Chapter-I

Effect of zoo visitor density on animal behaviour and faecal cortisol concentration of the Indian Blackbuck
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1.1 Introduction

Thousands of endangered mammals are held in captive condition for conservation in worldwide through the inbreeding programme where they are routinely exposed to the sight and sound of human audience. Over the past 20 years, great attention has been given to behavioural investigation undertaken in zoos. While basic research could be designed to test theories from ethology and behavioural ecology, but it is more applied and designed to tell us about how captive environment and the human audience could influence animal behaviour (Hosey, 1997). There has been conflicting evidence regarding the effect of zoo audiences on the lives of captive animals.

Behavioural research on zoo animals has always been an important supplement to better-known work in the field and laboratory. It is rarely argued that because of the zoo’s artificial nature the zoo’s influence on the behaviour of captive animals is difficult to assess. Recently, a number of researchers have emphasized the need for this kind of investigation (Burghardt, 1975; Moran and Sorensen, 1984). Much of the value learned from the study of animal in zoos, provided that the nature and influence of this particular environment on the animals behaviour are properly understood (Rumbaugh, 1988). Many studies, however, suggest that visitors are decremented to wild zoo animals, resulting in modulation of animal behaviour such as increased intragroup aggression, decreased exploration and stereotypes (Birke, 2002; Skyner et al., 2004; Davis et al., 2005; Hosey, 2005; Sekar et al., 2008). Venugopal and Sha (1993) reported that teasing of animals by zoo visitors may precipitate such behaviour. A decrease in social integration within captive groups and an increase in behavioural abnormalities during the presence of visitors have been reported (Mitchell et al., 1991). However, Hosey (2000) suggests that chronic exposure of captive animals to the zoo visitors reduced the stress in some species. It has also been suggested that some of these human–animal interactions could also prove enriching for the animal, especially when the zoo visiting public feed them (Hosey, 2000).
Enforced olfactory, auditory, or visual exposure to predators, aggressive conspecifics, and humans are known to induce fear behaviour and concomitant physiological responses in domestic and non-domestic animals (Blanchard et al., 1998). Moreover, inappropriate husbandry practices may lead to behavioural and physiological stress (Riley and Spackman, 1977; Wielebnowski et al., 2002a). Stress, defined as any physical or psychological stimulus that disrupts the homeostasis of the organism may contribute to this poor reproductive performance. Stress can also cause immunosuppression, disruption of metabolism and gastrointestinal dysfunction (Jayo et al., 1993; Monnikes et al., 1994). For example, in cheetahs and tigers, the occurrence of gastroenteritis has been linked to the captive environment and therefore may be associated with captive stress (Terio and Munson, 2000); and in primates, prolonged stress can suppress reproduction and can inhibit ovarian function and stimulate spontaneous abortion in females (Dunbar, 1989; Wasser et al., 1988).

Behavioural studies are the most common form of non-intrusive research used to assess the good healthy condition of animals in captivity (Mench and Mason, 2000; Mallapur and Choudhury, 2003). The presence of certain types of abnormal behaviours in an individual behavioural repertoire, for example, usually used to indicate that the individual is under stress (Moontnick and Baker, 1994). It is reported that chronic exposure of captive lion-tailed macaques exhibited several types of abnormal behaviours, of which some behavioural patterns were specifically exhibited owing to zoo visitors’ presence (Mallapur et al., 2005).

The zoo animals react to captive environments with behaviours modulated by adrenocortical response (Wells, 2005; Cockrem, 2005). Activation of the hypothalamic-pituitary adrenal (HPA) is considered to be associated with physiological stress (De Vries, 2002). Activation of the HPA axis during stress enhances the release of adrenocorticotropic hormone (ACTH) from the anterior pituitary, stimulating the synthesis and secretion of glucocorticoids from the adrenal cortices (Melmed and Kleinberg, 2003; Steward, 2003). Chronic stress and heightened levels of HPA axis hormones (i.e. glucocorticoids, ACHT and corticotrophin releasing hormone) can have detrimental effects including inhibition of normal reproductive function (Dobson and Smith, 1995), suppression of the immune system and atrophy of tissues (Steward, 2003). Elevated levels of glucocorticoids resulting from chronic stress may also cause
depression, hypertension, gastrointestinal ulceration, electrolyte imbalances, calcium loss, bone mass reduction and inhibition of growth (Breazile, 1987). Thus, improving the health and general well being of cervides in captivity requires identifying stressful environmental conditions or management practices and developing mitigating strategies.

Glucocorticoids are reported to be stress hormone that are the end product of HPA activity and are also effective indices of stress in many vertebrates (fish: Bonga, 1997; Turner et al., 2003; reptiles: Knapp and Moore, 1997; birds: Silverin, 1997; mammals: Alexander and Irvine, 1998; Turner et al., 2002). Due to the potential effects on physiological status, the measurement of glucocorticoids could serve as a valuable tool in studies of evolutionary ecology, conservation biology, and wild animal welfare. Blood samples for various measurements are restricted to use in wild captive species, because animals must typically be captured, thus potentially compromising an accurate assessment of stress (Le Maho et al., 1992; Cook et al., 2002). However, in many free-ranging cervid species, these endocrine studies are not practical and may even be dangerous due to excessive stress caused by capture and restraint (Schwarzenberger et al., 1996). Consequently, non-invasive monitoring of faecal steroid metabolite assays are now being used in a variety of disciplines, viz., animal science, behavioural ecology, conservation biology, ornithology, and primatology (Goymann et al., 2002; Ganswindt et al., 2003; Good et al., 2003). It is reported that the majority of adrenal and gonadal metabolites are excreted in faeces rather than in urine in the domestic cats (Shille et al., 1990; Graham and Brown, 1996).

In the light of these advantages, immunoassay has been widely used to measure faecal glucocorticoids and has been extensively adopted in mammals like bighorn sheep (Miller et al., 1991), domestic sheep (Palme and Mostl, 1997), chimpanzee (Whitten et al., 1997), cheetah (Jurke et al., 1998), African wild dog (Monfort et al., 1998), common marmoset (Sousa and Ziegler, 1998), spotted hyena (Goymann et al., 1999), red deer (Ingram et al., 1999), spider monkey (Davis et al., 2005), black rhinoceros (Turner et al., 2002; Carlstead and Brown, 2005) and pere davids deer (Li et al., 2007). Mostl and Palme (2002) reported that increases in levels of faecal cortisol might influence the behaviour and physiology of captive zoo animals. Stress-related behaviours in captive environments shows that the captive environment is a source of
stress (Hosey, 2005). Earlier studies have indicated that prolonged high cortisol levels could lead to loss of body weight, reproductive failure, immunosuppression, and a shorter life span (Clubb and Mason, 2003; Carlstead and Brown, 2005). Monitoring of adrenocortical and behavioural responses in captive stress has been emphasised in ex-situ conservation and animal welfare (Goymann et al., 1999; Mostl and Palme, 2002; Cockrem, 2005).

The present study investigated the behaviour and faecal cortisol concentrations in captive Indian Blackbuck under different conditions of zoo visitor density [zero level (i.e. zoo visitor absence or zoo holiday), low level, high level and extreme level (i.e. festival times)]. This study may help to understand the influence of zoo visitor density on the faecal cortisol concentration and the behavioural repertoire of this species.

1.2 Materials and Methods

1.2.0 Study area

This study was conducted in the conservation and breeding center of Arignar Anna Zoological Park (AAZP) (13°16'S and 79°54'E at an altitude of MSL+ 10m to 100m), Vandalur, Chennai, Tamilnadu, South India. Chennai has the distinction of having the first zoological park in India, which was started in 1855. In 1976, the zoo moved to the Vandalur Reserve Forest, an area of about 510 hectares near Chennai. The habitat of AAZP is considered a tropical evergreen shrub, a degraded forest mostly consisting of thorny bushes. Special features of this park include a lion and deer safari, bird aviaries, reptile and nocturnal house. Average annual rainfall is about 250 mm. Annual average temperature is 26°C. The annual average of people that visit the zoological park is 6-7 lakhs. The Blackbuck population in this park has fluctuated around 75 individuals since 2003 (14 adult males, 20 adult females, 16 sub-adult males, 18 sub-adult females and 7 young once). The primary predators of Blackbuck in this area are village dogs and jackals (Canis aureus) as well.

1.2.1 Blackbuck enclosure

All Blackbucks were housed in an outdoor enclosure of about 3.5 acres within a dry moat. Zoo visitor hours were 9 am to 6 pm every day except Tuesday (i.e. zoo holiday). The Blackbuck enclosure consists of three zones: i) the edge zone – the visitor area in which the distance between visitors and the Blackbuck is about 7 m², ii) the rear zone – the access area for zoo employees for the purpose of cleaning and feeding (twice
daily) and, iii) the enrichment zone - a central area with trees, food and water troughs and sheds.

1.2.2 Zoo visitor’s density

Visitor density can be defined as the number of people admitted to the Arignar Anna Zoological Park on a given day. Systematic data was not available on the number of visitors to the Blackbuck zone. The study was carried out in four different conditions: (1) zero visitor density where visitors were absent (i.e. zoo holiday), (2) low visitor density [weekdays; where the mean daily number of visitors to the zoo was 1262.46±149.81 (range: 528 to 1774)], (3) high visitor density [weekend; mean daily number of visitors to the zoo was 3381.66±204.38 (range: 2632 to 4677)] and (4) extreme visitor density [festival times; mean daily number of visitors to zoo was 10379.93± 2054.24 (range: 4851 to 36886)].

1.2.3 Behavioural observation

During behavioural observation, eight adult Blackbucks were monitored, whose individual identification was recognized by variation in coat colour, length of horn and some morphological characters (scar, broken horn, damaged ear pinna, etc.) (Table 1.0). Blackbucks were observed for 360 h over a period of approximately 12 months. Each animal was observed about 15 days separately for 6 h per day to record each of the behaviour at each visitor density. Observations were maintained at the same time of day (i.e. 10.00 am to 1.00 pm and 2.00 pm to 5.00 pm) for all animals, to prevent any inconsistent exposure to extraneous events in the environment (e.g. feeding and cleaning). Each Blackbuck behaviour (moving, resting, reproductive, social and aggressive behaviours) was recorded every 5 min over the 6 h per day for each condition using scan-sampling techniques (Altmann, 1974). At every sample point, the behavioural state of each individual was recorded systematically according to an ethogram. The following behavioural variables were monitored in each condition of zoo visitor density:

Moving behaviour: walk and run.

Resting behaviour: sitting, standing and lying down.

Social behaviour: grooming, horn bushing, playing and scent marking.

Reproductive behaviour: approach, Flehmen, mounting and sniffing.

Aggressive behaviour: fighting and chasing other individuals.
1.2.4 Collection of faecal materials

The faecal samples were collected for each condition of visitor density from each individual at intervals of every 5 episodes and were sealed in plastic bags. Samples were immediately stored at -20°C until further analysis.

1.2.5 Extraction of steroids for radioimmunoassay

Faecal samples were lyophilized for 24 h (Model LGA05, MLW. Leipzig, Germany). Dried faeces were then pulverized in a blender and all solid inert materials (e.g. seeds and rough dietary fiber) were removed. Steroids were extracted according to the modified method described by Palme et al. (1998), in which a proportion of the resulting powder (0.5g) was weighed and extracted with 5 ml of 80% methanol. After overtaxing for 30 s at high speed, the sample was shaken for 12 h on a mechanical shaker and then centrifuged at 700 x g for 15 min at 4°C; the resulting supernatant was collected into a clean tube. An aliquot of the supernatant (100 μl) was diluted 1:16 with assay buffer (20 mMol. trishydroxyaminomethan, 0.3 Mol. NaCl, 0.1% bovine serum albumin, and 0.1% Tween 80; pH 7.5) for radioimmunoassay (RIA) analysis.

Levels of Cortisol were measured in all faecal samples using a modified RIA, as described by Palme et al. (1998) and Huber et al. (2003). Intra and inter-assay coefficients of variation for Cortisol were calculated at less than 5.0% (n=24) and 14.4% (n=24) respectively.

1.2.6 Statistical methods

The total frequency of each behaviour was summed for each condition, providing an overall frequency count per Blackbuck for each behaviour. A Chi-square test was subsequently used for each behavioural category to determine whether the behaviour was influenced by zoo visitor density. The data for the faecal cortisol concentration was measured using the one-factor analysis of variance (ANOVA) (SPSS statistical software 11th version) to compare the cortisol level across the four categories of zoo visitor density. p<0.05 was considered as significantly different for all statistical tests.
1.3 Results

Visitor density had a significant effect on five of the eight Blackbuck behaviours that were recorded such as moving, resting, reproductive, social, and aggressive behaviours (Fig. 1.0). Animals spent significantly more ($\chi^2 = 34.19$, df= 21, $p<0.025$) time resting during the periods of zero and low levels of visitor density than during high and extreme densities. Extreme and high levels of visitor density influenced moving ($\chi^2 = 38.53$, df= 21, $p<0.01$) and aggressive ($\chi^2 = 31.73$, df= 21, $p<0.05$) behaviours more than the zero and low visitor densities. There was no significant effect of visitor density observed for the frequency of reproductive ($\chi^2 = 28.2$, df= 21, $p>0.100$) or social behaviours ($\chi^2 = 29.67$, df= 21, $p>0.100$).

The mean value of faecal cortisol was recorded at the zero (35.44±4.84 ng/g), low (55.07±5.57 ng/g), high (113.51±3.70 ng/g), and extreme (137.30±5.88 ng/g) levels of zoo visitor density. The one-way ANOVA with post hoc comparison (DMRT) test clearly showed that average Blackbuck faecal cortisol concentration was significantly higher ($F_{0.001, 3, 28} = 99.633$) during the extreme and high levels of zoo visitor density than during the zero and low levels (Tables 1.1 and 1.2).

1.4 Discussion

For many years, human audiences and tourists have been permitted into sanctuary, wildlife, and zoological parks, facilitating awareness about the importance of wild animal conservation and management (Cox, 1993). Since 1970s, research on the effects of zoo visitors on the welfare of wild zoo animals has intensified. Numerous studies have shown that characteristics such as visitor presence, density, activity, and position can affect zoo animal behaviour and, to some extent, physiology. It has reviewed the interactions between human audiences and captive wild zoo animals (Bolton, 1997). These studies showed variable results, where some zoo animals were tolerant of large number of visitors, and others were negatively impacted. For instance, the exposure to visitors resulted in higher levels of stereotyped locomotion, masturbation, and leg/hair pulling in captive mandrills. Furthermore, adult orangutans covered their heads with paper sacks and infants clinched to adults more often (Birke, 2002); there were also increased levels of urinary cortisol in spider monkeys (Davis et al., 2005) when they were exposed to high visitor densities. Investigation of green monkeys at a Mexico zoo, Fa (1989) found that locomotion and
aggressive behaviours were not seen, but the public fed these animals, such that zoo visitors were not perceived as stressful.

The finding of the present study indicates that the zoo visitors found to significantly influence the behaviour in captive Indian Blackbucks. The results of the present study are consistent with previous reports that the presence of visitors increases disturbance in a number of captive, wild animal species such as the following:

4. Sloth bear, *Ursus ursinus* (Forthman and Bakeman, 1992),
5. Chimpanzee, *Pan troglodytes* (Cook and Hosey, 1995),
7. Red deer, *Cervus elaphus* (Ingram *et al.*, 1999),
8. Roe deer, *Capreolus capreolus* (Dehnhard *et al.*, 2001),
14. White, *Ceratotherium simum* and Black rhinoceros, *Diceros bicornis* (Carlstein and Brown, 2005),
15. Spider monkey, *Ateles geoffroyii rufiventris* (Davis *et al.*, 2005),
16. Lion–tailed macaques, *Macaca silenus* (Mallapur *et al.*, 2005),
18. Peres davids deer, *Elephurus davidianus* (Li *et al.*, 2007),
19. Indian bison, *Bos Gaurus Gaurus* (Sekar *et al.*, 2008);

There are a few data on changes in the animal behaviour when the public are present, but levels of visitor-directed aggression are high, mostly as a consequence of people being bitten by monkeys they have provoked (Fa, 1991). In comparison, Fa (1992) reported that people-directed aggression is very low in semi-natural macaque
enclosures elsewhere in Europe; he also pointed out that feeding by the public has had an adverse effect on the reproduction of the Gibraltar macaque, and warned of the possibility of this as well as visitor-directed aggression in zoo primates.

It was suggested long ago by Morris (1964) that visitors might provide zoo animals with welcome variability in an otherwise monotonous existence, but the possibility that audience might be enriching for some zoo captive animals has not really been tested. However, the green monkeys, which showed to visitor-related increase in agonism were fed with thrown food by members of the public. Similarly, Cook and Hosey (1995) found that chimpanzees were willing to enter into fairly long interaction sequences with members of the public, when the only apparent reward was an occasional piece of food being thrown.

Aggressive behaviour which is considered to be the result of competition for resources and social dominance is largely related to social stress, and captivity usually amplifies the social stress in animals (Carlstead, 1996). The Blackbuck is engaged in more intergroup aggression and moved more during days of extreme and high visitors’ density than that of zero and low levels. Wormell et al. (1996) and Birke (2002) suggested that the aggressive behaviour might be increased or decreased under audience conditions. A detailed study by Mitchell et al. (1992) on golden bellied mangabeys showed that the number of animals increased visitor-directed and within-group aggression when the audience were present, but affiliative behaviour (e.g. grooming) was largely unchanged. Moreover, the male and female monkeys directed their aggression to different targets during human audience presence, implying that the humans are agonistic competitors. Mostl and Palme (2002) reported that aggressive behaviour is associated with glucocorticoid secretion and that increased cortisol may result an increased aggressive behavior. Creel (2001) reported that agonistic interaction provoked large and persistent increase in cortisol secretion.

Based on the present observation, as well as the one-way ANOVA test, it seems that elevated higher concentration of faecal cortisol levels are associated with extreme and high levels of zoo visitors density. Similarly, a high faecal cortisol was found in black rhinoceros in zoos where the animals have been kept in enclosures with a great degree of public exposure (Carlstead and Brown, 2005). Many researchers suggested that adrenocorticol hormones are the indicators of stress (Mostl and Palme, 2002). It is
also inferred from the present study that during extreme and high visitors density, the Blackbuck showed high faecal cortisol concentration as well as high frequency of moving and aggressive behaviours. Li et al. (2007) reported that the size of the animal enclosure, animal density and human audience could significantly influence the behaviour and adrenocorticol secretion in Peres David’s deer. Wingfield and Sapolsky (2003) reported that prolonged periods of high cortisol concentrations and social turbulence caused by severe chronic stress appear to be major factors that inhibit growth and suppress reproductive function. In contrast, significantly lower levels of faecal corticoids have been observed for white rhinoceros when the keepers were rated highly in terms of friendliness (Carlstead and Brown, 2005).

The present study, the sole concern was exploring the effect of zoo visitor density within the zoo setting on Blackbuck behaviour and levels of faecal cortisol. Further study is also needed to examine more closely the effects of visitor behaviour on the faecal cortisol and behaviour of zoo animals. In Indian zoos, disturbances by zoo visitors that are continuously high may be due to the lack of well-established conservation and animal welfare awareness programmes. Practices like shouting, teasing, feeding, and even physical harm are common animal experience in most Indian zoos (Venugopal and Sha, 1993). Such disturbances have been shown to adversely influence the behaviour of wild animals in captivity (Kratochvil and Schwammar, 1997; Birke, 2002). The problem may be solved in recommending to the zoo to set up a notice outside the enclosure or cage asking the public to co-operate and not attempt to tease or interact with the animals, which subsequently affect the animal welfare.

The findings of the present study suggest that Blackbucks are excited to the point of stress induction by extreme and high levels of zoo visitor density. The zoo visitors probably provide a unique and complex form of stimulation for the wild zoo animals, thereby leading to a potential negative impact on our subjects. Many researches suggested that adrenocortisol hormones are indicators of stress in many wild zoo animals (Mostl and Palme, 2002). The results of the present study further confirm the value of non-invasive faecal hormone monitoring as a powerful tool for evaluating adrenal activity in Blackbuck. Based on the present and previous results, it can be recommended to maintain a sufficient distance between zoo audience sites and animal enclosures so that the animals’ welfare is protected. Furthermore, assessment of
problems related to animal health, physiology and prevention to the problems would be greatly helpful to enhance the reproductive efficiency and animal-well being of captive animals.

1.5 Summary

- The present investigation indicates that the zoo visitor density significantly influenced the behaviour and faecal cortisol secretion in Blackbuck.
- The frequency of aggressive and moving behaviours, was greatly influenced during extreme and high levels of zoo visitor density.
- The results of the present study further confirm the value of non-invasive faecal cortisol monitoring as a powerful tool for evaluating adrenal activity in Blackbuck.
- The present study suggests that the long-term physiological stress and increase of conflict behaviour may subsequently affect survival and reproduction of Indian Blackbuck.
- Moreover, other measures of animal health and physiology may allow zoologists to support to determine what actually constitutes well-being for wildlife maintained \textit{ex situ}, which in turn may facilitate improved reproductive success and a higher likelihood of establishing self-sustaining captive population.
Table 1.0 Classification and special identification character of adult male Blackbuck

<table>
<thead>
<tr>
<th>Animal Number</th>
<th>Number of twists in horns</th>
<th>Special identification characters</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4*</td>
<td>Narrow horns are narrow with pointed tips; a large size mole in the lower abdomen on left side.</td>
</tr>
<tr>
<td>2</td>
<td>3*</td>
<td>Almost parallel horns with pointed tips.</td>
</tr>
<tr>
<td>3</td>
<td>3*</td>
<td>Left horn normal; right horn damaged and half bent.</td>
</tr>
<tr>
<td>4</td>
<td>4*</td>
<td>Slightly twisted narrow horns with their tips curve pointing each other</td>
</tr>
<tr>
<td>5</td>
<td>3*</td>
<td>Broad and compressed horn; tip of the right horn curved outward</td>
</tr>
<tr>
<td>6</td>
<td>3*</td>
<td>Long broad horns with their tips curved outward</td>
</tr>
<tr>
<td>7</td>
<td>3*</td>
<td>Broad horns with their tips curved inward</td>
</tr>
<tr>
<td>8</td>
<td>3*</td>
<td>Long narrow horns with their tips curved inward</td>
</tr>
</tbody>
</table>

* Thick solid horn
Table 1.1  ANOVA with post hoc comparison (one-way) of faecal cortisol concentration in four conditions of zoo visitor density

<table>
<thead>
<tr>
<th></th>
<th>Sum of Squares</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between groups</td>
<td>55235.067</td>
<td>3</td>
<td>18411.689</td>
<td>99.633</td>
<td>0.000*</td>
</tr>
<tr>
<td>Within groups</td>
<td>5174.261</td>
<td>28</td>
<td>184.795</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>60409.328</td>
<td>31</td>
<td>99.633</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The means were compared using DMRT. The means gain score of zero, low, high and extreme visitor density interns of faecal cortisol concentration of 8 identified male Blackbuck is statistically significant. * High statistically significant (p<0.001)

Table 1.2  Comparison of means from Table 1.1 using DMRT Post hoc tests

<table>
<thead>
<tr>
<th>VAR00001</th>
<th>Subset for alpha =0.05</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Zero visitor density</td>
<td>35.4475</td>
</tr>
<tr>
<td>Low visitor density</td>
<td>54.9875</td>
</tr>
<tr>
<td>High visitor density</td>
<td>113.5113</td>
</tr>
<tr>
<td>Extreme visitor density</td>
<td>137.3038</td>
</tr>
<tr>
<td>Significance</td>
<td>1.000</td>
</tr>
</tbody>
</table>

Means for groups in homogeneous subsets are displayed.
Means within a subset are not different from each other.
Fig. 1.0 Mean incidence of specific Blackbuck (n=8 animals) behaviours associated with different zoo visitor densities. Behaviours like moving (MB), resting (RB), social (SB), reproductive (REB) and aggressive (AB) during zero (ZVD), low (LVD), high (HVD), and extreme level of zoo visitor density (EVD). Behaviours significantly influenced by zoo visitor density is highlighted with an asterisk (*).