Short Communications

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Protein Fractionation analysis of Fowl Adenovirus Serotype 4 (FAV-4) Associated with Fowl Hydropericardium-Hepatitis Syndrome in Pakistan

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Abstract.- The primary objective of this study was to reveal the structural polypeptides of local fowl adenovirus serotype 4 (FAV-4) associated with hydropericardium-hepatitis syndrome (HHS) using 12.5% resolving gel. For the isolation of virus, livers samples were aseptically collected from four different field outbreaks in commercial broilers from Faisalabad. A 15-45% (w/v) sucrose gradient was used for purification of virus samples. Virus was confirmed by agar gel precipitation test. The protein fractions of local isolates were revealed by using 12.5% gel with discontinuous buffer system for sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). Twelve structural polypeptides having molecular weights from 13.2 kDa to 110.0 kDa were observed.

Key words: Fowl adenovirus 1 serotype 4, hydropericardium-hepatitus syndrome, SDS-PAGE.

Pakistan poultry industry during the past two decades showed outstanding development from backyard activity to a well established scientific and vivacious industry. The industry with a growth rate of 8-10% annually contributing 19% of the total national meat production and facilitating 1.5 million people for their livelihood. The current worth of Pakistan poultry industry is Rs. 200.00 billion (Anonymous, 2009). However, through this vigorous growth, Pakistan poultry industry has constantly been confronted with challenges in the form of different infectious diseases. Among these infectious diseases, hydropericardium-hepatitis syndrome (HHS) that has been caused by fowl adenovirus serotype 4 (FAV-4) is a serious threat to broiler industry of Pakistan (Khan *et al.*, 2005; Mansoor *et al.*, 2009). The most common clinical signs are accumulation of fluid (watery/jelly) in pericardial sac, nephritis, enlarged friable liver with large, round, basophilic intranuclear inclusion bodies (Ahmad *et al.*, 1989; Cheema *et al.*, 1989; Gowda and Satyanarayana, 1994).

Information about the structural polypeptides of HPS virus using 10% resolving gel has been delineated (Haq *et al.*, 1997), but structural protein featuring slight variation in molecular weights can not be separated using 10% resolving gel (Kumar and Chandra, 2004). This study was carried out to characterize FAV-4 from field outbreaks using Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) with 12.5% resolving gel to demonstrate the structural polypeptides of FAV-4. This will further help in development of proper diagnostic approach for confirmation of FAV-4.

Materials and methods

Broilers livers suspected to be naturally infected with HHS virus were aseptically collected from four field outbreaks in and around Faisalabad (Fig. 1). These samples were properly labeled, placed in sterile polythene bags, and stored at -70°C till further processing.

Purification of the virus from infected tissues was carried out by the procedure described by Mansoor *et al.* (2009).

Agar gel precipitation test (AGPT)

For the preparation of known hyperimmune sera, five, two-week old broiler birds were used (Kumar *et al.*, 1997). Three broiler birds were inoculated with 0.5 ml of HPS vaccine (Bioangara

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plus, Sana lab) using s/c route. Same vaccines was injected with a dose of 0.75, 1.0 and 1.5 ml weekly. Two birds were inoculated with PBS as control. The blood samples were collected 10 days post boosting. Sera were collected, inactivated by moist heat and stored at –20°C until used. Virus confirmation was done by agar gel precipitation (AGP) test (Kumar *et al*., 1997).

The total protein of purified HPS virus samples was determined by Biuret method (Gornall *et al*., 1949).

SDS-PAGE

Discontinuous buffer system with 12.5% gel concentration was used for the breakup of HPS virus proteins (Laemmli, 1970). Ready to use broad range protein markers (Sigma, USA) were dissolved in distilled water and loaded at 10µl/well. Electrophoresis was carried out at room temperature using 175 volt current for 1.5 hours. Gel was stained with coomassie brilliant blue for overnight. Then destained for 3 h in destaining solution, dried in gel dryer under vacuum (Rapidry, Japan) and photographed with a digital camera.

Results and discussion

Locally isolated FAV-4 virus samples were initially purified by filtration and then using sucrose gradient centrifugation. A clear viral band at the interface of 25% and 35% sucrose gradients was observed. The purified virus samples were confirmed by AGP test with only one sharp band of single identity with known hyperimmune serum.

The purified virus samples gave OD values of 0.309, 0.285, 0.297 and 0.311 at 540 nm. The total proteins (mg/ml) of four purified viral samples designated as 1, 2, 3 and 4 were 1.106, 1.020, 1.068 and 1.113, respectively.

Figure 2 shows polypeptides of four FAV-4 isolates resolved by SDS-PAGE in 12.5% acrylamide gel using discontinuous buffer system. The molecular weights of the separated proteins were 110, 85, 79.5, 70.4, 66, 50, 45.6, 41.8, 32.8, 20, 15.5 and 13.2 kDa

Liver samples collected from the clinically affected birds, were processed for the isolation and purification of FAV-4 through 15-45 % sucrose density gradient (Haq *et al*., 1997) and the presence

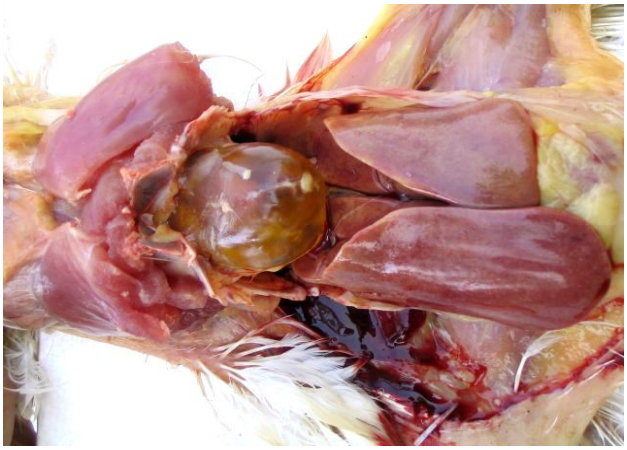

Fig. 1. A representative field case of HPS affected bird showing hydropericardium and hepatitis.

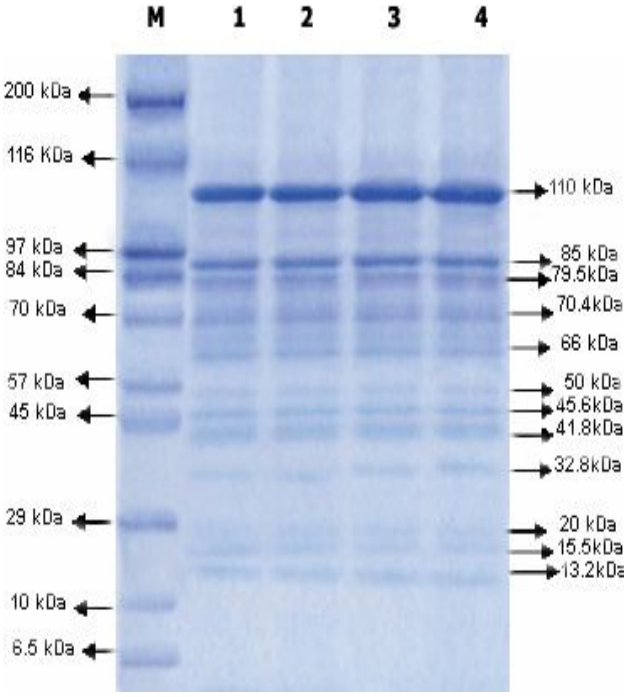

Fig. 2. SDS-PAGE of four purified local isolates of FAV-4 in 12.5% resolving gel, Lane 1, 2, 3 and 4: purified local isolates of FAV-4. Lane M: broad range molecular weight marker (Sigma).

of FAV-4 was initially confirmed by AGP test with one precipitation band of single identity. The sucrose gradient used in the present report was found comparable with another study (Ganesh *et al*.,

2001) which used 10-55% sucrose gradient for the purification of the FAV-4. The virus purification procedure which we adapted was also in agreement with egg drop syndrome virus purification, a fowl adenovirus, from clinical samples. In the present study, twelve structural polypeptides in FAV-4 ranging from 110.0 to 13.2 kDa were revealed using 12.5 % resolving gel. Previously it was reported that SDS-PAGE analysis of HPSV revealed 8 structural proteins using 10% resolving gel having molecular weights of 119.0 to 15.7 kDa (Haq *et al.*, 1997; Ganesh *et al*., 2001).

Eleven structural polypeptides, ranging from 100 kDa to 9 kDa in FAV serotype 1 have been demonstrated (Maiti and Sarkar, 1997). In FAV serotype 2, 11 structural polypeptides ranging from 97 kDA to 14 kDa have been reported (Nazerian *et al.*, 1991). However, Li *et al.* (1983) workers confirmed 14 structural proteins in chicken embryo lethal orphan (CELO) virus that is also a type 1 fowl adenovirus. The result of our investigation is in agreement with a previous report of Kumar and Chandra (2004) which described 12 polypeptides having molecular weight ranging from 110.0 to 13.8 kDa in 12.5% resolving gel.

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Acute Toxic Effect of Pesticides on Brine Shrimp and Opossum Shrimp

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Abstract.- The aquatic ecosystem as a greater part of the natural environment is faced with the threat of pesticide pollution. Pesticides enter the food chain and tend to accumulate in organisms at higher trophic level. The present investigation was undertaken to study the acute toxicity (LC_{50}) of organophosphate pesticide (methyl parathion) and pyrethroid pesticide (fenvalerate) on *Artemia* (brine shrimp) and mysids (opossum shrimp). The value of LC_{50} when exposed to methyl parathion was 0.00011 ppm for mysids and 0.11 ppm for *Artemia*. Similarly the LC_{50} of fenvalerate was found to be 0.0004 ppm for mysids and 0.18 ppm for *Artemia.* Pesticides appear to be highly lethal at very low concentration. High sensitivity is alarming as it may have implications on natural resources. Therefore, assessment of toxicity of other groups of pesticides on various marine organisms is required.

Key words: *Artemia*, mysids, pesticides, methyl parathion, fenvalerate

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Pesticides enter the environment by direct and indirect routes. Pesticides can be sprayed on crops, dispersed in the field as a granular substance, or dispersed from the aircraft. It is estimated that less than 0.1% of the pesticides applied to crops actually reaches the target pests. Most of the pesticide applied enters the environment, contaminating the soil, water, and air (Pimentel and Levitan, 1986). The pesticides have been found to be extremely toxic to several aquatic organisms (Mayer and Ellersieck, 1986; Ringwood, 1993). Carcinogenic and genotoxic pesticides are the most problematic as they effect on the genes of the organisms that may be transferred to next generation of the organisms (Giri *et al*., 2002). Pesticides are responsible for multiple effects on marine organisms, including human beings, affecting organ function, reproductive status, species survival, population size and ultimately biodiversity (Reichrtova, 1999; Porta and MacReady, 2000; Dutta and Arends, 2003). Acute toxicity bioassays are a convenient tool used extensively to assess the toxicity of physiologically active substances and also to evaluate the potential of chemical contamination on commercially and ecologically important species (Ahsanullah and Arnott, 1978). LC_{50} values for a number of pesticides in marine organism examples molluscs, crustaceans and fish have been reported (Linden *et al*., 1979; Persoone *et al.,* 1985; Babu *et al*., 1987; Clark *et al.,* 1989; Serrano *et al.,* 1995; Rico *et al*., 2010). *Artemia* and mysids form important links in the food webs of aquatic ecosystems (Mauchline, 1980; Sorgeloos, 1980; Mees and Jones, 1998). *Artemia* and Mysids are used frequently in laboratory toxicity tests (Vanhaecke and Persoone, 1984; Persoone and Wells, 1987; ASTM, 1990; Barahona and Sánchez-Fortún, 1996; Verslycke *et al*., 2004).

In the present study an attempt has been made to investigate the acute toxicity of organophosphate and pyrethroid pesticides on marine organisms. *Artemia* and mysids, were used as test animals. *Artemia* and mysids play a major role as they form a primary part of food chain in the marine food web. The results originated from the present study will be useful for agencies working on pollution management in the coastal area.

Materials and methods

Preparation of chemicals

Pesticides, methyl parathion 5% EC and fenvalerate 20% EC, were procured from Pakistan Agricultural Research Center. Stock solution of 100 ppm and appropriate working concentrations were prepared in filtered seawater.

Artemia

Artemia salina cysts were purchased from local market. *Artemia salina* cysts were hydrated in aquarium (19.5 cm length x 19.5 cm width x 20.3 cm height) containing six litre of aerated filtered sterile seawater at salinity 30 ppt. *Artemia salina* were exposed to a constant environmental temperature of 25±1°C and placed under a continuous illumination (60-watt florescent lamp). *Artemia* naupli were fed wheat flour. The naupli were not fed during the experiment. Naupli aged 48h-72h old were used for bioassay. Seawater was renewed daily to maintain water quality in the aquaria.

Mysids

The mysids (*Indomysis annandalei*) were collected at low tide from Sandspit backwaters (mangrove area) by handnet (2mm mesh). Mysids were transported in clean aerated seawater from the Sandspit to the laboratory ensuring minimum stress. Mysids were acclimatized in glass aquaria (90 cm length x 30 cm width x 32 cm height) containing clean aerated seawater, for forty-eight hours at room temperature 23±1°C in the laboratory. Mysids aged 48-72h old were used for bioassay. Mysids were fed adlibitum to avoid cannibalism. The animals were not fed during the experiments. Seawater was replenished everyday to maintain water quality in the aquaria.

Bioassay

Static bioassay system (Doudoroff *et al*., 1951) to evaluate LC_{50} was carried out in glass vials (5.5 cm length x 2.5 cm width) of 22 ml capacity, for *Artemia* and mysids. Initially all test organisms were treated with wide range of pesticide concentration in filtered seawater to evaluate the concentration at which mortality around 50% occurs. The experiment was repeated with five

concentrations of organophosphate and pyrethroid pesticides for test organisms. The five different concentration of pesticide for marine organisms ranged between 0.05-1 ppm for *Artemia* and 0.0001-0.0008 ppm for mysids. Concentrations were prepared with filtered seawater. The tests and controls for each experiment were in triplicate and the controls had only seawater. The other experimental conditions, such as, temperature 23°C-28°C, Salinity 30 ppt, pH 7.57, photoperiod 16 h light and 8 h dark were maintained throughout the experiment. Acute toxicity measured as mortality of organisms exposed to two pesticides, acting individually was estimated by determination of the 24 h LC_{50} (the concentration of the pesticides which kills 50% of the test animals after 24 h exposure). The LC_{50} values were determined by using Computer programme Biostat 2009 based on Finney programme 1952 (Probit analysis).

Result

The results obtained in the present experiments, depict that marine organisms are sensitive to both pesticides tested. The rate of mortality (%) is directly proportional to the concentration of pesticides (Figs. 1, 2). The variability in the degree of sensitivity is reflected by the lethal concentration values of pesticides. The value of LC_{50} when exposed to methyl parathion is recorded as 0.00011 ppm for mysids and 0.11 ppm for *Artemia.* Similarly the value of LC_{50} when exposed to fenvalerate is found to be 0.0004 ppm for mysids and 0.18 ppm for *Artemia.* LC_{50} value of a given organophosphate and pyrethroid pesticides varied significantly for these two organisms. Mysids appeared to be most sensitive to organophosphate and pyrethroid pesticides tested, where as *Artemia* showed resistant to these two pesticides.

Discussion

Result of the present study suggests that *Artemia and* mysids appear to be highly sensitive to organophosphate and pyrethroid pesticides and have low LC_{50} values. Our result is in agreement with Roast *et al.* (1999), Gartenstein *et al*. (2006) and Rao *et al.* (2007). The results obtained in the present study also suggest that organophosphate and pyrethroid pesticides toxicity differ from one organism to another. Interspecific variations may also be attributed to the chemical nature of pesticides. Number and types of esters present in organophosphates and synthetic pyrethroid and their stereochemistry regulates pesticide potency, spectrum of activity, and toxicology (Glickman and Casida, 1982; Gray and Soderlund, 1985; Bradbury and Coats, 1989; Reddy and Rao, 1992). For any species, sensitivity to a given pesticide varies with age, sex, nutritional background, health, stress and the microenvironment (Sanchez-Furton *et al*., 1995).

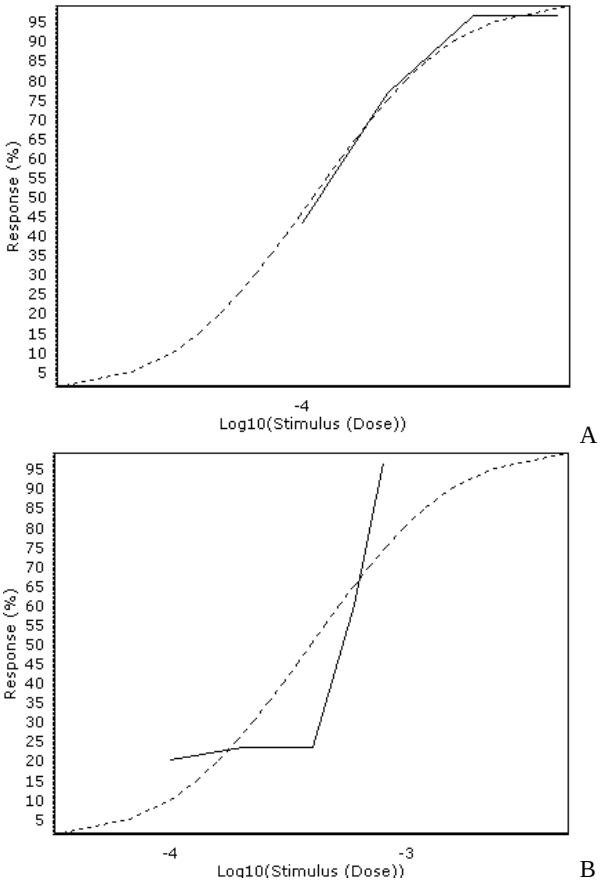

Fig. 1. Percent mortality of Mysids exposed to different concentration of pesticides. A, methyl parathion LC_{50} is 0.00011; B, fenvalerate LC_{50} is 0.00039.

In the present study *Artemia salina* appeared to be resistant compared to mysid, and have comparatively higher LC_{50} values for pesticides. This effect has also been observed earlier by Eisler

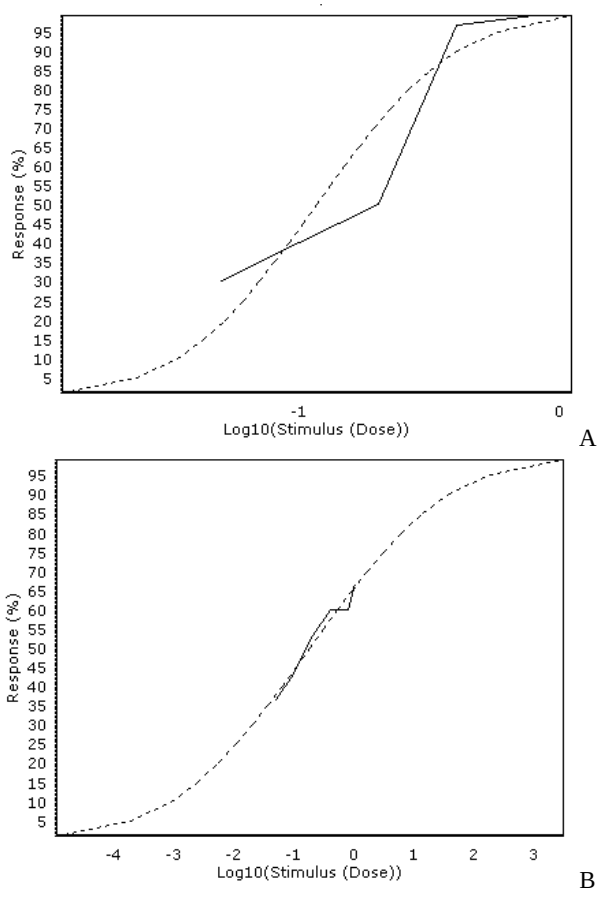

Fig. 2. Percent mortality of *Artemia* exposed to different concentration of pesticides. A, methyl parathion LC_{50} is 0.11 for *Artemia*; B, fenvalerate LC_{50} is 0.18

(1969) who reported that *Artemia* is 7-27 times more resistant to p, p-DDT compared to some marine decapod crustaceans. The observation of the present study that mysids are most sensitive to organophosphate and synthetic pyrethroid pesticides than other crustaceans is consistent with the results obtain earlier for many marine invertebrates (Clark *et al.*, 1989; Morton *et al.*, 1997; Roast, 1998; Hunt *et al.*, 2002). Clark *et al.* (1989) reported toxicities of permethrin, cypermethrin and flucythrinate to marine invertebrates and fish and found that crustaceans are more sensitive than fish, with LC_{50} of less than 0.02 ppb for permethrin and of less than 0.01 ppb for fenvalerate, cypermethrin and flucythrinate.

The Pakistan Environmental Protection Agency (PEPA) established the national industrial discharge standards in 1993. According to PEPA, the National Environmental Quality Standards (NEQS) relating to municipal and liquid industrial effluents for pesticide is 0.15 ppm (The Gazette of Pakistan, 1993). However, in our result the value of LC_{50} when exposed to methyl parathion and fenvalerate is recorded as 0.00011 and 0.0004 ppm for mysids, which is quite low. *Artemia*, on the other hand, showed resistance to these two pesticides. The present ecotoxicity data shows that very low level of pesticide in waterways will drastically affect the marine organisms and consequently on marine food web. As *Artemia* and mysids form a primary part of food chain in the marine food web. Long-term exposure of organisms to pesticides may have high risk of health hazard for the general public by consuming toxic fishes. The data obtained in the present study will be useful in determination of toxic limits (LC50) and evaluation of national discharge standards for pesticides established by PEPA.

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Analysis of Urea in the Blood Serum of Domestic Goats (*Capra hircus*) in Gilgit-Baltistan

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Abstract.- Urea is the end product of amino acid catabolism in mammals, which provides a non-toxic means for the removal of ammonia to maintain homeostasis. The urea present in the blood serum of ruminants appears to be actively transported across the rumen wall into the lumen and used as a nitrogen source. The ruminants are able to use the urea as a source for food protein. A blood urea analysis is extensively used as indicator of protein

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nutritional status in goats. The present study was proposed and conducted on goats (both male and female) age of two years (based on teeth composition) in four Districts of Gilgit-Baltistan *viz;* Ghizer, Baltistan (Skardu, Ghanche) and Hunza-Nagar. A total of four hundred and twenty two samples were collected from these districts and 167 male and 255 female goats were randomly selected for analysis of serum urea level from the three regions and serum was obtained by centrifugation. The quantity of urea in mM/liter of serum was determined by analyzing the blood serum of the goats in Micro-lab 300. The difference in urea contents of blood serum among areas is not significant in either males or females.

Key Words: Goat, blood urea, blood urea nitrogen, Gilgit-Baltistan.

The primary excretory form of nitrogen in mammals is urea, the end product of amino acid catabolism. The concentration of blood urea nitrogen (BUN) is related to the concentration of milk urea nitrogen (MUN), because the concentration of milk urea equilibrates rapidly throughout body fluids (Rook and Thomas, 1985). Urea provides a non toxic means for excretion of ammonia generated by amino acid catabolism and the intestinal micro flora. The production of urea occurs almost exclusively in the liver, and liver failure is frequently associated with a decrease in urea (Carloson, 2002). The urea present in the blood of ruminants appears to be actively transported across the rumen wall into the lumen and used as a nitrogen source (Schmidt-Nilson, 1958). Ruminants are able to utilize urea as a source for food protein (Blood and Henderson, 1963). Blood urea analyses are extensively used as an indicator of protein nutritional status in goats Gupta and Panda (1963). Urea is the principal non-protein nitrogen compound used by the ruminants and it is also used commercially in the feeds of ruminants, because it is economical, odorless, and high in nitrogen and plays major biological roles (Belaasco, 1954). Renal excretion of urea is rapid, chiefly by glomerular filtration and renal tubular secretion (Sollman, 1957). The recycling of urea in ruminants allows them to conserve nitrogen when their dietary intake is low, for sheep and goats in semi- arid and arid environments. Also, urea recycling enables ruminants to maintain their protein requirements when forage quantity is low (Schemidt-Nilson *et al.*, 1957). Urea recycling has been recognized as the major mechanism to improve the quality of protein supply (Houpt, 1959). Examining blood for its constituents is used to monitor and evaluate the health and nutritional status of animals (Gupta *et al.*, 2007).

The present study was aimed at evaluating the level of blood urea of goats feeding on pure organic food in nomadic conditions in Gilgit-Baltistan, as an indicator of protein digestion.

Materials and methods

The study was conducted in three major regions of Gilgit-Baltistan, namely Ghizer, Hunza-Nagar and Baltistan (Skardu and Ghanche), situated in the extreme north of Pakistan. The area has varying and harsh climatical conditions where the temperature ranges from -20°C in winter to 45°C in summer. The area remains out of the summer monsoon and annual rainfall recorded is not more than 200 mm. The important goat breeds found in Gilgit-Baltistan are the Kohi Ghizer, Jara Khail, Pamiri and Baltistani breeds. Goats of two years of age, both male and female at the two teeth stage, which were kept in organic feeding condition, were selected randomly. Twenty-five specimens were selected from each village randomly from different flocks. Blood samples were collected from four hundred and twenty two goats. The serum was extracted by using centrifugation at 4000 rpm for 5 minutes and stored at -20°C. The harvested serum was analyzed chemically using Micro lab-300.

Information regarding feed and fodder of goats in different regions was collected through questionnaires.

Results and discussion

Table I shows serum urea level in male and female goats of different regions of Gilgit-Baltistan.

The urea level in blood serum of goats of different regions of Gilgit-Baltistan, show a non-significant difference. The average urea level in male goats of Ghizer, Baltistan and Hunza-Nagir were 10.28±0.04 mM/L, 11.73±0.09 mM/L and

Table I.- Blood serum urea (mM/L) in male and female goats of different regions of Gilgit-Baltistan.

Regions	Male goats		Female goats		Total goats	
	No. of Observations	Serum urea (Mean±SEM)	No. of Observations	Serum urea (Mean±SEM)	No. of Observations	Serum urea (Mean±SEM)
Ghizer	84	10.28±0.04	138	11.25±0.44	222	10.88±0.03
Baltistan	39	11.73±0.09	56	11.43±0.76	100	10.64±0.56
Hunza-Nagar	44	9.34±0.08	44	11.08±0.80	100	11.36±0.61
F value		0.96		0.09		.477
P value		0.16		0.156		.621

9.34±0.08 mM/L, respectively. The results indicate lower urea in blood serum in Hunza-Nagar than in goats from Ghizer. However, the goats in Baltistan region showed a high level of urea in their main pool which indicates that either the goats take much proteineous food or their metabolism rate of amino acids is low.

The mean values of blood serum urea of female goats are 11.43±0.76 mM/L in Ghezir, 11.08±0.44 mM/L in Baltistan and 11.08±0.80 mM/L in Hunza-Nagar. In female goats slightly higher urea level was observed than compared with that of Ghezir and Huza-Nagar. Differences in urea level among female goats of the three regions were non-significant (Table I).

Similar type of study was carried out by Castro *et al.* (1977) on pygmy goats who showed higher urea concentration (17.80±3.13 mM/L) in their sera. According to Gray *et al.* (1988) the mean value of urea is 5.34±2.29 mM/L and 7.40 mM/L in Landrace Danish goat and cross-bred goat. Landrace Danish goats show less urea in their blood. Gray *et al.* (1988), showed urea concentration of 12.37 mM/l in the Black Bengal goats, Kaneko *et al.* (1989) reported 7.64-22.02 mM/L urea. According to Kaneko *et al.* (1989) in normal goats the value of urea is 3.57-7.14 mM/L. All these results deviate from the results obtain during the present study.

Carloson (2002) reported that the mean urea concentration in the blood of goats is 15.08±3.6 mM/L (6.07-23.92 mM/L). In these data, there is no significant difference between genders.Our results also fall within the same range as has been reported by Carloson (2002), although with an exception that there occurs a high concentration of urea in the serum of male goats.

On the bases of the present study it can safely be said that serum urea level has been observed higher in female goats than in male goats but the male goats of Baltistan region have higher concentration of urea in serum than the goats of the other regions. Statistical analysis shows non-significant differences between the genders for urea level. However, the inter-region variation in serum urea level is not significant. Although the main food source (*Artemisia* spp.) at pastures at which males and females feed is the same, the serum urea level indicates that females remain healthier than males. This may partly be due to females eating more, with a variety of other food sources available differently in each region of Gilgit-Baltistan.

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Feeding Habits of Common Quail (*Coturnix coturnix*) Migrating Through Rawalpindi, Pakistan

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Abstract.- This study was conducted to investigate the feeding habits of common quail (*Coturnix coturnix*) in rural areas of Rawalpindi, Pakistan during September 2008 to May 2009. A total of 28 birds were captured (12 males and 16 females), gizzards were removed for feeding analysis. Out of 28 samples, two gizzards were empty and the remaining 26 samples contained 84.30% plant seeds *viz*., millet (*Pennisetum americanum*; 17.9%), sorghum (*Sorghum bicolor*; 17.2%), ground nut leaves (*Arachis hypogaea*; 10%), pohli (*Carthamus oxyacantha*; 9.8%), swank (*Echinochloa crusgalli;* 8.7%), baru (*Sorghum halepense*; 6.6%), and sesame (*Sesamum indicum*; 1%). The debris and stone contents in gizzards were 10.6 and 5.20%, respectively. The results of this study indicated that plant contents represented the major food items of the common quail in Rawalpindi, Pakistan.

Key words: Common quail, feeding habits, food items.

The common or grey quail (*Coturnix coturnix*), a small game bird belonging to family Phasianidae, is widely distributed across Europe, Asia and Africa. Its population migrates seasonally and sporadically breeds in Punjab, Sindh and Balochistan provinces of Pakistan (Roberts, 1991). Due to its extremely wide range, the species enjoys a conservation status of 'Least Concern' (IUCN, 2011). However, overall degradation of wild habitats due to intensive agriculture, urbanization, pollution and over-hunting pose potential threats to this game bird. The wild quails are also sold at lucrative prices for consumption as food served at home and in restaurants. It is generally perceived that selection of breeding habitats by migratory bird species are determined by food availability at its stopovers and destinations during migration (Roberts, 1991; Cramp and Simmons, 1980; Thomaides *et al*., 2001). Studies to determine diet composition of a bird species are considered very important for devising conservation strategies to maintain its population in a particular habitat/area (Thomaides *et al*., 2001).

Rawalpindi district of Potohar area represents an ecology comprising major agricultural landscapes, which are one of the favorite habitats falling in the range of this species. The only information from Pakistan documented by Roberts (1991) reveals that food of common quail predominantly consists of seeds including fallen cereal grains in stubbles, as well as seeds of grasses and chicory. However, a report from Rajasthan area of India has shown that 90% of its food comprises of weed seeds (grasses and legumes), 18% cultivated grains (predominantly millet) supplemented with 8% insects and arachnids

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(Mukherjee, 1963). Due to lack of sufficient information on diet composition of the common quail visiting agro-ecosystem of Potohar area, present study was carried out to investigate this important trait of its life history with an aim to identify the food items which play major role in population distribution of this game bird.

Materials and methods

The study was conducted in agro-ecosystem of Rawalpindi district, Pakistan situated between 33°36′0″N and 73°02′0″E with an average rainfall ranged between 1384.2 mm in 2008 to 401 mm in 2009 (GOP, 2011), while the temperatures ranged from 16.9-46°C during summer and -02-24.7°C during the winter months.

In total, 28 common quails were captured during September 2008 to May 2009. The birds were flushed from agricultural fields by trained dogs and captured by setting nets with the help of local hunters. Immediately after capturing the birds were scarified for collection of their gizzard contents which were preserved in formalin (10%) and transported to the Laboratory, Department of Wildlife Management, Pir Mehr Ali Shah Arid Agriculture University Rawalpindi for further analysis. Individual sample was assigned a specific number; habitat and date of collection. Simultaneously, all relevant plant species, possibly contributing to the diet of the bird were collected as reference material to identify the gizzard contents.

The food contents recovered from each gizzard were placed in sieve to separate the food items and further observation was taken with 10 x hand lens. Identification of separated food items was carried out by using Stereo-microscope. Plant species were identified according to reference specimens collected from the field and with the available literature and converted to percentages of the total contents.

The weight of each constituent of food was taken and the proportions were made for each constituent based on percentage weight of each crop content. The frequency of occurrence was calculated that how many times each constituent of food appeared in the crop (Robert, 1964; Hansen *et al.*, 1971).

$$\text{Percentage of occurrence} = \frac{\text{Total number of each food item}}{\text{Total number of guts analyzed}} \times 100$$

Results and discussion

The data on the food items consumed by common quail captured during September 2008 to May 2009 from different areas of agro-ecosystem of Rawalpindi district, Pakistan is given in Table 1. Out of 28 samples, strangely two gizzards were found empty which seems uncommon and 26 samples (84.30%) contained food contents. The analysis of 26 gizzards revealed occurrence of millet seeds (*Pennisetum americanum*; 17.9%), sorghum seeds (*Sorghum bicolor*; 17.2%), Ground nut leaves (*Arachis hypogaea*; 10%), pohli seeds (*Carthamus oxyacantha*; 9.8%), swank (8.7%), baru seeds (6.6%) and sesame seeds (*Sesamum indicum*; 1%). The debris and stone contents in gizzards were 10.6% and 5.20% respectively. Seeds of millet, sorghum, swank, baru and seasame were found during the months of September to November as this is the growing season of these plant species. However, stones/grit were found during all months of the study period.

In our study, plant seeds were the major food items of common quail in the agricultural landscape of Rawalpindi, Pakistan. This might be due to lower insect populations and high availability of plant material during the study period as food resources (Wilson *et al*., 1999). It is pertinent to mention that food of common quail consists largely of seeds including fallen cereal grains in stubbles, as well as grass seeds and chicory (Roberts, 1991). The findings of our study are inline with the previous report on the feeding habit of common quail in Rajasthan, India in which plant material made up 90% of the total food items and the rest were either stones or insect body parts. In similar studies, the food items of European quail (*Coturnix coturnix coturnix)* (Penev, 1983; Michailov, 1995; Combreau *et al*., 2001; Tsachalidis *et al*., 2007) and Bobwhite quail (*Colinus virginianus*) (Jennings, 1941) were also predominantly plant material. A remarkable amount of debris (hairs, cotton fibers) and stones were recorded in this study.

Table I.- Food items consumed (g) by common quail (*Coturnix coturnix*) from September 2008 to May 2009 in Rawalpindi, Pakistan

Food Items		Months						Total (n=28)	% weight	Frequency of occurrence
Common name	Scientific Name	Sept. (n=6)	Oct (n=3)	Nov (n=3)	Mar (n=4)	April (n=5)	May (n=7)			
Millet (seeds)	*Pennisetum americanum*	5.6	6.7	0.0	0.0	0.1	0.0	12.4	17.90	18.0
Sorghum (seeds)	*Sorghum bicolor*	6.5	6.0	8.6	0.0	0.0	0.0	21.1	17.20	19.0
Ground nut (leaves)	*Arachis hypogaea*	0.6	0.5	2.2	3.0	2.0	4.0	12.3	10.00	19.0
Pohli (seeds)	*Carthamus oxyaacantha*	0.0	0.0	0.0	3.0	5.1	4.0	12.1	9.84	14.0
Swank (seeds)	*Echinochloa crusgalli*	1.8	6.0	2.9	0.0	0.0	0.0	10.7	8.70	11.0
Baru (seeds)	*Sorghum halepense*	1.9	3.6	2.6	0.0	0.0	0.0	8.1	6.59	13.0
Sesame (seeds)	*Sesamum indicum*	0.2	0.5	0.5	0.0	0.0	0.0	1.2	0.97	7.0
Debris	---------	3.0	1.0	1.5	2.2	3.3	0.0	11.0	10.60	19.0
Stones	---------	1.4	0.5	1.8	0.5	1.2	1.0	6.4	5.20	12.0

It is concluded that plant seeds represent the main food items of the common quail in Rawalpindi, Pakistan. However, no previous report is available on the feeding habits of common quail in this region. The present study provides baseline information regarding food habits of this bird for future studies.

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Prevalence of Amphistome Cercariae in Freshwater Snails of Punjab

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Abstract.- Seven species of snails *Indoplanorbis, Bulinus, Physa, Lymnaea, Gyraulus, Bellamaya* and *Oncomelonia* belonging to class *Pulmonata* and *Prosobranchia* were collected from water bodies of four districts *i.e.* Gujranwala, Sheikhupura, Lahore and Kasur of the Punjab province, Pakistan. Of 10341 snails, 11.67% (*Indoplanorbis, Bulinus* and *Physa*) were found to be shedding amphistome cercariae. Season wise prevalence indicated that it was highest during summer and lowest during winter. The most favourable temperature was 30±31°C, maximum day light for 14 h and pH ranging from 7.44 to 7.51.

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Key words: Fresh water snails, pulmonates, Prosobranchs, amphistome cercariae.

Snails have been known to play an important role as intermediate hosts for helminth parasitic diseases of animals like paramphistomosis, fasciolosis and schistosomiasis. Paramphistomosis is one of the most pathogenic disease in domesticated animals causing heavy economic losses to livestock industry. Geoclimatic conditions of Punjab and utilization of rivers as a source of irrigation water play a role in dissemination of these snails. Some studies have been done on different fresh water snails of Pakistan (Akhtar and Khan, 1989; Akhtar, 1990; Maqbool *et al*., 2003) but no work has been done on the seasonal incidence of various species of snails. The present study describes the seasonal distribution of various species of snails and the factors affecting the population of freshwater snails.

Materials and methods

Snails and aquatic plants were collected throughout the year from June 2003 - May 2004. The collection was from water bodies of four different areas in four districts of Punjab province namely Gujranwala, Sheikhupura, Lahore, and Kasur. Each area was subjected to study once per month.

Temperature and hydrogen ions concentration (pH) of water bodies were estimated (under field conditions) during the period of the study. The samples were collected by using a scoop net

The snails were washed thoroughly in running or still water and cleaned from mud, debris and ciliates. They were classified according to shell morphology as described by Gohar and El –Gindy (1960) and Malek (1984). Infection was seen in snails as per technique described by Malek (1984).

Results and discussion

A total of 7 different genera of snails *i.e. Indoplanorbis, Bulinus, Physa, Lymnaea, Gyraulus, Bellamaya* and *Oncomelonia* from different districts of Punjab province were collected. Of these, *Indoplanorbis, Bulinus* and *Physa* are related to our study.

In districts, it was found that the incidence snails was the highest at Gujranwala followed by Sheikhupura, Lahore whereas the lowest at Kasur (Table I). Generally, the seasonal incidence was the highest in summer and autmun (Table II).

It was generally observed that the population dynamics of different snails were variable in various districts. Regarding the seasonal incidence of the collected snails, the peak incidence of Indoplanorbid snails were found predominated in autumn and sharply decreased during spring and began to increase in summer and again decrease in winter. This observation is in agreement with that of Kendall (1950), Castro-Trejo *et al*. (1990), Mage *et al*. (2002), Pfukeni *et al*. (2005a,b), Keyyu *et al*. (2005), Dreyfuss *et al*. (2007), Sri-aroon *et al*. (2007); and Liang *et al.* (2010). *Bulinus* reached maximum number during summer and spring and the lowest in winter. This species of snail has the ability to survive on the effect of fluctuating temperature in different seasons. In the present study the highest incidence of *Physida* snails was found in summer and autumn which gradually decreased in winter. These results are in accordance with the results of Chingwena *et al*. (2002), Curtis *et al*. (2004), Pfukenyi *et al*. (2005a,b), Mavenyengwa *et al.* (2006), Phiri *et al*. (2007) and Arindam and Santra (2009), *Lymnaea* reached maximum number during autumn and was the lowest in winter. The number then started increasing in spring and decreased again in winter. These incidences concided with results of Ashmawy *et al*. (1993), Szmidt-Adjide *et al*. (1994), Chingwena *et al*. (2002), Pfukenyi *et al*. (2005 a,b); Mavenyengwa *et al*. (2006), Morakot and Sakchai (2006) and Sri-aroon *et al*. (2007)

Temperature had a significant effect on the population. The results of the present study indicated that autumn followed by spring temperatures, lie within the optimum temperature regime required for breeding and reproduction of aquatic snails. It was found that the high incidence of some species of snails belonging to pulmonate class occurred during spring. These results were in accordance with those of Dreyfuss *et al*. (2007), Sri-aroon *et al*. (2007), Arindam and Santra (2009), Rondelaud *et al.* (2007) and Liang *et al.* (2010). They reported that a temperature of 20°C is

Table I.- District wise prevalence (%) infection of various genera of snails in Punjab.

District	*Indoplanorbis*	*Bulinus*	*Physa*	*Lymnaea*	*Gyralus*	*Bellamaya*	*Oncomelonia*	Overall
Gujranwala	1076	213	525	273	312	311	292	3002
	(17%)	(12%)	(20%)	(26%)	(8.65%)	(0%)	(9%)	(15.5%)
Sheikhupura	1014	181	461	255	270	274	249	2704
	(15%)	(10%)	(17%)	(22. %)	(7.03%)	(0%)	(8%)	(12.6%)
Lahore	949	149	413	232	246	241	210	2440
	(12%)	(7.4%)	(13%)	(19%)	(5.69%)	(0%)	(6%)	(10%)
Kasur	918	126	256	201	215	202	177	2195
	(9.5%)	(4%)	(11%)	(17%)	(3.25%)	(0%)	(5%)	(8%)
Overall	3957	669	1755	961	1043	1028	928	10341
	(13.4%)	(9%)	(16%0	(22%)	(6.42%)	(0%)	(7%)	(12%)

Table II.- Seasonwise prevalence(%) infection of snails in different districts of Punjab.

Season	Districts				
	Gujranwala	Sheikhupura	Lahore	Kasur	Overall
Summer	1253	1144	1031	933	4361
	(17%)	(15%)	(13%)	(11%)	(14%)
Autumn	604	544	490	455	2093
	(18%)	(16%)	(12%)	(10%)	(14%)
Winter	834	732	563	577	2796
	(9%)	(9%)	(6%)	(4%)	(7%)
Spring	311	284	266	230	1091
	(12%)	(7%)	(6%)	(3%)	(7%)
Overall	3002	2704	2440	2195	10341
	(15%)	(13%)	(10%)	(8%)	(12%)

optimum constant for snail growth and this temperature was recorded in autumn and spring. They further reported that growth and reproductive activities of *Physida* snails was the greatest in autumn and spring while there was considerable mortalities during early summer.

In the present study it was also observed that the oviproduction process was not affected by the length of the day light period. Most snails present in winter were young, whilst in the summer season many old snails were collected. These results concided with those of Krailas *et al.* (2003). They reported that oviproduction by snails is not affected by the increasing period of day light whereas darkness has no harmful effect.

The average pH recorded in this study was slightly alkaline (7.2-7.6) and near to values recorded by Maqbool *et al*. (2003). The relation of snails to the water depth was also observed. *Bulinus* species were widely distributed in small canals and ditches. Pfukeni *et al*. (2005a,b) and Phiri *et al*. (2007) also recorded similar snails which were found clinging to water plants near the surface or in the bottom substrate. *Lymnaea, Phsa* and *Indoplanorbis* snails were seen floating on the surface. The same observations were recorded by Gohar and El-Gindy (1960). They explained that *lymnaea* snails needed a high rate of oxygen consumption. Philippi (1970) and Sheikh *et al*. (1984) reported that *Physida* species come to the surface water regularly for aerial breathing.

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Dubas Bug, *Ommatissus lybicus* (Tropiduchidae: Hemiptera) - A New Record From Panjgur, Balochistan, Pakistan

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Abstract: - Dubas bug, or the Old world date bug, or plant–hopper of Middle East *Ommatissus lybicus* Bergevin (Tropiduchidae: Hemiptera) has been recorded in several countries in the near East and North Africa. Dubas bug, locally known as Sheragoo (due to secretion of honeydew) is recorded for the first time from Pakistan. Its seasonal distribution, host plant, habitat, habits and natural enemies in Pakistan have been discussed in this paper.

Key words: Dubas bug*; Ommatissus lybicus*, Tropiduchidae, Hemiptera, date palm, honeydew, Sheragoo, biphagous, bivoltine.

Date palm is one of the important fruits of Pakistan. The major growing areas are restricted to some districts such as Panjgur and Turbat of Balochistan, Sukker and Khairpure of Sind and D.I. Khan of Khyber Pakhtoonkhawa. Factors such as improper fertilization, imbalanced nourishment and biotic stress, *i.e.*, red palm weevil, mites and mainly the presence of Dubas bug *Ommatissus lybicus* Bergevin, adversely affect the date yield (GOB, 2006).

Dubas bug, so called from the honeydew, Arabic, *dibis* (Hussain, 1963), or the Old world date bug (Klein and Venezian, 1985), is also known as the Planthopper of Middle East (Howard, 2001). Dubas bug was first noted as pest of date palm in Basra area of Iraq, between 1919-1920 (Ramachandra, 1922) and was named as new Fulgorid, Cinixii, group but the description,

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drawing and typical damage symptoms (secretion of honeydew) assumed that it be the same pest (Dowson, 1936). Since its first record in 1919 from Iraq, it was reported from Iran (1937), Sudan (1980), and Israel (mid 1980s) (Afchar, 1938; Askari and Bagheri, 2005; El-Haidari, 1982; Klein and Venezian, 1985). This notorious pest is now distributed in several countries in the near East and North Africa (Alfieri, 1933, 1934; Dowson, 1936; Hussain, 1974; Gharib, 1966; Hussein and Ali, 1996; Waller and Bridge, 1978; El-Haidari, 1982; El-Haidari and Al-Hafidh, 1986).

Date palm is the only host of this bivoltine pest and it is not a diseases vector (Gassouma, 2004; Howard, 2001). It only causes direct damage to date palm by draining sap. In case of heavy infestation, this pest produces extremely large quantities of honeydew, which cover the leaves and support sooty mold that growth on the honey dew and reduce the photosynthetic activities (Dowson, 1936; Mokhtar, and Al-Mejeni 1999; Klein and Venezian, 1985; Elwan and Al-Tamiemi, 1999; Gassouma, 2004). However, sooty mold was not observed on palm infested with dubas bug in the Arava valley, Israel (Klein and Venezian, 1985).

Dubas bug is considered a major pest of date palm in several countries of Old World (Kelin and Venezian, 1985), and it is the ranked one among pests of date palm in Iraq (Heil, 2007). In case of heavy infestation, the dubas bug might reduce the crop yield to level less than 50% (Gassouma, 2004). However in Iraq no damage and no losses in production were detected in the presence of this pest in large numbers on date palms (Ramachandra, 1922; Kelin and Venezian, 1985).

The genus *Ommatissus* has 11 species which differ based mainly on the extent and position of the dark marking on the face, lorae and genae, and on male genitalia (Asche and Wilson, 1989). In this study, host range, distribution and re-description of Dubas bug was carried out to establish whether the *Ommatissus lybicus* species present in Panjgur is the sameas that found in other parts of the world. In this manuscript, this species is presented as a first record from Pakistan.

Materials and methods

During the course of 2008-10 in the month of June and October surveys of infested date orchards in five Union Councils (UCs) were carried out. In each UC, 10-15 orchards were selected at random. The number of Dubas bug adults were collected from infested plants in selected area using an aspirator and preserved through wet and dry methods for identification and re-description. Both nymphs and adults were also collected by using yellow sticky traps. Nymphal instars were preserved in alcohol for description purpose. Sampling was done on weekly basis.

Specimens were identified according to Asche and Wilson (1989), while the illustrations were prepared using a Nikon microscope (SMS-1500 with 30x 1-11.25x), measurements of different body parts were taken by using an ocular micrometer in No if microscope (XSZ 107 BN, with 10x). Line drawings of important body parts were prepared with the help of stedler pen (0.2mm).

Ommatissus lybicus Bergevin
(Fig. 1)

Ommatissus binotatus var. *lybicus* Bergevine, 1930:20

Identification characters

Lorae dark brown; penis without sub-apical tooth or spine

Re-description of female

Body yellowish green or yellowish earth; 5-7mm long; about 2.7 times longer than wider at thorax. Forewing one times longer than wider. Eyes about 1.4 times longer than wider in dorsal view; distance between eyes is 1.1 times the eyes length. Vertex basal width to median length ratio is 1.35-1.61: 1.1. Frons spot quadrilateral; 1.3 times longer than wider. The frons ratio of median length to width at eyes is about 1.14-1.1. Pronotum 1.1 times wider than longer; width about 1.8 times shorter than distance between two dots on pronotum. Pronotal spot circular, 2.2 times wider than longer. Forewing one times longer than wider; about 1.1 times longer than body size; tegmina and wings translucent. Thorax 1.3 times wider than longer. Hind legs are shown in Figure 1D and genitalia are shown in Figure 1E.

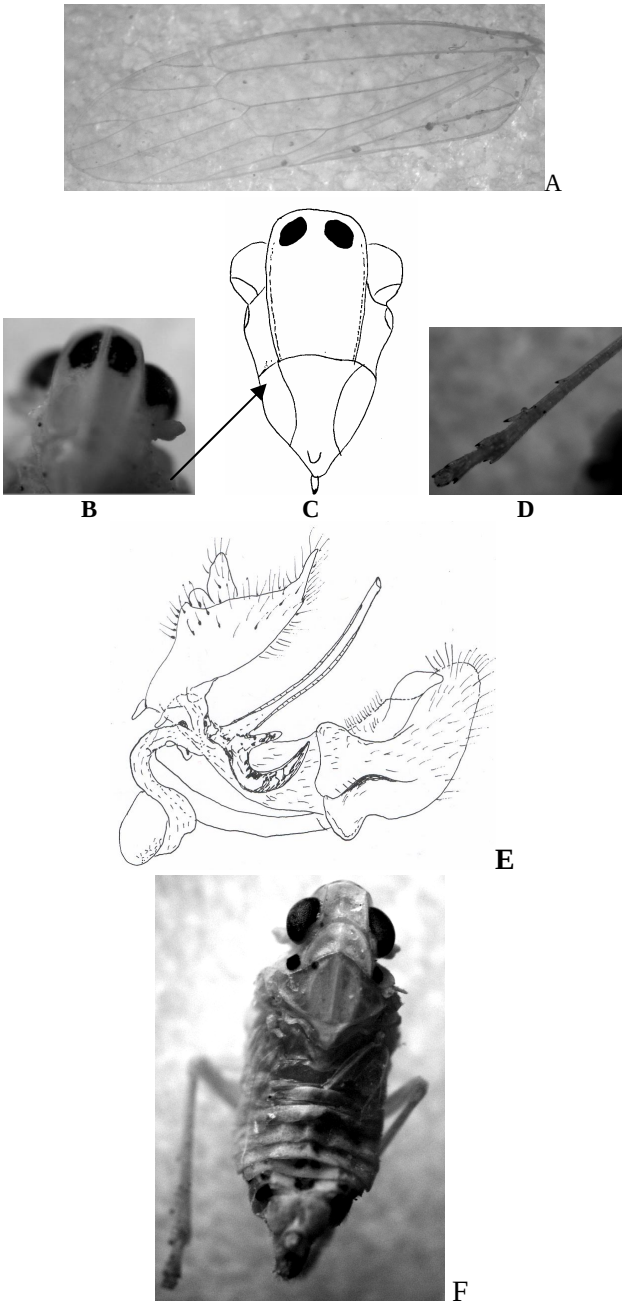

Fig. 1. *Ommatrissus lybicus*; A, fore wing venation; B, two spots on upper frons; D, spines on meta leg; E, male genitalia; F, adult (wings removed).

Measurements (in mm at 10X): Dorsal eye length: 0.38; Eye width: 0.28; Distance between eys: 0.43; Head width: 0.93; Maximum thorax width: 1.25; Maximum thorax length: 1; Distance between two dots (Pronotum): 0.63; Pronotum width: 0.35; Pronotum length: 0.31; Pronotum spot size: 0.6; Frons spot size (length): 0.28; Frons spot size (width): 0.13; Frons spot size length: 0.13; Frons spot width: 0.1.

Male

About 1.7 times shorter than female. Abdominal segment 7-8, lack four spots.

Description of nymphal instars

1st instar	1.1.23mm, 3 grey spots on each abdominal segment; eyes red; wing buds not developed.
2nd instar	1.73-2.25mm;2 grey lines along the dorsal side of the body; wing buds extending downward
3rd instar	2-2.23mm;wing buds covering 1st abdominal segment and part of 2nd segment
4th instar	3-4mm; wing buds covering 1-2 part of 3rd segment.
5th instar	2.6-3mm; wing buds covering 1-3 part of 4th segment.

Each instar with 16 white waxy filaments (with several finer filaments) at caudal part of body.

Remarks

Specimens collected from Pakistan were compared with the description given by Asche and Wilson (1989) and Kelin and Venezian (1985) and found to be similar.

Material examined

Khuda Badan, Tusaap, and Gramkan, 23-vi-2009,50 male and 50 female, date palm; Tusap, 1-vii-2008, 20 male and 30 female, date palm; Gramakan, Khuda Badan, Washbood, 20-x-2010, 40 male and 60 female, date palm.

Seasonal distribution

Dubas bug has two distinct generations (summer and over-wintering) in a year (Ba-Angood *et al*., 2009; Klein and Venezian, 1985). Payandeh *et al*. (2010) described that the nymphs of Dubas bug were active from April to May and August to October, at first and second generations respectively. But in our studies the nymphs of 1st (over-wintering/spring) and 2nd (summer) generations emerged on first week of April, and 4th week of August in 2009, while during the second

year (2010) the first instar nymphs of respective generations came out during the last week of March and 3rd week of August, respectively. The pest hibernate and aestivated in egg stage during the first and second generation, respectively (Askari and Bagheri, 2005). During the current survey in 2008, 2009 and 2010 it is now distributed in the whole date growing area of Panjgur. Dubas bug being weak flyer and with shorter period of adult stage (15-20 days), can be dispersed and distributed in the orchards through wind and plant material.

Host plant

Lepseme in 1947 reported that *Chamerops humilis* L. is an alternate host of the Dubas bug while Gossama in 2004 reported that date palm is the only host of this pest. However, during the course of current studies the Dubas bug (*Ommatissus lybicus*) was collected from *Nanorrhops richieana* (Family Palmacae) locally called Pish/Dazz about 15 -18 Km apart from date growing area in district Punjgur of Balochistan. Dubas bugs were found feeding on all varieties (Kehraba, Jan-sore, Mozavati, Rabbai, Sabzoo and Abe-dandan) but with different degree of infestation in relation to variety and agronomic practices. Kehraba was most preferred. The name of wild species Dazz was further confirmed from "Flora of Balochistan" written by Bickle, and Forest Department, Balochistan.

Damage and loss

On the basis of honeydew droplets from plants recorded on water-sensitive paper (WSP) and oily appearance of leaves, the cultivar Kehraba was more infested and susceptible as compared to other cultivars of the area. According to date growers, severe hopper infestation could result in premature fruit shedding (30% growers), delay in fruit maturity (20% growers), reduction in fruit shelf life (5% growers), reduction in post-harvest storage period (40% growers), and change in taste from sweet to bitter (50% growers) and fruit weight loss (100% growers). Majority of the growers reported that severe hopper infestation leads to 25-30% loss in the yield.

Habits and habitats

Being sap feeders, the nymphs and adults suck the sap from leaflets, midrib of fronds, and in case of severe infestation can be found on the fruit stalk and fruit. The nymphs and adults prefer the shady and green part of date palm. The adults lay eggs in the leaflets and remain dormant on an average of 62.70 and 147.60 days during summer and over-wintering generation. The bug completed the whole life cycle on fronds as described by Jasim and Al-Zubaidy (2010).

Natural enemies

During the current survey in 2008-2010, different bio-control agents like *Coccinella septumpuntata* and spiders was found to be feeding on adults. Immature of *Chrysoperla* sp. and *Coccinella septumpuntata* were feeding on various nymphal instares of Dubas bug. Ants (unidentified) were also observed in colonies of Dubas bug.

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Prevalence of Human Malaria Infection in Pakistani Areas Bordering Iran: District Turbat, Pakistan

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Abstract: This paper reports the prevalence of malarial parasites in the human population of District Turbat. Out of a total of 5912 blood samples 46.4% were found positive for malarial parasite. The incidence of *Plasmodium falciparium* was 69.4% and *P. vivax* was 30.5%. The incidence was higher (72.1%) in males. Age wise, the prevalence of the disease was 70.7% and 69.2% for age group 21-years and above and 11-20 years. The prevalence was higher 87.5% in May. No case of *P. malariae* and *P. ovale* was detected.

Key words: Human malaria, slide positivity rate, *Plasmodium vivax, P. falciparum*

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Malaria is one of the most devastating diseases in the world. Over 3 billion people live under the threat of malaria in 24 endemic countries and it kills over a million each year, mostly children (Korenromp, 2005).

An outbreak of malaria occurred in February, 2004, in the rural districts of Sindh and Balochistan provinces, *falciparum* malaria accounted for about 85% of cases (IRIN, 2004). Pakistan could be witnessing upcoming malarial endemicity in various rural areas, owing to the deep, stagnant flood waters providing breeding sites for large numbers of mosquitoes. Cases of malaria in Pakistan have also been imported in the past few decades because of the influx of Afghan refugees. In the many camps that the Afghan refugees occupied in Pakistan, 150 000 cases of malaria were diagnosed and treated each year, about 30% of which were due to *Plasmodium falciparum* (Rowland and Nosten, 2001).

Hozhabri *et al*. (2000) observed slide positivity rate 5.9% with 65% cases of *P. falciparum* and 35% of *P. vivax* in children, at Jhangra, Sindh. Bhalli and Samiullah (2001) presented a review of *falciparum* malaria. Akbar (2002) found high incidence of *falciparum* as compared to *vivax* (65% vs 35%) among 100 positive children for malaria at Baqai Medical University. Murtaza *et al.* (2004) studied 3.1% slide positivity with 58% *P. falciparum* and 42% *P. vivax* in Sindh. Mahmood *et al.* (2006) studied 348 patients with fever at Civil Hospital and Ankle Sria Hospital Karachi from August 2003 to December, 2005 and observed 35% positivity rate, with *P. falciparum* 88.5% and *P. vivax* 9%. Malaria in NWFP was studied by Saleem *et al*. (2006) and observed that cerebral malaria was more common in males (64%) and most vulnerable group was pregnant women. Nizamani *et al*. (2006) observed an average slide positivity rate 2.4% in Sindh and *P.*

falciparum ratio was found increasing in many districts of Sindh. In south Punjab, 41% were found to be infected by *Plasmodium* species (Shehzadi *et al.*, 2011). Prevalence of malarial parasite in human blood in Karachi was studied and out of 2457 samples, 311 of the samples were found to be positive (Faiz *et al.*, 2011).

In Balochistan Province too, cerebral malaria is a major community problem. Khadim (2002) observed 665 (11.7%) positive cases of malaria out of 5650 patients at CMH, Zhob during 2000-2001. Cases were seen throughout the year with maximum cases of *falciparum* malaria during the months of July to November. Malaria Control Program Balochistan (MCPB, 2004, 2005, 2006) observed slide positivity rate 5.7, 1.0, 5.3, 1.1, 9.6, 27.2, 13.3, 7.3, and 13.5% in 2004, 4.7, 0.5, 6.6, 1.5, 12.9, 32.4, 10.2, 7.5 and 13.5% in 2005, 5.7, 3.8, 17.5, 2.5, 42.2, 29.5, 7,6.8, and 12.9% in 2006 in the districts of Lasbella, Qilla Abdullah, Mastung, Khuzdar, Kohlu, Zhob, Kharan, Sibi and Turbat. Shaikh *et al.* (2005) studied endemicity of malaria in Quetta from January 1994 to December, 1998 and observed 34.8% positive smears, with 66.8% *P. vivax* and 30.7% *P. falciparum.* Farooq *et al.* (2008) studied 505 suspected malaria patients from district Khuzdar at CMH Khuzdar, from August 2003 to December, 2004, and observed higher prevalence of *P. falciparum* (69%) than of *P. vivax* (24%) and 7% mixed infection. Keeping in view the high mortality rate of malaria infection in Turbat areas published in local newspaper, the present investigation was carried out to know exactly the positivity rate and the dominant *Plasmodium* species.

Materials and methods

This study was conducted during July 2004 to June 2006 by adopting active case detection (ACD) and passive case detection (PCD) techniques (Paniker, 2002) in the areas of District Turbat to detect malaria cases from amongst those subjects who were suspected to be malaria patients. PCD technique was applied on 4795 patients presenting themselves to 15 health facilities (2 district hospitals, 6 basic health unit and 7 private clinics) with symptoms of shivering and fever or a history suggestive of malaria. ACD technique was applied by door to door visits on monthly basis with the help of head of the locality to 1117 subjects with sign or symptoms of malaria, 24 visits during two year study and blood films of both thin and thick were prepared. Blood slides were stained with Giemsa's stain (Manson-Bahr and Bell, 1987), and species of malarial parasites were identified using keys of Chiodini *et al.* (2001) and Paniker (2002).

Results and discussion

A total of 5912 blood smears were prepared from the age groups ranging from 1 to 21 years and above residing in 12 different localities of Turbat *viz.* Turbat city, Tump, Mand, Bulida, Hironk, Saami, Tajabaan, Hoshab, Shapuk, Gujar, Jussak and Gwarkop (PCD, 4795; 2334 +ve; *P. falciparum* 1643, *P. vivax* 691), (ACD, 1117; 412+ve: *P. falciparum* 265, *P. vivax* 147).

However, variations were observed among different localities having different environment and hygienic conditions.

Table I shows high slide positivity rate (SPR of 64.8% (PCD) in Saami and 57.1% (ACD) in Hironk area. The high SPR of 58.5% (PCD) and 59.1% (ACD) were observed both in the month of August (Table II). The overall SPR was 48.6% (PCD) and 36.8% (ACD), wherein *P. falciparum* was observed to be higher (70.3% with PCD and 64.3% with ACD) compared with that of *P. vivax* **(**29.6% with PCD and 35.6% with ACD.

Table III shows SPR in children (1-10 years) 43.4% with PCD and 27.0% with ACD. In the age group of 11-20 years this was 49.6% with PCD and 45.3% with ACD. In the age group of 21 years and above these values were 50.6% with PCD and 34.5% with ACD. High SPR was recorded in males 73.9% with PCD, 62.3% with ACD compared to and 26.0% with PCD, and 37.6% with ACD in female (Table IV). Statistical analysis showed no association between types of infection and age groups. Therefore, it can be said that any type of infection can occur in to any age group of people independently.

In the present study no case of mixed infection was seen. A mixed infection (0.4%) was noted in Sanjavi area of Balochistan (Yasinzai and Kakarsulemankhel, 2009a). In south Punjab, a higher mixed infection (*P. falciparum* and *P. vivax*) was accounted for 24.3% (Shehzadi *et al.*, 2011).

Table I.- Area-wise slides positivity rate (SPR) of malaria infection in Turbat District.

Areas	No. of slides examined		Total number of +ve		*P. v.* (%)		*P. f.* (%)	
	PCD	ACD	PCD (%)	ACD (%)	PCD	ACD	PCD	ACD
Turbat city	540	197	275 (50.9)	39 (19.7)	83	14	192	25
Tump	594	84	216 (36.3)	47 (55.9)	55	11	161	36
Mand	501	110	278 (55.4)	51 (46.3)	96	16	182	35
Buleda	532	122	251)47.1)	40 (32.7)	75	13	176	27
Hironk	288	63	183 (63.5)	36 (57.1)	48	09	135	27
Saami	336	58	218 (64.8)	28 (48.2)	52	16	166	12
Tajabaan	252	71	152 (60.3)	35 (49.2)	48	06	104	29
Hoshab	506	134	175 (34.5)	49 (36.5)	48	21	127	28
Shapuk	432	96	228 (52.7)	31 (32.2)	59	12	169	19
Gujar	356	77	169 (47.4)	23 (29.8)	58	18	111	05
Jussak	260	38	75 (28.8)	14 (36.8)	28	4	47	10
Gwarkop	198	67	114 (57.5)	19 (28.3)	41	07	73	12
Total	4795	1117	23334 (48.6)	412 (36.8)	691 (29.6)	147 (35.6)	1643 (70.3)	265 (64.3)

Abbreviations: PCD, passive case detection technique; ACD active case detection; *P.v., Plasmodium vivax*; *P.f., Plasmodium falciparum.*

Table II.- Month-wise slide positivity rate (SPR) in Turbat District.

Months	No. of slides examined		Total number of +ve		*P. v.* (%)		*P. f.* (%)	
	PCD	ACD	PCD (%)	ACD (%)	PCD	ACD	PCD	ACD
July 05-05	476	102	274 (57.5)	49 (48.0)	116	118	158	31
August	490	137	287 (58.5)	81 (59.1)	65	11	222	70
September	562	121	277 (49.2)	42 (34.7)	79	20	198	22
October	418	93	175 (41.8)	33 (35.4)	50	13	125	20
November	293	81	145 (49.4)	21 (25.9)	59	10	86	11
December	267	47	153 (57.3)	26 (55.3)	68	14	85	12
January 05-06	271	30	113 (41.6)	11 (36.6)	39	06	74	05
February	288	54	129 (44.7)	14 (25.9)	41	08	88	06
March	439	119	183 (41.6)	37 (31.0)	69	19	114	18
April	470	143	226 (48.0)	43 (30.0)	47	12	179	31
May	463	118	203 (43.8)	30 (25.4)	25	07	178	23
June	358	72	169 (47.2)	25 (34.7)	33	09	136	16
Total	49795	11117	2334 (48.6)	412 (36.8)	691 (29.6)	147 (35.6)	1643 (70.3)	265 (64.3)

For abbreviations, see Table I.

Table III.- Age-wise slide positivity rate (SPR) in Turbat District.

Age (Years)	No. of slides examined		Total number of +ve		*P. v.* (%)		*P. f.* (%)	
	PCD	ACD	PCD (%)	ACD (%)	PCD	ACD	PCD	ACD
1-10	1059	233	460 (43.4)	63 (27.0)	151	20	309	43
11-20	1976	406	982 (49.6)	184 (45.3)	299	59	683	125
21 above	1760	478	892 (50.6)	165 (34.5)	241	68	651	97
Total	4795	11117	2334 (48.6)	412 (36.8)	691 (29.6)	147 (35.6)	1643 (70.3)	265 (64.3)

For abbreviations, see Table I.

Table IV.- Sex-wise slide positivity rate (SPR) in Turbat District.

No. of slides		Total No. of +ve		Number of Male +ve				Number of Male +ve			
PCD	ACD	PCD (%)	ACD (%)	PCD (1725:73.9%)		ACD (257:62.3%)		PCD (609:26%)		ACD (155:37.6%)	
				P.v.	*P.f.*	*P.v.*	*P.f.*	*P.v.*	*P.f.*	*P.v.*	*P.f.*
4995	1117	2334(48.6)	412(36.8)	498	1227	86	171	193	416	61	94

For abbreviations, see Table I.

During present study, no case of *P. malariae* and *P. ovale* was observed, as the same was also not observed in Multan (Yar *et al.,* 1998) and also not seen in Karachi (Faiz *et al.,* 2011).

High SPR of *P. falciparum* was also observed in some part of the province *viz.*, Sanjavi (87.2%) (ACD) and 83.7% (PCD) (Yasinzai and Kakarsulemankhel, 2009a) and 55.1% in Musakhel (Yasinzai and Kakarsulemankhel, 2009b). In south Punjab, *P. vivax* was found to be more prevalent (39.0%) than *P. falciparum* (36.6%) (Shehzadi *et al.,* 2011). In Karachi, *P. falciparum* was observed to be dominating (90.99%) compared to *P. vivax* (9.0%) (Faiz *et al.,* 2011).

In conclusion, it is revealed from our results that infection with *P. falciparum* was found to be more prevalent in Turbat areas.

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