Indian Journal of Animal Sciences **80** (10): 961–64, October 2010

Mortality due to infectious canine hepatitis in sloth bears (*Melursus ursinus*) in captivity

R SINGH[1], K P SINGH[2], A K SHARMA[3], S NANDI[4] and R S CHAUHAN[5]

Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh 243122 India

Received: 10 January 2009; Accepted: 8 June 2010

ABSTRACT

Sloth bears (3 males and 2 female) of a rescue facility in Agra district of Uttar Pradesh, which died acutely due to emaciation, anorexia, non-specific nervous signs, and widely spread petechial hemorrhages/congestion on serosal surfaces, were subjected to histopathological examination. The clinical blood picture in one of the cases showed leucocytosis (15.9×10^3/µl) associated with marked absolute neutrophilia. The hemoglobin (15.4 g/dl), packed cell volume (43.4%), ESR (5 mm/h) and the platelet counts (290×10^3/µl) were within the normal range. The derived MCV (64 FL), MCH (22.7 pg), and MCHC (35.5 g/dl) values were not much changed. Serum ALT and AST were significantly high: 136 U/L and 1159 U/L, respectively, reflecting hepatic injury, however, total bilirubin in serum remained unaffected. In addition, total protein (3.9 g/dl: albumin 1.94 g/dl, globulin 1.96 g/dl) and alkaline phosphates (306 U/L) values deviated from the reference range showing damages to the liver and other body organs. The urea and creatinine levels were high, 79 mg/dl and 1.7 mg/dl, respectively, indicating renal damage. The histopathological changes in different organs showed necrosis of parenchymatous organs together with edema and hemorrhages, presence of intranuclear inclusions of Cowdry type A in hepatocytes, renal tubules, lymphoid tissues, and in wide spread vascular endothelial cells of the organs, including brain. The inclusions were sparingly few in liver, lung, stomach and intestine compared with spleen, brain and kidneys, where these were in abundance. In pancreas, these were almost not traceable. Interestingly, the intertubular vascular endothelial cells had plenty of inclusions.

Key words: Bear, Captivity, Histopathology, Hematology, ICH, Serum chemistry, Sloth bear

Canine adenovirus-1, a dsDNA icosahedral virus of the family Adenoviridae, causes clinical disease in dogs, other canidae, Ursidae (bears) and in avian species. Serological evidences show widespread neutralizing antibodies in animals ubiquitously, indicating sub clinical form of infection (Zarnke *et al*. 1989, Gese *et al*. 1997, Zarnke *et al*. 2004), whereas sporadic outbreaks with some deaths in young dogs also occur in kennels. The virus through oronasal route initially multiply in tonsils and regional lymph nodes (Greene 2006). The disease has been well documented in wild animals (Olson *et al*. 1988, Whetstone *et al*. 1988). The surveys in free ranging coyotes (*Canis latrans*) indicated a prevalence of antibodies to ICH to the extent of 97%, 82%, 54%, and 33%, for adults, yearlings, old pups, and young pups, respectively (Gese *et al*. 1997) and in wolves (*Canis lupus*) in Alaska 54/57 (94.7%), 87/116 (81%) and 84% out of 1122 cases tested, respectively (Zarnke and Ballard 1982, Stephenson *et. al*. 1982, Zarnke *et al*. 2004) suggesting wide spread clinical infection of ICH virus. A few reports also described isolated clinical cases as well as in outbreak forms in wild life parks (Collins *et al*. 1983, Kritsepi *et al*. 1996). In India, no such reports are available in bears and, therefore, this article describes an outbreak of ICH in bears (*Melursus ursinus*) kept in a rescue facility, which was confirmed based on the presence of wide spread intranuclear inclusion bodies of Cowdry type A in different tissues, serum biochemistry and hematology.

Present address: [1,2]Principal Scientist (e mail: rajendra_singh5747@rediffmail.com, [2]e mail: karam.singh @rediffmail.com),[4]Senior Scientist (e mail: snandi03 @yahoo.com), CADRAD; [3]Principal Scientist, Pathology Division (email: anilsharma@ivri.up.nic.in)

[5]Director, Institute of Biotechnology, Pantnagar, Nainital (e mail: rs_chauhan123@rediffmail.com).

MATERIALS AND METHODS

Clinical history

During March 2007, mortality in 5 sloth bears (3 females and 2 males, aged 3–7 years) in Agra Rescue Facility of Uttar Pradesh was reported. The bears died acutely (within12–24 h) exhibiting non- specific nervous signs of depression and disorientation. The postmortem report showed diffuse meningeal congestion in all the cases. The carcasses were less in flesh. The serosal surfaces of liver, stomach, intestines,

mesenteries, lungs, heart, kidneys, urinary bladder, lymph node and spleen had congestion and petichae to ecchymotic hemorrhages. One of the bears, in addition, showed watery nasal discharge, congestion and foci of consolidation in lung. Based on the clinical signs, postmortem lesion and clinical findings, the practicing veterinarian tentatively diagnosed the outbreak as leptospirosis/rabies or canine distemper.

Histopatholgy: Tissues (liver, stomach, intestine, pancreas, lung, heart, kidneys, urinary bladder, spleen, lymph node and brain) in 10% formalin submitted for the laboratory diagnosis were cut into thin pieces, processed to obtain 4–5 µm thick paraffin sections. The sections were stained with routine hematoxylin and eosin stain. Shorr's method of staining was carried out to demonstrate the intranuclear inclusions for differentiation.

RESULTS AND DISCUSSION

Clinical findings: Clinically, all 5 ailing bears were anorectic, emaciated and showed nonspecific nervous signs, which later died acutely within 12–24 h. Blood analysis in one of the clinical cases showed leucocytosis (15.9×10^3/µl) associated with marked absolute neutrophilia (15 000/µl). The hemoglobin (15.4 g/dl), total erythrocytes count (6.78×10^6/µl), packed cell volume (43.4%) and platelet counts (290×10^3/µl) were in the reference range. The derived MCV (64 FL), MCH (22.7 pg), and MCHC (35.5 g/dl) were not much changed. Serum enzyme activities of ALT and AST were significantly high: 136 U/L and 1159 U/L, respectively, reflecting hepatic injury. The total billirubin (0.40 mg/dl) in serum, however, remained unaffected confirming the earlier finding for ICH (Greene 2006). The serum levels of urea and creatinine were high, 79 mg/dl and 1.7 mg/dl, respectively, compared to the reference range in canines (15–45 mg/dl and 0.54–1.4 mg/dl, respectively) indicating kidney injury. Total protein (3.9 g/dl: albumin 1.94 g/dl, globulin 1.96 g/dl) and alkaline phosphates (306 U/L) values deviated from the reference range.

Microscopic examination: Gross changes of widespread congestion, peticheal to ecchymotic hemorrhages over the serosal surfaces in different organs were in concurrence with microscopic lesions. In general, the microscopic lesions of edema, hemorrhages and necrosis were present in liver, kidneys, brain, lymphoid tissues and lungs in association with intranuclear inclusions in vascular endothelial cells, hepatocytes and mesenchymal cells. The liver though grossly congested, yet showed widespread fatty changes and necrosis of hepatic cells in centrilobular and perilobular areas (Fig.1A). The sinusoids contained with RBCs and a few neutrophils, macrophages and lymphocytes. At the junction of the necrosed and viable hepatocytes, a few apoptotic hepatocytes and scattered swollen and abnormally large hepatocytes with large, solid intranuclear deep acidophilic to basophilic inclusions of variable sizes (2–3 cells/20) resulting into margination of the chromatin material (Fig.1B) were also visible. A few inclusions were present in the endothelial cells of periportal vessels along with mild peri-vascular infiltration of mononuclear cells. The hepatic cell injury was also corresponded by high activity of liver specific enzymes, pointing towards infectious canine hepatitis (ICH). The inflammatory cells in liver sinusoids were found to be

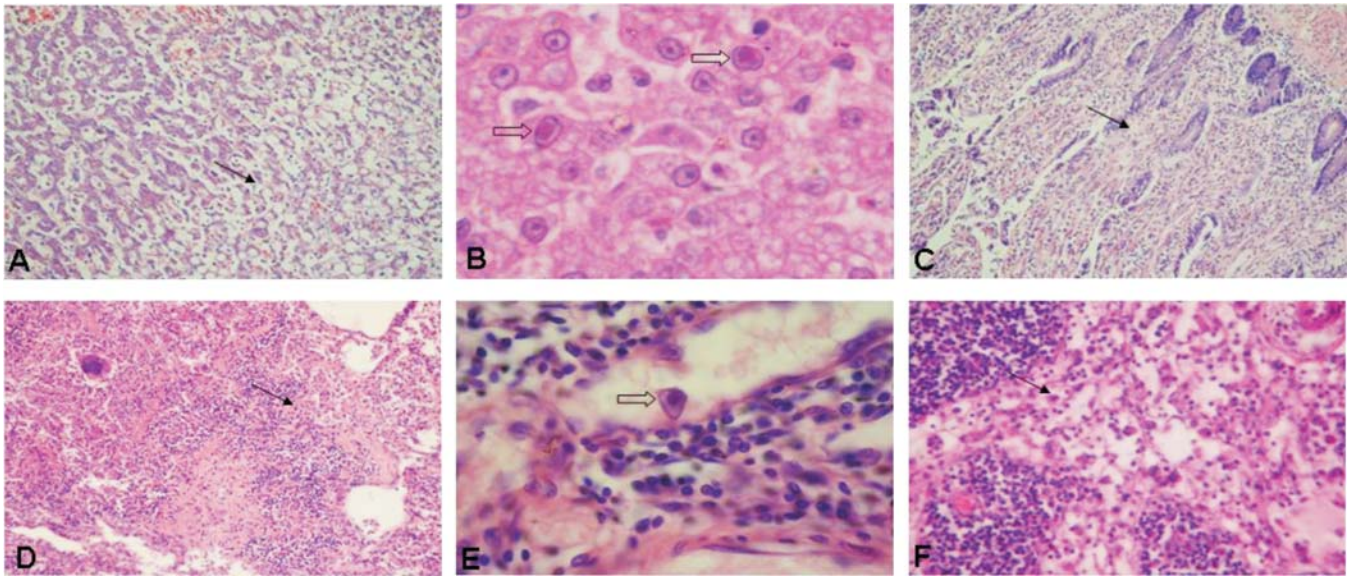

Fig. 1 (A-F). Sloth bear: Liver with periacinar necrosis, fatty changes, leucocytic infiltration and dilated synusoids (**A**), large intranuclear acidophilic inclusions (open arrow) and apoptotic hepatic cells (**B**); intestine with necrotic mucosa and mononuclear cell infiltration (**C**); lung showing chronic inflammatory exudate in the parenchyma (**D**, arrow) and presence of inclusion in endothelial cells (**E**, open arrow); lymph node oedema and inclusions in reticuloendothelial cells (**F**). All the pictures are stained with H&E. (Figs. A, C, D×10; Figs B, E, F×40).

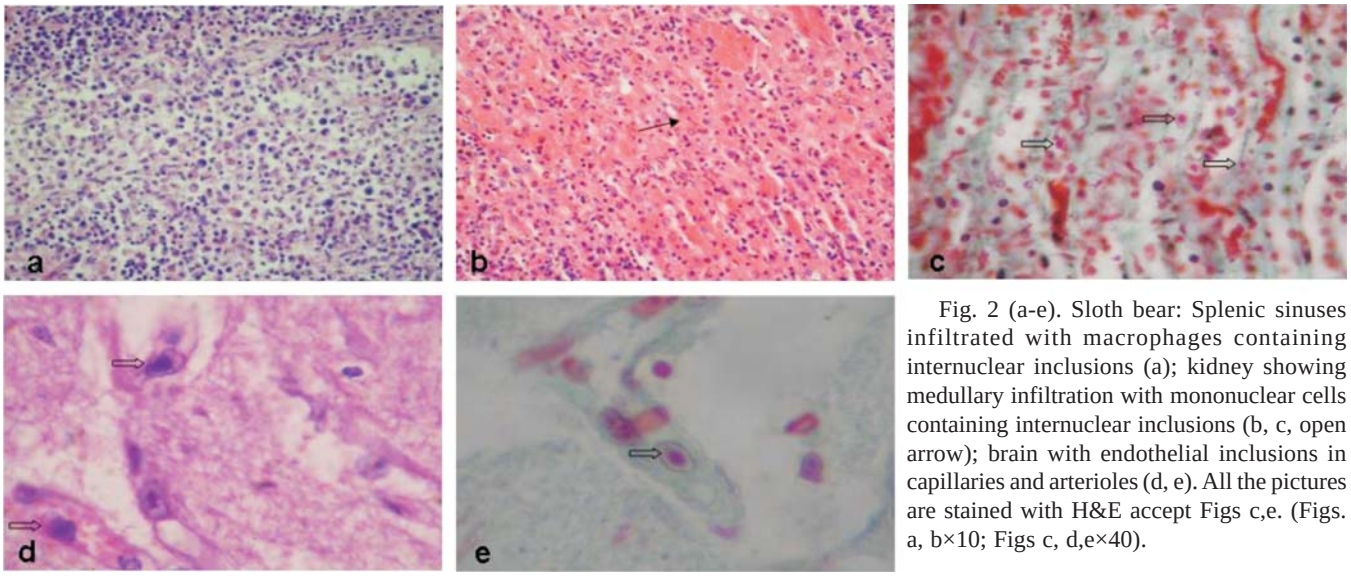

Fig. 2 (a-e). Sloth bear: Splenic sinuses infiltrated with macrophages containing internuclear inclusions (a); kidney showing medullary infiltration with mononuclear cells containing internuclear inclusions (b, c, open arrow); brain with endothelial inclusions in capillaries and arterioles (d, e). All the pictures are stained with H&E accept Figs c,e. (Figs. a, b×10; Figs c, d,e×40).

associated with the removal of necrotic debris (Geene 2006). The Kuffer's cells did not show the presence of intra nuclear inclusions indicating late phase of the disease process (Jubb *et al*. 2004). Kritsepi *et al.* (1996) reported similar changes in liver in one out of three 10–years-old brown grizzly bears (*Ursus arctos*) in a wild life park in Greece, which died acutely showing anorexia, lethargy, posterior ataxia, icterus and wide spread hemorrhages. The lamina propria of the stomach and the intestine showed moderate engorgement of blood vessels and infiltration with few mononuclear cells (macrophages, plasma cells and fibroblasts). The capillary/ arteriole endothelium in lamina propria, sub-mucosa and serosa behaved similarly as in other organs, and had occasional presence of inclusions. The glandular epithelial cells of intestine showed necrotic changes and goblet cells hyperactivity (Fig.1C). Pancreas showed severe engorgement and hemorrhages within the connective tissue and focal necrosis of pancreatic cells in various lobules. The capillary endothelial lining cell, however, did not show any inclusions. Lung showed mild vascular engorgement and interalveolar thickening with engorged capillaries. The alveoli in some areas contained sero-fibrinous exudate and few mononuclear cells admixed with RBCs. The capillary and arteriole lining cells were swollen due the presence of intranuclear inclusions. Many endothelial cells that contained inclusions were seen detached into the lumen of the vessels (Fig.1E). In one case, the bronchioles and the surrounding parenchyma were infiltrated with large number of macrophages, lymphocytes and plasma cells in the form of nodules (Fig. 1D). Heart musculature revealed edema and hemorrhages within the inter-cardiac muscle bundles. The vessels lining endothelium revealed intranuclear inclusions. The parenchyma of kidney in medulla was similarly engorged/ hemorrhagic and vessel contained mononuclear cells (macrophages, lymphocytes) and sloughed endothelial cells with intranuclear inclusion. Scattering of inclusions were also visible in the medullary tubules and the vessels, while a few in glomeruli (Figs 2 b, c). The lining cells of the medullary tubules that degenerated and sloughed into the lumen having proteinaceous material, were also visible in the pelvis region. Focal infiltration of mononuclear cells was seen in the intertubular connective tissue. The endothelial cells of blood vessels in urinary bladder also contained intranuclear inclusions. Lymphoid tissue (lymph node and spleen) showed severe engorgement/hemorrhages of the blood vessels having swollen and proliferated endothelial cells (Fig.1F). The medullary sinuses were oedematous and filled with fibrinous exudate admixed with large number of activated macrophages (debris laden) and polymorphs. The reticulo-endothelial cells contained intranuclear inclusions, which were plenty, particularly in spleen (Fig.2a). The lymphoid tissue showed lymphocyte proliferation. The brain showed engorgement and perivascular ring hemorrhages around the arterioles. The endothelial cells in majority of arterioles and capillaries, particularly in brainstream were proliferated, abnormally swollen, oval to oblong in shape and many of them contained intranuclear acidophilic inclusions (eosinophilc granular mass to discrete mass) (Figs 2d,e). The degenerated endothelial cells were present amidst mononuclear cells into the arteriolar lumen. The parenchyma surrounding the affected vessels was edematous, showed mild microglial cell reaction and neuronal degeneration. The capillary endothelial cells were swollen to cause occlusion of the lumen.

Based on clinical signs, hematology, biochemical and presence of specific intranuclear inclusions, the outbreak was confirmed of infectious canine hepatitis. The report seems to be the first of its kind in India.

ACKNOWLEDGEMENT

The authors are thankful to Dr G Sudhakar, Deputy

Conservator of Forest, National Chambal Sanctuary Project, Agra, for providing the morbid samples of bears along with history for carrying out the diagnosis.

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