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Heavy Metal Contamination of Drinking Water Nearby Hadiara Drain

Farzana Rashid,[1]* Hira Fatima,[1] Ijaz Ali,[2] Nadia Sharif,[1] Husna Malik[1] and Sumera Sajjad[1]

[1]Lahore College for Women University, Lahore, Pakistan

[2]Institute of Biotechnology and Genetic Engineering(IBGE), Peshawar, Khyber Pakhtunkhwa, Pakistan

Abstract.- The present study was conducted in the surrounding areas of Hadiara drain, a major drain flushing out in river Ravi. Various physical and chemical parameters were analyzed in the drinking water randomly collected from five areas located around Hadiara drain including Raja Bolay Village (RBV), Ghanakar Village (GV), Deu Khurd (DK), Kahna Nau (KN), and Green Cap Housing Society (GCHS). The results were compared with standard value of World Health Organization for drinking water. It was observed that, the pollutants emanating from local tannery and textile effluents discharged into Hadiara drain increases the value of electrical conductivity and dissolved oxygen in water which is above the WHO standard value. Among the heavy metals tested Cr was higher than the recommended values in all areas studied except in RBV and GCHS. The study concluded that the use of polluted water degraded the ground water quality.

Key words: Drinking water, Heavy metals, Hadiara drain, Chromium, Nickel, Copper

The importance of groundwater for the existence of human society cannot be over emphasized. Besides, it is an important source of water for the agricultural and industrial sector. Till now it had been considered as dependable source of non-contaminated water (Shyamala *et al*., 2008). Groundwater crisis is not the result of natural factors. It has been caused by human actions. Ground water contamination can be associated with different sources, such as seepage of agrochemicals, sewage and industrial wastewater. Seepage from unlined sewage and industrial wastewater channel causes a great threat to ground water quality (Bashir *et al*., 2001).

Several industries are located along Hadiara drain, a major drain coming from India and finally flushing out in river Ravi. These industries pass out their waste water in this drain through different discharge points. The pollution in drain is increasing day by day resulting under ground water contamination. People living in the areas around Hadiara drain use underground water for drinking purpose by digging up well (Nabi *et al.*, 2001; Khan, 2001). Textile and leather industries are the major industries established along Hadiara drain. Only one or two industries have their effluent treatment plant, rest of these industries discharge their effluent without any treatment, resulting increase in the pollution of the drain ultimately leading to the contamination of groundwater. Heavy metals of discharged effluents presence in water are considered serious pollutants because of toxicity, persistence and non degradable conditions in the environment, thereby constitute threat to human beings and other forms of biological life (Aina *et al*., 2009; Mohiuddin *et al*., 2010; Yousafzai *et al*., 2009). Ground water is getting contaminated day by day, and hence this study was undertaken to analyze drinking water (ground water) collected from surroundings of Hadiara drain in order to check the concentration of heavy metals such as Cr, Ni and Cu.

Materials and methods

The sampling area started from the Raja bolay village at the distance, off 23 km from main Ferozepur road Lahore along Hadiara drain up to Green cap housing society (GCHS) at Ferozepur road Lahore. The total size of study area was 15 km that is basically consisting of many textile and leather tanning industries, which discharge their waste water into the drain. Fifty drinking water samples were collected randomly from five different sites including Raja bolay village (RBV), Ganaker Village (GV), Deu Khurd (DK), District Kahna Nau (KN) and in the surrounding of Hadiara drain. All

* Corresponding author: fari_67@yahoo.com

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Table I.- Physicochemical analysis of drinking water samples.

Sampling sites	pH	EC (µS/cm)	TDS (mg/l)	DO	Cr^{6+} (mg/l)	Ni^{++} (mg/l)	Cu^{++} (mg/l)
1. RBV	7.3 ± 0.03	9100.6 ± 0.03	766.7 ± 0.03	2.7 ± 0.03	0.02 ± 0.003	0.2 ± 0.03	0.2 ± 0.03
2. GV	7.8 ± 0.03	2742.5 ± 0.03	2422.3 ± 0.03	2.5 ± 0.03	0.06 ± 0.03	0.7 ± 0.03	1.003 ± 0.03
3. DK	6.9 ± 0.03	1928.2 ± 0.03	1568.3 ± 0.03	2.7 ± 0.03	0.06 ± 0.03	0.9 ± 0.03	0.8 ± 0.03
4. KN	7.1 ± 0.03	1785.1 ± 0.03	1446 ± 0.03	2.7 ± 0.03	0.07 ± 0.03	0.8 ± 0.03	0.2 ± 0.03
5. GCHS	7.2 ± 0.03	406.9 ± 0.03	320.7 ± 0.03	2.6 ± 0.03	0.04 ± 0.03	0.3 ± 0.03	0.3 ± 0.03
6. WHO	6.5-8.5.	1000	1500	-	0.02	0.02	1.0-2.0

EC, electrical conductivity; TDS, total dissolved solids; DO, dissolved oxygen
RBV, Raja Bolay Village; GV, Ghanakar Village; DK, Deu Khurd; KN, Kahna Nau; GCHS, Green Cap Housing Society; WHO, World Health Organization.

the drinking water samples were obtained from hand pump and domestic motors coming from ground at the depth of 80-100 feet and after discharging of water from the tap for 3 min in 500 ml of sterile polythene bottles without any air bubbles. The temperatures of the samples were measured in the field at the time of sample collection and kept at 4ºC for future analysis.

Drinking water samples were analyzed for various important parameters such as pH , electrical conductivity, dissolved oxygen (DO), total dissolved solids (TDS), chromium (Cr), nickel (Ni) and copper (Cu). atomic absorption spectrophotometer (M Series AA Spectrometer FS95 Furnace Auto sampler GF 95Z Zeeman furnace) was used for heavy metal analysis.

Results and discussion

Table I shows physico-chemical analysis of drinking water including concentrations of Cr, Ni and Cu. The mean pH value of all drinking water samples was within the range of standard value of WHO which is 6.5-8.5 (Table I).

The mean EC values of all the drinking water samples except from GCHS were greater than WHO standard value of drinking water (1000 µS/cm). Lawson (2011) has attributed highest value of EC in the drinking water samples of RB with the mean value of 9100±289.65 µS/cm. Higher EC value may due to more salts and minerals.

Mean value of TDS in drinking water samples of RBV was measured as 766.7±23.33 mg/l and GCHS was measured as 320.7±6.07 mg/l and both of these are lower than WHO standard value of TDS for drinking water. Highest value of TDS was found in drinking water samples of GV (2422.3±173.02 mg/l). According to Lawson (2011) high levels of TDS are due to the presence of potassium, chlorides and sodium ions which have little or no short-term effects but makes water hard. The DO ranged between 2.5±0.03 to 2.7±0.04 mg/l.

The mean concentrations of Cr in the water samples ranged between 0.02±0.003 to 0.06±0.003 mg/l, as against 0.02 mg/l permissible limit of WHO.

Ni concentrations varied between 0.2 to 0.9 mg/l as against the permissible limit of 0.02 mg/l of WHO. Many studies have been published regarding nickel sensitivity in humans. It is considered as carcinogenic to human. Das *et al*. (2006) reported that high-dose of nickel in rats and dogs significantly decreasing their body weights.

The copper concentration of all the drinking water sample was within the WHO permissible limits. The maximum value of mean concentration of Cu was found in the GV *i.e.* 1.003±0.13 mg/l. The minimum value of mean concentration of Cu was found in drinking water samples of KN which is 0.23±0.03 mg/l.

Conclusions

The present study concludes that untreated effluents from textile and tanning industries are polluting Hadiara drain with Cu, Ni and Cr and affecting physical parameters of water. Exposure to these contaminants may be the cause of respiratory, skin, kidney and liver diseases among the population residing nearby Hadiara drain.

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The Prevalence of Common Mutations in *rpsL* Gene Associated with Resistance to Streptomycin in Random and Multiple Drug Resistant Isolates of *M. tuberculosis* from Pakistan

Rubina Tabassum Siddiqui[1]* and Javed Anver Qureshi[2]

[1]Health Biotechnology Division, National Institute for Biotechnology and Genetic Engineering, P.O.Box#577, Jhang Road, Faisalabad, Pakistan

[2] Institute of Molecular Biology and Biotechnology, Center for Research in Molecular Medicine, The University of Lahore, Lahore, Pakistan

Abstract.-Mutations in *rpsL* gene associated with resistance to streptomycin were screened in multiple drug resistant and randomly collected isolates of *M. tuberculosis* from Pakistan. Mutations were detected by PCR amplification of *rpsL* gene followed by dot-blot hybridization with allele-specific radioactively-labeled probes and by PCR-RFLP. Out of 45 isolates, 31 were resistant to streptomycin, while 14 were sensitive by conventional drug sensitivity assay. The rpsL43 mutation was found in 7 out of 31 isolates tested (22.58%) while rpsL88 was found in 4 out of 31(12.90%) isolates. The rpsL43 was also found in 27% isolates collected randomly, while rpsL88 was found in 5.4% isolates. All susceptible isolates had no mutation when probed with rpsL43 or rpsL88. The presence of rpsL43 mutation in randomly collected isolates indicates a high level of resistance to streptomycin.

Key words: *M. tuberculosis*, streptomycin, rpsL43, rpsL88, dot blot hybridization, PCR-RFLP

Tuberculosis remains one of the major health concerns as Pakistan ranks 5th among high burden countries in the world (WHO, 2012). The control of the disease is further complicated due to the emergence of multi drug resistant (MDR) strains which are defined as having resistance to at least isoniazid and rifampin; the most potent first line drugs (American Thoracic Society, 2000). MDR tuberculosis cases have to be treated with second line drugs which are more toxic, more expensive and less effective. Rapid detection of resistance/susceptibility to key drugs used for the treatment is essential for effective therapy and avoidance of further spread of resistant *M. tuberculosis* strains.

Mycobacteria develop natural resistance to drugs by spontaneous mutations and the rate of mutation for each drug is different (Riley, 1993). For example mutation rate for resistance against streptomycin is 1 in 10^8, 1 in 10^7 for ethambutol, and 1 in 10^9 for cycloserine (Gangadharam, 1984). *M. tuberculosis*, the causative agent for TB, is a slow grower. At present, combination therapy is used to treat the disease to avoid drug resistance as monotherapy induces the selection of drug-resistant populations (Petrini and Hoffner, 1999).

Streptomycin, an aminocyclitol glycoside antibiotic, is one of the first line drug used for the treatment of TB (WHO, 2010) though Centre for Disease Control, Atlanta, USA has recommended to use it as second line drug due to increasing

* Corresponding author: tabassum.rubina@gmail.com

resistance observed (CDC, 2003). The drug binds to the 16S rRNA, interferes with translation proof-reading, and thereby inhibits protein synthesis (Moazed and Noller, 1987). Mutations associated with streptomycin in *M. tuberculosis* have been identified in the 16S rRNA gene (*rrs*) and *rpsL* gene encoding ribosomal protein S12 (Finken *et al.*, 1993; Telenti and Iseman, 2000). The most common mutation is an AAG to AGG change in codon 43 resulting in lysine to threonine substitution. Mutations also occur in codon 88 and these result in lysine to arginine or lysine to glutamine amino acid replacement. Additional mutations are expected to be involved in resistance as mutations in *rrs* and *rpsL* do not explain resistance in all strains. Accordingly, *gid*B gene which encodes 7-methylguanosine methyltransferase is found associated with streptomycin resistance (Okamoto *et al.*, 2007).

Mutations associated with drug resistance depend on geographical location in which the specific isolate is found (Sandgren *et al.*, 2009). It is therefore important to analyze *M. tuberculosis* isolates from different regions and to determine region-specific mutations to be used as molecular markers for rapid evaluation of drug resistance. The data about mutations associated with drug resistance in Pakistan is only sparsely available. The aim of the present study was to screen common mutations in *rpsL* gene in isolates with known sensitivity and randomly picked isolates of *M. tuberculosis* from Pakistan.

Materials and methods

M. tuberculosis isolates (80 in number) originating from all four provinces of Pakistan were included in the study. Drug sensitivity data of isolates (43 in number) from Khyber Pakhtnkhwa was available while that of the remaining 37 isolates randomly collected from the other three provinces (Punjab, Sindh and Balochistan) was not available. DNA from all these isolates was extracted using CTAB method (van Embden, 1993). These isolates were screened by PCR followed by dot blot hybridization for mutations in codon 43 and codon 88 of *rpsL* gene; known to be involved in streptomycin resistance (Victor *et al.*, 1999). Briefly, a 50ng sample of DNA from each isolate was amplified in a 100µL reaction mixture using forward
(STR52: 5'-GTG AAG ACCGCGGCTCTGAA-3')
and reverse
(STR34: 5'-TTCTTGACACCCTGCGTATC-3')
primers. These primers amplified 272bp region of the *rpsL* gene around codon 43 and 88. The amplification was done in a thermal cycler set to denature DNA at 95^{o}C for 5 min., followed by 30 cycles of denaturation (94^{o}C for 1 min.) annealing (60^{o}C for 45 sec.) and synthesis (72^{o}C for 45 sec.). The reaction was incubated at 72^{o}C for 10 min. for final extension. Amplification products were run on 1.2% agarose gel. The diluted PCR product was applied on the nylon membrane (Hybond N+ from Amersham) using dot blot apparatus. The DNA was fixed to membrane by UV cross linker (Stratagene). Radio labeled (^{32}P dTTP) oligonucleotide probes specific to wild type
(rpsL43 wt: 5'-ACCACTCCGAAGAAGCCGAA-3'
and
rpsL88 wt: 5'-CGGGTGAAGGACCTGCCT -3')
and mutant DNA sequence at codon 43 and 88 was allowed to hybridized with dot blot having PCR products. The membrane was autoradiographed and x-ray film was developed in darkroom using developer and fixer from Fuji Colour Company.

PCR-RFLP to detect mutation at codon 43 of rpsL *gene*

A mutation at codon 43 of rpsL gene removes the *Mbo*II restriction site. The PCR products (10 µl) were restricted in 20 µL reaction at 37°C for 1 hour. The digested PCR products were run on 1.2% agarose gel and the DNA bands were visualized after staining with ethidium bromide. In this particular case a mutation at codon 43 of the *rpsL* gene removes the *MboII* cleavage site hence DNA from isolates carrying the mutation at this position remains undigested (normal cut/mutant does not cut).

Results

Of 43 isolates, 31 were streptomycin resistant while 12 were found to be sensitive to streptomycin by proportion method (Canetti, 1969). All the isolates showed specific amplification giving 272bp

product. Restriction of PCR product with *Mbo*II was found to be a rapid screening method for mutation at codon 43 of *rpsL* gene. The PCR products were also analyzed by dot blot hybridization using wild type and mutation specific probes (Fig. 1). The rpsL43 mutation was found in 7 out of 31 streptomycin resistant isolates (22.58%) while rpsL88was found in 4 out of 31 (12.90%) isolates tested. The rpsL43 was also found in 27% (10 out of 37) isolates collected randomly, while rpsL88 was found only in 5.4% isolates. All susceptible isolates had no mutation when probed with rpsL43 or rpsL88.

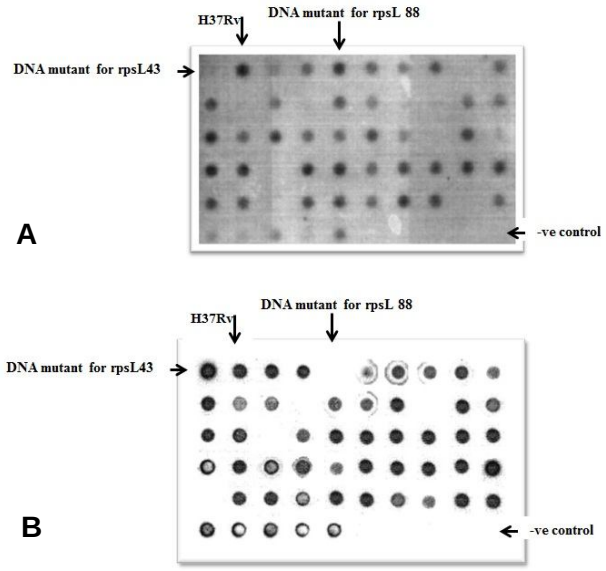

Fig. 1. Dot blot hybridization of 43 wild type probe (A) and 88 wild type probe (B) with PCR product of a region of *rpsL* gene. Amplification products from H37Rv and "no DNA" were applied as positive and negative control, respectively.

Discussion

Mutations associated with streptomycin resistance in *M. tuberculosis* were detected in *rpsL* gene. Mutations in codon 43 and 88 of the *rpsL* gene were found in 22.58% and 12.90% of the resistant isolates respectively. This low frequency of mutations in *rpsL* gene suggests that additional mutations are involved in resistance against streptomycin in Pakistan. The frequency of mutations associated with drug resistant is geographically related. Our frequency of finding *rpsL* gene mutations in MDR strains was much lower to those found in China where 81.4% of mutation happened in codon 43 of TB-rspL determined by *Mbo*II RFLP analysis (Huang et al, 2003). A study carried out on 14 drug resistant isolates from North India found no mutation at rpsL43 or rpsL 88 or in *rrs* gene (Siddiqi *et al.*, 2002). Thus, low frequency of mutations in *rpsL* gene is similar to those in North India. Recent data shows the low prevalence of Beijing strain in Pakistan and North India. It appears that different strains differ in mutations to avoid fitness cost due to different mutations. For examples countries such as Latvia where Beijing strain is dominant, RFLP by *Mbo*II and nucleotide sequencing of the rpsL gene fragment detected a single nucleotide substitution K43R in 40 (61%) of the 66 streptomycin-resistant *M. tuberculosis* isolates. In contrast, *rpsL* mutations were found in 48 % of the German isolates but only in 24% of the isolates from Sierra Leone (Dobner, 1997).

We conclude that the predictive values of mutations in *rpsL* gene vary significantly with the origin of the *M. tuberculosis* isolates and further work on understanding mechanism of resistance in *M. tuberculosis* isolates from this region is required before application of genotypic method in testing mutations associated with drug resistance against streptomycin in the country.

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Feeding Habits of a Freshwater Catfish, *Clupisoma naziri* (Pisces: Schilbidae) from Khyber Pakhtunkhwa Rivers, Pakistan

Umar Khan[1]*, Zaigham Hasan[1], Mian Inayatullah[2] and Arif Jan[1]

[1]Department of Zoology, University of Peshawar, Peshawar-Pakistan

[2]Department of Entomology, The University of Agriculture, Peshawar-Pakistan

Abstract.- Gut content analysis was used to evaluate the daily dietary habit of *Clupisoma naziri* in Indus and Kabul rivers, Khyber Pakhtunkhwa Pakistan. A total of 216 adult *C. naziri* were collected from summer till winter of 2011, 33.8% of examined catfish were found with empty stomach, while 66.2% fish had small aquatic and terrestrial insects. Of all the items 63.4% of food was comprised of terrestrial insects, while the rest was from aquatic sources. Furthermore, several algae taxa were also found in the diet of adult *C. naziri* as an important ingredient of its daily diet including; Cyanophyceae (46.5%), Bacillariophyceae (29.6%), Chlorophyceae (16.9%).

Keywords: Feeding Biology of *Clupisoma naziri*, catfish, gut contents, feeding intensity.

* Corresponding author: afg_durrani@yahoo.com

Clupisoma naziri belongs to family Schilbeidae found as native catfish species in Indus and Kabul rivers, Pakistan. This fish is distributed in Asia, *i.e.*, Pakistan (Mirza and Awan, 1973) and Afghanistan (Coad, 1981). In India, it is known as "Indus garua". It is popular for its flavour and regarded as delicious fish for its oil contents.

Being a potamodromous fish, during the months of May, June and August, it migrates upstream in the lower reaches of river Swat, Kabul and Indus. At this time total catch increases; being consumed in the region. As winter approaches, it gradually disappears from river Swat and Kabul due to its downstream migration in the river Indus.

The current status of natural aquatic resources reveals that in near future; this important fish species may be affected by several environmental impacts. These include use of pesticides by farmers around the area and the construction of the Warsak dam on river Kabul and Terbela dam on river Indus, which may have important negative impacts on the migration of potamodromous fishes. The migratory channels will get more blocked with the construction of the proposed Munda dam on river Swat (Craig, 2001).

To preserve the population of this fish species from further decline; it should be cultivated. However, in Pakistan, currently fish culture system is limited mostly to carp fish (Rab *et al.*, 2007). For propagating such fish species in captivity; more information on its biology, including reproduction and feeding is necessary (Turan *et al.*, 2005). The aim of this study was to find out detailed information about its daily diet and food quality in nature. Moreover, the morphology of mouth,

dentation, gills and stomach, were also studied as they are directly associated with food intake.

Materials and methods

The survey was conducted from June to the end of December 2011. Fish samples were collected from Peshawar fish Market, which is the biggest fish market in KP Province and fishes are brought to this market from all parts of the Province. Full record of each sample fish was noted, including place and catching time. Preferably, those fish were sampled which were brought to market early in the morning. Sampled fish was fully packed with crushed-ice providing -5°C to -12°C.

During this survey 216 fish samples were procured for diet analysis. Morphometric parameters like Total Length (TL), Total Weight (TW), eye diameter, length of upper jaw were measured. Morphological information of parts like mouth, teeth, gills, and stomach were also recorded. After that, stomach was dissected and its contents were transferred into a petri-dish (Zacharia and Abdurahiman, 2010).

Hard and identifiable material consisting of insects and their hard parts were preserved in 70% ethyl alcohol (Janjua and Gerdeaux, 2011) and soft contents, consisting mostly of algae were preserved in 2% buffered formalin (Ali *et al.*, 2010) for further microscopic examination. The invertebrates were identified with the help of identification keys (Borror *et al.*, 1981), whereas the algae were identified using the available literature (Smith, 1950; Prescott, 1961; Siddiqi and Faridi, 1964; Tiffany and Britton, 1971; Akiyama and Yamagishi, 1981).

Diet composition

Fish gut contents were analyzed using the Frequency of occurrence method (Hyslop *et al.*, 1980) which was calculated as the percentage of non-empty stomachs containing a particular prey type (Olowo and Chapman, 1999).

Frequency of occurrence

$$Oi = Ji/P * 100$$

Where, "Ji" is number of fish containing "prey i" and "P" is the number of fish with "prey i" in their stomach.

The monthly average weight (g) of food contents of *C. naziri* was obtained by using following statistical formula:

$$\bar{X} = \frac{\sum X}{N}$$

Total weight of each filled stomach was divided by 100g of the total body weight of each fish to get the value of X .

Results

Head of sampled *C. naziri* was moderate in size and oval blunt in shape. Head length was found to be 15.3% of the total body length. Eyes were comparatively smaller than *Eutropiichthys vacha*. Eyes were latterly situated in the middle of the head. Mouth was subterminal over hanged by bluntly slightly pointed snout. Upper jaw covered the lower jaw. Mouth opened at 40° angle with thin lips and surrounded with eight barbles, *viz.*, one pair of Nasal, one pair of maxillary and two pairs of mandibular barbles. Villiformes teeth were numerous and sharp. These were arranged like a shoe brush and were backwardly directed. Vomerine and palatine teeth were present on the roof of oral cavity. Vomerine and palatine teeth were close to each other and created uninterrupted band. Gill rackers were comparatively larger and pointed, continuously decreasing from top to lower end. Gills rackers were ±14, ±10, ±8, ±8 on first, second, third and fourth arch respectively.

Gills form strong sieve, could strain very firmly. Stomach was J-shaped having thin walls, however, very muscularised and possessed a well developed grinding function

The species presented some degree of food intake in all periods of the year. However, the weight of food contents was not the same in all the seasons: there was a decreasing trend from summer to winter (Fig. 1).

Eighteen different types of invertebrate prey and seven algae taxa indicated that *C. naziri* is predominantly insectivorous. The two-third (*i.e.* 66.2%) fish had small aquatic and terrestrial insects

in their stomach (Table I), with 63.4% of the diet being composed of terrestrial insects and36.6% from aquatic sources. Algae were found in high proportions including Cyanophyceae (*Lyngbya* spp. - 29.6%, *Oscillatoria* spp. - 16.9%), Bacillariophyceae (*Diatoma* spp.14.8%, *Cymbella* spp. 9.2%, *Synedra* spp. 5.6%), Chlorophyceae (*Ulothrix* spp. 9.2%, *Scenedesmus* spp. 7.7%). Remaining 7% contents mainly phytoplankton could not be identified due to their changed colour and partial digestion (Table II).

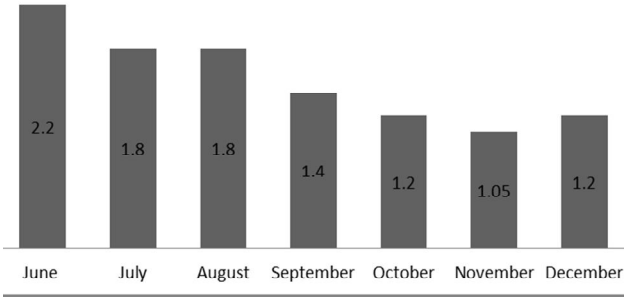

Fig. 1. Monthly average weight (g) of food contents of *C. naziri*.

Discussion

Clupisoma naziri on the basis of present study appears to be an insectivorous fish as 66.2% stomachs mainly contained small insects (both terrestrial and aquatic insects). It has been observed that *C. naziri* does not feed on small fishes, which may be due to its oral morphology; small mouth with short cleft. It is also important to note that algae are also an important component of its diet. Its feeding habits are much different from *Eutropiichthys vacha*, from the same subfamily (Schilbeinae) in Khyber Pakhtunkhwa Rivers, which feeds on small fishes (Khan *et al.*, 2013) and crustaceans (especially prawn).

With 63.4% food composed of terrestrial insects means that not only the river itself but the area surrounding the river also plays a great role in providing food to the fish. The reason for the frequent occurrence of "beetles" in the stomach contents of *C. naziri* is that they occur frequently in terrestrial environment (40% of all known insect species (Erwin, 1982) and are taken by rain and excess irrigational water and ultimately to the rivers/water bodies.

Table I.- Frequency of occurrence of different food items consumed by *Clupisoma naziri*.

Food items	No. of stomachs (n)	Frequency of occurrence (%)
True bugs (Hemiptera)*	19	13.4
Dragonfly naiad (Anisoptera: Odonata)*	18	12.7
Grasshoppers (Acrididae: Orthoptera)	17	12.0
Dung beetle (Scarabaeidae: Coleoptera)	9	6.3
Unidentified beetles (Coleoptera)	3	2.1
Flat headed borer (Buprestidae: Coleoptera)	11	7.7
Weevils (Curculionidae: Coleoptera)	11	7.7
Leaf beetle (Chrysomelidae: Coleoptera)	9	6.3
Water strider (Gerridea: Hemiptera)*	8	5.6
Unidentified beetles (Coleoptera)	7	4.9
Water beetles (Dytiscidae: Coleoptera)*	7	4.9
Ants (Formicidae: Hymenopetra)	6	4.2
Rove beetles (Staphylinidae: Coleoptera)	4	2.8
Ground beetles (Carabidae: Coleoptera)	4	2.8
Unidentified lacewings (Chrysopidae:Neuroptera)	4	2.8
Lady beetle (Coccinellidae:Coleoptera)	2	1.4
Yellow wasp (Vespidae:Hymenoptera)	2	1.2
Red wasps (Vespidae: Hymenoptera)	2	1.2

Animal groups marked with * are aquatic. The rest are terrestrial.

Table II.- Frequency of occurrence of different species *of* algae in the stomach of *Clupisoma naziri*

Food items	Number of stomachs (n)	Frequency of occurrence (%)
Cyanophyceae		
Lyngbya	42	29.6
Oscillatoria	24	16.9
Bacillariophyceae		
Diatoma	21	14.8
Cymbella	13	9.2
Synedra	8	5.6
Chlorophyceae		
Ulothrix	13	9.2
Scenedesmus	11	7.7
Unidentified	10	7.0

Similar to *Clupisoma garua* (Afsar, 1990) and *E. vacha* (Abbas, 2010; Khan *et al.*, 2013); there is no discontinuation in the feeding intensity; though stomach content decreases from summer to winter.

Conclusions

The majority of food (63.4% terrestrial insects) comes from terrestrial source; thus there is a strong probability of the pesticide-affected insects that might be injurious to the catfish and indicates that the population of the catfish might have been affected by excessive use of pesticides by farmer. Therefore, further research work is required to elaborate this aspect.

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Seroprevalence of *Toxoplasma gondii* Infection in Human Population of Mohmand Agency Khyber Pakhtunkhwa, Pakistan

Mudassir Shah,[1, 2,*] Muhammad Zahid,[1*] Aftab Alam Sthanadar,[1, 3] and Pir Asmat Ali[1]

[1]Department of Zoology, Islamia College University, Peshawar, Pakistan

[2]Government Degree College, Dara Adam Kheil, FR Kohat, Pakistan

[3]Department of Zoology and Animal Sciences, Post Graduate College Dargai, Malakand, KP, Pakistan

Abstract.- Across Pakistan, there is very scarce information regarding the seroprevalence of *Toxoplasma gondii* infection in general human population of Mohmand Agency. A total of 580 blood samples were collected from randomly selected localities of the study area and were tested by using Latex agglutination Test. A level of high prevalence (33.15 %) was observed in females as compared to males' population. Over all the prevalence rate recorded was 28.44%. In relation to age, the highest seroprevalence rate was detected in age group of 25-34 years. In relation to localities, seroprevalence was recorded high (33.33 %) in rural areas as compared to urban areas. The present study aimed to explore the frequency of infection in the human population and to provide an awareness initiative for locals about the deleterious consequences of the disease. The study will further open gates for future studies about toxoplasmosis.

Key words: *Toxoplasma gondii*, Latex Agglutination Test, seroprevalence, serum.

Toxoplasma gondii causative agent of toxoplasmosis is an intracellular protozoan parasite distributed world wide (Shah *et al.*, 2013a; Aldebret *et al*., 2011; Blader and Saeij, 2009; Ferguson,

* Corresponding author: mzahidsafi75@yahoo.com

2009). Infection usually occurs by ingestion of tissue cyst in meat and food or water contaminated by cat's faeces (Schlundt *et al*., 2004; Chaudhary *et al.*, 2006) and also from mother to offspring during pregnancy (Hajsoleimani *et al*., 2012; Sibley *et al*., 2009). *Taxoplasma gondii* can also be transmitted through blood or leucocytes from infected donors (Zhou *et al*., 2011; Bodaghi *et al.*, 2012).

Prevalence rate of *T. gondii* varies in different countries. Poland shows high prevalence 60% and Serbia city shows 58% (Hasan, 2011). Infections are often asymptomatic or have mild symptoms (Sarkar *et al*., 2012; Goz *et al.*, 2007). In USA about 10% and in Europe up to 30% AIDS patients are estimated to die from toxoplasmosis (Hill and Dubey, 2002). About 33% of the human population may harbour this parasite. Prevalence rate changes according to the climate, geographic factors, nutritional factors, sociocultural habits and transmission route (Shah *et al*., 2013b). Improved animal husbandry practices as well as increased awareness of the risks of consuming undercooked meat have resulted in decreased prevalence of toxoplasmosis world-wide (Weiss and Dubey, 2009).

The seroprevalence of *T. gondii* in the Southern Punjab has been reported to be 35.89% in males and 25.9% in females (Tasawar *et al.*, 2012). It was also found that *T. gondii* infection increased with age, lower educational level, populous living conditions and soil-related occupations (Shah *et al.*, 2013a,b; Khan *et al* 2011),

This study aims at determining the incidence of toxoplasmosis in general human population of Mohmand Agency, Pakistan. This will further open gates for the molecular diagnosis of toxoplasmosis in the region.

Materials and methods

Sera isolated from blood samples of 580 subjects from different localities of Mohmand Agency, Pakistan were used on spot for the presence of anti-toxoplasma antibodies, using Latex Agglutination Test Kits according to the manufacturers protocol (Antec Diagnostic Products, UK).

The results were expressed in percentages. The values between different age groups, sex groups and area wise were recorded and relevantly expressed in percentages. Microsoft Excel (version-10) was utilized by Windows-08, Corei3 computer (Release 16.0 standard version, Dell Microsoft Corporation).

Results and discussion

Table I shows age-wise, gender-wise and area-wise prevalence of toxoplasmosis in Mohamand Agency. Latex out of 580 blood samples 165 (28.44%) blood samples were detected seropositive for toxoplasmosis. Lowest prevalence (8.18%) was found in the age group of 05-14 years. whereas the highest seroprevalence (39.47%) was detected in the age group 25-34 years.

Table I.- Age wise, sex wise and area wise prevalence of toxoplasmosis in human population of Mohmand Agency.

	Total No. of cases	Total No. of positive	Percentage of positive (%)
Age (Years)			
5-14	110	9	08.18%
15-24	140	45	32.14%
25-34	190	75	39.47%
35-44	140	36	25.71%
Total	580	165	28.44%
Gender			
Male	200	39	19.50%
Female	380	126	33.15%
Total	580	165	28.44%
Area wise			
Urban	220	45	20.45%
Rural	360	120	33.33%
			53.78%

Out of 220 blood samples collected from urban areas 45 (20.4 %) were tested seropositive for *T. gondii*, whereas 33.33% blood samples from rural areas were found seropositive. A high prevalence rate was observed in females (33.15%) compared to males (19.5%).

The seroprevalence reported in this study is lower than 58% reported from Busrah province (Al-mousawi and Shani, 2011). Overall the spread of the disease varies among countries depending on the socioeconomic conditions, traditions and customs of

the people living there (Shah *et al.*, 2013a). The infection rate in the present study is lower than 39% reported from Valdivia province, Southern Chile (Munoz-zani *et al*., 2010), 52.1% from Malgasy population (Dromigny *et al*., 1996), 73.3% reported from Brazil (Cavalcante *et al*., 2006), 60% reported from Abbotabad, Pakistan (Ally and Idris, 2004), and 63% seroprevalence of toxoplasmosis reported from Punjab (Bari and Khan, 1990). The prevalence rate is also lower than 44.8% reported from Malaysia (Nissapatorn *et al*., 2004) but is higher than 12.3% reported from China (Yue *et al*., 2010). The prevalence rate was higher in the present study because of the warm and humid climatic conditions which is similar to previous studies carried out in Pakistan (Shah *et al*., 2013a). Anti-*Toxoplasma gondii* antibodies were found to be more common in females then in males. A significant gender difference was observed in the present study, which is similar to the work done in China (Yue *et al*., 2010) but no significant gender difference was observed in the work done in Iran (Sharif *et al*., 2010) which showed 23.5% prevalence in males and 20.1% in females.

In the present study the seroprevalence of toxoplasmosis is high (39.47%) in people of age group 25-34 years, which is almost similar to 33.3% reported from Nigeria (Uneke *et al.*, 2007). A low prevalence rate of 8.18% was observed in the age group of 05-14 years which is less than 23.1% reported from Iran (Sharif *et al*., 2010). The infection rate became low in the age group 35-44 years which has also been reported from other laboratories (Tasawar *et al.*, 2012; Uneke *et al.*, 2007). Increase in infection rate occurs with age from 05-14 to 25-34 in the present study which was also observed in Korea (Shin *et al*., 2009). Seroprevalence of 25.71% was observed in age group 35-44 years in the present study which is nearly similar to 17.33% of age group 41-50 years old from Pakistan (Ahmed *et al*., 2012).

In the present study population of rural areas were more exposed to the infection than urban areas. This is because the people of rural areas were more likely to have contact with soil compared to people of urban areas. The present report is consistent with that of the previous work *viz.*, 1.66% in urban and 4.49% in rural from Malaysia (Hao, 2013), and Korea (Shin *et al*., 2009). However, no significant difference was observed in the infection rate among the inhabitants of rural and urban areas in China (Yue *et al*., 2010) and in Switzerland (Studenticova *et al*., 2006). Infection rate of 33.33% detected in rural area is higher than 8.2% reported from Mexico (Alvarado-Esquivel, 2009) and 20% from Nigeria (Uneke *et al.*, 2007). In rural areas most of the people are illiterate and they have no awareness regarding the sanitation and personal hygienic conditions. Majority of the people are living in mud houses, thus having more contact with the soil (Shah *et al.*, 2013a).

T. gondii infection is controlled by washing of raw fruits and vegetables (Esquivel *et al*., 2011; Selseleh *et al*., 2012). Health education is also extremely important for women to prevent maternal toxoplasmosis. Women should be educated regarding their eating habits and the value of good hygiene at the first visit for antenatal care. Women should avoid eating raw and undercooked meat (Sakikawa *et al*., 2011; Cabanas *et al.*, 2012). Education of farmers, which help in reduction of *T.gondii* infection, reduce environmental contamination of oocyst and reduce number of cats which sheds oocysts in the surrounding are included in preventive methods (Abu-Dalbou *et al.*, 2010).

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Some Interesting Facts About Tongue Rolling Trait in Humans

Hafiz Muhammad Tahir,[1]* Jafar Aqeel,[1] Rabia Mishal,[1] Muhammad Mohsin Ahsan,[1] Muhammad Bilal[2] and Shafaat Yar Khan[1]

[1]Department of Biological Sciences, University of Sargodha, Pakistan

[2]Atta ur Rehman School of Applied Biosciences (ASAB), National University of Sciences and Technology, Islamabad, Pakistan

Abstract.- Present study was conducted to determine the prevalence of tongue rolling trait in the population of district Sargodha, Pakistan. We also tested the hypothesis that tongue rolling is not genetically controlled but people learn this trait with age and experience. To record the prevalence of rollers and non rollers, data of 1734 individuals (682 males and 1052 females) was collected. To study the effect of age and experience on tongue rolling, data of school students of two different age categories was collected. Observed frequencies of roller males and females were 68.32% and 45.82%, respectively. Significantly higher number of roller males was recorded in age category B (12-13 years) compared to age category A (7-9 years). However, difference was non-significant in the two age categories for females. We also recorded 19 % roller offspring from families in which both parents were non rollers. Results of the study indicate that tongue rolling is a complex trait which might involve non-genetic influence or may be controlled by multiple genes.

Keywords: Tongue rollers, complex trait, genetic traits.

Most of the genes follow Mendelian pattern of inheritance. However, inheritance of some traits involves complex genetic phenomena such as co-dominance, sex-linked genes and polygenic inheritance (Batul, 2010). Tongue rolling is the genetic trait quoted very frequently by biologists to demonstrate basic genetic principles (McDonald, 2011). The ability to roll tongue is considered to be due to the involvement of a dominant gene (R). People having recessive (r) allele are unable to roll their tongues (Hsu, 1948). Liu and Hsu (1949) reported the involvement of recessive gene in tongue rolling.

Present study was aimed at recording the prevalence of rollers and non rollers in the population of district Sargodha, Pakistan. Komai (1951) and Whittinghill (1970) claim that tongue rolling is not genetically controlled but people learn this trait with age and experience. In the present study, we have also tested this hypothesis by studying the tongue rolling trait among selected families.

Materials and methods

To record the prevalence of tongue rolling trait data of 1734 individuals (682 males and 1052 females) was collected randomly from different localities of district Sargodha. The age of individuals that were included in random data collection ranged from 18-56 years. Standard method described by Liu and Hsu (1949) was used for data recording. The individuals who were only slightly able to roll edges of their tongues were considered as non rollers.

To study the effect of age and experience on tongue rolling trait data of school students was collected. Students were grouped into two categories. Age of students in category A was between 7-9 years. However, students of category B were between 12-13 years. From each category data of 200 students was recorded. Data was collected separately for male and female students. The data obtained from these two age categories was compared using Chi-squared test.

Sturtevant (1940) and Komai (1951) reported the possibility of roller offspring even if both parents are non roller. To test this hypothesis we planned to study this trait in all members of selected families. We randomly collected data of 171 families. Breakup of the selected families was as under: Both parents rollers, 58; One parent roller and other non roller, 46; and both parents non rollers, 67.

* Corresponding author: hafiztahirpk1@yahoo.com

Results

Out of 682 males 68.32% (466) were roller and remaining 31.67% (216) were non rollers. However, there were 45.82% (482) roller females compared to 54.18% (570) non rollers. Statistically roller males were significantly higher than roller females (Chi-square value = 42.303; P > 0.001, Table I). Out of total non rollers (combined for males and females), 7% individuals were able to slightly roll edges of their tongues.

Number of roller males in age category B was significantly higher than the number of males in age category A (Table II). However, statistically non-significant difference was recorded in the number of females in two different age categories (Table II).

Highest percentage of roller offspring (87%) was recorded from the families in which both parents were roller (Table III). It is also depicted in the Table III that 63% roller offspring were observed in families in which only one parent was roller. We also recorded 19 % roller offspring of non roller parents (Table III).

Discussion

Among different tongue movements, which help in eating process, tongue rolling is used as an important classical indicator in human population genetics (He *et al*., 2012). The frequency of tongue rolling trait which is thought to be controlled by dominant gene varies among different populations. In Sargodha population frequency of roller males and females was 68.32% and 45.82%, respectively. Similar results have been reported by some other researchers. For example He *et al*. (2012) reported 63.6% rollers in Han nationality Shaanxi, China, Onyije (2012) reported 57.2% rollers from Southern Nigeria, Odokuma *et al*. (2008) reported 61.49% rollers from Urhobo tribe of Africa and Sturtevant (1940) reported 65% rollers in European population.

In the present study we recorded significant increase in tongue rolling trait with age, at least among males (Table II). This suggests that learning is crucial for tongue rolling trait. Our finding is supported by the observation of Komai (1951) who reported 20% increase in rollers from age 6-7 to 12 among Japanese school children. However, the possibility of involvement of multiple genes cannot be excluded from this finding. Matlock (1952) and Martin (1975) observed numerous pairs of monozygotic twins who differed in tongue rolling trait suggesting non-genetic influences on tongue rolling.

Table I.- Prevalence of roller and non roller population in district Sargodha, Pakistan.

Sex	Total	Roller (%)	Non roller (%)	P value
Male	682	466 (68.32)	216 (31.67)	$\geq$ 0.001
Female	1052	482 (45.82)	570 (54.18)	

Table II.- Effect of age and experience on tongue rolling in males and females.

Age category	Males (%)		Females (%)	
	Rollers	Non rollers	Rollers	Non rollers
A	90 (45)	110 (55)	39 (78)	61 (122)
B	138 (69)	62 (31)	44 (88)	56 (112)
Chi-square test	11.75		0.51	
P-value	0.001		0.47	

Table III.- Impact of family history on tongue rolling trait.

Parents	No. of families	% of roller offspring	% of non roller offspring
Roller x Roller	58	87	13
Roller x Non roller	46	63	37
Non roller x Non roller	67	19	81

Finally, results of family studies showed 19% roller offsprings from non-roller parents. Komai (1951) has also observed roller offsprings among families of non roller parents. This outcome is not possible if tongue rolling is considered an autosomal dominant trait. It is concluded from the study that tongue rolling is a complex trait which involves some non genetic influence or may be controlled by multiple genes.

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Recent Record of *Scotophilus heathii* From Wheat-Rice Based Agroecosystem of Punjab

Muhammad Shahbaz,[1] Arshad Javid,[1] Muhammad Mahmood-ul-Hassan,[2] Syed Makhdoom Hussain,[3] Sana Ashraf[1] and Muhammad Idnan[1]

[1]Department of Wildlife and Ecology, University of Veterinary and Animal Sciences, Lahore
[2]Department of Zoology and Fisheries, University of Agriculture, Faisalabad
[3]Department of Zoology, Wildlife and Fisheries, Government College University, Faisalabad

Abstract.- Present study was focused to explore core rice producing belt of central Punjab to ascertain the presence or absence of Asiatic Greater Yellow House bat *Scotophilus heathii*. Three districts namely Gujranwala, Hafizabad and Mandi Bhaudin were surveyed from January 2011 through December 2012 and a total of 33 *S. heathii* were captured. The external body, cranial and bacular measurements of the captured specimens were compared with available literature. The average head and body length of all 33 captured specimens was 73.7±4.4 mm, forearm length was 60.6±4.1 mm, greatest skull length (n = 6) was 18.7±1.7 mm while baculum (n = 1) was 3.075 mm long. The cranial and bacular features of the species which are important traits for mammalian identification are reported for the first time in country and this is also the first record of the species from study area.

Key words: cranial measurements, baculum size, agro-ecosystem, Gujranwala

Worldwide, genus *Scotophilus* is represented by 12 species with distribution ranges from South Africa to Senegal, Sudan, Somalia and Madagascar, Arabia, Afghanistan to Indonesia, Philippines and Taiwan (Koopman, 1993). In Indian subcontinent this genus is represented by two species namely greater Asiatic yellow house bat

* Corresponding author: arshadjavid@gmail.com

S. heathii and lesser Asiatic yellow house bat *S. kuhlii*. Both the species have been recorded from territorial limits of Pakistan (Bates and Harrison, 1997; Srinvasulu *et al.*, 2010; Javid *et al.*, 2014). In Pakistan, *S. heathii* is common and wide spread throughout the Indus plains and has been reported from Kohat (Khyber Pakhtunkhwa), Islamabad, Sanghoi, Bhattu Hisar, Multan Lahore and Sialkot (Punjab), and Kashmoor, Sakkur, Jacobabad (Wroughton, 1916), Mirpur Sakro (Lindsay, 1927), Dadu, Landi, Malir, Karachi (Sindh) (Siddiqui, 1960; Taber *et al.*, 1967; Walton, 1974).

S. heathii roosts in variety of habitats, in crevices and hollows of old buildings, tree hollows, banyan and peepal trees, abandoned wells, ruins (Sinha, 1986), leaves and crowns of coconut and palm (Phillips, 1980). The species lives singly or in colonies that vary up to 50 individuals (Sinha, 1986). The species is often mixed with its congener in the Indian region the larger species were initially recognized as *S. kuhlii* and smaller as *temmnickii* and *wroughtoni*. However, larger specimens of this genus were later referred to as *S. heathii* and both the species were correctly recognized (i) larger ones as *S. heathii* and (ii) smaller ones as *S. kuhlii* (Hill and Thonglongya, 1972; Corbet and Hill, 1992; Koopman, 1993; Bates and Harrison, 1997; Simmons, 2005).

Apparently *S. heathii* is a common and widespread species worldwide however during surveys in Sri Lanka only one specimen was collected during 1990s (Bates and Harrison, 1997). The present study is unique as *S. heathii* has never been reported from the study area prior to this survey.

Materials and methods

This two year study extending from January 2011 to December 2012 was conducted in areas of wheat-rice based agro-ecosystem of Punjab province. These areas are comprised of three districts namely Gujranwala, Hafizabad and Mandi-Bhaudin in central Punjab, which is most populated province of Pakistan. 19 sampling stations (Table I) were fixed in these three districts and netting efforts were made to ascertain the presence or absence of *Scotophilus heathii*. Three nights in each month, one night in each district was spent throughout the study period and bats were captured with the help of mist and hand nets. For locating potential bat roosts in the study area old and undisturbed buildings, ruins, abandoned wells, farm houses, tree groves and forest plantations were searched. People of the study area were also interviewed for getting maximum information about the exact location of bat roosts.

Table I.- Sampling sites in three districts of wheat-rice based agroecosystem of Punjab.

Sampling sites	District	Longitude	Latitude
Civil hospital, Gujranwala	Gujranwala	74°11.583 E	32°10.203 9 N
Qadirabad colony	Gujranwala	73°41.563 E	32°17.490 N
Kelaske village	Gujranwala	73 º58.668 E	32 º10.803 N
Gakhar mandi town	Gujranwala	74º08.791 E	32°18.340 N
Rasul nagar village	Gujranwala	73º46.720 E	32º19.68 N
Ali pur chattha town	Gujranwala	74º09.361 E	32º11.272 N
Verpal chattha village	Gujranwala	73º58.803 E	32º 10.803 N
District katchery, Hafizabad	Hafizabad	73°34.918 E	31 º58.018 N
Kot sarwar, village	Hafizabad	73 º30.189 E	31 º55.148 N
Nothain village	Hafizabad	73 º32.770 E	31 º56.241 N
Kaleki mandi village	Hafizabad	73°42.697 E	32°04.150 N
Sukheki mandi village	Hafizabad	73º34.108 E	31º61.116 N
Pindi bhattian town	Hafizabad	73 º16.895 E	31 º55.148 N
Jalal pur bhatian village	Hafizabad	73º22.51 E	32º02.39 N
Farid town	Mandi Bhaudin	73º30.46 E	32º35.123 N
Phalia town	Mandi Bhaudin	73º58.13 E	32º43.601 N
Malikwal town	Mandi Bhaudin	73º45.08 E	32º35.102 N
Head rasul Rest House	Mandi Bhaudin	73º31.148	32º40.096
Mano chak	Mandi Bhaudin	73º45.29	32º25.308

Once the bat specimens were captured, they were euthanized, placed in cotton bat bags and each specimen was weighed up to 0.1 g (Pesola balance 10050, Swiss made). Each bat was preserved in a plastic jar in absolute alcohol and brought to laboratory for further observations and analysis. The external body measurements were taken using a digital vernier caliper (0-150 mm) following Bates and Harrison (1997). For cranial and bacular measurements, the skulls and bacula were prepared and measured following Bates *et al.* (2005).

Table II.- Body mass (g), external, cranial and bacular measurements (mm) of *Scotophilus heathii* captured from wheat-rice based agro ecosystem of Punjab, Pakistan.

External body measurements	Present study (n=33)	Walton (1974)	Roberts (1997)	Bates and Harrison (1997)	Cranial measurements	Present study (n = 6)	Bates and Harrison (1997)
Body mass	30.9±5.4	-	(36-39)	-	Breadth of braincase	9.3±0.9	10.2 (9.7-10.8)
Head and body length	73.7±4.4	142	83 (72-92)	82.5 (67-93)	Zygomatic breadth	13.7±1.0	15.6 (14.5-16.9
Ear length	14.6±1.8	-	16 (14-7)	16.9 (13.0-20.2)	Postorbital constriction	5.1±0.8	5.5 (5.2-5.9)
Tragus length	8.16±0.73	-	-	-	Condylo-canine length	17.8±1.8	20.2 (19.0-21.3)
Thumb length	9.1±0.93	-	-	-	Condylo-basal length	18.3±1.8	-
Claw length	2.5±.39	-	-	-	Greatest length of skull	18.7±1.7	23.4 (21.7-25.2)
Forearm length	60.6±3.4	58	-	60.7 (55.4-65.8)	Maxillary too throw	6.8±1.0	7.7 (7.1-8.4)
3rd metacarpal length	59.7±2.5	-	-	59.4 (53.7-64.8)	Anterior palatal width	6.1±1.0	-
3rd metacarpal: 1st phalanx	19.9±1.5	-	-	-	Posterior palatal width	8.4±1.3	10.0
3rd metacarpal: 2nd phalanx	15.7±1.6	-	-	-	Mandibular too throw	7.9±1.0	8.8 (8.1-9.6)
4th metacarpal length	58.7±2.4	-	-	58.2 (54.0-63.9)	Mandible length	14.1±1.9	16.3 (14.8-18)
4th metacarpal: 1st phalanx	15.9±.96	-	-	-	**Bacular measurements**	**n = 5**	-
4th metacarpal: 2ndphalanx	11.6±1.4	-	-	-	Total length of vacuum	1.6±0.11	-
5th metacarpal length	54.5±1.7	-	-	54.6 (50.3-59.2)	Length of shaft	1.4±0.23	-
5th metacarpal: 1st phalanx	11.1±2.1	-	-	-	Length of proximal branch	0.20±0.02	5-3
Wingspan	355±30.1	-	-	-	Length of distal branch	0.04±0.01	-
Tibia length	23.9±1.2	-	-	-	Width of proximal branch	0.87±0.07	-
Calcar length	6.3±0.93	-	-	-	Width of distal branch	0.43±0.01	-
Hind foot length	12.7±1.5	13	12 (11-13)	12.0 (9.0-15.0)	Height of baculum	0.37±0.05	-
Tail length	53.3±4.3	60	55 (51-60)	59.1 (43.0-71.0)	-	-	-
Penis length	7.7±0.90	-	-	-	-	-	-

Mean±SD. Values in brackets show ranges.

Results and discussion

There is dire need to identify bats for better ecological research and conservation of taxa. The insectivorous bats are pest control agents and there is a strong relationship between bat morphology and prey selection (Nadeem *et al.,* 2013; Weterings and Umponstira, 2014). Bats identification on the basis of external body measurements and skull parameters is still a highly reliable method (Hill and Smith, 1984; Vaughan *et al.,* 2000; Jacobs *et al.,* 2006) and application of character matrices and identification keys are authentic tools to identify different chiropteran species (Daniel, 2009; Srinivasulu *et al.,* 2010). Roberts (1997) and Bates and Harrison (1997) are the only sources of literature in Indian region. *Scotophilus heathii* has been reported from sea level to an elevation of 1,500 m (Molur *et al.,* 2002). During present survey, a total of 33 *S. heathii* specimens were captured from an elevation of 1700 m. The external body, cranial and bacular

measurements of captured specimens were compared with Walton (1974), Roberts (1997) and Bates and Harrison (1997) (Table II). The average head and body length of all 33 *S. heathii* was 73.7±4.4 mm while the forearm was 60.6 ± 3.4 mm long. The lengths of 3rd, 4th and 5th metacarpals were 59.7±2.5, 58.7±2.4 and 54.5±1.7 mm, respectively. The average wingspan of the captured specimens was 355±30.1 mm and tail was 53.3±4.3 mm long. The average greatest skull length of six *S. heathii* was 18.7±1.7 mm. Average total length of baculum of five specimens was 1.6±0.11 mm (Table II). The baculum of *Scotophilus* lies in the glans penis and it is duckbilled shaped (Harrison and Brownlow, 1978). The bacular shape of one of the specimens is represented in Figure 1.

Fig. 1. Baculum of *Scotophilus heathii* showing its shape

Average values for head and body length and tail length of all the 33 specimens captured during the present survey are within the ranges given by Roberts (1997) and Bates and Harrison (1997) while the same measurements are smaller than the specimens' recorded by Walton (1974). The mean values of forearm length and hind foot length of the currently captured specimens are within the ranges given by Walton (1974), Roberts (1997) and Bates and Harrison (1997). Similarly, the mean values of the 3rd, 4th and 5th metacarpals fall within the ranges mentioned by Roberts (1997) and Bates and Harrison (1997). Mean breadth of braincase, mandibular tooth row and mandible length of six *S. heathii* captured during present study are within the ranges recorded by Bates and Harrison (1997) (Table II).

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Impact of Organic Acid on Some Liver and Kidney Function Tests in Japanese Quails, *Coturnix coturnix japonica*

Tariq Hayat[1], Asad Sultan[1], Rifat Ullah Khan[1], Sarzamin Khan[1], Zahoor ul Hassan[1], Rafi Ullah[1] and Tariq Aziz[2]

[1]Faculty of Animal Husbandry and Veterinary Sciences, The University of Agriculture, Peshawar, Pakistan; Livestock and Dairy Development, Peshawar (FATA), Pakistan

Abstract.- This study was conducted to investigate the outcome of organic acids on modulating liver and renal physiological functions in quail (*Coturnix coturnix japonica*). For this purpose, one group of 30 birds was offered potable drinking water, while other three groups, each of 30 birds were given 1, 2 and 3 ml of organic acid blend (citric acid 80g, lactic acid 52g, phosphorous 92g, copper sulphate 10g) per liter of drinking water, respectively for four weeks. Serum samples were used for estimation of liver and kidneys on weekly basis. Alanine aminotransaminase (ALT), aspartate aminotransaminase (AST), urea and creatinine showed significantly lower values in the treated groups compared to the untreated quails. The finding of the study suggested that addition of organic acids in the drinking water did not have any deleterious effects on health of liver and kidney in qauil.

Keywords: Quail, organic acid, liver enzymes, urea, creatinine.

After imposing ban on the use of antibiotics in animal feed, the researchers and poultry scientists are looking for alternative technologies to maintain poultry production (Khan *et al*., 2010, 2012a,b). Treating drinking water of the poultry farm has recently been advocated to improve the performance, digestibility and blood biochemistry of broilers (Adil *et al*., 2010; Khan and Naz, 2013; Gilani *et al*., 2014).

* Corresponding author: rifatullahkhhan@gmail.com

Organic acids (OA) possess growth promoting properties by maintaining cellular integrity of gut lining and improving digestive process by maintaining normal gut flora and hence can potentially be used as alternative to antibiotic growth promoters. They are generally considered to be safe and play a significant role in increasing the profitability of the poultry production and also by providing healthy and nutritious poultry products to the consumers. Therefore, most of the member states of EU have approved their use of organic acid in the feed for animal production (Patten and Waldroup, 1988; Ricke, 2003).

Organic acids such as citric acid, numeric acid and formic acid enhance the digestibility of protein and amino acid by increasing gastric proteolysis. Feeding of organic acid, reduces gastric pH which may boost the action of pepsin (Kirchgessner and Roth, 1988) arising proteolysis which activate the release of gastrin and cholecystokinin hormones and regulate the digestion and assimilation of protein (Henry *et al*., 1987). They play a significant role in better utilization of the available nutrient resulting in improved growth rate and feed conversion efficiency (Denli *et al*., 2003). Apart from that, they also improve the digestibility of minerals like phosphorus, calcium, zinc and magnesium provide as media in the conciliator metabolism (Kirchgessner and Roth, 1988).

The aim of the present study was to find the effect of organic acid on some blood parameters of Japanese quail.

Materials and methods

The present research study was approved by the Departmental Board of Studies for Procedures involving animal ethics, welfare and other practices. A total of 480 Japanese quails (*Coturnix coturnix japonica*), 14-day-old (average weight 40g) were reared in cages under optimal environmental and management practices. A commercial diet having 20% crude protein (CP) and 3,000 kcal of metabolizable energy (ME) was offered (Chand et al., 2014). Birds were randomly divided into four groups (OA-0, OA-1, OA-2 and OA-3) each having 4 replicates of 30 quails. The birds in group OA-0 were offered untreated drinking water while those in group OA-1, OA-2 OA-3 were given 1, 2 and 3 ml of Aciflex® (citric acid 80 g, lactic acid 52 g, Phosphorus 92 g, Cupper sulphate 10 g/litter) per liter of drinking water. The experiment lasted four weeks.

At the end of each week, a total of 48 birds (3 birds from each group) were slaughtered and blood samples were collected for serum separation. The serum samples were stored at -18°C until biochemical assays. Serum ALT (alanine amino-transaminase) and AST (aspartate amino-transaminase) activity was determined by using commercially available kits (AMP diagnostic, Austria and Labtest Diagnostics S.A). For determination of serum urea and creatinine, the kits from Linear Chemicals, Spain, were used. All parameters were determined spectrophoto-meterically.

The data was subjected to one-way analysis of variance (ANOVA) as described by Steel *et al.* (1997). To find the significance difference, Duncan's multiple range test was used (Duncan, 1955).

Results and discussion

The results of organic acid on serum ALT, AST, urea and creatinine for four weeks are shown in Table I. It is evident that serum ALT decreased significantly with the rising level of organic acid supplementation in each week. No change in AST concentration was observed in AST concentration during second and third week, however, it decreased significantly ($P<0.05$) during third and fourth week of the supplementation. Urea and creatinine concentrations decreased significantly ($P<0.05$) during first, second and third week. Urea concentration however showed lowest ($P<0.05$) concentration and creatinine concentration increased significantly ($P<0.05$) after four weeks.

The use of OA in the poultry production is generally considered as safe reliable alternative to antibiotic. The utilization of OA has been approved to improve the growth rate and FCR in broiler chicks (Adil *et al*., 2010). The organic acids in the non-dissociated (nonionised) type can infiltrate the bacterial cell and then interrupt in their normal functions (Dhawale, 2005). Apart from the antimicrobial activity, organic acids decrease the pH

of digesta, augment the secretion of pancreas, and have effects on the gastro intestinal mucosa (Dibner and Buttin, 2002). In this way, the intestinal mucosa is protected from the colonization of various pathogenic organisms and to improve the digestibility of the various minerals. A lot of work has been done in the broiler and layer birds regarding the use of organic acid. However, a slight potential affects of organic acid in the Japanese quails; so, this study was designed to test the effects of organic acid on the serum biochemical profile of the intestine.

The organic acids blend affected ALT activity of the quails in a dose dependent response. During wk 1 and 2 of the experiment a significantly lower values were noted supplying OA at 3 ml/L of drinking water as compared to the control group. However, with increasing the duration of the experiment, *i.e*., during wk 3 and 4, quails given OA at all doses *i.e*. 1, 2 and 3 ml/L of drinking water showed significant lower values in the ALT activity than the control group. Although no parallel study was found in the accessible literature regarding the effects of OA supplementation in the serum biochemical profile of the quail, Yesilbag and Colpan (2006) reported a negative effect (enhanced effect) upon the ALT activity of laying hens receiving OA at 1.0 and 1.5% in their diet. These divergent results might be due to the breed difference of the experimental animals or to the composition of OA mixture, as they fed OA blend including propionic acid and formic acids, and their ammonium salts; while in the present study we used citric acid, lactic acid, phosphorus, and copper sulphate. Similarly, Adil *et al*. (2010) reported a non-significant effect of organic acid supplementation in the ALT activity of the broiler chicks.

The supplementation of OA blend (Aciflex®) showed significant decrease in the aspartate AST activity of the quails. In contrast to our results, Yesilbag and Colpan (2006), Abdel-Fattah *et al*. (2008) and Adil *et al*. (2010) reported a non-significant affect of organic acid blend on the AST activity of broiler chicks and laying hens. Both ALT and AST are hepatocellular leakage/necrosis enzymes, with the alteration in the hepatocytes membrane permeability, the leakage of cytosolic enzymes occurs in the extracellular fluid and subsequently in the blood. The magnitude of the increase in the serum activity depends upon the

Table I.- Effect of Aciflex® supplementation on alanine aminotransferase (U/l) asartate aminotransferase (U/l), urea and creatinine concentration of Japanese quail.

Aciflex®) (ml/L)	Week 1	Week 2	Week 3	Week 4
Alanine aminotransferase				
0	23.71± 0.24^{a}	24.96± 0.44^{a}	25.27± 0.39^{a}	24.83± 0.31^{a}
1	23.56± 0.33^{a}	24.80± 0.28^{ab}	23.72± 0.26^{b}	24.53± 0.37^{ab}
2	23.11± 0.26^{a}	23.97± 0.36^{ab}	25.05± 0.29^{a}	23.67± 0.32^{bc}
3	22.36± 0.16^{b}	23.82± 0.28^{b}	24.09± 0.29^{b}	23.37± 0.27^{c}
P-value	0.0029	0.0079	0.0025	0.0575
Aspartate aminotransferase				
0	26.89± 4.97^{a}	27.63± 4.01^{a}	61.53± 10.58^{a}	80.51± 11.03^{a}
1	26.12± 2.13^{a}	26.67± 4.19^{a}	56.91± 5.08^{a}	62.17± 10.02^{ab}
2	26.72± 1.78^{a}	24.99± 1.73^{a}	46.92± 6.55^{ab}	67.44± 8.78^{ab}
3	25.47± 3.20^{a}	22.56± 1.76^{a}	34.69± 4.08^{b}	44.94± 3.21^{b}
p-value	0.9899	0.6882	0.0473	0.0508
Urea				
0	50.45± 0.20^{a}	54.89± 2.91^{a}	51.39± 0.81^{a}	51.25± 0.55^{a}
1	50.10± 0.15^{a}	50.87± 0.45^{ab}	49.62± 0.42^{b}	48.16± 0.37^{b}
2	49.21± 0.22^{b}	49.76± 0.30^{b}	48.11± 0.31^{c}	47.21± 0.35^{b}
3	49.13± 0.29^{b}	49.08± 0.27^{b}	47.26± 0.32^{c}	45.38± 0.50^{c}
P-value	0.0001	0.0379	0.0000	0.0000
Creatinine				
0	1.38± 0.18^{a}	2.38± 0.05^{a}	2.10± 0.51^{a}	2.37± 0.51^{a}
1	1.0± 0.11^{ab}	1.47± 0.15^{b}	1.23± 0.15^{b}	1.43± 0.11^{b}
2	0.89± 0.16^{b}	1.37± 0.14^{b}	1.12± 0.16^{bc}	1.30± 0.15^{b}
3	0.68± 0.12^{b}	1.32± 0.14^{b}	0.38± 0.08^{c}	0.50± 0.07^{c}
P-value	0.0104	0.0000	0.0013	0.0003

Means within a column with different superscripts are significantly different (P<0.05)

number of hepatocytes affected, the severity of the injury, the nature of insult and serum half life of the enzyme (Latimer and Mahaffey, 2003). The decrease in the activity of the enzymes might be due scavenging effect of organic acid mixture against the free radicals generated during the normal metabolic processes in the body.

The supplementation of OA blend in the drinking water significantly improved the kidney functions of the quails. During wk 1 and 2 of the experiment, the supplementation of OA blend (Aciflex®) at the rate of 2 and 3 ml/L of drinking water showed significant effect on blood urea nitrogen (BUN) values. With increasing the duration of the experiment the effect of organic acid at lowest dose *i.e.*, at 1 mL also showed significant different as compare to control. Also a significant effect of organic acid was noted in the serum creatinine values in the groups offered organic acid at 2 and 3 ml/L of drinking water. During the week 1, the supplementation of organic acid mixed drinking water at 1 ml/L of drinking water had no significant effect as compared to the values observed in the quails in control group. However, with increasing the duration of organic acid supplementation *i.e.* week 2 and onward, these effects became significant at this dose too.

In the present study, OA slightly decreased the serum concentration of uric acid and creatinine. This reduction could be related to the better utilization of protein and amino acid digestibility. This is supported by a number of reports which concluded that organic acidification increased the utilization and digestibility of protein (El-Kerdawy, 1996; Abdel-Azeem *et al*., 2000; Abdo, 2004).

In conclusion, the present study suggested that organic acid improved the health of the birds through decreasing the concentration of liver enzymes and end products of protein digestion.

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