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Diversity of Intestinal Parasites in Male and Female Students and Workers of Education Department of Swat, Pakistan

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Abstract.- Fecal samples of 420 individuals including 263 males and 157 females were examined for parasites. The parasites were found in 227 (65.9%) individuals, 93.9% helminthes and 6.06% protozoans. The most common helminths were *Ascaris lumbricoides, Trichuris trichura* and *Taenia saginata*, while among protozoans *Entamoeba histolytica* had the highest prevalence. The infection with one parasite species was 40.7%, with two 19.2%, with three 5% and with four 0.95%. The prevalence rate of *A. lumbricoides, T. trichura, Enterobius vermicularis,* hook worm, *T. saginata, Hymenolepis nana, E. histolytica* and *Giardia* species were respectively 40%, 19%, 8%, 3.6%, 12.8%, 10%, 4.4% and 1.69%. Males were found to be more infected than females.

Key words: Protozoan and helminth parasites, parasitosis.

The helminth parasite are widely distributed. More than 3.5 billion people are infected with intestinal worms; of these 1.47 billion have roundworms, 1.3 billion are infected with hookworms and 1.05 billion with whipworms. It is estimated that about 400 million school-age children are infected with these helminth parasites. Children between 5 and 15 years have hookworm infection which tend to grow with increasing age. Therefore, adolescent girls and women of childbearing age are generally infected with hookworms (Chan *et al.*, 1994; Luong, 2002).

This report deals with the prevalence of intestinal parasitic infections in the male and female students and workers of Education Department of Swat, Pakistan.

Materials and methods

The study area Swat lies between 34°34–35°55 N and 72°08 –72°50 E, with an elevation range from 4500 m to 6000 m.

A total of 420 individuals including 263 male and 157 females participated in this study. The participants were instructed to collect about 10 g of the personnel single stool specimens in wide-mouth plastic bottles with 10% M.I.F (merthiolate, iodine, formaldehyde) preservatives. The specimens were brought to the laboratory for parasitic examination. The faecal samples were examined for the presence of adult or segmental stage of parasites. Wet mount techniques in a fresh normal saline solution and Lugol's iodine solution were used for microscopic examination. The floatation, centrifugation and sedimentation techniques were used to confirm the negative cases.

Results

Out of 420 samples 277 (66%) were found positive for parasitic infections. The mean age of male and females were 18.9 and 20.7 years, respectively.

Table I shows the total number of infected cases, single infections of 100 males and 71 females multiple infections of 78 males and 28 females. More than half of the individuals had single infection. Helminths were found more prevalent than protozoan parasites in single as well as in the association with other species of parasites. Table II shows prevalence of nematodes, cestodes and protozoans in male and female. Male are found to be more infected with cestodes and *Giardia* sp., whereas females were more infected with nematodes and *Entamoeba histolytica*.

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Table I.- Prevalence of single and multiple parasitic infections in individuals (staff, students and workers) related to Education Department of Swat, Pakistan.

Types of parasites	Male	Female	Total	%
Single parasite species	100	71	171	61.7
Helminths	95	64	159	57.4
Protozoans	5	7	12	4.33
Multiple parasite species	78	28	106	38.3
Helminths	68	25	93	33.5
Protozoans	0	0	0	0
Helminths + Protozoans	10	3	13	4.69
Total infected individuals	178	99	277	

Table II.- Prevalence of nematodes, cestodes and protozoans in male and female individuals related to Education Department of Swat, Pakistan.

Parasites	Male	Female	Total	%
Nematodes				
Ascaris lumbricoides	108	56	164	39.8
Trichuris trichura	49	30	79	19.1
Enterobius vermicularis	18	16	34	8.25
Hook worm sp.	8	7	15	3.64
Cestodes				
Taenia saginata	42	11	53	12.8
Hymenolepis nana	37	5	42	10.1
Protozoans				
Entamoeba histolytica	9	9	18	4.36
Giardia sp.	6	1	7	1.6
Total No. of infections	*277*	**135**	**412**	
Mean±SD		35.7± 34.6	17.8± 16.8	52.7± 51.5

Discussion

Present study for intestinal parasites in Swat, Pakistan revealed 65.9% of parasitosis. The prevalence of *A. lumbricoides* infection was reported 68.7% by Stoddart (1999), 24.1% Akhtar *et al.* (1993), and 31.0% in Kurram Agency by Ali (1993).

The present study shows 39.8%, 19.1%, 9.81%, 3.64%, 12.8%, 7.98%, 5.98% and 3.03% prevalence of *A. lumbricoides, T. trichura, Enteroblus vermicularis*, hook worm, *Taenia saginata, Hymenolepis nana, Entamoeba histolytica* and *Girdia* sp. in human population of Swat. *T. trichura* infection has been reported to to 19.1% as against 22.5% by Stoddart (1999) and 7.8% by Ali (1993). The prevalence of *Enterobius vermicularis* has been reported to be 8.25% by Pal and Malik (1979) and 13.8% by Jamil (1999). The prevalence of hook worm has been reported to be 6.1% by Pal and Subhani (1989), 4.23 and 4.27% in Larkana and Shikarpur by Shaikh *et al.* (2000, 2003).

Cameron (1960) observed that in the areas where annual rainfall is between 30 and 40 inches hook worm infection is light. The eggs of *T. trichura* are much less resistant to temporary drought and heat than are *A.lumbricoides* eggs and will not survive in direct sun rays and intense cold (Faust *et al*., 1976)

The prevalence rate of *T. saginata* was reported to be 7.1% by Akhtar *et al*. (1993) Stoddart (1999) reported 2.65% infection rate in Chitral district including Balanguru village where the incidence was 10.6% which is comparatively high rate of infection recorded in Pakistan after the present investigation. Pal and Subhani (1989) recorded 3.5% prevalence in Dir district. This cestode and other parasites have never been reported from other parts of Pakistan including Multan. The absence of *T. saginata* from these regions of the country is related to the traditional consumption of well cooked meat (Ansari *et al*., 1968). A very high incidence of this tapeworm (25-75%) is found in humans of Africa, Tibet and Syria, where semi-cooked meat is consumed (Chandler and Read, 1961).

During the past, Farooqi (1964) reported 3.4%, Pal and Subhani (1989) 66%, Stoddart (1999) 5.98% and Jamil (1999) 7.2% prevalence of this cestode. Highest prevalence rate of *H. nana* was recorded in Pakistan; Pal and Malik (1979) reported 21.6%, Siddiqi and Bano (1979) 18.0%, Akhtar *et al*. (1993) 17.2%, and Shaikh *et al.* (2000, 2003) 21.5% and 20.9%. Farooqi (1964) recorded 3.5% prevalence in Multan and 8.7% in Peshawar. The infection rate of this parasite was 11.6% in Multan medical students (Farooqi, 1965). In Islamabad school children it was recorded as 11.9% by Pal and Malik (1979). In Peshawar school children the prevalence was 14.5% (Siddiqi and Bano, 1979). Bilqees *et al*. (1982) reported 60.5% of *E. histolytica* in Karachi. Nawaz and Nawaz (1983) recorded 12.6% and Ansari and Sapru (1964) 4.6% in Peshawar University.

The prevalence of *Giardia* species is similar to findings reported earlier from Pakistan. In hospital children in Bahawalpur it was reported as 4% by Farooqi (1964), 5.4% by Haleem *et al.* (1965) and 5.0% by Baqai *et al.* (1985). The highest infection rates of this parasite were 43.7%, 41.9%, 50.8%, 38.5%, 39.1% recorded by Baqai *et al.* (1985) from Karachi, Pal and Malik (1979) from Islamabad, Kamran *et al*. (2005) from Karachi, Shaikh *et al*. (2000) from Larkana and Shaikh *et al*. (2003) from Shikarpur.

Comparatively low temperature prevails in Swat district. Farming is the principal occupation, and until recently most farmers used night soil as fertilizer because of its ready availability. Raw manure flowed into the rivers or is widely used in vegetable gardens. Such conditions provide a favourable environment for the development of helminth infections.

Half of the infected individuals were found with one species of parasite, while the other half were found multiple infections. In the present study helminths are dominating than protozoan parasites. Most of the helminth parasites detected were soil-transmitted; this is an agreement with the rate of soil-contamination with helminth parasitic eggs.

However, different parasites having variable distribution in different parts of the country. *A. lumbricoides, T. trichura and T. saginata* were found to be more prevalent infections, on the other hand *E. vermicularis, H. nana, E. histolytica, Giardia* species and hookworm sp. were found comparatively with low rate While *Fasciola hepatica* is reported only from Swat district, KPK Pakistan (Khan *et al*., 2014).

Our findings than other studies conducted in other parts of the country shows that intestinal parasitic infections remain highly endemic and appear to be due to faecal contamination of drinking water, unhygienic living conditions, poor sanitary behaviour, lack of health care and health education.

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Bacteriological Analysis of Street Vended Raw Milk in Multan

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Abstract- The milk samples collected from various zones of Multan city were examined for microbial load. Most of the milk samples except for the milk collected from Gulgasht Colony (2.5×10^7cfu ml^{-1}), Ghantaghar (2.5×10^6 cfu ml^{-1}) and B. Z. University (7.8×10^5 cfu ml^{-1}) were within the standard limits. However, 100% milk samples were found to harbor *Staphylococcus aureus* and moulds, while *Eschrichia coli* and *Salmonella* were detected in 78.57% and 42.85% samples, respectively indicative of unhygienic practices adapted from farm to consumers.

Key words: Raw milk, *Salmonella, S. aureus; E. coli.*

Milk contains around 100,000 molecular components of nutritional and nutraceutical importance. Its good nutritional profile and high perishability is equally good for rapid growth of different spoilage and pathogenic microorganisms. Most of the pathogenic microorganisms enter raw milk from different sources such as milking equipment, soil, feed, air, feces, grass and infected teats if the animal is mastitic (Torkar and Teger, 2008; Parekh and Subhash, 2008). Milk contaminated by spoilage and pathogenic microorganisms becomes unfit for human consumption unless it is processed properly. It becomes difficult otherwise to meet the self-satisfaction, safety and hygienic demands of consumers (Nanu *et al.*, 2007).

Microbial load and type of the microorganisms depend upon the number of important factors such as cleaning in place of animals, equipments and utensils, and physical factors including ambient temperature, storage and worker's as well as animal health (Swai and Schoonman, 2011; Zelalem and Fye, 2006). Common microbial genera found in fresh milk are *Lactococcus, Lactobacillus, Streptococcus, Staphylococcus* and *Micrococcus.* Total coliforms are abundant in the excreta of animals and act as source of contamination in foods of animal origin including milk. *Escherichia coli, Staphylococcus* and *Salmonella* have frequently been reported as common contaminants of the milk (Quinn *et al*., 2002; Ackers *et al*., 1998; Makita *et al*., 2012; Cody *et al*., 1999).

Pakistan is ranked as the third largest milk producing country in the world with annual production of 49.512 million tons and consumption of 39.945 million tons obtained from 166.7 million animals (GOP, 2010). This milk, however, is grossly contaminated during its transportation from the cattle farms to consumers.

This research work was focused on the evaluation of raw milk transported to Multan to determine the level of microbial contamination.

Materials and methods

A total of 300 samples of street vended raw milk (collected from street vendors) and 30 samples of pure milk (directly drawn from animals under possible hygienic conditions) from various locations of Multan city were collected in 500 mL, labeled sterile glass bottles in early mornings during the second quarter of the year, 2013. The tightly closed bottles having milk samples were kept in an ice box to maintain the temperature at 4°C and transported to the laboratory within 2 hours of the collection and analyzed immediately. Initially, 1 mL of milk

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sample was dispensed into a test tube containing 9 mL of the sterile water (Awan and Rehman, 2005) then serial dilutions were prepared and vortexed on a vortex mixer (Heidolph Reax top, D-91126).

Serially diluted samples were aseptically poured in pre-sterilized petri plates followed by plate count agar medium (Oxoid) prepared according to the instructions of the manufacturer. The total bacterial counts were enumerated using a colony counter (IRmeco, Hamburg Germany) after the plates were incubated at 37ºC for 48 h (Harrigan and MacCance, 1976).

The serially diluted samples were examined for *Staphylococcus aureus*, *E. coli*, *Salmonella* and yeasts/molds according to the procedures described in Awan and Rehman (2005). All samples were assayed in triplicate. Statistical model 8.1 and completely randomized design (CRD) was applied to analyze the data. LSD test was applied to determine the homogenous groups.

Results and discussion

Table I shows the total plate count (TPC), *E. coli*, *S. aureus*, yeast and mould in street vended raw milk samples collected from different parts of Multan city. These samples were found heavily contaminated with spoilage and pathogenic microorganisms as compared to the pure milk samples collected from the countryside directly from the animals. The highest mean value for TPC was observed in milk samples from Gulgasht Colony, while the lowest mean value was recorded in pure milk samples of goat. None of the pure milk samples exceeded the permissible limits (5.0×10^5 cfu ml^{-1}) set by Food and Agricultural code (Section 35781) while street vended raw milk samples from BZ University (7.8×10^5), Ghantaghar (2.5×10^6) and Gulghast Colony (2.5×10^7) exceeded the said limits (5.0×10^5 cfu ml^{-1}). The highest microbial load found in the present study was well in line with the findings of researchers in Mali (Bonfoh *et al.*, 2003) and in Nigeria (Edward and Inya, 2013). The possible means of microbial contamination in milk include unhygienic utensils, dairy farm environment, bedding and farming system of animals, water used for cleaning of utensils and adulteration of milk. An added factor resulting in extensive microbial growth in milk is the time consumed during the transportation of milk from one place to other (Bonfoh *et al.*, 2003). The milkmen fail to maintain chilling temperatures during milk transportation. Moreover the opening of milk pans during door to door distribution can be expected to further deteriorate the microbial quality of milk.

E. coli was found in all the street vended milk samples. The mean recorded value was 5.5×10^3 cfu ml^{-1} (Table I). Highest prevalence level was recorded in the milk samples collected from Gulgasht Colony (4.0×10^4), while the pure milk samples showed the lowest load. The results of pure milk samples clearly indicated that the *E. coli* present in milk samples may not have originated from animal body, but may be attributed to the *E. coli* containing water used for washing of milking utensils, cleaning of udders of the milking animals and adulteration of the milk (Chye *et al.*, 2004). A high coliform count is also related to manure, barnyard mud, personal hygiene of milking men and old bedding (Afzal *et al.*, 2011). The extent of *E. coli* contamination in the present study was found higher than reported in earlier studies in Malyasia (Edward and Inya, 2013), India (Srujana *et al.*, 2011) and in Quetta, Pakistan (Shafee *et al.*, 2013).

The mean value of milk contamination by *S. aureus* recorded in the present study was 6.7×10^3cfu ml^{-1}(Table I). The maximum prevalence was recorded in BZ University (3.9×10^4), while the least load was found for Cantt zone (4.0×10^2). Despite high loads of *S. aureus*, none of the milk samples exceeded 10^4 cfu ml^{-1}, an extremely hazardous level for the human body (Han *et al.*, 2005). *S. aureus* positive milk from all zones indicated that the milk vended in the city areas either has been collected from mastitis infected animals or the milk has been contaminated through the supply chain system. Furthermore, the bedding (place of sitting of animals) by the diseased animals might also be one of the reason of mastitis and eventually the contamination of milk by *S. aureus*. A total count of *S. aureus* in the range of 10^8-10^9 cfu g^{-1} was observed on milking animal's beddings (Bramley and McKinnon, 1990).

Bulk milk is often contaminated with *Salmonella* from animal manure and the environment. Out of the total including street

Table I.- Colony count (cfu ml^{-1}) of TPC, *E. coli, S. aureus* and yeasts, moulds and *Salmonella* of street vended raw and pure milk samples.

Samples (n=12)	TPC	*E. coli*	*S. aureus*	Yeasts	Moulds	*Salmonella*
Street vended milk						
BZ University	$7.8\times10^{5\,c}$	4.0×10^{2h}	3.9×10^{4a}	$7.8\times10^{5\,c}$	4.0×10^{2h}	3.9×10^{4a}
Shah RukneAlam	$2.2\times10^{5\,f}$	$8.0\times10^{3\,c}$	6.9×10^{3d}	$2.2\times10^{5\,f}$	$8.0\times10^{3\,c}$	6.9×10^{3d}
LabarBasti	$1.5\times10^{5\,h}$	7.0×10^{2g}	$7.0\times10^{3\,c}$	$1.5\times10^{5\,h}$	7.0×10^{2g}	$7.0\times10^{3\,c}$
Band Bosan	$1.5\times10^{5\,h}$	1.5×10^{2j}	4.5×10^{2k}	$1.5\times10^{5\,h}$	1.5×10^{2j}	4.5×10^{2k}
Ghantaghar	2.5×10^{6}b	1.2×10^{4b}	3.5×10^{3h}	2.5×10^{6}b	1.2×10^{4b}	3.5×10^{3h}
Bahadurpur	2.5×10^{4k}	3.2×10^{2i}	1.4×10^{3j}	2.5×10^{4k}	3.2×10^{2i}	1.4×10^{3j}
Qasimpur	7.6×10^{4i}	4.0×10^{3f}	4.5×10^{3f}	7.6×10^{4i}	4.0×10^{3f}	4.5×10^{3f}
Cantt	$1.6\times10^{5\,g}$	7.0×10^{3d}	4.0×10^{2l}	$1.6\times10^{5\,g}$	7.0×10^{3d}	4.0×10^{2l}
Gulgasht colony	2.5×10^{7a}	4.0×10^{4a}	5.0×10^{3e}	2.5×10^{7a}	4.0×10^{4a}	5.0×10^{3e}
17 Kassi	$2.4\times10^{5\,e}$	4.7×10^{3e}	4.0×10^{3g}	$2.4\times10^{5\,e}$	4.7×10^{3e}	4.0×10^{3g}
Pure milk samples						
Cow	7.2×10^{4d}	ND ^{k}	1.9×10^{4b}	7.2×10^{4d}	ND ^{k}	1.9×10^{4b}
Buffalo	7.4×10^{4i}	ND ^{k}	1.7×10^{3i}	7.4×10^{4i}	ND ^{k}	1.7×10^{3i}
Sheep	5.5×10^{4j}	ND ^{k}	4.5×10^{2k}	5.5×10^{4j}	ND ^{k}	4.5×10^{2k}
Goat	1.0×10^{4l}	1 ^{k}	4.1×10^{2kl}	1.0×10^{4l}	1 ^{k}	4.1×10^{2kl}
Average	$\mathbf{2.1\times10^{6}}$	$\mathbf{5.5\times10^{3}}$	$\mathbf{6.7\times10^{3}}$	$\mathbf{2.1\times10^{6}}$	$\mathbf{5.5\times10^{3}}$	$\mathbf{6.7\times10^{3}}$

The means having different letters for a single element are significantly different from each other (P<0.05). ND, Not detected; and – signs represent positive and negative, respectively.

vended and pure milk samples, 43% showed positive signals for this pathogenic microorganism. Pure milk samples showed negative results for *Salmonella*. It was observed that 60% of the milk samples under study were contaminated with *Salmonella*, whereas, only 2 and 1.4% of the milk samples were found contaminated in India and Malaysia, respectively (Sharma and Joshi, 1992; Chye *et al.*, 2004).

The raw milk samples were infested with yeast and mould colonies resulted from all milk samples with the mean values 1.4×10^{3} and 5.2×10^{2} cfu ml^{-1}, respectively. The highest mean values recorded for yeast and mold were in milk samples from Shahrukne Alam (3.9×10^{3}) and Cantt. (2.3×10^{3}), respectively while the minimum were recorded from Bahadurpur 4.0×10^{1} and Gulgasht 2.5×10^{1}, respectively. The results of the present study are lower in comparison with that reported from Nigeria (Edward and Inya, 2013) but higher than reported from Sardinia by Fadda *et al*. (2004). Perhaps cultural practices for animal's bedding and hygienic milking conditions were better than those adopted in Nigeria while lower than in Sardinia.

Conclusions

The results of present study clearly indicated that the overall microbial quality of raw milk in Multan is inferior compared to that of pure milk. The possible means of contamination in milk by pathogenic microbes are dirty adulteration, unhygienic conditions of the farm and the personal involved. The transport of milk from one place to another without refrigeration may also be a major reason for the high microbial loads. Strict regulations should be adopted by the regulatory agencies in order to control microbial contamination in milk. Moreover, the milkmen and other personnel involved in the dairy business should be educated regarding the consequences of microbial contamination and ways to reduce it.

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Conflict of interest declaration

The authors declare no conflict of interest.

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New Evidence on Morphology and Distribution of the Southern Grey Shrike (*Lanius meridionalis*) in Maghreb

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Abstract.- In North Africa, are living two subspecies for Southern Grey Shrike *Lanius meridionalis*: *L. m. algeriensis* and *L. m. elegans*. This paper attempts to find out whether there are biometric differences between the two subspecies and between the genders. Results show that there are no significant differences between sexes or between subspecies, at least despite clear signal in plumage coloration.

Key words: *Lanius meridionalis.*

The shrike is a small to relatively large bird with a more or less sharply hooked bill and strong legs, feet and claws adapted for catching prey (Hutchins *et al*., 2002), They inhabit open habitats, especially steppe and savannah (Karakaş, 2011).

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A polytypic species of the southern grey shrike, *Lanius meridionalis* is distributed in a vast belt stretching over northern Africa, the middle East and parts of Asia (Lefranc and Worfolk, 1997). It is split from the great grey shrike *Lanius excubitor* (Isenmann and Bouchet, 1993; Isenmann and N., 1994; Lefranc and Worfolk, 1997). Ten subspecies of *L. meridionalis* are distributed in Palearctic and Africa (Harris and Franklin, 2000; Lefranc and Worfolk, 1997). Studies based on molecular techniques (Gonzalez *et al*., 2008; Klassert *et al*., 2008; Olsson *et al*., 2010) shows that the subspecies *L. m. meridionalis* appears distinct from other subspecies studied. Two of these subspecies (*L. m. algeriensis* and *L. m. elegans*) have been proposed genetically closer to *Lanius excubitor* than to *Lanius meridionalis* (Klassert *et al*., 2008). They differ in plumage coloration and biometric variables (Cramp and Perrins, 1993; Lefranc and Worfolk, 1997; Panov, 2011). Based on measurements of specimens preserved in museums as skins, Cramp and Perrins (1993) showed that there were significant differences in the beak-head's length between males and females of both the subspecies. Intermediate forms between the subspecies called *dodsoni* have been described in Maghreb (Lefranc and Worfolk, 1997).

L. m. algeriensis is distributed in the North-west of Africa along Atlantic and Mediterranean coasts, and *L. m. elegans* has been reported from north and center Sahara (Lefranc and Worfolk, 1997).

In this article, we introduce new biometric information for subspecies *algeriensis* and *elegans*, and we try to check whether there are differences between them. This information is added to the relative plumage coloration and molecular techniques to find out whether all sources of analyses agree in their conclusions on the similarity or the difference between the two subspecies of grey shrike from the north and the south.

Material and methods

A total of 29 *L. m. algeriensis* birds (13 males, 11 females and 4 of unidentified sex) and 38 *L. m. elegans* (17 males, 16 females and 5 of unidentified sex). These specimens were collected from 29 localities in Maghreb (10 in Morocco, 7 in Algeria and 12 in Tunisia), between 1874 and 1954.

According to Svensson (1992) five morphological measurements were taken into account: length of wing (maximum chord), total body, tarsus, bill to skull, and tail. All were measured with a 1 mm precision caliper. All values are presented as mean ± standard deviation. Sex was recorded in the collection labels after identification of fresh individuals in the field. However, after skinning all measurements of the great grey shrike decreased by 2-5% (Kuczyński *et al*., 2003).

The variables in this analysis were all normally distributed (Kolmogorov–Smirnoff test). Therefore, we used ANOVA to analyze differences between subspecies and the total biometric differences between sexes of each subspecies.

Results and discussion

Plumage and geographic distribution

L. m. algeriensis (Fig. 1). Grey darker back, throat paler contrasting with grey chest, wing bar white is narrow, supercilium line broad.

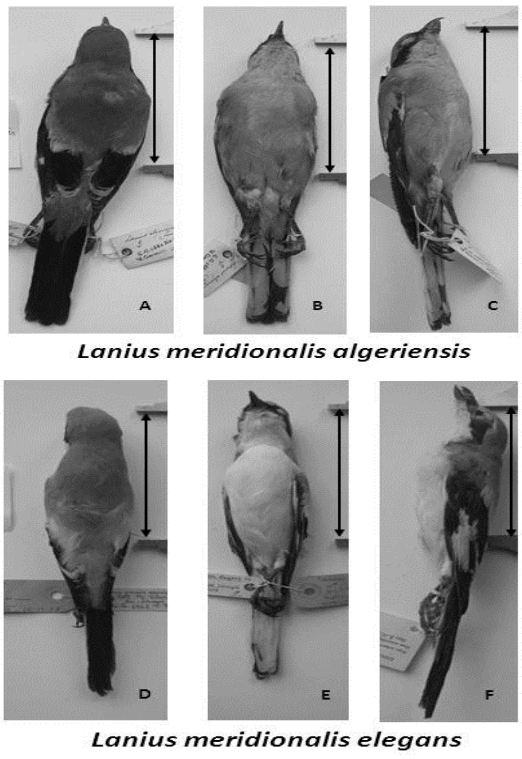

Fig. 1. Plumage color of the two subspecies: *L. m. algeriensis* and *L. m. elegans*. The distance indicated by arrows is 10 cm.

Table I. Comparison of variables measured for the two subspecies *L. m. algeriensis* and *L. m. elegans* according to sex.

Measurements (mm)	*L. m. algeriensis*		F	*P*	*L. m. elegans*		F	*p*
	Males (n=13)	Females (n=11)			Males (n=17)	Females (n=16)		
Wing length	99.99 ± 4.17 (94-109)	103.82 ± 2.99 (100-110)	0.001	0.94 ns	104.38 ± 3.76 (96-112)	104.29 ± 3.26 (99-112)	0.001	0.94 ns
Body length	215.68 ± 10.44 (210-250)	221.36 ± 11.20 (200-235)	1.17	0.29 ns	222.19 ± 8.94 (210-240)	225.24 ± 11.61 (210-240)	0.71	0.41 ns
Tail length	103.99 ± 8.46 (95-128)	105.18 ± 6.63 (91-112)	2.73	0.11 ns	106.25 ± 7.57 (97-116)	104.29 ± 6.44 (97-114)	0.63	0.43 ns
Tarsus length	29.68 ± 3.96 (20-36)	30.36 ± 1.29 (28-31)	0.48	0.49 ns	29.63 ± 2.96 (27-35)	30.24 ± 2.44 (26-34)	0.42	0.52 ns
Bill length	22.73 ± 2.15 (21-29)	23,18 ± 2.71 (19-28)	0.45	0.51 ns	22.88 ± 1.78 (20-25)	22.41 ± 1.94 (20-27)	0.51	0.48 ns

Ns, not significant.

L. m. elegans (Fig. 1). The back is pale grey, chest paler and white throat, outer rectrices white, wing bar white is wide.

The localities of the National Museum of Natural History specimens of Paris as well as personal observations of alive birds in Maghreb (Fig. 2) match with that given by Lefranc and Worfolk (1997), but for *L. m. elegans* expands its range in South-Western Morocco (Agadir, Tamanar and in Mogador), South of Algeria (Tamanrasset) and also in two localities in the South-East of Tunisia (Menzel and Medenine).

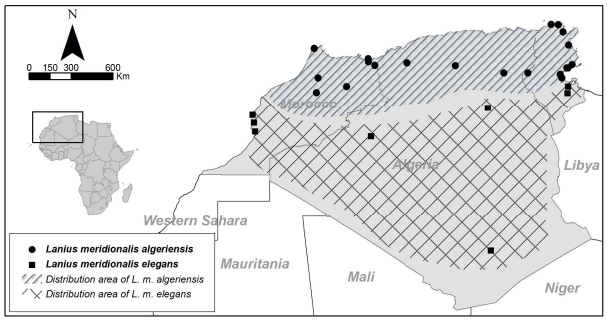

Fig. 2. Distribution of *L. m. algeriensis* and *L. m. elegans* in Maghreb based on personal observation and the localities of museum specimens analyzed.

Biometry

The analysis of biometric variables in this study showed a significant overlap between the sexes for each subspecies (Table I). It is probably for this reason; no significant differences were reported between the sexes within each sub-species.

These results differ from those of (Cramp and Perrins, 1993), who found significant differences between males and females in the length of beak-head for two subspecies.

We have gathered all males and females of each subspecies in order to perform a comparative study between them. No significant difference between biometric measurements of *L. m. algeriensis* and *L. m. elegans* was noted (all p values > 0.05). In all cases, degree of freedom is 1.22 for *L. m. algeriensis* and 1.33 for *L. m. elegans.*

Genetic analysis of (Olsson *et al*., 2010) with the use of mitochondrial DNA of *elegans* and *algeriensis* subspecies which are arranged differently in taxa that appear as follows: the first is nested among *elegans* and *koenigi*, another is nested with the *leucopygos* and the third is sister to *elegans-koenigi* clade. Thus, there is a special position in the relation *elegans-algeriensis*. Our data show differences in plumage and in geographical distribution of these two sub-species, but they do not correspond to differences in biometrics. This suggests doubts about whether the plumage coloration and distribution are sufficient criteria to distinguish the two as separate subspecies. To clarify this point we suggest a larger study that

includes biometrics information, morphological and molecular on shrikes of Maghreb region.

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Gross Morphometric Features of the Epididymis in Adult Male Pakistani Goats (*Capra hircus*)

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Abstract.- The current study was carried out to grossly measure the different parts of the epididymis in local nondescript breed of goat in Pakistan. A total of 100 male goats (body weight 20-40kg, age 1-3 years), slaughtered at different slaughter houses in Hyderabad were included in this study. The epididymis had the following mean measurements: left epididymis–length, breadth and thickness of head, body and tail were 3.49±0.75, 6.61±0.72, 1.68-0.26, 1.94±0.38, 0.54±0.11, 1.49±0.22, 0.67±0.18, 0.25±0.05, 1.27±0.20 cm, respectively. The circumference and weight of the tail was 4.78±0.64 cm and 2.46±0.91g. The right epididymis–length, breadth and thickness of the head, body and tail were 3.49±0.70, 6.58±0.70, 1.68±0.28, 1.94±0.41, 0.55±0.12, 1.47±0.22, 0.67±0.18, 0.25±0.05, 1.26±0.19 cm, whereas circumference and weight of the tail was 4.76±0.64 cm and 2.45±0.92 g, respectively. The current study demonstrated statistically no significant difference in the right and left epididymis parameters of the male goat.

Key words: Gross morphometry, epididymis, *Capra hircus.*

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Goats play a substantial role in the socioeconomic condition of the rural dwellers. Goat are relatively resistant to disease and thrives well in extensive system of animal management (Osuagwuh, 2002). Short gestation intervals and high rate of fecundity make the goat distinctive for selection as good source of animal protein (Osuagwuh and Apokodje, 1981).

Since the assessment of the male animal for breeding soundness is an essential characteristic of the successful reproductive operation in modern dairy goat management programme, it has been demonstrated that reproductive performance is economically very important in small ruminant husbandry enterprises. It effects the number of progeny produced per year (Greyling, 2000). Male fertility is considered an important factor of ruminant breeding programs as male ruminants can mate with a vast number of females (Katz, 2007). Therefore, the correct understanding of the reproductive biology particularly that of the male reproductive system is imperative in screening of male breeding stock for maintaining a good reproductive performance.

Epididymis is the basic segment of the excurrent duct system in the reproductive tract of male goat where maturation and storage of spermatozoa occur under androgenic control (Robaire *et al*., 2006). Grossly, the epididymis is comprised of the proximal region (the initial segment and the head), the body and the distal region (tail) (Robaire *et al*., 2006; Turner, 2008). Head and body of the epididymis are associated with sperm concentration and maturation whereas tail region is responsible for sperm storage, providing ample amount of male gametes available for ejaculation (Sullivan, 2004).

Several researchers investigated gross morphometric feature of the epididymis in many mammalian species. Maala and Malicdem (1997), grossly studied the epididymis in Philippine Carabao (*Babalis bubalis*); Rind *et al.* (2006) reported the biometric observation of the bovine epididymis. Currently, Olukole *et al.* (2009, 2014) investigated the epididymis of the domesticated adult African great can rat and African sideneck turtle (*Pelusios castaneus*). Likewise, Oyeyemi *et al*. (2012) and Ibrahim *et al*. (2012) recently reported morphometric assessment of the epididymis in sheep and goat. To our knowledge, detailed description of gross morphometry of the head, body and tail of the epididymis in male goat is not yet reported. On the other hand, research work on gross anatomy or morphometry of the reproductive organs in local male goats from Pakistan is very rare from existing literature. Recently we have reported the biometric feature of the testis in Pakistani adult male goat (Khan *et al.*, 2014). This study aims to document information on the gross morphometry of head, body and tail of the epididymis in the adult male goats to establish a base-line data on the anatomy of native animal stock in Pakistan.

Material and method

One hundred reproductive organs (including testicle with intact epididymis) of male goats of various ages range 1-3 years, and body weight (25-40 kg) slaughtered at different slaughter houses of Hyderabad district were collected. The reproductive organs/tracts, having no gross abnormalities or pathological lesions, removed from the carcasses, packed in polyethylene bags and brought to Department of Anatomy and Histology, Sindh Agriculture University, TandoJam, Pakistan within 2 h of collection. These reproductive organs were cleaned and the adhering tissue were removed and placed on the surgical table in their normal position. The epididymis was carefully separated from the testicular body and each portion of the epididymis *i.e.* head, body and tail was marked separately and then straightened out for taking the measurements as previously described (Rind *et al*., 2006; Oyeyemi *et al*., 2012).

For each part of the epididymis, it was first dissected, straightened out and the length was measured from the cranial to the caudal ends, along the longitudinal axis. The breadth included the greatest diameter between the lateral and medial borders. Similarly, the thickness was measured from the cranial to the caudal attachment of the head of epididymis to its free surface. The circumference of the tail of the epididymis was measured by encircling the tail its mid portion by a graduated nylon tape. Weight was recorded in grams with

triple beam balance.

The results on the various parameters of the epididymis of male goats were presented as mean ± SD. Dimensions and weight were analyzed between the right and left sides. Student's paired t-test was applied to compare the means between the right and left epididymis. The statistical significance was put at $p<0.05$. Data were analyzed using the SPSS version 16.

Table I. Gross morphometric feature of epididymis of adult male goat (n=100).

Segment of epididymis	Parameter	Side	Mean±SD
Head	Length (cm)	R	3.49±0.70
		L	3.49±0.75
	Breadth (cm)	R	1.94±0.41
		L	1.94±0.38
	Thickness (cm)	R	0.67±0.18
		L	0.67±0.18
Body	Length (cm)	R	6.58±0.70
		L	6.61±0.72
	Breadth (cm)	R	0.55±0.12
		L	0.54±0.11
	Thickness (cm)	R	0.25±0.05
		L	0.25±0.05
Tail	Length (cm)	R	1.68±0.28
		L	1.68±0.26
	Breadth (cm)	R	1.47±0.22
		L	1.49±0.22
	Thickness (cm)	R	1.26±0.19
		L	1.27±0.20
	Circumference (cm)	R	4.76±0.64
		L	4.78±0.64
	Weight (g)	R	2.45±0.92
		L	2.46±0.91

All values obtained were defined as mean±S.D. Data was analysed by using Student's paired `t' test; N, number of experimental animals; R, right; L, left

Results and discussion

The present study described the measurement of the epididymis in local nondescript breed of goats. Table I indicated the measurement of head, body and tail of the epididymis. Statistical analysis of the measurements obtained during current study demonstrated no significant difference (at 95 per cent confidence coefficient interval) in the length, breadth, thickness and weight of left or right epididymis. The nonappearance of significant difference among all feature of the epididymis during current study were in line with the findings of Olukole *et al.* (2009) on cane rat epididymis and Rind *et al.* (2006) on the bovine epididymis, respectively.

The results obtained in the current study on the length and width of the head and body of the epididymis were higher than that described by Farooqui *et al.* (2010) and Devi *et al.* (2013) on the biometry of epididymis in goat fetus and kids respecivly. Farooqui *et al.* (2010) reported that the length and width of left head epididymis in goat fetus was 7.3±0.81 and 4.5±0.48mm, whereas that of right epididymis was 7.05±0.57 and 4.5±0.52mm, respectively. In Asam goat kid, Devi *et al.* (2013) recorded the length of the body of the right and left epididymis as 3.80±0.01 cm and 3.56±0.01cm; the width of the body of right and left epididymis as 0.30±0.00 cm, 0.20±0.01 cm ; the thickness of the body of right and left epididymis as 0.09±0.00 cm, 0.10±0.00cm whereas the weight of the body of right and left epididymis were recorded 0.26±0.00 g, 0.25±0.00g respectively. Furthermore, the values obtained in the current study for the weight of the tail of the left and right epididymis were lower compared to that reported by Raji and Njidda (2014) in Red Sokoto goat and Ritar *et al.* (1992) in Angora goat .In Red sokoto goat, the weight of the left tail of epididymis was 3.06g and that of right was 2.96g whereas in Angora goat the combined weight of both left and right tail of the epididymis was 11.73±0.25 g. Ugwu (2009) also reported higher weight of epididymis tail in West African buck. Likewise, higher values than current finding have been observed in the weight of epididymis in dairy goat and Indian goat (Fielden and Berker, 1964; Jindal and Panda, 1980). The anatomical dimensions obtained in the present study for the adult male goats were greater than those reported by the aforementioned researchers, because they used goat kids or fetuses whereas in our study we used the adult animals .On the other hand,weight of the epididymis tail recorded in the current study was lower than that reported for Red Sokoto, Angora and Indian goats.This discrepancy in the measured weights of the tail of the epididymis might be due to different breeds of goats nourished under better mangemental conditions.

The correct understanding of normal

dimension of the reproductive tract is fundamental for successful reproduction. Also reproductive performance of an animal is strongly associated with the functional morphology of the genital organs (Siddiqui *et al*., 2005). The current morphometric appraisal of the epididymis are valuable for the functional assessment of epididymis in reproductive management of local goat population. Furthermore, data are characteristically essential as a reference in assessing congenital defects and organ pathologies or abnormalities that may affect the epididymis of male goats. Several studies have correlated the structural disturbance and/or depletion in physiological activities of the epididymis with male infertility factors; that include abnormality in composition of the epididymal plasma, reduction in sperm motility and biochemical changes in sperm membranes or structural alteration of the spermatozoa (Turner, 2008; Galloway, 1994; Oyeyemi and Ubiogoro, 2005). Also, each segment of the epididymis is well associated with the process of normal maturation of spermatozoa during their epididymal transportation from the testicle to the ductus deference. Furthermore, the head, body and tail of the epididymis are essentially associated with histological and functional characteristics of the epididiymis (McDonald, 1989). Further histological studies would be essential to determine the sequential histological changes of each segment of epididymis in local male goats; a better understanding of its reproductive functional morphology in association with the sperm storage and maturation.

In conclusion, this study demonstrated the anatomical dimensions of head, body and tail of the epididymis in adult local male goat in Pakistan. In addition, the current morphometric values could be used as baseline data in comparative reproductive biology and regional anatomy of male reproductive tract among species of the domestic animals and also within goat breeds under diverse environmental conditions.

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Conflict of interest

All authors have no conflict of interest with any one about this manuscript

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A Report on Uterine Leiomyoma in Nili Ravi Buffalo

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Abstract.- The basic aim of the present study was to report a rare clinical and histo-pathological condition of uterine leiomyoma in Nilli Ravi buffalo. A nine year old female buffalo with the history of repeat breeding and infertility was brought to Sahala slaughter house, Islamabad. Clinical examination through rectal palpation determined the presence of large, firm painless swelling in the right uterine horn with no foul smelling discharge. This condition was suspected to be a tumor or third degree endometritis. During necropsy, gross uterine examination revealed a firm, multilobular, well vascularized tumoural mass with 15.4 cm diameter in size. Histopathology of the tissue sections showed the characters of leiomyoma. As best of author's knowledge, this is the first report on uterine leiomyoma in Nilli Ravi Buffalo that exposes another factor of the infertility.

Key words: Leiomyeoma, uterus, nili ravi, infertility.

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Leiomyoma is a benign tumour of smooth muscles that can be originated in every organ (Cooper and Valentine, 2002). But uterine leiomyoma is more frequently reported than other organs. Uterine leiomyoma is rarely reported in cows and buffaloes when compared with mare, swine, sheep, cats and dogs (Lopez *et al.*, 1997). The etiology is unknown in all domesticated animals except in bitch. In bitch, uterine leiomyoma is considered to be originated due to hormonal imbalance and high extragenously administered estrogen (Baba and Catoi, 2007). The clinical manifestation of uterine leiomyoma includes uterine tumor with associated endometritis but depending on size, it is rarely detected in live animals unless cause clinical complications (Sharma *et al.*, 2012). Uterine leiomyoma is considered as important disease because it damages the uterine mucosa that leads to pregnancy failure. Hence, uterine leiomyoma contribute to the infertility of the animal (Azawi and Al-Sadi, 2010). Similar to cows, Uterine leiomyoma in river buffaloes breeds is rarely reported with its clinical signs and histopathological study as compared to other infectious diseases (Ashraf *et al.*, 2009). In this case, we report a rare condition of uterine leiomyoma which developed in a Nili Ravi buffalo and studied at macroscopic and microscopic level.

Materials and methods

A nine year old Nili Ravi buffalo, weighing approximately 500 kg, was brought to the Sahala slaughter house, Islamabad. Buffalo had the history of repeat breeding for more than two years with irregular estrus cycle. Other complains were of constipation and irregular feed intake. Clinical examination through rectal palpation was performed to check the conditions of ovaries, uterine horns, uterus body, cervix and vagina. After slaughtering, the reproductive tract was isolated for post mortem and histo-pathological examination. The abnormal mass was measured by utilizing vernier calipers. For histopathology, tissue samples taken from the uterus and the mass were fixed in 10% formalin solution and processed. All sections were stained with haematoxylin-eosin and some selected slides from the uterine mass and processed in the Pathology lab,

University of Veterinary and Animal Sciences, Lahore.

Results and discussion

During anti-mortem examination, trans-rectal palpation revealed the presence of hard, large painful swelling with no foul smelling discharge in the right horn of uterus. This condition was suspected to be a tumor or third degree endometritis. Gross examination of the isolated reproductive tract revealed a firm multilobular mass with 15.4cm in diameter obstructing the whole length of horn. There were also traces of pus present in between the lobular mass. The firm masses were reddish to white in color when dissected. Both right and left uterine horns had large sized multiple cruncles (Fig. 1A). On ovarian examination, both ovaries were found to be inactive with absence of any structure. All other structures in the reproductive tract i.e. cervix, vagina and vulva were normal in size and tonicity with no neoplastic mass. On histo-pathological examination, tumorous mass was revealed to be originated from muscular walls of uterus (Fig. 1B). Neoplastic tissue displayed marked hyper cellularity of smooth muscle cells in one or multiple layers embedded in connective tissue. The cytoplasm was mostly homogeneous, sometimes finely vacuolated and usually eosinophilic. With conspicuous, small, multiple nucleoli, nucleus had elongated shape with mild irregularity in borders. Mitotic figures were also present. Angiogenesis was remarkable with increased number of dilated blood vessels (Fig. 1C).

Reproductive tract tumors in cattle can occur in any part but uterus is considered as common site comparative to vagina or cervix**.** On the other hand, uterine leiomyoma are rare in buffaloes with a single case study to the author's knowledge (Azawi and Al-Sadi, 2010). In present study, uterine leiomyoma has been observed mainly in older buffalo that agrees with the studies related to cattle and other species (Nelis *et al.*, 2013)**.** Similar to previous studies in cattle, current study has also documented the history of infertility and clinical picture that include the hard tumorous swellings contiguous with endometritis (Avci *et al.*, 2010; Azawi and Al-Sadi, 2010; Enginler *et al.*, 2011; Sharma *et al.*, 2012). It has been observed that unlike sows with uterine leiomyoma, buffalo in this study did not exhibit the signs of pot-bellied abdomen (Miller and McDanielt, 1995). While concerning the histopathology as diagnostic approach, previous studies have reported smooth muscle cells and hyper cellularity along with increased angiogenesis that are in accordance with the present results (Baba and Catoi, 2007). These abnormal cellular proliferations are tenable to cause

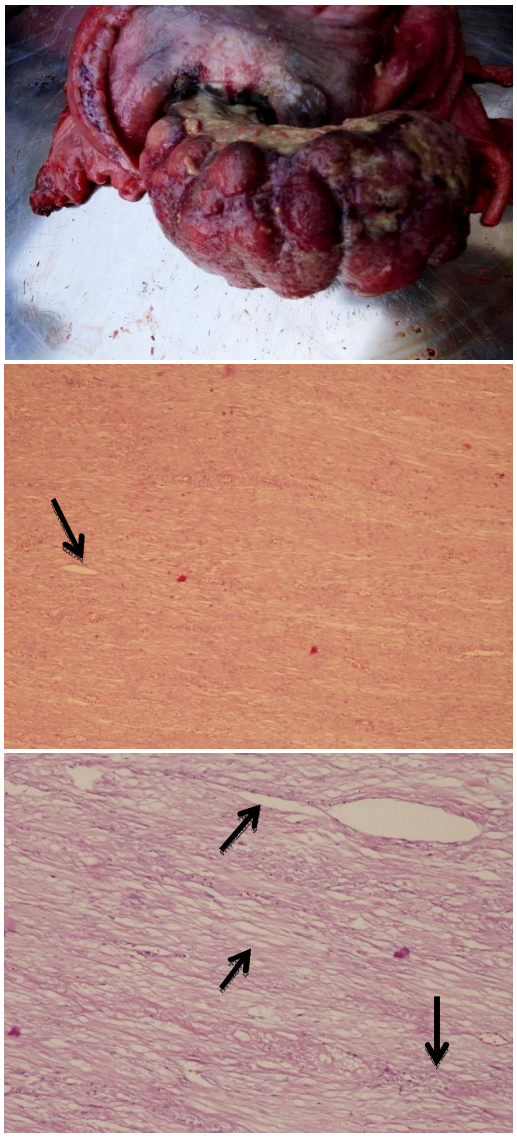

Fig.1. Moltilobular, tumoral mass protruded from the uterine endometrial wall Flat endometrial wall is also seen (A). B and C show Tumor mass, neoplastic cells forming bundles and congestion. Nuclear border irregularity is also evident

significant pathological changes in the uterine endometrium that could hinder the embryonic implantation and further lead to infertility (Makker and Goel, 2013).

This case study documents an interesting report which explains the incidence of uterine tumor in buffaloes that could also act as a risk factor for infertility. This case study would be informative for large animal clinicians and other veterinary related areas.

Conflict of interest

The authors have no conflict of interest.

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First Record of the Aphid Genus *Coloradoa* Wilson (Hemiptera: Aphididae) from Saudi Arabia, with some Morphological Notes on Variation in *C. rufomaculata* (Wilson, 1908)

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Abstract.- The aphid genus *Coloradoa* Wilson, is reported for the first time from the Kingdom of Saudi Arabia. The species *Coloradoa rufomaculata* (Wilson, 1908) was found feeding on *Ambrosia maritima* L. (Asteraceae) representing a new host plant. This species is widely dispersed in Europe, North America, eastern Asia, Japan, Africa, and the Middle East. A brief description of the species is provided with biometric data. These specimens were compared with descriptions in literature. Morphological variability in commonly used taxonomic characters has been discussed. Rostrum shape and antennae length have similarity with available literature and most important variable characters are siphunculi color, polygonal reticulation on 8th abdominal tergite and rostrum length.

Keywords: Aphididae, *Coloradoa rufomaculata*, *Ambrosia maritima*, feature variations.

The genus *Coloradoa* was erected by Wilson (1910) with the type species *Aphis rufomaculata* Wilson, 1908. This genus is a Eurasian genus (Halbert *et al.*, 2000) including 31 species (Remaudiere and Remaudiere, 1997). The known host plants for these aphids are members of the family Asteraceae (Durante *et al*., 2011). The genus *Coloradoa* is recognized as small greenish or

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reddish oval aphids, with the rostrum stiletto-shaped and body setae usually capitate, spatulate, fan-shaped or rod-shaped (Eastop, 1966; Blackman, 2010).

Coloradoa rufomaculata is a cosmopolitan species, widely distributed in Europe, North America, eastern Asia, Japan, Africa, and the Middle East (Eastop, 1966; Miyazaki, 1971; van Harten *et al*., 1994; Durante *et al*., 2011; Bodlah *et al*., 2011). *C. rufomaculata* was found feeding on *Chrysanthemum* sp. and *Artemisia* spp. in the USA (Palmer, 1952), on *Chrysanthemum* spp. in Yemen (Harten *et al*. 1994) and Iraq (Ali *et al*., 2012).

Wilson (1908) provided a description of *C. rufomaculata* based on type specimens indicating that in the live specimens the body is generally green, vertex of the head dusky, eyes light red, abdomen green and siphunculi dusky. Antennae are paler proximally and gradually black distally, dorsal hairs of head bottle-shaped and those on abdomen in partly open fan-shaped.

Several authors have noted variation of some of the above characters (Wilson, 1908; Zeck, 1941, Palmer, 1952; Cottier, 1953; Miyazaki, 1971). The purpose of this paper to provide a description of this species found in the Kingdom of Saudi Arabia (KSA), with comments on variability of certain described morphological characters.

Materials and methods

Specimens of *C. rufomaculata* were collected from Wadi Fig, Al Bahah Province, KSA, 19° 59' 254" N, 041° 31' 951" E, 2146 m a.s.1. The site was visited five times on 22.V.2013, 23.II.2014, 26.IV.2014, 5.VI.2014 and 13.VIII.2014. Hand collections and yellow water pan traps were used during each visit.

Aphids were mounted on microscopic slides using method of Blackman and Eastop (1984). Images of specimens were produced using a Leica microscope DM 2500®. Voucher specimens are deposited in King Saud University Museum of Arthropods (KSMA), Plant Protection Department, College of Food and Agriculture Sciences, King Saud University, KSA. The aphid species was identified according to Blackman (2010) and Dr. Susan Halbert, Division of Plant Industry, Florida Department of Agriculture and Consumer Services, Tallahassee, Florida U.S.A. confirmed the identification.

Abbreviation used: ANT I, II, III, IV, V and VI, first, second, third, fourth, fifth and sixth antennal segments; PT, process terminalis of last antennal segment; Siph, siphunculi; URS, ultimate rostral segment; 2HT, second segment of hind tarsi; RH, secondary rhinaria.

Coloradoa rufomaculata (Wilson, 1908)

Aphis rufomaculata Wilson, 1908
Coloradoa rufomaculata, Wilson, 19l0
Stephensonia lahorensis Das, 1918
Rhopalosiphum lahorensis Takahashi, 1924
Rhopalosiphum kiku Hori, 1929
Rhopalosiphum rufomaculatum Palmer, 1952

During the five visits to Wadi Fig, Al Bahah, nineteen apterous viviparous females were collected only on 22.V.2013 on *Ambrosia maritima* L. (Asteraceae) which is a new reported host for this aphid. No alatae were collected during the study. Eastop (1966) mentioned that alatae were not commonly collected, except during winter in Australia.

In the current study, *Coloradoa rufomaculata* was sympatric with *Macrosiphum euphorbia* (Thomas, 1878), and *Aulacorthum palustre* on *A. maritima*. Zeck (1941) similarly reported *M. sanborni* (Gillette, 1908), *Myzus persicae* (Sulzar, 1776) and *Aphis gossypii* Glover, 1877 with *C. rufomaculata* on *Chrysanthemum* sp.

Apterous viviparous female, in life green. Body pear shaped, 1.11-1.45mm excluding cauda (Fig. 1A). Antennae six segments, antennal tubercles not developed. Antennae 0.54-0.56 x as long as body. Terminal process of antennae 1.81-1.83 x as long as basal part of segment VI, 1.15-1.33 as long as segment III. Third antennal segment without secondary rhinaria, 1.36-1.58 x as long as segment IV. Antennal segments I, II and III paler than other segments. ANT IV slightly darker at proximal, V wholly black but ANT VI base black and PT paler. Distally tibia black, femora paler. Biometric data was showed in Table I. Siphunculi 8-16% dusky proximally and 84-92% black distally.

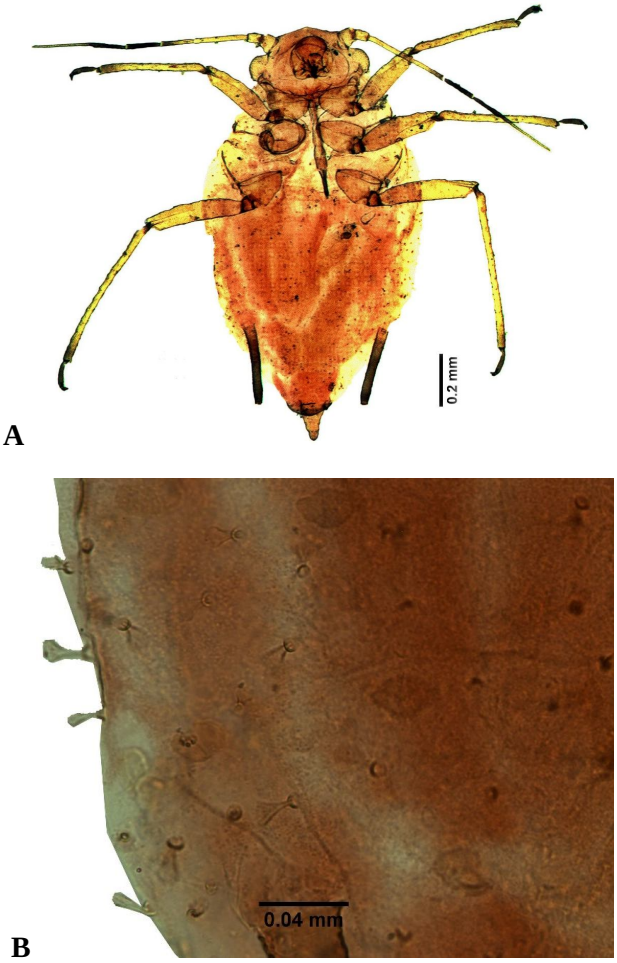

Fig. 1. *Coloradoa rufomaculata*. A: whole body; B: body setae.

Dorsal body setae fan-shaped (Fig. 1B). Frons of head with four frontal capitate hairs. Cephalic hairs fan-shaped and conspicuous, 10.4-20.8 µm. Front of head convex. Rostrum length extended to between mid and hind coxae, ultimate rostral segment stiletto-shaped, with 4-10 secondary hairs and 1.11-1.22 x as long as second segment of hind tarsi. First tarsal chaetotaxy with 3, 3, and 2 hairs. Tergite VIII with 2-5 (15.6-20.8µm) fan shaped hairs. Siphunculi slightly swollen apically with imbrication, 0.18-0.20 x as long as body, 1.64-1.69 as long as cauda, 5.6-7.0 times as long as their width and 1.46-1.55 as long as antennal segment III. Middle diameter of siphunculi 1.25-1.3x as long as middle diameter of hind tibia. Cauda triangular shape with 5 setae (10.4-31.2µm) four of them laterally and single preapical setae.

The population of *C. rufomaculata* collected in KSA varied slightly in certain characters such as body length, rostrum shape, antennal length and color as compared to previous descriptions (Wilson, 1908; Zeck, 1941; Palmer, 1952; Cottier, 1953). However, the siphunculi described as dusky in color by Wilson (1908); Zeck (1941); Palmer (1952) and Cottier (1953) were distinctly black in the KSA population and similarly reported for Japanese specimens **(**Miyazaki, 1971**)**. Rostrum length from the KSA specimens reached between the mid- and hind coxae, but Wilson (1908) in original description indicated that the rostrum barely reached the hind coxae (Table II). Eastop (1958) found polygonal reticulation on abdominal tergite eight, which was not reported in the available literature or in Saudi specimens.

Panigrahy and Patnaik (1991) reported on the variation of chromosomal number of two clones of *C. rufomaculata* on the host plant *C. coronarium* L. from two different localities in Chatrapur, Orissa India. Clone I had 8 diploid chromosomes, whereas clone II possessed 17 diploid chromosomes. Blackman and Eastop (2006) stated that “if two clones have different chromosomal numbers then how placed in one species". This obvious difference needs additional investigations such genomic analyses to reveal if cryptic species are involved or not.

Acknowledgements

The authors are grateful to Dr. Susan Halbert, Division of Plant Industry, Florida Department of Agriculture and Consumer Services, Tallahassee, Florida, U.S.A. for confirming of identification of aphids, and Prof. Dr. Boris Kondratieff, for reviewing the manuscript. We would like to thank the Acarology Laboratory, Plant Protection Department for using the Auto-Montage system. Also, we would like to take this opportunity to thank to Dr. Jacob Thomas, College of Science for assisting with the identification of host plant. We also thank the Deanship of Scientific Research, King Saud University for providing funding for this study.

Table I.- Biometric data (mm) for *Coloradoa rufomaculata* of apterae from Wadi Fig, Al Bahah Province, Kingdom of Saudi Arabia.

Specimen No	Body length	Antennal segments length					Cauda length	Siph		URS length	No of URS secondary setae	2HT length
		Total	III	IV	V	VI		Length	Width			
1	1.24	0.78	0.15	0.11	0.10	0.12+0.20	0.14	0.22	0.03	0.10	7	0.09
2	1.21	0.79	0.16	0.11	0.11	0.11+0.20	0.13	0.22	0.04	0.10	4	0.09
3	1.40	0.90	0.18	0.14	0.13	0.12+0.22	0.17	0.28	0.05	0.10	4	0.09
4	1.45	0.89	0.19	0.12	0.13	0.12+0.22	0.16	0.27	0.05	0.11	4	0.09
5	1.29	0.86	0.17	0.12	0.13	0.12+0.22	0.15	0.25	0.03	0.10	7	0.08
6	1.32	0.85	0.17	0.13	0.12	0.11+0.21	0.14	0.25	0.03	0.09	4	0.10
7	1.32	0.87	0.18	0.12	0.12	0.12+0.21	0.14	0.26	0.04	0.11	10	0.10
8	1.40	0.83	0.17	0.12	0.11	0.11+0.20	0.14	0.26	0.04	0.10	5	0.08
9	1.43	0.80	0.16	0.11	0.11	0.12+0.19	0.15	0.25	0.04	0.10	7	0.09
10	1.21	0.83	0.17	0.12	0.12	0.10+0.21	0.14	0.25	0.03	0.10	10	0.09
11	1.32	0.89	0.19	0.12	0.13	0.12+0.22	0.13	0.26	0.04	0.11	5	0.09
12	1.24	0.78	0.16	0.11	0.11	0.11+0.19	0.14	0.26	0.03	0.10	9	0.08
13	1.19	0.81	0.16	0.11	0.12	0.11+0.20	0.13	0.24	0.03	0.11	9	0.08
14	1.24	0.76	0.15	0.10	0.10	0.11+0.19	0.13	0.23	0.04	0.10	7	0.08
15	1.24	0.83	0.17	0.12	0.12	0.11+0.21	0.15	0.25	0.04	0.11	6	0.09
16	1.14	0.84	0.17	0.12	0.13	0.12+0.19	0.15	0.25	0.03	0.10	9	0.08
17	1.21	0.79	0.16	0.10	0.10	0.11+0.20	0.13	0.24	0.04	0.11	6	0.08
18	1.11	0.81	0.17	0.11	0.11	0.11+0.20	0.15	0.24	0.03	0.10	7	ND*
19	1.14	0.80	0.17	0.11	0.11	0.11+0.19	0.14	0.25	0.03	0.11	8	0.08

ND* = No data available due to broken legs

Table II.- Comparison of apterous viviparous females from Kingdom of Saudi specimens with reported specimens in literature.

Features	Authors					
	Wilson (1908)	**Zeck (1941)**	**Palmer (1952)**	**Cottier (1953)**	**Miyazaki (1971)**	**Saudi specimens**
Location	USA	Australia	USA	New Zealand	Japan	Saudi Arabia
Host Plant	*Chrysanthemums*	*Chrysanthemum* sp.	*Chrysanthemum* sp. *Artemisia ludoviciana, Artemisia vulgaris*	*Chrysanthemum* sp.	*Chrysanthemums*	*Ambrosia maritima*
Life color	Green	Bright green	Light apple green	Green	*ND	Green
Body length (mm)	1.40	1.70	1.40-1.50	1.30	ND	1.11-1.45
Antennae	0.80 mm in length, ANT light green at base shading to blackish at distal ends	ANT I, II, III pale, IV dusky, V and VI black	0.80-0.90 mm in length	0.83 mm in length, ANT I and II pale, III and IV colorless V and VI dusky	ND	0.76-0.90 mm in length, ANT I,II,III paler, IV slightly darker at proximal, V black, VI base black, PT paler
Rostrum	Barely reaching 3rd coxae	ND	attaining 2nd coxae	Extended between 2nd and 3rd coxae	ND	Extended between 2nd and 3rd coxae
Siph color	Dusky	Dusky	Dusky	Dusky	Siphunculi which is black, at most pale near the base.	Siphunculi which is black, at most pale near the base.
Cauda color	ND	Dusky	Dusky	Pale	ND	Dusky

*ND= No data available

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Comparative Effect of Different Pest Control Practices on the Population of Sucking Insect Pests and Yield of Sunflower (*Helianthus annuus*)

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Abstract.- Studies were conducted to investigate the comparative effect of different pest control practices *i.e*., chemical insecticide, cultural practices and neem seed aqueous extracts on the population of whitefly (*Bemesia tabaci)*, aphids (*Myzus persicae*) jassids (*Amrasca devastans*) and yield of sunflower. Findings of present study revealed that the highest per leaf population of whitefly (2.39), aphids (0.58) and jassids (1.15) was recorded in check plots, whereas, lowest per leaf population of whitefly (1.43), aphids (0.36) and jassids (0.78) was recorded in a combined application of insecticide + cultural practices + neem seed aqueous extracts. Similar trend was observed regarding sunflower yield per hectare in variable where plots were treated with a combined application of insecticide + cultural practices + neem seed aqueous extracts produced highest yield (5653 kg ha^{-1}), as against (4084 kg ha^{-1}) in control plots.

Key words: Sunflower, sucking insect pests, insect pest management

In Pakistan, sunflower (*Helianthus annuus*) is cultivated on an area of 397,306 ha, with the total production of 603,894 tons. Its average yield is 1520 kg ha^{-1}, while progressive farmers can obtain up to 3800 kg ha^{-1} (Anonymous, 2008-09). In the present changing agriculture and water constraint, area under sunflower has been increased significantly, especially in Sindh, since 2003 (Anonymous, 2005-06). Sunflower is grown for oil production or for the use in confectionaries, as its oil content ranges from 40-50% which comprises 90% oleic and 10% linoleic acids and protein contents from 20-30%

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(Malik *et al*., 2001). Seeds of sunflower are the third largest source of vegetable oil worldwide, following cotton seed and soybean, however, in Pakistan, sunflower is the second important source of vegetable oil after cotton seed (Thavaprakash *et al*., 2002). Sunflower oil is generally considered premium oil because of its light color, high level of unsaturated fatty acids and lack of linolenic acid, bland flavor and high smoke points (Nasim *et al*., 2011).

Among the several reasons of low production, susceptibility to insect pests and diseases is one of the major constraints. About 251 insect and mite species have been reported to attack sunflower around the globe (Rajmohan *et al*., 1974). In Pakistan, about 50 insect species have been known to damage the crop at various phases of its growth and development. Among them, 15 are considered potentially major destructive pests (Zahoor, 2000). Insect pests of sunflower are classified as seedling pests, sucking pests, chewing pests, soil insects and inflorescence pests (Basappa and Prasad, 2005). Among these, sucking pests like whitefly, jassids and aphids cause considerable losses to the crop at early stage of the crop growth. These sucking insect pests not only deteriorate the quantity but the quality of the crop as well.

The majority of farmers use chemical insecticides to control these insect pests in Pakistan, however, the indiscriminate use of these chemicals results in several problems such as insecticide resistance (Foster *et al.*, 2007), environmental hazards and adverse effects on natural enemies (Mani and Krishnamoorthy, 1997; Campiche *et al.*, 2006; Peng *et al.*, 2010). This threatening situation makes it imperative to study the efficacy of some effective pest management strategies which can minimize these losses. It is well documented that plant derivatives can be used as an alternative approach, for an instance, neem (*Azadirachta indica*) proved to have great potential for its commercial exploitation to control these pests. Neem derivatives carry repellent, deterrent, antiovipositional and growth inhibiting effects against insect pests (Mamoon-ur-Rashid *et al.*, 2012, 2013). Therefore, the present research work was conducted to reduce the yield losses caused by sucking insect pests and to devise an IPM program for their control.

Materials and methods

The experiments were conducted in the experimental area of Entomological Research Sub Station Multan. Sunflower variety S–278 was sown on Jan 22, 2013. The sunflower was sown in double row strips 90 cm apart. The experiment was laid out by following Randomized Complete Block Design, having eight treatments including a control one. Each treatment was replicated three times. Plot size was kept 3×4 m^2. The sowing was done by dibbler, 2-3 seeds were sown at one point with a distance of 12 inches. Thinning was done when the plants became 8-10 cm high by leaving one healthy plant. Weedicide Tweezer was sprayed at its recommended dose at the time of sowing before irrigation for weed management. The data were recorded on weekly basis.

The effect of three variables *viz.* insecticide (Confidor @ 620 ml hec^{-1}), cultural practices (hoeing) and neem seed aqueous extract @ 3% concentration was investigated. Details of treatments were as follows: T_0, check; T_1, insecticide; T_2, cultural practices; T_3, neem seed water extract; T_4, insecticide + cultural practices; T_5, insecticide + neem seed water extract; T_6, cultural practices + neem seed water extract; T_7, insecticide + cultural practices + neem seed water extract.

Insecticide (Confidor) was sprayed after 30 days of sowing and two times during crop growing season at an interval of 35 days with the help of knapsack sprayer.

A cultural practice, hoeing was done by hand khurpa around the stem of sunflower plants in order to dispose the eggs of insect pests to sunlight. Hoeing was done after 30 days of the sowing of sunflower crop and once during crop growing season.

Two kilograms of dry neem seed was collected from the neem trees in Entomological Research Sub Station and was grinded in electric mixer. Pulverized neem seed was suspended overnight in a cloth in a bucket of water at the rate of 50 gm litr^{-1} for the preparation of aqueous solution. In this way concentrated solution of 5% was prepared to be used in the experiment. After about 12 h the cloth was taken out and squeezed and

filtrate was collected. About 5 mg of soap was separately dissolved in a little water and added to the bucket. Solution was sprayed by knapsack hand operated sprayer after 30 days of sowing and then twice during crop growing season with an interval of 35 days.

Results and discussion

The data recorded on per leaf population of whitefly, aphid and jassids throughout the season in all the test treatments is given in Table I. The maximum per leaf population (2.39) of whitefly was recorded in T_0 (check), which was significantly different from all the test treatments. The minimum per leaf populations of whitefly (1.43) was recoded in T_7, which was statistically more or less similar to per leaf population (1.50) of whitefly recorded in T_1 (insecticide).

Table I.- Effect of different control practices on the population of whitefly, aphid and jassids throughout the crop season.

Treatments*	Mean per leaf population		
	Whitefly (*Bemesia tabaci)*	**Aphid *(Myzus persicae)***	**Jassid *(Amrasca devastans)***
T_0	2.39 a	0.58 a	1.15 a
T_1	1.50 ef	0.39 cd	0.96 b
T_2	1.98 b	0.49 b	1.09 a
T_3	1.81 bc	0.49 b	0.97 b
T_4	1.63 de	0.34 d	0.83 c
T_5	1.93 bc	0.47 bc	0.91 b
T_6	1.79 cd	0.49 ab	0.94 b
T_7	1.43 f	0.36 d	0.78 c
LSD Value	**0.168**	**0.925**	**0.075**

Each value is a mean of three replications. Means followed by the same letters are non –significant at α=0.05,

*T_0, check; T_1, insecticide; T_2, cultural practices; T_3, neem seed water extract; T_4, insecticide + cultural practices; T_5, insecticide + neem seed water extract; T_6, cultural practices + neem seed water extract; T_7, insecticide + cultural practices + neem seed water extract.

It is evident from the results that the application of insecticides reduced the population of whitefly when applied as a single dose or in combination with other test variables. However, the best results were obtained from the combination of all the control practices. The present findings are quite in agreement with those of Hassan *et al.* (1984), Balasubramnian and Chelliah (1986) and Aleem (1986) who effectively controlled whiteflies through insecticidal application. Application of neem seed water extract also had significant impact on the population of test insect as compared to check plots. Similar results have been reported by Basappa and Sriharan (1999) for the management of sucking insect pests in sunflower which strongly support the present findings.

Table II.- Effect of different control practices on the yield of sunflower.

Treatments	Mean yield in kg ha^{-1}
T_0 Check	4084 g
T_1 Insecticide	4450 f
T_2 Cultural practices	4725 de
T_3 Neem seed water extract	4692 e
T_4 Insecticide + Cultural practices	5382 b
T_5 Insecticide + Neem seed water extract	4751 d
T_6 Cultural practices + Neem seed water extract	5015 c
T_7 Insecticide + cultural practices + Neem seed water extract	5653 a
LSD Value	35.49

Each value is a mean of three replications. Means followed by the same letters are non –significant at α=0.05

The data revealed that the treatment T_0 (check) had maximum per leaf population (0.58) of aphid. The minimum per leaf population (0.34) of aphid was recorded in T_4, which was statistically similar to T_1 (insecticide) and T_7) having per leaf population 0.39 and 0.36 of aphid, respectively. The treatment T_6, T_2, T_3 and T_5 with 0.49, 0.49, 0.49 and 0.47 per leaf population of aphid was statistically similar.

The minimum per leaf population (0.78) of jassid was recorded in T_7, while the treatment T_0 (check) had maximum per leaf population (1.15) of jassid, which was statistically similar to T_2. While an intermediate per leaf population was recorded in T_1, T_3, T_5 (insecticide + neem seed water extract) and T_6 and was non-significantly different to each other. These findings are in line with those of Shah *et al.* (1984), Dhoble *et al.* (1985), Hassan *et al.* (1984), Aleem (1986), Balosubramanian and Chelliah (1986).

All the treatments had significant effect on

the yield of sunflower as compared to control. The minimum yield (4084 kg ha^{-1}) was recorded in T_0 (check). However, T_2, T_3 and T_5 (insecticide + neem seed water extract) had an intermediate yield. The maximum yield (5653 kg ha^{-1}) was recorded in T_7, which was significantly different from all the test treatments.

The highest per leaf population (2.39) of whitefly was recorded in T_0 and the lowest per leaf population (1.43) of whitefly was recorded in T_7.

It is clear from the data that application of combination of all practices increased the sunflower yield. These observations are in agreement with Jagadish *et al*. (2006). They found that the IPM module consisting of seed treatment with imidacloprid (5g kg^{-1}) + two sprays of NSKE 5% + two sprays of HaNPV at 250LE/ha provided significant reduction in population of all sucking pests and resulted in highest grain yield. The module also proved superior to chemical control in sunflower.

Cultural practice (hoeing) was tested for the purpose of exposing the eggs inside the soil to the sunlight, However, it did not show any role for the management of sucking pests, although cultural practice in combination with insecticide increased the yield of sunflower. The results of present study suggested that the combination of all three practices (T_7) can be used to get a better and safer control of the sucking insect pests to produce higher yield of sunflower.

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A Key to the Cicada Fauna of Pakistan Based on Structural Variation in the Timbals (Hemiptera: Cicadoidea)

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Abstract.- The, timbals (acoustic organs) of fifteen genera of the family Cicadidae of Pakistan are examined and illustrated. A key to the genera of cicadas of Pakistan is provided based upon diagnostic variations in timbal and timbal cover structure.

Keywords: Timbals, genera, Cicadidae, Pakistan

The sound system of cicadas has been used to distinguish genera and higher taxa of the Cicadoidea for many years (see review in Moulds, 2005). Although many of the structures used in the past have been shown to be incapable of grouping higher taxa (Moulds, 2005), the sound system can still be useful in distinguishing localized fauna. For example, Moulds (2005, 2012) employed cladistic methodologies analyzing Australian Cicadidae including the use of a few timbal characters.

This paper aims to explore the utility of using variations in timbal and timbal cover structure of the sixteen genera of Pakistani cicadas. Although the variations described here are shown to be useful in distinguishing genera, it should be noted that within species in a given genus there is diversity in the shape of the timbal cover, the number of ribs, and the surface of timbal as these are useful characters to distinguish species. A key is formulated of fifteen genera of Pakistani cicadas based on the timbal.

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Material and methods

Specimens of sixteen different genera of family the Cicadidae were collected from various localities of Pakistan between 2006-2012. The timbal was dissected from the cicada to produce the line drawings using a Wild Leitz binocular microscope.

Male specimens of the genus *Meimuna* Distant were not available for study as only a female specimen of *M. velitaris* (Distant, 1897) has been collected. Since the genus is part of the Pakistani cicada fauna (Ahmed and Sanborn, 2010), the generic characters (Sanborn, 2013) were used to insert the genus in the key. Three additional genera, *Chloropsalta* Haupt, *Klapperichicen* Dlabola and *Tanna* Distant, were only recently recorded (Ahmed *et al*., in press) for the country and are included in the key for completeness.

Results and discussion

The timbals of Pakistani cicadas are shown to be distinct and of value in identifying the local genera. The following key uses distinctive morphological characters of the timbal and timbal cover of the cicadas of Pakistan. As only males posses the sound producing apparatus in Pakistani cicadas, the key will only apply to males.

KEY TO THE GENERA OF CICADAS OF PAKISTAN ACCORDING TO TIMBAL VARIATIONS

1. Timbal without timbal cover..... 2
- Timbal with timbal cover..... 5

2. Timbal with more than four ribs..... 3
- Timbal with fewer than four ribs..... 4

3. Timbal elongate, ribs of significantly different lengths, longest rib greater than twice the shortest, upper part weakly sclerotized (Fig. 14).....*Tibicina* Kolenati, 1857
- Timbal approximately circular, longest rib les than twice the length of shortest, upper part broadly well sclerotized (Fig. 11) .....*Paharia* Distant, 1905

4. Timbal with long ribs present over anterior half; three ribs present (anteriormost rib shortest), each long rib closely abutting adjacent long ribs (Fig. 2) .... *Melampsalta* Kolenati, 1857
- Timbal with long ribs restricted to anterior third, four long ribs present (anterior two are shorter); long ribs clearly separated (Fig. 1) ....*Linguacicada* Chou *et al*., 1997

5. Timbal cover completely covering timbale..... 6
- Timbal cover not completely covering timbale.....11

6. Timbal cover transversely oval 7

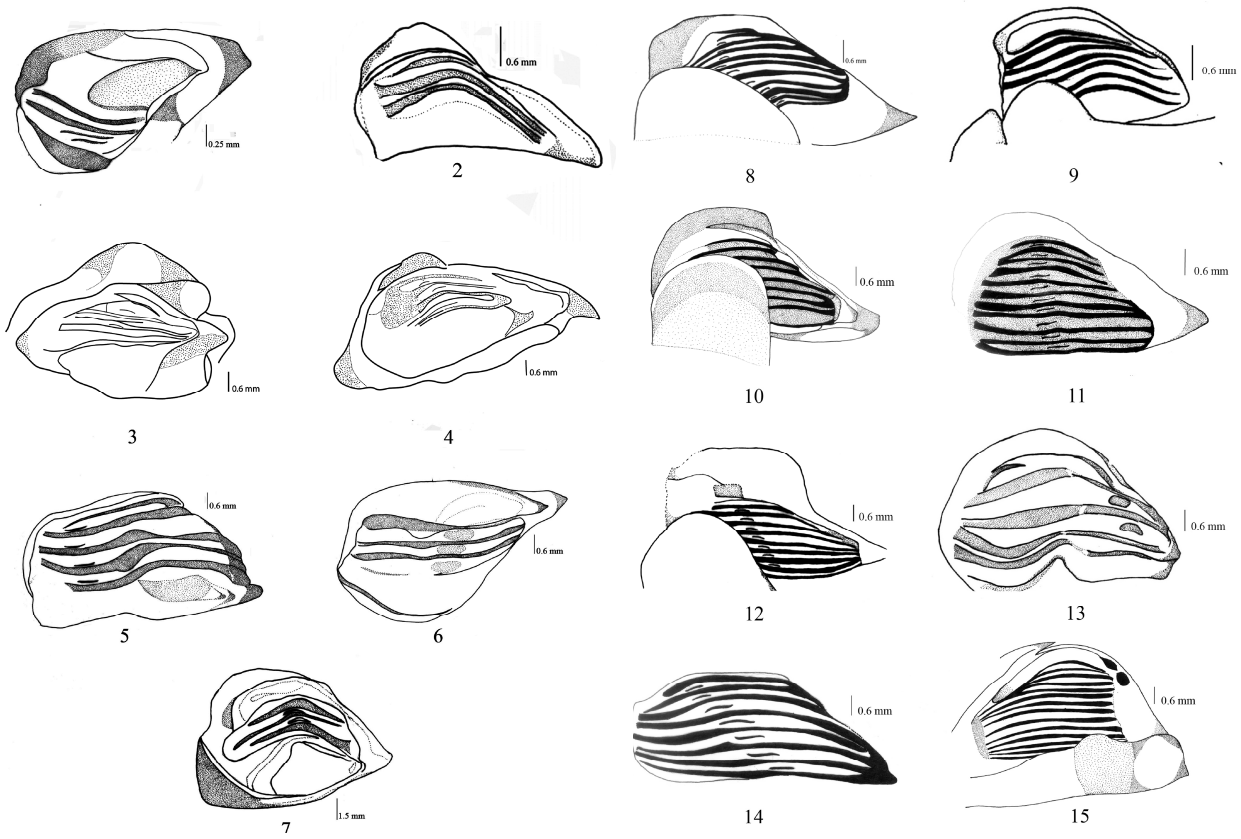

Fig. 1. Cicada fauna of Pakistan. 1) *Linguacicada continuata* (Distant, 1888), 2) *Melampsalta literata* (Distant, 1888), 3) *Pycna repanda repanda* (Linnaeus, 1758), 4) *Platypleura octoguttata octoguttata* (Fabricius, 1798), 5) *Haphsa nicomache* (Walker, 1850), 6) *Macrosemia saturata saturata* (Walker, 1858), 7) *Sonata obnubila* (Distant, 1888). 8) *Cicadatra persica* Kirkaldy, 1909, 9) *Shaoshia zhangi* Wei, Ahmad and Rizvi, 2010, 10) *Psalmocharias chitralensis* Ahmed and Sanborn, 2010, 11) *Paharia lacteipennis* (Walker, 1850), 12) *Klapperichicen turbatus* (Melichar, 1902), 13) *Tanna thalia* (Walker, 1850), 14) *Tibicina casyapae* (Distant, 1888), 15) *Chloropsalta ochreata* (Melichar, 1902).

- Timbal cover not transversely oval 9

7. Timbal cover very broad, 'dumbbell' shaped, laterally hollow, concealed by opercula apically, gray to chocolate brown, timbal with curved long ribs (Fig. 7) .. .. *Sonata* Lee, 2010

- Timbal cover not 'dumbbell' shaped, reaching ventrally to opercula..... 8

8. Timbal cover chocolate brown, covered with short golden pile at margin (Fig. 3) .... *Pycna* Amyot & Serville, 1843

- Timbal cover light brown, covered with scarce white pile, a depression laterally (Fig. 4) ....*Platypleura* Amyot & Serville, 1843

9. Timbal cover very thin, timbal with four long discontinuous long ribs and two broad, short intercalary ribs (Fig. 13) .....*Tanna* Distant, 1905

- Timbal cover longer than broad.....10

10. Timbal cover longer than broad, not extending ventrally over operculum, lobately produced apico-laterally, stout, scarce olive with gray brown dense pile, timbal with five long ribs, angulate (Fig. 5) .... *Haphsa* Distant, 1905

- Timbal cover extending laterally over operculum ....*Meimuna* Distant, 1905

11. Timbal cover reduced, timbal almost entirely exposed... 12

- Timbal cover more developed, timbal partially visible... 14

12. Timbal cover dark brown, lightly ochraceous between base and middle with scarce, long, dark bristle-like raised hairs (Fig. 12)..... *Klapperichicen* Dlabola, 1957

- Timbal cover not as above.....13

13. Timbal cover rectangular shaped, timbal much depressed internally, timbal with more than ten ribs (Fig. 15) ..... *Chloropsalta* Haupt, 1920

- Timbal cover crescent-like, timbal convex, slightly

depressed medially, timbal with fewer than ten ribs (Fig. 9) ..... *Shaoshia* Wei, Ahmed & Rizvi, 2010

14. Timbal cover concave basally, timbal scarcely exposed (Fig. 6) ..... *Macrosemia* Kato, 1925

- Timbal cover straight at base, majority of timbal exposedq 15

15. Timbal cover broadly oval, posterior half darker, timbal elongate (Fig. 10)..... *Psalmocharias* Kirkaldy, 1908

- Timbal cover narrowly oval, coloration uniform, timbal approximately circular (Fig. 8) .... *Cicadatra* Kolenati, 1857

Conflict of interest declaration

There are no conflicts of interest to be declared.

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Melamine Residues in Pet Food – Preliminary Report

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Abstract.- Eighty-four commercially available pet food samples including cat food (38 solid, 17 semi-solid) and dog food (20 solid, 9 semi-solid) were analyzed for melamine residues by competitive enzyme linked Immunosorbant assay. Among solid cat food, 78.94% (n=30) were contaminated with 14.43±1.27 (range >4-68 mg/kg) of melamine, while 82.35% samples of semi solid cat food (n=14) were found positive for melamine residue with mean value 6.43± 1.96 (range >2-13.14mg/kg). Similarly, 75% samples of solid dog food (n=15) were positive for melamine residues with mean value 8.50±1.73 mg/kg (range >4-49mg/kg). A total of 44.44% samples of semi solid dog food were positive for melamine with mean value 16.31±1.64 range of >2-34.25mg/kg. Out of all positive samples, 53% samples of pet food were contaminated beyond the Codex Alimentarius Commission (*i.e.* 2.5mg/kg). Analytical data showed existence of melamine residues in imported pet food**.**

Key words: Cat food, dog food, nephrotoxicity, CAC, pet.

Animal feed safety issue has gained great importance during passing few decades due to commercialization and rapid growth of animals industry. Among animals industry, pet food industry is flourishing exponentially because of their companionship with human beings particularly in western world. Pets are important not only for their loyal or playful characteristics but also seem to provide their owners with significant health benefit (Durrani *et al*., 2012). In the past, pets were usually raised upon wastes/remaining foods used by their owners. Later, for nutritionally balanced food

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requirement, formal pet food industry came into existence and now wide range of brands are available in market according to age and species of pet and being exported throughout the world. Pet food is usually prepared by using different ingredients *i.e.* cereals (corn, wheat gluten, corn gluten and rice protein) and meat portion. Sometime to elevate the apparent protein content, pet food's ingredients particularly grains protein including corn gluten and wheat gluten is adulterated with cheaper non protein nitrogen sources *i.e.* urea, cyanuric acid, ammelide, ammeline and melamine and its analogues (Ingelfinge,2008;Lakhshmi, 2012).

Melamine or 1,3,5-triazine-2,4,6-triamine is an organic base with chemical formulae ($C_3H_6N_6$). It is extensively used in plastic (Skinner *et al.*, 2010) and fertilizer industry due to its high nitrogen content (Gonzalez *et al.*, 2009). It is revealed that melamine contains 66 percent of nitrogen by mass. (Hau *et al.*, 2009). Melamine was considered to be non-toxic due to the reason that it is water soluble and efficiently (upto 90%) eliminated by kidneys within 24 h (Thompson *et al.*, 2008). The potential toxicity of melamine was taken into account seriously after consecutive outbreaks of pet's renal failure and associated deaths in Taiwan and United States during years 2004 and 2007 (Burns, 2007). The recalls in North America, Europe and South Africa came in response to reports of kidney failure in pets. The analysis of suspected food and pathological studies of kidneys of dead animals, it was identified that melamine is underlying cause of these out breaks (Chen *et al.*, 2009; Osborne *et al.*, 2009; Nilubol *et al.*, 2009; Park *et al.*, 2011). Melamine in combination with cyanuric acid becomes more toxic as compared to either melamine or cyanuric acid alone (Thompson *et al.*, 2008; Yhee *et al.*, 2009). It can precipitate in distal renal tubules and collecting ducts of kidney and chronic interstitial nephritis in animals and human beings (Dobson *et al.*, 2008; Bhalla *et al.*, 2009). In Pakistan, pet food is usually imported from Europe. As an emerging issue (melamine outbreaks), data regarding melamine residues in pet food is missing link in Pakistan. In view of foregoing, a preliminary study was conducted to assess the residue melamine in imported pet food marketed in Pakistan.

Materials and methods

Eighty-four commercially available pet food samples including cat food (38 solid, 17 semi-solids) and dog food (20 solid, 9 semi-solid) were collected from departmental stores of local market during a period of June to August 2011. The origin of pet food samples were from EU, UK and Korea. Samples were stored at 4°C till further analysis. Direct competitive ELISA kit (AgraQuant® Romer, Singapore) was used for the determination of melamine residues. For analysis, 2g of homogenized pet food samples were mixed with methanol: water (1:9; v/v) and vortexed vigorously for 2 min. The sample extracts were then filtered by using 0.45µm syringe filter (MiniSart® Germany). Filtrate was diluted with diluent solution. Dilution factor for solid and semi solid food were 100x and 200x respectively as per instructions given by ELISA kits manufacturers. Optical density (OD) values were recorded by using BioTek® ELISA Reader Elx808 (BioTek®, USA). The OD data was computed by using BioTek® Gen5 software (BioTek®, USA) for melamine quantification. Limit of detections for semi-solid and dry pet foods were 2-50mg/kg and 4-100mg/kg respectively.

Calibration curve was drawn by using linear regression equation of expected versus observed melamine. Recoveries for spiked pet food samples *i.e.* semi-solid and solid ranging from 0 to 3000ppb, showed good linearity *i.e.*, R^2 0.993 (solid) and 0.996 (semisolid) respectively (Fig. 1). For descriptive statistics, data was analyzed by using SPSS16 Software.

Results and discussion

A total of 75% pet food samples (n=63) were found positive for melamine ranging from >2-68mg/kg. Detailed results of all collected samples including cat and dog food samples are summarized in Table I. Data was further computed for frequency distribution of melamine in dogs and cat foods. Highest percentage of positive samples were found in the range of >5-10mg/kg and >20- 40mg/kg respectively (Fig.2).

Melamine does not exhibit systematic toxicity individually, but able to complex with other substances such as endogenous uric acid or cyanuric acid to form crystal in the tubules which cause

Table I. Melamine contamination (Mean±SE) in collected pet food samples.

Pets	Food type	n	Positive (%)	Mode (mg/kg)	Mean ±SE (mg/kg)	Range (mg/kg)	<MRL* n (%)	>MRL* n (%)
Cat	Solid	38	30 (78.94)	4.22	14.43±1.27	>4-68	-	30 (100)
	Semi Solid	17	14 (82.35)	5.40	6.43±1.96	>2-13.14	09 (64.28)	5 (35.72)
Dog	Solid	20	15 (75)	1.85	8.50±1.73	>4-49	-	15(100)
	Semi Solid	09	04 (44.44)	5.10	16.31±1.64	>2-34.25	1 (25)	3 (75)
	Total	84	63 (75)	-	6.20 ±0.97	>2-68	10(15.87)	53 (84.13)

*MRL- Maximum Residual Level established by CAC, 2010 *i.e.* 2.5mg/kg.

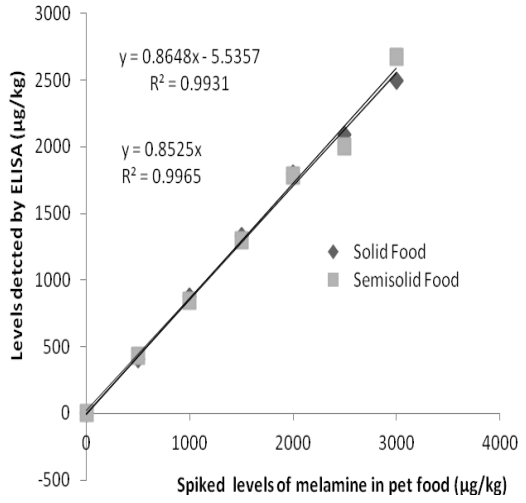

Fig. 1. Linear regression equation for melamine spiked samples solid and semi-solid pet food samples.

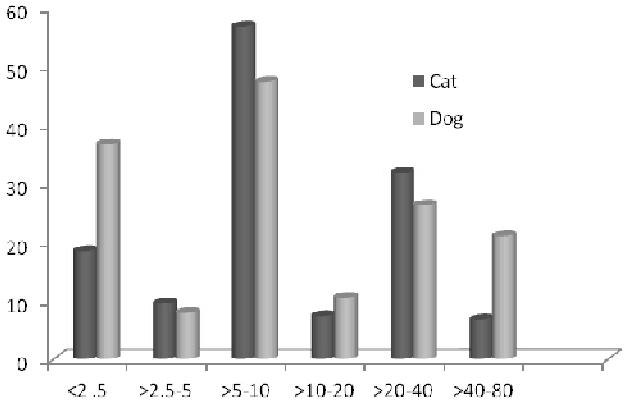

Fig. 2. Frequency Distribution of melamine (mg/kg) residue in collected dog and cat food samples.

kidney damage (Puschner *et al.*, 2007; Park *et al.*, 2011). Pathological studies of kidney of those affected/ died dogs and cats identified crystals of calcium oxalate and ammonium urate in the distal tubules and yellow discoloration of inner part of medulla with deposition of yellow green crystals in pelvic tubes (Skinner *et al.*, 2010; Cocchi *et al.*, 2010).

In fact, concurrent administration of melamine and cyanuric acids results in acute toxicity due to the formation of insoluble product *i.e.* melamine cyanourate at low pH *i.e.* 5.8. (Cianciolo *et al.*, 2008; Bhalla *et al.*, 2009). The general range of urine pH of cats and dogs is 5.5-7.0 depending upon the nature of diet. It is usually more inclined towards acidic values.Therefore, low urinary pH is a contributing risk factor for crystals formation in kidneys (Cocchi *et al.*, 2010). Highest level (68mg/kg) observed in present study showed a constant dietary exposure to melamine may cause stones formation and increases the incidence of urinary bladder in pets particularly male cats (Park *et al.*, 2011; Puschner *et al*., 2007). In present study, all samples were of imported brands and the countries of origin were supposed to be pioneers for declaring stringent legislations (*i.e.* EU & UK for melamine in infants and pet foods). However, these residual levels might be due to plastic packing materials leaching. Ludn and Petersen (2006) reported that continuous melamine migration may occur throughout the lifetime of the product when it is exposed to hot acidic foods. Furthermore, pH of present collected solid pet food samples were 5.8-6.4 whereas, pH of semisolid was 5.95- 6.8. Samples collection during warm climate (*i.e.* June to August, 2011) might be the reason for the melamine

existence in pet food samples. During these month temperature ranged between 44 to 48°C of Pakistan. Hence, melamine can be leached out from plastic packings to pet food due to improper storage, transportation and direct exposure to heat/ sunlight (Bradely *et al.*, 2005; Luden and Petersen, 2006).

In conclusion, data of present study revealed the presence of melamine residues in imported pet food and found far above the legislative limits. Findings of present study may be used as preliminary information to plan biological trials by keeping in view of other melamine related chemical compounds particularly cyanuric acid.

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Conflict of interest statement

The authors declare that they have no conflict of interest.

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Genetic and Clinical Features of Triploid Fetus: A Case Report in Han Chinese Population

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Abstract.- Triploidy is characterized by the presence of an additional set of chromosome in organisms. It has been proved lethal for humans' fetus and causing abortion at last trimester of pregnancy. Some of the triploidy neonates can survive up to several weeks with several physical and mental abnormalities. In current study we enrolled a 27-year-old pregnant woman for genetic counseling with a suspect of Edward's syndrome (trisomy 18) at her gestation period of 16 weeks, GTG-banded metaphase analysis revealed a karyotype of 69 XXX. Microsatellite based genotyping of fetus and parents revealed that triploidy is initiated during meiosis-I and inherited from mother. Therefore, we suggest that the diagnosis of triploidy should be performed at early stage of gestation to avoid the complication at late stages of pregnancy.

Key words: triploidy 69 XXX, digynic triploidy, diandric triploidy, prenatal Diagnosis, fetal diagnosis.

Triploidy syndrome is an extremely rare and lethal chromosomal disturbance in which individuals have 69 rather than 46 chromosomes. The majority of fetuses with triploidy are spontaneously miscarried during the pregnancy, and some of neonates with triploidy can survive for several hours after the birth with several physical defects like severe growth retardation and other multiple birth defects (Hassold *et al.,* 1980; Redline *et al.,* 1998). Despite the unknown etiology of triploidy, various studies have shown a possible maternal and paternal genetic factors contributing to the recurrent triploid pregnancies (Brancati *et al.,* 2003; Pergament *et al.,* 2000; Check *et al.,* 2009). Keeping in view all these possible risk factors, we initiated this study to evaluate the possible abnormalities and genetic background of aborted triploid fetus reported in Chinese Han population.

Patient and clinical findings

In current study a 27-year-old pregnant woman was referred for genetic counseling of abnormal maternal serum screening test at her gestation age of 16 weeks, screening tests were positive for Edward's syndrome (trisomy 18) with very low chorionic gonadotropin and normal alpha-fetoprotein levels (Table I). Several other serological tests were performed which revealed low level of creatinine, blood urea nitrogen and alkaline phosphates. Serum high density lipoprotein (HDL) level was little bit elevated (Table II). The other serological parameters were found normal in patient. The calculated risk for trisomy 18 was more than 1:10; amniotic fluid gradually reduced from gestation age of 14 to 20 weeks, and an ultrasound examination revealed severe oligohydramnios.

Table I.- Biochemical findings at second trimester of pregnancy.

Maternal Serum Markers	Concentration	Multiple of median	Reference
Alpha-fetoprotein	58.40 U/ml	1.44	0.40~2.50
Human chorionic gonadotropin	1.12 ng/ml	0.06	0.25~2.50

The maternal serum level of second trimester's pregnancy biomarkers were investigated in pregnant woman at her 16th week of gestation. The level of alpha-fetoprotein was found normal, while human chorionic gonadotropin was detected as lower at 0.06 MoM.

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Amniocentesis was performed at 21st week of pregnancy and the amniotic fluid was used to examine karyotype and genotype of the fetus. GTG-banded metaphase analysis revealed 69 XXX karyotype of fetus (Fig.1A), while parents have normal karyotype. To find out the inheritance pattern and exact cause of triploidy in fetus; a set of meiosis specific microsatellite markers was used (Table III). The molecular analysis of fetus and parents revealed that the extra haploid set of chromosome in fetus is resulted from nondisjunction at maternal meiosis-I. After detailed counseling and personal decision of the patient, the pregnancy was terminated at 25th week of gestation.

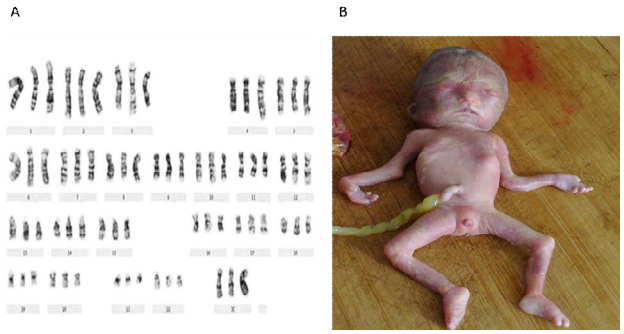

Fig. 1. Karyotype and Physical analysis of the triploid fetus; A, showed XXX69 karyotype of fetus; B, revealed overall physically abnormal fetus. The fetus had a small but almost normal appearance of placenta, weight 20g, and size 10cm×8cm × 0.7 cm, with two umbilical arteries and one vessel.

Table II.- Serological examination of mother at second trimester of pregnancy.

Biochemical Markers	Results	Reference Value	Unit
Creatinine (Cr)	37.13↓	50.00~120.00	μmol/L
Blood urea nitrogen (BUN)	1.58 ↓	2.14~7.14	mmol/L
Alkaline phosphates (ALP)	32.19↓	42.00~140.00	IU/L
High density lipoprotein (HDL)	2.74 ↑	0.90~2.30	mmol/L

Different biochemical tests were performed to find out the associated risk factors of abnormal fetus. The down arrows represent decreased serum level and up arrow reveal increased serum level of the certain biomarkers.

Table III.- Meiosis specific microsatellite markers used for genotype analysis of fetus and parents.

Markers	Father	Mother	Fetus	Interpretation
D8S1179	12/13	11/13	11/12/13	
D21S11	29/31	30/32.2	29/30/32.2	Mat MI
D7S820	8/8	9/12	8/9/12	Mat MI
CSF1PO	12/12	10/10	10/12	
D3S1358	15/16	15/17	15/17	
D5S818	10/13	11/12	10/11	
D13S317	9/12	8/11	8/9/11	Mat MI
D16S539	12/12	9/9	9/12	
D2S1338	20/24	20/24	20/24	
D19S433	13/14	14.2/15	14/14.2/15	Mat MI
VWA	16/18	17/20	17/18/20	Mat MI
D12S391	17/23	18/19	18/19/23	Mat MI
D18S51	13/16	13/16	13/16	
D6S1043	13/18	17/19	17/18/19	Mat MI
FGA	21/23	21/22	21/22	

Different microsatellite markers were used to find the inheritance of triploidy in fetus. Analysis of data revealed that error was occurred in maternal meiosis-I (Mat: MI) and inherited to fetus.

The pathological examinations revealed a female fetus, weight 370g, length 25cm, including intrauterine growth retardation, hypotonia, low-set ears, wide-set eyes (hypertelorism), and limb abnormalities (Fig.1B). Following several physical abnormalities were found in fetus; the left hand had bilateral overlapping of the third and fourth fingers, right hand had bilateral overlapping of second and third fingers, total syndactily of the feet, heart defects, with aplasia of the lungs. The fetus had a small but almost normal appearance of placenta, weight 20g, and size 10cm×8cm × 0.7 cm, with two umbilical arteries and one vessel.

Discussion

Triploidy is one of the most common chromosomal aberrations which causes abnormal fetus leading to spontaneous abortion, only rare triploid fetus can live after birth. Although the etiology of triploidy is not clear, the mechanisms of Triploidy may be maternal (digynic triploids) or paternal (diandric triploids). Digynic triploids results from error in first meiosis in oocytes; the resultant fetus has two sets of maternal chromosomes and a single set of paternal chromosomes. Digynic pregnancies usually have a

small placenta which secretes less human chorionic gonadotrophin (hCG), and fetal adrenal growth occurs under the influence of hCG, diagynic fetus usually has severe growth retardation associated with marked adrenal hypoplasia (Eidben *et al.,* 1996; McFadden *et al.,* 2002). Diagynic fetus can live upto second trimester, and some can survive few hours after the birth. Here we enrolled a pregnant woman with diagynic fetus and very low level of gonadotropin; fetus was aborted at 25th week of gestation. A very low level of maternal gonadotropin has been associated with Triplody (Schmidt *et al.,* 1994) and low chorionic gonadotropin and alpha-fetoprotein have also been reported in Down syndrome and trisomy 18 (Lehavi *et al.,* 2005; Ozturk *et al.,* 1990). Therefore, we suggest that the low level of gonadotropin can also be used as a biomarker for triploidy suspect during the second trimester of pregnancy in routine diagnosis.

Morphological analyses of the fetus represent complete abnormal body structure with incomplete growth of body parts and high power lens microscopic examination revealed an abnormal and incomplete internal body structure. Placenta and fetal membranes are appeared to be normal; umbilical cord contains two umbilical arteries and one vessel; these findings are consistent with previous reports (Hassold *et al.,* 1980; Redline *et al.,* 1998).

Unlike other aneuploidies, maternal age is not a risk factor for triploidy. Till now it has not been found any remarkable risk factors of triploidy pregnancies; therefore it indicates that a woman who has had one triploid pregnancy is not susceptible to carry another triploidy fetus. Although triploidy occurs randomly, few women may have recurrent miscarriages due to triploid fetus (Zaragoza *et al.,* 2000; Huang *et al.,* 2004). It has been identified that the maternal susceptibility locus on chromosome 19 is responsible for recurrent hydatidiform moles (Moglabey *et al.,* 1999).

In previous report total 13 embryos in IVF procedure were resulted in two additional triploid embryos, with an overall number of four triploid conceptuses in a woman, authors suggested that the event of triploidy occurred during maternal meiosis II (Pergament *et al.,* 2000). Huang *et al.* (2002) also reported triploid pregnancy with maternal origin; they speculated that the triploidy may be associated with the errors in both meiosis I and II.

In our present case, a total of 15 markers for 13 different chromosomes were used to analyze the origin of the extra-haploid chromosome. Seven markers revealed that the fetus inherited with two maternal alleles and one paternal allele, which prove maternal inheritance of triploidy that occurred due to failure of homologous chromosome or sister chromatids to separate properly in meiosis-I. More comprehensive studies are required to investigate the possible risk factors and exact underlying mechanism of triploidy occurrence. Therefore, we highly recommend that maternal diagnosis for triploidy must be done at early embryonic stage to avoid the complication of the pregnancy at late stage and to ensure the mothers' health during abortion.

Conflict of interest

All authors have declared that they have no conflict of interest.

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