Short Communications

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A Case of *Fasciola hepatica* Infection in Swat, Pakistan

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Abstract.- *Fasciola hepatica* was reported for the first time from the faecal sample of a 60 years old man during the survey of human intestinal parasitic infections in Swat, Pakistan. The man belonged to shepherd community of Swat.

Key words: *Fasciola hepatica*, Intestinal Parasites, Zoonotic infection

Human Fascioliasis is a zoonotic disease caused by *Fasciola hepatica* and *Fasciola gigantica*, which infests cattle and sheeps. In order to complete its life cycle it requires two hosts a definitive host the goat, sheeps and man are the accidental host while the intermediate host is the snail of different genera in different localities (Dittmar and Teegen, 2003).

The occurrence of *F. hepatica* in man since prehistoric is evident (Dittmar and Teegen, 2003; Farag *et al.*, 1979) but its role in man has always been considered as a disease of secondary importance (Malek, 1980; Boray, 1982). Review by Chen and Mott, 1990 was the first paper to highlight the importance of human fascioliasis as a public health issue.

It is reported that 2.5 million people have been infected in 61 countries especially from Bolivia, Peru, Egypt, Iran, Portugal, and France and that more than180 million people are at risk. (Haseeb *et al.*, 2002). The estimated number of people having Fascioliasis is 360,000 in Bolivia; 20,000 in Ecuador; 830,000 in Egypt; 10,000 in Iran; 742,000 in Peru and 37,000 in Yemen (Haseeb *et al.*, 2002). *F. hepatica* is found in parts of the United States, as well as in Great Britain, Ireland, Europe, The Middle East, The Far East, Africa and Australia (Crompton, 1999). In China, over 50 cases have been reported since 1959 (Xie and Yang, 1999). A case of ectopic parasitism: *F. hepatica* larvae burrow through human brain and mimic like cerebral aneurysm as reported in China (Ying *et al*., 2007).

Many human infections with *F. hepatica* were reported from Northern parts of Iran (Farid, 1971). The epidemics of human fascioliasis have been reported from France (Rondelaud, 1980), Egypt (Farag *et al.*, 1979) and Brazil (Amaral and Busetti, 1979). Rare cases have occurred in Japan (Yoshida *et al.*, 1974), central Europe, The Union of Soviet Socialist Republics (USSR) and England (Facey and Marsden, 1960). Approximately 50 cases have been recorded in Pureto Rico (Hillyer, 1981). Some cases have also been reported from countries in Asia including India (Narain *et al.*, 1997)

In Pakistan the intestinal parasitic infections are widely prevalent with variable distribution in different parts of the country. Various surveys have been conducted in different provinces of the country (Siddiqi and Bano, 1979; Pal and Malik, 1979; Nawaz and Nawaz, 1994; Chaudry *et al.*, 2004) but according to the literature available no case of *F. hepatica* has been reported except one case from Lahore (Qureshi *et al.*, 2005).

Materials and methods

Faecal sample of an out-patient, 60 years old (male) was collected during the survey of human intestinal parasitic infections in different occupational and age groups as well as gender in human population of Swat, Pakistan, during the autumn (August to September) 2006 from Civil Hospital Khwazakhela, Swat. The person was belonging to the shepherd community of Swat.

The wet mount techniques (WMT) including normal saline solution and Lugol's Iodine fresh smear preparation. The negative cases were

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confirmed by centrifugation and sedimentation procedures and techniques

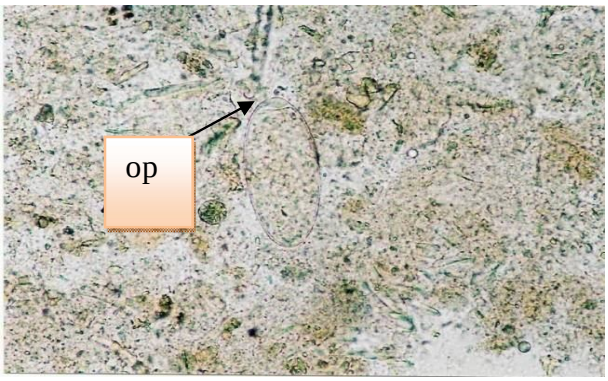

Fig 1. *Fasciola hepatica* egg; OP: operculum.

Results and discussion

This is the second report of *F. hepatica* from human in Swat, Pakistan but earlier a case was reported from Lahore (Qureshi *et al.*, 2005). When observed under microscope the ova was large, opperculated, oval in shape, brownish in colour, 140 micron in length and 80 micron in width containing a large unsegmented ovum in a mass yolk cells.

Human Fasciolasis, caused by *F. hepatica* is a sporadic disease of low economic importance but Chen and Mott (1990) highlighted the importance of this zoonosis identifying 2.594 cases from 42 different countries between 1970 and 1990. Hopkins (1992) and Rim *et al.* (1994) reported 2.4 and 17 million human infections worldwide with *F. hepatica.* World health organization (1995) recognizes fascioliasis as an emerging disease of humans.

In Swat, Pakistan out of 1041 faecal samples only one case has been found in an old person working as shepherd while Qureshi *et al*. (2005) worked on epidemiological aspects of human Fascioliassis in rural areas of Lahore, Pakistan.

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Length–Weight Relationships of Twelve Fishes from the River Padma near Rajshahi City, Bangladesh

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Abstract.- The objective of this study was to evaluate the length-weight relationships (LWRs) of freshwater fish species, which serves as a baseline for comparison to other relatively altered tropical Bangladeshi rivers. The LWRs of 12 species belonging to four classes, seven families and 11 genera (*Gagata youssoufi, Cirrhinus reba, Clupisoma garua, Ompok bimaculatus, Pangasius pangsius, Securicula gora, Ailia coila, Chanda nama, Parambassis ranga, Botia lohachata, Rhinomugil corsula* and *Labeo boga*) captured from the River Padma near Rajshahi City, Bangladesh, were studied. The allometric coefficient (b) of the LWRs indicated positive allometric growth in *P.* P.*pangsius*, *A. coila, C. reba, B. lohachata* and *C. nama* (b> 3.00), but negative allometric growth in *G. youssoufi, C. garua, O. bimaculatus, S. gora, P. ranga, R. corsula* and (b < 3.00). The results would be useful for sustainable management and conservation of the limited stocks in the Padma River ecosystem.

Key Word: Length-weight relationship, allometric, isometric, growth and Padma River.

Length–weight relationships have been used extensively for the conversion of growth-in-length equations to growth-in-weight for use in stock assessment models to estimate the stock assessment biomass from a limited sample size as indicators of fish condition, to compare the life histories of certain species among regions and other aspects of fish population dynamics (Binohlan and Pauly, 1998; Moutopoulos and Stergiou, 2002; Dubey *et al*., 2012; Mortuza and Misned, 2013; Nie *et al*., 2013). It also allows for the study of the ontogenetic allometric changes in fish growth (Teixeira-de Mello *et al*., 2006) and possible effects from parasites (Teixeira-de Mello and Eguren, 2008).

This study describes the length–weight relationships (LWRs) of 12 least concern and near threaten species according to IUCN read list from the Padma River, near Rajshahi city, Bangladesh (IUCN Bangladesh, 2000). These fish species were once abundant in rivers, streams, canals, reservoirs, lakes, ponds and beel, haor and baor swamplands of Bangladesh, India, Nepal and Sri-Lanka (Froese and Pauly, 2011), but the populations are in serious decline due to over-exploitation augmented by various ecological changes and degradation of their natural habitats. The LWRs of threatened fishes are the most important biological parameters to provide information on the growth and condition of fish species and the entire fish community, and are highly significant for management and conservation of natural populations (Sarkar *et al*., 2009; Muchlisin *et al*., 2010). LWRs have been reported for some commercially important fishes from the River Padma, but data for most of the endemic freshwater fish species are still missing. In this study, the parameters of the LWRs are presented for 12 such fish species collected from the river Padma near Rajshahi, Bangladesh.

Materials and methods

The Padma is one of the largest rivers and is part of the Ganga in Bangladesh. Monthly samples were collected from different fishing spots and fish markets of Rajshahi City, from June 2012 to May 2013. The fresh samples were immediately chilled in ice on site and fixed with 10% buffered formalin upon arrival at the laboratory then identified according to Jayaram (1981), Rahman (1989) and Talwar and Jhingran (1991). All morphometric measurements were executed according to Froese and Pauly (2011). All fishes were individually measured to the nearest 0.01 mm and weighed on a digital electronic balance (Bosch EP 628) to the

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Table I.- Parameters of the length-weight relationships for 12 fish species from the River Padma, near Rajshahi City, Bangladesh.

Family	Species	No. of species (n)	Length (mm) (min-max)	Weight (g) (min-max)	A	B	SE(b)	r2
Sisoridae :	*Gagata youssoufi*	130	17-45	0.13-1.22	0.7683	2.838	0.0778	0.9515
Schilbeidae :	*Clupisoma garua*	160	80-305	2.8-301	0.6908	2.813	0.0121	0.9761
	Ompok bimaculata	185	55-225	1.56-95	0.6153	2.849	0.0085	0.9644
	Ailia coila	210	51-170	0.31-23	0.8315	3.065	0.0079	0.9179
Pangasidae :	*Pangasius pangsius*	120	77-1177	3.40-16000	0.3047	3.000	0.817	0.9511
Cyprinidae :	*Cirrhinus reba*	155	73-245	3.49-58.57	0.4601	3.178	0.0312	0.9741
	Securicula gora	170	71-224	2.58-70.00	0.7071	2.942	0.0104	0.9623
	Labeo boga	150	80-290	8-360.1	0.1242	2.843	0.0149	0.853
Ambassidae :	*Chanda nama*	200	21-90	0.12-8.7	0.6989	3.010	0.0082	0.8649
	Parambassis ranga	190	21-89	0.13-10.5	0.6669	2.920	0.0101	0.9216
	Rhinomugil corsula	195	89-261	6.5-198.31	0.6987	2.984	0.0068	0.8779

nearest 0.01 g. The LWR was estimated using the expression $W = a L^b$ (Ricker, 1973), where W is total weight (g) and L = total length (mm). The parameters 'a' and 'b' were estimated by linear regression after logarithmic transformation of weight and length data:

log W = log a + b log L), where, W is weight of fish (g), L is length of fish (cm), a, y Intercept or the initial growth coefficient, b, slope or the growth coefficient.

The statistical significance level of r^2 and the SE for standard error of b ($P < 0.001$) were calculated for all 12 species.

Results

The data from 2015 specimens of 12 fish species belonging to seven families and 11 genera were used for the LWR calculations (Table I). The observed maximum total length among all individuals sampled during the study was 1177 mm, which was a specimen of *P. pangasius* having a total weight 16 kg and the minimum total length 17 mm with total weight 0.13g was that of a specimen of *G. youssoufi*. The regression coefficients (b) values ranged from 2.813 for *G. youssoufi* to 3.178 for *C. reba*, whereas the 'a' values ranged from 0.01242 to 0.8315 for *L. boga* and *A. coila* respectively. The calculated 'b' value of the LWR indicated positive allometric growth in *P. pangasius, A. coila, C. nama B. lohachata* ($b > 3.00$), but the rest of the species showed negative allometric growth ($b < 3.00$). All LWRs were highly significant ($P < 0.001$), with coefficient of determination (r^2) ranged from 0.838 for *G. youssoufi* to 0.976 for *C. garua*.

Discussion

Using the value of 'b' obtained in this study, fish representing both isometrical and allometical growth were observed in the study samples (Andreu-Soler *et al*., 2005). Most of the estimates for 'b' values obtained in the present work show a similar trend with those of Sani *et al*. (2010) in tributaries of the Yamuna and Ganga by Sarkar *et al*. (2009) in Ganga basin, Pet *et al*. (1996) in Sri Lankan reservoirs, Ahmed and Saha (1996) in Kapati Lake, Bangladesh, and by Sivakami (1987) and Ramakrishniah (1988) in the Nagarjunasagar reservoirs. However, in the present study, the higher value of b (3) for some species may be due to the dominance of juveniles and an incomplete coverage of the known size range. Differences in the slopes of the length-weight estimates for the same species in different regions can be affected by environmental

conditions or developmental state of the fish (Le Cren, 1951) or, according to Froese (2006), by the range lengths used in the length-weight relationships.

The coefficient of determination (r^2) ranged from 0.853 (*L. boga*) to 0.9741 (*C. reba*), nine of 12 regressions presented r^2 values higher than 0.90. All linear regressions were statistically significant ($P < 0.001$). The calculated standard error of b (SE) ranged from 0.0068 to 0.0817, thus indicating a tendency towards positive allometry, which is in accordance with the majority of fish species (Froese, 2006).

In conclusion, this study provides an important baseline study on the LWRs of 12 least concern and near threatened fish species from the River Padma near Rajshahi City, Bangladesh. These results can be an effective tool for fisheries management and conservation to initiate early management strategies and regulations for conservation of the remaining stocks of the endangered species in the Padma River. This study also provides valuable information for the online database, as well as providing an important baseline for future studies within the Ganga-Brahmaputra basin have almost all been subjected to perturbations of various origins.

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Treatment of *Eimeria tenella* Infection in Broilers by Using Sugar Cane Extract

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Abstract.- The objective of this study was to observe the effects of oral administration of sugar cane extract (SCE) on *Eimeria tenella* oocysts infection in commercial broiler chickens. The present paper describes the growth promoting effects of SCE in broiler chickens. For this purpose one hundred and sixty (160) day old chicks were divided into 4 groups, *i.e.* A, B, C and D, each of 40 chicks. Group A was non-infected and non-medicated control (healthy control), group B was infected with *Eimeria tenella* sporulated oocysts on day 21 (20000 oocysts per chick) and was considered as infected control. Chicks of group C were orally administered with SCE (500mg/ kg body weight) on day 21 and then challenged with 20,000 oocysts per chick of *E. tenella* oocysts. Chicks of group D were orally administered SCE at the dose rate of 500mg/kg body weight for three consecutive days and then challenged with 20,000 oocysts per chick of *E. tenella* oocysts. The results indicated that maximum reduction of oocyst per gram feces OPG counts (98.73%) occurred in members of group D which were administered SCE (500 mg/kg) for three consecutive days and then challenged with *E. tenella* oocysts. Group C (administered SCE for a single day @ 500mg/kg) was placed at no.2 and the reduction in this group occurred as (83.54%) as compared to group B. Mean body weight gains were calculated by taking difference between average weight records on day 21 and day 35 of age (final weight record). The difference of each group was compared with healthy control and percentage of weight gain of all the groups were recorded and compared.

Key words: Chickens, *Eimeria tenella,* sugar cane extract.

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Avian coccidiosis in broilers and layers is considered as one of the most important manage mental problem in which various species of genus *Eimeria* are involved but *E. tenella* is one of the most pathogenic and causes caecal coccidiosis in young birds and broilers (Dalloul and Lillehoj, 2006; Mansori and Modiranei, 2012).

Coccidiosis seriously impairs the growth and feed utilization of infected animals resulting in loss of productivity (Yun *et al*., 2000a,b).Conventional disease control strategies relied heavily on chemoprophylaxis (Laurent *et al*., 2001; Al-Idreesi *et al*., 2013). Increasing regulations and bans on the use of anticoccidial drugs coupled with the associated costs in developing new drugs and live vaccines increased the need for the development of novel approaches and alternative control strategies. *Eimeria tenella* primarily invades and resides in the linings of caecum of exposed chickens (Vervelde *et al.,* 1996; Yun *et al.,* 2000a,b; Fetterer and Allen, 2001; Yumauchi *et al*, 2006a,b). Infective sporozoites enter the caecal mucosa by penetrating crypt epithelial cells. Immunity does not prevent sporozoite invasion of cells but does prevent sporozoite development (Vervelde *et al*., 1996; Laurent *et al*., 2001*).*

The effectors of immune responses to primary and challenge coccidial infections are primarily T-Cells residing in gut-associated lymphoid tissues (Lillehoj and Trout, 1996). Humoral immune responses also occur but antibodies play a minor role in resistance and immunity to coccidia (Lillehoj, 1987). In avian coccidiosis, it is clear that IFN- y is produced by the host at sites of infection. Chemoprophylaxis and anticcocidial feed additives have contributed a lot in controlling many infectious diseases but frequent consumption of various chemicals and antibodies have complicated the problem by the development of antibiotic resistant strains and environmental pollution (Farzana *et al.,* 2009; Asma *et al*., 2008).

To prevent the-emergence of drugs resistance, new drugs have been developed and administered with existing drugs on rotational basis. However, this has resulted in the increased cost of poultry products. Furthermore, drug or antibiotic residue in the poultry products is potentially harmful

to the consumer. Therefore, there is need for stringent regulation of anticoccidial drugs.

Materials and methods

The present study was designed to evaluate the effects of oral administration of sugar cane extract (SCE) as an alternate tool for protection against the development of *E. tenella* infection in chickens weight gain, oocysts shedding, morbidity and mortality of birds were observed and the following procedure was adopted.

Collection and sporulation of coccidial oocysts

The caecal material was collected from Veterinary Research Institute, Lahore and sporulation of coccidial oocyst was done as per technique described by Garcia (2009).

After completion of 80% sporulation, the mixture from the petri dishes was sieved through muslin cloth. The filtrate was centrifuged for 2 min at 1500 rpm, the supernatant was discarded and the sediment was mixed with saturated NaCI and again centrifuged. The upper layer of the supernatant was collected with pipette and added in 1:10 ratio of the distilled water to dilute the NaCl solution. The suspension was allowed to stand overnight to let the oocysts settle at the bottom. The supernatant was discarded and the sediment re suspended in 2% potassium dichromate solution.

The suspension was measured in graduated beaker and thoroughly mixed. Five samples of one ml each were withdrawn with a graduated pipette and transferred to a measuring cylinder containing 45 ml of distilled water (50 ml stock solution). It made 1:9 dilutions. The mixture was agitated thoroughly and again 3 samples of 0.1 ml each were withdrawn and each sample was spread on the glass slide in longitudinal fashion. Then oocysts were counted by McMaster egg counting technique (Coles, 1986).The average of 3 samples of 0.1 ml was calculated and multiplied by the 10 to calculate the number of oocysts per ml of the stock solution.

Sugar cane extract administration

SCE was produced from sugar cane (*Saccharum officinarum* L.) by following the methodology of Awais *et al*. (2011). The final concentration (100mg/ml) was constituted in 0.1 M phosphate buffered saline (PBS; pH 7.2). Sugar cane contains about 70% water in which sucrose is 11% to 16%, reducing sugars 0.4 to 2%, fiber 10% to 16%, organic non sugars and mineral matters 0.5% to 1%, forming about 88% by weight of juice in stem. The remaining 12% represents the insoluble cane fiber component. SCE was administered at the dose of 500 mg/kg (10ml of sugar cane juice) per kg body weight.

Experimental design

One hundred and sixty day old broiler chicks were purchased from the local hatchery. The chicks were reared under standard hygienic conditions. All the birds were vaccinated against New Castle Disease (ND) on day 1 and 20 of age. At the age of day 3 all the birds were randomly divided into four groups, *i.e.*, A, B, C and D, each of 40 birds.

Birds of group A remained uninfected and reared as non-infected, non-medicated; birds of group B were administered orally with 20,000 virulent sporulated oocysts of *Eimeria tenella* on day 20 of age and were kept as infected, non-medicated; while those of C were orally administered with a single dose of SCE (500mg/kg) and then challenged with the 20,000 oocysts per chick of *E. tenella* oocysts; and those of group D were orally administered SCE for 3 consecutive days and then challenged with *E. tenella* oocysts 20,000 oocysts per chick.

Weight gains were recorded on day 21, 28, 32 and 35 of age. OPG counts of feces for 3 groups (B, C and D) was carried out on day 5 to 7 (day 26 to 28 of age) of oral administration of SCE and challenge dose of sporulated oocysts of *E. tenella.* The OPG counts were performed by the McMaster egg counting technique (Coles, 1986).

Statistical analysis

Data was analyzed statistically using ANOVA, Analysis of variance for weight gain to compare the performance of different groups and also to evaluate the efficacy of two different administration of sugar cane extract (Steel and Torrie, 1982).

Results and discussion

Table I shows effect of SCE on OPG counts of infected broilers. Extent of hemorrhage and oocysts output in feces of chickens administered sugar cane extract was milder than that of infected group. These results are supported by (Mansori and Modirsaen, 2012). Similar findings have been reported by (Lillehoj, 1987; El-Abasy, 2002; Yamuchi *et al*., 2006a,b; Awais *et al*., 2011; Akhtar *et al*., 2012).

Table I.- Mean OPG counts in test groups (C and D) and infected control group (B).

Age of chicks (days)	Group A Control (un-infected) (n=40)	Group B infected (n=40)	Group C SCE for one day + infected (n=40)	Group D SCE for 3 days + infected (n=40)
26	0	5000	1500	1000
27	0	39500	6500	500
28	0	20000	11500	8000

Group A was non-infected non- medicated and remained free of infection. Group B was infected control. The findings of test groups were compared with the infected control group B which passed the maximum number of total OPG (39,500). Whereas members of group C passed 11,500 and 8,000.According to the reduction in OPG counts as compared to control group B, the maximum reduction (98.73%) was observed in group D which was administered sugar cane extract (500 mg/kg) for three consecutive days.

Group C was placed at number 2 and reduction in this group occurred as (83.54%). This group was administered with a single dose of sugar cane extract. The findings of the present study are supported by (EI-Abasy, 2002, 2003a,b) in terms of prophylactic effect of sugar cane extract given at the dose rate of 500mg/kg.

Table II shows the effect of SCE on the body weight of infected broilers. Body weight gain in group A from day 21 onward was (67.85%) by the end of experiment which was the highest among all the experimental groups. In group B only (60.47%) body weight gains was noted. Group C became 3rd in ranking order in terms of weight gains acquired (61.90%) mean weight per bird. In group D, mean body weight (360 gm) was (65.84%). This group was ranked as the highest in terms of body weight gains as compared to weight record of Day 21. These results are in accordance with those of Yun *et al*. (2000a,b) and Fetterer and Allen (2001).

Table II.- Comparative mean weight gain record of different groups.

Groups	Days of age				Total weight gain (g) (%)
	21	28	32	35	
Healthy control	342	658	900	1064	722 (67.85)
Infected control	400	639	854	1012	612 (60.47)
SCE-1 + challenge	400	633	887	1050	650 (61.90)
SCE-3 + challenge	360	675	835	1054	694 (65.84)

It was concluded that SCE has biological properties. It stimulates the growth rates in industrial broiler chickens.

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Heavy Metals in Wintering Great Bustard's Feces

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Abstract.- Feces is undigested food residues and therefore heavy metal content in feces of animal can reflect the heavy metal content of this animals' habitats. In Jan. 2013, the feces of wintering great bustard were collected from the intersection area of the Yellow, Wei and Luo River in the Shaanxi Yellow River wetland, China. This study determinate the contents of nine heavy metals - Mn, Cd, Cr, Cu, Ni, Pb, Zn, As, Hg in bustard' s feces and describes the threats of these metals in particular with Pb, Mn and Ni on this wintering bird. Among these, the content of Mn was the highest in great bustard feces (174.55 mg/Kg), followed by Zn (57.46 mg/Kg), Cr (19.53 mg/Kg), Cu (15.80 mg/Kg), Ni (5.70 mg/Kg), Pb (2.93 mg/Kg) and As (1.62 mg/Kg). The content of Cd and Hg were the lowest *viz.*, 0.35 mg/Kg, 0.05 mg/Kg, respectively. The present results obtained by atomic fluorescence morphology analyzer, DMA-80 mercury analyzer and inductively-coupled plasma emission spectrometer indicated that these methods were simple and reliable, and could be used for biological trace elements in long-term follow-up surveys.

Keywords: Great Bustard, feces, heavy metal, atomic fluorescence morphology analysis, plasma emission spectrometry.

The great bustard, *Otis tarda*, ranges across central and southern Europe, Western Russia and some temperate areas of central and eastern Asia to the Pacific, occupying open steppe grasslands and extensively cultivated fields (Del Hoyo *et al.*, 1996). Although widely distributed, most populations have suffered large declines and some went extinct in relatively short periods during the last century

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(Alonso *et al.*, 2003). At present, the main stronghold is found in Spain where 60–70% of the world's population (Alonso and Palacín, 2010), and the populations of the nominal subspecies are listed as Vulnerable (VU) worldwide by IUCN at 2012.

Many bird species are at, or near, the top of the food chain and consequently prone to accumulating environmental pollutants. Since levels of heavy metals in the feces of birds reflect the background abundance of these pollutants, this can be a useful indicator of heavy metal pollution in the environment. The great bustard is a key species in conservation of the lowland grassland ecosystem. Most of the studies on great bustards are based on population distribution (Martínez, 2008; Alonso and Palacín, 2010), diet (Lane *et al.*, 2001; Bravo *et al.*, 2012), habitat selection (Delibes *et al.*, 2012) and ecological studies (Raab *et al.*, 2011; Kessler *et al.*, 2013). Feces is undigested food residues and heavy metal content in feces of wild great bustard can reflect the heavy metal content of this birds' habitats. Very little published information is available in the literature. Therefore, AFS-9230 atomic fluorescence spectrometer, plasma emission spectrometer (IRIS Advantage) and DMA-80 mercury analyzer were utilized to determine the content of nine heavy metals *viz.*, Mn, Cd, Cr, Cu, Ni, Pb, Zn, As and Hg in the feces from wintering great bustard in the intersection area of the Yellow River, the Wei River and the Luo River in China in order to provide basic data for the biology and habitat protection of great bustard.

Materials and methods

Study area

The intersection area of the Yellow, Wei and Luo River in the Shaanxi Yellow River wetland which is located in the middle reaches of Yellow River basin of China, has open and wide topography and less human interference. This land with its elevation below 400m, is a semi-arid continental monsoon area of temperate zone with sufficient sunshine and appropriate rainfall (mostly from July to September); The annual average temperature is 13.5°C, annual precipitation ranged from 529 mm to 574 mm and frost period ranged from 160 to 167 days. The main crops here are wheat and beans providing food resource for wintering great bustard, thus this land has become one of the major wintering habitats for great bustard in China. Each year, as many as 300 great bustards migrate to here from northern Mongolia in mid to late October and return back to their breeding grounds in mid-March to early-April of the next year (Wu, 2012; Kessler *et al*., 2013).

Sample pretreatment

After the bustards population was found on wintering ground, feces of great bustard were collected and then taken to the laboratory for analysis. Sample (2 g) was weighed, in digestive tube to which 4 ml HNO_3 was added and covered with small funnel in the fume hood. The fumed was shifted to the digestion furnace for 24 h. The contents were dissolved at 80-90°C for 30 min, followed by 100-110°C for 30 min and at 120-130°C for 1 h. The above solution was placed in a cool ventilated place, and then kept at 100-110°C for 30 min after addition of 1 ml H_2O_2, and then the temperature was raised to 120-130°C for 1 h. Finally, the dissolves sample was cooled and transferred to 20 ml volumetric flask to constant volume. The sample was filtered and then stored in a fresh bottle for further analysis.

Main instruments and the standard equations

Atomic fluorescence morphology analyzer SA-10 is an element morphological detector based on high performance liquid chromatography (HPLC) and hydride generation atomic fluorescence spectrometry. Element As was determined by atomic fluorescence morphology analyzer (SA10, Beijing Titan Instruments Co., Ltd.). Element Hg was determined by DMA-80 mercury analyzer (Beijing Midwest Yuanda Technology Co., Ltd.). The sample can be measured directly without pretreatment. This method can avoid the loss of volatile mercury, mutual contamination and pollution of the environment in preconditioning, ensuring the accuracy of data analysis.

Inductively coupled plasma atomic emission spectrometry (ICP-AES) is a means of analysis method based on plasma atomic emission spectrometer, due to its low detection limit, high accuracy, wide linear range and advantages of determination of multiple elements at the same time,

therefore, shows the strong competitiveness. Determination of Mn, Cd, Cr, Cu, Ni, Pb and Zn was conducted by inductively coupled plasma emission spectrometer (IRIS Advantage, Thermo Electron Corporation), glass concentric spray chamber and 2D dispersion system.

Results

The content of heavy metal Mn were the highest in great bustard feces (174.55mg/Kg), followed by Zn (57.46 mg/Kg), Cr (19.53 mg/Kg), Cu (15.80 mg/Kg), Ni (5.70 mg/Kg), Pb (2.93 mg/Kg), As (1.62 mg/Kg). The content of Cd and Hg are 0.35 mg/Kg and 0.05 mg/Kg, respectively.

Discussion

Studies on heavy metal content are beneficial to understand the factors effecting the growth, survival, reproduction and development status of great bustard. The correlation coefficient reached 0.9998 in determining the trace heavy metals (Table I) with the atomic fluorescence morphology analyzer, DMA-80 mercury analyzer and inductively-coupled plasma emission spectrometer, which verified the high accuracy of this method. Therefore, the long-term tracking determination of great bustard feces by this method is significant for acquiring the change of heavy-metal content in great bustard's habitat.

Table I.- Emission wavelength and equation parameters of seven heavy metals.

Element	Wave length	Slope	Intercept	Correlation coefficient
Mn	279.5	68.8869	0.0216	0.9999568
Cd	226.5	78.8282	0.0667	0.9998761
Cr	283.5	26.6707	0.2876	0.9999585
Cu	324.7	32.4616	-0.0697	0.9999632
Ni	231.6	35.8338	0.0050	0.9999967
Pb	220.3	3.1926	-0.0499	0.9999348
Zn	206.2	5.7162	0.0373	0.9998187

The excessive content of element Pb in the body may lead to diseases of nervous system, hematopoietic organ and kidney of animal (Li and Ding, 2007; Yousafzai *et al.*, 2010). In this study, high Pb content in great bustard's feces reflects the environmental pollution due to growing number of transportation vehicles, agricultural mechanization and excessive utilization of pesticides and fertilizers in wintering habitat. Due to many farming vehicles and agricultural machinery in this sparsely-populated farming area, long-term accumulation can cause high levels of Pb in soil, resulting in the enrichment of crops with higher concentration of Pb in the bodies of animals (Wu *et al.*, 2013). Elemental Mn is involved in many biochemical reactions as a catalyst or ingredient of enzymes; excessive Mn can cause damage to the nervous, immune, reproductive system of animals (Hu and Shao, 2000). Ni stimulates the hematopoietic function, and it is the ingredient of some enzymes. Deficiency of Ni can cause slow growth and weak fecundity, while excess Ni can lead to degenerative changes in myocardium, brain, liver and kidney (Yousafzai and Shakoori, 2006). Further work on both two heavy metals is needed.

There has been increasing concern about the entry of potentially harmful substances into the food chain destined for endangered bird consumption (van Wyk *et al.*, 2001) because heavy metals can be responsible for a variety of acute and chronic toxic effects in vertebrates (Parmegianni, 1983). The applications of animal feces have been reported in DNA content analysis, animal pathogens and hormonal changes (Tian and Zhang, 2008; Yu *et al.*, 2011). Besides, the feces of animals is an important indicator of their health status and has its significance in animal breeding and protection.

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Morphometrics of Blyth's Horseshoe bat *Rhinolopus lepidus* Blyth, 1844

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Abstract.- A bat roost with 70 individuals of *Rhinolopus lepidus* was found from an underground cellar of an old temple at Rasul Nagar (32º 19.687 N, 073º 46.922 E), Gujranwala district, in the Punjab province. From the roost, 10 (4♂, 6♀) individuals were captured with the help of a hand net. The average head and body length of all the captured specimens was 42.36±1.1 mm, forearm length was 40.34±0.75 mm, greatest skull length (n = 2) was 17.5±0.49 mm while baculum (n = 2) was 4.35±0.38 mm long. The present record is first from the study area.

Key words: Forearm length, bacular length, range extension, Horseshoe bat.

Horseshoe bats are characterized by their muzzle possessing a noseleaf resembling a horseshoe (Bates and Harrison, 1997). Chiropterans are second largest and most diverse group of small mammals after Rodentia but least studied in Pakistan (Javid *et al.*, 2012a,b; Shahbaz *et al.*, 2014). The members of this group are unique in their vagrancy (Turmelle and Olival, 2009) and many species have changed their distribution range during past century (Taber *et al.*, 1967; Javid *et al.*, 2013).

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Family Rhinolophidae is represented by a single genus *Rhinolophus*. The genus comprises more than 77 species (Simmons, 2005) which are distributed throughout the Afrotropical, Australian, Indomalayan, Oceanian and Palaearctic regions (Csorba *et al.,* 2003; Stoffberg *et al.,* 2010). The genus is represented by five species in Pakistan (Roberts, 1997).

The taxonomy of least horseshoe bat, *Rhinolophus pusillus* Temminck, 1834 and Blyth's horseshoe bat, *R. lepidus* Blyth, 1844 is confusing (Csorba *et al*., 2003) and these two taxa have similar external morphology and cranio-dental measurements (Bates and Harrison, 1997). However, some features that help in identification of these species are the sella which has broad rounded base in *Rhinolophus pusillus*. The forearm length in *R. pusilus* is 34.9 mm to 37.8 mm, condylo canine length is 13.0 mm to 14.0 mm and the maxillary tooth row length is 5.4 mm to 6.0 mm while in *R. lepidus* the base of sella is not broadly rounded, forearm length varies from 37.0 mm to 41.8 mm, the greatest skull length is less than 17.0 mm and the maxillary tooth row length ranges from 5.6 mm to 6.8 mm (Srinivasulu *et al*., 2010).

Rhinolophus lepidus has wide range of distribution from Afghanistan to Pakistan, north India, Nepal, Burma, Thailand, southern China, Peninsular Malaysia and Sumatra (Simmons, 2005). In India the species has been reported from Rajasthan (Prakash, 1963; Sinha 1979; Senacha, 2003) and Madhya Pradesh (Wroughton, 1913; Brosset, 1962; Khajuria, 1980). From Pakistan the species was first recorded from Abbottabad in 1985 (Roberts, 1997). The present study was conducted to search for the presence of this species from central Punjab from where the species has never been documented prior to this survey.

Materials and methods

Three main rice producing districts of the Punjab province namely Gujranwala, Hafizabad and Mandi Bhauddin were surveyed from January 2011 to December 2012 to document the bat fauna in the agroecosystems of these areas. Three netting nights in each month and one night in each district were spent throughout the study period for collection of bats. Bats were captured with the help of mist and hand nets. Potential bat roosts in the area, old and undisturbed temples, buildings, ruins, abandoned wells, farm houses, tree groves and forest plantations were explored. Local people were also interviewed for getting maximum information about the bat roosts in the areas. A bat roost was found in an underground room of a temple at Rasul Nagar (32º19.687 N, 073º46.922 E). These bats were identified as *Rhinolophus lepidus* by pointed connecting process, curved forwards, tall bluntly pointed lancet and greyish to orange fur color. Out of the total 70, ten (4♂, 6♀) *R. lepidus* were captured with the help of a hand net on August 3, 2012.

The captured specimens were euthanized, placed in cotton bat bags and each specimen was weighed up to 0.1 g (Pesola balance 10050, Swiss made). These specimens were preserved in a marked plastic jar in absolute alcohol and given unique identification numbers. Details of sex, age, exact locality and district of capture were noted on the jar. The preserved specimens were brought to the laboratory for further study and analysis. The morphometric measurements were taken with the help of digital vernier calipers following Bates and Harrison (1997). For cranial and bacular measurements, the skulls and bacula were prepared and measured following Bates *et al.* (2005) and Javid (2011). Identification was made on the basis of morphological observations and comparisons of morphometric measurements given by Bates and Harrison (1997).

Results and discussion

Sinha (1980) encountered the species hanging from ceilings of dark temples. During present survey the species was observed in an underground room of a temple at Rasul Nagar, Gujranwala. Morphological observations of the specimens showed that the dorsal hair color of the captured specimens was grey-brown, ventrally the fur color was pale and the ears were pointed without tragus but with antitragal lobe.

The horseshoe structure is relatively narrow, 7.0 mm wide and does not cover the entire muzzle, lancet of horseshoe had pointed tip and had concave sides. Sella is small in size, 2.0 mm in length. Our findings are in agreement with the observations of

Table 1.- Body weight (g), external body, cranial and bacular measurements (mm) of *Rhinolophus lepidus* (mean and range when at least two specimens were recorded) captured from Rasul Nagar, Gujranwala.

External body measurements	Present study (n = 10)	Bates and Harrison (1997)	Cranial measurement	n = 2	Bates and Harrison (1997)
Body mass	6.42 (5.0-8.20)	-	Breadth of braincase	7.5 (7.3-7.7)	7.1 (6.5-7.8)
Head and body length	42.36 (41-44.6)	42.9 (35-54)	Zygomatic breadth	8.5 (83-8.7)	8.2 (7.6-8.8)
Ear length	13.49 (11-16)	16.9 (14.5-20.6)	Postorbital constriction	2.3 (2.2-2.4)	2.2 (1.8-2.6)
Forearm length	40.34 (39-42)	39.8 (37-41.8)	Condylo-canine length	14.2 (14-14.4)	14.6 (13.8-15.5)
3rd metacarpal length	31.28 (30-32.4)	30.4 (28.2-33.3)	Condylo-basal length	14.8 (14.6-15)	-
3rdmetacarpal: 1st phalanx	12.35 (11.7-13)	11.8 (10.0-13.3)	Greatest length of skull	17.5 (16.8-17.5)	17.2 (16.2-18.4)
3rd metacarpal: 2nd phalanx	19 (17-23)	17.3 (16-18.9)	Maxillary toothrow	6.75 (6.5-7)	6.1 (5.6-6.8)
4th metacarpal length	32.0 (30.8-33)	31.4 (29.6-33.8)	Anterior palatal width	4.3 (4.2-4.4)	4.0 (3.7-4.2)
4th metacarpal: 1st phalanx	9.68 (9-11)	8.7 (7.6-10.5)	Posterior palatal width	6.3 (6.2-6.4)	5.9 (5.7-6.3)
4th metacarpal: 2nd phalanx	11.55 (9-13)	10.8 (9.6-12.3)	Mandibular toothrow	5.6 (5.5-5.8)	6.6 (6-7.4)
5th metacarpal length	31.48 (30.4-32.7)	31.1 (29.4-33.4)	Mandible length	11.2 (11-11.4)	11 (10.0-12.1)
5th metacarpal: 1st phalanx	10.61 (9.8-11.5)	-	**Bacular measurements**	**n = 2**	
Wingspan	240.10 (220.9-250.2)	244 (232-256)	Total length of baculum	4.35 (4.06-4.61)	-
Tibia length	17.19 (16.5-18.1)	16.7 (14.9-18.4)	Length of shaft	3.85 (3.69-4.02)	-
Hind foot length	8.78 (8-10.5)	7.6 (5.5-10)	Length of proximal branch	0.53 (0.46-0.61)	-
Tail length	22.79 (21-26)	20.4 (14-28)	Height of baculum	1.30 (1.23-1.38)	-
Penis length	6.57 (6-7)	-	-	-	-

Roberts (1997) and Bates and Harrison (1997). The external and the craniodental measurements are as given in Table I.

Generally the baculum is short and lies in the glans but in some species like *R. lepidus* it is very long, above 75% of the penis length (Sinha, 1976). Average total length of baculum of two specimens captured during present study was 4.35 mm, while the average length of the penis (n = 4) was 6.57 mm.

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