Short Communications

Pakistan J. Zool., vol. 45(4), pp. 1147-1150, 2013

Blindness and Visual Impairment in Retinitis Pigmentosa: A Pakistani Eye Hospital-Based Study

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Abstract.- The aim of this work was to determine visual disability associated with retinitis pigmentosa (RP) in Punjab province of Pakistan. The analytical study was performed on all RP cases from Lahore during ophthalmologic consultation at the Layton Rahmatullah Benevolent trust (LRBT) hospital during Aug 2008-Jan 2011. Five hundred RP cases (142 women and 358 men) with average age of 25.56 years were identified from different areas of Punjab corresponding to hospital prevalence of 1.1/100. RP was bilateral in all cases and identified in 68% of the cases. Visual disability was very common in RP.

Key words: Retinitis pigmentosa, Blindness.

Visual impairment and blindness have very important impacts on the socioeconomic development of individuals and societies. Their consequences have significant public health issues with great impact in the developing countries, where 80% of the world blindness occurs (Congdon *et al*., 2003). Currently there are an estimated 37 million people with blindness and 124 million people with low vision worldwide (Resnikoff *et al*., 2004).

Prevalence of retinitis pigmentosa (RP) ranges from 1/3000 to 1/5000. RP is a genetic disorder with diffused retinal dystrophy and in most cases, initially affects the rods, with subsequent degeneration of cones (Eballe *et al*., 2010). RP is characterized by progressive degeneration of the retina usually starting in the mid-periphery of the fundus and advancing towards the macula and fovea. Classic clinical findings include bone spicules, pigmentation or pigment clumping, retinal arteriolar narrowing, waxy pallor of the optic nerve, epiretinal membrane formation, atrophy of the retinal pigment epithelium (RPE) and choriocapillaris (starting at the mid-periphery of the retina with preservation of the RPE in the macula until late in the disease), posterior sub-capsular cataract, epi-retinal membrane formation, and cystoid macular edema (CME) (Hamel, 2006). Transmission of RP is genetic and may be transmitted in various modes *i.e.,* autosomal dominant, autosomal recessive and X-linked recessive (Eballe *et al*., 2010). In the present study prevalence of visual disability associated with retinitis pigmentosa was determined from Punjab province of Pakistan.

Materials and methods

Patients affected with RP were screened by visiting the Layton Rahmatullah Benevolent trust (LRBT) Eye hospital, Lahore. Proforma to acquire preliminary information about the diagnosis and disease status in the patients were distributed in the low vision unit of the hospital. Case files of patients with RP were reviewed. The variables analyzed were: age, gender, main complaint and far visual acuity. The diagnosis of RP was made on the basis of an examination of the ocular fundus using a biomicroscope (fundoscopy) and far visual acuity. Electroretinography and genetic tests were not performed in our study due to inadequate level of technical expertise in hospital unit. The data collected were compiled. The chi-square test was used for comparison between different age groups and was considered significant at $P<0.05$. The classification of visual impairment was made using the analysis of visual acuity as defined by World Health Organization (WHO). Snellen chart is used for testing the vision, with the standard Snellen chart, vision is tested at a distance of 6 meters or 20 feet. The 'normal' visual acuity is 6/6 or 20/20.

Results

After the study of 45,000 cases, 500 cases of RP were identified from different areas of Punjab, Pakistan. These included 142 women and 358 men.

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0030-9923/2013/0004-1147 $ 8.00/0

The average age was 25.56 years with a range of 3-75 years. The most represented age group was 11-20 years, which included 168 patients (33.67%), followed by patients aged 21-30 years (119 cases, 23.80%). There were 93 patients (18.60%) >40 years and 51 patients (10.20%) <10 years. The main complaint was a drop in visual acuity, which was found in 395 patients (79%), followed by night blindness in 289 patients (57.8%).

Of those examined in LRBT, 62 patients (12.40%) were identified with a visual acuity of <6/7.5 (<0.3 Log MAR,) and are said to have mild vision loss or near normal vision. The prevalence of moderate or severe visual impairment were 20.00% and 14.80% respectively. There were 62 patients who had profound visual impairment (12.40%). The prevalence of near total visual impairment was 33.00% and that of total visual impairment was 7.40%. Thus in RP patients from LRBT, representing some areas of Punjab, the prevalence of low vision was 35% and that of blindness was 53% (Fig. 1).

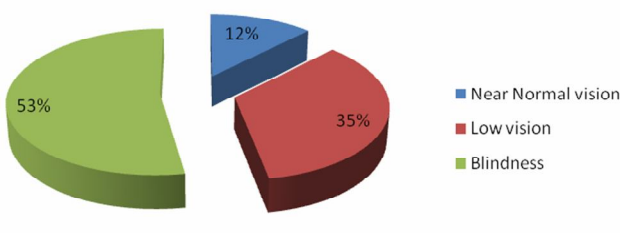

Fig. 1. Distribution of blindness and low vision in Punjab province of Pakistan

RP was bilateral in all patients, and isolated with no additional findings in 341 cases (68.20%). The major associated pathologies with RP were nystagmus (27 cases), age-related macular degeneration (12 cases), cataracts (6 cases), glaucoma (6 cases), unilateral macular holes (10 cases), high myopia (9 cases), gyrate atrophy (1 case), pigment retinopathy (2 cases), sluggishly reactive pupil (9 cases) and maculopathy (76 cases). Hearing loss was not reported to prompt consideration of Usher syndrome.

Discussion

This study focused on RP patients treated at Layton Rahmatullah Benevolent Trust Eye Hospital Lahore. The hospital prevalence of RP would clearly not be representative of the entire population of the province Punjab of Pakistan. Hospital prevalence obtained by Eballe *et al*. (2010) was 1.6/1000 in a Cameroonian hospital based study. In contrast, our prevalence (1.1/100) is much greater. In our series, male predominance of 71.60% is seen in contrast to findings of Cameroonian hospital based study in February, 2010 where RP was equally common in both men and women (p>0.05) (Eballe *et al.*, 2010). This gender bias is also reported in the findings of Kaya-Ganziami *et al*. (1994) and Ukponmwan *et al*. (2004) in Nigeria, where male predominance of 63.6% and 66.6% respectively have been reported. It is generally recognized that night blindness is one of the commonest complaints in RP (Kaya-Ganziami *et al*., 1994; Hruby, 1983; Noble *et al*., 1998; Heckenlively *et al*., 1988). Indeed, a drop in visual acuity and night blindness were the main reasons for consultation in our study.

The average patient age 25.56 years in our series is younger than that found in Japan, where an average age of 35.1 years was reported (Tsujikawa *et al*., 2008). The average age was 36.7 years at diagnosis in Nigeria (Ukponmwan *et al*., 2004). In our study, 53% of patients had significant bilateral blindness while low vision was found in 35% of patients, at the time of diagnosis. This is comparable to the data of Ukponmwan *et al.* (2004) who reported binocular blindness in 50% and low vision in 26.7% of patients in their Nigerian series (Ukponmwan *et al*., 2004). Similarly Grover *et al.* (1996) reported a visual acuity <1/10 in 25% of their patients suffering from RP patients, corresponding to severe low vision or blindness (Grover *et al*., 1996). Our data are also consistent with Cameroonian hospital based study where 57.5% of patients were reported with a significant bilateral decrease of visual acuity (Eballe *et al*., 2010).

Retintis Pigmentosa in this cohort was isolated in 65% of cases, and no case of Usher syndrome was noted. These findings are similar to those as reported by Eballe *et al.* (2010) who found 67.5% of isolated RP cases (Eballe *et al*., 2010). This is also reliable with a report by Kaya-Ganziami

et al. (1994) who found that 72.27% of cases of RP were isolated (Kaya-Ganziami *et al*., 1994) whereas in another study, only 47% cases (Grover *et al*., 1996). Maculopathy comprises 15.20% of associated pathologies in our series. Diabetic retinopathy is a specific microvascular complication of diabetes, and it accounted for 0.67% of total RP patients. Diabetic retinopathy is a major cause of blindness (Klein *et al*., 1998).

The most representative age group in Punjab was 11-20 years, having 33.60% of total RP patients. These data show that rate of RP is greater in teenagers or students in contrast to other age groups, in Punjab. There are several areas of concerns when considering the educational needs of a student with RP. There is a higher incidence of depression among individuals that suffer from low vision. Other researchers have reported that children with visual impairment "may have more difficulty developing positive self-esteem" (Griffin-Shirley and Nes, 2005)

The typical adaptation for RP focus on high illumination with no glare, absorptive lenses, infra-red viewing devices, prism glasses to increase visual fields and closed circuit television for maximum contrast. Educational consideration usually include teaching by organized search patterns using a grid pattern to aid the student in locating objects or visual targets. Students may need to be seated farther away to increase their visual field. Precautions should be taken to prevent retinal detachment (Levack, 1999). Although there is no cure for this progressive disease, nutritional approaches and gene therapy may hold promise (Smith, 2004). Knowledge and attitude of public towards diseases will help in formulating an effective strategy that will support public health policy makers (Hafeez *et al*., 2012). In a randomized controlled study, the progression of the retinal detachment has been reported as slower among the RP patients taking 15,000 international units of vitamin A (Berson, 1996).

Visual impairment and night blindness in RP is very common and becomes even more severe with aging. Early screening for the disease may be the best strategy for its management, including both appropriate treatment and genetic counseling.

Acknowledgements

We sincerely thank the staff of Layton Rehmatullah Benevolent Trust (LRBT) hospital for their help in the clinical evaluation and data collection.

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(Received 3 August 2012, revised 14 April 2013)

Pakistan J. Zool., vol. 45(4), pp. 1150-1153, 2013

Bacteriological Quality Assessment of Raw Milk Under Traditional Managemental System in Quetta, Pakistan

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Abstract. One hundred 100 raw milk samples (70 shops and 30 dairy farms) were collected from Quetta City and were subjected to microbiological assessment. Out of the 100 samples, 94 were found positive for bacterial contamination. Out of the positive samples, 25 different bacterial species were identified. The most frequent bacteria were *Escherichia coli* (60%), *Streptococcus faecalis* (26%), *Entrobacter cloacae* (16%), *Bacillus cereus* (10%), *Lactobacillus bulgaricus* (6%) and many of *Staphylococcus* and *Streptococcus* species. Statistical analysis revealed a significant association ($P<0.05$) in the occurrence of different bacteria in the raw milk samples sold under prevailing traditional managmental system. These results clearly indicate the heavy contamination of raw milk sold in shops and dairy farms in Quetta posing serious threat to public health.

Key words: Raw milk, bacterial assessment, contamination, traditional managemental system.

Milk is nutritious diet for humans containing all essential nutrients required for maintaining normal body functions. It is the compulsory part of daily diet for the expectant mothers as well as growing children (Javaid *et al.,* 2009). The milk obtained from healthy animal's udder is generally free from pathogenic bacteria but some of the animals in field condition may be suffering from sub-clinical mastitis excreting the causative agent in milk (FAO, 2008). Due to its complex biochemical composition and high water contents, milk serves as an excellent culture medium for the growth and multiplication of various kinds of micro-organisms. These bacterial contaminants may produce undesirable effects during the processing of milk. Usually milk is contaminated with different types of micro-organisms at milk collection centres.

It is a major part of human food and plays a prominent role in the Pakistani diet. Approximately 50 percent of the milk produced is consumed as fresh or boiled (Anjum *et al.,* 1989). Consumption of raw or inadequately pasteurized milk has been associated with several outbreaks of enteric infections associated with pathogens (Keene *et al.*, 1997). A variety of diseases may be potentially transmitted through milk. The source of pathogenic agents present in milk may be either from cow, human or by both (Khan *et al*., 2000).

Pasteurization is considered a safe way for the protection of important milk born diseases like tuberculosis caused by *Mycobacterium tuberculosis* and *Mycobacterium bovis*. Moreover, in some parts of the world, milk is still a significant source of certain diseases (Adam and Moss, 2008). In raw milk micro organisms can originate from different sources like air, feed, soil, faeces and grasses. (Torkar and Teger, 2008).

Pakistan is the world's 5^{th} largest milk producing country (Anonymous, 2007). where 71% milk is obtained from buffaloes, 24% from cows and 5% from camel and other animal species (Anonymous, 1994). The spoilage of raw milk by different pathogens not only cause economical losses to farmers but also acts as a potential source of transmission of highly zoonotic diseases.

This study was aimed at assessing the bacterial load of raw milk sold in the local market of Quetta for possible control of milk spoiling bacteria and spread of infections through its consumption.

Materials and methods

One hundred raw milk samples (70 milk shops and 30 dairy farms) were collected in sterilized container from different areas of Quetta City and were kept at 4°C in refrigerator. All the collected samples were processed within 24 h in the laboratory All the possible hygienic measures were

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adopted during collection, transportation and processing of all milk samples.

The milk samples were inoculated in Brain Heart Infusion (BHI) broth tubes and incubated at 37 C overnight. The inoculum from BHI broth was streaked onto the Brain Heart Infusion Agar, MacConkey's Agar, Blood Agar and Eosin Methylene Blue Agar plates in order to assess different growth and culture characteristics of all the pathogens present in the milk samples.

All the colonies on the culture plates were Gram stained for bacterial identification and once triple cloned were used for various bio-chemical tests *viz*., catalase, oxidase, o-nitrophenyl- -D-galactopyranoside (ONPG), argnine dihydrolase, lysine/ornithine decarboxylase, indole, citrate utilization, hydrogen sulfide, urea hydrolysis, Voges-Proskauer (VP), methyl red (MR), motility, gelatinase, carbohydrate utilization, nitrate reduction and growth at different temperatures for bacterial identification (Harley and Prescott, 1993; Harrigan, 1998). All the results were analysed using advanced bacterial identification Abis6 online software.

Results

Of the total 100 raw milk samples 94% were found positive for different bacterial contamination with 25 bacterial species. The highest bacterial contamination observed was for *Escherichia coli* (60%) followed by *Streptococcus faecalis* (26%), *Entrobacter cloacae* (16%), *Streptococcus bovis* (14%), *Bacillus cereus* (10%), *Streptococcus thermophilus* (10%), *Obesumbacterium proteus* (8%), *Streptococcus sanguis* I (8%) and *Streptococcus uberis* (8%). Conversely, the lowest contamination was noticed with *Staphylococcus saprophyticus* subsp bovis (2%), *Streptococcus saprophyticus* III (2%), *Streptococcus dysgalactiae* subsp equimitus (2%), *Streptococcus cremoris* (2%), *Streptococcus* group O (2%), *Streptococcus saprophyticus* I (2%), *Streptococcus faecalis* var malodortus (2%) and *Micrococus luteus* II (2%) followed by *Streptococcus milleri* (4%), *Streptococcus faecium* (4%), *Staphylococcus epidermidis* (4%), *Staphylococcus capitis* subsp capitis (4%), *Kluyera ascorbata* (4%), *Lactobacillus bulgaricus* (6%), *Streptococcus lactis* (6%) and *Streptococcus pneumoniae* (6%). Almost all the area of city is experiencing unhygienic milk, either from retail shops or directly from Dairy farms. These results reflect heavy contamination of raw milk samples in the target area (Table I).

Table I.- Bacterial quality assessment of bovine raw milk samples collected from Retail shops and Dairy farms in Quetta City, Pakistan.

Locality	Total no. of samples collected	No. of contaminated samples
Wahdat Colony	5	4
Brewery Road	21	20
Sairab Road	11	11
Jan Muhammad Road	14	13
Quarry Road	12	12
Joint Road	6	5
City	7	6
Satellite Town	6	5
Kasi oad	4	4
Sabzal Road	9	9
Qambrani Road	5	5

The chi square test revealed that there is significant association ($P<0.05$) in the occurrence of different bacteria in the milk samples obtained from dairy farms and retail outlets.

Discussion

It is evident from the results of the present study that bovine raw milk samples of the Quetta City are heavily contaminated with different bacterial species which pose serious threat to public health. The present findings corroborate with the screening carried out by Farzana *et al*. (2004) who tested 50 milk samples collected from various milk shops of Multan City and reported bacterial contamination in 40% of the samples. Similarly Muhammad *et al*. (2009) also reported supply of unhygienic and poor quality raw milk to Lahore City.

In the present investigation, *E. coli* were detected in 60% samples. These findings are in agreements with Swai and Schoonman (2011) who reported 57% of milk samples contaminated with *E. coli* in Tanzania. The present study highlights high count of members of enterobacteriacae in all the milk samples, which are concurrent with those of Hamida *et al.* (2009) and Fulya (2011). This might

be attributed to the unhygienic collection of milk from individual farm and careless handling during transportation. Furthermore, addition of coliform contaminated water to milk samples could be the likely reason of its high count. The coliform bacteria have minimum generation time 20 minutes and multiply at very rapid rate to reach unhygienic level.

This study indicates 15 different species of S*treptococcus*. Similarly Zadoks *et al.* (2004) screened 48 New York State dairy farms and isolated Streptococci (69%), Staphylococci (3%), and Gram-negative bacteria (3%). Among the streptococcal isolates the *Streptococcus uberis* was 81%, *Aerococcus viridans* 50% and *Streptococcus agalactiae* 31%. These results indicate the possible transmission of these bacterial pathogens from feedstuff and faeces. Three different species of *Staphylococcus* were also isolated during the present research. This organism is a typical bacterial pathogen causing serious chronic infections in mammary tissue of animals (form of mastitis). Its possible transmission could be during milking of the animals etc. The other contaminating bacterial species from the raw milk samples were *Bacillus cereus* (10%), *Obesumbacterium proteus* (8%) and *Kluyera ascorbata* (4%). Likewise Parkash *et al.* (2007), Griffiths and Phillips (2007) and Srujana *et al.* (2011) isolated *B. cereus, E. coli, Micrococcus luteus, Pseudomonas aeruginosa* and *Staphylococcus aureus* from raw milk samples.

The *Lactobacillus bulgaricus* were also isolated and identified (6%) from these samples which has also been reported by Aziz *et al.* (2009) from raw milk samples of cattle, buffalo and sheep in Punjab, Pakistan. The high *Lactobacillus* counts represent lack of cooling immediately after milking that may result in early milk spoilage.

This study also reported the presence of *M. luteus* with 2% detection. The species has also been reported by Adesina *et al.* (2010) with 6.7% prevalence evaluating microbial quality of cow milk in Nigeria, as this bacterium is found in soil, dust, water and air. The contaminated farm environment is the possible transmission of the bacterium reflected in the present study.

Acknowledgments

The authors are grateful to Mr. Abdul Haq Laboratory Assistant, for his cooperation during the entire period.

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(Received 11 August 2012, revised 4 June 2013)

Pakistan J. Zool., vol. 45(4), pp. 1153-1156, 2013

Analysis of Stomach Contents of Freshwater Catfish, *Eutropiichthys vacha* (Hamilton, 1822) from Khyber Pakhtunkhwa Rivers, Pakistan

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Abstract.- This study was carried out from summer till winter of 2011 to find out the diet of adult Catfish, *Eutropiichthys vacha*. A total of 216 fish stomach contents were analyzed to find out food items of this fish. Out of these, 63 (29.2%) stomachs were empty and the remaining 153 (70.8%) fish stomachs had prey. Stomach contents of 58% samples had small fishes, 36.8% had small aquatic and terrestrial insects and the remaining 5.2% had shrimps. In addition phytoplankton, consisting of Lyngbya and Oscilatoria and Diatoma (Bacillariophyceae), were also found as an important ingredient of diet of this fish. Some contents of phytoplankton could not be identified due to its partial digestion and change of colour. The feeding intensity was also studied to find out any cessation of feeding. The study confirmed that there is no discontinuation of feeding in this fish from summer to winter, as the weight of stomach contents remained unchanged during the study period. The structure of mouth, dentation, gills and stomach were also studied as they are associated with food intake.

Keywords: Catfish biology, *Eutropiichthys vacha*, gut content, feeding intensity.

Freshwater catfish *(Eutropiichthys vacha)* is an economically important fish of the family Schilbidae. This fish has gained popularity among consumers due to its high nutritional value (Hasan *et al.*, 2002). It is one of the most important food fish in Khyber Pakhtunkhwa (KP) province of Pakistan. *E. vacha* is a potamodromous fish, *i.e.*, migrates from one river to another for spawning and nursery ground (Chandra, 2010). The construction of large dams is one of the most important negative impacts on the migration of this fish as it cuts off river connection. In KP, construction of man-made barriers like Warsak dam on River Kabul and Tarbela dam on River Indus has blocked the upward migratory movements of many fish species including *Eutropiichthys* species. Construction of the proposed Munda dam on River Swat will be another barrier in its migratory movements and as a consequence, there will be overall decline of fish population in natural resources (Craig, 2001).

Therefore, it is mandatory to adopt alternative measures to prevent the population of *Eutropiichthys* species from further decline and enhance its population in natural resources. One of the alternatives is to propagate *Eutropiichthys* species in captivity. Although in Pakistan culture system mainly gyrates around carp fishes (Rab *et al.*, 2007) but in order to initiate *Eutropiichthys* species in captivity a pilot scale study is required on its biology, taxonomy, food and feeding habits, breeding and rearing techniques in captivity (Turan *et al*., 2005).

The aim of this study was to find out the normal diet of adult *E. vacha*. In this regard, all those structures were also studied which are associated with its food intake.

Materials and methods

A total of 216 *E. vacha* samples were procured from the fish market in Peshawar, from August through December 2011, which were used for diet analysis. *E. Vacha* is brought mostly to market from the KP'S rivers, *viz.*, Kabul River at Warsak Dam, Sardaryab River, Jahangira River etc. Fish is also brought from the Punjab to KP market as well.

In the laboratory, the length and weight of fish were recorded. Before analysis, the weights of filled and empty stomachs were recorded. Alongwith this, structures of fish like mouth, teeth, gills, and stomach were also studied. To analyze the

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stomach contents, a longitudinal cut was made across the stomach and the contents were transferred into petri-dish (Zacharia *et al.*, 2010). Identifiable material, *e.g.*, insects and their hard parts were preserved in 70% ethyl alcohol (Janjua *et al.*, 2011), while the remaining stomach contents, which consisted of phytoplankton were preserved in 10% buffered formalin (Ali *et al.*, 2010).

Fish diets were measured by frequency of occurrence (Hyslop *et al.*, 1980), which was calculated as the percentage of non-empty stomachs containing a particular prey type (Chapman *et al.*, 1988). Identification of the insects and other arthropods was done with the help of keys (Boror *et al.*, 1981).

Results

Morphological features

Head length was found to be 18.5% of the total body length. Eyes are larger with eye diameter 3.1 to 3.7cm. Mouth is sub-terminal with highly extended mouth cleft reaching behind the eye orbit. Upper jaw is slightly pointed and longer. Mouth opens with 30° angle and is surrounded by eight barbels, *viz.*, one pair of nasal, one pair of maxillary and two pair of mandibular barbels. Nasal barbels reaches just behind the head while maxillary barbels reach the base of pectoral fin. Mandibular barbels are comparatively smaller and do not extend beyond the head. Villiform teeth, palatine teeth and vomarine teeth are present. Villiformes teeth are numerous, short, sharply pointed. These are arranged just like a shoe brush and backwardly directed. Vomarine and palatine teeth are present on the roof of oral cavity. These are very near to each other and form uninterrupted band. Gill rackers continuously decrease from top to lower. Gill rackers are ±15, ±12, ±5, ±2 on first, second, third and fourth arch respectively. Decrease in gills rackers may help in pushing the prey into esophagus. Stomach is quite similar to sac like shaped. Stomach wall is thick and have well develop grinding function as much of animal break down found in stomach.

Feeding intensity from summer to winter

The weight of food contents varied 2.5 g to 3.8 g, from August through December. However, the proportion of various insects was high during summer months, which decreased in winter (Fig. 1).

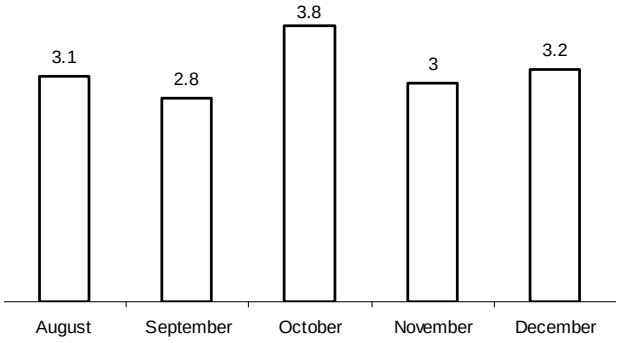

Fig. 1. Weight (g) of food content of *E. vacha* from August to December.

Food items

Food items, which were found in the stomachs of *E. Vacha* are given in Table I. During this study, 58% of fish stomachs had small fishes, fish scales, and fish spine. Small fishes were identified as *Hypophthalmichthys molitrix, Puntius ticto* and *Puntius sophore.* The largest fish, which was found in *E. vacha* stomach, was *H. molitrix* whose total length was 6.7 cm and width 3.1 cm. Small aquatic and terrestrial insects were found in 36.8% stomachs, while the remaining 5.2% had shrimps. The phytoplankton was also investigated. Frequency of occurrence of *Lyngbya* and *Oscilatoria* (Cyanophyceae) was 50.9%, Diatoma (Bacillariophyceae) was 28.8%, while 20.3% phytoplanktons remained unidentified due to their partially digested state.

Discussion

E. vacha is predominantly Piscivorouous as 58% of the stomachs had small fishes, fish scales and fish spine. Invertebrate animals were found in 36.8% of all stomachs. Some portion of phytoplankton remained unidentified because of its changed colour. The colour may be changed due to acid and different enzymes action in the stomach. Abbas (2010) worked on the same species *E. vacha* and found its feeding predominantly on crustaceans (prawn) and aquatic insects, and suggested it as corni-omnivorous. During present study only 8 out of 153 stomachs had crustaceans (shrimp). This

change in feeding habit might be due to the fact that the population of crustaceans in KP's river is comparatively very low.

Table I.- Frequency of occurrence of different animal species and phytoplanktons in the stomach of *Eutropiithys vacha.*

Food items	No. of stomachs (n)	Frequency of occurrence (%)
Animals		
Small fishes (Order Cypriniformes)	63	41.18
Fish scales	23	15.03
Fish spine	3	1.86
May-fly (Order Ephemeroptera)	11	7.19
Shrimp (Order Decapoda)	8	5.23
Stone fly (Order Plecoptera)	8	5.23
Grasshopper (Family Acrididae)	5	3.27
Ants (Family Formicidae)	4	2.61
Honey Bee (Family Apidae)	3	1.96
Dung beetle (Family Scarabaeidae)	5	3.27
Water beetles (Family Dytiscidae)	2	1.31
House fly (Family Muscidae)	2	1.43
Wasp (Order Hymenoptera)	2	1.31
Dragonfly naiad (Sub order Anisoptera)	2	1.31
Aphids (Family Aphidae)	1	0.65
Ground beetles (Family Carabidae)	1	0.65
Adult moths (Family Sphingidae)	1	0.65
Rove beetles (Family Staphylinidae)	1	0.65
Diving beetles (Family Dytiscidae)	1	0.65
Water strider (Gerridea)	1	0.65
Hopper (Family Cixidea)	1	0.65
Weevil (Family Curculionidae)	1	0.65
Water scavenger beetle	1	0.65
Unidentified beetles (Order Coleoptrea)	3	1.96
Phytoplanktons		
Cyanophyceae		
Lyngbya	51	33.3
Oscillatoria	27	17.6
Bacillariophyceae		
Diatoma	44	28.8
Unidentified	31	20.3

The study suggests that main food items of *E. vaha* found in KP rivers is not crustaceans but fish and small invertebrate animals. This study suggests that there is neither discontinuation in feeding intensity nor abrupt decrease in the weight of stomach contents. Only 63 stomachs were found empty throughout this study. Afsar (1990) worked on *Clupisoma garua* feeding habits and found no discontinuation in its feeding, and reported its high feeding intensity during September to October. Abbas (2010) work also confirmed that there is no cessation of feeding even during spawning season and concluded no discontinuation of feeding throughout the year. During this study, we also collected about 21 nematodes from the gut of *E. vacha*.

Conclusions

(i) *E. vacha* predominantly feeds on small fishes; therefore, the artificial food for *E. vacha* may include trash fishes. In addition to this *E. vacha* can also be fed with flesh, animal viscera etc.

(ii) *E. vacha* is comparatively aggressive fish and should not be reared in polyculture system except only if used in approved ratio to control overpopulation problem like Nile tilapia (45 fish / Hectare).

(iii) The daily feeding quantity of adult *E. vacha* may be between 3.2 to 4.5 g/100 g of body weight of fish.

(iv) As mentioned in discussion, during this study, 21 nematodes from the gut of *E. vacha* were also collected. Further study is required in this regard. These nematodes may probably reside near the region of gills. There are many fish-borne diseases in which fish plays the role of intermediate host for particular parasite.

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(Received 14 February 2013, revised 20 June 2013)

Pakistan J. Zool., vol. 45(4), pp. 1156-1160, 2013

Metals Concentration in Water, Fodder, Milk, Meat, Blood, Kidney and Liver of Livestock and Associated Health Impacts by Intake of Contaminated Milk and Meat

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Abstract.- Present study was aimed at determining concentrations of heavy metals (Cu, Cd, Pb, Ni and Cr) in water, fodder, milk, meat, blood, kidney and liver of livestock from two villages in the vicinity of a polluted drain. The aim was also to determine the health risk for the consumers due to intake of milk and meat of livestock from these villages. Order of metals concentration in water and fodder collected from these villages was Cu>Ni>Pb and Cu>Ni>Pb>Cd respectively. In livestock milk, meat and blood this order was Cu>Ni>Pb>Cd but in kidney and liver Cu>Cr>Ni>Pb>Cd, respectively. Cd was absent in water but Cr was absent in water, fodder, milk, meat and blood. The daily intake of Cd and Pb by milk and meat was higher than their permissible limits in both the villages. This may entail serious health hazards for the people consuming milk and meat of livestock from these villages.

Key words: Heavy/trace metals, bioaccumulation, polluted drain, contaminated milk, contaminated meat

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Metals are required for the appropriate functioning of biological system but the deficit and excess of metals in body cause many disorders. Heavy metals like lead, cadmium and copper are most toxic due to their accumulative nature (Aycicek *et al.,* 2008). They finally enter into the food chain and effect health of animals and humans as well (Kaplan *et al.,* 2010). During the last few decades heavy metal pollution due to municipal solid waste, pesticides and fertilizers has increased to a great extent which poses a risk to animal and human life (Rajaganapathy *et al.,* 2011). Build up of heavy metals in agricultural soils is of immense concern due to their risk to enter into food chain via consumption of the agriculture produce (Rattan *et al.,* 2005). Considerable quantities of lead and cadmium transfer into plants from soil contaminated with metals (Pugh *et al.,* 2002) and cause metal accumulation in grazing ruminants specifically cattle (Farmer and Farmer, 2000). The metal accumulation induces toxic effects in cattle (Dwivedi *et al.,* 2001) and the humans who feed on contaminated meat (Cui *et al.,* 2005).

The present study was carried out in two villages namely the Nurpur and Soara. Nurpur is located at Latitude 31.4435° N and Longitude 74.5669° E while Soara is located at Latitude 31.4126° N and Longitude 74.4372° E. The people of both the villages use untreated Hudiara Drain's water for the purpose of irrigation. Hudiara is a natural storm water drain which has its origin in Batala India and enters into Pakistan at Hudiara Village (Iram *et al.* 2009). On both sides of Hudiara Drain, various industries are present that discharge their effluents into it. About 100 industries are situated adjacent to Hudiara Drain covering 55km on the Indian side so the drain is earlier polluted before entering into Pakistan (WWF, 2007). After entering into Pakistan there are 112 small industries along the drain as it flows 63km through the Punjab into river Ravi (Kashif *et al.,* 2009). Around twenty seven villages in the vicinity utilize Hudiara Drain's water for irrigation and drinking purpose for cattle. Nurpur village is situated near the entry point of Hudiara Drain at Pakistan-India border near Lahore City. After entry into Pakistan, the drain receives effluents from various industries while flowing

towards the Soara Village. This study is aimed at determination of heavy metals in water, fodder, meat, milk, blood, kidney and liver of goat and cattle from both villages in the vicinity of Hudiara Drain and associated health risk due to the intake of contaminated milk and meat.

Materials and methods

Collection and preparation of different samples

Water samples were collected from Nurpur and Soara Villages and acidified to pH<2 with concentrated HNO_3. Fodder samples collected from these villages were washed with double deionized water at the site, collected in paper bags and transported at 4°C. Samples were then completely dried in an oven at 70°C, converted into powder by electric grinder and acid digested. Samples (1g) were digested using 20ml HNO_3 (65%) and 3.33ml H_2O_2 (35%) and kept at 200°C for 2 hr in a digester until a clear solution was obtained which was raised to 25ml by deionized water and then filtered by vacuum filtration assembly for analysis by Atomic Absorption Spectrophotometer (Khan *et al.,* 2010).

Milk samples were collected in polyethylene acid washed bottles (Bilandzic *et al.,* 2011) and stored at -18°C. Samples were acid digested following the same procedure as for fodder samples.

Blood sample were collected from animals at the time of slaughter, in vials washed with nitric acid. EDTA was added to the samples and stored at -18°C. 2ml HNO_3 (65%) and a few drops of H_2O_2 (35%) were added and then heated at 80°C until a clear solution was obtained (Alonso *et al.,* 2000).

Meat, liver and kidney samples were collected in zip lock bags separately and kept at -18°C (Miranda *et al.,* 2009). Samples were acid digested in a digester following the same procedure as for fodder samples.

Metal analysis

Copper, cadmium, lead, nickel and chromium were analyzed by Atomic Absorption Spectrophotometer, Shimadzu AA-7000F.

Determination of daily metal intake

Daily metal intake was calculated using the formula

DMI = (Metal concentration x intake) / Body weight
(Singh *et al.,* 2010)

Where metal concentration is the level of metal in particular sample, intake is amount of milk or meat taken daily and body weight is the average body weight of an average person. The DMI was derived using the local daily intake of meat that is 39g, milk 161 g (Akhter *et al.,* 2002) and body weight 70 kg.

Statistical analyses

Difference in metals concentrations in water, fodder, milk, meat and tissues of livestock in both the villages were analysed by t-test using SPSS software, Version 16.0

Results and discussion

Metals concentrations in water fodder, milk and different tissues of livestock at Nurpur and Soara Villages are presented in Table I. Order of mean metal concentration in water was Cu>Ni>Pb. Cd was not detected in any water samples of both the villages. The order of metals concentrations in fodder was Cu>Ni>Pb>Cd but in milk, meat and blood their order was Cu>Ni>Pb>Cd. Cr was absent in water, fodder, milk, blood and tissues of livestock at both the villages except in kidney and liver of livestock at Soara Village.

The daily metals intake due to milk and meat consumption at both the villages studied is presented in Table II. Daily metals intake was higher from milk as compared to meat.

Cu and Ni concentrations were significantly higher in Soara as compared to Nurpur Village ($P<0.001$). Concentration of Cu in water at Nurpur was 6.4 and at Soara 7.3 times higher than it's permitted limit of 1.3mg/l (USEPA, 2012) while Ni concentration in water from Nurpur was 70 times and at Soara 75 times greater than it's standard value of 0.020 (WHO, 1993). This shows that when water enters into Pakistan at Nurpur, it is already contaminated with metals from the industries of India which discharge their effluents into Hudiara Drain before entry into Pakistan. The concentrations of Cu, Pb and Ni in fodder at Soara were higher than their concentrations at Nurpur. This was due to the fact that Hudiara Drains's water was used for the cultivation of fodder, so high concentration of

Table I .- Concentrations of metals (mg/kg) in water, fodder, milk, meat, blood, kidney and liver of the livestock from Nurpur and Soara Villages.

	Concentrations of metals (mg/kg)									
	Cu		Cd		Pb		Ni		Cr	
	Average ±SD (Range)		Average ±SD (Range)		Average ±SD (Range)		Average ±SD (Range)		Average ±SD (Range)	
	Nurpur	Soara	Nurpur	Soara	Nurpur	Soara	Nurpur	Soara	Nurpur	Soara
Water	8.3±0.01*** (8.29-8.31)	9.5±0.01*** (9.49-9.51)	ND	ND	ND	1.3±0.01 (1.29-1.31)	1.4±0.01*** (1.39-1.41)	1.5±0.01*** (1.49-1.51)	ND	ND
Fodder	10.8±0.01*** (10.79-10.81)	15.01±0.01*** (14.9-15.01)	1.2±0.01* (1.19-1.21)	0.5±0.01* (0.49-0.51)	0.9±0.01*** (0.89-0.91)	1.5±0.01*** (1.49-1.51)	2.3±0.01*** (2.29-2.31)	2.9±0.01*** (2.88-2.91)	ND	ND
Milk	5.6±0.1* (5.5-5.7)	5.3±1.9* (3.4-7.2)	0.6±0.4 (0.2-1.0)	0.6±0.4 (0.2-1.0)	0.1±0.01** (0.09-0.11)	1.1±0.4** (0.7-1.5)	4.8±3.4* (1.4-8.2)	5.6±3.9* (1.7-9.5)	ND	ND
Meat	7.2±1.6 (5.6-8.8)	7.9±1.2 (6.7-9.1)	0.6±0.2*** (0.4-0.8)	1.0±0.1*** (0.9-1.1)	0.9±0.1*** (0.8-1.0)	4.5±4.3*** (0.2-8.8)	2.4±2.1* (0.3-4.5)	4.5±4.3* (0.2-8.8)	ND	ND
Blood	12.4±5.7* (6.7-18.1)	13.1±2.0* (11.1-15.1)	0.2±0.02** (0.18-0.22)	0.7±0.1** (0.6-0.8)	1.7±0.01*** (0.69-1.71)	6.0±5.9*** (0.1-11.9)	4.3±2.0* (2.3-6.3)	6.5±2.2* (4.3-8.7)	ND	ND
Kidney	108.2±94.1* (14.1-202.3)	12.7±3.6* (9.1-16.3)	0.8±0.7 (0.1-1.5)	0.9±0.01 (0.89-0.91)	0.9±0.1 (0.8-1.0)	1.0±0.1 (0.9-1.1)	0.8±0.6** (0.2-1.4)	2.4±0.4** (2-2.8)	ND	4.5±0.01 (4.49-4.51)
Liver	105.0±90.7* (15-195)	31.2±28.0* (3-59)	1.7±0.7 (1-2.4)	2.1±0.2 (1.9-2.3)	2.7±0.9** (1.8-3.6)	4.8±1.2** (3.6-5.0)	3.2±0.4 (2.8-3.6)	3.2±1.0 (2.2-4.2)	ND	17.0±0.01 (16.68-17.01)

ND = Not detectable *** P<0.001, **P<0.01, *P<0.001

Table II.- Daily metal intake (mg/kg/day) by consumption of milk and meat of livestock from Nurpur and Soara villages.

Metals	Metal intake (mg/kg/day)				Permissible Limit (mg/kg/day)
	Nurpur Village		Soara Village		
	Milk	Meat	Milk	Meat	
Cu	0.01	0.004	0.012	0.0015	1.3
Cd	0.0015	0.0001	0.0015	0.0005	0.001
Pb	0.003	0.0005	0.0026	0.0023	0
Ni	0.0112	0.0035	0.0128	0.0003	0.6

metals in water from Soara contributed to the high concentrations of meals in fodder from Soara as compared to Nurpur.

Feeding of livestock on contaminated feed and rearing them in proximity to polluted surroundings introduce heavy metal pollution into their milk, meat and tissues (Sedki *et al.*, 2003). Copper was present in the milk, meat, blood, kidney and liver of livestock. Highest concentration of copper was recorded in kidney and liver of livestock at both Nurpur and Soara Villages. When copper enters into the organism's body, in the first phase it is accumulated in the liver and kidneys so a higher amount of copper was recorded in liver of livestock. In the second phase, copper redistributes itself from liver to other body organs (WHO, 2004). Highest concentration of copper was detected in the liver as compared to other tissues of livestock as reported in other studies (Swaileh *et al.*, 2009; Okoye and Ugwu, 2010). Copper concentrations in milk, meat, blood, kidney and liver were below the permissible limit of 200mg/kg (ANZFA, 2001). Cadmium is a highly toxic heavy metal. The highest amount of cadmium was found in the liver of livestock which was 3.4 and 4.2 times higher than its standard value of 0.5mg/kg (FAO/WHO, 2000) at Nurpur and Soara Villages, respectively. Kramarova *et al.* (2005), Akan *et al.* (2010) and Farmer and Farmer (2000) reported cadmium in kidney and liver of livestock.

Lead and Nickel were detected in all the samples of milk, meat, blood, kidney and liver of livestock. Highest amount of lead was detected in liver, meat and blood. Gonzalez-Waller *et al.* (2006), and Farmer and Farmer (2000) reported highest level of lead in liver of livestock. The Pb in

liver of livestock at Soara was 4.8 and at Nurpur 2.7 times higher than the permissible limit of 1mg/kg (ANZFA, 2001). Ni concentration in livestock at Soara was greater than livestock at Nurpur in almost all the samples. Chromium was only detected in kidney and liver of livestock at Soara Village but it's value was 2.8 times greater in liver of livestock than it's permissible limit of 6.3 mg/kg (NUTTAB, 2010).

Daily intake of copper, cadmium, lead and nickel by humans was higher through milk when compared to meat due to it's higher intake. The daily intake of Cu and Ni from milk and meat at both Nurpur and Soara villages was within their permissible limits of 1.3mg/kg/day and 0.6mg/kg/day respectively (WHO, 1996). The daily metal intake of Cd from meat at Nurpur and Soara was within it's permissible limit but from milk it was 1.5 times higher than it's permissible value of 0.001mg/kg/day (US NSF, 2003). The intake of lead is not acceptable so its limit is 0mg/kg/day (US NSF, 2003). Pb intake from milk and meat at both Nurpur and Soara exceeded it's permitted limit.

Conclusions

Water of Hudiara Drain entering from India was heavily polluted. At Nurpur village, high metals concentrations were recorded which further increased at Soara Village due to addition of metals from numerous industrial units after entry into Pakistan. Contaminated water in both the villages introduced metal contamination in fodder for livestock feeding, resulting in high concentrations of metals in milk and meat. Daily metal intake of Cd and Pb exceeded their permitted limits and may cause risk for the consumers by intake of milk and meat from both the villages.

Acknowledgement

Authors are thankful to Department of Chemistry, GC University, Lahore for providing facilities for metal analysis.

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(Received 19 February 2013, revised 13 May 2013)

Pakistan J. Zool., vol. 45(4), pp. 1160-1164, 2013

Toxicity of Synthetic Pyrethroid Pesticides, Fenpropathrin and Fenvalerate, on Killifish *Aphanius dispar* Juveniles

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Abstract.- Pesticides pose a continuous threat to coastal areas receiving direct pesticide applications or rainfall run-off from agricultural land. Pesticides can be acutely toxic to fish and other marine organisms and its effect on these organisms can be assessed using toxicity bioassays, a convenient tool used extensively worldwide. *Aphanius dispar* (killifish) was exposed to different concentrations of fenvalerate, fenpropathrin (synthetic pyrethroid pesticide) to determine (24 h) $LC_{50.}$ In addition, biochemical changes occurred due to exposure to pesticides, *e.g.* total tissue protein content, was also determined. The present results reveal that the LC_{50} values for two pesticides tested were (0.0165 ppm for fenvalerate and 0.0014 ppm for fenpropathrin) indicating high sensitivity of fish juveniles and that *A. dispar* responded differently to different pesticides. The two pesticides tested appear to have reduced total protein in muscle tissue of *A. dispar* indicating impairment of protein synthesis and severe physiological distress on fish juveniles. Long-term exposure of organisms to pesticides may pose a high risk of health hazard to the general public via consuming these toxic fishes.

Key words: fenvalerate, fenpropathrin, pesticides, fish, *Aphanius dispar*

The increasing use of pesticides in agriculture has contributed significantly to improved national earnings and plays a vital role in controlling malaria (Boateng *et al.*, 2006). However, this positive trend

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from the use of pesticides has a negative effect on fish and other marine life, which is of great concern. When pesticides enter aquatic ecosystems, the environment costs can be high. Pesticides are one of the many factors contributing to the decline of fish and other marine species. Various studies have shown that pesticides can be acutely toxic to fish (Moore and Waring, 2001; Madhab *et al*., 2002; Eder *et al.*, 2004). There are numerous biochemical indices of stress that have been used to assess the health of non-target organisms exposed to toxic chemicals in marine ecosystem (Nimmi, 1990). Proteins are important organic substance required by organisms in tissue building. Biological activities are regulated by enzymes and hormones, which are also proteins. Assessment of protein activities can be considered as a diagnostic tool to determine the physiological status of cells or tissues (Manoj, 1999). The pesticide induced changes can affect the biological diversity and alter the functioning of marine ecosystem (Lam and Gray, 2003; Vasseur and Cossu-Leguille, 2003). The total protein content in fishes exposed to pesticides have been reported previously (Singh and Agarwal, 1994; Atamanalp *et al*., 2002; Borges *et al*., 2007; Korkmaz *et al*., 2009; Shoaib *et al*., 2012).

In the present study, an attempt has been made to investigate the toxicity (24 h LC_{50}) of fenpropathrin and fenvalerate pesticides to the killifish and affect on total protein content.

Materials and methods

Pesticide used

Pesticides, fenpropathrin 20% EC and fenvalerate 20% EC were obtained from Pakistan Agricultural Research Center.

Test organism

Juvenile fishes (*Aphanius dispar*) mean weight 112±1mg were collected at low tide from Sandspit (mangrove area). The fishes were kept in clean aerated seawater in glass aquaria (60cm Length x 30cm width x 29.5 cm height) at temperature (23 1 C), with salinity 30‰, pH 7.6, photoperiod (16 h light and 8 h dark). Fishes were acclimatized to laboratory conditions for 48 h. During this period, fishes were fed *ad libitum* two times a day. Medium of aquaria renewed daily.

Bioassay

Standard bioassay methods (APHA *et al*., 1971) were followed to determine toxicity of pesticide using static bioassay system (Doudoroff *et al.*, 1951). Bioassays to evaluate LC_{50} were carried out in glass jars (20.5cm length x 13.5cm width) of two liters capacity. The other experimental conditions, such as temperature 25.5±2.5 C, Salinity 30‰, pH 7.6, photoperiod (16 h light and 8 h dark) were maintained throughout the experiment. Concentration gradient (0.001-0.08 ppm) for the two test pesticides were prepared with filtered seawater. Acute toxicity (24 h LC_{50}, the concentration of the pesticides, which kills 50% of the test animals after 24 hour exposure) was measured by exposure to two pesticides separately. The tests and controls for each experiment were in triplicate and the controls had seawater only. Fish, both in control and test, were not fed during 24h exposure experiment. Fish were considered dead when they become immobile and ceased all respiratory movement. At the end of exposure period (24h) fish were removed from jars and stored at -20 C for total protein analysis in muscle tissues of fish. Dead fishes were discarded. The LC_{50} values were determined by using computer programme (Biostat, 2009) based on Finney Method 1952 (Probit analysis).

Total protein content of fish muscle tissues was analyzed by Biuret method (Tietz, 1995; Randox kit TP 245).

Result and discussion

The present results reveal that the LC_{50} values for two pesticides tested were 0.0165 ppm for fenvalerate and 0.0014 ppm for fenpropathrin (Fig.1) indicating high sensitivity of fish juveniles and that *A. dispar* responded differently to different pesticides. It was evident that the rate of fish mortality (%) was directly proportional to the concentration of pesticides. Fenvalerate and fenpropathrin treatment affected total protein level significantly. Fish groups exposed to fenvalerate and fenpropathrin showed decline in protein levels by 20% and 28%, respectively.

Low values of LC_{50} observed for both pesticides suggest that synthetic pyrethroid have toxic effect on fish exposed even for a very short period (24h). The results reported here are in

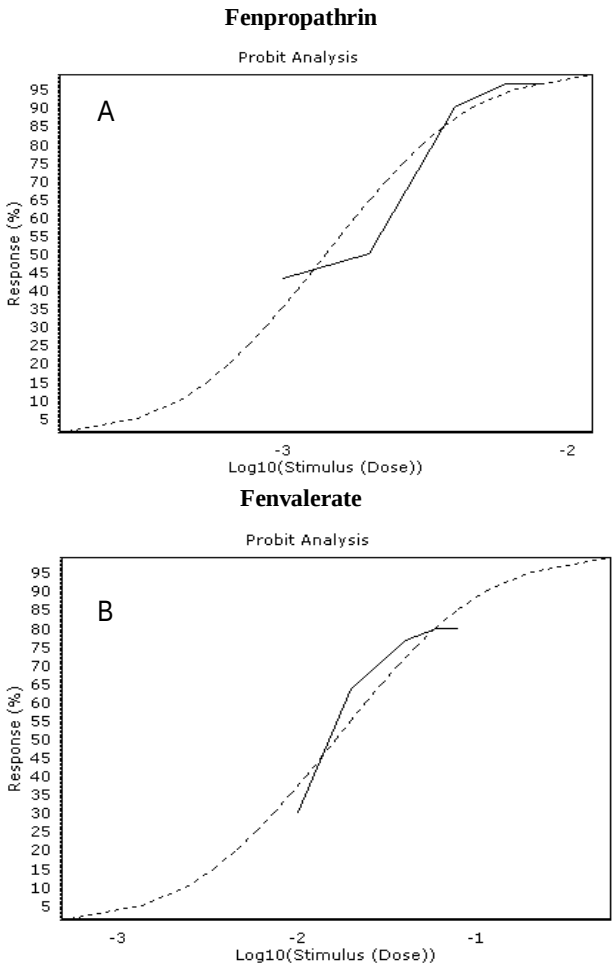

Fig. 1. Probit analysis curve showing response of fish (*Aphanius dispar*) exposed to different concentrations (dose) of pesticides; A and B.

agreement with previous reports (Saha, 2003; Aydin *et al.*, 2005; Giddings *et al.*, 2009; Korkmaz *et al.*, 2009). Synthetic pyrethroids are more toxic, particularly to fish, than organophosphates and carbamates (Bradbury *et al.*, 1985), as they are readily absorbed through the gills of fish even at very low concentration in water (Clark *et al.*, 1985). In addition, pyrethroids are metabolized and eliminated slowly in fish than in mammals or birds (Bradbury and Coats, 1989), hence they are more toxic to fish than to other organisms.

The differences observed in the toxicity of two pyrethroid pesticides (fenvalerate and fenpropathrin) to *A. dispar* may be attributed to the differences in pesticide absorption and degree of fish susceptibility/tolerance, biotransformation, excretion (Omitoyin *et al.*, 2006), and difference in metabolic response of organisms to different pesticides (Johnson and Toledo, 1993). In addition, the toxicity of pesticides generally varies from species to species (Pickering *et al.*, 1962), bioassay techniques, experimental conditions and purity of pesticides used (Chambers and Yarbrough, 1974). It has been shown that the magnitude of pesticide toxicity also depends on length and weight, corporal surface/body weight ratio and breathing rate (Singh and Narain, 1982; Murthy, 1986; Alkahem *et al.*, 1998). The fish juveniles appeared to be more susceptible to pesticides, for example, as reported for *Heteropneustes fossilis* (Dutta, 1995) and *Micropterus salmoides* (Pan and Dutta, 1998). The juveniles of killi fish used in the present study showed similar response.

Pesticides are known to affect the protein levels in tissues of non-target species, for example, fish (Khattak and Hafeez, 1996; Sancho *et al.*, 1997; Jadhav, 2002; Tilak, 2005; Jaroli and Sharma, 2005; Shoaib *et al.*, 2012). Reduction in tissue protein levels of fish exposed to synthetic pyrethroid, as observed in the present study, is in conformity with observation made earlier for fish exposed to fenvalerate (Reddy and Bashamohideen, 1988), cypermethrin, permethrin and fenvalerate (Singh and Agarwal, 1994), fenitrothion (Sancho *et al.*, 1997), endosulfan and thimet (Jadhav, 2002), cypermethrin (Korkmaz *et al.*, 2009). Reduction in protein levels in tissues of fish is an indicator of their general state of health. The depletion in total protein content may be attributed to: i) utilization of protein to mitigate the energy demand when the fish is under stress (Rao *et al.*, 1987; Baskaran *et al.*, 1989); ii) increased proteolytic activity (Ravinder *et al.*, 1988; Jenkins *et al.*, 2003; Radha *et al.*, 2005); iii) reduced protein synthesis owing to liver cirrhosis (Garg *et al.*, 1989; Ravichandran *et al.*, 1994; Kumari and Kumar, 1995); iv) shrinkage and lysis of RBCs (Das *et al.*, 2004).

In summary, the present study indicates that pyrethroid pesticides have drastic effect on marine fish, with low LC_{50} values, causing mortality and alteration in tissue protein levels. Release of agricultural wastes would tend to expose marine

fish, particularly juveniles, in the coastal waters. This would impose threats not only to the ecology of the area but also to the fishery industry. Possible accumulation of pesticides in fish will be transferred to predatory organisms and also to human health. The data obtained in the present study will provide informs the degree to which fish is prone to pesticide pollution and therefore it may be helpful in proposing executable pollution mitigation plans.

Acknowledgements

The authors would like to thank Dr Shahida Akhtar, Ex-Director, Pakistan Agriculture Research Centre, for her support and guidance. Financial assistance provided by the Centre of Excellence in Marine Biology, University of Karachi is thankfully acknowledged. Field and laboratory studies have been supported by DelPHE project (#82) awarded to PJAS.

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(Received 9 February 2013, revised 23 April 2013)

Pakistan J. Zool., vol. 45(4), pp. 1164-1167, 2013

Supplementation of GABA-B Receptor Antagonist (CGP 55845), Following Hypoxia Ischemia Encephalopathy Moderately Affects the Hematological and Serum Biochemical Profile in Albino Mice

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Abstract.- Present study was conducted to report the effect of 1mg GABA-B receptor antagonists (CGP55845; 3-N [1-(S)-(3,4-dichlorophenyl) ethyl] amino-2-(S)-hydroxypropyl-P-benzyl-phosphinic acid) / kg body weight/ml of solvent injected for 12 days following hypoxia ischemia encephalopathy on postnatal day 10 on hematological and serum biochemical profile of albino mouse. Blood samples from 34 albino mice (18 CGP55845 treated and 16 saline treated) were collected from direct cardiac puncture and various hematological parameters such as blood glucose, packed cell volume (PCV), total WBC (TWBC) count, total RBC count and serum biochemical parameters, such as cholesterol, aspartate transaminase, alanine transaminase (ALAT) high density lipoprotein, low density lipoprotein, total protein and triglycerides were determined. Glucose, MCV, TWBC and PCV concentrations in male and TWBC and PCV concentrations in females decreased significantly in CGP55845 treated albino mice. Triglycerides and ALAT were significantly increased in the CGP55845 treated albino mice.

Key words: GABA-B receptor antagonist, hypoxia ischemia, serum triglycerides, transaminases, lipoproteins.

Hematological profile provides information about the severity of the disease and the responses to the treatment, metabolic state of an animal and it also helps in establishing a prognosis (Satue *et al*., 2009; Aigner *et al*., 2012). Hypoxic-ischemic encephalopathy (HIE) is a condition in which patients suffer from pure hypoxic event without the involvement of cardiac vascular collapse and is a common cause of neonatal brain injury (David and Geer, 2006). In neonates hypoxic-ischemic (HI) injury either results from birth asphyxia, *i.e.*, premature separation of placenta, compression of umbilical cord, excessive contraction of uterus and excessive anesthesia to the mother, which depresses oxygenation of blood (Guyton and Hall, 2000). Out of every 1000 live full-term births, 1-3 are affected by perinatal HIE (Graham *et al*., 2008). 15-20% of affected new born die in postnatal period, while 25% of the survivors suffer from severe and long lasting neuropysiological consecution such as cerebral palsy and epilepsy, mental abnormalities, cognitive problems, increased hyperactivity and visual perceptive dysfunction (Chilai and Yang, 2011).

γ–amino butyric acid (GABA) is the major inhibitory neurotransmitter and plays a vital role in regulating the activity of neuronal cells (Bettler *et al*., 2004). GABA brings about its function through GABA-A which is ionotropic and GABA-B metabotropic receptors. GABA-B receptors are widely used in the treatment of neurologic and psychiatric disorders including absence seizures, γ–

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hydroxybutyrate toxicity and more recently used for the treatment of autoimmune limbic encephalitis (Eduardo and Benarroch, 2012). Activation of GABA-B receptors produces anesthetic effects in animals with neuropathy and chronic inflammation (Pin and Prezeaul, 2000). GABA-B antagonists have antidepressant activity (Cryan and Kaupman, 2005), cognition improvement (Froestl *et al*., 2004) and beneficial effects in rat models of absence epilepsy (Manning *et al*., 2003). GABA-B receptor antagonist CGP 55845 selectively involve in behavioral learning, increasing swimming time in rat modified forced swim test. The compound inhibits GABA-B responses to baclofen and potentiates the hypoglycemic response to glucose in vitro (Slattery *et al*., 2005). Present study was conducted to study the effect of HI stress followed by GABA-B receptors antagonist injection and its effects on the hematology and serum biochemical profile of albino mice.

Materials and methods

Subjects

Adult albino mice (both male and female) were used during these experiments. Breeding pairs of albino mouse were purchased from veterinary Research Institute, Ghazi Road, Lahore. Mice cages were maintained filled with wood chips at the core Animal Facility at BioPark of Bahauddin Zakariya University, Multan. In breeding colony, all albino mice were housed in individual cages, standard mouse diet and water was available *ad libitum*. Room temperature was maintained at 22±1°C, room was illuminated at an intensity of about 200 1x in 2m from 8 a.m. to 7 p.m. Albino mouse was housed in individual cages. All the experimental protocol was approved by the ethical committee of Institute of Pure and Applied Biology at Bahauddin Zakariya University, Multan.

Murine model of hypoxia ischemia encephalopathy

On postnatal day 10, isoflurane inhalation (3%) was used to anesthetize pups. By using poly propylenedalcon USP 6 suture, the right common carotid artery was ligated. During the surgery temperature was maintained at 36 °C by keeping pups on a hot plate. The surgical procedure was completed within 10 min. Mice were then placed in a hypoxic chamber for 25 min with constant supply of 8% oxygen balanced with nitrogen. The hypoxic chamber was kept on hot plate to maintain the ambient temperature inside the chamber at 36 °C. Pups were returned to their mothers for recovery after hypoxic exposure.

Experimental design

On 18-20th day of life, mice were separated from their parents and fed on normal mouse diet until 13th week of life when they started receiving intra peritoneal injections of the GABA-B antagonist (1mg/kg body weight/ml of solvent) for 12 days. Separate control groups were maintained in parallel. They also underwent the hypoxic ischemic insult on postnatal day 10 and after 13 weeks of life, they received intraperitoneal saline injection for 12 days.

Blood and serum collection

Following the intra peritoneal injections for 12 days, mice were anesthetized with 3% Isoflurane and blood was sampled either from retro-orbital sinus or through direct cardiac puncture. Blood was divided into two parts; one for hematological and the other for serum biochemical profiling.

Hematological and serum biochemical profiling

Hematological parameters *viz.*, blood glucose level, mean corpuscular volume (MCV), packed cell volume (PVC), total red (TRBC) and white blood cell (TWBC) count and serum biochemical parameters such as cholesterol, aspartate transaminase (ASAT), alanine transaminase (ALAT), high density lipoprotein (HDL), low density lipoprotein (LDL), total protein and triglycerides were determined in blood samples by using Hitachi 902 automatic analyzer (Japan) following Khan *et al*. (2011).

Statistical analysis

All the data are expressed as Mean±SD. Statistical package Minitab (version 16, Pennsylvania) was used for the analysis of results. Two sample t- tests was applied to compare various

Table I.- Effect of GABA-B receptor antagonist (CGP55845) on the various hematological and serum biochemical parameters of albino mice following hypoxic- ischemic insult. Data is expressed as Mean ± Standard deviation. P-value indicates the results of 2 sample t-test.

Parameters	Male mice		Female mice	
	Control (n=16)	CGP55845 treated (n = 18)	Control (n=9)	CGP55845 treated (n = 10)
Hematological profile				
TWBC count	8153 3599	6377 6041	8945 ± 1702	4717 ± 2373**
TRBC count	6785000 ± 1531276	6839412 ± 1903850	736166 ± 171164	710333 ± 169296
PCV (%)	38.99 7.02	21.10 6.03***	39.52 ± 7.95	21.43 ± 3.04**
Glucose (mg/dl)	210.2 45.2	169.7 59.8*	203.9 ± 47.8	164.7 ± 73.4
MCV (fp)	58.81 ± 9.75	25.6 ± 17.0***	57.9 ± 26.3	33.09 ± 5.82
Serum biochemical profile				
Total protein (g/dl)	4.900 0.79	5.73 4.64ns	4.900 ± 0.8	6.44 ± 4.44
Triglycerides (mg/dl)	136.8 95.6	317 207*	148 ± 106	326 ± 230
Cholesterol (mg/dl)	100.7 48.5	193 137	82.6 ± 23.7	127.6 ± 82.5
HDL (mg/dl)	23.83 4.4	20.4 10.3	14.40 ± 2.24	14.40 ± 2.24
LDL (mg/dl)	71.7 ± 48.1	162 ± 188	47 ± 33.3	51 ± 40.9
ASAT (μ/l)	145 103	299 278	182 ± 160	311 ± 361
ALAT (μ/l)	41.6 19.3	90.9 45.1*	37.4 ±21.7	109 ± 51.3

P < 0.05 = Non significant (ns), P> 0.05 = Least significant (*), P> 0.001 = highly significant (***)

parameters of hematology and serum biochemical profile of albino mouse between CGP55845 treated and their respective untreated controls following hypoxia ischemia encephalopathy.

Results and discussion

When various hematological parameters were compared between CGP55845 treated and saline control albino mouse, it was observed that glucose, MCV and PCV in the males and PCV and TWBC in the females were significantly decreased after CGP55845 treatment (Table I). Among serum biochemical parameters, triglycerides and ALAT were significantly increased (Table I).

The data shows a severe effect of hypoxic ischemic insult and CGP35348 supplementation on WBC confirming the findings of Brown *et al.* (2001) who showed that increased WBC count in blood is an indicator of myocardial infarction and stroke.

The decreased MCV levels may indicate a decrease in size of erythrocytes due to stressful conditions (Rao and Vidyunmala, 2009). This parameter showed significant statistical difference when compared between CGP55845 and saline treated (control) (P = 0.002) (Table I) albino mice.

Ambali *et al*. (2010) reported significant elevation in the level of PCV in mice due to any stress resultant in hemoconcentration understudy following HI insult. The PCV value indicates oxygen carrying capacity of the blood which measures the degree of stress on animal health (Larson *et al*., 1985).

Glucose is the primary source of energy for the body cells. Blood glucose level outside the normal range may be an indicator of medical condition or illness (Walker *et al*., 2006). In present study, the glucose level was significantly decreased in CGP55845 albino mice when compared with saline treated (control) albino mice (P = 0.040) (Table I). The present work is the first of its kind and the results can be used as a baseline reference data which will be helpful in monitoring the health status of the subjects suffering from hypoxic ischemic insult.

Plasma triglycerides are single most important predictor of HDL cholesterol level. Increased triglyceride level associated with decreased HDL level (Phillips *et al*., 1981). Data analysis revealed that Triglyceride level was significantly higher when compared between

CGP55845 and saline treated (control) albino mice (P = 0.040) (Table I).

ALAT is an enzyme found in highest amount in liver and typically used to detect liver injury (Pratt, 2010). It is most commonly used indicator of cell necrosis, and its elevated level is due to the liver cells injury resulting in leakage of ALAT into the circulation (Dofour *et al.*, 2000; Kew, 2000).

Acknowledgement

The authors are grateful to Higher Education Commission (HEC) of Pakistan for providing research grant for this study under Indigenous PhD Scholarship Scheme.

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(Received 19 February 2013, revised 25 June 2013)

Pakistan J. Zool., vol. 45(4), pp. 1167-1169, 2013

Management of Stored Grain Infested with Red Flour Beetle *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) in Southern Punjab, Pakistan

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Abstract.- A survey was conducted in Bahawalpur, Multan, Lodhran, Khanewal and Vehari districts in southern Punjab to determine the damage and the control measures adopted by farmers, house owners and shop keepers to control the stored grain pest *Tribolium castaneum.* Metal bins, earthen bins and polyethene bags were used for storage of grains. For control of pests 43% people used phosphine tablets, 26% used botanicals, 22% used cleaning method, 0.33% used mercury, and 0.12% used sodium chloride salt as their control measure, while 0.29% people did not adopt any control measure.

Keywords: *Tribolium castaneum*, red flour beetle.

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Tribolium castaneum (Herbst) is a very important and major pest of stored grain (Howe, 1965). Synthetic insecticides and fumigants are mostly used to control the insect pests of stored grains, which is wrought with the risk of development of resistance in insects (Zettler and Cuperus., 1990; White, 1995; Ribeiro *et al.*, 2003; Saeed *et al.*, 2012), increasing the storage cost, harmful effects to end users and toxic residual effects on stored food material.

Safe storage of grains and other food items against insect pest damage is an important task (Haq *et al*., 2005). Though chemicals have provided effective control of pests, but their adverse effects on human life and the ecosystem have always stressed upon for alternative methods for the control of various arthropod pests (Risk *et al.*, 2001). Plants such as *Cassia fistula* (L.), *Azadirachta indica* (A. Juss), *Chrysanthemum coronarium* (L.) *Calotropis procera* (Ait), and *Lantana camara* (L.) have shown insecticidal, growth regulating, repellant and antifeedant properties (Deka and Singh, 2005; Singh and Singh, 2005; Prakash and Rao, 2006; Kestenholz *et al*., 2007; Neoliya *et al*., 2007; Sankari and Narayanswamy, 2007). They are traditionally widely used as protectants for storing grain due to their easy biodegradable nature (Dwivedi and Garg, 2003). Present study was designed to assess the behavioral responses of society to this insect as a pest, its basic knowledge about the pest, and the management strategies adopted for storage of food commodities at domestic and commercial levels.

Materials and methods

An assessment questionnaire was devised to conduct the survey on identification of stored products pest *T. castaneum*, damage done by this pest, storage of grains and the control measures. The survey was done in 5 districts *i.e.* Bahawalpur, Multan, Lodhran, Khanewal and Vehari in southern Punjab. The data were collected from the 500 people comprising 100 shopkeepers, 75 housewives and 325 males including house owners and farmers with age groups ranging from 20-70 years.

Results and discussion

The analysis of the collected information showed that 95% persons could identify the insect (Table I). For storage purpose 60% people used metal bins, 30% used polythene bags and 10% used earth bins. To control the pest 43% people used phosphine tablets, 26% used botanicals, 22% used cleaning method, 0.33% used mercury, 0.12% used sodium salt as their control measure, while 0.29% people did not adopt any control measure.

Table I.- Data obtained from interviewees storage, pests and control measures.

	Total	Frequency	Percentage
Identification of *T. castaneum*			
Yes	500	477	95.4
No	500	23	4.6
Categories of people			
Housewives	75	73	97.3
Shopkeepers	100	90	90.0
House owners	325	319	98.2
Methods of grain storage			
Metal bins	500	301	60.2
Bags	500	148	29.6
Earthen bins	500	51	10.2
Measures adopted for pest control			
Tablets (Phosphine)	500	210	42.0
Botanicals	500	125	25.0
Cleaning	500	105	21.0
Salt	500	55	11.0
Mercury	500	3	0.6
Nothing	500	2	0.4

In Pakistan, 5-7% of the food grains are lost due to poor storage conditions (Jilani and Ahmad, 1982). Rajashekar *et al.* (2012) reported that fumigants including phosphine tablets rapidly kill all kinds of insect infestation in storage bins. According to Kenkel *et al.* (1994), 75% of the consumers are very much concerned with the food safety as reported by national and regional surveys. Synthetic insecticides have been used by people in different areas for many years for efficient control of insect pests (Salem *et al., 2007*). Their massive

and indiscriminate use, however, resulted in increasing resistance of the insect pests along with hazardous residual effects (Mukerjee *et al.*, 1973; Hamid *et al.*, 1988; Bell, 2000; Norman, 2000, Phillips and Throne, 2010). The use of certain plants and spices can be useful in protecting the stored grains against pest population. It is done by mixing the grains with the plant protection material (Tapondjou *et al.*, 2002; Tripathi *et al.*, 2009). Farmworkers have inherited the knowledge and researchers have experienced the usefulness of plant material including leaves, seeds, bark and oils of some traditional plants as grain pest control agent (Saxena *et al.*, 1988; Akinneye *et al.*, 2006).

Better storage facilities can increase the food safety worldwide and thus be helpful in reducing food scarcity.

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(Received 25 April 2013, revised 5 June 2013)

Pakistan J. Zool., vol. 45(4), pp. 1170-1172, 2013

Comparative Efficacy of Various Indigenous and Allopathic Drugs Against Ovine Fasciolosis

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Abstract.- A total of 110 animals were used in 11 controlled experiments to compare efficacy of plants, *Saussurea lappa*, *Fumaria parviflora*, *Caesalpinia crista* and an allopathic drug triclabendazole. Efficacy of drugs was measured by determining the difference of eggs per gram of faeces (EPG) pre and post-treatment. *Saussurea lappa*, after a single dose of 60, 70 and 80 mg/kg body weight, reduced EPG by 47.36, 48.57 and 55.55 percent, respectively. After the second dose the respective reduction in EPG was 81.57, 85.71 and 94.44 percent. *Fumaria parviflora* at a rate of 60,70 and 80 mg/kg body weight was 41.66, 45.71 and 63.88 percent, respectively, whereas efficacy at two dose levels with the same dose rate was 83.33, 91.42 and 97.22 percent respectively. *Caesalpinia crista* at 60, 70 and 80 mg/kg body weight was 38.23, 45.71 and 51.42 percent dose levels. Allopathic drug i.e Triclabendazole at 10mg/kg body was 88.89 percent effective on 18th day and 100% effective on 28th day. The efficacy order was triclabendazole, *Fumaria parviflora* (80 mg/kg), *Saussurea lappa* and *Caesalpinia crista*. No toxic effects were seen in any of these indigenous plant origin drugs.

Keyword: Sheep, fasciolosis, indigenous drugs, efficacy

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Fasciolosis is a wide spread disease of ruminants and causes heavy losses. In Pakistan the incidence of both *Fasciola hepatica* and *F. gigantica* has been recorded (Kendall, 1966). These parasites wander in the liver parenchyma, cause haemorrrhage and damage the tissues which leads to cirrhosis in chronic cases (Irfan, 1968). The flukes are voracious blood suckers and each fluke can suck 0.2 ml of blood/day (Jennings *et al*., 1956). Fasciolosis thus causes anaemia, diarrhoea, loss in weight, low milk and meat production and even death (Khan and Maqbool, 2012).

The incidence of fasciolosis is increasing in Pakistan because more areas are coming under water logging and salinity (Khan and Maqbool, 2012). In the absence of pasture dressing techniques, the affected animals have to be treated with anthelmintics. Anthelmintic treatment is a regular practice in enzootic areas, but it fails to eradicate the disease. Allopathic drugs are neither completely effective, nor do they retain their efficacy by their continuous administration due to the development of resistance but also adversely affect milk and meat production of animals (Chartier *et al*., 2001).

Indigenous drugs like *Saussurea lappa*, *Fumaria parviflora* and *Caesalpinia crista* have been used since ancient times to treat worm infestation. Not much work has been to prove their efficacy against fasciolosis in sheep. The present study was designed to report the efficacy of these indigenous drugs against fasciolosis.

Materials and methods

Study area

The current study was conducted in and around Dhadar of Bolan district. This city is situated near the bank of Bolan River and has been prone to flash floods during the monsoon every year.

Animals used

A total of 110 sheep naturally infected with fasciolosis were used in these studies. These sheep were of various ages (1 to 3 years) of both sexes and of Balochi breed. All animals were kept under

similar feeding and managemental conditions throughout the course of experiment. The studies were conducted during late summer and autumn months. A detailed history of each animal was recorded.

Grouping of animals

Positive sheep were randomly divided into 5 main groups; A, B, C, D and E. The first three groups were further subdivided into 3 subgroups of 10 animals each. The first three subgroups, A1, A2 and A3 were given *Saussurea lappa* at rate of 60, 70 and 80 mg/kg body weight. Animals in subgroups of B i.e B1, B2 and B3 were given *Fumaria parviflora* at rates of 60, 70 and 80 mg/kg body weight.Sheep in subgroups C1, C2 and C3 were given *Caesalpinia crista* at the rate of 60, 70 and 80 mg/kg body weight. Animals in group D were given triclabendazole at recommended dose rate *i.e.*, 10 mg/kg body weight. While animals in group E were not given any treatment and acted as positive control. Ten animals in group were negative for fasciolosis and no treatment was given and acted as negative control.

Preparation and administration of indigenous drugs

Indigenous plant products were dried under shade, finely ground into powder form and were stored in air tight glass bottles at 4°C. Gum tragacanth was also powdered and 2% w/v aqeous solution was prepared and store at 4°C. At the time of medication, a calculated amount of drugs was weighted and suspended in 100 ml of 2% gum solution and were given orally.

Parasitological techniques

Faecal samples were collected separately from animals in a clean polythene bags and were labeled. Faeces were examined by direct smear, simple sedimentation and zinc sulphate floatation technique (Soulsby, 1982). Counting of egg (EPG) was performed through McMaster egg counting technique as described by Kelly (1974).

Faecal egg counts were carried out on zero, 3rd, 7th, 18th day post treatment. Animals found positive were given a second dose of respective drug on day 18th and their faecal egg count were performed on day 21st and 28th. Side effects of drugs, if any were also recorded.

Results and discussion

Efficacy of drugs was determined on the basis of reduction in egg counts after treatment.

Efficacy of Saussurea lappa

Saussurea lappa at rate of 60 mg/kg body weight was 47.36%, 81.57% effective at one and two dose levels, respectively. At higher doses of 70 and 80 mg/kg body weight were 48.57% and 55.55% effective at one dose level and 84.71% and 94.44% effective at two dose levels, respectively (Table I). The results are in agreement with Kailani *et al*. (1995), Maqbool *et al*. (2004) and Deeba *et al*. (2009).

Table I.- Efficacy of various indigenous and allopathic drugs against fasciolosis is sheep

Drug	Efficacy (%)		
	Dose mg/kg	18th day	28th day
Saussurea lappa	60	47.36	81.57
	70	48.57	85.71
	80	55.55	94.44
Fumaria parviflora	60	41.66	83.33
	70	45.71	91.42
	80	63.88	97.22
Caesalpinia Crista	60	38.23	73.52
	70	45.71	80
	80	51.42	85.71
Triclabendazole	10	88.89	100.00

Efficacy of Fumaria parviflora

Fumaria parviflora at the rate of 60, 70 and 80 mg/kg body weight caused 41.66, 45.71 and 63.81 % reduction in egg per gram of faeces at one dose level, respectively. Whereas at two dose level it was 83.33, 91.42 and 97.22%, respectively on 28th day post-treatment. Similar results were also reported by Kailani *et al*. (1995) and Maqbool *et al*. (2004), Hussain *et al*. (2008).

Efficacy of Caesalpinia crista

Caesalpinia crista at the rate of 60, 70 and 80 mg/kg body weight, respectively, caused 38.23, 45.71 and 51.42 percent reduction in EPG count at

one dose level, whereas at two dose levels this drug caused 73.52, 80 and 85.71 percent reduction in EPG count. Nearly similar results were reported by Kailani *et al*. (1995), Maqbool *et al*. (2004) and Sabber *et al*. (2007).

Efficacy of triclabendazole

Allopathic drug *i.e.* triclabendazole at the recommended dose of 10 mg/kg caused 88.89% reduction on the 18th day, and after administration the second dose on the 18th day caused a 100% reduction in EPG count on the 28th day post treatment.

Almost similar results were also reported by Craig and Huey (1984), Richard *et al.* (1990) and Maqbool *et al*. (2004).

Group E

An 30% increase occurred in the egg counts of all control groups.

The results obtained from this study indicated that all drugs were effective at one dose level and highly effective at two dose level. The efficacy order was triclabendazole, *Fumaria parviflora*, *Saussurea lappa* and *Caesalpinia crista*. No side effects were noted with any of indigenous drug.

Although allopathic drugs are effective against fasciolosis but produce several adverse effects. Hence the development of newer, effective, safer and economical drugs has remained an active area of research.

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(Received 25 April 2013, revised 17 May 2013)

Pakistan J. Zool., vol. 45(4), pp. 1172-1177, 2013

First Report on the High Magnitude of Seasonal Weight Changes in the Raccoon Dog Subspecies *Nyctereutes procyonoides viverrinus* in Japan

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Abstract.- In this study, data on seasonal weight changes in the raccoon dog subspecies (*Nyctereutes procyonoides viverrinus*) were obtained from an individual recaptured by trapping and from dead individuals collected by radio tracking between August 2006 and April 2007 in a high-altitude area (1300–1700 m) of Japan. The rate of weight gain in 2 individuals from spring to autumn was 75–84% and that of another individual for one month from November to December was 34%. The magnitude of seasonal weight changes in the raccoon dogs in the study area was much larger than those previously reported in Japanese raccoon dogs from an area with a mild climate and was similar to those in Finnish raccoon dogs (*N. procyonoides ussuriensis*) in areas with cold climates, indicating that Japanese raccoon dogs could plastically change their fat reserves according to climate changes.

Keywords: Adaptation, canid, carnivore, plasticity.

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The raccoon dog (*Nyctereutes procyonoides*), a medium-sized carnivore endemic to eastern Asia, is classified into 5 or 6 subspecies (Ellerman and Morrison-Scott, 1951; Ward and Wurster-Hill, 1990). One of the subspecies, *N. procyonoides ussuriensis* (hereafter referred to as the Ussuri subspecies), originally from the Amur and Ussuri regions of Siberia and eastern China, has spread rapidly into many European countries after being introduced into western Russia during the first half of the 20th century (Kauhala and Kowalczyk, 2011).

Raccoon dogs in different areas have adapted to different climates, habitats, and diets, as shown by their differences in body size, fat reserves, fur thickness, and behavioral and dental characteristics (Kauhala and Saeki, 2004). In Japan, which has a much milder climate than the Russian Far East and Finland, the adaptability of the Japanese subspecies (*N. procyonoides viverrinus*) to cold weather is believed to be lower than that of the Ussuri subspecies (Kauhala and Saeki, 2004). In addition, the Japanese subspecies has no need to be inactive in winter, whereas the Ussuri subspecies is usually inactive during harsh winters in cold climates such as that found in Finland (Kauhala and Saeki, 2004; Kauhala *et al*., 2007). These differences probably lead to differences in the ability to accumulate fat between the 2 subspecies. For example, no evidence of the accumulation of large fat reserves typical of the Ussuri subspecies has been found in the Japanese subspecies; the rate of weight gain from spring to autumn was approximately 60–70% in the Ussuri subspecies and approximately 20–30% in the Japanese subspecies (Korhonen *et al*., 1991; Kauhala, 1993; Kishimoto, 1997).

In Japan, however, only a few studies have been conducted in areas where winters are harsh, and it is not clear whether the ability to accumulate fat is also lower in such areas. Recently, Seki and Koganezawa (2011) reported that the Japanese subspecies restrict their movements in low air temperatures and heavy snow in winter in a high-altitude area of Japan; this behavior has been also observed in the Ussuri subspecies in Finland (Kauhala *et al*., 2007). Therefore, fat accumulation is expected to be higher in the Japanese subspecies in those areas than in areas with milder winters. An investigation of seasonal weight changes in the Japanese subspecies in areas with harsh winters would help us assess the plasticity of its fat accumulation in response to climatic change.

Japanese mountains are steep, and access is often difficult, particularly in winter. This is probably one reason why few studies have been conducted in areas of Japan where winters are harsh. Fortunately, however, data on seasonal weight changes in the Japanese subspecies were obtained from individuals recaptured by trapping and from dead individuals collected by radio tracking. In this paper, I report the results of the first observation of the high magnitude of seasonal weight changes in the Japanese subspecies.

Materials and methods

Study area

Fieldwork was conducted in Nikko National Park, Tochigi Prefecture, Japan. The study area is located in the cool temperate zone, and altitude ranged from 1300 to 1700 m (montane to subalpine zone; 36°45′ N, 139°25′ E). According to data collected between 1971 and 2000 by the Nikko Weather Station (1292 m), located approximately 7 km east of the study area, the mean annual temperature was 6.7°C (–4.2°C in February and 18.5°C in August). Snow cover during the study period was continuous from late December to late March, with a depth of approximately 20–60 cm (Seki and Koganezawa, 2011).

Trapping and radio tracking

Trapping of raccoon dogs was conducted using a maximum of 19 Havahart live traps (26.5 × 31.5 × 81.5 cm; Woodstream Corp., USA), baited with fried chicken, sausages, bananas, or plums, from August to December 2006 and from April to November 2007. There were 40 trap sites, covering an area of approximately 200 ha. The traps were checked at dawn and closed during daylight hours. The mean trapping effort (number of traps × number of nights traps were set) was approximately 62 trap nights per month (SE = 9, range 17–138).

Between August and November 2006, 3 adult males and 4 adult females (hereafter referred to as AM1–3 and AF1–4, respectively) were captured (Table I). The captured animals were immobilized with ketamine hydrochloride (10–20 mg/kg) at the trap sites and the sex, body sizes and weights of each individual were recorded. I classified each individual as an adult (>1 year) by the degree of wear on the incisors (Hata, 1973). Radio collars (Advanced Telemetry System, Inc., USA) weighing 50–120 g were attached. The collars represented about 1–2% and 2–3% of adult body weight at trapping and in spring, respectively. The animals were released at the trapping sites after they had recovered from anesthesia. During handling, the raccoon dogs were kept warm using quilts and rags to prevent hypothermia. Capture and handling were carried out to minimize stress of the animals, according to the approved guidelines (Japan Ethological Society, 2002).

Collared raccoon dogs were located using a portable receiver (FT-290mkII, Yaesu Radio Co. Ltd., Japan) and a hand-held four-element Yagi antenna (H-4EL, Ham Center Sapporo, Japan). Locations of animals were estimated by triangulating compass bearings taken from a minimum of two separate locations. By conducting continuous radio tracking, I determined whether collared individuals moved. I inferred that collared individuals had died if no movement was detected for several days and searched for dead individuals by radio tracking.

Body weight estimation

Weights of recaptured animals were determined by calculating the trap weight with and without the animal.

Three collared individuals (AF2–4) died during the study period. AF2 and AF4 were found drowned in a pond at a fish farm, approximately 3 km from their trapping sites, on December 4, 2006, and March 26, 2007, respectively, by farm staff. These dates were considered the days that the individuals died because the drowned individuals had not been observed by staff before those dates. The individuals were weighed after wiping off excess water. The weight of AF3, which was found dead in the study area on April 29, 2007, was also measured. Because AF3 had not moved since April 19, 2007, that date was considered the day that the individual died. Weight loss in AF3 from April 19 to April 29 was assumed as low, because I did not observe any putrefaction, visible insects such as maggots, or signs that the body was fed on by carnivores. Although radio tracking confirmed that AM1 moved until June 2007, AM3 moved until July 2007, and AM2 and AF1 moved until October 2007, radio signals were not received after then, probably because of the battery life.

Comparison of the weight gain rate among the subspecies

To compare the results in the study area with those in other areas, I reviewed 15 data sets of the rate of weight gain from spring to autumn in raccoon dogs (9 studies). In this review, I used not only the data sets of adults in both spring and autumn but also those of subadults and individuals, whose ages were not provided, from October onwards. However, the influences of the differences in ages on the results were considered to be low, because the offspring of raccoon dogs attain adult body weight between October and December (Ikeda, 1983; Kauhala, 1993). If I could not find the mean values of the body weights in the literature, I used graphs to read the values. In addition, there were some cases in which body weights were measured from spring in the first year to autumn in the second year. In this case, I computed the ratio between the weights in spring and autumn in the first and second year, respectively, and the mean of the values recorded in the first and second year was computed.

Results and discussion

The seasonal weight measurements of raccoon dogs are shown in Table II. The weight of AF3 was 2.0 kg on April 19, 2007, 38% of measured weight on November 16, 2006. Reportedly, in Mt. Nyugasa, Nagano Prefecture, which has a climate similar to that of the study area, an adult male weighing 2.5 kg was radio tracked for 3 months after April 27 (Yamamoto, 1994). These results indicate that in areas of Japan where winters are harsh, the threshold of the weight needed by raccoon dogs for survival at the end of April might

be between 2.0 and 2.5 kg. However, because the value could change on the basis of several factors

Table I.- Body measurements and date of capture, recapture, and death of adult raccoon dogs between August 2006 and April 2007.

Racoon dog number	BW (kg)	HB (mm)	T (mm)	Date of		
				Capture	Recapture	Death (cause)
Mal 1	5.2	545	155	8/22	9/1, 10/25, 10/26, 11/9, 4/6	-
Male 2	5.6	550	200	10/26	-	-
Male 3	4.7	520	220	11/2	11/9, 11/15, 11/16	-
Female 1	5.6	510	220	10/26	-	-
Female 2	4.7	450	170	11/2	-	12/4 (drowning)
Female 3	5.2	515	195	11/16	-	4/19 (unclear)
Female 4	5.9	540	150	11/17	-	3/26 (downing)

BW, body weight measured in the first trapping; HB, head and body length measured in the first trapping; T, tail length measured in the first trapping; the data for BW, HB, and date of capture of raccoon dogs, except for female 2, were derived from Seki and Koganezawa (2011).

Table II.- The seasonal weight measurements of adult raccoon dogs between August 2006 and April 2007.

Raccoon dog number	Body weight (kg)						
	August	September	October	November	December	March	April
Male 1	5.2	5.2	6.5	7.0	-	-	4.0
Female 2	-	-	-	4.7	6.3	-	-
Female 3	-	-	-	5.2	-	-	2.0
Female 4	-	-	-	5.9	-	3.2	-

Table III.- Comparison of the rate of mean weight gain from spring to autumn among the 3 subspecies.

Raccoon dog subspecies	Weight (kg)		RW (%)	T (°C)	IT	Latitude/Longitude	Country	Reference
	Spring (period, n)	Autumn (period, n)						
viverrinus	3.6 (Mar–Apr, 2)	6.5 (Nov, 2)	79	6.9	W/R	36°45' N, 139°25' E	Japan	Present study
	4.3 (Apr–May, 10)	5.6 (Aug–Oct, 10)	34	15.0	C/FE	35°42' N, 139°32' E	Japan	Kishimoto, 1997
	4.7 (Apr, 8)	6.0 (Oct, 8)	28	-	C/FE	63° N, 28° E	Finland	Korhonen *et al*., 1991
albus	3.7 (Mar–Apr, 3)	6.9 (Oct, 3)	85	6.9	W/R	43°46' N, 142°28' E	Japan	Kitao *et al*., 2009
	4.7 (Mar, 3)	6.7 (Oct–Nov, 3)	43	6.9	C/FA	43°46' N, 142°28' E	Japan	Kitao *et al*., 2009
ussuriensis	4.3 (Apr, 9)	8.6 (Oct–Nov, 10)	100	2.0	W/R	62°33' N, 29°9' E	Finland	Mustonen *et al*., 2007
	5.0 (Mar–Jun, 133)	8.5 (Oct–Nov, 62)	70	1.1–5.4	W/H	61°14' N, 25°10' E	Finland	Kauhala, 1993
	4.5 (Mar, 8)	7.0 (Dec, 17)	56	-	W/H	62–63° N, 29–30° E	Finland	Nieminen *et al*., 2011
	4.9 (May, 15)	8.2 (Nov, 15)	67	-	C/FE	-	Finland	Korhonen, 1988
	4.9 (May–June, 10)	7.5 (Nov, 10)	53	-	C/FE	63° N, 28° E	Finland	Korhonen *et al*., 1991
	8.4 (Jun, 28)	11.3 (Dec, 28)	35	-	C/FE	63° N, 28° E	Finland	Asikainen *et al*., 2005
	8.0 (Jun, 28)	10.8 (Dec, 28)	35	-	C/RF	63° N, 28° E	Finland	Asikainen *et al*., 2005
	7.3 (Jun, 28)	11.0 (Dec, 28)	50	-	C/FA	63° N, 28° E	Finland	Asikainen *et al*., 2005
	5.9 (Mar–Apr, 10)	8.8 (Oct–Nov, 10)	49	-	C/OF	-	Finland	Korhonen, 1987
	5.1 (May–Aug, 10)	6.6 (Oct–Dec, 10)	30	-	C/FE	-	Finland	Korhonen, 1987
	5.1 (May–Aug, 10)	7.4 (Oct–Dec, 10)	43	-	C/FA	-	Finland	Korhonen, 1987

RW, rate of mean weight gain from spring to autumn; T, temperature; IT, individual type; W, wild free-ranging individual; C, captive individual; R, recaptured individual; FE, fed individual; FA, fasted individual for some period; H, hunted individual; RF, restrictively fed individual; OF, overfed individual; values of temperatures in the studies of Japan were obtained from data collected between 1981 and 2010 by Nikko Weather Station (36°44′ N, 139°30′ E) for present study, Fuchu Weather Station (35°41′ N, 139°29′ E) for

Kishimoto (1997), and Asahikawa Weather Station (43°45′ N, 142°22′ E) for Kitao *et al*. (2009).

such as the climate, condition of pregnancy, and the incidence of diseases, more cases needed to be studied to reach a conclusion. The weight of AM1, which was recaptured over several months, increased from 5.2 kg in August to 7.0 kg in November and decreased to 4.0 kg in April. Of the individuals that drowned, the weight of AF2 increased from 4.7 kg in November to 6.3 kg in December and that of AF4 decreased from 5.9 kg in November to 3.2 kg in March. Although the sample size was small in this study, the results are similar to those of previous findings (Korhonen, 1988; Korhonen *et al*., 1991; Kauhala, 1993; Kishimoto, 1997), which indicated that both wild and captive raccoon dogs reached their minimum and maximum body weights in spring and autumn, respectively. This pattern is probably caused by the habits of raccoon dogs: they fatten themselves from late summer to autumn before entering winter dormancy (Kauhala and Kowalczyk, 2011) and voluntarily decrease food consumption during the periods between winter and early spring, even though feed would be readily available (Korhonen *et al*., 1991; Kishimoto, 1997).

No significant difference in the minimum and maximum weights of the Japanese subspecies between years has been reported (Kishimoto, 1997). Thus, assuming little between-year difference in the magnitude of seasonal weight changes in the animals in the study area, the rate of weight gain from spring to autumn would be 75% for AM1 and 84% for AF4. The comparison of the results in the study area to those in other areas is shown in Table III. The values in the study area are higher than those reported previously for the captive individuals of the Japanese subspecies and are similar to those for the wild and some captive individuals of the another Japanese subspecies (*N. procyonoides albus*; hereafter referred to as the *albus* subspecies) and the Ussuri subspecies, *i.e.*, the rate of weight gain in raccoon dogs from spring to autumn was approximately 30% for captive individuals of the Japanese subspecies (Korhonen *et al*., 1991; Kishimoto, 1997) and 56–100% for wild and some captive individuals of the *albus* and Ussuri subspecies (Korhonen, 1988; Kauhala, 1993; Mustonen *et al*., 2007; Kitao *et al*., 2009; Nieminen *et al*., 2011). In addition, it is noteworthy that out of the Ussuri subspecies in captivity, there were individuals in which the rate of weight gain was approximately 30% similar to that in the Japanese subspecies (Korhonen, 1987; Asikainen *et al*., 2005). Furthermore, the rate of weight gain in AF2 for 1 month from November to December was 34%. These results indicate that the ability of the Japanese subspecies to accumulate fat is not necessarily lower compared to that of the Ussuri subspecies and that the Japanese subspecies could plastically change its fat reserves according to climatic changes or habitat differences (*i.e.*, wild or captive). The fact that the rate of weight gain in the *albus* subspecies in an area with a cold climate from spring to autumn was 85% for wild individuals and 43% for captive individuals (Kitao *et al*., 2009) also supports the above hypothesis. Fluctuations in the rate of weight gain according to climatic changes were also reported for other medium-sized carnivores such as the raccoon (*Procyon lotor*) (Mugaas and Seidensticker, 1993) and the European badger (*Meles meles*) (Kowalczyk *et al*., 2003).

Although the possibility of speciation in the Japanese raccoon dog has been discussed (Kauhala and Saeki, 2004), the discussion focused mainly on comparing the Ussuri subspecies and the Japanese subspecies inhabiting areas with warm climates. In addition, Kauhala and Saeki (2004) compared seasonal weight changes in the two subspecies by using the data for both wild and captive individuals of the Ussuri subspecies but only the data for captive individuals of the Japanese subspecies. Thus, further studies on seasonal weight changes in wild individuals of the Japanese subspecies in both areas with warm and cold climates should help us to understand the differences in the abilities of the Japanese and Ussuri subspecies to accumulate fat. In this study, I showed that the magnitude of seasonal weight changes in the Japanese subspecies was higher in the study area with a cold climate than in an area with a mild climate and was particularly similar to those in the wild individuals of the Ussuri subspecies. In addition, the Japanese subspecies in the study area were reportedly more carnivorous than those in areas with mild climates; this is similar to the food habit of the Ussuri subspecies (Seki,

2011). Although the Japanese subspecies is considered to be adapted to mild climates (Kauhala and Saeki, 2004), some populations inhabit high-altitude areas with cold climates. Determination of the ecological and morphological traits of these populations would be important in a discussion of speciation in the Japanese subspecies.

Acknowledgements

I am most grateful to Professor Masaaki Koganezawa, Utsunomiya University, for his support during this study and to Assistant Professor Takuya Kato, Nippon Veterinary and Life Science University, for introducing me to a paper on seasonal weight changes in raccoons. I also sincerely thank the staff at the fish farm for their efforts to notify me of the 2 raccoon dogs that died in a pond.

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(Received 25 April 2013; revised 17 June 2013)

Pakistan J. Zool., vol. 45(4), pp. 1177-1179, 2013

Little Gull: A New Bird Record for Pakistan

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A little gull *Hydrocoloeus minutus* (*Larus minutus*, revised) was sighted and photographed at the Rawal Lake, Islamabad on May 26, 2013. At 6 in the morning, shortly after a rain and thunderstorm, the author visited the eastern shore of the Rawal Lake, just south of where the small Korang River enters the lake. The lake is a favorite spot for bird watchers in Islamabad, and is part of the Margalla Hills National Park (but under pressure of nearby housing development and of pollution).

A small gull was noticed next to two bigger ones, which looked like the regularly sighted black-headed gulls *Chroicocephalus ridibundus*. Its bill was black, with a white patch on the head above it, a grey-blackish cap and a white front. The legs seemed dark red; the wing was light grayish with a distinctly darker under-wing and tail. When flying, a dark zigzag band over the upper wings, reminiscent of a Kittiwake (*Rissa* species), as well as a dark band on the under-tail (not shown on the upper side) were clearly noticed (Fig. 1). This bird looked only

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A

B

C

D

E

F

G

Fig. 1A-F. Photographs of a little gull, *Hydrocoloeus minutus*, taken May 26, 2013, at Rawal Lake, Islamabad. Note the head pattern (F). A black tail band and a clear head pattern are obvious with the bird in flight (A and E).

marginally bigger than the whiskered terns (*Chlidonias hybrida*) and behaved in flight very much like it as it joined the group of terns from time to time. Likely it was a juvenile or first summer (they take three years to reach maturity). Several excellent photographs were subsequently taken by Mr. Riaz Khan, who is a local bird photographer.

The next day, the bird could not be found anymore.

The species has not been mentioned so far in literature on birds of Islamabad (Ward, 1994; Pyhala, 2001), or in guides covering the whole of Pakistan (Roberts, 1991; Grimmett *et al.*, 2008; Mirza, 2007/2013). It has, however, been reported as an accidental vagrant in the Indian subcontinent in winter in the Rann of Kutch and in spring in the Kashmir/Ladakh region (distribution maps in Ali and Ripley, 1969; Kazmierczak, 2000). A juvenile female specimen was collected in Ladakh on 21 September, 1936 by Walter Koelz[1]. There has also been a sighting record from Mumbai, India. (Magrath, 1910)

The little gull breeds in Asia in Siberia, possibly Sea of Okhosk and China. According to Olsen and Larsson (2004) the migration of the East-Siberian populations is poorly documented. The Ladakh record indicates that juvenile vagrants occasionally may take up a southward migration soon after they learn to fly, possibly while accompanying other gulls. Our solitary bird being in company of black-headed gulls supports this suggestion.

Other vagrant or irregular spring migrants that have recently been recorded at the Rawal Lake include greater sand plover *Charadrius leschenaultii*; black stork *Ciconia nigra*; greater painted snipe *Rostratula benghalensis*; Pheasant-tailed jacana *Hydrophasianus chirurgus* (up to 5 at a time!); terek sandpiper *Xenus cinereus*; glossy ibis *Plegadis falcinellus*; little tern *Sternula albifrons* and white-winged tern *Chlidonias leucopterus*.

Acknowledgements

My thanks are due to Mr. Steve Norman who confirmed the identification and to Mr. Riaz Khan for the photographs of this bird. Further sincere thanks are due to Prof. Z.B. Mirza, for reviewing and enhancing the note above.

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(Received 26 June 2013, revised 7 July 2013)

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Pakistan J. Zool., vol. 45(4), pp. 1180-1182, 2013

Otolith Biometry - Total Length Relationships in the Population of Hazar Bleak, *Alburnus heckeli* (Battalgil, 1943) Inhabiting Lake Hazar, Elazig, Turkey

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Abstract.- In this study, the relationships between otolith biometry-total fish length of 110 Hazar bleak, *Alburnus heckeli* (Battalgil, 1943) (Cyprinidae) specimens from Lake Hazar were examined. Total length and weight of specimens ranged between 8.8 and 11.0 cm and between 4.4 and 13.80 g, respectively. The length, breadth and weight of otoliths of each specimen were measured which were respectively 1.48-2.86 mm, 1.02-2.43 mm and 0.0005-0.002 g. There was strong relationship between the otolith length and total length of fish.

Key words: *Alburnus heckeli*, Hazar Bleak, otolith biometry.

Information on the relationship between otolith dimension and the total length of fish is useful to determine the size of fish if the lengths of otoliths encountered in predator stomach are measured. Moreover the length of a fish can be verified, if the age determined from the otolith lies outside the expected values (Eecheverria, 1987). The size and shape of otoliths have been used for age determination in fishes (Aydin *et al.*, 2004; Akyol *et al*., 1997; Sahin and Günes, 1998; Sen *et al.*, 2001; Aydin *et al.*, 2004; Metin ve Ilkyaz, 2008; Bostanci and Polat., 2009; Ilkyaz *et al.*, 2011; Jawad *et al*., 2012; Basusta *et al*., 2013a). The hazar bleak, *Alburnus heckeli* (Battalgil, 1943) is pelagic and endemic species inhabiting Lake Hazar (Elazig, Turkey) of upper Tigris River (Geldiay and Balık, 1996). This species is assessed as data deficient (DD) due to lack of the biological data by the International Union for Conservation of Nature (IUCN) (Bogutskaya, 1997). Although there are some information about comparative age determination and growth characteristics of the hazar bleak (Gökerti and Basusta, 2010; Bal and Calta, 2012), the relationship between fish length and otolith dimensions has not been investigated for this species. Therefore, this study provides the first information on the otolith biometry-total length relationships of *Alburnus heckeli* inhabiting in the Lake Hazar, Elazig, Turkey.

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Material and methods

Fish samples were caught by a cast-net from Lake Hazar (latitude: 38° 29’ N; longitude: 39° 25’ E) which is a tectonic lake located in Elazig province of eastern Anatolia, Turkey during February-October 2011. The specimens were identified according to Geldiay and Balik (1996). Total length was measured to the nearest 1 mm and the weight of each specimen was determined with a digital scale nearest to the 0.01g and then the otoliths of the fish samples were removed. Otolith length (OL), breadth (OB) and weight (OW) were measured from each specimen nearest 0.001mm and 0.0001g, respectively. The otoliths were dried, cleaned with 96% ethyl alcohol (Chugunova, 1963), and their dimensions measured from anterior to posterior edge of the greatest distance (Aydin *et al*., 2004; Basusta *et al.*, 2013b).

The otolith dimensions-total length relationships were examined by using the following equation:

$$y=a+bx$$

where y is otolith length, x is fish length, a is intercept value, b is coefficient value (Sen *et al*., 2001; Aydin *et al*., 2004; Mahmood *et al*., 2012).

Results and discussion

A total of 110 specimens of *Alburnus heckeli*

have been examined. Total length and weight of specimens ranged between 8.8 and 11.0 cm and 4.4 and 13.80 g, respectively. The length, breadth and

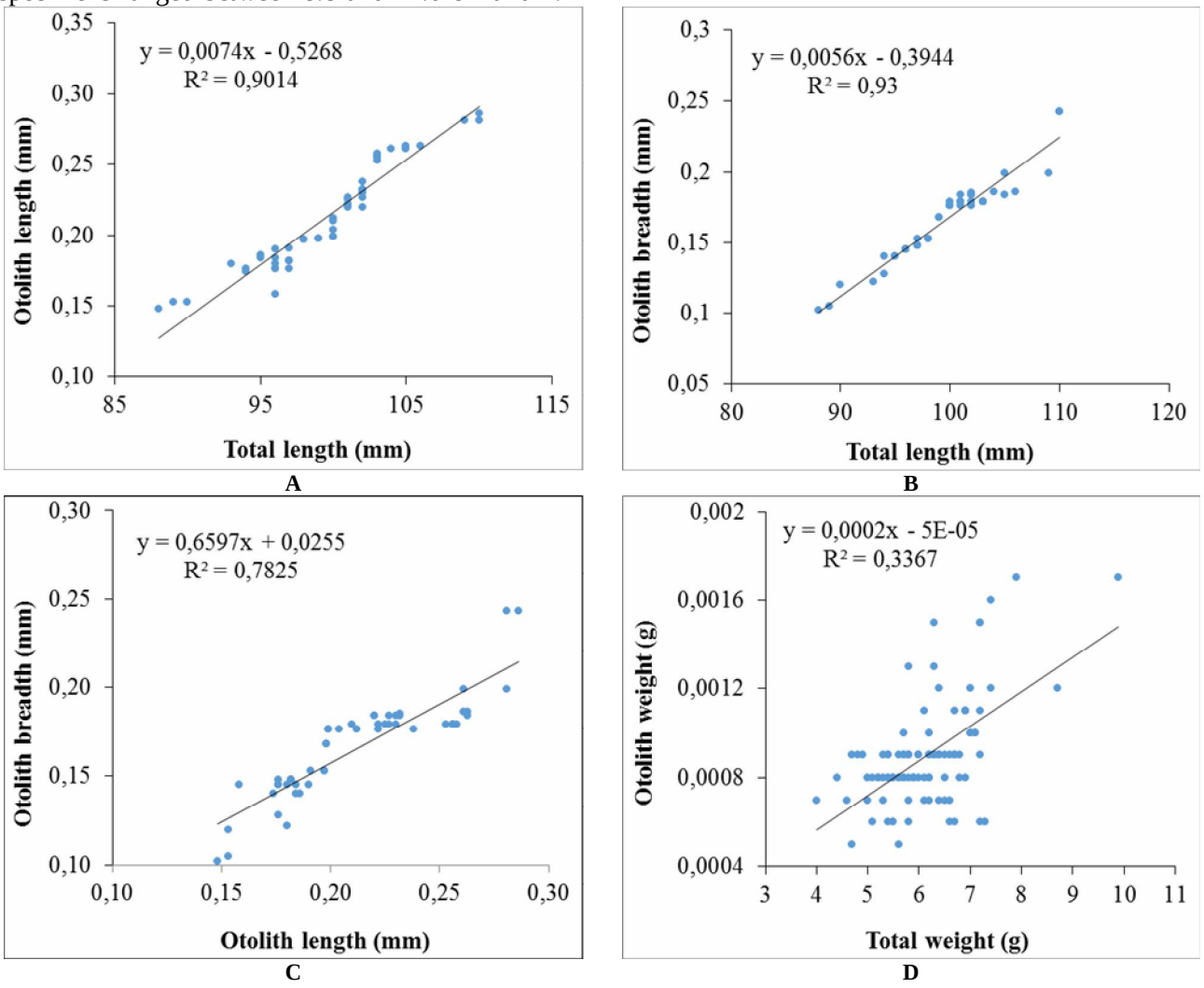

Fig. 1. Total length-otolith length (A), total length-otolith breadth (B), otolith length-otolith bread (C) and total weight-otolith weight relationship in *Alburnus heckeli*.

weight of otoliths were 1.48-2.86 mm, 1.02-2.43 mm and 0.0005-0.002 g, respectively.

The relationship among total length with otolith length and otolit breadth were as y=0,0074x-0,5268 (R^2 = 0,9014); y=0,0056x-0,3944 (R^2 = 0,93), respectively (Fig.1A,B).

The relationships between otolith length and otolit breadth and total weight and otolith weight were as y=0.6597x+0,0255 (R^2 = 0,7825) and y=0,0002x+-5E-05 (R^2 = 0,3367), respectively (Fig. 1C,D).

There was a strong relationships between the otolith lengths and total length obtained (R^2 = 0.9014) and a moderate relationship between the otolith lengths and otolith breadths (R^2= 0.7825). Harkönen (1986) reported that there is a high correlation between fish length and otolith length and that is generally a linear relationship. Brander (1974) asserted that otolith mass has a direct relationship to the fish age.

This is the first information on otolith dimensions-total length relationship of *Alburnus*

heckeli inhabiting Lake Hazar, Elazig, Turkey and also this study also provides a trusty worthy tool for stomach contents research and for determination of size of fish in archaeological samples.

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(Received 18 June 2013, revised 17 July 2013)