Short Communications

Pakistan J. Zool., vol. 41(5), pp. 413-414, 2009.

Recent Records of Smooth-Coated Otter (*Lutrogale perspicillata*) from Sindh, Pakistan*

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Abstract.- Mammals have no parallel in animal kingdom in terms of their adaptability and specializations. Smooth-coated otter (*Lutrogale perspicillata*) is classified as Vulnerable on the 2005 IUCN Red List of mammals for Pakistan, and is listed on Appendix II of CITES. Direct sightings and indirect records confirmed the presence of smooth-coated otter at Nara Canal and Chotiari Reservoir, District Sangahr and Haleji lake, District Thatta, Sindh. It has been a first record of the direct sightings by any scientific research team in more than a decade. The population seems to comprise of few individuals. It is facing extremely high risk of extinction due to multitude of detrimental factors.

Key words: Nara Canal, status of smooth-coated otter, Sanghar, Thatta.

Smooth-coated otter found in Pakistan is known as Sindh otter, *Lutrogale perspicillata sindica*, and is endemic sub-species to Pakistan. It is found in rivers and their tributaries, lakes, streams, ponds, marshes and riverine forests where tall *typha* grass provides it with cover to remain hidden from its enemies. The smooth concave upper edge to the nose differentiate it from other species of otter - the common otter (*Lutra lutra)* found in Pakistan.

It is distributed from Indonesia through Southeast Asia into China, India, and Pakistan with a small population in Iraq. In Pakistan, it is distributed throughout the lower Indus riverine system and upto the outer foot hills of Punjab. It has also been reported from Keti Bunder, Thatta district, Sindh, and is believed to enter in man-made canal systems, storage reservoirs, swamps and lakes. It prefers to live in warmer water that is heavily silt laden and has a smooth flow. Historically, it was known to inhabit a wide range , being common in the lower reaches of the Indus, particularly around Sukkur and the east Nara, Sindh (Roberts, 1997).

Materials and methods

Robust surveys were conducted under WWF Indus for All Programme to confirm the presence or absence of otter at programme sites. After the confirmation of their presence use of most appropriate wildlife survey techniques, with modifications where necessary, such as track count and line transect were adopted to collect baseline data about the status and distribution of the animal so that more comprehensive research to estimate precise population of otters at potential areas can be done in the future. Boats were used to reach reed beds located inside the Chotiari reservoir.

Results and discussion

Presence of Smooth-coated otter using indirect signs *i.e.* tracks (Fig. 1A) was confirmed at two of the Indus for All Programme sites *viz.* Nara Canal and Chotiari Reservoir, District Sangahr and Haleji lake, District Thatta. However, direct sightings (Fig. 1B) were successful only at Chotiari reservoir. Six cubs of smooth-coated otter were observed, photographed and released back. After Roberts (1997) this has been a first record of the direct sighting of smooth-coated otter by any research team in more than a decade.

The current population of Smooth-coated otter in the province of Sindh, Pakistan, comprises only of few individuals, and the future survival of the animal seems bleak. Fresh foot-prints of smooth-coated otter were observed in January, 2008 at three localities *viz*. Nara Canal, Chotiari Reservoir and Haleji lake; however, direct sightings were successful only at Chotiari Reservoir, Sanghar. Roberts (1997) reported it from Sukkur and east

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0030-9923/2009/0005-0413 $ 8.00/0

Nara, Sanghar Distrcit. Study conducted by Gachal *et al*. (2007) depicts an alarming picture about the status of the animal, as presence of the species was confirmed at only two of the 15 sites *viz*. Haleji lake and Sajawal in Sindh. However, we did not record any direct or indirect evidence of the presence of otter at Sajawal. Its presence is also needs to be confirmed at Keenjhir lake after few locals reported the animal around certain points.

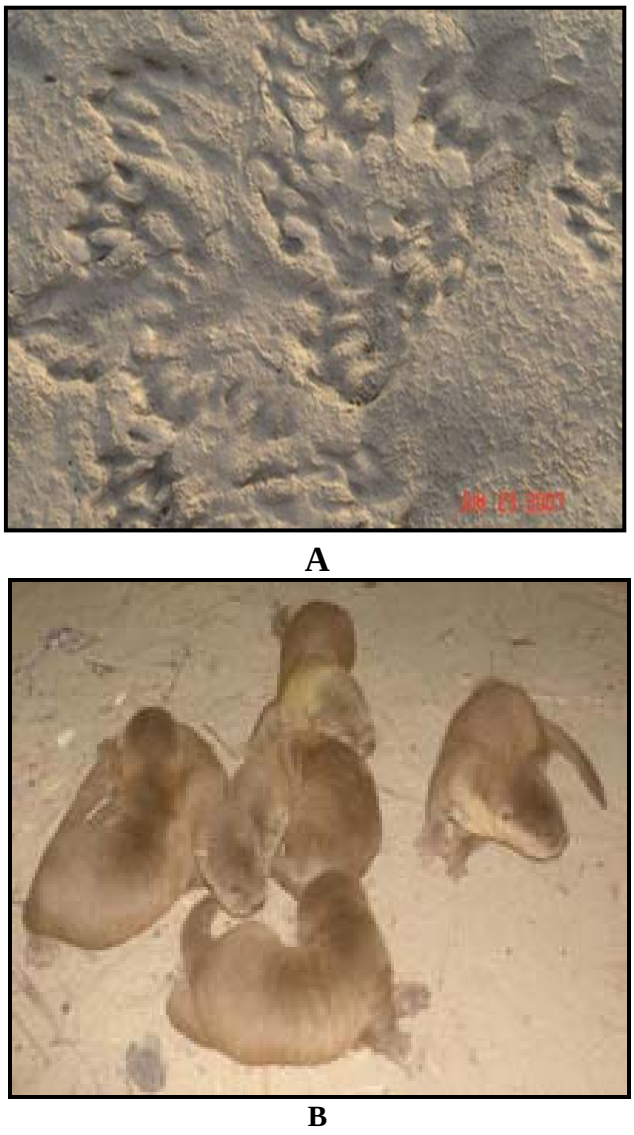

Fig. 1. *Lutrogale perspicillata;* A, otter track; B, cubs.

Threats

Multitudes of factors are threatening the survival of the species such as habitat loss for the reclamation of the land for various purposes. Construction of dams for mega hydro-electric projects in the upstream has greatly reduced water flow in the Indus River; consequently, riverine forests whose existence rely upon inundation of river Indus are shrinking at unprecedented levels. Furthermore, habitat modification and alterations are also playing havoc to the otter population. Nonetheless, poaching outpace all other threats, as otter is mainly hunted for the demand of its skin and misconception about the medicinal value of its skin and fat. Auyorvedic practitioners consider the cushions made of otter skin as a remedy to piles and a cap made of otter skin as a cure for migraine.

Conclusion

There is a pressing need to prioritize the effective conservation and management of smooth-coated otter. It could be achieved by creating awareness among the masses, implementation of laws that exist, strict ban on hunting and trade, declaring areas where otters are confined as protected areas.

Acknowledgements

Authors offer deep sense of gratitude to "Indus for All Programme" of WWF Pakistan, specially, Dr. Ghulam Akber, Regional Director, and Mr. Rab Nawaz, Natural Resource Manager, Indus for All Programme, WWF.

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(Received 5 July 2008, revised 21 April 2009)

Pakistan J. Zool., vol. 41(5), pp. 415-416, 2009.

Occurrence of the Norway Rat, *Rattus norvegicus,* in Rawalpindi and Islamabad

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Abstract.-The Norway rat which was recently reported (outside Karachi and coastal areas of Sindh) from the Lahore city railway station has now been found to be widely distributed in Rawalpindi city and in parts of Islamabad capital city.

Key words: House rate, house mouse, body weight, body measurement.

The Norway rat *Rattus norvegicus,* a native to Central Asia, has a cosmopolitan distribution, being mainly confined to major cities and especially the seaport cities (Brooks, 1973). In Pakistan rat was largely confined to Karachi and Pasni on the Mekran coast. Recently, it has been recorded from the railway station of Lahore city (Roberts, 1997). In 2008, we recorded *R. norvegicus* from different parts of Rawalpindi and Islamabad cities. In Rawalpindi, the rat was collected from Satellite Town, Shamsabad, Sadiqabad, Raja Bazar, Sadar and Lalazar colony, while from Islamabad it was taken from Aabpara, G and I sectors. However, there are unconfirmed reports of its occurrence in the Pirwadhai and Nullah Leh areas of Rawalpindi and from the Bari Imam Shrine area, and the settled localities on the outskirts of the campus of Quaid-i-Azam University, Islamabad. Although the present picture of the distribution of the Norway rat is incomplete and fragmentary, yet it indicates that the rat has established itself over a sizeable area of the two cities. It is a general impression among the residents of Sadiqabad and Satellite Town that the rat began appearing in these localities some 15-20 years ago.

Table I provides essential statistics on the body weight and body measurements of the adult specimens of the two sexes of the Norway rat taken from Rawalpindi and Islamabad cities. Study skins and skulls of some of these specimens are housed in the Biodiversity Lab of the Department of Zoology of the Arid Agriculture University, Rawalpindi.

Table I.- Data on the body weight (g) and body measurements (mm) of the two sexes of the Norway rats collected from Rawalpindi and Islamabad cities.

	N	O.R.	$\bar{X}$	s	V*
Male					
Body weight	24	227-462	325	61.485	18.91
Body length	24	185-254	218	18.697	8.56
Tail length	24	150-219	183	20.156	11.01
Hindfoot length	24	30-50	43	4.665	10.85
Ear length	24	18-30	23	3.153	13.71
Tail length / Body length	24	0.664-1.020	0.84	0.109	12.98
Female					
Body weight	6	233-390	299	53.479	17.88
Body length	6	180-245	198	23.878	12.06
Tail length	6	160-200	179	15.626	8.73
Hindfoot length	6	40-45	42	1.862	4.43
Ear length	6	17-25	22	3.141	14.28
Tail length / Body length	6	0.816-0.994	0.909	0.0617	6.79

*Coefficient of variation

The introduction of *R. norvegicus* to the guild of commensal murids of Rawalpindi and Islamabad is a definitely not a good news for the people of the two cities. Like *Rattus rattus* and *Mus musculus*, *R. norvegicus* shares several diseases with man and thus is a health risk. Moreover, it is a formidable pest with a predilection for cereal grains, meat, fish and nuts (Roberts, 1997; Brooks, 1973). By virtue of the fact that *R. norvegicus* is essentially a temperate zone species, it is expected to establish itself better in the warm-temperate climate of Rawalpindi-Islamabad than in the subtropical climate of Lahore and perhaps for this reason it may have a competitive edge over *R. rattus*, which is basically a tropical species (Greaves, 1982).

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(Received 10 February 2009, revised 14 April 2009)

Pakistan J. Zool., vol. 41(5), pp. 416-417, 2009.

A Rare Skink, *Novoeumeces blythianus*, Recorded from Nag Valley, Kharan, Balochistan

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Abstract.- Orange-tailed skink is a rare species. It was reported from Khyber Pass near Afghan border and coastal areas of Pakistan only. Now we have recorded this species from Nag Valley, district Kharan, Balochistan.

Key words: Orange-tailed skink, *Eumeces* Thal desert, Khyber Pass.

Orange-tailed skink (*Novoeumeces blythianus*) belongs to the genus *Novoeumece*s of the family Scincidae. The genus *Novoeumeces* was recently created for three Old World species that formerly had been put under the genus *Eumeces* (Griffith *et al*., 2000). Three species of this genus have been recorded from Pakistan, the Mole skink (*Novoeumeces indothalensis)* from Thal desert of Punjab, Red-striped skink (*Novoeumeces schneiderii zarudnyi*) from Astola Island, and orange-tailed skink (*Novoeumeces blythianus*) from the Khyber Pass area of northwestern Pakistan near the Afghan border, and from the coastal areas of Pakistan (Minton, 1966; Khan, 2006).

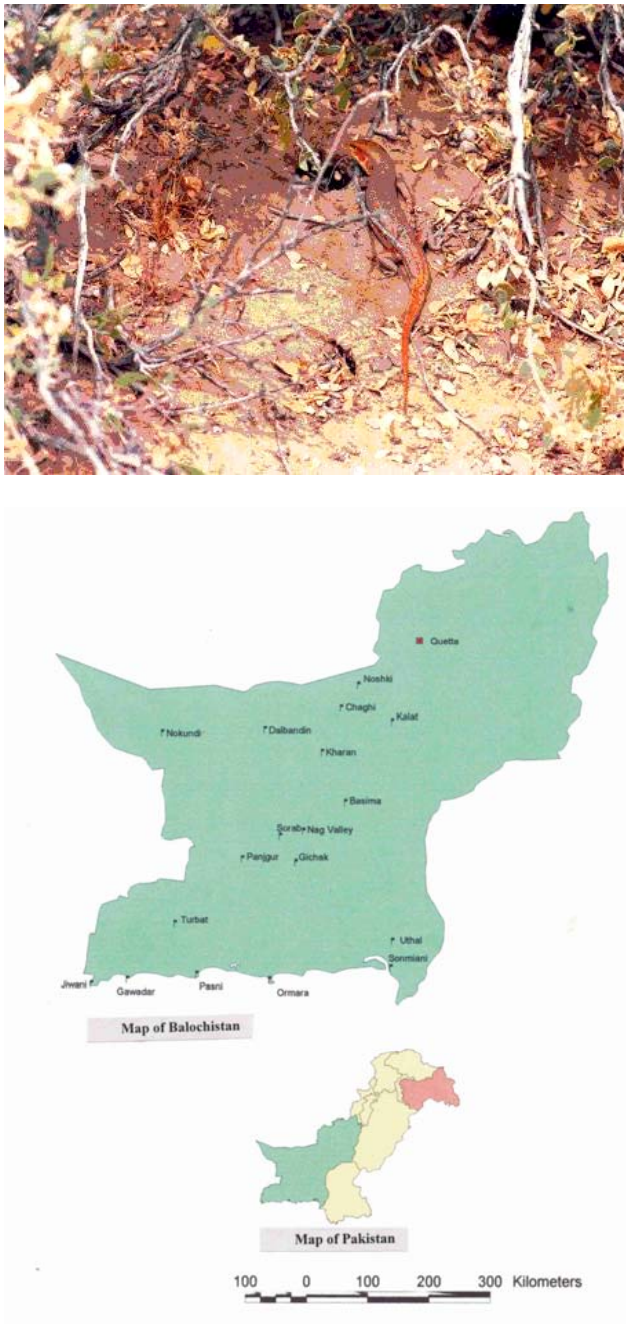

Fig. 1. Orange-tailed skink in Nag Valley along with a map of Balochistan province showing the location of Nag valley.

We recorded *N. blythianus* from the Nag Valley area during a Houbara Expedition in April 1998. Nag Valley is located 27°41 N and 65°14 E southwestern Kharan between Besima, and Panjgur in Balochistan (see map). This narrow valley,

having an area of about 1500 km^2, is a known breeding area of Houbara bustard in Pakistan (Nadeem *et al*., 2004). Its altitude varies between 1100m and 1600m. The climate of the area is generally arid with a maximum precipitation of about 200mm per annum. The minimum and maximum temperatures of Nag Valley recorded in April averaged 16°C and 30°C, respectively. The rainfall is usually erratic and irregular. The rains are mostly received during winter and very rarely in early spring. Rainfall seems to exhibit a cyclic fluctuation with 2-3 years of drought followed by more generous rain. Windstorms (speed 10-20mph) are constant feature of Nag Valley and they lower the temperature during summer (Nadeem *et al*., 2004).

The skink was observed under a shrub (*Zygophyllum eurypterum*) near a hole (Fig. 1). It was snapped and shot with a handycam for 20-30 seconds till it suddenly entered the hole. Another specimen was sighted under a *Zygophyllum* shrub in May 1999. Only two accidental sightings of the skink perhaps indicated it was rare in the area, or at least rarer than the other reptilian species frequently seen in the area *e.g. Agrionemys horsfieldii, Varanus griseus, Trapelus megalonyx, Trapelus agilis, Calotes versicolor, Eremias fasciata Acanthodactylus cantoris, Psammophis schokari, Boiga trigonata ,* and *Spalerosophis diadema* during the five years (1997-2001) of our Houbara Surveys. The flora of the area was characterized by *Zygophyllum eurypterum, Rhazya stricta, Pennisetum divisum, Convolvulus spinosus, Otostegia aucheri, Astrogalus stocksii, Cymbopogon jwarancusa, Haloxylon ammodenderon, Haloxylon griffithi, Fagonia indica* and *Peganum harmala.*

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(Received 13 March 2009, revised 20 April 2009)

Pakistan J. Zool., vol. 41(5), pp. 417-420, 2009.

Caligus mauritanicus Brian, 1924 a Parasite of Common Cultured Dentex, *Dentex dentex* L. from Aegean Sea and Parasites of Cultured Fishes of Turkey

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Abstract.- *Caligus mauritanicus* Brian, 1924 (Copepoda, Caligidae) was reported on the cultured common dentex, *Dentex dentex* (Linnaeus, 1758) from the East Aegean Sea in July-August 2008. This parasite is reported for the first time from cultured common dentex in Turkey. Its prevalence and mean intensity were 20% and 5.6, respectively. This study also provides a checklist of 28 protozoans, 17 helminths (11 monogeneans, 4 digeneans, 1 acanthocephala, 1 cestoda) and 11 parasitic crustaceans (copepod and isopod) reported from ten freshwater and marine fish species cultured in Turkey.

Key words: *Caligus, Dentex,* cultured fish.

Aquaculture activities in Turkey started with carp and trout farming in 1970s and continued with gilthead seabream/seabass farming in the Aegean Sea and Levantine Sea in the midst of 1980s; and tuna rearing in the Aegean Sea and the Mediterranean Sea in early 2000s. Initial attempts have been made to introduce *Seriola dumerili, Argyrosomus regius, Puntazzo puntazzo, Dentex dentex, Salvelinus fontinalis, Salmo trutta labrax, Epinephelus* sp. *Pagrus auriga, Pagrus pagrus* into aquaculture systems.

Several parasites have been reported from *Oncorhynchus mykiss, Cyprinus carpio, Sparus aurata, Dicentrarchus labrax, Seriola dumerili, Argyrosomus regius, Puntazzo puntazzo, Dentex dentex, Salvelinus fontinalis* and *Salmo trutta labrax* cultured in Turkey (Table I). *Cyprinus carpio* was

the most parasitized fish, with 29 species of protozoon (17) and metazoon parasite (12) (Öktener, 2003, 2005, 2009; Öktener and Trilles, 2004; Öktener *et al*., 2004; Oğuz and Öktener, 2007; Tokşen and Esat, 2008). In terms of host-parasite distribution, parasites of the other cultured fish may be ranked as follows: *Oncorhynchus mykiss* (18 parasites), *Dentex dentex* (9 parasites), *Dicentrarchus labrax* (7 parasites), *Sparus aurata* (6 parasites), *Salvelinus fontinalis* (2 parasites), *Salmo trutta labrax* (2 parasites), *Seriola dumerili* (2 parasite), *Argyrosomus regius* (1 parasite), *Puntazzo puntazzo* (1 parasite).

Among the parasites reported from cultured fish of Turkey, the dominant taxa are the protozoan parasites with 28 species, followed by the helminths with 17 species (11 monogeneans, 4 digeneans, 1 acanthocephala, 1 cestod) and parasitic crustaceans with 11 species.

This parasitological survey was carried out with the aim of identifying the composition of the

Table I.- Parasites of cultured freshwater and marine fish in Turkey.

Host	Parasites	
	Protozoans	**Metazoans**
Cyprinus carpio Lin.,1758	*Chilodinella cyprini*, *Trypanosoma* sp., *Trypanoplasma* sp., *Sphaerospora* sp., *Eimeria* sp., *Trichodina nigra*, *Trichodina perforate*, *Trichodina acuta*, *Trichodina mutabilis*, *Trichodina domerquei*, *Trichodinella* sp., *Trichodinella subtilis*, *Apiosoma piscicolum*, *Ichthyophthirius multifilis*, *Costia necatrix*, *Cryptobia branchialis*, *Epistylis* sp	Monogenea: *Gyrodactylus* sp., *Dactylogyrus* sp., *Dactylogyrus vastator*, *Dactylogyrus anchoratus*; Digenea: *Diplostomum spathaecum*, *Clinostomum complanatum*, *Sanguinicola* sp., Acanthocephala: *Pomphorhynchus laevis*; Cestoda: *Caryophyllaeus laticeps*; *Argulus foliaceus*, *Lernaea cyprinacea*, Arthropoda: *Ergasilus* sp
Oncorhynchus mykiss (Walbaum, 1792)	*Chilodinella cyprini*, *Chilodinella piscicola*, *Hexamita salmonis*, *Eimeria truttae*, *Trichodina nigra*, *Trichodina domerquei*, *Trichodinella* sp., *Tripartella* sp., *Ambiphrya* sp., *Apiosoma piscicolum*, *Ichthyophthirius multifilis*, *Costia necatrix*, *Epistylis* sp	Monogenea: *Gyrodactylus* sp., *Dactylogyrus* sp; Digenea: *Clinostomum complanatum*, *Crepidostomum farionis*; Acanthocephala: *Pomphorhynchus laevis*
Salvelinus fontinalis (Mitchill, 1814)	*Costia necatrix, Trichodina* sp	-
Salmo trutta labrax Pallas, 1814	*Ichthyophthirius multifilis*, *Costia necatrix*	-
Sparus aurata Lin., 1758	*Trichodina* sp., *Costia* sp., *Oodinium* sp.	Monogenea: *Microcotyle chrysophrii, Furnestina echeneis*; Arthropoda: *Ceratothoa oestroides*.
Dicentrarchus labrax Lin., 1758	*Amyloodinium ocellatum*	Monogenea: *Diplectenum aequans*; Arthropoda: *Caligus minimus*, *Lernanthropus kroyeri*, *Nerocila orbignyi*, *Ceratothoa oestroides*, *Ceratothoa italica*
Dentex dentex Lin., 1758	*Amyloodinium ocellatum*, *Trichodina* sp., *Epistylis* sp., *Riboscyphidia* sp., *Ceratomyxa* sp.	Monogenea: *Gyrodactylus* sp., *Microcotyle* sp.; Arthropoda: *Clavellotis* sp., *Caligus mauritanicus*
Puntazzo puntazzo (Cetti, 1777)	-	Monogenea: *Lamellodiscus ignoratus*
Argyrosomus regius (Asso, 1801)	-	Monogenea: *Benedenia sciaenae*
Seriola dumerili (Risso, 1810)		Monogenea: *Zeuxapta seriolae*; Arthropoda: *Pennella instructa*

parasitic fauna of common dentex under farming systems in Turkey, so as to develop prevention and control measures in advance of any possible outbreak of infection.

Materials and methods

Seven common dentex, *Dentex dentex* (Linnaeus, 1758) (Teleostei, Sparidae) bought from farming system in the Aegean Sea Coasts of Turkey (38° 26' N; 26° 42' E) were examined for ectoparasites and endoparasites on July-August 2008. Common dentex (600-800 g) were selected. The gill arches and intestine of fish were examined using a dissecting microscope (Wild M5). Collected parasites were preserved in 70% ethanol. Identification was done using the specific keys of Brian (1924), Radujkovic and Raibaut (1989), Capart (1959) and Yamaguti (1963).

Results and discussion

A female of *Caligus mauritanicus* Brian, 1924 (Copepoda, Siphonostomatoida, Caligidae) (Fig. 1), measuring 4-7 mm was collected from gill filaments. The parasites had 20% prevalence and 5.6 mean intensity.

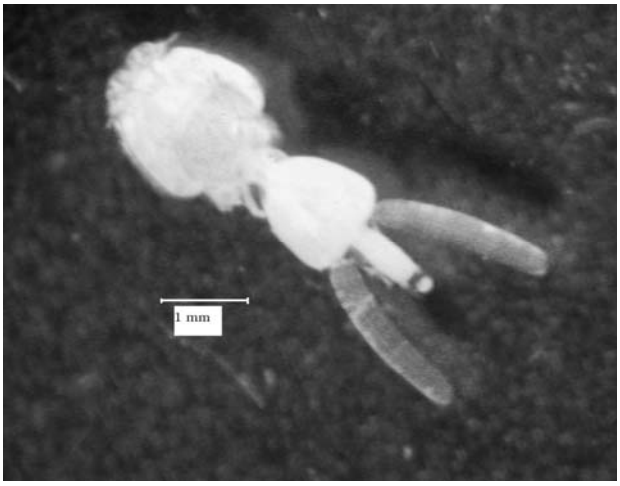

Fig. 1. *Caligus mauritanicus*, the complete specimen (scale bar 1mm).

Geographical distribution

Mauritania, Congo, Senegal, Angola, Monaco, Tunisia, Adriatic Sea, Mediterranean Sea, New Caledonia (Brian, 1924; Capart, 1959; Yamaguti, 1963; Radujkovic and Raibaut, 1989; Boxshall and Huys, 2007).

Hosts

This euryxenic species can be found infesting diverse fish species, including *Dentex dentex, Sciaena umbra, Argyrosomus regius, Lichia amia*, *Arius heudeloti*, *Pagrus* sp. *Campogramma glaycos*, *Dentex* sp. *Pomatomus saltator*, *Cynoglossus* sp. *Caranx* sp. *Apsilus fuscus*, *Caranx rhonchus*, *Dentex gibbosus*, *Plectorhynchus mediterraneus, Pagrus pagrus, Polydactylus quadrifilis, Trachurus trachurus, Trigla lyra, Umbrina* sp. (Brian 1924; Capart 1959; Yamaguti 1963; Radujkovic and Raibaut 1989).

Remarks

The only caligids previously reported from Turkish waters are *Caligus pageti* Russel, 1925 on *Mugil cephalus, Liza saliens, L. ramada, Chelon labrosus* from the Aegean Sea; *Caligus minimus* Otto, 1821 on *Dicentrarchus labrax* from the Aegean Sea; *Pseudocaligus apodus* Brian, 1924 on *Mugil cephalus, Liza saliens, L. ramada, Chelon labrosus* from the Aegean Sea; *Caligus* sp on *Sardina pilchardus* from the Sea of Marmara; *Lepeophtheirus europaensis* Zeddam, Berrebi, Renaud, Raibaut, Gabrion, 1988 on *Platichtys flesus* from Ekinli Lagoon; *Caligus bonito* Wilson, 1905 on *Coryphaena hippurus* from the Aegean Sea (Oğuz and Öktener, 2007; Öktener, 2008).

Morphological differences with regard to previous descriptions have not been observed. *Caligus mauritanicus* according to the general drawings by Brian (1924), Radujkovic and Raibaut (1989), Capart (1959), Yamaguti (1963) is in accordance with my specimens and also by the detail of leg 1 , leg 4 and type of furca. This parasite is reported for the first time from cultured common dentex and Turkey.

Acknowledgement

I am grateful to Prof. Dr. Raul Casto Romero of University of Antofagasta, Chile for identification of *Caligus mauritanicus*.

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(Received 17 April 2009, revised 15 May 2009)

Pakistan J. Zool., vol. 41(5), pp. 420-421, 2009.

A New Species *Temiorchis sindhensis* (Trematoda: Brachycoeliidae) from Frog (*Rana cyanophlyctis*) of Pakistan

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Abstract.- A new trematode is described from the small intestine of frog (*Rana cyanophlyctis*) and named as *Temiorchis sindhensis*. The present specimens are compared with all the previous species and found different in several morphological characters. Species name refers to the locality of the host.

Keywords: Trematode, Frog, *Temiorchis sindhensis* new species, Pakistan.

The genus *Temiorchis* Mehra *et* Negi, 1925 is known to occur in amphibians and reptiles (Yamaguti, 1971). Two species of the genus have been recorded from Pakistan (Bhutta and Khan, 1975; Bilqees and Khan, 2003). Present is regarded a new species *Temiorchis sindhensis* because it shows distinct diagnostic features as compared to the previous species.

Six frogs (*Rana cyanophlyctis*) collected from Oderolal Station, Sindh, Pakistan were examined for helminth parasites. Four trematodes were recovered from the small intestine of one frog. For a detail study these specimens were fixed in F.A.A. under glass slide pressure, dehydrated in graded series of alcohols, stained with Mayer's carmalum, cleared in clove oil and xylol and mounted permanently in Canada balsam. Measurements are in millimeters. Specimens are in the collection of the senior author.

Temiorchis sindhensis, new species

Host	Frog (*Rana cyanophlyctis*)
Location	Small intestine
Locality	Oderolal Station, Sindh, Pakistan
No. of specimens recovered	4 from a single host
No. of hosts examined	6

Temiorchis Mehra *et* Negi, 1925
Syn. *Centrovitis* Bhalerao, 1926

Body elliptical, elongate and spinulate measuring 2.00-3.40 by 0.64-0.76. Oral sucker smaller as compared to acetabulum measuring 0.10-0.16 by 0.15-0.18. Pharynx well developed 0.075-0.09 by 0.09-0.10. Oesophagus measuring 0.12-0.3 by 0.3 by 0.03-0.06. Ceca short, well surpassing acetabulum, the left ceca measuring 0.60-1.05 by 0.09-0.32. Acetabulum in anterior third of the body 0.15-0.21 by 0.16-0.22. Distance between

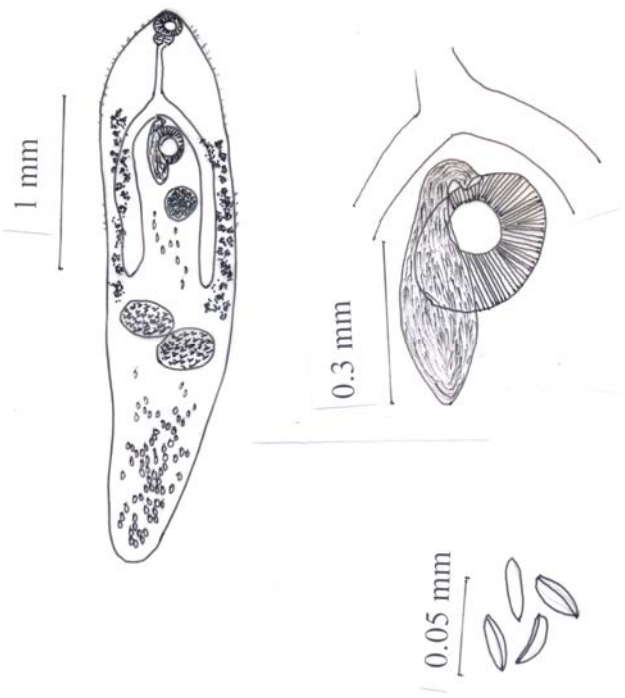

Fig. 1. *Temiorchis sindhensis*, new species; A, ventral view of holotype; B, eggs enlarged.

Acetabulum and cecal bifurcation 0.067-0.11. Testes oval, the anterior measuring 0.16-0.31 by 0.21-0.22, and the posterior 0.17-0.40 by 0.22-0.25. The two testes are either close by or at a distance of 0.035-0.045 from each other. The distance between anterior testis and ovary 0.18-0.21. Cirrus pouch well developed, enclosing tubular seminal vesicle, well developed prostatic complex and cirrus. Genital opening just behind cecal bifurcation. Ovary rounded to oval, a little left of median line measuring 0.105-0.30 by 0.06-0.13. Distance of ovary and acetabulum varies between 0.052-0.15. Vitellaria bunch like, lateral to ceca commencing almost at the level of cecal bifurcation Uterine coils reaching to posterior extremity. Excretory vesicle tubular. Eggs oval, small, numerous, measuring 0.026-0.027 by 0.095-0.11.

Discussion

Mehra *et* Negi (1926) erected the genus *Temiorchis* with *T. ranarum* as its type species in *Rana tigrina* from India.

Later Bhutta and Khan (1975) reported the *T. ranarum* from *Rana tigrina* from Sahiwal and Lahore, Pakistan.

Bilqees and Khan (2003) added another species to the genus namely *T. fatimaae* from *Rana cyanophlyctis* from Karachi, Sindh. Both oral sucker (0.26 by 0.31) and acetabulum (0.27 by 0.29) in *T. ranarum* are bigger as compared to present species. The length of eggs in *T. mehrai* Rai, 1962 from *Bufo melanostictus* (0.028-0.031) and *T. vitelloconfluentum* Rai, 1962 from *Rana tigrina* (0.028-0.035) are larger as compared to the length in the present specimens. It differs from *T. fatimaae* in the body shape, moreover the eggs in the present species are much smaller (0.026-0.027 by 0.009-0.11) as compared to *T. fatimaae* (0.041-0.045 by 0.022-0.026). Also the oesophagus is not as long as *T. fatimaae* and the testes are more posteriorly placed as compared to *T. fatimae*.

In view of these differences a new species is proposed. The new species is named *T. sindhensis* referring to the locality of the host.

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(Received 4 February 2009, revised 27 April 2009)

Pakistan J. Zool., vol. 41(5), pp. 421-424, 2009.

PCR Detection of *Salmonella enteritidis* and *Salmonella typhimurium* in Poultry Feed

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Abstract.- This research was undertaken to detect *Salmonella enteritidis* and *Salmonella typhimurium* in commercial poultry feed by using PCR method in Pakistan. Five different types of commercial poultry feed samples *i.e;* layer starter (LS), layer grower (LG), layer finisher (LF), broiler starter (BS), and broiler finisher (BF) were randomly collected through double blinding strategy from each of the 20 feed mills. A total of 100 samples of feed were subjected to PCR test. Each feed sample was tested for the detection of two important serovars of *S. enterica* serotype I; *S. enteritidis* and *S. typhimurium*. The positivity percentage of *S. enteritidis* in the poultry feed was 20, 15, 10, 15 and 10 for LS, LG, LF, BS, and BF, respectively. *S. typhimurium* was detected from LS, LG, LF, BS, and BF feed samples at the rate of 15, 10, 10, 10, and 10 percent, respectively. All feed samples tested and found positive were mutually exclusive for either *S.entreritidis* or *S. typhimurium*.

Key words: layer, broiler, *Salmonella enterica serotype I*, double blinding strategy.

Salmonellosis has been declared by the World Health Organization (WHO) and Food and Agriculture Organization (FAO) as one of the most common and important zoonoses, and has been included in the list of commonest zoonoses of the terrestrial animal health code of Office of International Epizootics (Anonymous, 2004). With the expansion of poultry industry, the pandemicity of *Salmonella enteritidis*, lack of effective national preventive control measures, and due to unawareness of biosecurity protocols, and the disease has increased dramatically in the developing countries for the last two decades (Gupta, 1999). Globally, there are more than 1500 serovars of *S. enterica* serotype I. The major pathogens include *S. enteritidis, S. typhimurium, S. newport, S. typhi, S. paratyphi A & C,* which account for salmonellosis in animals and food poisioning and typhoid fever in humans (Porwollik *et al*., 2004). There is much evidence for the possible transmission of *Salmonella* pathogens from poultry feed to poultry meat, eggs, and consequently to humans (Gupta, 1999), therefore, researchers are finding methods in producing *Salmonella* free poultry meat and eggs for its consumers (Hagren *et al*., 2008).

The PCR is considered an easy, simple and reproducible test (Hein *et al*., 2006). Antonio *et al*. (2000) have reported that *S. enteritidis, S. typhimurium* and *S. vircho* could easily be distinguished through the gene profile by using PCR method. It is also an easy method to differentate one serovar from the other serovars of *S. enterica* serotype I. PCR method takes an average of 6-8 hours for the detection and confirmation of *Salmonella* spp. whereas the conventional methods require 8 days with significant increase in the expenses (Myint *et al*., 2006).

In Pakistan, there is lack of reliable data about the microbial contamination of poultry meat primarily because the conventional methods of *Salmonella* detection are too lengthy, time consuming and expensive. Therefore, an effort was made for the detection of *S. enteritidis* and *S. typhimurium* in five types of commercial poultry feed by using the PCR. The results were available within 8 hours as compared to one week in the conventional method of culture (Malorny *et al*., 2008; Hagren *et al*., 2008). The futuristic vision was to determine the possible role of *S. enteritidis* and *S. typhimurium* if any, in the transmission cycle through poultry feed to poultry meat, eggs and finally its zoonotic impact in terms of public health hazard (Hald *et al*., 2004).

Materials and methods

A total of 100 poultry feed samples of layer starter (LS), layer grower (LG), layer finisher (LF), broiler starter (BS), and broiler finisher (BF) were collected from 20 random feed mills (Fewtrell *et al*., 2004). All samples were collected in sterilized plastic bottles and were trasported immediately to the PCR laboratory, School of Biological Sciences, University of the Punjab, Lahore, for further processing through polymerase chain reaction (Wang and Yeh, 2002). Each feed sample was tested for two serovars *i.e.*, *S. enteritidis* and *S. typhimurium*. The data obtained were statistically analysed according to Roger *et al*. (2003).

Following primers were used for PCR.

Primers used for *Salmonella enteritidi* (Set-1)

Primers Sequence		Expected Size
5'-AGTGCCATACTTTTAATGAC-3'	(Reverse primers)	316 bp
5'-ACTATGTCGATACGGTGGG-3'	(Reverse primers)	

Primers used for *Salmonella typhimurium* (Set-2)

Primers Sequence		Expected Size
5'-GTGAAATTATCGCCACGTTCGGGCAA-3'	(Reverse primers)	284 bp
5'-TCATCGCACCGTCAAAGGAACC-3'	(Reverse primers)	

Results and discussion

Table I shows the positivity rate for *S. enteritidis* in poultry feed samples. It was 20%, 15%, 10%, 15% and 10% for LS, LG, LF, BS and BF, respectively. The percentage of positivity from the same feed samples for *S. typhimurium* for types of LS, LG, LF, BS and BF was 15%,10%,10%, 10%, and 10%, respectively. The positivity rate of each serotype was mutually exclusive in feed samples.

S. enteritidis was detected 20% more as compared to *S. typhimurium* (15%) in the tested feed samples. The results are comparable to Charlton *et al.* (2005). However, there was no significant differences in the positivity rate between the layer feed samples and broiler feed samples with respect to *S. enteritidis* and *S. typhimurium*. However, it was biologically significant and in agreement with other studies (Veldman *et al.*, 1995) and disagree to Cox *et al.* (1983).

Table I.- Different poultry feed samples found positive for *Salmonella enteritidis* and *Salmonella typhimurium* after PCR amplification.

Feed samples	n	*Salmonella entertidis*	*Salmonella typhimurium*
Layer starter (LS)	20	4	3
Layer grower (LG)	20	3	2
Layer finisher (LF)	20	2	2
Broiler starter (BS)	20	3	2
Broiler grower (BG)	20	-	-
Broiler finisher (BF)	20	2	2

P >0.05

The results of the present study are matching to Veldman *et al.* (1995) in which 10% of 360 feed samples were contaminated with *Salmonella* species and quite similar to Boqvist and Co-workers (2003) in which, 555 samples were collected from animal feed stuff and feed mills in Sweden between 1993 and 1997. The most prevalent isolate from feed mills was *S. typhimurium* (n=91) followed by *S. dublin* (n=82). As per investigation (Nashed, 1986) the feed and litter contamination with *S. typhimurium* remain alive and viable at 37°C in the feed up to 6 weeks and at an ambient temperature for up to 71 weeks. A very high percentage of *Salmonella* organisms were reported in mash, pelleted and meat meal samples at a percentage of 58, 0 and 92, respectively.

It is interesting to note that pelleted feed sample had zero positivtity of *Salmonella* organism, because of the advanced processing techniques of the feed. This is an encouraging aspect that the pelleted feed instead of mash feed should be used in order to avoid the salmonellae infection as a commercial poultry feed. Furthermore an extraordinary high rate of *Salmonella* pathogen contamination (92%) has been reported by Cox and co-workers (1983), probably because the samples were derived from meat and/or bone meal. According to a report by Rouse and coworkers (1988), *S. typhimurium* was isolated after the contamination of sterilized feed with meat meal prepared from commercial broiler carcasses.

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(Received 14 February 2009, revised 11 March 2009)

Pakistan J. Zool., vol. 41(5), pp. 424-428, 2009.

Detection of Canine Distemper Virus from Lymphopenic Dogs by RT-PCR Amplification of Nucleoprotein Gene

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Abstract.- This study involves detection of canine distemper virus (CDV) from 45 clinically suspected dogs showing respiratory signs and lymphopenia. Some dogs manifested digestive system involvement but none of them showed any nervous symptom. Nucleoprotein (NP) gene was amplified by RT-PCR from conjunctival swabs. Only 10 of 45 (22.2%) dogs were found positive to CDV infection. The difference in lymphocyte count in the blood of distemper positive and negative dogs was insignificant ($P = 0.203$). It was, however, significant ($p = 0.0056$ or $p < 0.05$) in distemper positive dogs when repeated thrice at an interval of ten days showing rapid recovery. The results suggest that canine distemper is independent of age and sex. It may involve secondary infections and lymphopenia is the clinical sign that can help in presumptive diagnosis at an early stage of infection.

Key words: Canine distemper virus, Conjunctival swab, Lymphopenia

Canine distemper (CD) is an acute multisystemic disease. The morbidity and mortality ranges from 25% to 70% and 0% to 100%, respectively (Dungworth, 1993). The disease is most commonly observed in puppies when they have lost their maternal antibodies (Kim *et al.,* 2006). The prevalence of canine distemper virus (CDV) based on clinical cases has been reported to be 11% in Pakistan (Jafri and Rabbani, 1999). The high cost of vaccine, vaccine failure, and lack of awareness about importance of vaccine, countrywide pet registration policy and laboratory based disease diagnostic facilities are some of the factors which result in perpetuation of the CDV in Pakistan.

Upon exposure to aerosol or other secretions containing virulent CDV, the virus localize in bronchial lymph nodes and tonsils. It appears in blood mononuclear cells two to three days post infection. Within the first week of infection, the virus replicates in multiple lymphoid tissues leading to immunodeficiency which may eventually cause death of the dog (Dungworth, 1993). Widespread viral replication commonly causes clinical signs of pyrexia and severe leucopenia, leading to appearance of sub-clinical, respiratory or/and digestive, nervous and cutaneous lesions (Gebriella *et al*., 2006).

It is rarely possible to diagnose the CDV infection in mild or sub-clinical cases (Kim *et al.*, 2006). Clinical signs similar to other respiratory and enteric diseases of dogs further complicate the diagnosis (Gebriella *et al.,* 2006). However, in

severe cases, conjunctivitis, pustular ocular and nasal discharge, illness of three weeks or longer, fever and diarrhea help in suggesting presumptive clinical diagnosis of CD. This requires laboratory based confirmation.

Owing to distribution of the virus through out body tissues and fluids, various samples like blood, serum, nasal and conjunctival swab, cerebrospinal fluid, urine, tracheal washing and skin biopsy can be used for etiological diagnosis of CDV (Appel, 1969; Frisk *et al.,* 1999; Kim *et al.,* 2006). However, the clinical utility of tissue and fluid samples varies depending upon days of infection (Kim *et al.,* 2006). Among these, conjunctival swab is the specimen from which viral shedding start first and last longer during the course of disease (Kim *et al.,* 2006). Various laboratory procedures used for detection of CDV antigen are laborious, time consuming and of limited value (Frisk *et al.,* 1999). Recently, reverse transcription polymerase chain reaction (RT-PCR) has been considered fairly reliable diagnostic test for CDV infection (Frisk *et al.,* 1999; Kim *et al.,* 2006).

After exposure of virus in dog, as lymphopenia is immediate systemic effect and it starts shedding from conjunctival swabs within one to two days post infection. The present study was undertaken to determine relationship of antigen detection and clinical diagnosis in terms of lymphopenia. The study describes the detection of CDV nucleoprotein gene from clinically distemper suspected dogs along with lymphopenia by RT-PCR using conjunctival swabs. It also reveals post infection recovery status of dogs in terms of lymphocyte count.

Materials and methods

Collection of samples

During 2007, blood and conjunctival swabs were collected from each of 45 dogs, clinically suspected for CD on the basis of respiratory signs such as purulent occulonasal discharge, conjunctivitis, bronchitis, and bronchopneumonia. A brief history regarding age, sex, body temperature, vaccination record, clinical findings and secondary bacterial, protozoan or worm infestations was taken. Five milliliters of peripheral whole blood from cephalic vein was collected into a vaccutainer (Venoject$^{(R)}$, Belgium). The conjunctival swabs were taken by swabbing sterilized cotton sticks in microfuge tube using 0.5 ml of sterilized 0.9% saline solution. Anti-coagulant added whole blood and conjunctival swabs were also taken from each of five non vaccinated healthy dogs as negative controls for lymphocytes and RT-PCR. The commercially available multivalent vaccine (Hexadog, Merial, France) served as positive control for RT-PCR. The conjunctival swabs were stored at -20°C till further use. All the samples were collected from Pet Centre of University of Veterinary and Animal Sciences (UVAS) and private clinics in Lahore.

Lymphocyte count

Immediately after collection, the anti-coagulant added whole blood of all the suspected and healthy dogs was subjected to lymphocyte count using hematological analyzer (Abacus, Austria). The lymphocyte count was repeated thrice at a regular interval of 10 days to observe the pattern of further decrease or increase in number of lymphocytes using single factor ANOVA test.

Reverse transcriptase polymerase chain reaction

Canine distemper viral RNA was extracted from conjunctival swab (200µl) using "One Step RNA Reagent" (Bio- Basic Inc., Canada). The extracted RNA was dissolved in 20µl DEPC water (Diethyl pyrocarbonate) and used for reverse transcription by RevertAid First Strand cDNA Synthesis Kit (Fermentas, EU). Briefly, 1µl of Random Hexamers (0.2ug/µl) and 4µl of DEPC water were added to the extracted RNA in equal volume. Following addition of 1µl of Ribolock (20U/ul), 4µl of 5X Reaction Buffer, 1µl of 10mM dNTPs and incubation at 25°C for 5 minutes, 1µl of M – MuLV Reverse Transcriptase (200U/µl) was added and thermocycler (Thermo Electron, Finland) was set up as one cycle each as follows: incubation at 25°C for 10 minutes, 42°C for 1 hour and 70°C for 10 minutes. The synthesized cDNA was chilled by placing microfuge tubes in ice and stored at -20°C until further use.

The complete nucleoprotein coding genome region of CDV RNA was amplified using the primer pair described by Yoshida *et al.* (1998) with minor

modification. Oligonucleotide (e-oligos, GeneLink, USA) primers used were a 21mer (sense) (5'GTGTCAGAAATAGCATCCAAG3', nt 1287-1307) and 26mer (antisense) (5' GTGGGATCCAGACTGGTCTTGAATAT3', nt 1705-1680). The PCR amplification was performed in a total volume of 25 µl by adding 5µl of extracted cDNA, 2 µl (10 pmole/µl) of each of primer pair, 12 µl of PCR master mix (Eppendorf, Germany) and 6 µl of DEPC water. The thermocycler was set up as follows: an initial denaturation step at 94 °C for 5 min followed by 30 cycles of 30 seconds each at 94°C, 59°C and 72°C. Amplification was terminated by a final extension at 72°C for 10 min. The amplicons were resolved on 1.5% agarose gel containing 0.5 µg/ml of ethidium bromide after electrophoresis at 3.5 volt per centimeter. The bands were observed under UV light and photographic records were made.

Results and discussion

The suspected dogs, 75.5% male and 24.4% female, were of different ages ranging from three months to 42 months. Nearly all the dogs (91.1%) had body temperature above 103°F. The vaccinated and non-vaccinated dogs were 48.8% and 28.8%, whereas, 22.2% of dogs were having no information about vaccination. The total lymphocyte count of five normal dogs ranged from 1×10^3 to 4.24×10^3 μl^{-1}. In suspected cases, the count was insignificant (p=0.203) with respect to distemper positive (22. 2%) and negative dogs (78.8%) at the first examination. It was in the range of 0.21×10^3 to 2.33×10^3 μl^{-1} in distemper negative and 0.05×10^3 to 1.96×10^3 μl^{-1} in distemper positive dogs. Although, the variable but overall declining pattern of lymphocyte count can be used to suggest presumptive clinical diagnosis of CDV infection but it is not compulsory that all lymphopenic dogs are distemper positive. Lymphopenia is characteristic feature of many other viral infections in the acute phase (Oldstone, 1996) such as measles (Okada *et al.,* 2000), influenza (Tumpey *et al*., 2000), severe acute respiratory syndrome (SARS) (Peiris *et al.,* 2003), dengue virus infection (Fadilah *et al.,* 1999), cytomegalovirus infection (Einsele *et al.,* 1993). In many cases a correlation of disease severity and degree of lymphocyte depletion was observed. Based on this, the determination of lymphocyte numbers can have a prognostic value for the development of the disease. The mechanism of this lymphocyte depletion in the acute phase of virus infections is unknown. A non-specific over-activation of the innate immune system to a rapidly replicating virus in a naïve host might be involved (Schobesberger *et al.,* 2004).

The difference in lymphocyte count at each interval of 10 days was significant ($p = 0.0056$ or $p < 0.05$) in distemper positive dogs showing rapid recovery. It was in the range of 0.22×10^3 to 1.82×10^3, 0.59×10^3 to 2.3×10^3 $\times10^3$ and 1.5×10^3 to 3.2×10^3 μl^{-1}, on second, third and fourth examination, respectively (Fig. 1). The increasing pattern of lymphocyte was not similar in all the dogs that may be due to different age, immune status, remedial measures, care and patient management.

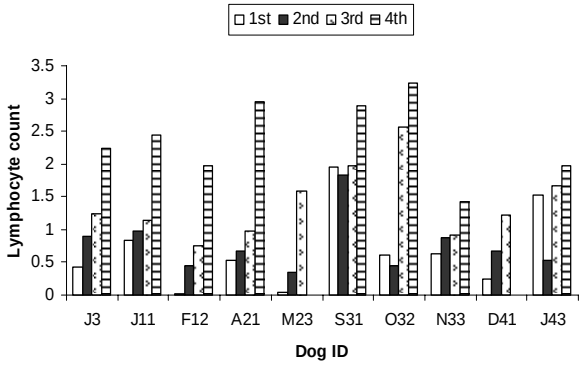

Fig. 1. Recovery pattern of lymphocyte (10^3/ul) in distemper positive dogs at an interval of ten days.

Keeping in view the pattern of lymphocyte increase in distemper positive dogs, it seemed that after infection it takes several weeks to regain the lymphocyte number. Moreover, the response of individual to various infections varies from each other. It depends upon strain of virus involved, feeding, medication, environmental and management practices. Schobesberger *et al.* (2004) revealed that CDV-infection induced severe decrease in lymphocytes 3 days post infection and the declining pattern remains till 24 days post infection with some dogs showing a transient recovery. Nearly all the lymphopenic distemper

positive dogs (73.3%) were having secondary infections such as *E. coli, Salmonella* spp., *Toxocara canis, Ancylostoma caninum, Uncinaria* spp. and *Babesia* spp. It may be due to CDV induced immunosuppression caused by direct or indirect virus-mediated T and B cell cytolysis and dysfunction of $CD4^+$ T cells (Schobesberger *et al.,* 2004). It has been shown that peripheral blood cytokine production is minimized either due to depletion of cytokine producing cells or by virus-induced down regulation of cytokine production (Iwatsuki *et al.,* 1995, Gröne *et al.,* 1998) consequently resulting in a reversible massive cytolytic (Zurbriggen and Vandevelde, 1994) virus-induced immune suppression, which can persist for several weeks, rendering animals highly susceptible to opportunistic infections (Krakowka *et al.,* 1980).

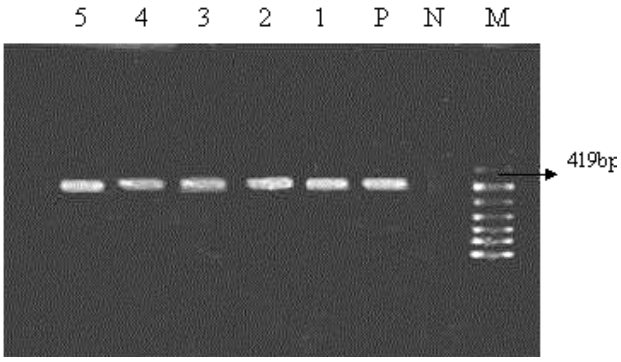

Fig. 2. Gel electrophoresis of RT-PCR product from CDV infected dogs using conjunctival swabs. Lane M, molecular size marker GeneRuler 100bp DNA, Lane N, negative control; Lane P, positive control (Hexadog vaccine); Lanes 1, 2, 3, 4, 5, nucleoprotein gene amplification from dogs positive to CDV infection.

Although all the suspected dogs were lymphopenic and exhibiting digestive system involvement (75.5%) like vomiting and diarrhea, only 10 (22.2%) were found positive to CDV infection (Fig. 2). None of suspected dogs showed any nervous symptoms. This supports the statement of clinical finding similarity with other respiratory and enteric diseases of dogs like canine hepatitis, canine parvovirus, corona virus, herpes virus, parainfluenza and leptospirosis. Moreover, the sensitivity of the RT-PCR depends upon selection of primers used for amplification of target position in viral genome (Frisk *et al*., 1999), nucleic acid extraction method and nature of sample (Saito *et al.,* 2005). For conjunctival swab, it has been reported that virus appears early and eliminate later from conjunctiva than any other fluid sample (Kim *et al.*, 2006). During the early course of disease, conjunctiva and eye get infected at the time of generalized viremia (Appel and Summers, 1995). Additionally, CDV replicating in conjunctival sac or orbital cavity does not subject to immunity avoiding rapid elimination (Kim *et al.,* 2006). Moreover, there is a widespread ocular involvement in CDV infected conjunctival epithelium, corneal epithelium and iris in dogs (Kim *et al*., 2006).

Two dogs, each male (M23) and female (D41), died. Male showed nervous signs like muscle twitching, ataxia, seizures and in-coordination in body movement, while female died for unknown reasons. It was assumed that nervous system perhaps already invaded by the virus before any supportive medical care was given. The virus can invade the central nervous system (CNS) approximately 10 days post infection (Greene and Appel, 1998) resulting in a demyelinating disease.

The present study confirms the usefulness of RT-PCR as a sensitive, diagnostic tool for ante-mortem detection of canine distemper virus (Frisk *et al*., 1999).The results demonstrate that CDV-induced lymphopenia is an early event after infection and the degree of lymphocyte depletion correlates with the severity of disease. However, all lymphopenic dogs may not be distemper positive. It may be suggestive of presumptive clinical diagnosis of distemper and requires necessary laboratory test for the presence of antigen. Furthermore, CDV infection even in vaccinated dogs (2 of 10) requires phylogenetic analysis of prevalent and vaccinal strains.

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(Received 9 October 2008, revised 2 June 2009)