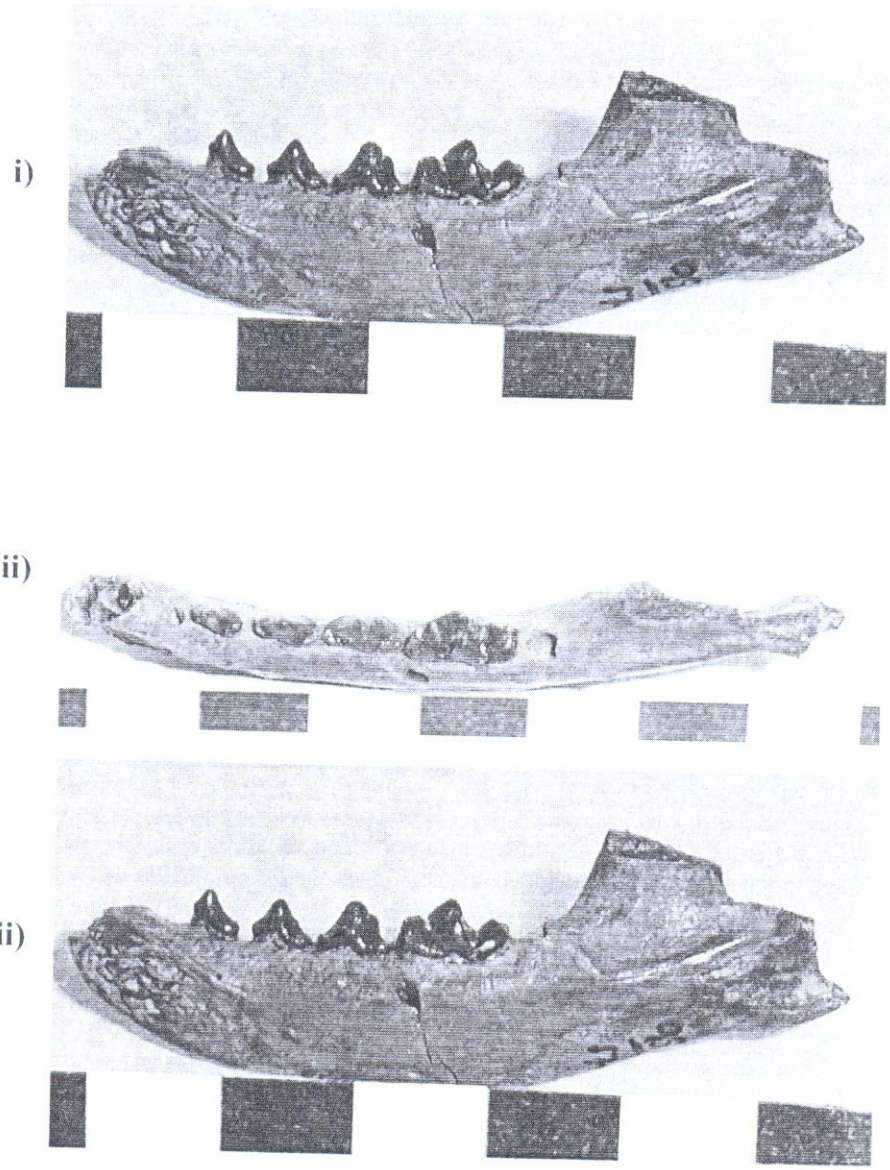

Fig. 1: *Martes lydekkeri* (Colbert). A right mandibular ramus having P_2–M_1 (P.U.P.C. No. 95/17), collected from Padhri, Jhelum district, the Punjab province, Pakistan. i) Inner view, ii) Crown view, iii) Outer view.

Material Studied

P.U.P.C. No. 95/17 (Fig. I), A right mandibular ramus having P_2–M_1.

Locality

Padhri, Jhelum district, the Punjab province, Pakistan.

Horizon

Middle Siwaliks.

Description

The specimen under study includes the right mandibular ramus with P_2–M_1. The ramus as well as the teeth is in an excellent state of preservation. The mandible includes, the anterior portion of vertical ramus, and horizontal ramus with symphysis. The length of preserved mandibular ramus is 70.5 mm, while the height of vertical ramus is 21 mm and the length of symphysis is 21 mm. Depth of ramus below canine is 10.3 mm, below P_2 is 11.2 mm, while below M_1 is 12 mm, respectively.

P_2 is smaller than P_3, while P_3 is smaller than P_4. In all premolars the protoconid is well developed and sharp. The teeth are at early stage of wear. All premolars are broad at the base and narrow anteriorly. The paraconid from $P_{2\text{-}4}$ is small and rudimentary. The posterior accessory cusp is well developed in $P_{2\text{-}3}$, while it is much prominent in P_4. M_1 is also excellently preserved, showing large trigonid and small talonid. In trigonid, the protoconid is large and higher than that of paraconid and metaconid. In M_1, the paraconid is also broad than metaconid. The former is also vertically higher than the latter. The talonid is also well preserved, consisting of large hypoconid and small entoconid. Anteriorly the ramus is showing hallow space instead of canine, indicating that it has large canine.

DISCUSSION

Martes lydekkeri was originally described as *Mustela lydekkeri* having been compared with *Mustela palaeosinensis*, rather than with modern *Mustela*. However, since the Siwalik specimen has a well-developed metaconid, as in *M. palaeosinensis* as well which structure lacking in the modern *Mustela*.

In type specimen of *Mustela lydekkeri*, the jaw in the British Museum, described by Lydekker as *Mustela* sp., was referred to the species *Martes lydekkeri*.

After careful re-examination of the Amer. Mus. specimen and its comparison with Lydekker's description and figure and with Pilgrim's description, Colbert (1933) concluded that both the specimens belong to same species as *Martes lydekkeri*. Moreover, Colbert (1933) compared Amer. Mus. specimen with Zdansky's figures of the type of *Sinictis dolichognathus* and conclude that *Martes lydekkeri* is not of the genus *Sinictis*, because it is considerably smaller than the typical Sinictis, but also it is characterized by a distinctly basined talonid, whereas *Sinictis* is defined as having a trenchant talonid.

The specimen under study has greater anteroposterior and transverse values of M_1 than that of Amer. Mus. collection. Similarly the specimen described in present work also have greater values of protoconid heights, whereas the height of metaconid is exactly

similar in P.U.P.C. No. 95/17, while other two specimens under study have greater values of metaconid heights. Length of talonid has lesser value in P.U.P.C. No. 95/17 than that of type specimen. Depth of ramus below protoconid is greater in P.U.P.C. No. 95/17 than that of Amer. Mus. collection (Table 1).

The chief distinction of Siwalik form from other primitive species of *Martes* is its larger protoconid, otherwise it is almost identical in its shape and structure with the modern *Martes* (Colbert, 1933).

In lower jaw I_1 is minute, I_{1-3} are wider, canines are slightly curved, the first lower molar is also very small P_{2-3} are double rooted, single cusped teeth, P_4 has a small posterior accessory cusp, M_1 is equal in size with *Martes Martes*, while M_2 is a small nearly rounded tooth, double rooted, with two small cusps. There is also a gradual decrease from postglacial to recent (Anderson, 1970).

Table I: Comparative dental measurements (mm) of lower first right molar referred to *Martes lydekkeri* (Colbert).

	P.U.P.C. No. 95/17	Amer. Mus. No. 19407
Length of M_1	10	9.9
Width of M_1	5.4	4.4
Height of protoconid	5.5	4.8
Height of metaconid	04	3.2
Length of talonid	04	4.5
Depth of ramus below protoconid	12	9.8

The mandible is deeper and shorter below premolars in the fossil material (Kurten, 1965). In material under study the incisors and canines are missing. P_{2-3} are single cusped and double rooted, P_4 is also with small posterior accessory cusp. The space left by canine in the material under study also suggests that it is large and slightly curved.

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DISTRIBUTION OF FRESWATER FISHES IN BALOCHISTAN PROVINCE OF PAKISTAN

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Abstract: There are 68 species of freshwater fishes (excluding exotic species and marine species entering freshwater along the Makran coast) known from Balochistan province of Pakistan. They belong to 41 genera, 16 families and 9 orders of teleostean fishes. Their distribution in Balochistan is discussed. The distribution of genera, families and orders in various ichthyogeographical provinces *viz*; Gedrosia, Yaghistan and Mehran are tabulated. The fish fauna is richest in Mehran province and poorest in Gedrosia.

Key words: Freshwater fishes, Balochistan, Pakistan, Gedrosia, Yaghistan, Mehran, Ichthyogeography.

INTRODUCTION

Balochistan is the largest province of Pakistan. Its area is approximately 350,000 km^2 (Gils and Baig, 1992). Its fish fauna was studied by McClelland (1842), Day (1880), Gunther (1889), Zugmayer (1912-13), Mirza (1972) and many others. Some additions were made by Nazneen *et al.*, (1989), Nazneen and Begum (1993), Coad (1996) and Ahmad *et al.* (1997). There are 68 freshwater native species of fishes belonging to 41 genera, 16 families and 9 orders of teleostean fishes. Several marine species enter into the streams and rivers in the coastal areas for the shorter and longer distances. Since the present paper deals with the zoogeographical aspects, the exotic and marine species of fishes are not taken into consideration. Among the ichthyogeographical provinces recognized by Mirza (1994) three provinces: Gedrosia, Yaghistan and Mehran are represented in Balochistan.

SYSTEMATIC ACCOUNT

ORDER OSTEOGLOSSIFORMES
FAMILY NOTOPTERIDAE

1. *Chitala chitala* (Hamilton)
   This species is rarely found in the Hab and Porali rivers
2. *Notopterus notopterus* Pallas
   This species also is rarely found in the river Hab.

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ORDER CLUPEIFORMES
FAMILY CLUPEIDAE

3. *Gudusia chapra* (Hamilton)
This species was reported from Lasbela by Zugmayer (1913).

ORDER CYPRINIFORMES
FAMILY CYPRINIDAE
Subfamily Cultrinae

4. *Chela cachius* (Hamiton)
This small fish is found in the rivers Nari and Bolan.

5. *Salmophasia bacaila* (Hamiton)
This beautiful fish is widely distributed in the streams and rivers in eastern side of Balochistan, east of central Brahui and Hala ranges. It has been recorded from the river Zhob by Shahjehan and Jan (2001). Its record from Lasbela by Zugmayer (1913) is doubtful.

Subfamily Rasborinae

6. *Barilius modestus* (Day)
This species has been recorded from the river Zhob.

7. *Barilius pakistanicus* Mirza and Sadiq
This species is widely distributed in eastern rivers from Zhob to Kolachi.

8. *Barilius vagra* (Hamilton)
This species also is widely distributed in the eastern parts of Balochistan.

9. *Devario devario* (Hamilton)
This species has been recorded from rivers Porali and Hab and also from the rivers of Sindh-Balochistan hills.

10. *Rasbora rasbora* (Hamilton)
This is a rare species and has been recorded from Hab River only (Nazneen and Begum, 1997).

Subfamily Aspidoparinae

11. *Aspidoporia morar* (Hamilton)
This species is widely distributed in southern and southeastern parts of Balochistan and also in adjoining coastal areas of southeastern Iran.

Subfamily Barbinae

12. *Cirrhinus mrigala* (Hamilton)
This is found in the streams and rivers of Sindh-Balochistan hills

13. *Cirrhinus reba* (Hamilton)
This species also is found in Sindh-Balochistan hilly areas.

14. *Cyprinion microphthalmus* (Day)
This species is mainly distributed in the western Balochistan. It also extends into Iran and Afghanistan.

15. *Cyprinion milesi* (Day)
This species is also mainly distributed in the western Balochistan, west of central Brahui-Hala ranges. It extends into Iran also.

16. *Cyprinion watsoni* (Day)

This is the commonest species of the genus. It extends into the hilly areas of NWFP, Sindh, Punjab and Azad Kashmir.

17. *Gibelion catla* (Hamilton)

This is a rare species in Balochistan. It has been recorded from the river Bolan only (probably transplanted) by Mirza and Naik (1967).

18. *Labeo caeruleus* Day

This species is found in the Sindh-Balochistan hilly areas.

19. *Labeo calbasu* (Hamilton)

This species has been recorded from Bolan River by Siddiqi (1962).

20. *Labeo diplostomus* (Heckel)

This species is widely distributed in the eastern Balochistan from the river Zhob upto Hab River. It has also been recorded from rivers Porali, Hingol and Rakhshan as *Labeo dero.*

21. *Labeo dyocheilus pakistanicus* Mirza and Awan

This species is distributed in the rivers Zhob, Nari, Bolan, Kolachi etc. It is not found in the areas west of central Brahui Range. It has not been collected from the coastal areas.

22. *Labeo gedrosicus* Zugmayar

This species is endemic to Mashkhel drainage system of the north-western Balochistan. It was described by Zugmayer in 1912. Since then it has never been collected.

23. *Labeo macmahoni* Zugmayer

The status of this species is uncertain. A new subgenus *Tariqilabeo* was created by Mirza and Saboohi (1990) to accommodate this species. According to Kullander *et al.* (1999), it is a synonym of *Crossocheilus diplochilus* (Heckel).

24. *Labeo rohita* (Hamilton)

This species was recorded by Day (1880) from rivers of Sindh-Balochistan hills. It is not found in the western Balochistan.

25. *Naziritor zhobensis* (Mirza)

This is the Zhobi Mahseer. It was described as *Tor zhobensis* by Mirza (1967) from river Zhob. Its range was extended to the rivers Kunder, Gomal, Shahur, Tochi, Kurram and Kabul. It has not been recorded from Sindh, Punjab and Azad Kashmir. It is thus endemic to Balochistan and NWFP. Its biology has not been properly studied.

26. *Puntius conchonius* (Hamilton)

This species has been recorded from southeastern Balochistan. Along the coastal areas it has been recorded from Lasbela by Nazneen *et al.* (1989) and from Hab by Nazneen and Begum (1993).

27. *Puntius punjabensis* (Day)

It has been recorded in Balochistan from Lasbela only by Zugmayer (1913).

28. *Puntius sophore* (Hamilton)

This species is quite common in eastern Balochistan and coastal areas. It has been recorded from Lasbela and Basol stream near Pasni as *Barbus stigma* by Zugmayer (1913), and from the river Bolan by Siddiqi (1962) etc.

29. *Puntius terio* (Hamilton)

It has been reported from rivers/streams of Sindh-Balochistan hills by Day (1880).

30. *Puntius ticto* (Hamilton)

This species has also been recorded from rivers/streams of Sindh-Balochistan hilly areas.

31. *Systomus sarana* (Hamilton)

It has been collected from the river Bolan and Sindh-Balochistan hills.

32. *Tor macrolepis* (Heckel)

It is the famous Golden Mahseer of Pakistan, Afghanistan and India. It was wrongly reported as *Tor putitora* (Hamilton) from Pakistan, Kashmir and the Indus system in India. It was recognized as a subspecies: *Tor putitora macrolepis* (Heckel) by Mirza and Bhatti (1996) and *Tor macrolepis* (Heckel) by Mirza (2003).

Subfamily Garrinae

33. *Garra gotyla* (Gray)

This species is found in the rivers Zhob, Nari, Bolan and Kolachi in the eastern Balochistan. It has not been collected from the western Balochistan.

34. *Garra rossica* (Nikolsky)

This is a small sized fish which is restricted to the northwestern Balochistan (Lora-Pishin and Maskkhal drainage systems). Its record from the river Kunder by Mirza *et al.* (1994) is doubtful.

35. *Crossocheilus diplochilus* (Heckel)

This is the most widely distributed species in almost all the drainage systems in Pakistan and extended into Afghanistan and Iran.

Subfamily Schizothoracinae

This is the subfamily restricted to the northwestern Balochistan in the rivers Kundar, Zhob, Nari and Bolan only. Only three species of this subfamily, *viz*; *Racoma labiata*, *Schzocypris brucei* and *Schizothorax plagiotomus* are found in Balochistan. The record of *Schizopygopsis stoliczkae* from Ziarat valley was due to wrong identification of *Schzocypris brucei*. *Schizopygopsis stolizkae* Steindachner in Pakistan is found only in the Northern Areas (Baltistan).

36. *Racoma labiata* McClelland and Griffith

This species is distributed in headwaters of the river Zhob, Nari and Bolan only.

37. *Schizocypris brucei* Regan

This snow-carp is distributed in the headwaters of Zhob, Kundar, and Nari rivers (Ziarat valley and Shah karez).

38. *Schizothorax plagiostomus* Heckel

This widely distributed snow carp species has been recorded from the head waters of Zhob, Nari and Bolan rivers.

FAMILY COBITIDAE

39. *Botia birdi* Chaudhuri

It has been recorded from the rivers Zhob, Nari, Bolan, Kolachi, Mula and other water bodies in the eastern Balochistan.

FAMILY NEMACHEILIDAE

40. *Acanthocobitis botia* (Hamilton)

This beautiful loach is distributed in the rivers Zhob, Nari, Bolan Kolachi and Hab.

41. *Nemacheilus corica* (Hamilton)

This species has been recorded from the river Zhob by Mirza *et al.* (1994).

42. *Schistura anambarensis* (Mirza and Banarescu)

This species has been recorded from the river Anambar (Loralai District) and Sasol stream (Khuzdar District).

43. *Schistura arifi* Mirza and Banarescu

This loach is endemic to river Zhob.

44. *Schistura balochiorum* (Zugmayer)

This loach has been collected from Panjgur and Harnai

45. *Schistura harnaiensis* (Mirza and Nalbant)

This loach has been described from Harnai. It is very closely related to *S. balochiorum*.

46. *Schistura kessleri* (Gunther)

This loach is widely distributed in western Balochistan.

47. *Schistura machensis* (Mirza and Nalbant)

It has been collected from Mach (Bolan river drainage) and Harnai (Nari river drainage). It is endemic to Balochistan.

48. *Schistura pakistanicus* (Mirza and Banarescu)

It is found in rivers Zhob, Kunder and Gomal. It appears to be endemic to Gomal drainage system.

49. *Triplophysa brahui* (Zugmayer)

This loach is endemic to the Lora-Pishin River in Pakistan and Afghanistan.

ORDER SILURIFORMES

The catfishes are only found in the eastern Balochistan. This order is completely absent in the western Balochistan, west of the central Brahui-Hala ranges.

FAMILY BAGRIDAE

50. *Rita rita* (Hamilton)

This species has been recorded from the rivers Bolan and Mula only.

51. *Mystus cavasius* (Hamilton)

This species has been recorded from Bolan River only.

52. *Mystus gulio* (Hamilton)

This catfish is partly marine. It was recorded from Lasbela by Zugmayer (1913).

53. *Sperata sarwari* Mirza, Nawaz and Javed

This valuable species was recorded from river Bolan as *Mystus seenghala* by Siddiqi (1962).

FAMILY SILURIDAE

54. *Ompok pabda* (Hamilton)

This species has been collected from southeastern Balochistan.

55. *Wallago attu* (Schneider)

This is the famous mullee. It has been recorded from southeastern part of Balochistan.

Its record from Lora-Pishin by Zugmayer (1913) is not correct.

FAMILY AMBLYCIPITIDAE

56. *Amblyceps macropterus* Ng

It was recorded as *Amblyceps sp.* from the river Gaj by Day (1880). It has now been described as *Amblyceps macropterus* by Ng (2001).

FAMILY SISORIDAE

57. *Glyptothorax naziri* Mirza and Naik

It has been collected from rivers Zhob and Anambar.

58. *Glyptothorax punjabensis* Mirza and Kashmiri

This species has been collected from river Zhob only.

ORDER BELONIFORMES
FAMILY BELONIDAE

59. *Xenentodon cancila* (Hamilton)

It has been recorded from Sonmiani by Ahmad *et al.* (1997).

ORDER CYPRINODONIFORMES
FAMILY APLOCHEILIDAE

60. *Aplocheilus panchax* (Hamilton)

This species was recorded from southeastern part of Balochistan in the river Nullini (Day, 1880).

FAMILY CYPRINODONTIDAE

61. *Aphanius dispar* (Ruppell)

It is widely distributed in the southeastern part of Balochistan and extends into coastal areas reaching Panjgur.

ORDER CHANNIFORMES
FAMILY CHANNIDAE

62. *Channa gachua* (Hamilton)

This is widely distributed in southeastern Balochistan and the coastal areas. It extends into the river Pakhshan and southeastern Iran.

63. *Channa marulia* (Hamilton)

It is restricted to the Nari and Bolan rivers.

64. *Channa punctatus* (Bloch)

It is also found in the southeastern Balochistan.

65. *Channa striata* (Bloch)

It was recorded from Sindh-Balochistan hills by Day (1880). Its record from Balochistan needs confirmation.

ORDER PERCIFORMES
FAMILY CHANDIDAE

66. *Chanda nama* (Hamilton)

It was also recorded from Sindh-Balochistan hills by Day (1880). Its record from Balochistan needs confirmation.

FAMILY GOBIIDAE

67. *Glossogobius giuris* (Hamilton)

It is widely distributed in the east and southern Balochistan.

ORDER MASTACEMBELIFORMES
FAMILY MASTACEMBELIDAE

68. *Mastacembelus armatus* (Lacepede)

It is widely distributed in the eastern Balochistan from the river Zhob in the north and the river Porali in the south.

Table I: Distribution of higher taxa of freshwater fishes of Balochistan in Gedrosia, Yaghistan and Mehran ichthyogeographical Provinces of Pakistan

	I	II	III
ORDER OSTEOGLOSSIFORMES	-	-	+
I: FAMILY NOTOPTERIDAE	-	-	+
Genus1. *Chitala*	-	-	+
2. *Notopterus*	-	-	+
ORDER CLUPEIFORMES	+	-	+
II: FAMILIY CLUPEIDAE	+	-	+
3. *Gudusia*	-	-	+
ORDER CYPRINIFORMES	+	+	+
III: FAMILY CYPRINIDAE	+	+	+
Subfamily Cultrinae	-	+	+
4. *Chela*	-	+	+
5. *Salmophasia*	-	+	+

Subfamily Rasborinae	-	+	+
6. *Barilius*	-	+	+
7. *Devario*	-	-	+
8. *Rasbora*	-	-	+
Subfamily Aspidoparinae	+	-	+
9. *Aspidoparia*	+	-	+
Subfamily Barbinae	+	+	+
10. *Cirrhinus*	-	+	+
11. *Cyprinion*	+	+	+
12. *Gibelion*	-	-	+
13. *Labeo*	+	+	+
14. *Naziritor*	-	+	-
15. *Puntius*	+	+	+
16. *Systomus*	-	-	+
17. *Tor*	+	+	+
Subfamily Garrinae	+	+	+
18. *Crossocheilus*	+	+	+
19. *Garra*	+	+	+
Subfamily Schizothoracinae	-	+	+
20. *Racoma*	-	+	+
21. *Schizocypris*	-	+	-
22. *Schizithorax*	-	+	+
IV: FAMILY COBITIDAE	-	+	+
23.*Botia*	-	+	+
V: FAMILY NEMACHEILIDAE	+	+	+
24. *Acanthocobitis*	-	+	+
25. *Nemacheilus*	-	+	+
26. *Schistura*	+	+	+
27. *Triplophysa*	+	-	+
ORDER SILURIFORMES	-	+	+
	-	-	+
VI: FAMILY BAGRIDAE	-	-	+
28. *Rita*	-	-	+
29. *Mystus*	-	-	+
30. *Sperata*	-	-	+
VII: FAMILY SILURIDAE	-	-	+
31. *Ompok*	-	-	+
32. *Wallago*	-	-	+
VIII: FAMILY AMBLYCIPITIDAE	-	-	+
33. *Amblyceps*	-	+	+
IX: FAMILY SISORIDAE	-	+	+
34. *Glyptothorax*	-	-	+
ORDER BELONIFORMES	-	-	+

X: FAMILY BELONIDAE	-	-	+
35. *Xenentodon*	+	-	+
ORDER CYPRINODONTIFORMES	-	-	+
XI: FAMILY APLOCHEILIDAE	-	-	+
36. *Aplocheilus*	+	-	+
XII: FAMILY CYPRINODONTIDAE	+	-	+
37. *Aphanius*	+	-	+
ORDER CHANNIFORMES	+	-	+
XIII: FAMILY CHANNIDAE	+	-	+
38. *Channa*	-	-	+
ORDER PERCIFORMES	-	-	+
XIV: FAMILY CHANDIDAE	-	-	+
39. *Chanda*	+	-	+
XV: FAMILY GOBIIDAE	+	-	+
40. *Glassogobius*	-	+	+
ORDER MASTACEMBELIFORMES	-	+	+
XVI: FAMILY MASTACEMBELIDAE	-	+	+
41. *Mastacembelus*			

I Gedrosia Ii Yaghistan III Mehran (after Mirza, 1994)

DISCUSSION

The freshwater fish fauna of Balochistan is composed of the South Asiatic, High Asiatic and West Asiatic elements. The Central Brahui Range extending from north to south in the form of Hala Range is the main dividing line. In the east of this line the rivers mostly belong to the Indus drainage system except the river Hab and the river Porali which are coastal rivers falling into the Arabian Sea directly. In the north-eastern part, the snow-carps (subfamily Schizothoracinae) are distributed in the rivers Kundar, Zhob and headwaters of the rivers Nari and Bolan. This area is included in the Yaghistan ichthyogeographical province which extends into NWFP drained by the rivers Gomal, Shahur, Tochi, Kurram and Kabul. The south-eastern Balochistan is a part of Mehran ichthyogeographical province. It lacks Schizothoracine fishes and the Zhobi mahseer, *Naziritor zhobensis* (Mirza), which is distributed in the river Kunder, Zhob, Gomal, Tochi, Kurram and Kabul in the Yaghistan ichthyogeographical province.

The western Balochistan west of the Central Brahui and Hala ranges is included in the Gedrosian ichthyogeographical province which extends into the southeastern Iran also (Coad, 1996). The fish fauna of this province is mainly South Asiatic and West Asiatic. There are no snow-carps. The only central Asiatic representative is *Triplophysa brahui* (Zugmayer), which is endemic to Lora-Pishin River in Balochistan and Afghanistan. In the rivers of the coastal part of Gedrosia there are several species of marine origin, but their systematics and distribution have not been properly studied after

Zugmayer (1913). Thus the fishes of marine origin are not discussed in this paper. Within Gedrosia three ichthyogeographical divisions are possible. In the north-east the river Lora-Pishin has poorest fish fauna. It is composed of the genera *Cyprinion*, *Crossocheilus* and *Garra* (subgenus *Discognathus*) among the *Cyprinidae* and *Triplophysa* and *Schistura* among the *Nemacheilidae*. In the Mashkhel river system the genera *Labeo* (*Bangana*), *Aspidoparia*, *Aphanius* and *Channa* are added but *Triplophysa* is not found. *Triplophysa* is restricted to the Lora-Pishin only in Balochistan. In the coastal areas the genus *Tor* is found in the river Hingol, the genus *Puntius* was recorded by Zugmayer from Pasni (Basol stream). Several genera of marine origin, viz., *Glossogobius*, *Boleophthalmus*, *Periophthalmus*, *Hilsa*, *Acanthopagrus*, *Mugil*, *Liza* etc are added.

The Yaghistan ichthyogeographical province extends into the river Gomal, Shahor, Tochi, Kurram in NWFP with several additional species. The Mehran ichthyogeographical province extends into Indus plain and submontane Indus region where several more fish taxa are added (Table I). The record of *Aplocheilus panchax*, *Channa striata* and *Chanda nama* by Day (1880) from Balochistan needs confirmation.

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