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Non-invasive urine collection- floor pit method in captive sloth bears (*Melursus ursinus*)

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Abstract
Urine plays an important role in understanding metabolic conditions, reproductive status and stress physiology. We report here an effective non-invasive method of urine collection from captive Sloth bears (*Melursus ursinus*). The role of hormonal profiling and urinalysis are described. The pertinency of this method and its purpose for conservation are discussed.

Keywords: sloth bear, non-invasive, sample collection, urine

1. Introduction
The process of removing waste products from the body followed by cellular metabolism is known as excretion [1]. Like amphibians and marine fishes, mammals excrete urea as toxic wastes hence called ureotelic animals [2]. Urine plays an important indispensable role in clinical diagnosis [3]. Non-invasive urine samples are widely used for urinalysis and physiological assessment like reproduction, stress and behaviour [3-5]. The nature of being stable and obtainability in large quantities non-invasively makes it most important biofluid as compared to others [6]. Since Sloth bears (*Melursus ursinus*) are highly threatened schedule 1 species and considered as vulnerable by IUCN, it is very significant to understand their age-based physiology. There is a severe lack of scientific information about the basic biology of *M. ursinus* even after its wide distribution in Indian subcontinents. There have been defined reports of hematology and biochemistry values in *M. ursinus* but no urologic values [7, 8]. Furthermore, urinalysis have various beneficial aspects still it's a very neglected aspect in veterinary science [3]. It is hoped that the application of this method will facilitate to evaluate health profile of sloth bears in captive settings.

2. Material and Method
The method reported here was developed for captive Sloth bears under rehabilitation at Agra Bear Rescue Facility, Uttar Pradesh, India. A pit of ≤ 45.72 cm length, 2 cm wide and 1.5 cm deep was made at the end on the concrete flooring of animal's enclosure (figure 1). The pits were made in such a way that the urine will drive downward and get accumulated within it by the effect of gravitational force (figure 1). The ideal time for collection of urine samples was after the morning feeding of individuals. Prior to sample collection, the floor along with the pit was cleaned with disinfectants and distilled water. The pit then wiped by using sterile medical cotton rolls and allowed to air-dry. Soon after the urination, urine was collected from the pit via sterile injection and immediately transferred to an air-tight screw cap vial (figure 2). Urinalysis was performed soon after the collection by using urine test strips and UA analyzer supplied from IDEXX laboratories to avoid the disintegration of casts and other cells. Urine sample filled vials were stored frozen at -20˚C for later analysis.

3. Results and Discussion
Standard urinalysis parameters viz. colour, clarity, sedimentation, pH, leucocyte, protein, glucose, ketone bodies, urobilinogen, bilirubin and blood cells were determined to evaluate metabolic conditions. Presence of air bubbles, hairs and accidental contamination by faeces and debris from enclosure floors suspected to be an artifact.

In this scenario, consecutive urinalysis needs to be performed with an uncontaminated sample. In addition to this, presence of leucocytes in urine needs to be confirmed under microscopic examination. However, there have been limited reports of hematology and biochemistry values in sloth bear but no urinalysis values [7, 9]. In most wildlife species and especially in *M. ursinus* repeated blood collection is inconceivable for hormonal analysis [10]. Furthermore, chemical immobilization induces stress in an animal which in turn affects physiological and hormonal profiles [4, 10]. Hence by this method, the animal was allowed in its familiar enclosure for the purpose of urine collection which neither effect it's physiological nor psychological conditions. Quantitative enzyme immunoassays generated urinary oestradiol and progesterone metabolites in *M. ursinus* [11]. Urinary hormone assays and behavioural profiling in *M. ursinus* illustrated the reproductive physiology and behavioural ecology of this iconic species [11, 12]. Earlier studies on the estrous cycle of *M. ursinus* has also observed an exhibition of wild instincts in captive conditions [13]. Reproductive status evaluation by hormonal monitoring for insemination and ovulation could bring successful reproduction in these animals. Even more, corticosteroid studies could be done to gather information about stress levels and so as necessary modification could be done in habitat and enrichments to adrenal activity diminution. By this method, we standardize the process of non-invasive urine sample collection is found to be less complicated in *M. ursinus*. Hence it is believed that the information obtained by this method would be invaluable for ameliorated veterinary care and in situ conservation.

Fig 1: Yellow coloured arrow indicating the floor pit made at animal's enclosure (a) and accumulation of urine in the pit after urination (b)

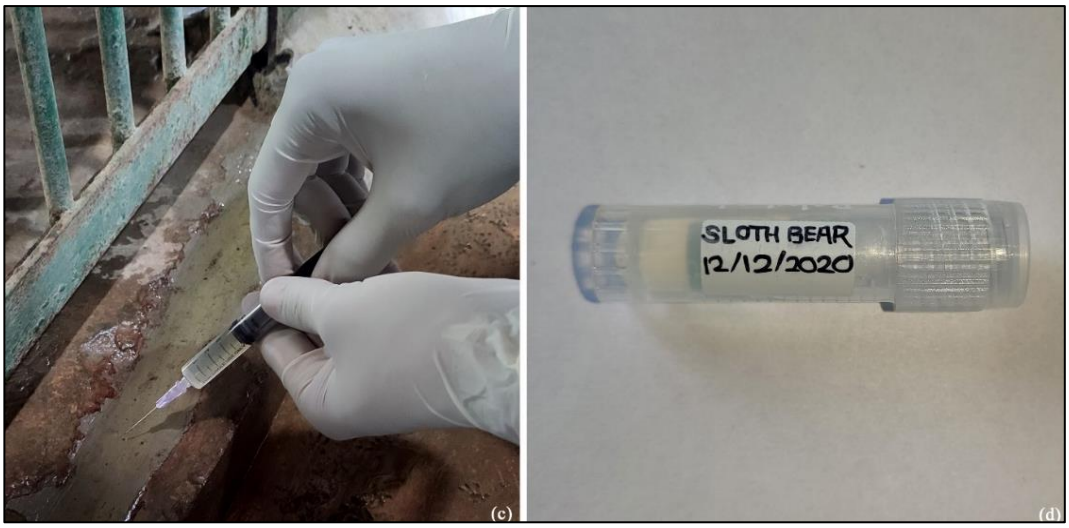

Fig 2: Urine sample collection (c) and storage in labelled air-tight screw cap vial (d)

4. Conclusion

The collection of urine sample by non-invasive method found to be an effective way to monitor metabolic condition and reproductive endocrinology. Procedure of collection may vary with respect to the local condition and the species involved. Advancement in understanding the urologic values of sloth bear will facilitate to offer high quality veterinary care and management.

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