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Case Report

Concurrent helminthosis engendered gastroenteritis in a leopard *Panthera pardus*

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Summary

The necropsy of a leopard (*Panthera pardus*), succumbed to a chronic ailment exhibited a mixed parasitic gastroenteritis. Gross internal examination of carcass revealed the presence of round and tapeworms in the stomach and intestines with diffuse catarrhal and hemorrhagic gastroenteritis. The detailed examination of the intestinal content revealed the presence of *Toxocara canis* and *Spirometra* species eggs. Also, the gross morphological investigation of round and tapeworms approved the presence of both species. Histo-pathological examination showed sloughing of intestinal epithelium, hemorrhages, and ulcerative areas with the infiltration of polymorphonuclear cells admixed with mononuclear cells. Lungs revealed the accumulation of eosinophilic edematous fluid in the alveolar spaces along with inflammatory cells. These parasites are pathogenic to precious wild felids and often pose a threat of zoonotic transmission due to spill-over infections. The present case study is an attempt to put on record a case of parasitic gastroenteritis in a captive leopard.
Keywords: Leopard; parasitic diseases; *Spirometra* species; *Toxocara canis*; zoonosis

Introduction

Out of 250 wild carnivore species distributed throughout the world, 60 species are recorded from India (Acharjyo, 2004). The main purpose of keeping the wild carnivores in captive state in zoological/ wildlife parks is associated with education, exhibition and gene conservation (Khatun *et al*., 2014). In natural habitat, wild animals sustain in a balanced system with the parasites due to some natural resistance (Thawait *et al*., 2014). Whereas, captivity leads to stress further ensuing depressed immune state of the wild animals, eventually rendering them vulnerable to various infectious diseases including parasitic, bacterial and viral (Moudgil *et al*., 2013). Helminth parasites, if present in heavy numbers are often capable to cause mortality and morbidity in wild captive animals (Acharjyo, 2004). Also, certain helminths infecting the wild animals especially carnivores hold significant zoonotic potential. Wild animals are quite potent to spill over the infection to other animals, humans and birds as well (Otranto *et al*., 2015). The transmission of parasites from wild animals to domestic animals and human beings is mostly a result of constricting the boundaries meant for the wild animals. So, domestic animals and human population could easily pick the infection at the close vicinity of national parks, wildlife sanctuaries and zoological/ wildlife parks etc. and thereby wild animals can act as a potent mode of disease transmission (Singh *et al*., 2017).

Geo-helminths could be considered as most potent parasitic invaders of wild animals in captivity rather than bio-helminths as they get optimum conditions for development and can quickly lead to re-infection (Panayotova-Pencheva, 2013). Toxocariasis in wild felids is an important parasitic disease which can affect any age group (Despommier, 2003) and leads to neurotoxocarosis in human beings which often act as precipitation factor for the de-

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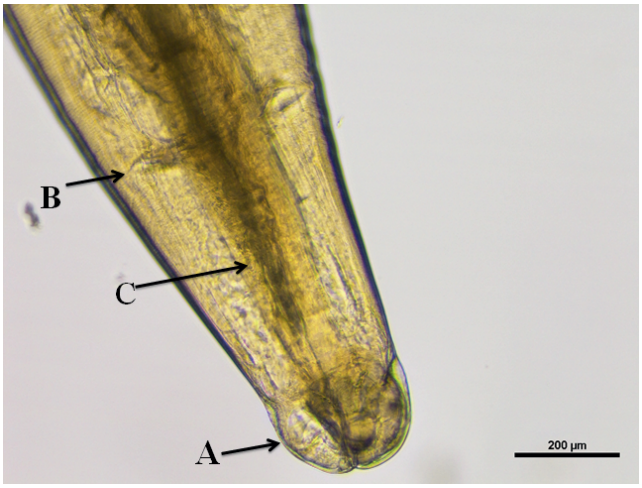

Fig.1. Photomicrograph of anterior end of *Toxocara canis* showing triradiate lips (A), cervical alae (B) and oesophagus (C) (10×)

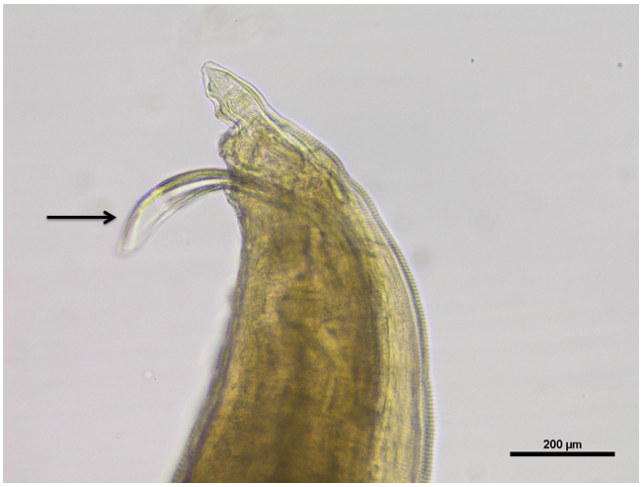

Fig. 2. Photomicrograph of posterior end of male *T. canis* showing sub equal spicules (arrow) (10×)

velopment of epilepsy (Xinou *et al*., 2003). *Toxocara* species has earlier been reported sporadically in leopards of various zoological parks of India during coprological and necropsy investigations (Nashirudullah & Chakraborty, 2001; Singh *et al*., 2006; Mahali *et al*., 2010; Thawait *et al*., 2014). Similarly, *Spirometra* species was also recovered during a necropsy of a leopard in a forest in Shimoga, Karnataka (Ananda *et al*., 2011). The highest incidence of spirometrosis in wild felids is associated with consumption of intermediate hosts including tadpoles, snakes, birds and alligators (Arjun *et al*., 2017). The parasitic load in hosts can lead to low fertility, decline in body weight, heavy morbidity and mortality. Thus, the present study is an attempt to highlight the presence and pathological aftermaths of concurrent helminthosis in precious wild felid *Panthera pardus* in India.

Material and Methods

A captive male leopard, approximately 19 years old weighing 35 kg was maintained at Gopalpur Zoological Park, Himachal Pradesh. A thorough clinical examination of the leopard showed that the animal was debilitated, anorectic, anemic and showed respiratory distress. The blood sample was collected for hemato-biochemical examination from tail vein of the animal after tranquilization with injection Xylazine and Ketamine @ 1mg/kg body weight and 5 mg/kg body weight respectively through intramuscular route.

The hematological parameters considered for analysis included hemoglobin (Hb), packed cell volume (PCV), total erythrocyte count (TEC), total leukocyte count (TLC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC). The serum biochem-

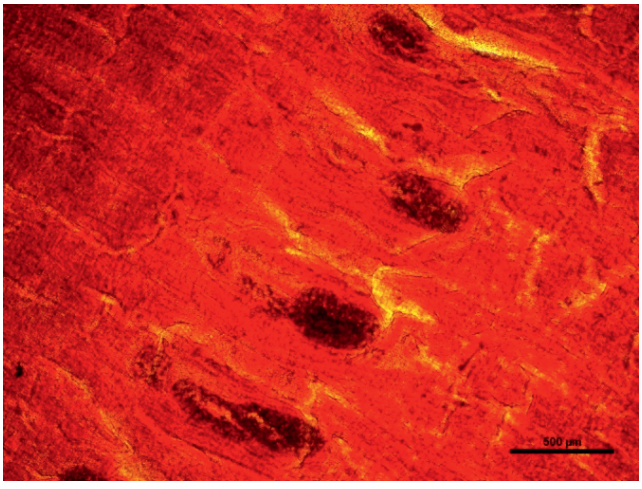

Fig. 3. Photomicrograph of gravid proglottid of *Spirometra* species exhibiting spiralled uterus (4×)

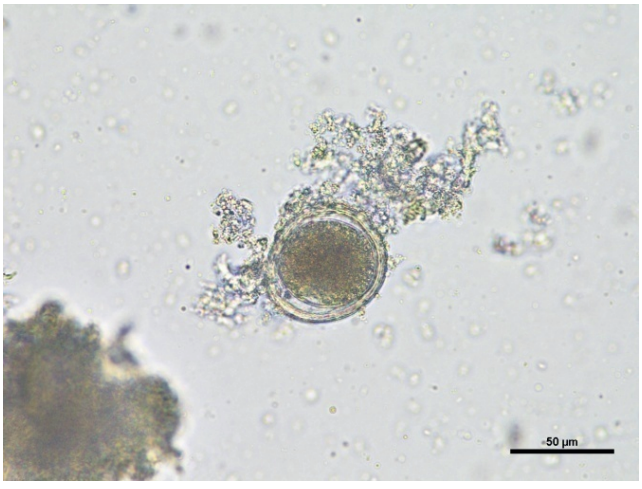

Fig. 4. Photomicrograph of unembryonated *Toxocara canis* egg (40×)

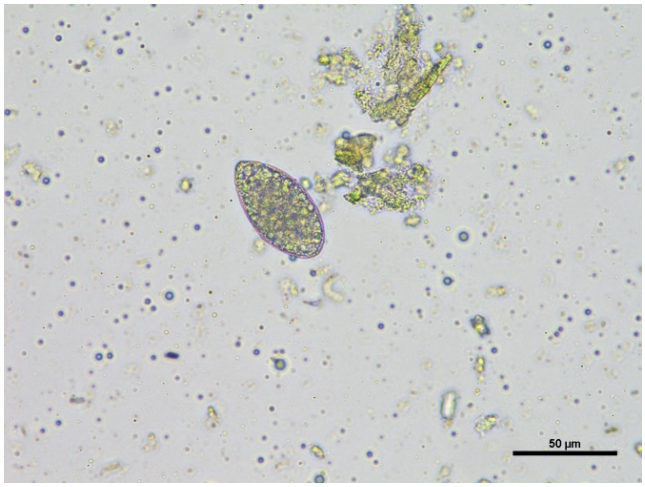

Fig. 5. Photomicrograph of *Spirometra* species egg with pointed ends (40×)

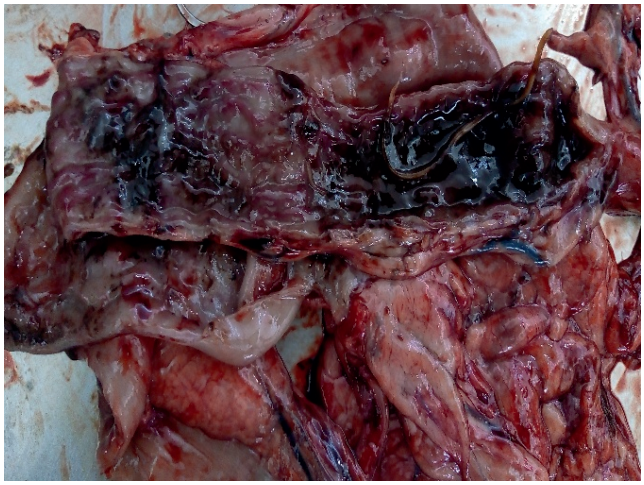

Fig. 6. Intestinal mucosa showing presence of round worms along with catarrhal to haemorrhagic exudate

ical parameters included alanine aminotransferase (ALT), aspartate aminotransferase (AST), total protein, blood urea nitrogen (BUN) and creatinine. The animal was treated symptomatically but not responded to the treatment and succumbed to the infection.

The leopard was presented to the Department of Veterinary Pathology, DGCN COVAS CSKHPKV, Palampur for necropsy examination. A thorough external and internal examination of the animal was performed for the presence of any injury, ectoparasites and other associated pathological alteration. The gross lesions were recorded after detailed necropsy examination. On examination of gastrointestinal system the presence of round and tapeworms were evident. The parasites were removed gently, washed in normal saline and sent to the Department of Veterinary Parasitology for species identification. The nematodes were cleared in lactophenol in order to assess the morphological and morphometric characteristics of males and females (Zajac, 1994). The length and width of roundworms was measured in millimetres (mm) and was expressed considering mean ± standard deviation. The cestodes were stained with Borax carmine stain as per the method of Urquhart *et al*. (1996). The smears prepared from the intestinal contents revealed the presence of eggs. The morphometric analysis targeting the size of the eggs was performed as per Kazacos & Turek (1983). The length and breadth of the eggs was expressed as mean ± standard deviation in micrometers. The organs showing pathological changes were collected and fixed in 10% neutral buffered formalin for histopathological examination. The fixed tissues were embedded in paraffin, sectioned at 4 – 5 microns and stained with Haematoxylin and Eosin as per the protocol given by Luna & Lee (1968).

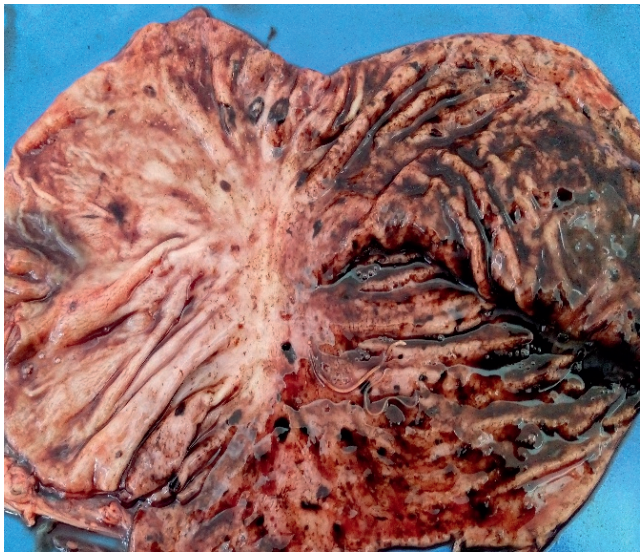

Fig. 7. Thickened gastric mucosa along with multifocal erosive and ulcerative areas

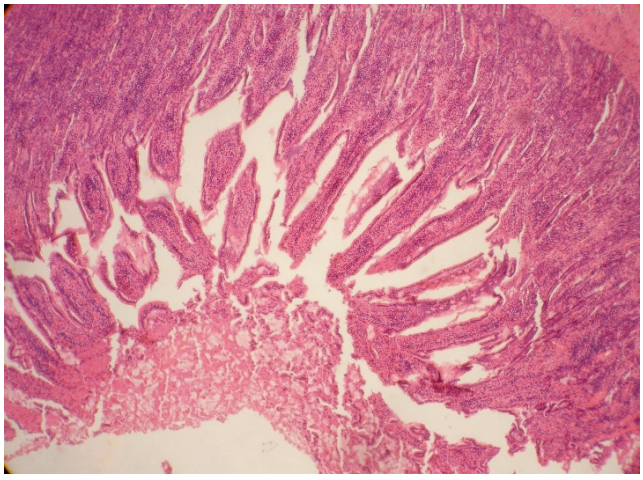

Fig. 8. Photomicrograph showed denuded intestinal epithelium, homogenous pink catarrhal exudates and mononuclear cells admixed with few neutrophils (40×)

Ethical Approval and/or Informed Consent

No experimental animals were used in this study.

Results and Discussion

In the past, only sporadic cases of parasitic infections during scatological and necropsy investigations have been reported in wild felids from different parts of India and there is a woeful paucity of comprehensive studies involving pathological upshots. Parasitic diseases reported in wild animals mainly include infections due to gastrointestinal parasites (Singh *et al*., 2006) and haemoprotozoans.

In the present case study, the body temperature of the leopard was observed 96.6° F. The respiration rate and heart rate were 16/min and 92 beats/ min, respectively. The Hb and PCV concentration observed in leopard's blood was 7.7 g/dl and 25% respectively, which were lower than the normal reference values as given by Sabapara *et al.* (2008). WBCs count reported was 13.3×10^9/L (higher than normal), whereas the total erythrocyte count (TEC) was 5.17×10^{12}/L (normal) compared with the reference values given by Sabapara *et al*. (2008). The platelet count obtained was 513×10^9/L, which was higher than the reference values given by Salakij *et al*. (2009). The results of erythrocytic indices were MCV-48.4 fl, MCH-14.8 pg, and MCHC-30.8 g/dl. On biochemical analysis of serum sample, no statistically significant alterations in the values of glucose (101 mg %), protein (6.2 g/dl) and creatinine (1.9 mg) were observed, whereas the values of blood urea nitrogen (100.4 mg %) was elevated from the normal reference values. The values of liver specific enzymes ALT (51 IU/L), ALP (18 IU/L), AST (30 IU/L) and GGT (5 IU/L) were almost normal as compared with the reference values given by Singh (2005). The variations in the values of hemato-biochemical parameters, reduced bone density on radiographic examination, enlarged kidneys and shrunken liver lobes might be an outcome related to the effect of age.

The results obtained from parasitological investigation showed that the leopard was infested with intestinal *Toxocara canis* and *Spirometra* spp. The adult worms of *Toxocara canis* were identified on the basis of gross morphological examination. Grossly, the size of the male worms (n=6) were measured 67.14 ± 4.26 mm (61.6 – 71.8 mm) × 1.28 ± 0.08 mm (1.2 – 1.4 mm) (length × width), whereas, female parasites (n=6) were measured 86.88 ± 3.92 mm (81.2 – 90.8 mm) × 1.72 ± 0.11 mm (1.6 – 1.8mm) (length × width), respectively. The distinctive morphological features included three developed triradiate lips (one dorsal and two subventral) with cervical alae and filariform oesophagus (Fig. 1) in adult parasites of *T. canis*. The caudal end of the male parasites possessed two subequal spicules with large one 2.16 ± 0.29 mm (1.8 – 2.4 mm) and smaller one 1.12 ± 0.08 mm (1 – 1.2 mm) (Fig. 2), respectively; whereas, female posterior end had a tapering blunt tail. The findings were in concordance with the observations of Radwan *et al*. (2009), who ascertained the prevalence of *Toxocara* species in wild animal population based on morphological studies. *Toxocara* species parasites had also been earlier reported from wild felids (including leopards) from different zoological gardens of India (Moudgil *et al*. 2015) and heavy burdens of these parasites had also been incriminated for mortalities of the infested captive wild animals. In case of cestode parasites, mature proglottids were broader than long and in gravid proglottids, numerous ovoid eggs with pointed ends were observed in the spiralled uterus (Fig. 3). The cestodes were identified as a species of *Spirometra* described by Yamaguti (1959). *Spirometra* species had also been earlier reported from wild felids kept in captivity from different parts of India (Moudgil *et al*. 2015). The intestinal content smears revealed the presence of two types of eggs; first, subglobular ascarid eggs with thick, finely pitted shell and round embryonic mass and second, unembryonated ovoid yellowish-brown eggs with pointed ends. The ascarid egg size (n=10) was 89.9 ± 3.07 µm (86.2 – 94.8 µm) × 75.3 ± 1.76 µm (72.8 – 78.2 µm) (length × breadth) (Fig. 4); whereas ovoid cestode eggs measured 59.9 ± 2.19 (56.8 – 62.4 µm) × 35.1 ± 0.95 µm (33.6 – 36.2 µm) (Fig. 5). The morphometric observations of the eggs of ascarids and cestodes substantiating to be of *Toxocara canis* and *Spirometra* species were in concordance with morphometric values reported by Brooker & Bundy (2014); Soulsby (1982); Muller-Graf (1995); Zajac & Convoy (2012), respectively.

The infection of wild felids with ascarids could be attributed to the housing conditions, especially the floors. In case of soil or wooden floors, the fecal material of the animals either remains clogged or attached to the surface (Moudgil *et al.*, 2017). The conditions lead to survivability of the eggs for a longer time even in harsh environmental conditions, eventually leading to transmission of infection to susceptible animals (Bowman, 1999; Singh *et al*., 2006). The presence of direct life cycle of the ascarids and short generation period for the infective stages could be considered as a reason for persistence of ascarid infection in well sanitized cages (Bowman, 1999; Moudgil *et al*., 2014). On the other hand, in case of *Spirometra* species a wide variety of animals and birds act as second intermediate hosts containing the plerocercoid stages (Soulsby, 1982) and consumption of any such intermediate host could have resulted in infection to the leopard.

The detailed necropsy examination of the leopard showed edematous and diffusely congested lungs with scanty frothy exudates in trachea. A heavy load of adult creamish white round worms was present in the stomach. The mucosa of the stomach was thickened and showed multifocal areas of erosions and ulcerations (Fig. 7). On opening the intestine, off dull white colored round worms and tapeworms were seen. The mucosa of intestinal loops containing these worms showed catarrhal to hemorrhagic enteritis (Fig. 6).

Histopathologically, stomach revealed denudation of mucosa, indicating ulcerative lesions, areas of diffuse hemorrhages along with the infiltration of mononuclear cells (MNCs). The small intestine exhibited denudation and clubbing of villi, homogenous pink catarrhal exudates with area of hemorrhages, cellular debris and

inflammatory cells especially MNCs admixed with few neutrophils (Fig 8). Eosinophilic edematous fluid was accumulated in the alveolar spaces of the lungs along with the infiltration of PMNCs admixed with MNCs. The histological picture of spleen revealed the presence of depleted lymphoid follicles, which is a strong indication of immunosuppression.

The hematological and biochemical parameters are reliable indicators of the health status of the animals (Ohaeri & Eluwa, 2011) and may prove important in subclinical and clinical infections. The decline in hematological parameters like Hb and PCV, which is important for causing anemia and hypoproteinaemia in the leopard is unclear. However, some of the researchers believe that oxidative stress and lipid peroxidation mechanisms of tissue damage could be the most appropriate cause of anemia in ascarid infections (Salem *et al*., 2015).

Most of the wild animals are endangered and already at the verge of extinction due to habitat destruction (forest fire), loss of genetic diversity, improper feeding and hunting (Sengar *et al*., 2017). The pace of this mechanism is further exacerbated by many diseases caused by a variety of pathogenic agents including parasites. The health status of captive wild felines is often influenced by various factors including age, feeding, environment, sanitation and irregular deworming which increases the risk of parasitism. The parasites (nematodes and cestodes) observed in the present study apart from inflicting serious health hazards and even mortalities of the animals also hold significant zoonotic potential. The present necropsy study suggests the necessity of regular deworming in captive wild animals and emphasizes on rising trends of parasitic infestations which are often overlooked.

Conflict of Interest

The authors declare that they have no conflict of interest regarding the publication.

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References

Acharjyo, L.N. (2004): Helminthiasis in captive wild carnivores and its control in India. Review. *Zoos' Print J.,* 19(7): 1540 – 1543

Ananda, K.J., Rani, B.K., Ramesha, K., Swethashree, M.J., Ranjitha, V.H., Nagaraj, K.H., Prasad, R.V. (2011): Tapeworm infection in a leopard: A case report. In *Compendium of 21st National Congress of Veterinary Parasitology*, *Mumbai*, *January 5–7, 2011*. Mumbai, India pp. 125

Arjun, M.S., Ravindran, R., Zachariah, A., Kumar, M.A., Varghese, A., Deepa, C.K., Chandy, G. (2017): Gastrointgestinal Parasites of Tigers (*Panthera tigris tigris*) in Wayanad Wildlife Sanctuary, Kerala, India. *Int. J. Curr. Microbiol. App. Sci*. 6(8): 2502 – 2509. DOI: 10.20546/ijcmas.2017.608.296

Bowman, D.D. (1999): Georgi's Parasitology for Veterinarians. 7th Edition, WB Saunders, Philadelphia

Brooker, S.J., Dap, B. (2014): Soil transmitted helmiths (Geohelminths). In: Farrar, J., Hotez, P.J., Junghanss, T., Kang, G., Lalloo, D., White, N.J. (Eds.) *Manson's Tropical Diseases*, 23rd Edition, Saunders Ltd

Despommier, D. (2003): Toxocariasis: clinical aspects, epidemiology, medical ecology and molecular aspects. *Clin. Microbiol. Rev*., 16 (2): 265 – 272. DOI: 10.1128/ CMR.16.2.265-272.2003

Kazacos, K. R., Turek, J.J. (1983): Scanning electron microscopy of the eggs of *Baylisascaris procyonis*, *Baylisascaris transfuga* and *Parascaris equorum* and their comparison with *Toxocara canis*and *Ascarissuum. P. Helm. Soc. Washington,* 50: 36 – 42

Khatun, M.M., Begum, N., Mamun, M.A.A., Mondal, M.M.H., Shakif-Ul-Azam, M. (2014): Coprological study of gastrointestinal parasites of captive animals of Rangpur recreational garden and zoo in Bangladesh. *JoTT.,* 6(8): 6142–6147. DOI: 10.11609/JoTT.o3093.6142-7

Luna, H.T., Lee, G. (1968): Manual of histopathological staining methods of the Armed Forces Institute of Pathology, 3rd edn. Plackiston Division McGraw Hill Book Co., London

Mahali, A.K., Panda, D.N., Panda, M.R., Mohanty, B.N., Sahoo, B. (2010): Incidence and seasonal variation of gastro-intestinal parasitic infections in captive carnivores in Nandankanan Zoological Park, Orissa. *J. Vet. Parasitol.,* 24: 111 – 115

Moudgil, A.D., Singla, L.D. (2013): Role of neglected wildlife disease ecology in emergence and resurgence of parasitic diseases. *Trends Parasitol. Res.,* 2: 18 – 23

Moudgil, A.D., Singla, L.D., Pallavi. (2015). Parasitosis in wild felids of India: An overview. *JoTT*., 7(10): 7641 – 7648. DOI: 10.11609/JoTT.o4236.7641-8

Moudgil, A.D., Singla, L.D., Singh, M.P. (2014): *In vitro* study on targeting developmental embryonation pattern of eggs of ascarid species of wild animals. *Appl. Biol. Res*., 16: 237 – 241. DOI: 10.5958/0974-4517.2014.00016.0

Muller-Graf, C.D.M. (1995): A coprological survey of intestinal parasites of wild lions (*Panthera leo*) in the Serengeti and Ngorongoro crater, Tanzania, East Africa. *J. Parasitol*., 81: 812 – 814

Nashiruddullah, N., Chakraborty, A. (2001): Parasites of captive wild carnivores of Assam State Zoo. *Intas Polivet*, 2: 173 – 181

Ohaeri, C.C., Eluwa, M.C. (2011): Abnormal biochemical and haematological indices in trypanosomiasis as a threat to herd production. *Vet. Parasitol*. 177: 199–202. DOI: 10.1016/j.vetpar.2011.02.002

Otrantoa, D., Cantacessi, C., Pfeffer, M., Dantas-Torres, F., Brianti, E., Deplazes, P., Genchi, C., Guberti, V., Capelli, G. (2015): The role of wild canids and felids in spreading parasites to dogs and cats in Europe Part I: Protozoa and tick-borne agents. *Vet. Parasitol.,* 213: 12–23. DOI: 10.1016/j.vetpar.2015.04.022

Panayotova-Pancheva, M.S. (2013): Parasites in captive animals: a review of studies in some European zoos. *Zool. Gart*., 82:60 – 71.
Radwan, N.A., Khalil, A.I., El-Mahi, R.A. (2009): Morphology and occurrence of species of *Toxocara* in wild mammal populations from Egypt. *Comp. Parasitol*.,76(2): 273 – 282. DOI: 10.1654/4367.1
Sabapara, R.H., Jani, R.G., Bhuva, C.N. (2008): Hematological reference intervals for Indian leopards (*Panthera pardus*). *Vet. World*., 1(6):173 – 174
Salakij, C., Salakij, J., Narkkong, N.A., Prihirunkit, K., Kamolnorranath, S., Apibal, S. (2009): Hematology, cytochemical and ultrastructural characterstics of blood cells in leopard (*Panthera pardus*). *Comp. Clin. Path*., 18(2): 153 – 161. DOI: 10.1007/s00580-008-0774-7
Salem, N.Y., Yehia, S.G., El-Sheriff, M.A. (2015): Haemato-biochemical and mineral status in dogs with intermittent diarrhea and unthriftiness. *Res. J. Vet. Pract*., 3(4): 89 – 92. DOI: http://dx.doi.org/10.14737/journal.rjvp/2015/3.4.89.92
Sengar, A., Shrivastav, A. B., Singh, K. P., Rokde, A. (2017): Non-invasive assessment of gastrointestinal parasites infection in free ranging wild herbivores and adjoining livestock of Panna Tiger Reserve, Madhya Pradesh, India. *Vet. World*., 10(7): 748 – 751. DOI: 10.14202/vetworld.2017.748-751
Singh, A.B. (2005): Tigers blood: Haematological and biochemical studies. *Intech*., 229 – 241
Singh, K.P., Shrivastav, A.B., Gupta, S.K., Agrawal, S., Singh, K. (2017): Occurrence of *Gnathostoma spinigerum* in tigers and leopards. *J. Parasit. Dis. Diag.n Ther*., 2(2): 1 – 3
Singh, P., Gupta, M.P., Singla, L.D., Singh, N., Sharma, D.R. (2006): Prevalence and chemotherapy of gastrointestinal helminthic infections in wild carnivores of Mahendra Choudhury Zoolgoical Park, Punjab. *J. Vet. Parasitol.,* 20: 17 – 23
Soulsby, E.J.L. (1982): *Helminths, Arthropods and Protozoa of Domesticated Animals*. 7th Edition, London, UK: Bailliere Tindall.
Thawait, V.K., Maiti, S.K., Dixit, A.K. (2014): Prevalence of gastrointestinal parasites in captive wild animals of Nandanvan zoo, Raipur, Chhattisgarh. *Vet. World.,* 7: 448 – 451. DOI: 10.14202/vetworld.2014.448-451
Urquhart, G.M., Armour, J., Duncan, J.L., Jennings, F.W. (1996): *Veterinary Parasitology*. 2nd Edition, Oxford, UK: Blackwell Science Ltd
Xinou, E., Lefkopoulos, A., Gelagoti, M., Drevelegas, A., Diakou, A., Milonas, I., Dimitriadis, A.S. (2003): CT and MR imaging findings in cerebral toxocaral disease. *Am. J. Neuroradiol*., 24: 714–718
Yamaguti, S. (1959): *Systema Helminthum*. Inter Science New York, NY, 338 – 361 pp.
Zajac, A.M. (1994): Faecal examination in the diagnosis of parasitism. In: Sloss, M.W., Kemp, R.L., Zajac, A.M. (Eds) *Veterinary Clinical Parasitology*. AMES, IA: Iowa State University Press
Zajac, A.M., Convoy, G.A. (2012): *Veterinary Clinical Parasitology.* 8th Edition, Ames, Iowa: Wiley Blackwell, pp. 368