

Short Communications

Pakistan J. Zool., vol. 41(2), pp. 155-156, 2009.

First Report of the Caridean Shrimp *Leandrites celebensis* (De Man, 1881) From Sindh Waters

Q.B. Kazmi*, R. Sultana and H. Khatoon
Marine Reference Collection and Resource Center,
University of Karachi (QBK, HK) and PCSIR
Research Laboratory Complex, Karachi (RS)

Abstract.- Four genera of Caridean shrimps belonging to the family Palaemonidae have been reported from Pakistan. The genus *Leandrites* and species *L. celebensis* is an addition to this faunal list. The specimens were collected from shallow brackish water in the Indus deltaic region, they are being briefly described and illustrated here.

Key words: *Leandrites*, carideans, Indus delta.

A small number of caridean shrimp *Leandrites celebensis* (De Man, 1881) has been found in author's collections of caridean shrimps obtained during an Higher Education Commission Pakistan funded research project (2006-2007). The shrimps were collected from Ghorabari in the Indus deltaic region. The sample also contained *Palaemon semmelincki* (De Man, 1881) which is already reported by Kazmi and Kazmi (2002) but the genus *Leandrites* is now reported from here for the first time. Since *L. celebensis* has been described in detail by Kemp (1925) as *Palaemon hornelli*, Holthuis (1950), Bruce (1987) and Jayachandran (2001) it is only being briefly described here. Four males measuring 4.5 to 5mm and eight females measuring 2.2 to 3.5mm in carapace length were collected from Ghorabari (Ambro Creek), (Longitude N24° 25.157', latitude E67° 37.164') with air temperature (9.00 am): 28°C, (2.00 pm) 30°C, sea water temperature: 29-30°C, salinity: 35-35ppt, and depth: 12-20 ft.

Description

Rostrum (Fig. 1A) nearly straight reaching, beyond level of distal end of antennal scale, armed with 11-13 dorsal teeth, and 3-4 teeth on distal half of ventral margin. First and second pereiopods normal (Fig. 1B, D). Second pereiopods overreach antennal scale by length of chela and half of carpus.

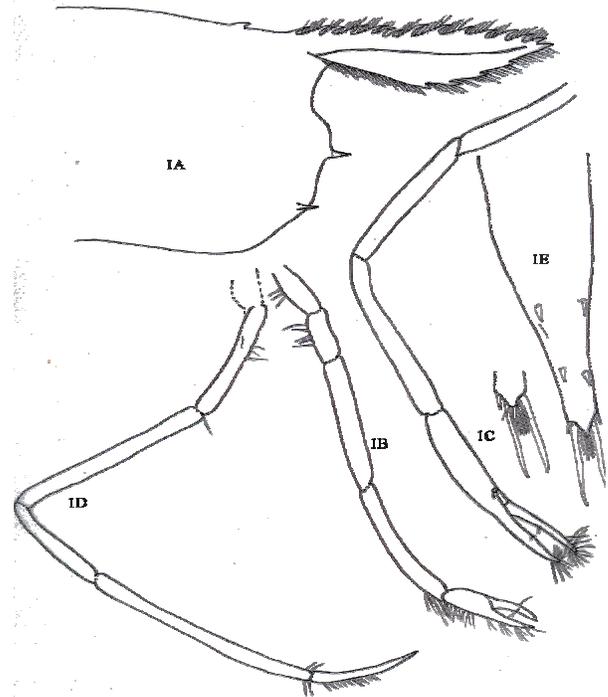


Fig. 1. *Leandrites celebensis*; A, rostrum and anterior margin of carapace; B, C, First and second legs; D, fifth leg; E, telson and ventral view of tip.

Remarks

The specimens at hand show no difference from those described by earlier workers except that the rostral dentation has a lesser number of teeth than found in the Australian specimens (Bruce, 1987). A pair of stout setae at the base of submedian pair of telson visible in the ventral view (Fig. 1E) are not mentioned by the earlier workers.

Distribution

The species is a shallow brackish water form. Previously reported from Celebes, Singapore, North

* Corresponding Author: qbkazmi@yahoo.com
0030-9923/2009/0002-0155 \$ 8.00/0
Copyright 2009 Zoological Society of Pakistan.

Australia and S. India. The present report extends its range to northern Arabian Sea in Pakistani waters.

Acknowledgements

The first author (QBK) is grateful to Dr. A. J. Bruce of the Australian Museum for improving the text. She is working under the Higher Education Commission Expansion Program, Pakistan.

References

- Bruce, A.J., 1987. *Beagle Rec. N. Terr. Mus. Arts Sci.*, **4**: 57-60.
 Holthuis, L.B., 1950. *Siboga Exped.*, **30**: 1-268.
 Kazmi, Q.B. and Kazmi, M.A., 2002. *J. Wildl. Fish.*, **1**: 11-14.
 Kemp, S., 1925. *Rec. Indian Mus.*, **27**: 249-343.
 Jayachandran, K.V., 2001. *Palaemonid prawns. Biodiversity, taxonomy, biology and management.* Science Publishers, Inc. USA, pp. 624.

(Received 31 November 2007, revised 1 September 2008)

Pakistan J. Zool., vol. 41(2), 156-158, 2009.

Erythrocyte Morphology and Comparative Erythrocyte Measurements in Some Lacertid Lizards From Turkey

M. Sevinç*, İ.H. Uğurtaş and A. Akkaya
 Department of Biology, Faculty of Science and Art,
 Uludağ University, Nilüfer-Bursa, Turkey
 *E-mail: smurat@uludag.edu.tr

Abstract. - General morphology and size of erythrocytes of some lacertid lizards, *Ophisops elegans*, *Lacerta cappodocica*, *Anatololacerta danfordi* and *Podarcis muralis*, living in Turkey was studied using Wright's technique. The longest erythrocytes and nuclei were measured in *O. elegans* and the shortest ones in *L. cappodocica*. The widest erythrocytes were recorded in *P. muralis* and the narrowest ones in *L. cappodocica*. The widest nuclei were found in *O. elegans* and the narrowest ones in *L. cappodocica*.

Key words: Erythrocyte morphology, erythrocyte size, Lacertidae.

The previous studies on the blood of reptiles

described the structures, often comparing them with those of the other vertebrates (Saint Girons, 1970). Various authors have described the circulating blood cells of different reptile species (Ryerson, 1949; Hartman and Lessler, 1964; Szarski and Czopek, 1966; Saint Girons, 1970; Canfield and Shea, 1988; Cannon *et al.*, 1996; Alleman *et al.*, 1999; Sevinç *et al.*, 2000, 2004; Sevinç and Uğurtaş, 2001, 2008; Arıkan *et al.*, 2004; Tosunoğlu *et al.*, 2004; Martinez-Silvestre *et al.*, 2005; Carvalho *et al.*, 2006; Metin *et al.*, 2006). There are a few studies on reptilian blood cells in Turkey (Sevinç *et al.*, 2000, 2004; Atatür *et al.*, 2001; Sevinç and Uğurtaş, 2001, 2008; Uğurtaş *et al.*, 2003; Arıkan *et al.*, 2004; Tosunoğlu *et al.*, 2004; Metin *et al.*, 2006). Our aim was to describe erythrocyte morphology and measure the erythrocyte and nucleus sizes of some lacertid lizards (*Ophisops elegans*, *Lacerta cappodocica*, *Anatololacerta danfordi* and *Podarcis muralis*) which live in Turkey. This study is the first of its kind on Turkish species of these lacertid lizards.

Materials and Methods

In this study, 20 (12 ♀, 8 ♂) individuals of *O. elegans*, 15 (7 ♀, 8 ♂) of *L. cappodocica*, 10 (4 ♀, 6 ♂) of *P. muralis* and 11 (6 ♀, 5 ♂) of *A. danfordi* (Lacertidae) were examined. Three or five blood smears were prepared per individual. The smears were air-dried and stained immediately with Wright's stain technique. On each slide 100 mature erythrocytes and their nuclei were measured by means of an ocular micrometer at a magnification of 1600x (Hartman and Lessler, 1964).

Results

The erythrocytes of the lizards are nucleated oval cells, and their nuclei are also oval and centrally located like those of the other reptile species. The cytoplasm of mature erythrocytes appeared both light and dark pink and was homogeneous under Wright's stain. The nuclei of mature erythrocytes are chromophilic.

In the present study, the longest erythrocytes were recorded in *O. elegans*, the shortest ones in *L. cappodocica*, the widest ones in *P. muralis* and the narrowest ones in *L. cappodocica*. The longest and widest nuclei were found in *O. elegans*, the shortest

and the narrowest ones in *L. cappodocica*. According to these results, *L. cappodocica* has the smallest erythrocytes and nuclei sizes of the all examined species. Mean erythrocyte and nucleus measurements of examined species with standard deviation were given in Table I.

Table I.- Mean erythrocyte dimensions of examined species with standard deviations.

Examined species	EL (µm)	EW (µm)	NL (µm)	NW (µm)
<i>O. elegans</i>	16.91 ± 1.00	8.33 ± 0.66	6.86 ± 0.64	3.49 ± 0.41
	13.48 ± 0.70	7.56 ± 0.53	5.99 ± 0.50	3.20 ± 0.30
<i>L. cappodocica</i>	14.00 ± 0.92	8.79 ± 0.67	6.11 ± 0.66	3.34 ± 0.41
	15.46 ± 0.54	8.56 ± 0.47	6.60 ± 0.54	3.27 ± 0.31
<i>P. muralis</i>				
<i>A. danfordi</i>				

EL, erythrocyte length, EW, erythrocyte width, NL, nucleus length, NW, nucleus width.

Discussion

Reptiles are a heterogeneous group of vertebrates with regard to their blood cell morphology. Normal blood morphology needs to be described for representative species of the 4 major orders of reptiles. Reptilian blood cell investigations carried out by various authors reported that the sizes of erythrocytes vary in members of the 4 orders of reptiles (Hartman and Lessler, 1964; Szarski and Czopek, 1966; Saint Girons, 1970; Canfield and Shea, 1988; Alleman *et al.*, 1999; Sevinç *et al.*, 2000, 2004; Uğurtaş *et al.*, 2003; Sevinç and Uğurtaş, 2008).

Within the class Reptilia, the largest erythrocytes are seen in *Sphenodon punctatus*, turtles and crocodylians. Saint Girons (1970) reported erythrocytes and nuclei measurements of *Lacerta agilis* and *L. vivipara*. He measured only erythrocyte and nucleus length. Erythrocyte size of *Lacerta rudis* was measured by Sevinç *et al.* (2000). Sevinç and Uğurtaş (2001) reported erythrocyte and nucleus measurements (both length and width) of *L. rudis bithynica* (= *L. saxicola bithynica*). Sevinç and Uğurtaş (2008) reported that the longest erythrocytes were found in *L. sicula* and the shortest ones in *L. trilineata*.

In reptiles, lizards have more erythrocytes than snakes, and turtles have the fewest ones. Since the lizards have the smallest erythrocytes of all reptiles, and turtles the largest, there may be an inverse correlation between the number of erythrocytes and their size (Ryerson, 1949).

In the present study, erythrocyte morphology and the results of erythrocytes and nuclei sizes are in agreement with the other results carried out by Hartman and Lessler (1964), Szarski and Czopek (1966), Saint Girons (1970), Canfield and Shea (1988), Alleman *et al.* (1999), Sevinç *et al.* (2000), Sevinç and Uğurtaş (2001, 2008), Martinez-Silvestre *et al.* (2005), Carvalho *et al.* (2006) and Metin *et al.* (2006).

References

- Alleman, A.R., Jacopson, E.R. and Raskins, E.R., 1999. *Am. J. Vet. Res.*, **60**: 507-514.
- Arikan, H., Kumlutaş, Y., Turkozan, O., Baran, İ. and Ilgaz, C., 2004. *Amphibia-Reptilia*, **25**: 465-470.
- Atatür, M.K., Arikan, H., Çevik, İ.E. and Mermer, A., 2001. *Turk. J. Zool.*, **25**: 149-152.
- Canfield, P.J. and Shea, G.M., 1988. *Anat. Histol. Embryol.*, **17**: 328-342.
- Cannon, M.S., Freed, D.A. and Freed, P.S., 1996. *Anat. Histol. Embryol.*, **25**: 11-14.
- Carvalho, R.L., Antoniazzi, M.M. Jared, C., Silva, A.M.J., Santos, A.A. and Egami, M.I., 2006. *Comp. Clin. Pathol.*, **15**: 169-174.
- Hartman, F.A. and Lessler, M.A., 1964. *Biol. Bull.*, **126**: 83-88.
- Martinez-Silvestre, A., Marco, I., Rodriguez-Dominguez, M.A., Lavin, S. and Cuenca, R., 2005. *Res. Vet. Sci.*, **78**: 127-134.
- Metin, K., Türkozan, O., Kargın, F., Başımoglu Koca, Y., Taşkavak, E. and Koca, S., 2006. *Acta Vet. Brno*, **75**: 49-55.
- Ryerson, D.L., 1949. *J. Ent. Zool.*, **41**: 49-55.
- Saint Girons, M.C., 1970. In: *Biology of the Reptilia* (ed. C. Gans), vol. 3, pp. 73-91. Morphology C. Academic Press, New York.
- Sevinç, M. and Uğurtaş, İ.H., 2001. *Asiatic Herpetol. Res.*, **9**: 122-129.
- Sevinç, M. and Uğurtaş, İ.H., 2008. *Pakistan J. Zool.*, **40**: 59-60.
- Sevinç, M., Uğurtaş, İ.H. and Yıldırımhan, H.S., 2000. *Turk. J. Zool.*, **24**: 207-209.
- Sevinç, M., Uğurtaş, İ.H. and Yıldırımhan, H.S., 2004. *Asiatic Herpetol. Res.*, **10**: 217-223.
- Szarski, H. and Czopek, G., 1966. *Bull. Acad. Pol. Sci. Cl. II. Ser. Sci. Biol.*, **14**: 437-443.

Tosunoğlu, M., Ayaz, D., Tok, C.V. and Dülger, B., 2004. *Asiatic Herpetol. Res.*, **10**: 230-234.

Uğurtaş, I.H., Sevinç, M. and Yıldırımhan, H.S., 2003. *Zool. Stud.*, **42**: 173-178.

(Received 7 November 2008, revised 20 January 2009)

Pakistan J. Zool., vol. 41(2), pp. 158-160, 2009.

Prevalence of Anaemia in Pregnant Women and General Population of Hyderabad District and Its Adjoining Areas (Sindh, Pakistan)

R. Bhatti and D. M. Shaikh

University of Sindh, Jamshoro (RB) and
Isra University, Hyderabad (DMS)

Abstract- The study on 600 pregnant women attending Liaquat University of Medical and Health Sciences was carried out to evaluate the percentage of anaemia cases present in the District Hyderabad and its surrounding adjoining areas among pregnant women. Haemoglobin (Hb) levels less than 10 g /dl was observed in 59% of the cases and 41% were with maximum of 11.5 g/dl. The 90% cases belonging to middle or poor class were classified as anaemic and were at the risk of abortion. Eleven cases of still birth were also reported as they had less than 5g /dl. During second phase of study, 1460 healthy individuals, including 380 males, 660 females and 420 children's, aged from 12 to 50 years, belonging to different socio-economic and educational groups were studied. The results showed that percentage of haemoglobin among non-pregnant woman was below 10 g /dl, whereas males had 13.8 g/dl and children had average of 9.5 g/dl. The main cause of anaemia's seems socioeconomic status of general population and improper knowledge of maintenance of pregnancy.

Keywords: Anaemia, iron deficiency, pregnancy, population.

Lack of dietary iron is the worlds leading nutritional deficiency and the most common cause of anaemia. About 4-5 billion people (66-80% of the

worlds population) may be iron deficient, out of which two billion people (over 30% of the worlds population) are anaemic mainly due to iron deficiency (WHO, 2003). Nutritional anaemia is the result of deficiency of one or more essential nutrients (Iron, folate and vitamin B₁₂). This anaemia causes wide-ranging symptoms *viz.*, fatigue, weakness, cognitive deficits to serious heart complications, developmental disorders, and pathology of hereditary blood disorders.

This present study was based on determination of haemoglobin contents and frequencies of different blood groups in pregnant women, as well as in general mixed population including different ethnic groups and tribes of Hyderabad districts and its adjoining areas.

Materials and methods

Pregnant women (600) attending Antenatal Clinic Liaquat University of Medical and Health Sciences Hospital, Hyderabad were included in this study. Controls comprised apparently healthy women, teachers, students, house wife and para-medical staff. The questionnaire was also developed and used to collect additional information regarding the nutritional intake and socio-economic status of every individual. Similar type of subjects were selected and included regarding family size, total income, and family head.

Venous blood was used for estimation of haemoglobin by cyanmethaemoglobin method (Tharp, 1986).

Results and discussion

Majority of the subjects (66%) for this study came from rural (n=321), thar (n=33) and hilly areas (n=42), where no medical facilities exist, whereas 34% were from urban areas (n=204). Most of them were uneducated house wives (Table I); and 59% were below the WHO health standard and 41% just on the margin (Table II). Almost 60% of the pregnant women had haemoglobin below 10 g/dl and remaining 40% had marginally above 11 g/dl (Table III). According to WHO (2003), standard lower limit of normal haemoglobin of pregnant women is 11 g/dl. In our study prevalence reached upto 72% excluding marginal 168 cases having just 11.5 g/dl. Severe anaemic cases (6%) had

Table I.- Socioeconomic conditions and occupation of pregnant women in different age groups included in this study.

Sr. No	Age group (years)	No. of subject (%)	Socioeconomic conditions*			Occupation	
			Poor	Fair	Good	House wife	Working
1.	15 - 20	108 (18%)	93	12	3	102	6
2.	21 - 25	153 (25.5%)	108	45	00	147	6
3.	26 - 30	276 (46%)	183	78	15	270	6
4.	31 - 35	63 (10.5%)	42	21	00	63	00
	Total	600	426	156	18	582	18

*Poor earning < Rs. 3000; Fair earning Rs. 3000 – 4000; Good earning Rs. 3000 – 7000.

Table II.- Percentage of mild, moderate and severe anaemic subjects.

Hb level	Category type of anemia	Age groups					Total (%)
		15-20	21-25	26-30	31-35	Total	
> 10.0 g/dl	Negative	42	63	114	27	246	41
9.0 to 9.9 g/dl	Mild	21	18	42	9	90	15
5.0 to 8.9 g/dl	Moderate	39	69	99	21	228	38
<5 g/dl	Severe	6	3	21	6	36	6
	Total	108	153	276	63	600	100

haemoglobin less than 5 g/dl (Table III). Eleven cases of still birth were reported (Table III). All types of abortions were not differentially related to haemoglobin content. Table IV shows prevalence of anaemia in general population of Hyderabad. Of the total of 1460 subjects examined 59% were found anaemic. The percentage of anaemia in other cities of Pakistan has also shown similar pattern (Heince *et al.*, 1972). Hashmi *et al.* (1973) have reported 63.8% cases with <11 g/dl Hb in pregnant women attending the out patient clinic at Jinnah Postgraduate Medical Centre, Karachi. However, Mukhopadhyay *et al.* (2004) have reported 62-88% pregnant women of India.

Table III.- Percentage of abortion against haemoglobin content (n=600)

Hb g/dl (level)	No. of subjects	No. of Abortions	(%) of Abortions
2 – 6	168	57	34 (11*)
7 – 10	264	132	50
11 – 13.9	168	72	43

* Still birth.

Table IV.- Prevalence of anaemia in general population of Hyderabad.

Subjects	No.	Mean Hb % (+ SD)	No. (%) anaemia
Male	380	13.8±2.329	98 (25.78)
Female	660	9.6±1.862	427 (64.69)
Children	420	9.5±3.035	341 (81.19)
Total	1460	10.97±2.408	866 (59.31)

The cause of anaemia in general is socioeconomic attributable to under-nourishment and iron deficiency (Pollitt, 1991; Nokes *et al.*, 1998; Guyatt, 2000).

References

- Guyatt, H.L., 2000. *Parasitol. Today*, **16**: 153-158.
- Hashmi, J.A., Nisa, A.N., Malik, F., Saeed, M. and Chaudhery, A.S., 1973. *Pakistan J. med. Res.*, **12**: 22-33.
- Heince, J.D., Siddiqui, A.R. and Aziz, M.A., 1972. *Pakistan J. med. Res.*, **11**: 26-35.
- Karim, S.A., Khurshed, M. and Rizvi, J.H., 1988. *J. Pakistan med. Assoc.*, **38**: 271-272.
- Mukhopadhyay, A., Bhatla, N., Kriplani, A., Pandey, R.M. and Sakena, R., 2004. *J. Obstet. Gynaecol. Res.*, **30**: 409-417.

- Nokes, C., Bosch, C.V. and Bundy, D.A.P., 1998. *The effects of iron deficiency and anaemia on mental and motor performance, education achievements, and behavior in children. An annotated bibliography.* International Nutritional Anaemia Consultative Group, Washington DC.
- Pollitt, E., 1991. *Food Nutr. Bull.*, **13**: 110-118.
- Tharp, G.D., 1986. *Experiments in physiology.* MacMillan, London.
- World Health Organization, 2003. *Health topics A-Z. Iron deficiency anaemia. Daily versus intermittent iron supplementation in pregnant women: Hematological and pregnancy outcome.* Geneva, Switzerland.

(Received 6 June 2006, revised 2 February 2009)

Pakistan J. Zool., vol. 41(2), pp. 160-163, 2009.

Detection of *Mycobacterium avium* subsp. *paratuberculosis* in Domestic Ruminants in Lahore, Pakistan

Zafar Iqbal Chaudhry*, Farhan Anwar Khan, Saba Badar, and Muhammad Shahid

Department of Pathology, University of Veterinary and Animal Sciences, Lahore

*E-mail: zafar1949@yahoo.com

Abstract.- Two thousand tissue samples of terminal ileum and mesenteric lymph nodes (MLN) cattle (*Bos taurus*) and buffalo (*Bubalus bubalis*) obtained randomly from abattoirs in Lahore district were used for detection of *Mycobacterium paratuberculosis* and *Mycobacterium bovis* using acid fast staining and PCR analysis. Acid fast staining revealed the presence of acid fast bacilli in 17.4% intestinal and 16.4% MLN tissues in buffalo, while in cattle 19.2% intestinal and 17.8% MLN were found positive for bacilli. In PCR analysis, 12.8% and 12.4 % intestinal and MLN tissues were positive for *Mycobacterium paratuberculosis* in buffalo. However, in cattle, PCR analysis showed respectively, 14.2% MLN and intestinal tissues positive for *Mycobacterium avium* subsp. *paratuberculosis*. Both types of tissues from cattle (5.8%) and buffalo (5%) were also positive for *M. bovis* by PCR. It is concluded that infections by various mycobacterium species can be differentiated by PCR amplification, which is not possible by acid fast staining technique.

Key words: Paratuberculosis, Bovine tuberculosis, PCR, Acidfast staining.

Paratuberculosis, a chronic granulomatous bowel disease of ruminant animals caused by an intracellular organism *Mycobacterium avium* subsp. *paratuberculosis* (MAP) is cause of severe economic losses all around the world. The disease affects both domestic and wild ruminants. Animals become infected early in life but often do not develop clinical disease until 3 years of age (Chiodini *et al.*, 1984). During this sub clinical phase of infection, some *Mycobacterium avium* subsp. *paratuberculosis* are shed in faeces, due to which the infection spreads throughout the herd. During clinical phase of the infection, faecal shedding of the microorganism is high and can exceed 10^{10} organisms per gram of faeces (Kurade *et al.*, 2004). The terminal stages of the disease is characterized by chronic diarrhea, rapid weight loss, diffuse edema, decrease milk production and infertility. Ability of the *Mycobacterium avium* subspecies *paratuberculosis* to survive at pasteurization temperature and its potential role in the etiology of a human's chronic granulomatous ileocolitis (Crohn's disease), indicate the need for the control of the disease in animal population (Naser and Shfran, 2000; Grant *et al.*, 2001).

Conventional diagnostic methods employed currently include culturing of the organism and ELISA. Though culture is regarded as the gold standard for *Mycobacterium paratuberculosis* diagnosis but a long incubation period of 12-16 weeks makes the technique more laborious. ELISA is quite rapid test but with a limitation of low sensitivity, as antibodies may not be detectable during early stages of infection (Collins, 1996).

The molecular diagnosis based on the detection of IS900 specific sequence of MAP by polymerase chain reaction (PCR) is considered a method with high specificity, sensitivity and rapidity (Huntley *et al.*, 2005). In Pakistan, paratuberculosis has been a neglected subject, till now, little is known about its prevalence and detection. So, keeping in view the economic and public health significance of the disease, present project was designed to detect paratuberculosis in ruminant animals from an abattoir of Lahore.

Materials and methods

Collection of samples

Two thousand tissue samples, one thousand each of terminal ileum and mesenteric lymph nodes, were collected over a period of one year (May 2007-April 2008) from an abattoir of Lahore from 500 cattle and 500 buffalo, randomly and transported to the laboratory in ice box. Lesions if present were recorded and scored in the intestines and mesenteric lymph nodes *i.e.*, congestion, hemorrhage, mucosal thickening or corrugations. Slaughter house was visited twice a week and 20 animals were examined daily, over a period of one year (10 cattle and buffalo each).

PCR analysis and acid fast staining

The samples were subjected to the Ziehl-Neelsen's acid fast staining (Quinn *et al.*, 1994) for recording presence of bacilli. The PCR was carried out as described by Sivakumar *et al.* (2005) and Aradaib *et al.* (2005) for primer set 1 and primer set 2, respectively, for detection of *Mycobacterium avium* subsp. *paratuberculosis*. All the samples were also subjected to PCR for detection of *M. bovis* as described by Shah *et al.* (2002) using primers given in Table I. For DNA extraction GENTRA PUREGENE[®], USA DNA purification kit was used and the procedure described by the manufacturer was adopted.

Results and discussion

Out of 500 buffalo, 285 (57%) animals were emaciated and 215 (43%) were apparently in normal body condition. Seventy eight (15.6%) animals were suffering from diarrhea of varying intensity. After slaughtering, intestine from 145 (29%) animals and mesenteric lymph nodes from 156 (31.2%) animals showed different gross lesions *i.e.*, congestion, hemorrhages, mucosal thickening of various degree. Acid fast staining of smears from intestinal and mesenteric lymph nodes scrapings revealed the presence of rosy red rods in 87 (17.4%) and 82 (16.4%) samples, respectively. Sixty four tissue samples (12.8%) from both intestine and MLN were positive with PCR by using primer set 1 and primer set 2 except, two tissue samples of MLN which did not produce amplicon with primer set 2. Twenty five (5%) samples were positive to PCR for

detection of *Mycobacterium bovis* (Table II).

In cattle, 295(59%) animals showed emaciation and 205(41 %) animals were in a good body condition. Diarrhea was observed in 96 (19.2%) animals. Sixty four (29.8%) tissue samples of intestine and 178 (35.6%) mesenteric lymph nodes showed gross lesions. Out of total 500 tissue

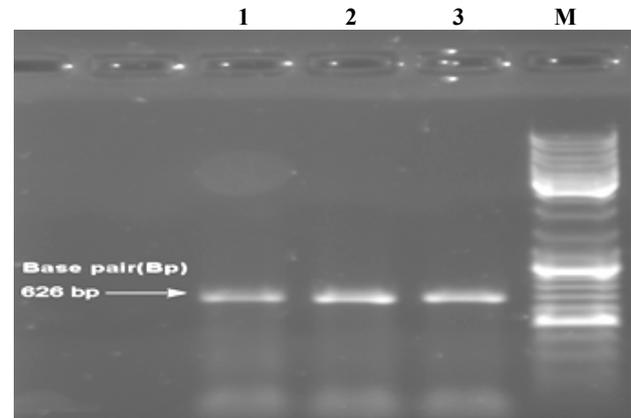


Fig. 1. Amplification of IS900 sequence of *M. paratuberculosis*. Lane 1, positive control; Lanes, 2, 3, experimental samples; M, marker DNA.

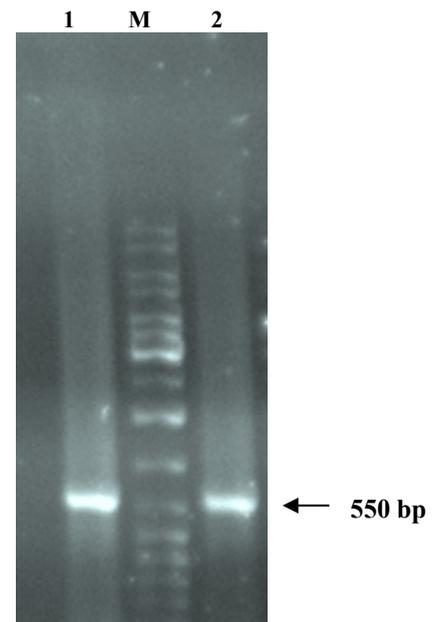


Fig. 2. Amplification of IS1311 sequence of *M. paratuberculosis*. Lane 1, positive control; Lane 2, experimental sample; M, marker DNA.

Table I. Different primer sets used along respective product sizes.

Primer set	Primer sequence		Product size (bp)	Target region	Reference
Primer set 1	5'GTTATTAACGACGCCAGC3' (F) 5'ACGATGCTGTGTTGGGCGTTAG3' (R)		626 bp	IS900	(Sivakumar <i>et al.</i> , 2005)
Primer set 2	5'TGAACGGAGCGCATCACGAA3' (F) 5'TGCAGCTGGTGATCTCTGAT3' (R)		550 bp	IS1311	(Aradaib <i>et al.</i> , 2005)
Primer set 3	5'TCGTCCGCTGATGCAAGTGC3' (F) 5'CGTCCGCTGACCTCAAGAAG3' (R)		500 bp	IS1311	(Shah <i>et al.</i> , 2002)
				<i>M.bovis</i>	

Table II.- PCR and Acid Fast Staining analysis of Intestines and Mesenteric Lymph Nodes (MLN) of Cattle and Buffaloes.

Techniques	Buffalo		Cattle	
	Intestine (n=500)	MLN (n=500)	Intestine (n=500)	MLN (n=500)
Acid Fast Staining	87 (17.4%)	82 (16.4%)	96 (19.2%)	89 (17.8%)
PCR Primer set 1	64 (12.8%)	64 (12.8%)	71 (14.2%)	71 (14.2%)
PCR Primer set 2	64 (12.8%)	62 (12.4%)	71 (14.2%)	71 (14.2%)
PCR Primer set 3	25 (5.0%)	25 (5.0%)	29 (5.8%)	29 (5.8%)

samples of terminal ileum, 96 (19.2%) were positive with acid fast staining and smears from mesenteric lymph node tissues showed bacilli in 89 (17.8%) samples. When samples were subjected to PCR by using primer set 1 and primers set 2, 71 (14.2%) tissue samples from both intestine and MLN were positive for paratuberculosis while 29 (5.8%) samples were positive for bovine tuberculosis (Table II).

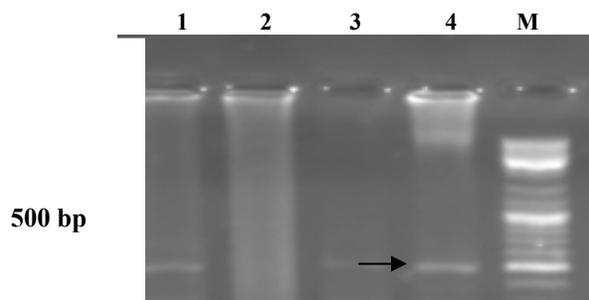


Fig. 3. Amplification of insertion sequence of *Mycobacterium bovis*. Lane 1, positive control; Lanes 2, 3, 4, experimental samples; M, marker DNA.

Upon postmortem examination, intestinal tissues from (29%) buffalo and cattle (31.2%) manifested gross abnormalities of mesenteric lymph nodes and intestine. The lymph nodes were enlarged and edematous. The intestines were thickened with

pronounced mucosal corrugations. Few animals (2.2% buffalo and 2.8% cattle) were suffering from MLN enlargement only without intestinal involvement. These lymph nodes may be enlarged due to some other infection. In their study, Sivakumar *et al.* (2006) have reported that out of 1000 buffalo, only 20 (2%) buffalo showed the lesions of paratuberculosis in intestine and mesenteric lymph nodes. Acid fast staining of the smears revealed the presence of acid fast bacilli in (17.4%) intestinal and (16.4%) MLN samples from buffalo. Similarly in cattle, mesenteric lymph nodes demonstrated acid fast bacilli in a lower number (17.8%) of samples than intestines (19.2%). PCR amplification using primer targeting IS 900, unique to *Mycobacterium avium* subsp. *paratuberculosis* amplified DNA from (12.8%) intestinal and mesenteric lymph node tissues of buffalo. When IS 1311 was targeted (an insertion sequence present in both *Mycobacterium avium* subsp. *avium* and *Mycobacterium avium* subsp. *paratuberculosis*), the results were not different significantly ($P < 0.5$). In cattle 14.2% tissues (both MLN and intestines) were positive to PCR targeted against both IS900 and IS1311. Both sets of primers were able to detect MAP infection. Results obtained in this study support the findings of Sevilla *et al.* (2005) who reported that primers targeted against IS 900 and IS 1311 can amplify DNA of MAP. Results of the

present study indicate a higher degree of infection in cattle compared with buffalo. This may be due to the reason that buffalo are more resistant to paratuberculosis than cattle (Mukherjee and Lahiri, 1966).

PCR is a more sensitive technique than acid fast staining but the results obtained in present study showed a higher number of positive samples with acid fast staining compared with PCR and as acid fast staining can not differentiate if the bacterium observed is the causative agent of tuberculosis or paratuberculosis so all the samples were also screened for *Mycobacterium bovis* by PCR targeting against *M. bovis* specific fragment generating an amplicon of 500bp. In buffalo (5%) and in cattle (5.8%) animals were positive for *M. bovis* and were suffering from intestinal tuberculosis.

References

- Aradaib, I. E., Abbas, Z. A., Abbas, B. and El- Sanousi, S. M., 2005. *Vet. Res. Commun.*, **29**: 381-385.
- Collins, M.T., 1996. In: *The veterinary clinics of North America food animal practice. Paratuberculosis (Jhone's disease)* (ed. R. W. Sweeny). W. B. Saunders Co., Philadelphia, Pa. pp. 357-371.
- Chiodini, R.J., Kruiningen H.J. and Merkal, R.S., 1984. *Cornell Vet.*, **74**: 218-262
- Grant, R.I., Rowe, M.T., Dundee, L. and Hitchings, E., 2001. *Int. J. Dairy Tech.*, **54**: 2-13.
- Huntley, J.F.J., Whitlock, R.H., Bannantine, J.P. and Stabel, J.R., 2005. *Vet. Pathol.*, **42**: 42-51.
- Naser, A.S. AND Shafran, I., 2000. *Int. J. Dairy Tech.*, **95**: 1094-1095.
- Quinn, P.J., Carter, M.E., Markey, B.K. AND Carter, S.G.R., 1994. In: *Clinical veterinary microbiology*, Wolfe Publishing, London, pp.144-155.
- Sevilla, I., Singh, S.V., Garrido, J.M., Aduriz, G., Rodríguez, S., Geijo, M.V., Whittington, R.J., Saunders, V., Whitlock, R.H. and Juste, R.A., 2005. *Rev. Sci. Tec. Off. Int. Epiz.*, **24**: 1061-1066.
- Shah, D.H., Verma, R., Bakshi, C.S. and Singh, R.K., 2002. *FEMS Microbiol. Lett.*, **214**: 39-43.
- Sivakumar, P., Tripathi, B.N. and Singh, N., 2005. *Vet. Microbiol.*, **108**: 263-270.
- Mukherjee, A. and Lahiri, A., 1966. *Indian Vet. J.*, **37**: 349-353.
- Kurade, N.P., Tripathi B.N., Rajkumar, K. and Parihar, N.S., 2004. *Vet. Pathol.*, **41**: 378-387.

(Received 3 July 2008, revised 10 February 2009)
