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Occurrence of extrahepatic biliary tract pathologies in rehabilitated captive dancing sloth bears (*Melursus ursinus*) and its diagnostic challenges

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Abstract
Sloth bears are large, charismatic mammals that belong to the order carnivora and the family Ursidae. Each mammalian species possesses the unique size and structure of their extrahepatic biliary tract. While gall bladder is present in many species, others are lacking this organ. Sloth bears have a well-developed gall bladder and bile ducts with Penta lobed liver. Since they possess a gall bladder; they are more prone to cholecystolithiasis, cholecystitis, varied degree of sludge formation, and cholangiocarcinoma. In the sloth bears at Wildlife SOS, Agra Bear Rescue Facility, such cases were encountered and provided with disease-specific treatment to stabilize the health condition. Trans abdominal ultrasonography examination was conducted to evaluate liver, gall bladder and ascites to diagnose the extrahepatic biliary tract pathologies along with haemato-biochemical indices and visible external symptoms such as inappetence, general weakness, bulged abdomen, hair loss, allergic dermatitis on limbs, passing mucoid brownish black diarrhoeic faeces and icteric mucous membrane. This study aims to disseminate knowledge on extrahepatic biliary tract pathologies of sloth bears that will help wildlife veterinary professionals, wildlife neophytes to enhance their early diagnosis and treatments as well as scientists in evolutionary biology and oncology for conducting further studies.

Keywords: Biliary tract, carcinoma, ultrasonography, gallbladder, sloth bears

1. Introduction
The sloth bear *(Melursus ursinus)*, is listed under schedule I of the wildlife protection act 1972, and it is classified as vulnerable in the IUCN red list of threatened species [6]. Sloth bears forage on mango, fig, ebony, and other fruits (frugivory), and also on some flowers. However, ants and termites (myrmecophagy), dug out of their cement-hard nest mounds, are a year-round staple. Also, sloth bears climb trees and knock down honeycombs, beetles, grubs, ants, and other insects round out their diet. During food shortages, sloth bears will eat carrion. This bear species is commonly found in the Indian subcontinents and frequently got poached for body parts and also the cubs used to train for illegal street performance by the calendar gypsies. Due to their poor handling, hygiene with malnourishment; they are always in the stage of compromised health. Wildlife SOS is an NGO that started working along with all the state forest departments and rescued all the performing bears from the illegal captivity; housed them in a well-established rescue facility for further care and rehabilitation. However, they are receiving good quality freshly prepared balanced porridge which is further supplemented with multivitamins, minerals, raw honey with propolis, protein source as diet in morning and evening; seasonally available fruits as part of enrichment in noontime food., 45% of the annual mortality happening due to hepatic disorder.

There have been relatively few reports of tumours in bears (*Ursus* sp.). The most prevalent tumours are hepatic neoplasms i.e. hepatocellular and biliary carcinoma [1, 7, 20, 21, 28]. This captive sloth bears populations are more prone for various degree of extrahepatic biliary tract pathologies while aging and cholangiocellular carcinoma at any stage of life. In this article we dealt with the ultrasonography aided diagnostic approach along with haemato-biochemical indices and visible external symptoms for early diagnosis and provide diseases specific treatment and also to create awareness among the scientists in evolutionary biology and oncology, making further research on molecular genetic level to understand the status of p53 gene amplification in this species.

p53 is one of the most well-studied and crucial tumour suppressor genes. p53 serves as the "guardian of the genome" and the "cellular gatekeeper" [12, 14] and any possibility for gene therapy to minimize the occurrence of malignancy in this species as part of conservation medicine.

2. Materials and methods

In captive bears, neoplasia commonly involves the hepatobiliary and gastrointestinal [2, 3] which led to the compelling need of acquiring more adequate clinical approaches for the evaluation of the hepatic system. Obtaining liver tissue samples by liver biopsy (percutaneous, laparoscopic, surgical, trans jugular) is the most direct approach to the evaluation of hepatic fibrosis in human medicine. However, liver biopsy is associated with potential morbidity and mortality and has several limitations, including sampling error and high inter-observer variability [2, 25]. So, by considering captive management of wild animals, The non-invasive method of health evaluation by using ultrasonography technique, collection blood samples for hematobiochemical analysis is more beneficial and less risk to the patient, that can be achieved after chemical immobilization [2] or by positive reinforcement training [4, 18, 24]. Routine observation for any symptoms and correlate the same perfectly with clinical findings will also be mandatory for an exact diagnosis.

2.1. External symptoms

The routine monitoring of the animal activities and visual evaluations always remains a preliminary clue for any further diagnostic approaches. The animal suffering from gall bladder disease/cholangiocarcinoma always exhibits symptoms of bulged abdomen (Figure 1 & 2), icteric mucus membrane due to generalized jaundice, facial/peripheral oedema as according to the severity of the condition (Figure 3 & 4), intermittent vomiting, passing mucoid brownish block diarrhoeic faeces, rarely passing grey colour semisolid faces (Figure 7 & 8). The faecal analysis has always revealed the presence of a parasitic infestation. in the initial stage, there won't be any marked changes in food intake, but as days progress the gradual inappetence and intermittent anorexia will develop, which will lead to leathery and general weakness. Nonparasitic allergic dermatitis lesions around the lips, limbs, and perianal region were also noticed in the bears which have hepatic pathology (Figure 5 & 6).

Fig 1 & 2: Bulged abdomen in Sloth bears due to ascitic fluid

Fig 3: Generalized jaundice

Fig 4: Facial/peripheral oedema

Fig 5 & 6: Dermatic lesion on limbs

Fig 7 & 8: Faeces appearance and condition in bears affected with hepatic disorders

2.2. Hematobiochemical analysis

The blood samples can be obtained from the cephalic vein or saphenous vein or jugular vein for the hematobiochemical analysis. The level of SGPT, SGOT, GGT, ALP, LDH, and total bilirubin is always in the elevated range in animals suffering from hepatic carcinoma. The total protein value will be in the lower range. Low sodium, levels were also observed in most of the cases. In few cases, mild elevation of phosphate ions was noticed.

2.3. Transabdominal ultrasonography

Ultrasonography is a well-recognized safe, fast, repeatable method to evaluate gastrointestinal (GI) disease in people. Reference to its use in veterinary medicine is limited, During the previous 20 years, great advances in veterinary abdominal ultrasonography equipment and expertise have occurred and ultrasonography has been commonly in small animal practice and Ultrasonographic descriptions of many chronic GI disorders have been published [13, 23]. Sloth bear has well-developed a hepato- biliary system which consists of Penta lobed liver, gall bladder, and bile ductus (gross image). We can adopt the transabdominal ultrasonographic technique as the same is performed in small animals [5, 27]. Any good quality B mode ultrasonography unit with a 3 -5 MHz curve leaner probe is enough to do the ultrasonography in sloth bear (Figure 9 & 10). With the help of ultrasonographic scanning of the abdomen, we can easily find out asities due to peritoneal effusions (Figure 11 & 12), any abdominal mass, gall bladder abnormalities including wall thickening, various degree of proliferation (Figure 13, 14, 15 & 16), presence of pericholecystic fluid (Figure 17 & 18), various stages of sludges (Figure 19 & 20), and hypertrophy of gall bladder and gall stone. Documented photographs of gross PM lesions are incorporated here in this article for better understanding (Figure 21, 22, 23 & 24).

Fig 9 & 10: Ultrasonography machine used for diagnosis in Sloth bear with multi transducer option

Fig 11 & 12: Ascitic fluid present in the abdomen of a Sloth bear appreciated in ultrasonography

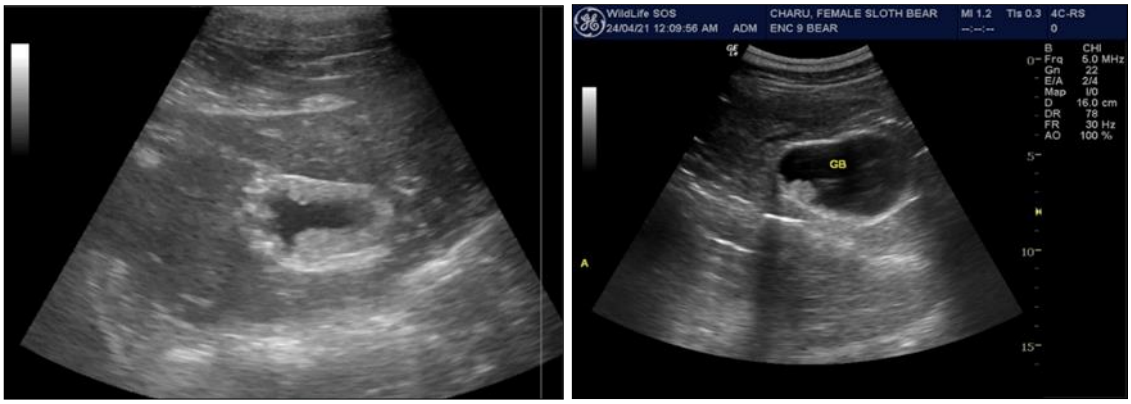

Fig 13, 14, 15 & 16: Various digress of proliferative lesions on the gallbladder wall appreciated in ultrasonography

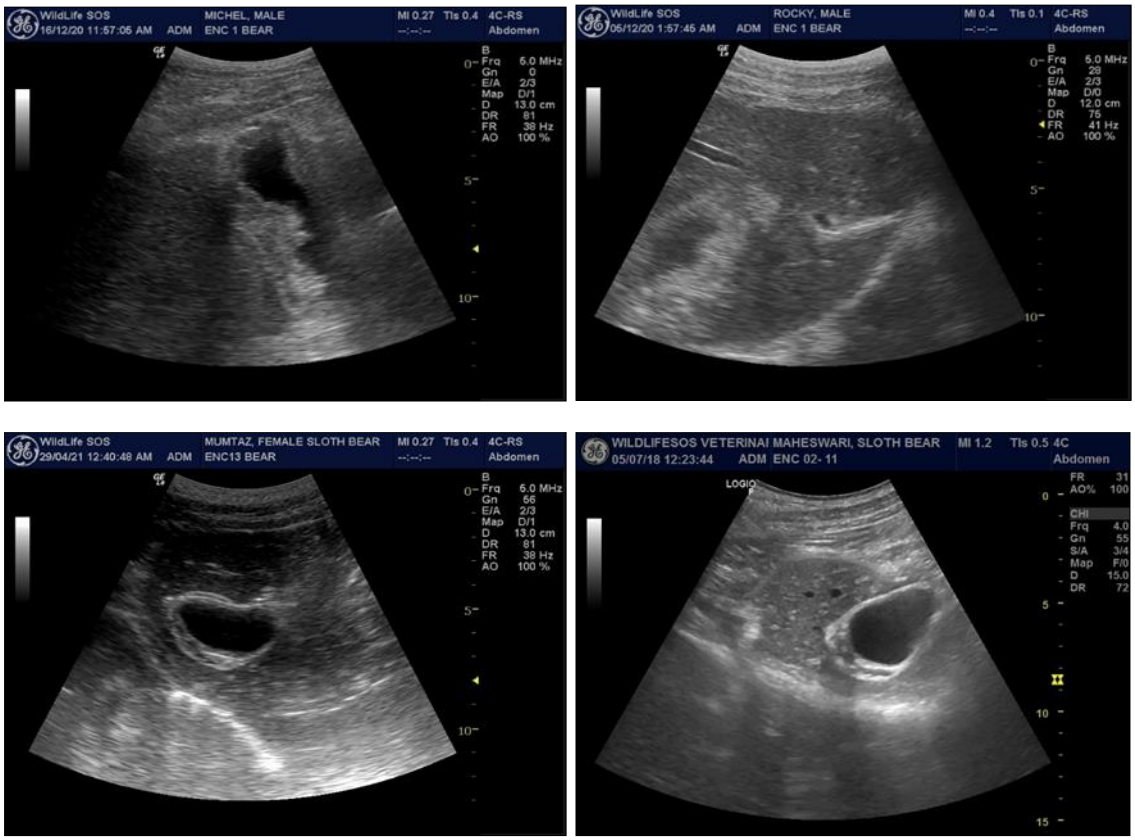

Fig 17 & 18: Arrow showing presence of pericholecystic fluid with hyperechoic gall bladder wall

Fig 19 & 20: Arrow showing presence of cholelith (gall stone) showing sludge inside the gall bladder

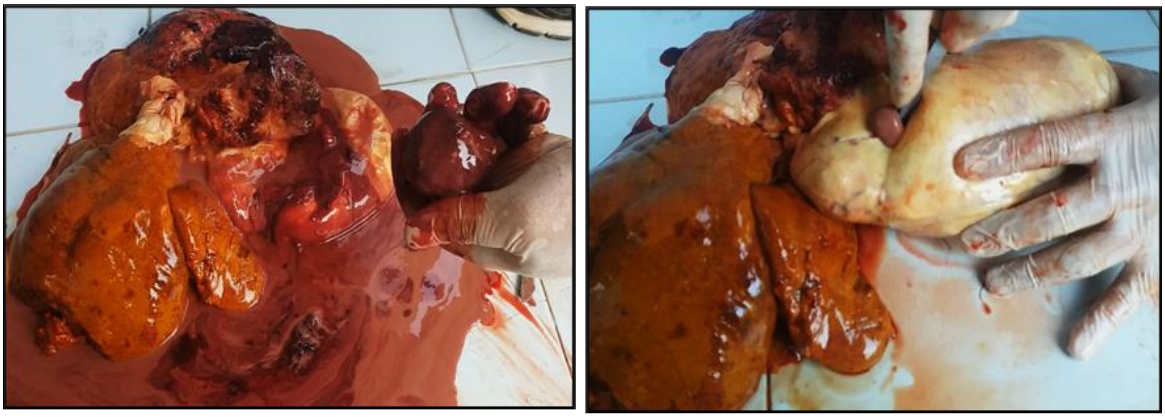

Fig 21 & 22: Post mortem examination revealed the presence of cholelith inside the gall bladder and abnormal enlargement of the gall bladder due to sever cholecystitis

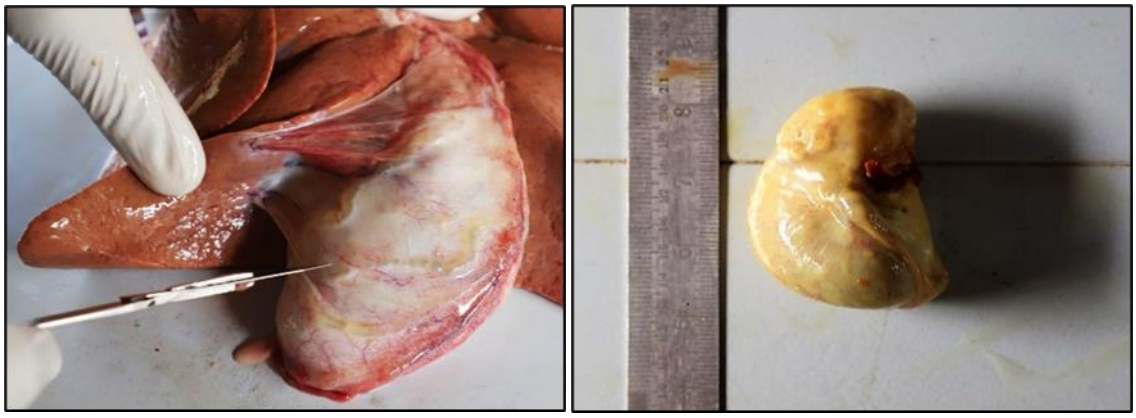

Fig 23 & 24: Congested gall bladder with oedematous wall and icteric, shrunken gallbladder with thick wall and tumour mass

3. Discussion

The detailed record of cancer occurrence in captive animals remains an important source of information for studies in comparative oncology, as well as providing data on the Etiology of neoplastic diseases [17]. Wildlife cancer statistics are, however, highly scattered in the scientific literature and hence challenging to access. Moreover, tumours in wildlife are most commonly detected during post mortem examination and therefore hard to confirm without histopathological examinations. However, even such analyses can be inaccurate because of high levels of autolysis [17, 19]. Reported the highest tumour prevalence in the carnivores. Similar high cancer prevalence in this group of mammals was mentioned by [15, 16]. More cancer prevalence in the mammal digestive system was recorded by [17] and [15]. In the year 2017, [1] reported that the biliary adenocarcinoma was the leading cause of death for adult sloth bears housed in U.S. zoos with no apparent gender predilection [7]. Reported Biliary and hepatic carcinomas in bears at the San Diego zoological gardens [11]. Also reported the hematobiochemical changes, assists, and jaundice as we mentioned in our findings [22]. Stated that tumours of the liver, bile ducts, and pancreas quite frequently develop in bears, usually in older animals, and also recorded unspecific symptoms such as vomiting, anorexia, weight loss, and abdominal swelling. Hyponatremia is a common finding in patients with decompensated cirrhosis due to abnormal regulation of body fluid homeostasis [8].

4. Conclusion

In zoos, bears are considered to be animals that have few problems and are relatively free from disease [9]. This may be attributed either to “an extraordinary resistance against all

kinds of diseases or to an unbelievable ability to conceal symptoms of sickness and pain [26]. This statement suggesting that the challenges in disease diagnosis in bears, and insists they need of multi-facet diagnostic approach. The literature reports also evidencing the incidence of extrahepatic biliary tract pathologies in the captive bear population around the globe which is not particular to any location, age, sex, and any specific feeding behaviour exactly. So, this overall reported incidence suggesting that the high chances for the genetic predisposition for neoplasms in this species may be weakening their Cancer defence mechanisms. Using an evolutionary and comparative approach to study cancer defence mechanisms has implications in human health and disease. It can provide new insights into cancer treatment (e.g., p53, immunotherapy) and prevention [10].

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