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New Records of Mantispid Flies (Neuroptera: Mantispidae) from Pakistan

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Abstract.- Collection of mantispid flies was carried out from three different regions of Pakistan. Taxonomic identification and genitalia studies revealed three species out of 30 colleted specimens. All the three species i.e. *Mantispa styriaca*, *Mantispa scabricollis* and *Nampista auriventris* are new records for Pakistan. Taxonomic keys, drawings of taxonomic characters for wings, thorax (pronotum) and head spots are provided for all explored species to facilitate future studies on this group.

Keywords: Mantispidae, mantispid flies, Neuroptera.

Mantispid flies belong to the family Mantispidae of order Neuroptera. Worldwide total number of species known for this family exceeds four hundred (Ohl, 2007). Family Mantispidae was first time split into two genera, *i.e.*, *Symphasis* and *Mantispa* by Banks (1892). However, recently it has been divided in to four subfamilies *viz*, Symphrasinae, Drepanicinae, Calomantispinae and Mantispinae (Velasco and Ramos, 2008). In Asian and European countries only the subfamily Mantispinae is reported uptil now. Within Asia, 36 species are reported from China (Ohl, 2004), five from Russia (Zakharenko, 1987), 15 from Japan (Kuwayama, 1925), 17 from India, three from Iran (Aspöck *et al.*, 1980, 2001; Mirmoayedi, 2002), one from Iraq, four from Turkey (Dobosz, 2007), one from Oman (Aspöck *et al.*, 2001) and one has been recorded from Saudi Arabia (Ohl, 2004). The Mantispidae of Middle East were poorly explored in the past. Today's knowledge of Mantispid fauna of this region is restricted to the studies of Aspöck *et al.* (1980, 2001), Mirmoayedi, (2002), Ohl (2004, 2007), Ozbay *et al.* (2005) and Ari *et al.* (2008). Taxonomic studies on this important group of insect predator have been neglected in Pakistan. There is no record showing species composition of mantispid flies endemic to Pakistan. Keeping this in view it was planned to initiate taxonomic work on mantispid fauna of the country by undertaking surveys in different regions of Pakistan.

Materials and methods

Collection was done during day as well as night time by aerial netting and mounting light traps. Collected specimens were killed using ethyl acetate. Drawings of taxonomic characters for wings, thorax (pronotum) and head spots were made for all specimens. Abdomens of male and female specimens were dissected to pull out genitalia which were thereafter treated with 10% KOH solution. Photographs of male genitalia were made by a JVC digital camera mounted on Olympus CH40 microscope. Genitalia of males were thereafter preserved in small plastic vials with glycerin. Identified specimens along with preserved genitalia are deposited in National Insect Museum Islamabad, Pakistan as reference collection.

Results

As a whole thirty specimens were collected from three regions of the country with varying habitats. Taxonomic identification and genitalia studies revealed three species under two genera *i.e.*, *Mantispa styriaca* (Poda, 1761**)**, *Mantispa scabricollis* McLachlan, 1875 and *Nampista auriventris* Handschin, 1960. All the three species are reported from Pakistan for the first time which emphasizes need for further extensive future surveys to unhide the mantispid fly fauna of Pakistan. Habitat and host details are provided for all the collected specimens.

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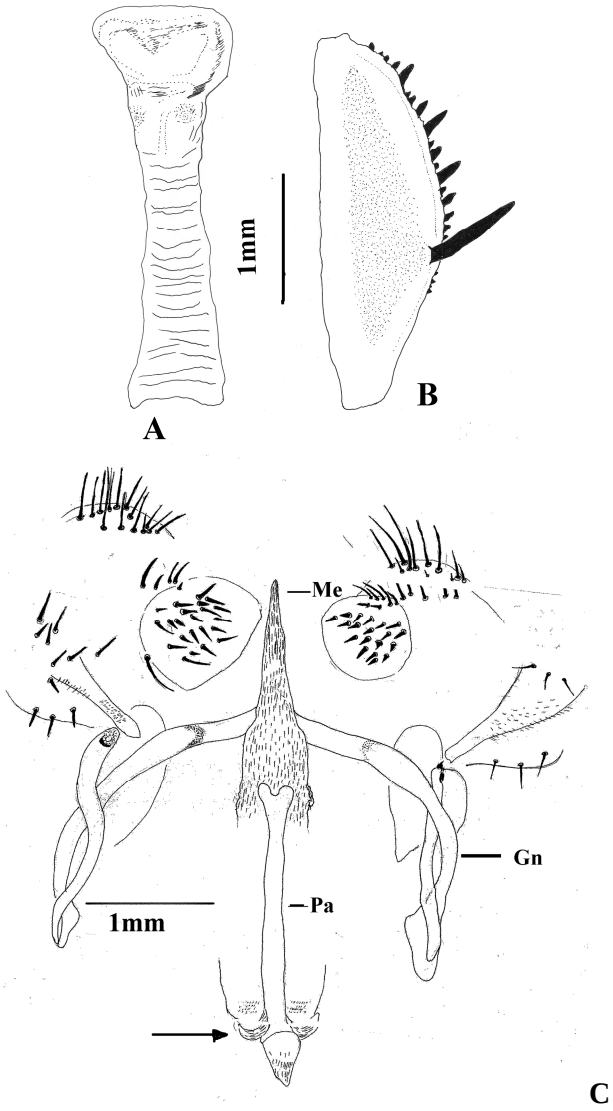

Fig. 1. *Mantispa styriaca*; A, pronotum; B, femur of fore leg; C, base of paramer, dome shaped with lateral wings (showed by an arrow). Me, mediuncus; Pa, Paramer, Gn, gonarcus.

1. *Mantispa styriaca* (Poda, 1761)

Material examined

Punjab: Islamabad (33º43´N 75º05´E), 20.ix.2006, 2♂ 8♀, collected during day time (between 11-12 O'clock) when they were observed sitting on leaves of *Dalbergia sissoo* surrounded by high grassy vegetations and maize fields with stagnant irrigated water; AJ&K: Kotli (33º30´N 73º55´E), 23.iv.2008, 4♂, collected during day time (between 12-15 O'clock) when they were flying over trees of *Dalbergia sissoo* with close proximity of a flowing perennial river.

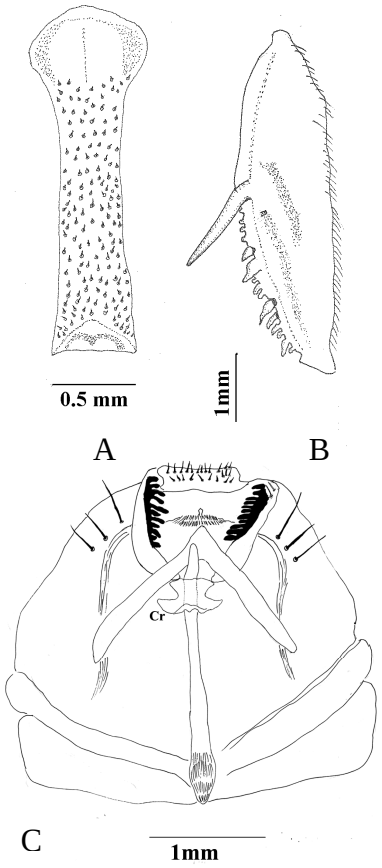

Fig. 2. *Mantispa scabricollis;* A, pronotum with spine like bristles; B, femur of fore-leg; C, crown like structure base of gonarcus (Cr).

2. *Mantispa scabricollis* McLachlan, 1875

Material examined

Sindh: Islamkot (24º42´N 70º13´E), 27.iii.2008, 10♂, collected at night by light trap mounted in a sandy desert with no rain received over a period of last two months.

3. *Nampista auriventris* Handschin, 1960

Material examined

Sindh: Islamkot (24º42´N 70º13´E),

27.iii.2008, 6♂, collected at night by light trap mounted in a sandy desert with no rain received over a period of last two months.

A taxonomic key has been prepared for Mantispidae fauna of Pakistan to facilitate future studies over this group in this region of the world.

KEY FOR IDENTIFICATION OF *MANTISPA* SP.

1	Pronotum without spiny bristles (Fig.1A,B) ..... 1´
1´	Pronutum covered entirely by short spiny bristles (Fig. 2A,B) 2
2(1´)	Base of gonarcus contains a crown like structure (Fig. 2c)..... 2´
2´	Base of gonarcus without a crown like structure.....3
3(2)	Gonarcus curved, like an arch, with pointed median part.....*Mantispa scabricollis*
3´	Gonarcus curved, but it's median part, not pointed (Fig. 1C).....4
4	Base of paramer, dome like, with two lateral wings (Fig. 1C)..... *Mantispa styriaca*
4´	Base of paramer, dome like, without two lateral wings (Fig. 3) .....*Nampista auriventris*

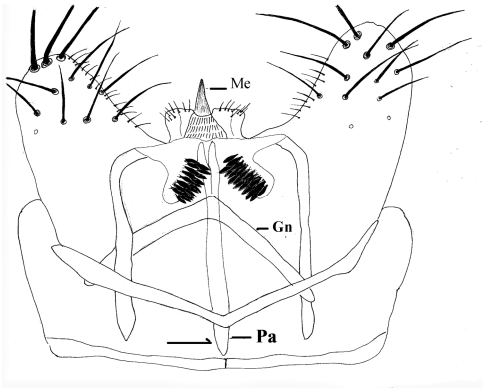

Fig. 3. *Nampista auriventris* : Base of paramer, dome shaped without lateral wings(arrow). Me, mediuncus; Gn, gonarcus; Pa, paramer.

Discussion

In spite of taxonomic problems which encountered this section of Neuropterology and because of existing dispute on nomenclature of some species between different authors (Kuwakawa, 1925; Poivre, 1983; Abraham and Papp, 1994; Aspöck, 1994; Aspöck and Aspöck, 1994) study of Mantispidae fauna observed a leaping progress during past twenty years. During eighties authors like Aspöck *et al.* (1980) and Zakharenko (1987) reported 350 species worldwide. Twenty years later known species under this family throughout world reached to 410 species (Ohl, 2004).

Although lot of work has been carried out over Mantispidae fauna of China, India and Iran by different authors (Ohl, 2004), but no data was available for Mantispidae of Pakistan. *Mantispa scabricollis* is already reported from Iran but it is known neither from India nor from China which are the adjacent countries to Pakistan. *Mantispa styriaca* is already recorded from India and Iran but it is still not reported from China. Similarly *Nampista auriventris* is not recorded up till now from Iran, China and India but is already reported from Oman, Uzbekistan and Tajikistan (Ohl, 2004).

Pakistan has an important geographical position with total area of 881,640 km^2. It has abundance of Oriental, Palearctic and Ethiopian fauna. Its Oriental representation of species is continuous with those of Indian Punjab and Rajisthan and Palearctic is continuous with those of Iranian Baluchistan, eastern Afghanistan and Russia (Separated by few miles) and north western and eastern China. It has a definite Ethiopian influence which runs along southern coastal areas of Sindh and eastern Mekran in Baluchistan (Qadri, 1968).

Taxonomic work over Mantispidae fauna of Pakistan needs more investigations through country wide surveys. It is highly probable that the total number of species under this family in different parts of this country is much more than reported in this study.

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Seroprevalence of Anti-*Toxoplasma gondii* Antibodies in Captive Birds in Lahore, Pakistan

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Abstract.- The seroprevalence of anti-*Toxoplama gondii* antibodies in 200 captive birds in urban and peri-urban areas of district Lahore was studied by latex agglutination test. Of 200 captive birds, 20 (10%) tested positive for anti-*Toxoplasma gondii* antibodies. The highest seroprevalence of anti-*Toxoplama gondii* antibodies was observed in turkeys (16%) followed by ducks (12%), pigeons (8%) and quails (4%).

Key words: Toxoplasmosis, captive birds, anti-*toxoplasma* antibody, latex agglutination test.

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Toxoplasmosis is one of the more common parasitic zoonosis world-wide and tends to be more prevalent in tropical climates.The etiological agent of toxoplasmosis, *Toxoplasma gondii,* is an intracelluler protozoan (Smith, 1995) which utilizes felids as definitive hosts (Torada, 2001) and wide range of warm blooded intermediate hosts including birds, human and other mammals (Hill *et al*., 2005). Nearly one-third of humanity has been exposed to this parasite (Dubey and Beattie, 1988; Resendes *et al*., 2002; Dubey *et al*., 2003). Toxoplasmosis is a significant problem in congenitally infected infants and immunosuppressed individuals.

Infected birds are considered as important source of *Toxoplasma gondii* worldwide. Infections and antibody titers have been documented in turkeys with improved isolation procedures and serological test (Quist *et al*., 1995). The rate of toxoplasmosis in turkeys as one of the intermediate host of *Toxoplasma gondii* is a good indicator of environmental contamination because of eating habits from the ground. Turkeys developed antibodies to *T. gondii* detectable by MAT, Enzyme linked immunosorbent assay (ELISA), Latex agglutination test (LAT) or Indirect haemagglutination (IHAT) but not by the DT (Dubey *et al*., 1993).

Pigeons are highly susceptible to oral infection with oocysts. Experimental oral infection with only 50 oocysts results in seroconversion, while feeding with 500 oocysts has proven to be lethal to 100% of pigeons assayed (Biancifiori *et al*., 1986). Nobrega and Reis (1942) also isolated *T. gondii* from pigeons. As well pigeons are source of *T. gondii;* the high seroprevalence in pigeons could be related to large population of homeless cats in the city and may suggest the significant role of urban pigeons in epidemiology of toxoplasmosis (Piasecki and Wieliezko, 2004).

Clinical toxoplasmosis has also been reported in domestic ducks (Boehringer *et al*., 1962) from Argentina. Recently, 48 ducks were examined in Egypt by modified agglutination test with 50% positive results (El-Massry *et al*., 2000).

Quails also responded serologically to *T. gondii.* High levels of *T. gondii* antibodies were detected in the sera of quails after performing

several serological tests including LAT at USAD zoonotic disease laboratory (Dubey *et al*., 1994). Quails that survived initial *T. gondii* infection remained infected and infection was subclinical in many avian species (Parenti *et al.*, 1986). Experiments on Japanes quails showed alteration inhemogram and subclinical evolution of this infection (Munhaz *et al*., 2004).

Serodiagnosis has been a reliable tool to diagnose toxoplasma infection in both humans and birds, such as IHA (Nieto and Melendez 1998), indirect immunoflorescence (IFAT) (Van-Der-Puiji *et al*., 2000), ELISA (Hashemi-Freshaki, 1996) and LAT (Ahmed, 1999). The serological screening of *T. gondii* infection in birds is an indirect means to assess the prevalence of *T. gondii* oocysts in soil as the avian population feeds directly from the ground. As a result of serological and parasitological surveys it became clear that *T. gondii* infection was common in some avian species (Sabin and Feldman, 1948).

The prevalence of toxoplasmosis in birds is not very well known making it impossible to assess their potential significance to public health. In view of economic importance of birds in Pakistan and their potential role in the zoonotic transmission of toxoplasmosis, the aim of this study was to determine the seroprevalence of *T. gondii* infection in birds.

Material and methods

A total of 200 serum samples (Ducks n =50, Turkeys n=50, Pigeons n=50, and Quails n=50) were collected at random from Quail Research Institute, University of Veterinary and Animal Sciences, Lahore and local pigeon shops. Under aseptic measures, 1-2 ml of blood was drawn by veni-puncture with the help of disposable syringes and was transferred to a screw capped sterile clean test tube slowly to avoid hemolysis (Benjamin, 1986). The samples were left for about an hour for blood clotting to occur. The clotted blood was then separated with a fine loop and serum samples were centrifuged at 3500 rpm for at least 5 minutes. The supernatant clean sterile serum, was aspirated with a Pasteur pipette and put in a screw capped vial and was stored at -20°C until process for analysis (Samaha *et al*., 1993). All the serum samples were analyzed for specific IgG *Toxoplasma* antibodies using LAT. For this purpose the commercial test kit namely "*Toxoplasma* Latex" manufactured by Quimica Clinica Apelicada, SA Amosta, Spain was used (Sydney and Kenneth, 1982).

Results and discussion

Data on seroprevalence of anti-*Toxoplasma gondii* antibodies in various captive birds are shown in Table I. Of 200 captive birds, 20 (10%) tested positive for anti-*Toxoplasma gondii* antibodies. The highest seroprevalence was observed in turkeys (16%) followed by ducks (12%), pigeons (8%) and quails (4%). Davidson *et al*. (1985) in South eastern states, El-Massry *et al*. (2000) from Giza (Egypt) and Quist *et al*. (1995) from West Virginia (USA) performed experiments on turkeys and reported 10-20% prevalence. The results of the present study are broadly consistent with the findings of Tsai *et al*. (2006), who examined 665 pigeon serum samples from Tiwan and reported 4.7% and 6% prevalence in eastern and northern areas, respectively. Harold *et al*. (2009) tested 495 serum samples of wild pigeons from Israel and recorded similar prevalence. Yan *et al*. (2009) tested serum samples of 394 ducks from Chinaand Burridge *et al*. (1979) tested 16 wild ducks in US. They recorded 16% and 6% seropositivity for anti-*Toxoplasma* antibodies, respectively. Butty (2009) reported higher prevalence of toxoplasmosis (76.6%) in turkeys from ten regions in Ninevah governorate. However, Literak and Hejlicek (1993) carried out an isolation experiment in 60 domestic ducks and only one bird (1.7%) was found positive. This variation in result could be due to different environmental, geographic and management conditions and also due to different ways of rearing.

Among 50 pigeons examined one gave an antibody titer of 1:256 which suggested the possible recent contact, one showed an antibody titer of 1:128 which was due to acquired or evolving immunity, whereas two gave an antibody titer of 1:16 which indicated residual or non-specific immunity as reported by Tsai *et al*. (2006).

Among 50 ducks examined one gave an antibody titer of 1:256 which suggested the possible recent contact, two showed an antibody titer of 1:128 which was due to acquired or evolving

immunity, whereas three gave an antibody titer of 1:16 which indicated residual or non-specific immunity as reported by Bartova *et al*. (2004).

Table I.- Distribution of anti-*Toxoplasma gondii* antibodies in captive birds by using Latex Agglutination Test (LAT).

Bird spp.	No. tested	Antibody titer (reciprocal)			Seropositivity	
		16	128	256	No.	%
Pigeons	50	2	1	1	4	8
Ducks	50	3	2	1	6	12
Turkeys	50	3	2	3	8	16
Quails	50	1	1	0	2	4
Total	200	9	6	5	20	10

Among 50 turkeys examined three gave an antibody titer of 1:256 which suggested the possible recent contact, two showed an antibody titer of 1:128 which was due to acquired or evolving immunity, whereas three gave an antibody titer of 1:16 which indicated residual or non-specific immunity as reported by El-Massry *et al*. (2000).

Among 50 quails examined, no quail gave an antibody titer of 1:256 which suggested no possible recent contact, one showed an antibody titer of 1:128 which was due to acquired or evolving immunity, whereas 1 gave an antibody titer of 1:16 which indicated residual or non-specific immunity as reported by Dubey *et al*. (1994).

In general low titer indicated post exposure and probable immunity and high titer of 1:256 strongly suggested present infection.

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Evaluation of Rearing *Cyprinus carpio* Fry on Freshwater Rotifer, *Brachionus calyciflorus**

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Abstract.- *Cyprinus carpio* fry reared on monogonont rotifer, *Brachionus calyciflorus*, cultured on mixed algae showed better results as far as their weights, lengths, specific growth rate (SGR) and condition factor were concerned. However, the fry reared on commercial diet had better survival rates than other two groups.

Key words: *Brachionus calyciflorus, Cyprinus carpio*, fry, rotifer.

Many small invertebrates such as rotifers, cladocerans, and copepods, form a large part of fish food (Tucker, 1988; Dominguez-Dominguez *et al*., 2002). The food webs, starting from algae cause bigger and indirect routes of carbon leading to fish (McCormick and Cairns, 1991). Rotifers directly use suspended organic substances (Pourriot, 1965) and indirectly consume dissolved organic substances when eating bacteria and protozoa (Arndt, 1993). Rotifers have an important position in the food chains of water bodies due to their large population density and extraordinary production rates (Wallace, 2002; Wallace and Snell, 2010).

While aquaculture can be a risky business, it can be very profitable. Zooplankton, particularly, rotifers, are required, because they are the preferred first-food of fish fry (Amornsakun *et al*., 2003; Hagiwara *et al*., 2001) and because they contribute to faster growth and survival of the cultured fish (Shamsaie *et al*., 2007). Rotifers of genus *Brachionus* have been extensively utilized as initial food for raising fish fry (Wallace, 2002; Hagiwara *et al*., 2007) and crustaceans in aquaculture (Lubzens *et al.,* 1989, 2001). *Brachionus calyciflorus* is one of the live food organisms used for the mass production of larval fish. This rotifer occurs in several strains of different sizes, which makes it fit for fish fry of various sizes. It is possible to isolate *B. calyciflorus*, to produce by batch culture and 'feed-back' culture systems. It can be supplemented with foods having EFA for better survival and growth of many fish species (Arimoro, 2006). Rotifers have great nutritional value for planktivorous fish because their proteins speed up growth of fish larvae and juveniles (Lim and Wong, 1997; Kitto and Bechara, 2004)

The aim of this study was to use *B. calyciflorus* to rear *Cyprinus carpio* fry and to explore their role in growth and survival of fry.

Materials and methods

Culturing rotifers for fish fry

A culture station comprising of 4 jars, of 1-gallon each, was set up (Wilkerson, 1998; Hoff and Snell, 1999). The jars were marked 1–4. A portion of the rotifer (*Brachionus calyciflorus*) starter culture: *i.e.*, 10–20 rotifers / ml (37850 to 75700 rotifers/gallon), was poured in each jar. The rotifers of jar 1 and 2 were fed on a mixed culture of algae, mainly *Nannochloropsis*, *Chlorella*, *Tetraselmis* etc. The rotifers of jar 3 and 4 were fed on *Chlorella* only. Optimum feeding rate was about 100 cells of algae per individual rotifer.

Rotifers were harvested when their densities were in the range of 150–200 individuals per ml. To obtain rotifers for feeding fish fry, water was siphoned from the culture container through a 55-µm sieve. Then fresh water was used to back flush the rotifers off the sieve in a plastic tub. About 50% of the container volume was drained daily to capture the rotifers. It was repeated until rotifer density dropped to the range of 75–100 individuals per ml.

Feeding rotifers to fish fry

Fry of *Cyprinus carpio*, about one day old, were used for experiment. Nine glass aquaria of 20-L capacity were used to rear fish fry, in triplicate per dietary treatment. About 200 fish fry were put in each aquarium. Fry of the 1st group were fed on rotifers reared on mixed culture of algae. Fry of the

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2nd group were fed on rotifers reared on *Chlorella* only. Fry of the 3rd group were fed on commercial diet Biokyowa-C 1000 (Kyowa Hakko Kogyo Co. Ltd., Tokyo, Japan). This product comprises 55% protein, 10% fat, 4% fibers, and 17% ash. Fry were fed at a rate of 3–5 rotifers per ml ($6x10^4$ to $1x10^5$ rotifers in each aquarium). The experiment was continued for 10 days during which time 10–20% of the water was replaced per day in each aquarium. Each day10 fry were randomly removed from each aquarium to estimate their weights and lengths. Fry weight was determined in mg by Sartorius digital scale (Model PT-120, 0.001 accuracy). Fry length was measured to the nearest mm.

Average increase in weight and length per day, condition factor, and specific growth rate, were determined according to FAO (1986).

The rotifer culture water was never put into the fry aquarium. Fry were fed normally twice a day.

Statistical analysis

EXCEL 2007 was used for all calculations (MEAN, STDEV, SEM, r values, average increase in weight and length per day, condition factor and specific growth rate, etc) and to make the graphs. ANOVA and correlation were determined by using MINITAB 13 for Windows.

Results

Table I shows the growth parameters of fry fed on mixed algae, rotifers and commercial diet. The average increase in weight of fry per day fed on mixed algae was 9.62±0.21 mg, while average increase in length per day was 1.59±0.03 mm. When fed on rotifers the average increase in weight and length of fry was 7.52±0.13 mg and 1.02±0.01 mm per day, respectively.

Feeding on *Chlorella* resulted in an increase of 6.02±0.05 mg in weight and 0.72±0.01 mm length per day.

Figure 1 shows the pattern of growth over a period of ten days fed on algae, rotifers and commercial diet. A positive correlation (Pearson) was observed among the weights and lengths of the fish fry reared on the three food types (Fig. 2).

Analysis of variance for average weights of the fry showed a no significant difference (P=0.608) among the weights of fry reared on three food types. However analysis of variance for average lengths of fry showed that lengths had slight significant difference at P=0.056.

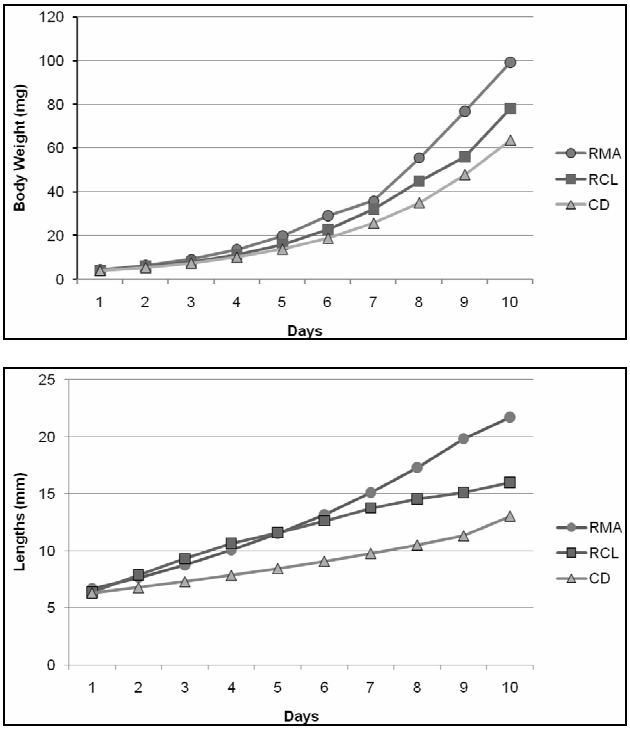

Fig. 1. Body weights and body lengths of *Cyprinus carpio* reared on three types of feed. RMA, rotifers reared on mixed algae; CRL, rotifers reared on *Chlorella*; CD, commercial diet (control).

Discussion

Food supply during early life of fish fry plays a very important role in survival and growth. Improper food supply may cause mass mortality of fish fry and juveniles (Houde, 1978). Various food regimes are needed for various species in their early lives. The food regimes utilized for fish production in Japan had been described (Watanabe *et al*., 1983). Generally, rotifers are the initial food for newly hatched fish having body length more than 2.3 mm.

In this study of *Cyprinus carpio* fry fed with rotifers showed better results as far as their weights, lengths, specific growth rates (SGR) and condition factors were concerned (Shamsaie *et al*., 2007). However, fry reared on a commercial diet had better

Table I.- Growth performance of *Cyprinus carpio* fry reared on mixed algae, *Chlorella* and commercial diet.

Diets	Fry fed on					
	Commercial diet		Mixed diet		*Chlorella*	
	Day 0 (n=3)	Day 10 (n=3)	Day 0 (n=3)	Day 10 (n=3)	Day 0 (n=3)	Day 10 (n=3)
Body weight (mg)	2.91±0.06	99.15±2.05	2.9±0.07	78.11±1.33	2.91±0.11	63.58±0.63
Average Increase in weight (mg/day)	9.62±0.21		7.52±0.13		6.07±0.05	
Body length (mm)	5.85±0.07	21.70±0.33	5.81±0.04	16.01±0.04	5.84±0.04	13.02±0.10
Average increase in length (mm/day)	1.59±0.03		1.02±0.01		0.72±0.01	
Condition factor (final)	9.75±0.64		19.02±0.33		28.42±0.80	
Specific growth rate (%/day)	35.29±0.41		32.94±0.15		30.87±0.28	

* – Means **(±1sd).**

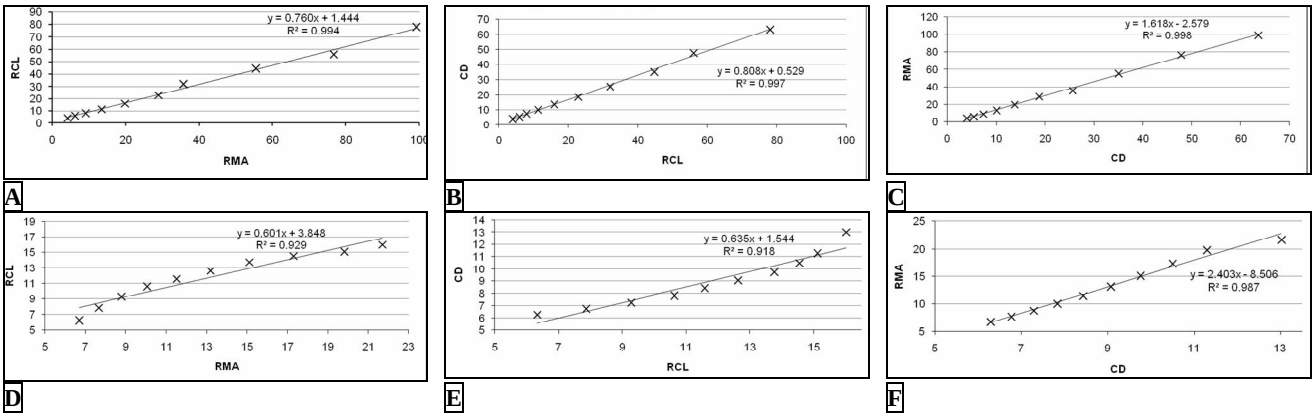

Fig. 2. Correlation between weights (A, B, C) and lengths (C, D, E) of *Cyprinus carpio* fry fed on Rotifers reared on mixed algae (RMA), and (RCL), and Commercial diet (CD). A, D, correlation of RCL and RMA, B, E, RCL and CD; C, F, CD, RMA.

survival rate than those fed on rotifers. This was contrary to the work of Shamsaie *et al*. (2007) these workers reported that use of rotifers as live food for fry provided better survival rate than the other diets they used. Rotifers have been proved to be better initial live food for fish fry (Amornsakun, 2003; Ludwig, *et al*., 2008). Rotifers cultured on mixed algae (*Nannochloropsis*, *Chlorella*, *Tetraselmis*) were a better initial food when compared to rotifers cultured on *Chlorella* only (Kobayashi, 2008). This may be because *Nannochloropsis* and *Tetraselmis* contain essential fatty acids [EFA: *i.e*., docosahexaenoic acid (DHA); eicosapentaenoic acid (EPA); arachidonic acid (ARA or AA)] which are required for survival and growth of fish larvae (Kanazawa *et al*., 1979; Watanabe, 1993; Rodriguez *et al*., 1997). First feeding fry have a large neurosomatic index, and high demand for EFA, which must be fulfilled by the food to avoid neural dysfunction (Bell *et al*., 1995; Sargent *et al*., 1997).

Microalgae may have different compositions of fatty acids depending upon culture technique and species (Volkman *et al*., 1989). High concentrations of vitamin B_{12} are present in *N. oculata*, and play an important role in the survival of fish larvae. Vitamin B_{12} also is found to be vital for increasing resistance to diseases in fish larvae (Marini, 2002). Rotifers enriched by fatty acids, either by live food (Gatesoupe, 1991) or commercial food, are thought to be the better initial diet for fish fry (Kenzo *et al*., 2003; Rawles *et al*., 2007). This finding is in agreement with the work presented here; rotifers fed on mixed live algae possess high concentrations of fatty acids. Thus rotifer is the best initial feed for fish fry.

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Prevalence of ABO and Rh Blood Groups in Students of University of Sargodha, Punjab, Pakistan

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Abstract.- The aim of the present study was to record the prevalence of ABO and Rh blood groups among the graduate and post graduate students of University of Sargodha. A total of 1341 individuals (449 males and 892 females) were screened for blood group testing. Blood group B was the most common in both sexes. The AB blood group was least common. About 90% individuals were Rh positive. The frequencies of ABO and Rh blood groups are similar to those reported from other regions of Punjab. Data generated with this study will be helpful to the health planners, blood banks and blood donating societies in the area.

Keywords: ABO blood groups, Rh factor, blood transfusion.

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In humans, approximately 400 red blood antigens and 19 blood groups systems have been identified (Rehman *et al.,* 2005; Khattak *et al.,* 2008). Both ABO and Rh blood group systems are considered important during blood transfusions. Although the blood group systems are same among all human beings but the prevalence of ABO and Rh groups is different in various parts of the world and in different races. The study of ABO blood group system is of immense interest, due to its medical importance in different diseases and its role in blood transfusion, forensic pathology and its association with different diseases like duodenal ulcer (Hoffbrand and Pettit, 2006), diabetes mellitus (Garraty *et al.,* 2000) and urinary tract infection (Rehman *et al.*, 2005). Blood group system is considered one of the strongest predictors of national suicide rate and a genetic marker of obesity (Mollison *et al.,* 1993; Hein *et al.,* 2005). Study of blood group is helpful to know the genetic history of a person (Sokolov, 1993). For example, protein intake is very high among the individuals of blood group O and they generally secrete higher stomach acids and experience more incidence of gastric ulcer disease than the other groups. However, individuals of blood group A are associated with vegetarian food sources and secrete smaller amounts of stomach acid and have lesser chances for gastric ulcers, heart diseases, cancer and diabetes (Viola and Carolyn, 1991). From above review it is clear that blood group determination is very important in clinical practice. Keeping in view of this fact present study was planned. Aim of this study was to investigate the prevalence of ABO and Rh blood group systems in district Sargodha and to compare the results with other studies conducted in Pakistan and in other parts of the world.

Materials and methods

The study was conducted during the month of February, 2011 and March, 2012. Only the data of graduate and post graduate students (both males and females) belonging to Sargodha region were included in this study. Each donor was informed about the purpose of the study. Blood samples were taken under aseptic conditions using finger prick method. ABO and Rh blood groups were determined by agglutination test using antisera-A, antisera-B and antisera-D. A total of 1341 (892 females and 449 males) individuals were screened for the blood group testing. The number of the females screened for the ABO and Rh blood groups was high as in University of Sargodha ratio of females students is very high.

Results

A total of 1341 students were screened for blood group testing. Percentage of A, B, AB and O blood groups among male and female students is given in Table I. In both sexes the most common blood group was B, followed by O and A. AB blood group was lowest in frequency. Out of the total samples, 90.43% males comprised of Rh positive and remaining 9.57 were Rh negative. However, in females frequency of Rh positive and Rh negative was 88.91% and 11.09%, respectively (Table II).

Table I.- Frequency of ABO and Rhesus blood groups among graduate and postgraduate students of University of Sargodhar.

	Male		Female	
	No.	%	No.	%
Blood group				
A	98	22.0	163	18.0
B	179	40.0	369	41.0
AB	53	12.0	119	13.0
O	119	26.0	241	274.0
Rh blood group				
+	402	90.0	793	89.0
-	43	10.0	99	11.0

Discussion

ABO blood group system is considered the most important during blood transfusions as the most of deaths from blood transfusion occurs when incompatible type of ABO blood is transfused (Sarban, 2009). It has also been found that susceptibility of certain diseases is linked with the ABO blood groups. According to Stayboldt *et al.* (1987) gastric cancers are more common among the persons of A blood group. Furthermore, coronary heart disease, ischemic heart disease, venous thrombosis and atherosclerosis are also more frequent among the individuals with A blood

group. The individuals of blood group O are more susceptible to gastric and duodenal ulcers (Hoffbrand and Pettit, 2006). Khan *et al.* (2010) also found higher incidence of male infertility among the individuals of O blood group.

Table II.- Comparison of distribution of rhesus (Rh) blood groups in the current study and other parts of the world.

Study area	Rh positive (%)	Rh negative (%)
Bahrain	94.5	4.5
Britain	83	17
India (Punjab)	97.3	2.7
Iran	88.7	11.3
Kenya	96.1	3.9
Nigeria	95.67	4.33
Present study	**89.67**	**10.33**
Saudi Arabia	92	8
USA	85	15
Yemen	92.9	7.1

Note: Data of different countries for comparison was taken from Khattak *et al.* (2008).

In our study the most common ABO blood group was B, followed by O and A. Most of the studies conducted in Pakistan described the similar general pattern of distribution of ABO blood group (Rehman *et al.*, 2005; Anees and Mirza, 2005; Hussain *et al.*, 2001). Khan *et al.* (2004) conducted a study to record the prevalence of ABO blood group system in Bannu region NWFP. According to this study frequency of B was highest (36.23%), followed by A (31.03%), O (25.07%) and AB (7.67%). In Punjab and NWFP B blood group is most prevalent (Rahman and Lodhi, 2004; Khan *et al.*, 2005; Majeed and Hayee, 2002). However, in Sind and Baluchistan O group is predominant (Amjad *et al.*, 2002; Hussain *et al.*, 2001; Khaskheli *et al.*, 1994). The difference in the distribution of blood group is expected due racial variation in different provinces.

Das *et al.* (2001) reported the blood group O (38.75%) as most common from South India, followed by B (32.69%) and A (18.85%). Another study from India (Punjab) showed highest prevalence of B (37.6%) blood group, followed by O (31.2%) and A (21.9%). Shaik and El-Zyan (2006) reported O (34.7%) as most common blood group from Iran. It was followed by A (33.1%) and B (23.3%). Distribution pattern of ABO blood group was same in Saudi Arabia, Oman, Kuwait and Bahrain with O as the most common blood group followed by A and B. O blood group is characteristic of these countries. Prevalence of AB blood group was less than 5% in these countries (Sarban, 2009).

Of the total 1341, about 90% individuals were Rh positive in the present study. A comparison of Rh blood groups of the current study and with other studies in Pakistan and studies from other parts of the world is given in Table III. It is clear from the comparison that there is shortage of Rh negative blood group in the population.

It is concluded from the study that, like other regions of Punjab, the most common blood group in the Sargodha region is B. AB blood group is the lowest in frequency. About 90% of the studied population was with Rh positive blood group. This information is important for the blood banks and blood donating societies as well as health planners.

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Potato: A New Host Plant of *Tuta absoluta* Povolny (Lepidoptera: Gelechiidae) in Turkey

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Abstract.- Tomato moth (*Tuta absoluta* Povolny) damaging the tomato plant was first observed in Turkey in 2010. The population of *T. absoluta* was monitored weekly and the damage was assessed in the potato growing areas of Karapinar Station of Konya Source of Soil and Water Research Institute. The pheromone traps were also used to assess the population during the blooming period. The number of mature males trapped in one week rose up to 224.

Key words: *Tuta absoluta*, Potato, a new host plant.

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Tomato moth (*Tuta absoluta* Povolny) is a serious pest which damages the vegetative and generative parts of the plant in Latin American countries such as Peru, Argentina and Brazil to many European, African and Asian countries. Its presence in tomato was reported in greenhouses in Izmir (Kilic, 2010), in Antalya (Erler *et al*., 2010) and reported in tomato greenhouses of Mersin province with a maximum fruit infestation rate of 38.4% (Karut *et al*., 2011). Even though the main host of *T. absoluta* is tomato, some other solanaceous crops, weeds and potato have been reported as the hosts in several countries (Galarza, 1984; Notz, 1992; CIP, 1996; Pereyra and Sánchez, 2006).

Potato, a carbonhydrate source crop highly important as a human nutrition, has been mainly grown in Nigde and Nevsehir provinces of Turkey. Unfortunately, due to some diseases such as the potato wart disease especially the seeding material production has been shifting to other regions such as Konya where the leading location of these new growing areas.

The main objective of this study is to determine adult population of *T. absoluta* on potato using pheromone traps in Karapinar Research Station potato growing area, Konya, Turkey.

Materials and methods

To monitor adult populations of *T. absoluta*, three delta type pheromone traps were placed in two potato fields in Karapinar Research Station with 2 km distance from each other. One of the fields planted potato in previous year was a 7 hectares and the other one was a 0.1 hectare. There was no other host for *T. absoluta* in the research area.

Two pheromone traps (Production 1 and 2), obtained from TRECE Inc., were placed in production field and one (Experimental) was placed in research field at the flowering stage of the potato, and they were monitored weekly till the harvest season. The capsules and the sticky parts of the pheromone traps were replaced monthly.

Results and discussion

Population development and crop damage of *T. absoluta* were investigated on potato being a new host for the pest in Turkey. It was observed that

when in absence of tomato as host, *T. absoluta* fed on potato and caused damage in economical level.

Adult population development of the pest determined through the pheromone traps is shown in Figure 1.

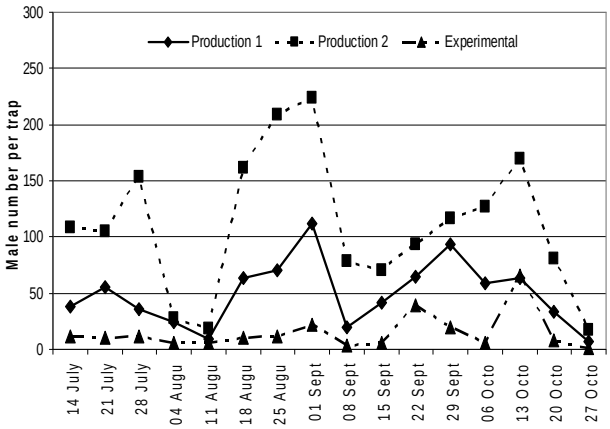

Fig. 1. Population development of *Tuta absoluta* on potato, in the two production fields and one experimental field.

To monitor *T. absoluta* populations on potato three pheromone traps were placed in two different potato production field, during the flowering time on July 7, 2011. The number of adults caught by traps indicated that there were three peaks, first one was the late July (28.07.2011), second one was at the beginning of the September (01.09.2011) and the last one was at mid-October (13.10.2011) (Figure 1). The variations in the number of captured moths between the traps could be related to the different sizes of the fields.

Results revealed that *T. absoluta* damages potato and has three generations in a year. It is assumed either they do not have diapauses thus they would move to the tomato growing greenhouses where, adjacent to the potato fields, or to the over-wintering sites. This may be explained why they cause damage on potato in Karapinar district where adjacent to Cumra where district having tomato greenhouses and fields.

Tuta absoluta, an economically important pest of tomato, can feed and cause damage on potato during absence of tomato. After the ban of potato growing in Nigde and Nevsehir provinces due to the potato wart disease, *Synchytrium endobioticum* (Schilbersky) Percival), the demand for potato growing in Konya region is become very important. Possibility of economical losses of potato due to *T. absoluta* in Konya and the other regions concern growers. Therefore, control actions against *T. absoluta* must be taken before its spreading throughout the potato growing areas to preclude from its harm. Results shows that population level is substantial and has a capability to threaten the potato production. Moreover, the pest can also spread to the tomato greenhouses in Konya when the winter is not so harsh. Since the pest has been detected in Turkey recently, besides potato monitoring other possible hosts is crucially important to prevent its spreading and building large populations. Authorized governmental units should inform growers and have them take necessary actions against the pest.

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Occurrence of Pathogenic Bacteria in Small Mammals - Inhabiting Poultry Farms of Rawalpindi/Islamabad, Pakistan

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Abstract.- This study was designed to determine the prevalence of pathogenic bacterial species in black rat (*Rattus rattus*), house mouse (*Mus musculus*) and mongoose (*Herpestes javanicus*) residing at poultry farms in Rawalpindi/Islamabad. For this purpose, sixty seven specimens (black rat 46; house mouse 16; mongoose 5) were captured to check the presence of bacterial species in faecal matter, urine and blood of these species. The faecal matter of black rat was found to be contaminated with *Escherchia coli* (89.13%; 41), *Salmonella* spp. (58.69; 27), *Proteus* spp. (32.6%; 15). *Salmonella* spp. (30.4%; 14) was also isolated from urine samples of black rat. Similarly the faecal matter of house mouse was contaminated with *E. coli* (37.5%; 6), *Salmonella* spp. (25%; 4), and Proteus spp. (18.75%; 3). Mongoose urine was contaminated with *Klebsiella* spp. (20%; 1). However the blood samples of all the species captured from the poultry farms were found negative for *Salmonella* spp., *E. coli*, *Klebsiella* spp. and *Proteus* spp. In conclusion, black rat, house mouse and mongoose are the main reservoirs for the bacterial species at poultry farms of Rawalpindi/Islamabad.

Keywords: *Salmonella*, poultry farm, mongoose.

The vertebrate pests residing at poultry farms, can cause considerable damage to the food, food products, buildings, stored products, and also serve as potential source of pathogenic diseases in human and animals. These animals transmit the bacteria through faeces, urine, and hair remnants (Padula *et al*., 2000; Mehmood *et al*., 2012).

Rural livestock, spilled feedstuffs, water and availability of shelter favours the formation of vertebrate pest colonies in the vicinity of poultry farms (Meerburg *et al*., 2006; Leirs *et al*., 2004) which are potential risk for the birds (Henzler *et al*., 1998). These pests serve as reservoir of bacterial species which can transmit diseases to the environment, poultry feed and animals (Rose *et al*., 2000). The bacterial incidences can be controlled through pest control measures in hen houses (Henzler *et al*., 1998).

In previous studies, bandicoot rat and shrews such as bandicoot rat present at poultry farms are a source of pathogenic bacterial infections (Mehmood *et al*., 2011, 2012). To our knowledge the occurrence of bacterial species in house mouse, black rat and mongoose residing at poultry farms has not been studied. Therefore present study was designed to identify the occurrence of pathogenic bacteria in black rat, house mouse and mongoose inhabiting poultry farms in Rawalpindi/Islamabad.

Materials and methods

The study was conducted from November 2007 to October 2008. A total of 67 animals (house mouse, black rat and mongoose) were captured by live traps from seven poultry farms of Rawalpindi/Islamabad. Traps were baited at night with chicken feed, butter chapatti and carrots and checked for trapped animals each morning during sampling. The samples were immediately transported to Animal Physiology laboratory, Department of Zoology, Arid Agriculture University, Rawalpindi. Captured animals were euthanized with chloroform. The samples were dissected and blood samples were taken by cardiac puncturing. Urine samples were taken by rubbing sterilized cotton swab from urinary bladders of the dissected animals. Faecal samples were taken on a sterilized spatula by cutting large intestine. All samples were taken individually and aseptically after dissection to avoid contamination from external sources (Mehmood *et al*., 2011, 2012).

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Table I.- Occurrence of *Salmonella* spp. *Escherichia coli, Klebsiella* spp. *and Proteus* spp. in Black Rat (*Rattus rattus*), House Mouse (*Mus musculus*) and Mongoose (*Herpestes javanicus*) in the faecal matter (F), urine (U) and blood (B) at poultry farms of Rawalpindi/Islamabad, Pakistan.

Species	Total No. of animals captured	*Salmonella* spp. (%)			*Escherichia coli* (%)			*Klebsiella* spp. (%)			*Proteus* spp. (%)		
		F	U	B	F	U	B	F	U	B	F	U	B
Black rat	46	58.69 (27/46)	30.4 (14/46)	0	89.13 (41/46)	30.4 (14/46)	0	0	0	0	32.6 (15/46)	2.17 (1/46)	0
House mouse	16	25 (4/16)	0	0	37.5 (6/16)	0	0	0	0	0	18.75 (3/16)	0	0
Mongoose	5	0	0	0	0	0	0	0	20 (1/5)	0	0	0	0

Culture media was prepared by adding 63g of SS agar and trip in one litre of distilled water. The solution was heated with frequent agitation and allowed to boil for one minute to completely dissolve the agar. The mixture was cooled to about 50°C, mixed and poured into sterilized Petri dishes. The Petri plates were allowed to dry for two hours for further bacterial culture (Mehmood *et al*., 2011, 2012).

All samples were subjected to incubation in Selenite broth (Oxoid). After incubation at 37°C for 24 hours, samples were checked for bacterial growth. A loopful of each sample was inoculated onto a plate of SS agar (Oxoid) at 37°C for 36 hours. After inoculation period bacterial species grew as dew drop colonies and were identified using biochemical tests (Mehmood *et al*., 2011, 2012).

Results and discussion

The data on the occurrence of *Salmonella* spp., *Escherichia coli, Klebsiella* spp. *and Proteus* spp. in faecal matter, urine and blood of black rat, house mouse and mongoose residing at poultry farms in Rawalpindi/Islamabad are given Table I. The faecal matter of black rat was found to be contaminated with *Escherchia coli* (89.13%), *Salmonella* spp. (58.7) and *Proteus* spp. (32.6%). *Salmonella* spp. (30.4%) was also isolated from urine samples of black rat. Similarly the faecal matter of house mouse was contaminated with *Escherchia coli* (37.5%), *Salmonella* spp. (25%), and *Proteus* spp. (18.8%). Mongoose urine was contaminated with *Klebsiella* spp. (20%). However the blood samples of all the species and faecal matter of mongoose captured from the poultry farms were found negative for *Salmonella* spp., *E. coli, Klebsiella* spp. and *Proteus* spp.

It is well recognized that rodents are the reservoirs of pathogenic bacteria at poultry farms (Meerburg *et al*., 2006; Hiett *et al*., 2002; Heuer *et al*., 2001; Mehmood *et al*., 2011, 2012) and have definite association with the occurrence of bacteria in poultry meat (Arsenault *et al*., 2007) and eggs (Cogan and Humphrey, 2003) which are the hazards for human health. Mehmood *et al*. (2011) have reported the presence of *E. coli* (62%), *Proteus* spp. (13%) and *Salmonella* spp. (69%) in faecal matter and urine samples of house shrew *Suncus murinus*. In Japan, occurrence of *Salmonella* spp. was reported in rat liver (14.6%), intestine (10.6%), and in environmental samples (floor litter, egg belt, fan and attic litter). Several bacterial species like *E. coli* (7%), *Salmonella* spp. (20%), *Proteus* spp (15%) and *Klebsiella* spp (275%) were reported to be present in urine and faecal samples of bandicoot rat (*Bandicota bengalensis*)captured from the poultry farms of Rawalpindi and Islamabad (Mehmood *et al*., 2011). Isabel *et al*. (2004) reported 6% occurrence of *Salmonella* spp. in black rat at poultry farms in Argentina. In other studies occurrence of *Salmonella*, *Escherchia coli* was recorded overall 23.5% from liver, spleen and intestine of house mice, poultry farms in Japan (Henzler and Optiz, 1992). These studies suggest that bacteria carrier pests are associated with the infection of *Salmonella* and *Klebsiella* in the poultry and poultry products

(Meerburg *et al*., 2006; Pocock *et al*., 2001; Rose *et al*., 2000; Leirs *et al*., 2004).

The horizontal transmission of the bacterial species in rodent colony is common and rapid (Welch *et al*., 1941). Davies and Breslin (2003) believed that susceptible rodent populations in commercial poultry farms are supposed to be a reservoir of many pathogens. Moreover, the risks for salmonella presence in laying hens is highly associated with poor rodent control and a poor standard of cleaning and disinfection (Henzler and Optiz, 1992). The higher rodent population density in a poultry facility has high risk of the *Salmonella* spp. transmission in poultry products (Meerburg *et al*., 2006; Pocock *et al*., 2001).

The risk of bacterial infections from rodents to the birds on the poultry farm can be reduced by applying suitable control measures (Van De Giessen *et al*., 1998). In conclusion, it is recommended to adopt suitable strategies to eliminate the small mammals populations from the poultry farms which serve as potential reservoir of bacterial species, which can infect the poultry and poultry products and can cause human health problems.

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Isolation and Growth of Human Keratinocytes from Plucked Hairs

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Abstract.- Hair follicle is part of epidermis that is embedded into dermis. The multipotent stem cells of hair follicle have the ability to differentiate into different type of cells including keratinocytes, muscles, melanocytes and neurons etc. Keratinocytes are usually grown on feeder layer or serum free medium. In the present study hair follicle cells were isolated and grown by two different methods *viz.* direct outgrowth and enzymatic treatment. In the case of direct outgrowth method, the hair was made to adhere to plastic surface for 24 h and after

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5-6 days in culture medium cells started migrating out. In enzymatic treatment method only a few cells got adhered to plastic surface and they did not grow further. In conclusion we can say that direct outgrowth method is more suitable way to isolate and grow cells from plucked hair which is non-invasive source to get keratinocytes and stem cells that have multi differential potential.

Keywords: Keratinocytes, multipotent stem cells, hair follicles, epidermis.

The human epidermis is a thin non-vascular layer comprising epithelial keratinocytes which is produced by multipotent stem cells continuously. Skin fibroblasts reside in thicker dermis layer below epidermis which is constantly shedding its cells (Fuchs, 2007). Different type of cells including keratinocytes, fibroblasts, endothelial cells and melanocytes can be cultured from same skin sample. The hair follicle is a part of epidermis that is deeply embedded in dermis and may comprise rapidly dividing cells, slow dividing cells or those at resting phase. In the bulge of hair follicle there are stem cells that finally differentiate into keratinocytes. The cells which surround the hair follicle and are continuous with epidermis can be easily isolated after plucking of hair (Amoh *et al.*, 2009). The stem cells of hair follicles not only have the ability to differentiate into keratinocytes but they can also differentiate into other cell types. These stem cells also form epidermal keratinocytes after wound healing (Ito *et al.*, 2005). After the injury some cells come out of follicle, migrate and proliferate and help in wound repair (Taylor *et al.*, 2000).

Culturing of keratinocytes is not easy as traditionally there was use of feeder layer to grow keratinocytes and there was rapid apoptosis in cells without feeder layer (Rheinwald and Green, 1975; Tenchini *et al.*, 1992). Currently efforts have been made to grow the cells in serum free low calcium medium. This approach has an advantage that there is no contamination of feeder cells but have disadvantage that cell proliferation is very low (Aasen and Belmonte, 2010).

In the present study we have described two methods to isolate hair follicle cells and then grow them for specific period of time in serum based medium without feeder layer.

Materials and methods

Reagents

Phosphate-buffered saline (PBS), Penicillin/streptomycin cocktail, 0.25% (wt/vol) (PAA, Austria-P11-010) Trypsin/EDTA (PAA, Austria-L11-004). DMEM (GIBCO, Invitrogen), Heat-inactivated FBS (PAA, Austria-A11-104), Glutamine (200mM Stock), 2-Mercaptoethanol, PBS without calcium and magnesium, Gelatin 0.1% (wt/vol) solution, Dimethyl sulfoxide (DMSO).

Isolation and growth of cells

A non-coated 100-mm bacterial plate was prepared containing phosphate buffered saline (PBS) and antibiotics (penicillin/streptomycin). Tweezers was used to gently pull out human hair from the occipital part of head and immediately placed in PBS to avoid drying of cells. After washing of cells in PBS, hair were shifted to DMEM medium. While hairs were submerged in medium, external part of the hair were cut off leaving behind only the bulb and the outer root sheath (ORS).

Direct outgrowth method: The hairs were placed in coated culture plate (NUNC). Few drops of DMEM (with 15% FBS) were added to keep the hair moist for 24 h at 37^{o}C in humidified environment and allowing them to stick to the culture dish. Medium (5 ml) was added to culture dish and incubated again at 37^{o}C in humidified environment with change in medium after 2-3 days until the outgrowth of epithelial keratinocytes became visible.

Enzymatic digestion method: Hair were pulled out in anagen growth phase (10-12 hair), rinsed immediately with PBS, and placed in Petri dish. the ORS area was cut into three pieces with the help of scalpel. 1 ml trypsin was added into 1.5 ml eppendorf and hair pieces were shifted to it for 15 min with gentle shaking. After 3 min. the mixture was transferred to 15 ml Falcon tubes and 5ml completed medium was added into it. Vigorously pipetted up and down to obtain single cells from plucked hair, and then centrifuged at 200xg for 5

min. The pellet was re-suspended in complete medium and the number of cells were counted by hemocytometer and added in 6 well plate followed by incubation at 37°C in humidified environment for 3 days.

Results

Direct outgrowth

When plucked hair bulbs were directly incubated in complete DMEM medium, there was outgrowth of epithelial keratinocytes appeared after 4-6 days. The cells were observed migrating out around the hair ORS region. The cells started dividing in the medium and there was increase in number (Fig. 1B). There was outgrowth of cells from hair ORS and cells showed typical morphology of epithelial keratinocytes (Fig. 1C)

Enzymatic digestion

When cells were digested with trypsin and plated in culture plate, they were observed to be present as single cell suspension (Fig. 2A). When medium was changed after 4 days of incubation only few cells were observed to adhere to the plastic surface (Fig. 2B) and most of the cells were still present in suspension form. Morphologically adherent cells were spindle shaped. Although the medium was changed after every 3 days for 20 days but they didn't further divided and no increase in number of cells was observed.

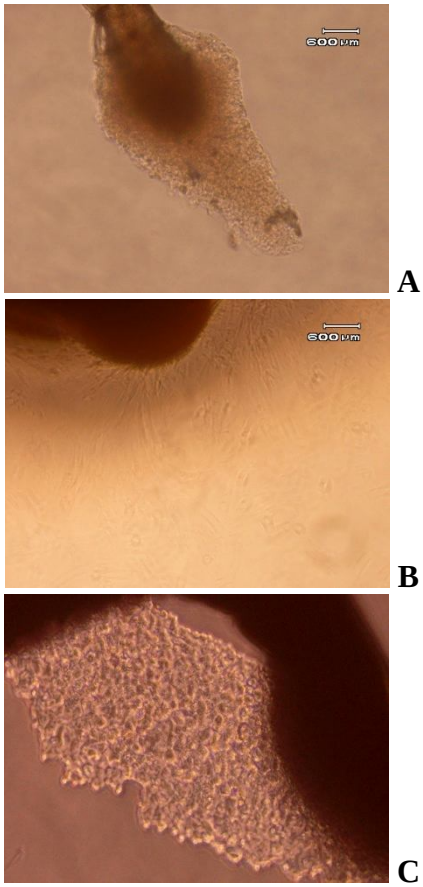

Fig. 1. Direct outgrowth of human keratinocytes from plucked hairs.

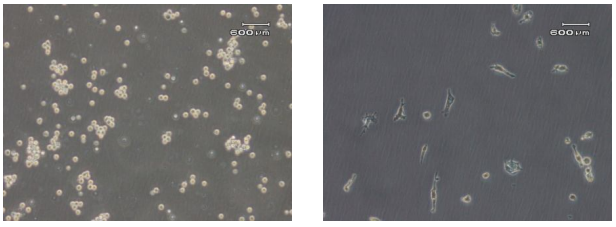

Fig. 2. Growth of hair follicle keratinocytes after enzymatic digestion.

Discussion

Initially, researchers used to think that adult mammalian stem cells can only differentiate into their tissue of origin but new research reports proves that tissue based stem cells are much more plastic than previously thought (Bjornson *et al.*, 1999; Clarke *et al.*, 2000). It was also thought that multipotency of adult stem cells is due to their cellular fusion *in vivo*, which actually is not the case (Jiang *et al.*, 2002). Each hair follicle passes through three stages: growth (Anagen), involution (catagen) and rest (telogen). Stem cells in the hair bulge can generate epidermis and sebaceous gland (Cotsarelis *et al.*, 1989, 1990; Tumbar *et al.*, 2004). These cells are also reported to differentiate into melanocyte lineage (Morris *et al.*, 2004; Taylor *et al.*, 2000; Nishimura *et al.*, 2002). In addition nestin positive cells in bulge area can also give rise to smooth muscle cells, melanocytes and neurons (Amoh *et al.*, 2005). Recent studies have shown that stem cells in hair follicle also serve as local reservoir of mast

cells precursors (Kumamoto *et al.*, 2003). All these findings suggest that these hair follicle stem cells are unique type of adult stem cells that can differentiate into variety of cells. In addition hair follicle cells are easily accessible.

There are various ways to culture keratinocytes (Detmar *et al.*, 1993). Most often direct outgrowth culture approach is used for plucked hair (Well, 1982; Moll, 1996). In addition keratinocytes are also obtained and cultured by enzymatic digestion of hair (Limat and Noser, 1986). Both of these approaches have advantages and disadvantages. In our study we could get growing cells in case of direct outgrowth method. After few days of culture, cells started moving out from hair ORS (Fig. 1) while in case of enzymatic digestion, although we could get good number of isolated cells but only few of them get attached to plastic surface. In addition the attached cells could not divide further. This was probably due to enzymatic treatment of cells and they might have lost important cell surface proteins. It is also reported that cells isolated after hair plucking give transient amplifying cells that can be grown for short period of time. In order to get the stem cells for long term culture mostly tissue micro dissection is done to isolate complete bulge and dermal papilla (Aasen and Belmonte, 2010).

Isolation of cells from plucked hair is non-invasive procedure to isolate and culture cells that can be used in different basic research experiments and clinical applications. In the present study we described two different methods to isolate cells from plucked human hair. The cells were isolated and grown successfully as outgrowth culture.

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