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Ultrasonographic study of gallbladder pathologies in sloth bears: A focus on animal welfare undertaken by Wildlife SOS, Agra, India

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ABSTRACT

The diagnosis of gallbladder pathologies in sloth bears (*Melursus ursinus*) is a critical aspect of veterinary care as they may lead to significant clinical complications. Traditional diagnostic methods often rely on minimally invasive or non-invasive procedures that require chemical immobilization, often stressful for the animals including huge cost involvement of tranquilizing drugs and equipments. This study explores the use of operant conditioning techniques to encourage bears to comply voluntarily with ultrasonography for non-invasive detection of gallbladder pathologies. Ultrasonographic examination of 48 bears was successfully performed by adopting positive reinforcement technique, revealing that 22.9% were diagnosed with cholecystitis, 6.3% with sludge, and 2.1% with a gallbladder polyp. The ultrasonographic findings included hyperechoic non-acoustic shadows indicative of sludge, an irregular and hypoechoic thickened gallbladder wall suggestive of cholecystitis, and an echogenic mass protruding from the inner lining of the gallbladder mucosa, consistent with a polyp. Supportive medications along with nutritional management were adopted to enhance the hepatic function. This study demonstrates the feasibility of using operant conditioning as a part of a co-operative care program for the effective and non-invasive diagnosis of gallbladder pathology through ultrasonography in bears, potentially improving animal welfare and diagnostic accuracy by reducing the need for chemical immobilization.

Key words: Gallbladder pathologies, non-invasive ultrasonography, operant conditioning, sloth bear

INTRODUCTION

Interaction with captive animals promotes physical and mental stimulation, and any carefully tailored behavioral strategy that meets individual or group needs in ex-situ environments contributes to improved animal welfare (Hediger, 1950). Associative learning, including both classical and operant conditioning is fundamental to foster positive human-animal interactions (Rault et al., 2020) our understanding of the underlying processes that govern the positive perception of humans by animals is incomplete. We cover the potential mechanisms involved in the development and maintenance of positive human-animal relationships from the perspective of the animal. This encompasses habituation, associative learning, and possibly attachment or bonding based on communication and social cognition. We review the indicators from the literature to assess a positive human-animal relationship. We operationally define this positive relationship as the animal showing voluntary approach and spatial proximity (seeking, and the application of positive reinforcement in operant conditioning effectively enhances the likelihood of desired behavioural responses (Young and Cipreste, 2004). Positive reinforcement involves rewarding a response or behaviour with something the subject desires (such as foods of preference), thereby increasing the frequency of that behaviour (Jill and MacPhee, 2010; Keller et al., 2020). Shaping is a procedure within operant conditioning and corresponds to a process that can be used to train a certain behavior, through a series of small, selectively reinforced steps (Cipreste, 2014). Wild animals maintained in captivity can be effectively conditioned through structured training techniques that facilitate routine handling and promote voluntary participation in husbandry and veterinary procedures (Skinner, 1951). The implementation of animal training protocols must be systematically planned based on predefined objectives, species-specific behavioral and physiological knowledge, and individual-specific characteristics (Cipreste et al., 2022). This approach facilitates the execution of veterinary procedures in captive animals by promoting their cooperation and reducing stress during handling (Coleman et al., 2005; Mattison, 2012; Ward and Melfi, 2013; Rault et al., 2020; Atchison, 2023). Many facilities are starting to train primates to voluntarily cooperate with veterinary, husbandry, and research procedures, such as remaining still for blood draws or injections. Such training generally reduces the stress associated with these procedures, resulting in calmer animals and, ultimately, better research models. However, such training requires great investments in time, and there can be vast individual differences in training success. Some animals learn tasks quickly, while others make slower progress in training. In this study, we examined whether temperament, as measured by response to a novel food object, correlated with the amount of time it took to train 20 adult female rhesus macaques to perform a simple task. The monkeys were categorized as "exploratory" (i.e., inspected a novel object placed in the home cage within 10 sec. As such, it serves as a viable alternative to traditional chemical (Laule and Desmond, 1998) or physical (Desmond and Laule, 1994) restraint methods, with the potential to minimize trauma associated with these conventional practices (Waiblinger et al., 2006). We briefly explain why an increased understanding of the human–animal relationship (HAR). Ultrasonography has a wide range of utility related to reproduction assessment, assisted reproduction technologies, anatomic, physiologic, and morphological studies (Stefanello et al., 2009). A thorough understanding of the species-specific anatomy is essential before performing ultrasonography, as the absence of such knowledge or prior experience may hinder accurate identification of pathological changes (Hildebrandt and Saragusty, 2015). This study explores the use of operant conditioning within a cooperative care framework to facilitate non-invasive ultrasonographic diagnosis of gallbladder pathology in sloth bears, potentially enhancing diagnostic accuracy and animal welfare.

MATERIALS AND METHODS

Site

The study was conducted on forty-eight ($n = 48$) sloth bears housed at the Agra Bear Rescue Facility, Agra, Uttar Pradesh, India. The facility is managed by Wildlife SOS, an Indian non-profit conservation organization that has been actively involved in the rescue and long-term rehabilitation of sloth bears formerly exploited in the dancing bear trade. The bears included in this study ranged in age from 5 to 30 years and had an average body weight between 80 and 120 kg.

Exhibit layout

Two enclosures were specifically engineered to facilitate voluntary participation of animals enabling safe and efficient access to the abdominal region. The first structure comprised vertical metal bars with an overall height of 230 cm and width of 103 cm. A horizontal metal bar was positioned at a height of 112 cm from the ground, providing a stable surface for the bear to hook its forepaws and support itself comfortably during the procedure. The exhibit included two strategically placed 15 × 15 cm access ports located at 44 cm intervals from the centreline, with the upper port positioned at 40 cm height and the lower one aligned below, allowing access to different abdominal regions based on bear posture (Fig.1). The second enclosure layout supported abdominal access while the bear was in lateral recumbency, specifically accommodating individuals with musculoskeletal challenges who were unable to maintain a sitting posture for prolonged periods. This structure featured a wider metal-barred front measuring 230 cm in width and height. A single access port (22 × 22 cm) was positioned 10 cm above ground level, allowing unobstructed access to the lateral abdominal surface (Fig. 2).

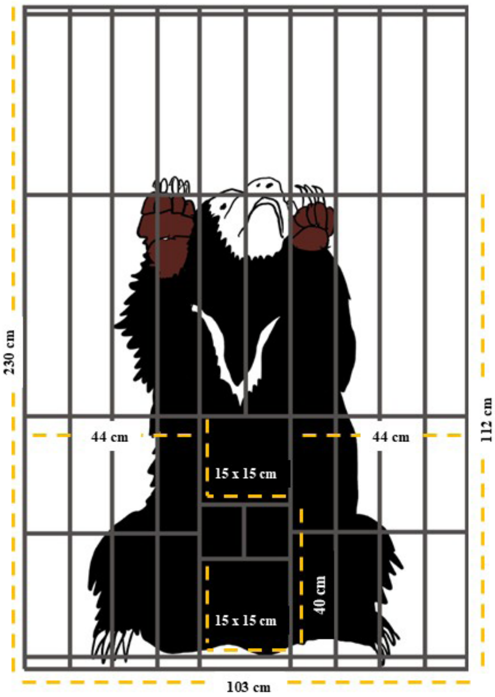

Fig. 1. Enclosure modification to facilitate abdominal ultrasonography in a seated (vertical stationing) posture

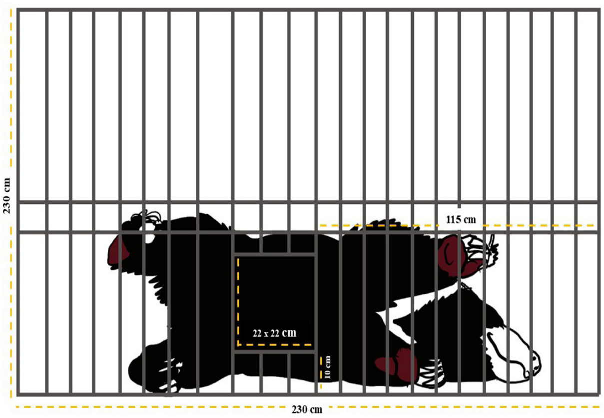

Fig. 2. Modified enclosure design facilitating abdominal ultrasonography in lateral recumbency

Behavioural training

A structured operant conditioning approach was employed using positive reinforcement techniques. A target stick was utilized to guide individuals to a designated location (stationing). Each subject underwent daily training sessions, with trials conducted twice, depending on the complexity of the behavior being taught. Each session was subdivided into three parts, lasting between 3 to 6 minutes, interspersed with 1 minute break. A single trial continued until the subject successfully performed the target behavior three times consecutively. A clicker served as the conditioned reinforcer; the click sound was delivered immediately following the desired behavior to signal the impending delivery of a primary reinforcer. Food rewards, used as positive reinforcers, were administered promptly following the appropriate behavioral response. A behavior was considered to be learned when the subject could demonstrate the target behavior reliably and confidently, completing three successful repetitions. Details of specific behaviors targeted and the reinforcement criteria are outlined below (Table 1).

Table 1. Criteria for reinforcement during operant conditioning of sloth bears

Behaviour	Criteria for reinforcement
Target	Bear touches target stick with nose
Sit	Bear has back (hind paws) end on ground front end (fore paws) held up by hooked into bar that divides mesh sections
Lay down	Bear turns head, shoulder, and hips in and lays on side (laterally)

Desensitization

The desensitization process was incorporated through step-wise exposure to ultrasonography-related stimuli depending on each individual's comfort and response. Initially, bears were acclimated to gentle abdominal touch by hand with calm behaviour positively reinforced. Once basic tactile contact was tolerated, light pressure using anon-functional PVC pipe was introduced to stimulate ultrasound probe contact. Subsequently, the bears were gradually exposed to presence and sound of a trimmer and the ultrasound unit (visual and auditory stimuli) followed by the application of ultrasound gel using gloved hands. This was followed by brief and progressively longer sessions with the actual probe and machine in operation. The procedure was considered successful when the bear remained relaxed and cooperative during full simulated scanning without signs of stress or avoidance.

Hepatobiliary ultrasonography

Due to the dense fur covering of sloth bears, abdominal fur was clipped to ensure optimal acoustic coupling during transcutaneous ultrasonographic examination. Abdominal ultrasound was performed (Fig. 3 and 4) on 48 bears using the LOGIQ e (GE Medical Systems, China) equipped with a 4C convex probe (1-8 MHz). Gallbladder wall thickness was measured, with values exceeding 3 mm considered indicative of increased wall thickness. Moreover, the gallbladder was assessed for wall echogenicity, presence of wall edema, and intraluminal contents (i.e., sediment, sludge, and gallstones). In addition, the liver was evaluated for echogenicity and overall morphological appearance.

RESULTS AND DISCUSSION

Gallbladder ultrasonography was successfully performed in 48 sloth bears. Out of these, 33 individuals (68.75%) showed no abnormality detected (NAD). Gallbladder lesions were detected in 15 bears (31.25%). The most common finding was cholecystitis, observed in 11 bears (22.91%), characterized by increased gallbladder wall thickness (>3 mm), hyperechoic wall margins, and occasionally pericholecystic fluid. Sludge was detected in 3 bears (6.25%), visualized as echogenic material without acoustic shadowing. A single case (2.08%) of a gallbladder polyp was identified as a well-defined echogenic intraluminal mass (Fig. 5). Representative ultrasonographic images of gallbladder abnormalities observed in the study are presented in Fig. 6-11.

Fig. 3. Operant conditioning-assisted ultrasonographic examination of the gallbladder in a seated sloth bear

Fig. 4. Abdominal ultrasonographic examination conducted under lateral recumbency.

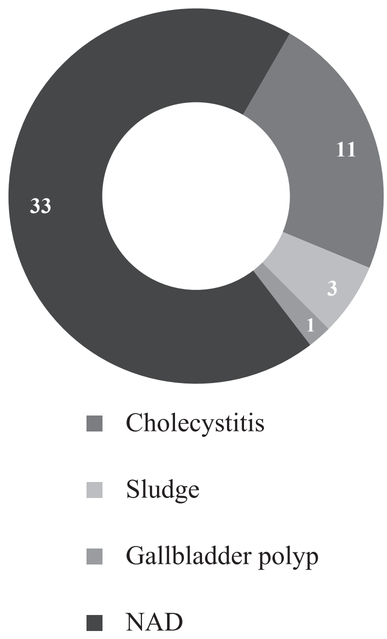

Fig. 5. Donut chart representing the distribution of gallbladder pathologies

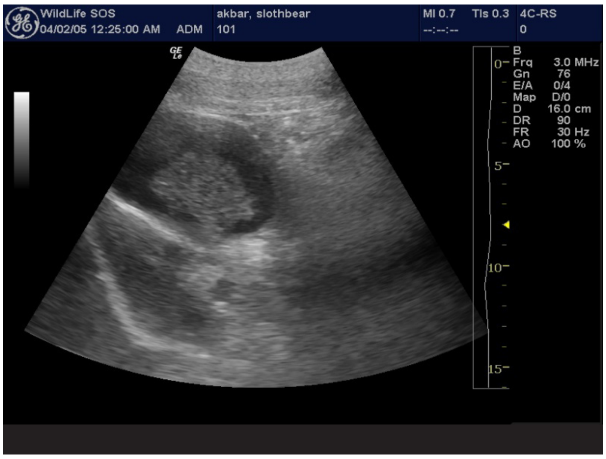

Fig. 6. USG showing presence of sludge in gallbladder

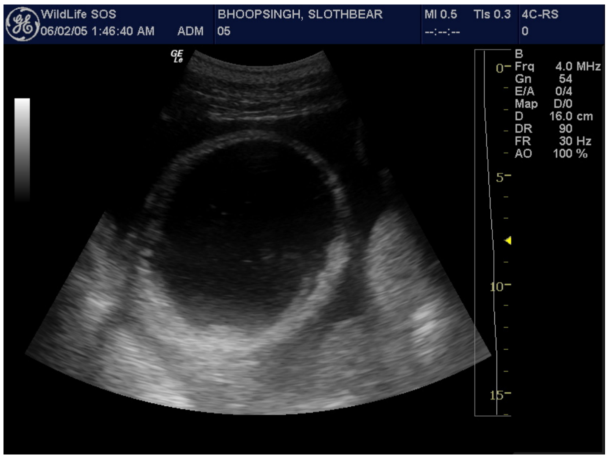

Fig. 7. Thickened gallbladder wall with abnormal bile consistency

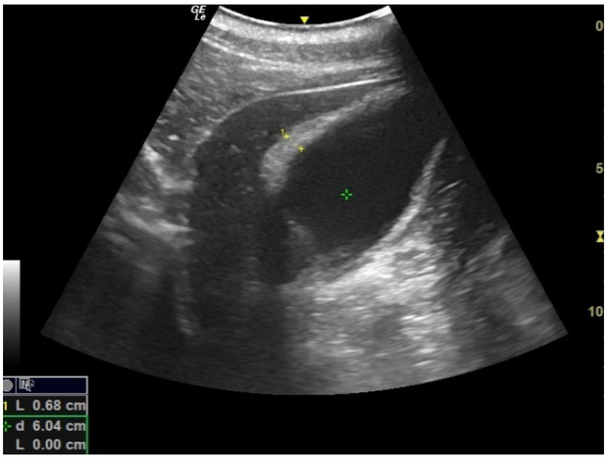

Fig. 8. Thickened gallbladder wall, suspected for cholecystitis

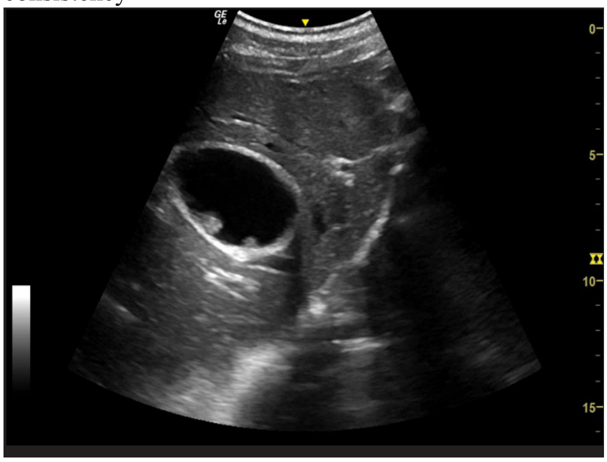

Fig. 9. Ball on the wall sign; gallbladder polyp

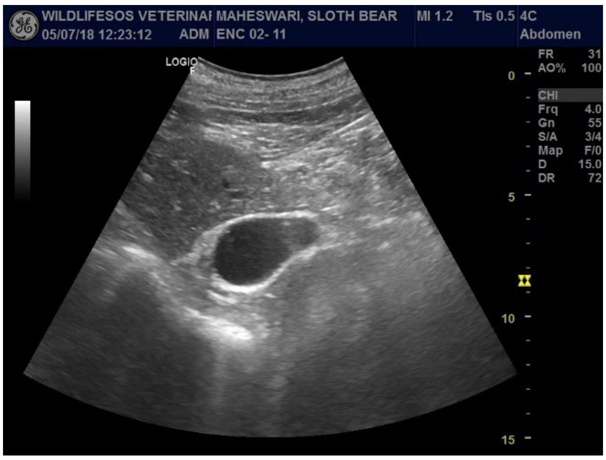

Fig. 10. Pericholecystic fluid with hyperechoic gallbladder wall

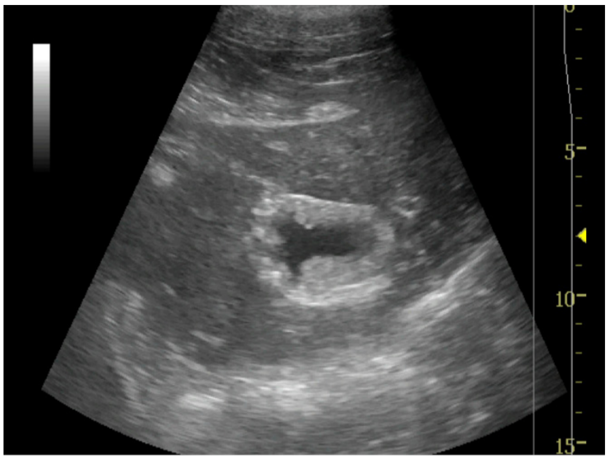

Fig. 11. USG showing hypertrophy of gallbladder wall

The predominance of cholecystitis (22.91%) is of clinical significance, possibly indicating subclinical inflammation or early biliary disease in a population previously subjected to compromised health. Detection of biliary sludge (6.25%) may suggest bile stasis or increased bile concentration. The single gallbladder polyp observed warrants continued surveillance for potential neoplastic transformation, although such findings are infrequently reported. However, the prevalence of subtle hepatobiliary alterations underscores the utility of routine ultrasonographic monitoring as a non-invasive diagnostic tool to inform preventive veterinary care in long-term captive populations.

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