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Some Observations on the Infestation of Bivalve, *Scapharca natalensis,* by Larval Nemetode, *Echinocephalus* (Nematoda: Gnathostomatidae)

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Abstract.- Larvae of *Echinocephalus* was reported from arkshell *Scapharca natalensis* from Clifton beach, Karachi infesting either foot, alimentary canal wall and genital duct. All the specimens of the host collected during October, 1978 were observed to be infested. About 37% of the specimens of *Scapharca natalensis* were observed to be infested with by 1 larval *Echinocephalus* per host while 11% were infested with 2 larvae per host. Only 8% of the specimens were infested by 3 larvae and 3 % by 4 larvae per host. No apparent ill effect of the parasite on host was noticed.

Key words: Larvae, *Echinocephalus,* host, bivalve, arkshell, seasonal distribution.

Larval *Echinocephalus* sp. is known to infest different bivalves and sea urchins (Cheng, 1978). Though life history of *Echinocephalus* is not well understood, it is known that larvae of this genus are found in different part of the viscera of the hosts. Adult of these parasitic nematodes spend their life in the spiral-valve region of marine rays and other fishes (Arya, 1986; Ko *et al.,* 1974). *Echinocephalus,* at present, is represented by ten valid species that are limited in host distribution primarily to marine and freshwater stingrays (Hoberg *et al.*, 1998). However, only a few species of *Echinocephalus* are known to specific level because most of the specimens were collected from body of bivalves and in larval condition the distinguishing characters are not sufficient enough to identify them to specific level (Milleimann, 1963). Abalone, sea urchins and bivalves serves as intermediate host and adult life is spent in elasmobranches (Milleimnan, op. cit, 1963; Gomez-del-Prado-Rosas, 1983, 1984). Adult *Echinocpehalus pseudouncinatus* is known to parasitize horned shark (*Heterodontus francisci*) and bat stingray (*Myliobatis californicus*) whereas its larvae are known to parasitized abalones (*Haliotis corrugata* and *H. fulgens* etc.). Pearse and Timm (1971) reported juveniles of *E. pseudouncinatus* from gonad of sea urchin. Similarly adult *Echinocephalus janzeni* was reported to infest marine stingrays of Central America (Hoberg *et al*., 1998). Adult *Echinocephalus overstreeti* recorded from stingray (*Taeniura melanopilos*) from Marquesas Island (Deardorff and Ko, 1975) and also infest thornyback ray *Urogymnus asperrimus* from Enewetak Atoll (Brooks and Deardroff, 1988). This species is also reported from Australian water infesting elasmobranches and mollusks (Beveridge, 1987; Beveridge, 1991). Adult *Echinocephalus diazi* infest stingray *Himantura schmardae* in Venezuela (Hungria, 1978). Baylis and Lane (1920) reported this species from ray *Myliobatis nichofi* and bivalve *Pinna* sp. Beberidge (1985) reported *E. uncinatus* from ray *Dasyatis pastinacea*. Milleimann (1963) described in detail the taxonomy of the genus *Echinocephalus* and given description of four species besides a number of larvae of unknown *Echinocephalus*. Ko (1975, 1976, 1977) have dealt with taxonomy and biology of *Echinocephalus sinensis* found in oyster *Crassostrea gigas* from Hong Kong. Recent review of the genus *Echinocephalus* was made by Hoberg *et al* (op. cit, 1998).

Anderson (2000) speculated that the intermediate hosts of *Echinocephalus* spp. are arthropods, probably marine crustaceans such as copepods and that molluscs, echinoderms and other marine organisms serve as paratenic or second intermediate hosts in which growth occurs.

No information about *Echinocephalus* is available from Pakistan except Bilqees and Fatima (1992) who reported *Echnocephalus* larvae from visceral mesenteries of a teleost fish *Protonibea*

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diacanthus from Karachi. Khan and Begum (1971) recovered *Echinocephalus uncinatus* from the mesentery of *Cyanoglossus sindensis* and *Lates calcarifer*. *Echinocepahalus. muraenesocis* was reported from conger pike Pakistan by Bilqees *et al.*, (1971), however, according to Deardroff *et al*., (1981) this species was not adequately described. Bilqees and Fatima (1992) reported *Echinocephalus muraenesocis* from *Muraenesox.* Larval *Echinocephalus* sp. were known to parasitize a number of teleost fish seabream *Argyops filamentosus, Pseudorhombus arsius* and *Trachinotus blochi* at Kuwait (Petter and Sey, 1997). Shipley and Hornell (1904) reported larvae of unknown *Echinocephalus* sp. infesting the adductor muscle of pearl oyster *Margratifera* from Sri Lanka. Rajyalakshmi (1994) reported *Echinocephalus scoliodonti* from *Rhizoprionodon acutus* (as *Scoliodon sorrakowah*) from India. Gupta and Garg (1976) reported *Echinocephalus uncinatus* from *Thryssa* sp. whereas Shafee and Natrajan (1976) reported *E. uncinatus* from eagleray *Aetobatus narinari* from Bay of Bengal (Rajyalakshmi *et al.*, 1984).

Consumption of raw bivalves and abalone etc. may lead to infestation in human causing larval migrans in which larval nematodes does not develop into an adult but penetrate through the gut wall and wanders aimlessly in the body before being overcome by the internal defense mechanism of the body (Bower, 2001). Present paper deals with incidence of larval infestation by unknown *Echinocephalus* sp. in bivalve *Scapharca natalensis* from Clifton, Karachi.

Materials and methods

Monthly collection of arkshell *Scapharca natalensis* was made from intertidal areas on the sandy beach of Clifton, Karachi, Pakistan between July 1978 and July 1979. The area is located on the eastern side of Manora Channel and characterized by long stretch of sandy micaceous beach extending upto Gizri Creek. *Scapharca natalensis* is found inhabiting the sand bed near the lower littoral zone.

The collected specimens of the arkshell *Scapharca natalensis* were kept alive in the laboratory in the running seawater. Dissection of the animals was made and live larval *Echinocephalus* were removed from the tissue. The larvae can be easily detected with their head embedded in the tissue while the body is wriggling. The specimens were persevered in neutralized 5% formalin after narcotization with isotonic $MgCl_2$ solution.

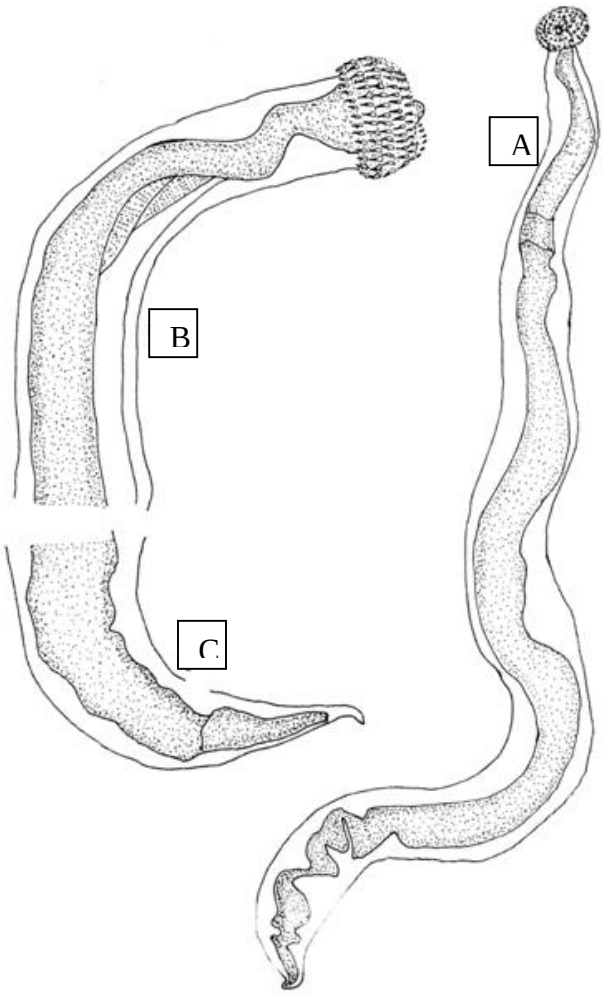

Fig. 1 *Echniocephalus* sp. (A) Complete Larva (B) Anterior portion (C) Posterior portion

Results and discussion

Larvae of *Echinocephalus* were found to infest the visceral organs of the arkshell *Scapharca natalensis* especially attached to either foot, wall of alimentary canal and genital duct. The larvae have an elongated body with rounded head Figure 1A. *Pro see* view also shows only six rows of hooks (Fig. 1B). The tail is pointed and alimentary canal is opened to side through anus a little behind the tail

(Fig. 1C). These characters are insufficient to identify the larvae found in *Scapharca natalensis* to specific level. Female larvae of a number of species of *Echinocephalus* have six rows of hooks such as larvae reported to *Echinocephalus uncinatus* by Millemann (1963) and *E. sinensis* from Hong Kong by Ko (1975).

Table I present the data of infestation of arkshell *Scapharca natalensis* by *Echinocephalus* sp. at Clifton. It is evident that 37% of the specimens of *Scapharca natalensis* were observed to be infested with by 1 larval *Echnocephalus* per host while 11 % were infested with 2 larvae per host. Only 8 % of the specimens were infested by 3 larvae and 3 % by 4 larvae. Though only 10 shells were collected during October 1978 but all of them were found to be infested with *Echinocephalus* larvae. Among these 4 were infested with 1 larvae and remaining with 4 larvae of the parasitic nematode. The size of larval *Echinocephalus* ranges between 0.6 to 2.4 cm.

Table I.- Monthly infestation in the *Scapharca natalensis* by *Echinocephalus sp.*

Months	No. of Specimens	Nil	1	2	3	>4
July 1978	21	9	6	5	1	0
August	17	6	5	4	2	0
September	9	3	4	1	1	0
October	10	0	4	0	0	6
November	22	8	11	2	1	0
December	8	3	1	4	0	0
January 1979	14	5	7	1	1	0
February	24	10	13	1	0	0
March	16	6	9	1	0	0
April	31	19	10	1	1	0
May	12	5	3	2	2	0
June	21	11	4	1	5	0
July	7	2	2	0	3	0
Total	212	87	79	23	17	6
Percentage infestation		41	37	11	8	3

Larval *Echinocephalus* was observed to be attached to different parts of the visceral mass of the host including to the genital duct. Ko (1975) reported similar infestation of *Echinocephalus sinensis*. During the present study a number of samples of fishes including rays and sharks were examined to trace the adult *Echinocephalus* but none revealed any nematode to this genus. In the absence of adult and based on basis of larval characters it seems impossible to identify the larvae to specific level. No apparent harmful effect of larval infestation was noticeable on the host during present study as most of the infested specimens survived for more than two months in laboratory without any apparent ill effects. During the present study 100% infestation observed during October 1978 may be on account of smaller sample size.

This species was not observed to infest any other bivalve found on Clifton beach although many species of bivalves were examined. Although primary host of this species was not traced but possibility of infestation of this species to human being seems to be remote because rays are not eaten in Pakistan and also bivalves are not utilized for food. Ko (1977) have carried out experiments on the infestation of kitten and other domestic animals though oyster *Crassostrea gigas* infested with *Echinocephalus sinensis* and concluded that there is strong possibility that human being can be infested if raw and improperly cooked hosts are consumed.

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Evaluation of Different Insecticides Against Rice Leaffolder *Cnaphalocrocis medinalis* (Guenee) Under Field Conditions

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Abstract.- Rice leaffolder is considered as a sporadic insect pest of rice in Pakistan. It can cause 30 to 40% leaf infestation and 30% yield loss. A field trial was conducted at Rice Research Institute Kala Shah Kaku Punjab Pakistan in which efficacy of certain insecticides *viz.*, Proaxis 60CS (gamma-cyhalothrin) @ 75ml, Karate 2.5EC (lambda cyhalothrin) @ 200ml, Padan 4G (cartaphydrochloride) @ 9Kg, Belt480SC (flubendiamide) @ 50ml, Virtako40WG (thiamethoxam + chlorantraniliprole) @ 40gm, Coragen 20CS (chlorantraniliprole) @ 50ml per acre was checked against rice leaffolder *Cnaphalocrocis medinalis* (Guenee). The experiment was laid out in RCBD with three replications. Insecticides were applied at economic threshold level (ETL) and observations were recorded after seven days of insecticide application. Yield data was taken before harvest of rice crop. All the insecticides effectively controlled the pest and increased grain yield was achieved as compared to control. Belt 480 Sc with 1.12% damaged leaves was the best amongst all treatments followed by Proaxis 60CS 1.28%, Karate 2.5 EC 1.83%, Padan 4G 2.37% Virtako 40WG 2.53%, Coragen 20CS 2.59% and in control 15.83% damage was observed. Highest paddy yield was achieved in case of Belt 480 Sc 3.33 t/h followed by Proaxis 60CS 3.18 t/h, Karate 2.5 EC 3.01 t/h, Virtako 40WG 2.87t/h, Padan 4G 2.75 t/h, Coragen 20CS 2.57t/h and control 2.21 t/h. All the new test insecticides proved very effective for the control of rice leaffolder.

Key words: Rice, *Oryza sativus*, Padan, virtako, coragen, Proaxis, Belt, Karate.

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Rice *(Oryza sativa* L.) ranks as second amongst the staple food grain crops in Pakistan and it has been a major source of foreign exchange earnings in recent years, and is the staple food for more than 2 billion people, mostly in developing countries (FAO, 1995). Pakistan grows a high quality of rice to fulfill the domestic demand and also for exports. Rice accounts 4.9% of the value added in agriculture and 1% of GDP (Anonymous, 2011).

Insects are important constraints to rice production and occur in all rice growing environments. The rice leaffolder (RLF), *Cnaphalocrocis medinalis* (Guenee) (Lepidoptera: Pyralidae), is a major insect pest of rice. Outbreaks of serious infestations of rice leaffolder, have been reported in many Asian countries including China, India, Japan, Korea, Malaysia, Sri Lanka and Vietnam (Wada and Shimazu, 1980; Heong, 1993).

In Pakistan, this pest multiplies enormously, with a severe incidence observed during August-September of the cropping season. The overall leaf infestation by *C. medinalis* was seen to be 25 % which reduced the grain yield up to 30% in major rice growing tracts of Punjab. However, at some locations the infestation was found to be 50% (Salim *et al.*, 1991). A close correlation very often exists between the intensity of leaffolder attack and loss in grain yield (Upadhyay *et al*., 1975). Bautista *et al.* (1984) have clearly shown that loss in yield due to the rice leaffolder is positively related to the percentage of damaged leaves. In their studies, 17.5% infested leaves resulted in 16.5% yield loss, and a loss of 21.3% yield occurred with 26.6% damaged leaves.

Though the integrated pest management practices like use of resistant varieties, fertilizer light traps etc. have substantial effect on management of this pest, insecticides still remain the major control measure against leaffolder. In different experiments conducted by farmers in Philippines, the rice yield increased from 0.44 to 3.19t/ha due to insect pest control by insecticides (Heinrichs, 1978; Litsinger *et al*., 1980).

In the present study the effectiveness of some insecticides have also been evaluated against rice leaffolder.

Materials and methods

To determined the efficacy of new insecticides against rice leaffolder field experiments in triplicate were laid out in randomized complete block design at Rice Research Institute, Kala Shah Kaku, Punjab, Pakistan during the kharif season 2010 and 2011.

Super Basmati was planted in plot size of 4x7m. Plant to plant distance was maintained at 9 inches. Fertilizer was applied as 133 kg nitrogen, 85kg phosphorus and 62 kg potash, per acre.

Seven insecticides *viz*., Proaxis 60CS (gamma cyhalothrin), Karate 2.5 EC (lambda cyhalothrin), Padan 4G (cartap hydrochloride), Belt 480SC (flubendiamide), Virtako 40 WG (thiamethoxam + chlorantraniliprole), Coragen 20CS (chlorantraniliprole) were checked against rice leaffolder *Cnaphalocrocis medinalis* (Guenee) at the doses of 75 ml/acre, 200 ml/acre, 9 kg/acre, 50 ml/acre, 40 g/acre and 50 ml/acre, respectively. Padan and lambda cyhalothrin were used as standards. Insecticides were applied at economic threshold level (ETL) of the pest and observations regarding damaged leaves were recorded after seven days of insecticide application. Yield was recorded at the time of crop maturity. Statistical analysis was done by using MStatC software. The infestation by rice leaffolder was calculated by the following formula.

$$\frac{\text{No of infested hills}}{\text{Total no of hills}} \times \frac{\text{No of damaged leaves}}{\text{Total no of leaves}} \times 100$$

Paddy yield was calculated by the following formula. Moisture was taken with the help of moisture meter.

$$\text{Yield at 14\% moisture} = \frac{\text{Yield X 100} - \text{Actual moisture}}{100 - 14}$$

Results and discussion

Table I shows the damage by rice leaffolder before and after the application of insecticides during kharif 2010 and 2011 which ranged from 1.12% to 15.83 % leaves in all the treatments.

All the insecticides effectively controlled the pest and in resulted increased grain yield compared

Table I.- Percentage damaged leaves in pre-treatment, post treatment application of different insecticides along with yield.

Name of product	Damaged leaves (%)		% control over check*	Yield (T/h)
	Pre-treatment	Post-treatment		
Belt 480SC	2.41±0.07	1.12±0.24 b	92.91	3.33±0.12 a
Proaxis 60CS	3.09±0.60	1.28±0.4 b	91.89	3.18±0.11 ab
Karate 2.5 EC	2.78±0.05	1.83±0.42 b	88.41	3.01±0.08 bc
Padan 4G	3.53±0.09	2.37±0.80 b	85.00	2.75±0.00 cd
Virtako 40 WG	3.08±0.31	2.53±0.31 b	83.98	2.87±0.09 c
Coragen 20 CS	3.23±0.28	2.59±0.69 b	83.60	2.57±0.06 d
Control	3.49±0.09	15.8±1.37 a		2.21±0.10 e
		LSD= 2.228		LSD= 0.2977

Mean±SE; Means sharing similar letters in a column are not statistically significant (P>0.05)
*These calculations were done with reference to control values.

to control. Belt 480 SC with 1.12 % damaged leaves (DL) was the best amongst all treatments. The damage in control plot was 15.83%.

A significant correlation was observed between different treatments and the yield. Highest paddy yield was achieved in the case of Belt 480 SC which was 3.33 t/h followed by Proaxis, Karate 2.5, Virtako, Padan and Coragen (Table I).

Belt (flubendamide) results with 1.12% DL is close to those of Sharma and Srivastava (2009) who observed 1.95% damaged leaves (DL) as compared to 10.66% in control with a combination treatment with flubendamide + fipronil. The results of our findings regarding damage in flubendamide and cartep hydrochloride treated fields agree with that of Kulagod *et al.* (2011), who stated that flubendamide gave better control and reduced leaf damage by rice leaffolder.

Results of our investigation agree with that of Kumar *et al*. (2010) who reported 2.37% leaf damage by rice field folder in field grown rice. Karthikeyan *et al*. (2007) reported reduction in damage done by yellow stem borer and also reduction in leaf damage caused by leaffolder by 47.7%, and increased grain yield after Padan treatment compared to control.

Karate 2.5EC with 1.83% damaged leaves is in line to that of Sharma and Srivastava (2008) who stated that Lambda cyhalothrin (Karate zeon 5CS) with 3.22% and 2.82% DL proved more efficacious than the monocrotophos (4.26% and 3.81% DL) for the control of leaffolder, *Cnaphalocrocis medinalis.*

Chlorantraniliprole and insect growth regulator (IGR) insecticides (tebufenozide and hexaflumuron) exhibited great efficacy against *C. medinalis* (Zheng *et al*., 2011). Toxicity test results showed that the mortality of *C. medinalis* fed on rice leaves treated by chlorantraniliprole was about 40% within 24h after treatment, reaching 100% within 96h after treatment. .The results altogether revealed that chlorantraniliprole had pronounced effectiveness against *C. medinalis* (Fang *et al*., 2009). These results conform to our findings that coragen 20 CS (chlorantraniliprole) proved very effective and showed 85.03% control over check against rice leaffolder.

Results of our finding agrees with that of Chalapathi-Rao *et al*. (2002) who stated that thiomethoxam effectively controlled rice leaffolder damage and increased grain yield over control was achieved.

It is therefore concluded that the new test insecticides proved very effective in the control of rice leaffolder.

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Sedative and Analgesic Effects of Xylazine in Rabbits

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Abstract.- An experimental study was carried out on nine rabbits to compare the sedative and analgesic effects and also to record some physiological effects of xylazine at three different intramuscular doses *i.e.* 6, 8 and 10 mg/kg body weight. Our results demonstrated that xylazine at 6 mg/kg produced medium sedation, whereas 8 and 10mg/kg of xylazine produced deep sedation in rabbits. In addition, the maximum skin analgesia occurred at 13.22±0.68 min with 10mg/kg body weight for the total duration of 56.55±0.76 min as against 23.77±0.79, 16.55±0.53 min for the total duration of 35.11±0.84, 15.88±0.44 min, 8 and 6 mg/kg, respectively. Furthermore, the current results demonstrated decrease in pulse rate, respiratory rate and body temperature in the experimental rabbits after 45, 60 and 75 min with 6, 8 and 10 mg/kg, respectively. Body temperature returned to base line after 75, 90 and 105 min with 6, 8 and 10mg/kg of body weight, respectively. On the other hand, some side effects such as salivation and tympany were observed after administration that were transient and did not pose any serious problem at any stage during the experiment. All side effects had returned to normal 120 min after administration. It can be deduced from the current study that xylazine is a potent and safe drug for sedation in rabbits.

Key words: Rabbit, xylazine, sedation, analgesia, dose response, physiological effects.

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Xylazine is a [2-(2, 6 dimethyle phenyl amino)-4H-5, 5 dihydro-1, 3-Thiazine] thiazine derivative classified as alpha$_2$ adrenergic agonist with sedative, analgesic and muscle relaxant properties (Paddleford, 1999). It has been used alone or in combination for minor surgical procedures.

Extensive studies have been carried out in various animal species *viz.,* sheep (Lin *et al.,* 1993), goat (Aithal *et al.,* 1997), mare (Skarda and Muir, 1996) and cattle (Aziz, 2008) to explore the sedative and analgesic role of xylazine. Tutunaru *et al.* (2011) and Baumgartner *et al*. (2012) have used xylazine in combination with general and/or inhalant anaesthetics in rabbits. Xylazine alone as sedative and analgesic has been very rarely used in rabbits. The present study aims at determining time dependent effect and functional significance of xylazine on the physiological factors in rabbits.

Materials and methods

Nine healthy male rabbits with mean age of 8.79±0.28 months and weight of 1188.89±81.98 g were purchased from Pakka Qilla, Hyderabad and were kept in Animal House, Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University, Tandojam. They were fed gram, chopped sorghum, burseem, barley grain and oats. Xylazine was administered intramuscularly at 6, 8 and 10 mg/kg body weight in thigh muscle of each of three rabbit. The pulse rate/minute, respiratory rate and body temperature was recorded first after 5 and 10 min and then every 15 min up to 120 min after administration of xylazine. The occurrence of analgesia was tested by deep pin pricking of skin at various body parts. Heart rate was recorded by auscultation of heart with the help of stethoscope and respiratory rate by observing abdominal movements during respiration while body temperature was noted per rectum with clinical thermometer.

Analysis of data was performed by using analysis of variance (ANOVA) and Duncan Multiple Range Test (DMRT).

Results and discussion

Table I shows the sedative and analgesic effects of xylazine administered at 6, 8 and 10 mg/µg body weight.

The deep and prolonged sedation has been demonstrated at 8 and 10mg/kg,whereas moderate sedation was observed at 6 mg/kg body weight. Our results further demonstrated that the total duration of sedation was dose dependent and was prolonged with increase in the dose of xylazine. Table I also shows that the onset of skin analgesia was more rapid with higher doses of xylazine. The difference in time for onset of skin analgesia and total duration of sedation in animals treated with different doses of xylazine were statistically significant ($P<0.01$). The result of our study are in line with those reported by various researchers in various animal species, such as sheep (Jam, 2005), small ruminants (Khan *et al.*, 2004), cattle (Aziz, 2008) buffalo (Al-Share, 1997 and sheeps (Coulson *et al.,* 1989).

Table II shows effects of three doses of xlazine on pulse rate, respiratory rate and change in body temperature of rabbits, after intramuscular administration of xylazine at 6, 8 and 10 mg/µg body weight. The pulse rate was significantly ($P<0.01$) decreased during first 5 min with all three doses of xylazine and this effect was dose dependent. Respiratory rate decreased significantly with all three doses of xylazine and returned to normal after 90, 105 and 120 minutes with 6, 8 and 10 mg/kg, respectively

Similar effects of xylazine have been reported by Nolan and Erhardt (1990) who reported that α2 agonist xylazine produced a dose-dependent decrease in mean arterial blood pressure in conscious rabbits when injected intrathecally through a cannula previously implanted under general anaesthesia. Likewise smiliar result have also been reported by Jam (2005) in sheep and Kumar and Thurmon (1979) in goats.

The finding of the current investigation were in agreement with that of Baniadam *et al.* (2004) who evaluated the effect of xylazine-ketamine on the cardiovascular and respiratory systems of sheep and reported that the mean arterial blood pressure decreased significantly 30, 45 and 60 min after anaesthesia and body temperature decreased significantly. Furthermore xylazine-ketamine combination had little depressant effect on the cardiovascular system, but decreased body temperature during anesthesia. These changes may

Table I.- Sedative and analgesic effects after administration of xylazine in rabbits.

	Xylazine dose		
	6 mg / kg (n=3)	**8 mg / kg (n=3)**	**10 mg / kg (n=3)**
Onset of sedation (min)	4.22± 0.14++**	2.77± 0.14 ++rr	1.44 ± 0.17 **rr
Total duration of sedation (min)	71.11± 0.65**++	88.77±1.44 ++rr	108.77 ± 1.28**rr
Onset of skin analgesia (min)	23.77±0.79 ++**	16.55± 0.53 +rr	13.22 ± 0.68 **rr
Total duration of skin analgesia (min)	15.88± 0.04++**	35.11±0.84++rr	56.55± 0.76**rr

Significant difference (P<0.01) between values for 6 and 8 mg / kg (++), for 6 and 10 mg / kg (**), and for 8 and 10 mg / kg (n)

Table II.- Mean pulse rate / minute, respiratory rate/min and temperature of rabbits after administration of xylazine

Time interval	**Xylazine dose groups**		
Minutes	**6 mg / kg (n=3)**	**8 mg / kg (n=3)**	**10 mg / kg (n=3)**
Pulse rate/min			
0 (Control)	202.44±0.33	202.33±0.44	202.11±0.38
05	195.77±0.52**++ΔΔ	186.33±0.57**++rr	180.66±0.37**rrΔΔ
15	185.55±0.64**++ΔΔ	180.22±0.59**++rr	175.44±0.44**rrΔΔ
30	180.33±0.40**++ΔΔ	174.77±0.66**++rr	171.33±0.47**rrΔΔ
45	173.66±0.52**++ΔΔ	166.66±0.55**++rr	160.22±0.52**rrΔΔ
60	178.66±0.47**++ΔΔ	160.88±0.51**++rr	152.33±0.66**rrΔΔ
75	190.22±0.83**++ΔΔ	172.33±0.52**++rr	147.33±0.57**rrΔΔ
90	202.66±0.37**++ΔΔ	186.44±0.44**++rr	177.33±0.81**rrΔΔ
105	202.77±0.27ΔΔ	202.55±0.41rr	191.66±0.52**rrΔΔ
120	202.88±0.30	202.66±0.28	202.22±0.36
Respiratory rate / min			
0(Control)	50.88±0.31	50.77±0.32	50.88±0.31
05	47.22±0.27**+ΔΔ	45.88±0.45**+rr	42.00±0.40** ΔΔ rr
15	45.22±0.43**++ΔΔ	42.55±0.41**++rr	39.77±0.36** ΔΔ rr
30	41.77±0.36**++ΔΔ	39.88±0.20**++rr	36.88±0.35** ΔΔ rr
45	40.22±0.36**++ΔΔ	37.22±0.70**++rr	35.00±0.28** ΔΔ rr
60	39.33±0.44**++ΔΔ	35.22±0.40**++rr	32.88±0.30** ΔΔ rr
75	42.77±0.36++ΔΔ	40.00±0.33**++rr	30.22±0.59** ΔΔ rr
90	50.88±0.35++ΔΔ	46.22±0.32**++rr	36.66±0.37** ΔΔ rr
105	50.88±0.30ΔΔ	51.11±0.38rr	46.66±0.33** ΔΔ rr
120	51.11±0.35	51.44±0.24	50.88±0.30
Temperature (°F)			
0(Control)	104.18±0.04	104.26±0.04	104.28±0.05
05	104.02±0.05**++ΔΔ	103.73±0.04**++rr	103.37±0.05** ΔΔ rr
15	103.82±0.05**++ΔΔ	103.40±0.06**++rr	103.06±0.04** ΔΔ rr
30	103.66±0.05**++ΔΔ	103.18±0.06**++rr	102.82±0.05** ΔΔ rr
45	103.31±0.07**++ΔΔ	102.97±0.06**++rr	102.48±0.08** ΔΔ rr
60	103.84±0.04**++ΔΔ	102.68±0.08**++rr	102.13±0.05** ΔΔ rr
75	104.20±0.03++ΔΔ	103.53±0.06**++rr	101.46±0.13** ΔΔ rr
90	104.26±0.03ΔΔ	104.31±0.04rr	103.13±0.07** ΔΔ rr
105	104.31±0.03	104.31±0.04	104.26±0.06
120	104.28±0.03	104.33±0.04	104.33±0.04

Significant difference (P<0.05) (*), and P<0.01 (**) from corresponding control value
Significant difference (P<0.05) (+), and (P<0.01) (++) between values for 6 and 8 mg / kg;
ΔΔ Significant difference (P<0.01) between values for 6 and 10 mg / kg;
rr Significant difference (P<0.01) between values for 8 mg / kg 10 mg / kg

not be important in healthy animals because pulse and respiratory rates as well as body temperature were normalized 120 min post injection. Therefore, this drug can be used safely in rabbits. On the other hand, some side effects were also observed with xylazine usage in our current study such as salivation, defecation, urination, tympany, staggering and muscle relaxation. Similar side effects of xylazine has been reported in many other animals species such as in sheep (Lin *et al.* 1993), goat (Aithal *et al.,* 1997), mare (Skarda and Muir, 1996) and cattle (Aziz, 2008).

In conclusion, current study demonstrated that xylazine is safe and potent drug, which can be used at different doses in rabbits.

Conflict of interest

The authors have no conflict of interest to declare.

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Parenteral Combined with Topical Treatment of Navel Ill in Cow Calves

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Abstract.- A total of 40 cow calves (≤15 days) infected with navel ill were selected. Ten calves (Group A) were given topical treatment (Pyodine 10 % and Neomycin Sulphate & Bacitracin Zinc) twice a day for five days, 10 calves (Group B) were given parenteral therapy (Amoxicillin and Meloxicam) for five days, 10 calves (Group C) were given both topical and parenteral therapy for five days, and 10 calves (Group D) were kept as a positive control. Among group A, B and C calves, the percentage recovery was 43, 43.45 and 84%, respectively. Among group (D) calves, one died and three had maggots in their wounds. Among 10 healthy calves (Group E), three calves became emaciated. It is concluded that in the early treatment of navel ill, parenteral therapy with topical application shows maximum recovery and calves show better growth when reared in hygienic environmental conditions.

Key Words: Navel ill, rearing of cow calves; chemotherapy of navel ill.

Infection of umbilicus and its associated structures occur commonly in the newborn calf. This infection may occur soon after the birth and may also result in omphalitis, omphalophlebitis, omphaloarteritis or urachitis of the urachus (Ganga *et al.,* 2011; Kasari, 1993). The predisposing factors

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that become source of navel infection include, when an umbilical cord is cut at a level very close to abdominal wall of calf, poor navel hygiene practices, and unfavorable environmental conditions such as parturition of cow in unhygienic calving pens, inadequate colostrum feeding within six hours after calving or having the umbilical stump sucked on by other calves if newborn calf is mixed with other calves after calving (Smith, 2003). Cord blood serum (CBS) is one of the allogenic sources of serum and is easy to be isolated and is free of bacterial and viral contamination. Human Umbilical Cord Blood Serum is a better alternative of Fetal Bovine Serum. (Aftab Ahmad *et al.*, 2012).

Clinical signs of infection are heat, swelling, purulent discharge or pain. Concurrent signs of systemic infection such as joint infection, pneumonia, diarrhea, meningitis, or uveitis may be noted (Trent and Smithy, 1984). It is also important to apply some disinfectant like 2% iodine or chlorhexidine solution to the umbilical stump just after parturition (Smith, 2003). Early treatment with antibiotics and supportive care may allow resolution of infection before development of abscess and distention of the urachus or the umbilical arteries and vein (Turner *et al.,* 1982). Antibiotics and pain killers are effective in most mild cases. Antibiotic treatment should continue until after the signs have disappeared which can take over a week even in mild cases (Laven, 2006).

There is lot of information on surgical treatment of navel ill but a very little information is available on the parenteral combined with topical treatment of navel ill in Pakistan. The present study describes the parenteral combined with topical treatment of navel ill in cows. It will provide baseline data for further study on the parenteral therapy of navel ill in calves in Pakistan.

Materials and methods

A total of 40 cow calves (≤15 days) infected with navel ill and 10 healthy calves were randomly selected from Kahna region of Lahore.

Calves showing symptoms of navel ill were divided into four categories *viz.,* normal *i.e.* showing no apparent clinical signs of disease, umbilical cord somewhat dry or wet. Mild infection *i.e.* little swelling of the umbilicus, plus or minus fever, heat and pain; less severe infection *i.e.* swelling of the umbilicus, fever, heat, pain and purulent discharge; and severe infection *i.e.* swelling on umbilicus, fever, purulent discharge, heat, pain and thickening of abdominal wall. The percentage infection of Navel ill was categorized on the basis of clinical signs as follows: Swelling on umbilicus, 20%; pain on palpation, 5%; heat, 5%; fever, 10%; purulent discharge or abscess formation, 35%; thickening of abdominal wall (Skin of umbilicus), 25%.

Chemotherapeutical trials

The calves of category four were selected for chemotherapeutical trials. Infected calves with navel ill were further divided into four more groups and the fifth group was of normal calves. Each group consisted of 10 calves. The antibiotic used for chemotherapeutic trial was Moxin (Breeze Pharma Pvt Ltd), which contains amoxicillin 150 mg per ml. The NSAID used was Melacam (Medi-vet), which contains Meloxicam 20 mg per ml. For topical application Pyodine solution (Brookes Pharma) containing 10% Povidine iodine and Cicatrin powder (GlaxoSmithKline) containing Neomycin Sulphate BP 3300 units per gram & Bacitracin Zinc BP 250 units per gram were used.

Calves of group A (n=10), group B (n=10), C (n=10), group D (n=10) and group E (n=10) were treated as show in Table I. Chi Square analysis (Stata Software), was used to analyze the data.

Results and discussion

When calves of group A (n=10) were treated with topical application of Pyodine solution and Cicatrin powder, the average recovery was 43.75% (Table I). Purulent discharge was the most affected clinical sign with the other clinical signs recovering only a small extent. The percentage recovery was checked graphically among the different breeds of calves within group A, where crossbred calves showed the maximum recovery (Table II). The average percentage recovery of group A among males was 41% and among females was 46.5%, but the overall recovery in group A was 43.75% (Table II). It is also important to apply disinfectant to the umbilical cord after parturition. After the cord is broken, 2% iodine or chlorhexidine solution should

Table I.- Average percentage recovery of group A, B and C after chemotherapeutical trials.

Description	Group A Topical treatment of 10% Povidine iodine and Neomycin sulphate + Bacitracin Zinc twice a day for 5 days (n=10)	Group B Parenteral therapy of Amoxicillin + Meloxicam for 5 days (n=10)	Group C Topical treatment of 10% Povidine iodine and Neomycin sulphate + Bacitracin Zinc and Parenteral therapy of Amoxicillin + Meloxicam for 5 days (n=10)	Group D Positive control
Average percentage recovery	43.75%	43.60%	83%	1 Died 3 had maggots in their wounds

Table II.- Percentage recovery among different breed of calves of Group A, B and C after chemotherapeutical trials.

Group A Calves	Percentage recovery after treatment	Group B Calves	Percentage recovery after treatment	Group C Calves	Percentage recovery after treatment
Friesian (Female)	30%	Friesian (Female)	29%	Friesian (Female)	80%
Crossbred (Male)	55%	Jersy (Male)	29%	Jersy (Female)	85%
Cholistani (Female)	60%	Crossbred (Female)	50%	Crossbred (Female)	95%
Sahiwal (Female)	45%	Crossbred (Male)	36.5%	Crossbred (Female)	95%
Crossbred (Male)	50%	Crossbred (Male)	50%	Friesian (Male)	65%
Friesian (Male)	35%	Friesian (Female)	40%	Crossbred (Male)	80%
Crossbred (Male)	40%	Crossbred (Male)	42.5%	Crossbred (Male)	85%
Crossbred (Male)	62.5%	Crossbred (Female)	60%	Crossbred (Male)	80%
Crossbred (Male)	25%	Crossbred (Male)	44%	Crossbred (Male)	70%
Sahiwal (Male)	35%	Crossbred (Male)	55%	Crossbred (Male)	95%
Average recovery (%)	43.75		43.60		83
Male	41%		41.47%		83%
Female	46.5%		48.08%		90%

be applied to the stump twice daily until the remaining umbilicus is dry (Smith, 2003). When calves of group B (n=10) were treated with parenteral therapy of Moxin and Melacam, the average percentage recovery was 43.60% (Table I). All other signs showed a marked reduction except purulent discharge which showed little change. When percentage recovery was checked graphically among different breeds of calves among group B, crossbred calves showed the maximum recovery calves (Table II). The average percentage recovery of group B among males was 41.47 % and among females was 48.08%, but the overall recovery in group B was 43.60% (Table II). Early treatment with antibiotics and supportive care may allow resolution of infection before development of abscess and distention of the urachus or the umbilical arteries and vein (Turner *et al.,* 1982). When calves of group C (n=10) were treated with topical application of Pyodine solution and Cicatrin powder plus parenteral therapy of Moxin and Melacam, the average percentage recovery was 83% (Table I). Almost all signs resolved. When percentage recovery was checked graphically among the different breeds of calves of group C, crossbred calves again showed maximum recovery (Table II). The average percentage recovery of group C among males was 76 % and among females

was 90 %, but the overall recovery in group B was 83 % (Table II). Ten calves with a history of swelling in the umbilical region were treated with Strepto-Penicillin and Meloxicam, administered intramuscularly for 5 days and routine dressing of the wound was carried out (Ganga *et al*., 2011). Early treatment with antibiotics and supportive care may allow resolution of infection before development of abscess and distention of the urachus or the umbilical arteries and vein (Turner *et al.*, 1982).

Calves in Group D were kept as a positive control and were all diseased. Among these calves, three had maggots in their wounds; one died from septicemia, two calves had a small umbilical hernia and the remaining calves had no further complications (Table I). Bacterial toxins kill the calf by sending it into shock, due to circulatory problems, and by causing multiple organ failure (Smith, 2003). When navel ill is not treated early, the infection may lead to many complications including involvement of the liver via the umbilical vein, umbilical hernias, wound myasis, and finally, death of the calf due to shock and septicemia. Continued infection may result in generalized omphalitis, omphalophlebitis, omphaloarteritis or infection of the urachus with extension to the bladder causing cystitis (Reef, 1987). Omphalophlebitis, omphaloarteritis, and urachitis are terms used to further describe the extension of inflammation or infection from the external umbilicus to the intra-abdominal segment of the umbilical vein, umbilical arteries, and urachus respectively (Kasari, 1993). Calves in Group E were kept as a negative control and were all healthy. Among these calves, three became thin and emaciated but all others remained healthy and showed no clinical signs of disease. The difference between the thin and the healthy calves was due to different calf rearing practices at different facilities. Calf rearing management practices including navel hygiene, cutting of navel stump six inch above from abdominal wall, proper navel dipping (dipping of navel cord with some antiseptic like Pyodine or chlorhexidine twice daily for three days), colostrum feeding within 6 h after parturition, parturition of cow in hygienic calving pens and rearing of calf in separate hygienic pens varied among different dairy farms and local small holder farms within the study area. Secondly different number and different breeds of calves were selected randomly from different places. The environmental conditions varied by facility and by difference in location. It is suspected that the significant association among male and female calves of different breeds was directly associated to this. Factors that predispose a calf to developing navel infection include an umbilical cord the breaks off or is cut at a level too close to the body wall, poor navel hygiene practices (like not dipping the navel with a disinfectant for a few days after birth), and unfavorable environmental conditions such as birth in a dirty calving area, inadequate colostrum or having the umbilical stump sucked on by other calves (Smith, 2003).

When chemotherapeutic trials were conducted among the three groups of calves, male calves were represented significantly more often than female calves. Three groups of calves also showed significant association due to different results from different groups after treatment. The results were significant asp value < 0.05 *i.e.* 0.000 (Table III).

Table III.- Statistical analysis of treatment between different groups A, B and C.

Tabulate var1 var2 [weight = var3], chi2

var1 / Breed	var2 Before treatment %age Infection	var2 After treatment %age Infection	Total Male & Female
Group A	159	41	200
Group B	314	86	400
Group C	234	166	400
Total	707	293	1000

Pearson chi2 (2) = 47.9650 Pr = 0.000

The maximum results obtained during chemotherapeutical trials were 83 % when topical and parenteral treatment was carried out at the same time. Intra-abdominal umbilical cord remnant infections were diagnosed in 21 calves during a 5 year period. According to this study, the urachal remnant alone was found in 15 calves, umbilical artery remnant alone in one calf, and the umbilical vein remnant alone in 4 calves. The dual occurrence of urachus and umbilical vein remnant was found in

one calf. All cases were approached surgically by ventral celiotomy (Trent and Smith, 1984). Omphalophlebitis also called navel ill with liver involvement was diagnosed by ultrasonography in 13 calves. In this procedure, the infected umbilical vein and the contracted umbilical vein were removed surgically (Steiner and Lejeune, 1993).

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Dystocia Due to Perosomus Elumbis (Acaudatus) in a Buffalo

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Abstract.- Perosomus elumbis (Acaudatus) is an occasionally found congenital anomaly characterized by partial or complete agenesis of lumbar, sacral and coccygeal vertebrae and ankylosis of the hind limbs. A seven years old BCS 3.5±0.5 pluriparous buffalo with dystocia due to malformed fetus was presented at Theriogenology clinic. The calf had a shortened trunk and weighed roughly 32.5 kg. There was an absence of the dorsal part of the vertebrae in the fetus and misshapened sacral vertebrae with the squeezed perineal region. Small and ankylosed limbs of the fetus were loosely attached to the trunk. The monster calf was forcefully extracted with partial fetotomy result in successfully resolved dystocia of a buffalo. The malformations were mostly confined to the abdominal region. This report presents information about the successful treatment of a distance case due to perosomus elumbis (Acaudatus) reflex and the estradiol local feathering for cervical dilation yielding acceptable results.

Key words: Perosomus elumbis, monster, dystocia, fetotomy, buffalo.

Dystocia may be of maternal or fetal origin and fetal cause's accounts for 40.84% in Murrah buffalo (Srinivas *et al.*, 2007). Major causes of bovine dystocia are abnormal fetus and monster conditions (Shukla *et al.*, 2007). Abnormal

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embryonic development may be congenital or genetic resulting in monsters conditions. Monsters may be due to congenital or infectious diseases that may affect the parturition (Arthur *et al.,* 2001). Development of the sexual organs of fetus suffering from monsters condition is distorted (Vegad, 2007). In farm animals most important and common cause of dystocia is related to fetal emphysema (Arthur *et al.,* 2001). Incomplete cervical dilation and inadequate expulsive maternal forces come under the term maternal dystocia (Jackson, 1995). A rare case is described in this report with Perosomus elumbis (Acaudatus) reflex in the fetus leading to dystocia in the dam. It is characterized by partial or complete agenesis of lumbar, sacral and coccygeal vertebrae and usually includes arthrogryposis of the hind limbs and malformations of the musculature. Cases of fetal monsters are rare but reported more commonly in cattle and rarely in buffaloes (Sharma *et al.,* 2010; Dennis and Leipold, 1986; Leipold and Dennis, 1986).

Case history and clinical signs

A seven year old pluriparous Nili Ravi buffalo (*Bubalus bubalis*) with average body condition score 3.5±0.5 (1-5) having normal gestation length and prolonged straining was brought to the Theriogenology clinic. Animal was brought with the history of anorexia and straining from last two days without any progression. After proper washing and disinfecting the perennial area with 2% potassium permanganate solution, vaginal examination was done by using vaginal speculum. Vaginal examination revealed presence of the abnormal fetus having ankylosed limbs and shortened trunk. Cervix was partially opened with only three fingers passing through it. The fetus was found dead as no reflex was being observed on inserting index finger into the mouth through the rectum. The temperature was subnormal at 36ºC. Overall health status of buffalo was good.

Results

After taking a complete clinical history and shown signs, animal was secured in a crush. The buffalo was administered calcium borogluconate (Mil-Fone C, Star, Pakistan; each 100 ml contains 26.6 g calcium borogluconate and 5.4 g boric acid, @ 150-250mg/kg; 300 ml I/V). Afterwards, for cervix dilation commercial preparation of estradiol (Stilbesterol dipropionate, Star, Pakistan @4-10mg/kg; 5ml; I/M) was administered along with local feathering of the cervix with the same hormone. Dexafar **(**Dexamethasone, Farvet, Netherlands @0.02-2mg/kg; 10ml; I/M**)** was also administered for initiation of mechanism of gluconeogensis, while atropine sulphate (Atrostar, Star, Pakistan@ 0.06-0.12mg/kg; 15ml; I/M) was given to stabilize the heart beat of the animal. The animal was left undisturbed for 45 to 60 minutes, after that cervix was found completely relaxed. Malformed dead fetus in anterior longitudinal presentation with dorso-sacral position was palpated per vagina with large head and small limbs. For proper application of traction forces, chains were applied on neck and fetlock joint of forelimbs when fully secured. Limbs and head came out of the birth canal on forceful traction, further traction was impossible. Further examination revealed very long neck of the fetusand abnormally large trunk still in the abdominal cavity with infirm consistency and coat. To squeeze and make the fetus in a maternal birth canal,in accordance evisceration was done. Large quantity of fluid came out of the fetus on partial fetotomy,as rib detached by using blunt edge sharp hook and then organs were removed by one using hand. Due to reduction in size, downward traction resulted in the successful solution of dystocia case. A lot of fluid coming out of utreus alongwith fetus having an incomplete vertebral column and ankylosed limbs (Fig. 1). After successful traction of dead fetus, buffalo was treated and survived with antibiotics and electrolytes for energy source having a fair prognosis of future fertility.

Discussion

The present monster has a small, flattenedand deformed pelvis with strongly ankylosed and flexed hind limbs and atrophy of the muscles of there are quarters. In most cases, caesarean section is the ultimate option for resolving Perosomus elumbis as suggested by Testoni *et al.* (2005). These findings are in accordance with those of Son *et al.* (2008) in Holstein calf which was successfully treated by surgery. According to the literature, C-section has

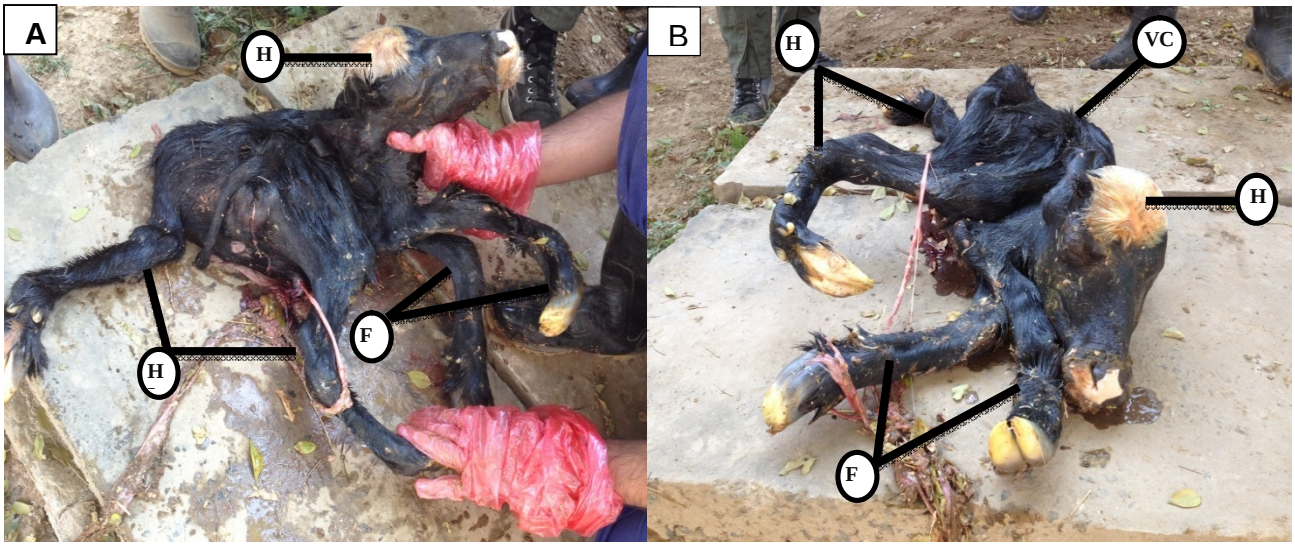

Fig. 1. A, Note the head(H) of the fetus with ankylosed front limbs (FL), hind limbs (HL) and atrophy of muscles of hindquarters; B, shows representing the pointed elongated head, ankylosed limbs and lack of development of the vertebral column (VC).

been performed mostly to correct these types of dystocia (Monfared *et al.*, 2013) which is not possible in field conditions. In the present report, partial fetotomy was performed which provide the evidence that dystocia due to Perosomus elumbis (Acaudatus) could be resolved successfully through it and estradiol local feathering for cervical dilation yields acceptable results. However, this case report will be helpful for the veterinarians to resolve dystociadue to Perosomus elumbis in field conditions.

Acknowledgement

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Conflict of interest

All authors have no conflict of interest with anyone about this manuscript.

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Evaluation of Oxidative Stress After Exposure to Different Doses of Deltamethrin and Cadmium in Swordtail Fish Gills

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Abstract.- In the present study, deltamethrin, a pyrethroid insecticide and cadmium were investigated for their potency to induce oxidative stress and effects on antioxidant systems in gill tissue of Swordtail fish (*Xiphophorus hellerii*). Animals were exposed to sublethal doses of deltamethrin and cadmium for 72 hours except the control group. Protein, malondialdehyde (MDA), reduced glutathione (GSH) levels and catalase (CAT) enzyme activities were determined. The results showed that total proteins MDA and GSH increased in each group, except for 0.2 ppm deltamethrin MDA and 0.1 ppm cadmium group where GSH was decreased. Catalase activity was decreased in all groups when compared to control group.

Keywords: Cadmium, deltamethrin, oxidative stress, swordtail fish, *Xiphophorus hellerii*

Pyrethroids, such as deltamethrin, are widely used as insecticides (Lone and Javaid, 1976; Laskowski, 2002), but their run off in the water bodies contaminate the aquatic habitat been. Oxidative stress has been reported after deltamethrin exposure in different fish tissue (Sayeed *et al*., 2003; Abdollahi *et al*., 2004).

Another pollutant is cadmium (Cd), a non-essential and toxic metal that can be found at elevated levels in the waters and sediments of the aquatic environment as a result of natural and anthropogenic activities such as industrial effluent, mining, metal production (Asagba *et al*., 2008). Interactions of heavy metals with pesticides offer an important premise of study to ecotoxicologist. These interactions may result in a potentiation, antagonistic or synergistic toxic response (Bouraoui *et al*., 2008).

The measurements of anti-oxidative enzyme activities in fish can demonstrate the oxidative damage induced by chemicals in aquatic ecosystems (Jin *et al*., 2010). Exposure to elevated metal and pesticide concentrations higher than the maximum levels allowed by governmental agencies, may stimulate the development of reactive oxygen species (ROS) like superoxide anion ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), and hydroxyl radical ($HO^{\cdot}$), which may damage DNA, lipids and proteins (Halliwell and Gutteridge, 1999). Oxidative stress is defined as a disruption of the prooxidant–antioxidant balance in favor of the prooxidants, leading to potential damage (Kochhann *et al*., 2009). The antioxidant defense system is formed by antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione-S-transferase (GST), and by the non-oxidant defense system, like glutathione (GSH), E vitamin, and uric acid (Trenzado *et al*., 2006).

Aquatic organisms including fish are exposed to a mixture of aquatic pollutants naturally. In this study, it is intended to point out the biochemical effects of multiple pollutants on fish in aquatic environments. We have compared and evaluated the synergistic or antagonistic toxicity profiles of 0.5 and 1 ppm cadmium and 0.2 and 0.4 ppm deltamethrin exposure of 72 h, as well as their mixture in terms of acute effects on biochemical parameters in Swordtail fish.

Materials and methods

Deltamethrin 2.5 EC (purity 2.5%, dissolved in 97.5% acetone) and cadmium chloride ($CdCl_2$) were used in this study. *Xiphophorus hellerii* Heckel, 1848 was obtained from local commercial aquarists. The animals were acclimatized for two weeks in stock tanks under laboratory conditions. Millipore water was used in this experiment. During the acclimatization period fish were fed *ad libitum*

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with pellet twice a day. The fish were randomly selected and divided into seven experimental groups, arranged aquarium tanks to hold 10 fish in each. The groups are 0.2 ppm Deltamethrin (D); 0.4 ppm D; 0.1 ppm Cadmium (Cd); 0.5 ppm Cd; 0.2 ppm D + 0.1 ppm Cd; 0.4 ppm D + 0.5 ppm Cd and the last one was used as control group (C). For each concentration, three replicates were maintained. Sublethal concentrations of D were selected based on 72-h LC_{50} value for *X. hellerii* (Khalili *et al*., 2012).

After 72 h, gills were removed. Tissues were homogenized in a buffer containing 1.15 % KCl, to obtain 1:10 (w/v) whole homogenate. The homogenates were centrifuged at 18,000g for 30 min at 4°C, then used for determination of malondialdehyde (MDA) and reduced glutathione (GSH) concentrations and catalase (CAT) activities. Protein concentrations were measured spectrophotometrically at 595 nm according to Bradford method (1976).

Concentrations of MDA were determined according to the method described by Ledwozyw (1986), the lipid peroxidation in the tissue samples were measured using the thiobarbituric acid reaction. The quantification of the thiobarbituric acid reactive substances was determined by comparing the absorption with the standard curve of MDA equivalents, generated by the acid-catalyzed hydrolysis of 1,1,3,3-tetramethoxypropane.

GSH concentration was measured with an assay using the dithionitrobenzoic acid (DTNB) recycling method described by Beutler (1974). In this method, the chromophoric product resulting from the reaction of the reagent DTNB and free sulphydryl groups possessed a molar absorption at 412 nm.

CAT activity was determined according to the method of Aebi (1974), and the principle of the assay was based on the determination of the rate constant of hydrogen peroxide decomposition by the CAT enzyme.

The SPSS 16.0 package program was used in analyses. Study data were given as arithmetic means and standard deviations. The one way analysis of variance and student t-test were used for the determination of the significance of differences between the groups. A value of $p<0.05$ was considered statistically significant.

Results

Statistically significant differences were observed in the MDA levels of the Cd treatment groups compared to the control ($p<0.05$). The MDA levels increased in the groups exposed to Cd, D+Cd and decreased in the 0.2 ppm D group ($p<0.01$), no statistical difference was seen in MDA levels of 0.4 ppm D, compared to the control group ($p\geq0.05$) (Fig. 1).

Compared to the control group, a statistically significant increase in GSH was seen in the groups which were administered both doses of DM, whereas a significant decrease was found in 0.2 ppm D + 0.1 ppm Cd group (Fig. 2). ($p<0.01$)

CAT activities showed a statistically significant decrease in all treatment groups compared to control group tissues ($p<0.05$) (Fig. 3).

Discussion

In this study it is aimed to explain the synergic and antagonistic effects of D and Cd from different sources on antioxidant systems of aquatic organisms in their natural environments, using biochemical methods. To gain insights into the combined toxicological effects of D and Cd, we have compared and evaluated the toxicity profiles of 0.5 and 1 ppm cadmium and 0.2 and 0.4 ppm exposure as well as their mixture in terms of the acute effects on 72 h tested biochemical parameters in Swordtail fish (*Xiphophorus hellerii* Heckel, 1848).

Two groups of antioxidants in aquatic animals are low molecular mass antioxidants (LMWA) such as GSH, ascorbic acid (vitamin C), and high molecular mass antioxidants (HMWA) such as CAT and SoD (Livingstone, 2001). Synthetic pyrethorid compounds and heavy metals cause oxidative stress by affecting antioxidant system in aquatic organisms. MDA is one of the products formed as a result of lipid peroxidation and a parameter widely used to show the oxidative damage (Topal *et al*., 2013). The high MDA level shows lipid peroxidation. It is the indication of the preservative effects of oxidative enzymes that lipid peroxidation either does not occur or it occurs at lower levels (Oruç *et al*., 2002; Atıf *et al*., 2005). In

our study MDA amounts have increased in all groups except for 0.2 ppm D and 0.4 ppm D groups in proportion to control (Fig. 1). A study with similar results were conducted by Pandey and colleagues, who reported that the lipid peroxidation in gills from *Channa punctata* exposed to multiple metal mixtures (Cd, Cu, Fe, Ni) was significantly increased and they suggested a persistent nature of metal-induced peroxidation damage in gill (Pandey *et al*., 2008). Souid and workers as related to Cd effect on oxidative stress biomarkers in *Sparus aurata* tissues, have defined that Cd increases the MDA, GSH and CAT activities in all tissues (Souid *et al*., 2013).

Cellular GSH amount is important in protecting the metabolic activities. GSH levels can decrease in the case of detoxification and oxidative stress. However, in the case of continuing stress, GSH/GSSG rate increases to oppose the oxidative stress with the effect of adaptive mechanisms. According to the findings of our study, GSH amounts have increased in all groups except for 0.2 ppm D + 0.1 ppm Cd group (Fig. 2). Although we have acquired similar results, Fırat and colleagues informed that there was a significant decrease of GSH in gills contrary to our findings (Fırat *et al*., 2009). Cd affects antioxidant levels in all aquatic organisms and causes oxidative stress. In *Haplobrachus occipitalis*, which is exposed to sublethal doses of Cd for 28 days, it has been observed that CAT specific activity increases substantially depending on dose in comparison with control. In the same study, GSH level has dropped as a result of the increased heavy metal concentration (Ezemonye and Enuneku, 2011). Stara and colleages reported that GSH level increased in all tissues of *Cyprinus carpio* under chronic exposure of a pesticide Simazine on the 14th and 28th days, but it reduced on the 60th day (Stara *et al*., 2012).

CAT is one of the important intracellular antioxidant enzymes in the defense mechanism. It has been reported that CAD react differently under the effect of toxic substances. In our study, it is determined that the activity of CAD decreases in all groups in proportion with the control (Fig. 3). According to Basha and colleagues, sublethal doses of Cd in *Oreochromis mossambicus* raised the

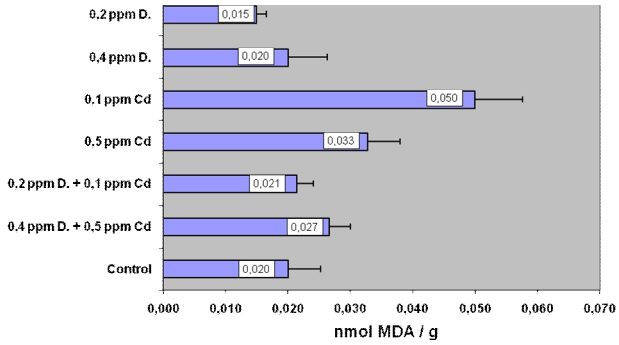

Fig. 1. Lipid peroxidation measured troughout MDA levels in gill of *Xiphophorus hellerii* in the control and experimental groups.

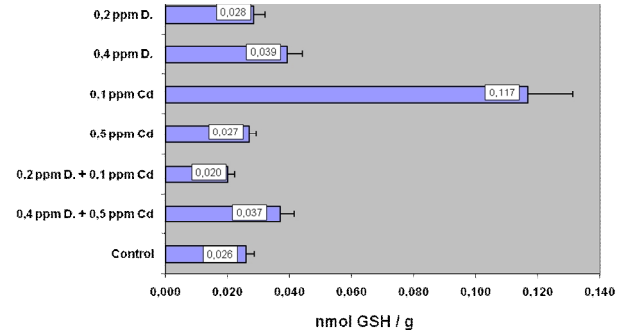

Fig. 2. GSH levels in gill of *Xiphophorus hellerii* in the control and experimental groups.

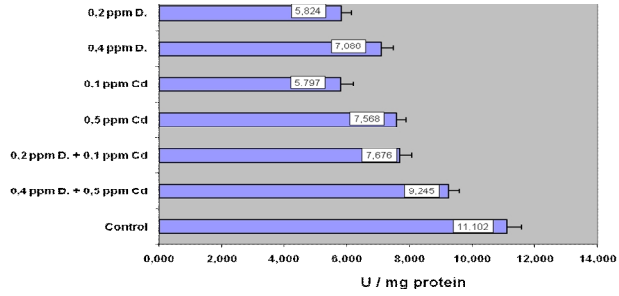

Fig. 3. CAT enzyme activities in gill of *Xiphophorus hellerii* in the control and experimental groups.

activity of CAD enzyme until the 15th day and then reduced on the 30th day (Basha and Rani, 2003). A reduction can occur in CAD activity because of the excess production of lipid peroxidation and free radicals. In a study, the CAT activity decreases, while lipid peroxidation levels increase in gill tissues of carps (*Cyprinus carpio*) exposed to

fipronil for 90 days in rice fields. Instability between antioxidant system occurring as a result of pesticide toxicity and pro-oxidant situation is one of the possible reasons for the changes in CAT activity (Clansen *et al*., 2012)

Consequently, the assayed parameters like antioxidant alterations and variations in enzyme activity can be used as good biomarkers of pollution in the water bodies affected with pesticides and heavy metals. In this study, oxidative stress resulting from D and Cd in gill tissues of *X. hellerii* is shown by the increase in lipid peroxidation levels. It is determined that reduced glutathione amount increases to resist oxidative stress. Oxygen radicals formed as a result of oxidative stress have also been observed to inhibit CAD enzyme activity. It was found that D and Cd had antagonistic effect on each other in the aquatic environment. D reduced the effect of Cd, but this was not enough to prevent to producing ROS. According to the results of this study, it can be seen that D and Cd in acute doses cause damage in fish gills.

Uncontrolled using of pesticides and heavy metals has been seen to cause important problems in aquatic organisms along with the targeted organisms. Toxic substances affect all organism groups in the food chain. Therefore, pollution studies observing aquatic organisms in every step of the food chain should become widespread. It is believed that the results of this study will be beneficial in environmental pollution studies, in which many variants are investigated all together.

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First Record of the Tribe Bulaeini (Coleoptera: Coccinellidae) With Generic and Species Descriptions From Pakistan

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Abstract.- *Bulaea lichatschovi* (Hummel, 1827), belonging to the genus *Bulaea* under the tribe Bulaeini is recorded from Sindh Province, Pakistan for the first time. Detailed descriptions, illustrations and distributions are provided. Food preferences of this species are also discussed.

Keywords: Coleoptera, Coccinellidae, Bulaeini, *Bulaea*, first record, Pakistan.

The family Coccinellidae, commonly called ladybirds or ladybugs, belong to the superfamily Cucujoidea, order Coleoptera, suborder Polyphaga (Kovar, 1996; Hunt *et al.*, 2007). Most species are brightly coloured and patterned. This family comprises about 360 genera and more than 6000 species up to the present (Vandenberg, 2002).

Numerous species of Coccinellids are major biological control agents of pests such as aphids, whiteflies, mealybugs, scale insects, thrips and mites in all parts of the world (Moreton, 1969; Hawkeswood, 1987; Majerus, 1994; Khan, 2001).

Among the Coccinellids, the members of the genus *Coccinela* have more potential to control different types of insect pests specially aphids. In Pakistan the seven spotted and eleven spotted ladybirds of the genus *Coccinella* have been studied in different aspects (Sattar *et al*., 2008; Sarwar and Saqib, 2010; Ahmad *et al*., 2011).

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Bulaea belongs within the tribe Coccinellini by earlier taxonomists such as Crotch (1874), Casey (1899), Watson (1956) and Fursch (1967). Savoiskaya (1969) proposed two tribes, Tytthaspidini and Bulaeini based on the morphology of larvae. The tribe Coccinellini was considered as polyphyletic (Savoiskaya and Klausnitzer, 1973). The genus *Bulaea* also includes phytophagous species and represents an independent shift to phytophagy according to our study (Giorgi *et al*., 2009). *B. lichatschovi* is known to eat leaves (Savoiskaya, 1970), as well as pollen (Capra, 1947; Savoiskaya, 1983), but unlike members of Epilachninae, the adults will consume aphids under laboratory conditions. The ancestral state in the transition to phytophagy seen in *Bulaea* is ambiguous, but was likely aphidophagy, pollinivory, or both. The mandible of adult shows little change from the standard aphidophagous type, unlike the highly elaborated biting and grinding surfaces found in the Epilachnine mandible (Samways *et al*., 1997). The species of this genus are found on sandy and desert places from Senegal through Egypt and Arabia to central Asia and Persia (Iran) (Crotch, 1874). Ozbek and Cetin (1991) reported *B. lichatschovi* (Hummel) from sugar beet, lentil alfalfa from different localities of Turkey. Kapur (1969) recorded this species from Rajasthan from India. In Pakistan this species was first time recorded from Sindh Province feeding on aphids as well as on pollens especially from deserts but rarely from agricultural fields.

Materials and methods

Coccinellids were collected from different localities of the Sindh Province. The specimens were mounted after boiled in 10% solution of KOH for 10-15 min. Various body parts were separated and mounted in Canada balsam after a brief dip in xylol. Different structures including genitalia were studied under a Kruss Binocular. Measurements and drawings of the body and other structures were made by using a micromillimeter scale and an ocular grid. The terminologies for various taxonomic structures including genitalia and procedures used by Innayatullah and Siddique (1978) and Gordon (1985) were generally followed. The taxonomic structures especially male and

female genitalia after illustration were preserved in microvial with glycerine and pinned with specimen. All diagrams are to the given scales and all measurements are in millimeters.

Tribe Bulaeini

Bulaeini Savoyskaya, 1969: 102

Diagnosis

Body medium to large; elongated-oval, strongly convex; head vertical; clypeus with broad anterolateral emarginations; gena never extending on to eye; mandible large with bifid apex; maxillary palp long, terminal segment with dorsal margin strongly produced, weakly securiform; pronotum convex anteriorly, never excavated anterolaterally. Sipho narrow, siphonal capsule weakly hook shaped with small, short adjacent arm while large thick opposite arm; paramere narrow, longer than median lobe; trabes bifurcated apically; genital plate weakly s-shaped; lateral plate large, oval; spermatheca with distinctly short cornu, ramus large tetragonal, nodulus absent.

Genus *BULAEA* Mulsant

Bulaea Mulsant, 1850: 36, 69. Type species: *Coccinella lichatschovi* Hummel, 1827

Diagnostic description

Length 3.8–6.0 mm; width 3.0–4.6mm; elongate-oval, strongly convex. Body with pronotum and elytra brownish yellow while ventrally light yellow with black pigments. Antennae with first segment tetragonal, narrow medially; second small, oval; third to seventh small elongated, eight to ten expanded distally, eleventh larger, rounded with apical margin straight; mandible large, strong.

Prosternum with anterior margin deeply concave with long entire hairs; prosternal carinae present; epipleuron deeply descending; tibia with spurs; tarsal claws appendiculate. Abdomenal postcoxal lines incomplete with oblique dividing line.

Male genitalia

Sipho thick basally, narrower distally; siphonal capsule very weakly hook shaped, with opposite arm distinctly large, broader, cylindrical while adjacent arm very small, narrow; median lobe narrow apically, slightly longer than parameres; trabes apically bifurcated.

Female genitalia

Genital plate elongated, poorly S-shaped; lateral plate distinctly large, rounded-oval; spermatheca deeply L-shaped, with cornu distinctly short than ramus, nodulus absent; infundibulum present; sperm duct very short.

Bulaea lichatschovii (Hummel, 1827)
(Fig. 1)

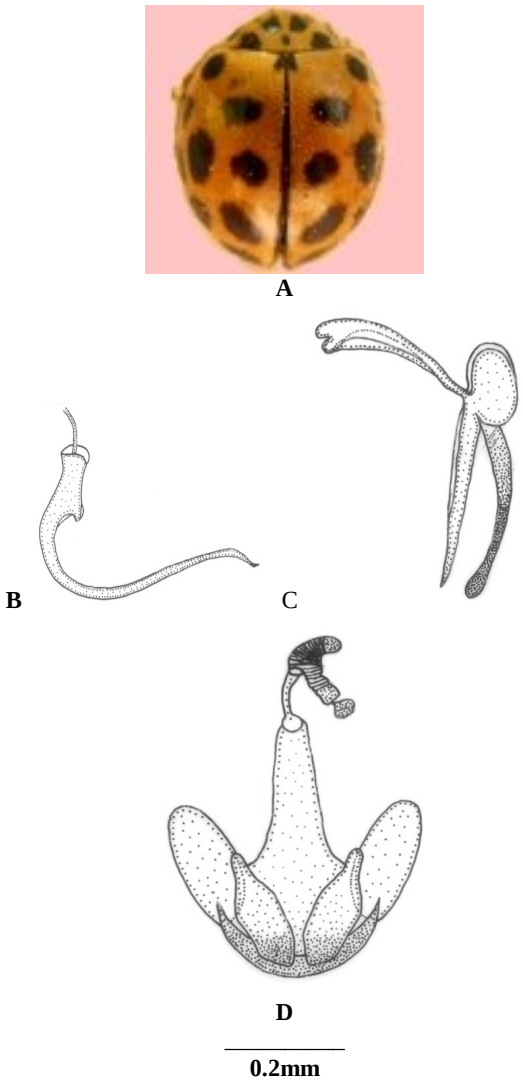

Fig.1. *Bulaea lichatschovii* (Hummel): A, adult (male); B-C, male genitalia: B, sipho; C, tegmen (lateral view); D, female genitalia

Coccinella lichatschovi, 1827.
Bulaea lichatschovi: Iablokoff-Khnzorian, 1982; Bielawski, 1975; 1984.
Coccinella pallid Motschulsky, 1949.
Coccinella bocanderi Mulsant, 1850.
Coccinella quinquedecimpunctata Chobant, 1980.

Redescriptions

Adult length 4–5.7mm; width 3.4–4mm; body elongate-oval, strongly convex. Head posteriorly black surrounding eye bifurcating medially, vertex, frons, clypeus, mouth parts legs and venter light yellow with black pigments; pronotum yellow with seven black spots, five arranged near to posterolateral margins while other two elongated spots arranged medially diverging from each other; elytra yellowish with nineteen black spots including one common spot at anterior junction. Eyes small, coarsely faceted; ligula anteriorly deeply notched; submentum laterally thick, anteriorly narrow with straight margin. Prosternal process with carinae broader anteriorly not reaching the anterior margin; epipleural inner margin narrow anteriorly as well as posteriorly before expanding, slightly emarginated medially. Postcoxal line arcuate; terminal segment deeply notched with entire small hairs.

Male genitalia

Sipho thick anteriorly while narrower distally with slightly s-shaped terminal end; siphonal capsule with opposite thick, large, tetragonal while adjacent arm very minute, pointed; median lobe narrow, deeply sharped apically, slightly longer than parameres; parameres strongly narrow medially, converged; trabes broadly bifurcated apically.

Female genitalia

Genital plates elongated, straight, slendrical; lateral plates distinctly large, elongated-oval; spermatheca with cornu distinctly short, ramus longer than cornu, nodulus absent.

Type depository

BMNH

Material examined

3males and 2 females Pakistan; Sindh: Mirpur Khas, Larkana, Sukkhar, Hyderabad, Karachi, 14.6.1972, 19.6.1973, on wheat, mustard., leg., Khan, M.I., lodged at Natural History Museum, Department of Zoology, University of Karachi.

Notes

Throughout the world only two species of the genus *Bulaea* have been recorded but from Pakistan this species is recorded for the first time.

Distribution

Afghanistan, Bulgaria, China, Central and West Asia, Greece, India, Mediterranean Region, Mongolia, North and Central Africa, Poland, Pakistan: Mirpur Khas, Larkana, Sukkhar, Hyderabad, Karachi.

Discussion

In Pakistan very little taxonomic work has been done on the family Coccinellidae. The genus *Bulaea* had been included in the tribe Coccinellini by the ancient taxonomists such as Crotch (1874), Casey (1899), Watson (1956) and Fürsch (1967). Savoiskaya (1969) created a new tribe, Bulaeini in the subfamily Coccinellinae based on the morphology of larvae.

Members of the genus *Bulaea* are mostly pollinivorous but sometimes aphidophagous. The genus *Bulaea* includes phytophagous species which represent an independent shift to phytophagy according to the study of Giorgi *et al*. (2009). *B. lichatschovi* is known to feeding on leaves, pollen and aphids. The mandible of adult *Bulaea* shows little change from the standard aphidophagous type, unlike the highly elaborated biting and grinding surfaces found in the Epilachnine mandible (Samways *et al*., 1997).

Similarly in the present study the members of the tribe Bulaeini were recorded mostly from desert areas of different localities of the Sindh Province feeding on different species of aphids but sometimes were found to be pollinovorous. The members of the tribe Bulaeini were also differentiated from the other tribes of the subfamily Coccinellinae by having clypeus with broad anterolateral emerginations; gena never extending on to eye; mandible large with bifid apex; maxillary palpus with terminal segment with dosrsal margin strongly produced anteriorly, weakly securiform; pronotum convex anteriorly, never excavated anterolaterally.

The distinguished generic character includes the shape of antennal segments. On species level, colouration of the body and the distinguished parts of male and female genitalia were focused distinctly. Among these characters, the bifid condition of mandibles indicated that these Coccinellids are aphidophagous. For the first time from Pakistan, only one species, *Bulaea lichatschovi* was recorded by the author from different localities of the Sindh Province.

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Seroprevalence of Antibodies to *Toxoplasma gondii* in Butchers and Buffaloes at Lahore, Pakistan

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Abstract.- The purpose of this study was to record the seroprevalence of *Toxoplasma gondii* antibodies in buthchers and buffaloes at Lahore , Pakistan. Blood samples of 50 butchers and 50 buffaloes were taken from main abattoir and local buffalo farms around Lahore, and assessed for seroprevalence of *T. gondii* by using a latex agglutination test (LAT). In the present study, 10% of butchers and 14% buffaloes were found seropositive for *T. gondii* infection. Butchers ranging in age from 51-60 (n=12) years and above exhibited the highest seropositivity 25% followed by those aging 41-50 years (n=10) 20%. Similarly in buffaloes 14% was also related to age by using LAT.

Key words: Toxoplasmosis, seroprevalence, butcher, buffaloes.

Toxoplasma gondii is an intracellular protozoan parasite (Dubey, 2009) which infects humans as well as a wide variety of mammals and birds Tenter *et al*. (2000), Sadia *et al*. (2012). It exhibits a predator-prey type life cycle and felines are the only definitive host (Tenter and Oswaldo, 2009). *T. gondii* is found throughout the world and tends to be more prevalent in tropical climates. *Toxoplasma* has a complex life cycle consisting of intestinal and tissue phases. The intestinal phase of the infection occurs only in felines and exhibits a typical intestinal coccidian life cycle consisting of merogony and gamogony. The tissue phase of the infection can be transmitted congenitally to offspring and to other intermediate hosts through carnivores (Al-Daoody, 2012). Ingestion of an infected animal will release the bradyzoites from the tissue cysts which then infect cells in the new host. The infection is acquired from under cooked meat. During the 1980s' toxoplasmic encephalitis emerged as a common complication associated with AIDS (Marques *et al*., 2008; Sukthana, 2006; Jones *et al*., 1999; Parmley *et al*., 1992). Congenital toxoplasmosis is more like to be symptomatic and can be particularly severe. The parasite can be transmitted to humans in several ways, including ingestion of raw or undercooked meat, contact with soil , eating poorly washed raw fruits and vegetable and ingestion of oocysts in cat faeces (Berger-Schoch *et al*., 2011). An investigation of recent outbreak has suggested that *T. gondii* could also be waterborne (Zahida *et al*., 2013; Jones and Dubey, 2010; Heukelbach *et al*., 2007; Dubey, 2004).

Due to the increasing risk of public health by ingestion of contaminated meat and milk, the development of reliable and sensitive method for diagnosing the toxoplasmosis becomes very important. As the butchers are considered to be at more risk for toxoplasmois and buffaloes are important source of meat in Pakistan, the aim of present study was to record the seroprevalence of toxoplasmosis in butchers and buffaloes in Lahore, Pakistan.

Materials and methods

A total of 100 serum samples (butchers n=50 and buffaloes n=50) were analyzed for anti-*Toxoplasma* antibodies. These samples were collected randomly from Lahore abattoir and local buffaloes farms. History of butchers and buffaloes was noted. Under aseptic measures, 8-10 mL of blood was drawn (intravenous) with the help of disposable syringes and transferred slowly to a screw capped sterile clean test tube to avoid hemolysis (Figueiredo *et al*., 2001).

The samples were left for about an hour for blood clotting to occur. The clotted blood was then separated with a fine loop and serum samples were centrifuged at 3500 rpm for 5 minutes. The sterile serums supernatants were stored at -20ºC until

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further analysis. The serum samples were analyzed for specific IgG Toxoplasma antibodies using Latex Agglutination (LA) commercial test kit "Toxoplasma Latex" from Quimica Clinica Apelicada,SA Amosta, Spain. The "*Toxoplasma gondii* Latex" was used according to manufacturer's instructions.

Results

Table I shows the prevalence of anti-*T. gondii* antibodies in sera collected from five age groups of butchers by latex agglutination test (LAT). Amongst the five groups of butchers, Group-5 (51-60 years and above) had the highest seropositive percentage *i.e.*, 25%, followed by Group-4. The rest of the age groups had no positive sample (Table I). Two butchers showed antibody titer at screening dilution of 1:16, two butchers gave antibody titer of 1:128 and one was positive at screening dilution of 1:256 (Table I). Overall seropositive percentage by using LAT was 10% in butchers at Lahore Abattoir.

Table I.- Prevalence of anti-*Toxoplasma gondii* antibodies in sera collected from different age groups of butchers and buffaloes by latex agglutination test (LAT).

Age (Years)	No. of individual	Antibody titer			Sero positive (%)	Sero positive (%)
		1:64	1:128	1:256		
Butchers						
Group-1 (15-20)	04	0	0	0	0	0
Group-2 (21-30)	06	0	0	0	0	0
Group-3 (31-40)	08	0	0	0	0	0
Group-4 (41-50)	10	1	1	0	2	20
Group-5 (51-60) & above	12	1	1	1	3	25
Total	50	02	02	01	05	10
Buffaloes						
Group-1(1-5)	10	0	0	0	0	0
Group-2 (5-10)	12	1	1	0	2	16.66
Group-3 (10-15)	28	1	2	2	5	17.85
Total	50	2	3	2	7	14

Table I shows the prevalence of anti-*T. gondii* antibodies in sera collected from three age groups of buffaloes by LAT. Buffaloes in Group-3 (10-15 years and above) had the highest seropositive percentage (17.85%), Group-2 (5-10 years) had 16.66% seropositive, while the Group-1 (1-5 years) had no positive case. Two buffaloes showed antibody titer at screening dilution of 1:16, three buffaloes showed antibody titer at 1:128 and two showed antibody titer at screening dilution of 1:256 (Table II). Overall seropositive percentage in buffaloes for *Toxoplasma* antibodies was 14%. History of butchers and buffaloes was noted (Table II). The data collected was analyzed and presented in terms of percentage so that prevalence of *T. gondii* antibodies and their relation with cats can be proved. There exist a positive correlation between the animal (cats) contact with the butchers and buffaloes (Tables II).

Discussion

In the present study, the overall prevalence of anti-toxoplasma antibodies in butcher was 10%. Among 50 butchers examined in the present study, an antibody titer of 1:256 was found in one butcher, which suggested the possible recent contact, an antibody titer of 1:128 was found in two butchers which was due to acquired or evolving immunity, an antibody titer of 1:16 was also found in two butchers, which indicated residual or non-specific immunity. In general, low titers indicated past exposure and probable immunity while high titer of 1:256. These results are in agreement with Chaudhry *et al*. (2006) who randomly collected fifty serum samples from butchers for analysis of *T. gondii* by LAT. Out of which,14 samples showed at 1:16 dilution. Out of them 10 were seropositive on 1:64 dilutions. It shows 20% prevalence of the toxoplasmosis in butchers.

Table II.- Animal Information and butchers information.

	n	Response frequency	%
Buffalo			
Group-1 (1-5)	50	10	20
Group-2 (5-10)	50	12	24
Group-3 (10-15)	50	28	56
Sex (all female)	50	50	100
Breed-Cross breed (Neeli and Ravi)	50	50	100
Contact with cats	50	50	100
Butchers			
Age Group			
Group 1 (15-20)		4	8
Group 2 (21-30)		6	12
Group 3 (31-40)		8	16
Group 4 (41-50)		10	20
Group 5 (51 and above)		22	44
Educational achievement			
Primary		30	60
Secondary		16	32
Tertiary		4	8
Year of service		(Average)	
1–5		3	6
6–10		5	10
11–20		7	14
21–30		10	20
>30		25	50
Type of training			
Formal training		7	14
On-the-job training		43	86
Safety information shared			
Keep clothes clean		Yes	8(12)
Keep surroundings clean		No	43(86)
Wash hands		No	0(0)
Wear protective gears		No	1(2)
Other/Don't remember		NA	0(0)
Begin work day with clean protective clothing			
Always		No	45(90)
Sometimes		Yes	5(10)
Last medical exam			
Current year		2	2(4)
1 year before		6	6(12)
2 years before		8	8(16)
3 years before		5	5(10)
4 years before		19	19(38)
Can't remember/No response		10	10(20)

Similar results were reported by Mazumder *et al*. (1988) and Samad *et al*. (1997) who used LAT for the detection of antibodies against *T.gondii* in butchers and found 24% and 50% cases positive respectively. Zaki (1995) also supported the results of present report by studying seroprevalence of *T. gondii* in domestic animals whereas findings of Zhang and Wei (2000) were in partial favor with the results of present report who evaluated the diagnostic value of LAT in 288 human serum samples for the detection of antibodies of *T. gondii* and found 8.9% seropositivity with positive titers (>or=1:32)

As far as the age of butchers and anti-toxoplasma antibodies titer relationship is concerned the highest percentage seropositivity was observed in Group-5, followed by Group-4. This might be due to the continuous exposure to the contaminated environment at abattoir and livestock farms and availability of the final hosts such as cats. These findings are in agreement with the results of Chaudhari *et al*. (2006) and Samad *et al*. (1997). The older butchers had higher serpositive cases for anti-*Toxoplasma gondii* antibodies than younger butchers, this statement supports the study conducted by Jones *et al*. (2009), Pawelec *et al*. (2002) and Young (2000).

It was also noted that those butchers who had been in close and prolonged contact with the animals at abattoir showed highest seropositive results of toxoplasmosis. These findings are in agreement with those of Fayomi *et al*. (1987) who had shown the highest prevalence of toxoplasmosis (87.0%) among the staff of slaughterhouse. Overall prevalence of anti-toxoplasma antibodies in buffaloes was recorded as 12%. Prevalence of anti-toxoplasma antibodies were studied by researcher around such as El-Ridi *et al*. (1990) in Egypt (20.0%), Samad *et al*. (1997) in Bangladesh (12.09%) and Zaki (1995) in Pakistan (25%). This variation in the results could be due to difference in environmental and geographical conditions, different ways of consuming meat like undercooked, uncooked and unhygienic conditions *e.g*., contamination of food items by flies and other insects carrying oocysts and intimate association with pets (Al-Daoody, 2012; Edelhofer and Prossinger, 2010; Torada, 2001; Wallace, 1972).

The seroprevalence of *T. gondii* in buffaloes varied with different age groups. The highest seropositivity of (17.85%) was found in Group 3 (10-15 years and above) followed by Group -2 (16.66%) and no seropositive case was recorded in

Group-1 (1-5 years). The present study revealed that the prevalence of antibodies increased with age. These findings are in concomitant with (Santos *et al*., 2010, 2013; Figueiredo *et al*., 2001) that infection was higher in older animals than younger animals.

Among 50 buffaloes examined in the present study, anti- body titer of 1:16 was recorded in two buffaloes who indicated residual or nonspecific immunity, 3 showed antibody titer of 1:128 which was due to acquired or evolving immunity, whereas two buffalo were positive at antibody titer of 1:256 which suggested no recent contact with parasite.

From the data, it was concluded that seroprevalence of toxoplasmosis in buffaloes was low (14.0%) in Lahore, Pakistan. Clinical disease is not unusual in immunocompromised individuals and ruminants. The prevalence of Toxoplasma infection in humans and animals is often associated with infection in pets (Al-Daoody, 2012; Edelhofer and Prossinger, 2010). Toxoplasmosis is zoonotic infection occurring in man, in domestic animals, wild animals and birds. Keeping in view of these findings, proper system of management and health practices should be adapted to reduce the exposure rates.

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