

ORIGINAL ARTICLE

Assessment of stress due to hot ambience in donkeys from arid tracts in India

Kataria N.^{1*}, Kataria A.K.²

¹ *Department of Veterinary physiology, College of Veterinary and Animal Science, Rajasthan University of Veterinary and Animal Sciences, Bikaner – 334 001, Rajasthan, India*

² *Apex Centre for Animal Disease Investigation, Monitoring and Surveillance, College of Veterinary and Animal Science, Rajasthan University of Veterinary and Animal Sciences, Bikaner – 334 001, Rajasthan, India*

*Phone 0091 – 151- 2546399

*Email- nalinikataria@rediffmail.com

Received September 7, 2010

To assess the stress due to hot ambience in donkeys from arid tracts in Rajasthan state, India, serum prolactin and cortisol levels were determined by radioimmunoassay. The blood samples to harvest the serum were collected from the same animals during moderate (maximum temperature of 28°C - 29°C) and hot (maximum temperature of 45°C- 46°C) ambiances. During hot ambience the animals showed significantly ($p \leq 0.05$) higher levels of serum prolactin and cortisol when compared to the moderate ambience. The mean rise in prolactin was 4.42 times whereas cortisol levels were 4.22 times higher. Further a multiple fold rise in serum prolactin clearly suggested that it can also be used as an indicator of stress in donkeys along with the cortisol.

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Stress can disturb the physiological functioning of an animal. Extreme hot ambience causes significant stress for all animals. There are many measurable parameters of stress and cortisol hormone is considered as a key indicator. Cortisol is released in response to stress and it is responsible

for many stress related physiological changes in the body. Higher and prolonged levels of cortisol in the blood have been shown to have negative effects on the body leading to a condition of chronic stress. Research now indicates that the prolactin (PRL) hormone, released from the anterior pituitary, may

also be an indicator of stress in animals (Kataria and Kataria, 2010). Prolactin has also been shown to influence cortisol secretion (Drago *et al.*, 1985). Stress induced PRL secretion in animals and humans signifies its role in general adaptation syndrome (Fava and Guaraldi 1987) and it plays a role in the correction of stress, the immune system and thermal regulation (Ahmadzadeh *et al.*, 2006). Prolactin is now being used as a stress marker in humans (Sobrinho, 2003) and animals (Kataria and Kataria, 2010).

Donkeys are amongst the oldest companion of man. They are very hardy animals and can work incessantly with little rest and on poor forage. They are mainly used as a pack and draught animals. Although they are major asset to human lives in arid areas but their physical needs are always ignored in the name of being hardy animals. It is important to protect these animals from harsh elements of the climate i.e. extreme heat and cold, so that they can be saved from stress. It is important to know the basic physiological attributes of these animals in order to reduce likelihood of the diseases. It is a melancholy that despite of immense significance of these animals in the livelihood of poor people, very little scientific attention has been paid to detect the stress.

Proper management of these animals is important to protect them from ill effects of high ambient temperature. The emerging concepts of management for these animals include early detection of stress and its timely alleviation. If levels of stress are continuously elevated for prolonged periods, physiological modulations may turn into pathological consequences. It is unfortunate that little attention has been paid to either the stress factors or health of the donkeys. To ensure optimum management of these animals in arid tracts, it is vital to assess levels of stress.

Therefore the present study was carried out to assess stress due to hot ambience in donkeys from arid tracts.

MATERIALS AND METHODS

Serum prolactin (PRL) and cortisol levels were determined in donkeys found in the arid tracts of Rajasthan state, India. The animals were maintained in the similar type of management and feeding conditions. Blood samples were collected as a part of routine health check-up during moderate ambience (maximum temperature of 28°C - 29°C) and hot ambience (maximum temperature of 45°C-46°C). These animals were free from endo-parasites as assessed by routine faecal examination. The samples were collected from adult male animals. Same animals (20) were used in the study.

All the samples were collected in sterile tubes without anticoagulants for serum separation. The serum PRL was determined by immunoradiometric assay using RIA kit (IRMA CT, RADIM, Italy) as per the manufacturers protocol. The method employed the use of two anti-PRL monoclonal antibodies which recognised two different epitopes of the molecule. One antibody was adsorbed in solid phase in the coated tube (mouse monoclonal anti-PRL antibody) and the other as radioactive conjugate labelled with iodine-125 (¹²⁵I anti-PRL mouse monoclonal antibody in serum matrix). The serum samples and labelled antibodies were incubated simultaneously in the coated tubes. The amount of bound conjugate was directly proportional to the hormone concentration in the sample and standard. At the end of the incubation period, the unbound material was removed by an aspiration and washing cycle (Tris-HCl and Tween 20). The radioactivity in the tubes was measured in a ¹²⁵I Gamma counter (ECIL, India).

Serum cortisol was determined using the Gamma coat (¹²⁵I) cortisol radioimmunoassay kit

procedure based on the competitive binding principles of radioimmunoassay (DiaSorin, USA). Serum samples and standards were incubated with cortisol tracer in antibody-coated tubes (Rabbit anti-cortisol serum coated) where the antibody was immobilised onto the lower inner wall of the Gamma Coat Tube. After incubation the contents of

the tubes were decanted and the tubes were placed in a ^{125}I Gamma counter (ECIL, India).

Statistical significance for individual parameter between healthy and affected group was analysed according to Snedecor and Cochran (1967).

RESULTS AND DISCUSSION

The mean \pm SEM values of serum PRL and cortisol in the donkeys are presented in table 1.

Table 1. Serum levels of prolactin and cortisol in donkeys

Ambiences	Prolactin (pmol/L)	Cortisol (nmol/L)
Moderate (n=20)	747.91 \pm 19.6	24.63 \pm 2.09
Hot (n=20)	3311.30 \pm 130.21 ^d	103.97 \pm 5.43 ^d

1. Figures in the parentheses indicate number of animals.
2. Superscript 'd' indicates that a given parameter differs significantly ($p \leq 0.05$) from moderate mean value.

There is paucity of literature regarding information on serum PRL levels in the donkeys. The mean value of serum PRL in donkeys was compared with the values available in other animals (Ahmadzadeh *et al.* 2006 and Kataria and Kataria, 2010). Earlier workers have reported the use of commercially available human radioimmunoassay (RIA) kits (Al-Qarawi and El-Mougy, 2008) for PRL determination as carried out in the present study.

During hot ambience significantly ($p \leq 0.05$) higher levels of serum PRL and cortisol were observed as compared to the moderate ambience. The mean rise in serum PRL and cortisol levels during hot ambience was 4.42 fold and 4.22 fold,

respectively. A significant ($p \leq 0.05$) correlation ($r=0.967$) between the PRL and cortisol was observed during hot ambience which showed similar pattern of changes in markers of stress. Hot ambience associated variation in serum PRL was accompanied by a rise in serum cortisol levels, a well documented marker of stress in animals (Kataria *et al.* 2000). Research in sheep also related the higher serum PRL levels with stress in animals (Kataria and Kataria, 2010).

Rising trends of serum PRL and cortisol in donkeys was in accordance with earlier reports in stressed animals (Ahmadzadeh *et al.* 2006). The reported average increase in PRL levels in affected sheep was 4.72 fold while reports in stressed rats

suggested a 10-14 fold increase in PRL secretion (Kjaer *et al.*1994). Though cortisol is an important marker of stress (Kataria *et al.*, 2000), in the present study it appears that PRL is also significant for physiological adjustments of stress in donkeys. Stress factors probably produce a complex set of hormonal and metabolic changes which are evoked by anxiety or handling (Reis *et al.*1998).

Hot ambience produces a cascade of changes in the physiological mechanisms of the animals to withstand the stress. Higher cortisol concentration substantiated the significance in meeting the energy crisis during physical stress, since cortisol increases glucose supply, by the glucogenolytic and gluconeogenetic properties (Kataria *et al.*, 2000). In animals elevated blood concentrations of cortisol have been accepted as an indicator of acute stress. Stress is an important stimulus for the release of CRH and hence the release of ACTH (Kataria *et al.*, 2000). Heat stress through photoperiodic response is generally coupled with low quality nutrition and is found to be associated with increased PRL secretion (Brown and Forbes 1980). It is important to understand the patho-physiology of stress induced PRL release. The stress-induced PRL release is a rapid and strong response of the body (Noel *et al.*1972), also indicating towards defensive behaviour (Drago *et al.* 1982) and immuno modulatory influence on the immune system (Reifen *et al.* 1997).It can be inferred that increase in serum PRL level can also be used as a marker of coping strategies to stress (Sobrinho 2003) along with cortisol. Therefore PRL can also serve as a sensitive marker of both physical and psychological stress (Gala 1990). Kataria and Kataria (2010) suggested that a multiple fold rise in serum PRL was an indicator of stress in different pathologies in sheep

It was concluded that the increase in serum PRL and cortisol levels in donkeys was to attenuate the homeostatic disruption. The rising levels of the hormones were suggestive of stress adaptation to unfavourable ambience. Higher serum PRL during hot ambience suggests its use as an indicator of stress in donkeys along with cortisol.

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