Animal Reproduction Science 172 (2016) 173–181

Contents lists available at ScienceDirect

Animal Reproduction Science

journal homepage: www.elsevier.com/locate/anireprosci

Non-invasive monitoring of reproductive and stress hormones in the endangered red panda (*Ailurus fulgens fulgens*)

Neema Raja Beaulah Budithi[a], Vinod Kumar[a], Suneel Kumar Yalla[a], Upashna Rai[b], Govindhaswamy Umapathy[a,*]

[a] *Laboratory for the Conservation of Endangered Species, CSIR-Centre for Cellular and Molecular Biology, Uppal Road, Hyderabad 500 007, India*

[b] *Padmaja Naidu Himalayan Zoological Park, Darjeeling 734101, India*

ARTICLE INFO

Article history:
Received 28 March 2016
Received in revised form 18 July 2016
Accepted 26 July 2016
Available online 26 July 2016

Keywords:
Red panda
5α-Pregnan-3α-ol-20-one
Cortisol
Testosterone
Pregnancy detection
Faecal hormone

ABSTRACT

The red panda (*Ailurus fulgens fulgens*) is classified as endangered due to its declining population, habitat fragmentation and poaching. Efforts are being made to breed them in captivity as part of nationwide conservation breeding program. This study aimed to standardize Enzyme immunoassays (EIAs) to monitor reproductive (Progesterone metabolite, Testosterone) and stress hormone (Cortisol) in red panda. For this purpose, we collected 1471 faecal samples from four females and one male over a period of one year from Padmaja Naidu Himalayan Zoological Park, Darjeeling, India. HPLC confirmed the presence of immunoreactive 5α-pregnan-3α-ol-20-one, testosterone and cortisol metabolites in faecal samples. Using 5α-pregnan-3α-ol-20-one EIA, we were able to monitor reproduction and detect pregnancy in one of the females, which successfully conceived and delivered during the study period. We were also able to monitor testosterone and cortisol in faecal samples of the red panda. Faecal testosterone levels were found in higher concentration in breeding season than in non-breeding season. Faecal cortisol concentrations showed a negative relationship with ambient temperature and peaked during winter months in all animals. Standardization of EIAs and faecal hormone monitoring would facilitate red panda conservation breeding programs in India and elsewhere.

1. Introduction

The red Panda (*Ailurus fulgens fulgens*), an endemic species of temperate forests in the Himalayan region, is categorized endangered by IUCN Red List 2015 due to its declining population by 50% over three generations (Glatston et al., 2015). It is also listed under Schedule I species of the Indian Wild Life (Protection) Act 1972. Red panda belongs to the family of Ailuridae and comprises of two subspecies, *A. fulgens styani* and *A. fulgens fulgens*. *A. fulgens styuni* is distributed along south central China and these two subspecies are parted by the Salween (Nu Jiang) river (Roberts and Gittleman, 1984; Choudhury, 2001). Red panda is mainly confined to the Himalayas in India, Nepal, Myanmar and southern China. In India, their distribution is restricted to the North Eastern parts (Northern West Bengal, Sikkim, Arunachal Pradesh and Meghalaya) and south of the Himalayas (Choudhury, 2001). The major threats to

* Corresponding author at: CSIR-Centre for Cellular and Molecular Biology (CCMB), Uppal Road, Hyderabad 500007, India.
E-mail address: guma@ccmb.res.in (G. Umapathy).

http://dx.doi.org/10.1016/j.anireprosci.2016.07.016
0378-4320/

Table 1
Details on the duration of sample collection and mating/parturition date of the study animals in Darjeeling zoo, India.

S.No	Name of the Animal	Sex	Age of the animal (yrs on Jan 2016)	No.of Samples collected	Mating dates	Date of Parturition
1	Sheetal	Female	12.6	401	29.01.2013	28.06.2013
2	Janaki	Female	5.7	397	15.01.2013 29.01.2014	–
3	Sambridhi	Female	7.6	294	23.01.2014	04.07.2014
4	Smile	Female	3.7	325	–	
5	Rahul	Male	13.7	54	–	

its survival include habitat loss and poaching for its fur, mostly in China and Myanmar (Choudhury, 2001).

The red panda is usually nocturnal and solitary in nature (Roberts and Gittleman, 1984). It mainly feeds on bamboos which are its primary sustenance (mostly tender leaves, shoots) and it also sometimes feeds on small mammals, bird eggs, fruits and acorns (Choudhury, 2001). The red panda reaches sexual maturity at the age of 18–20 months and the first births have been recorded between 24 and 26 months of age (Roberts and Kessler, 1979). The red panda breeds during January – March in the northern hemisphere captive populations (Roberts and Kessler, 1979; Wei et al., 2005) while its breeding is in June-August in the southern population (Spanner et al., 1997). The gestation length varies widely from 114 to 158 days (Northrop and Czekala, 2011; MacDonald et al., 2005). In captivity, the red panda populations are not self-sustaining due to poor reproductive success and high mortality rate (Glatston and Roberts, 1988).

Understanding of reproductive physiology is very important for the successful captive breeding program and it is generally assessed by circulating hormones in the blood. Collection of blood samples on a regular basis from endangered animals is not always possible due to stress caused by anesthesia. Alternatively, faecal steroid hormone analysis has been practiced for a decade in a variety of animals (Schwarzenberger et al., 1996) including black rhinoceros (Schwarzenberger et al., 1993a), okapi (Schwarzenberger et al., 1993b), felids (Brown et al., 1994; Umapathy et al., 2013), Asian elephants (Kumar et al., 2014) musk deer (Mithileshwari et al., 2016), and chelonians (Umapathy et al., 2015).

Previously, radioimmunoassay was used for faecal hormone assays but due to the radioactive hazards, ELISA has become the preferred choice. Further, EIAs were now developed against major metabolites of parent hormone, as very little parent hormone is present in the faecal samples (Brown et al., 1994; Schwarzenberger et al., 1996; Umapathy et al., 2013). Earlier studies have shown that 5α-pregnan-3α-ol-20-one is one of the major metabolites of progesterone in big cats (Umapathy et al., 2013), musk deer (Mithileshwari et al., 2016), primates (Heistermann et al., 2001), Asian elephants (Ghosal et al., 2012), and it could be used to monitor reproductive status of many wild animals. Previous studies on faecal steroid hormone analysis in red panda were carried out using radioimmunoassay (RIA) against parent progesterone (Spanner et al., 1997; MacDonald et al., 2005; Wei et al., 2005). In this study, progesterone was measured using antibodies raised against one of major progesterone metabolites, 5α-pregnan-3α-ol-20-one to monitor reproduction in the red panda. Further, as a part of the conservation breeding program of the red panda, the present study aimed to validate and standardize enzyme immunoassays (EIAs) to assess the fertility status, pregnancy detection and stress level in captive red panda using faecal steroid hormones.

2. Materials and methods

2.1. Sample collection and study animals

Faecal samples of females (n = 4) and male (n = 1) were collected every day in the morning between 8–9 h from January 2013 to April 2014 at Padmaja Naidu Himalayan Zoological Park, Darjeeling, West Bengal, India. All study animals were caged separately except one female (Janaki) with another female (Sambridhi). Males were caged adjacent to female enclosures and were allowed into female enclosures during breeding season for mating. The males were separated after successful mating recorded through CCTV cameras that were installed in all directions and monitored for 24 h. Red pandas are known to defecate in a particular place of the enclosure, and using video footage, we collected the faecal samples of identified individual. Following collection, the samples were lyophilised and sent to laboratory for steroid hormone analysis. The details of the study animals are given in Table 1. All the enclosures in exhibit/display and conservation breeding centre are open enclosures provided with feeding enrichment area and also with aerial walkways, perches, sunny spots, logs and bamboo for climbing and resting for the animals. These enclosures are also equipped with a minimum of three nesting boxes made of wood covered by tin from outside. The animals were fed twice a day with cut and uncut fruits, milk, honey and eggs along with the bamboo hung upright in the enclosures, and drinking water was available ad libitum.

2.2. Extraction of faecal steroid metabolites

The faecal steroid metabolites were extracted by the procedure described previously (Brown et al., 1994; Umapathy et al., 2013). Dried faecal samples were pulverized and 0.2 g of the powder was weighed and boiled for 20 min with 5 ml of 90% ethanol. The samples were then centrifuged at RCF of 500*g* for 10 min and the supernatant was collected into a fresh glass tube. The pellet was again suspended in 5 ml of 90% ethanol, vortexed, centrifuged and the supernatant was pooled, dried completely in an oven at 40° C and resuspended in 1 ml of absolute methanol. The final faecal extract was used for the EIAs. Extraction efficiencies of faecal steroid metabolites were