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ASSOCIATION BETWEEN THE LEVELS OF STRESS MARKERS AND THE ONSET OF KANGAROO DISEASE (LUMPY JAW DISEASE) IN CAPTIVE KANGAROOS

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A B S T R A C T

Kangaroo disease (lumpy jaw disease; LJD) is a disease of the oral cavity in Macropodidae that may be caused by stress-related factors; however, detailed information about its pathogenesis is lacking. Therefore, in this study, we evaluated markers of stress in kangaroos with and without LJD to determine the factors that cause an LJD outbreak. We evaluated the oxidative stress value, antioxidant activity, and plasma cortisol concentration in blood samples. Additionally, we measured the cortisol concentration in saliva samples. The oxidative stress value and serum cortisol concentrations were statistically significantly different between the two groups, but the antioxidant activity and saliva cortisol concentrations did not differ significantly. Relatively large variations were observed for each value within individuals.

Keywords: Antioxidant activity, cortisol, kangaroo, lumpy jaw disease, oxidative stress.

INTRODUCTION

Kangaroo disease (Lumpy Jaw Disease; LJD) is an oral disease that affects animals of the Macropodidae family. This disease is known by several other names, including lumpy jaw, actinomycosis, nocardial mycosis, and dermatophilus congolensis (Antiabong *et al.*, 2013; Blanden *et al.*, 1987). Infection is considered a cause of the disease, and the main pathogenic bacteria are thought to be staphylococcus (*Bacteroides* sp.), streptococcus (*Corynebacterium pyogenes*), necrobacillosis (*Fusobacterium necrophorum*), mycobacteria, and actinomyoes (Antiabong *et al.*, 2013; Blanden *et al.*, 1987). LJD occurs in both wild and captive environments, but it is said to be more common among captive animals (Antiabong *et al.*, 2013; Bradley *et al.*, 1980; King and Bradshaw, 2010). In both wild and captive animals, the disease occurs in areas that are susceptible to contamination by feces and develops when lacerations or abrasions become infected after animals come into contact with feces (Antiabong *et al.*, 2013; Blanden *et al.*, 1987). The most commonly infected tissue is the periodontium due to the specific process by which kangaroo molars erupt, grow, and fall out. When a molar erupts or drops out, the outer skin is broken, creating an environment that is susceptible to infection (Antiabong *et al.*, 2013; Blanden *et al.*, 1987). The chronic symptoms include local swelling of the infection site, abscess with a frequent discharge of foul-smelling green pus, lack of appetite, and loss of vitality. Furthermore, pain in the oral cavity and other factors can leave animals unable to eat, causing death in some cases. Treatments that can elicit a complete cure have yet to be found, so palliative care is generally provided in the form of administering antibiotics, anti-inflammatory agents, and other drugs; discharging pus under anesthesia; and tooth extraction (Antiabong *et al.*, 2013; Bradley *et al.*, 1980; King and Bradshaw, 2010). A relationship between the onset of the disease and stress has been strongly suggested in captive animals, but many details related to the mechanism of onset remain unclear. Therefore, the objective of this study was to examine one possible cause of LJD by comparing the differences in the levels of stress-related markers in healthy and LJD animals raised in zoos.

MATERIALS AND METHODS

Venous blood and saliva samples were taken from eastern grey kangaroos (healthy group: n = 12, LJD group: n = 13) and yellow-footed rock-wallabies (healthy: n = 1, LJD: n = 12) at Itozu no Mori Zoological Park and Hibiki Animal World in Kitakyushu, Fukuoka Prefecture, Japan. The blood samples were transferred to tubes containing heparin and then centrifuged (3,000 revolutions, 5 min, 4°C) to separate the plasma. The plasma was used to measure the antioxidant activity, oxidative stress, and blood cortisol levels. Saliva samples were collected by placing a cotton swab in each animal's mouth for 2 to 3 min. The cotton swabs were then placed in spitz tubes and centrifuged (3,000 revolutions, 15 min), and the extract was dispensed into Eppendorf tubes to measure the salivary cortisol. All samples were kept in frozen storage at -30°C until the measurements were performed. A free radical analyzer (FREE Carpe Diem, Wismerll Co., Tokyo) was used to perform Biological Antioxidant Potential (BAP) tests. The Reactive Oxygen Metabolites Test (d-ROMs test) was performed using the same free radical analyzer. The blood concentrations of several hydroperoxides are expressed in numerical values, and the arbitrary unit U.CARR is used to express the results. We divided the antioxidant activity by the oxidative stress (BAP/d-ROMs) to determine the latent antioxidant activity. The blood cortisol levels were measured using an electro-chemiluminescence immunoassay (ECLIA). Additionally, salivary cortisol was measured with an enzyme-linked immunosorbent assay (ELISA) using a cortisol enzyme immunoassay kit (Arbor Assays, Michigan USA). The means and standard deviations were calculated for all samples measured. A t-test was used to compare the healthy and LJD groups. In all cases, $p < 0.05$ was considered a statistically significant difference.

RESULTS AND DISCUSSIONS

The LJD group had significantly higher d-ROMs and blood cortisol levels than the healthy group (Figure 1, 2). The BAP levels were not significantly different between the two groups (Figure 3).

The value of BAP/d-ROMs was significantly lower in the LJD group compared with the healthy group (Figure 5). The salivary cortisol levels were not significantly different between the groups (Figure 4), but the LJD group tended to have lower levels (Figure 5). The d-ROM levels were significantly higher in the LJD group compared with the healthy group.

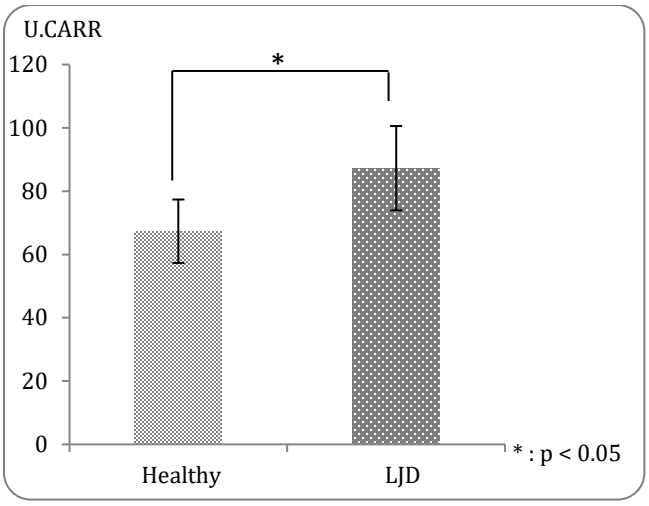

Figure 1. BAP levels in kangaroos with and without lumpy jaw disease (LJD).

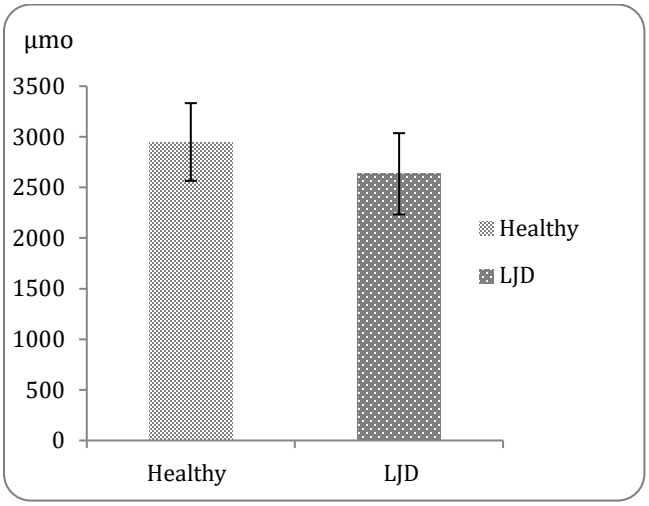

Figure 2. d-ROMs levels in kangaroos with and without lumpy jaw disease (LJD).

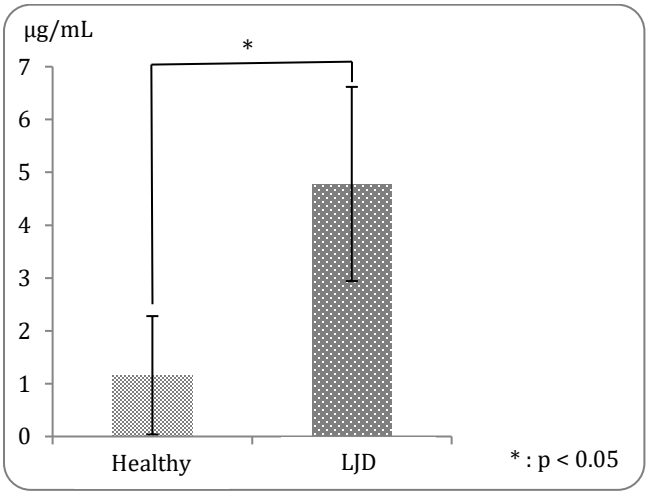

Figure 3. Serum cortisol levels in kangaroos with and without lumpy jaw disease (LJD).

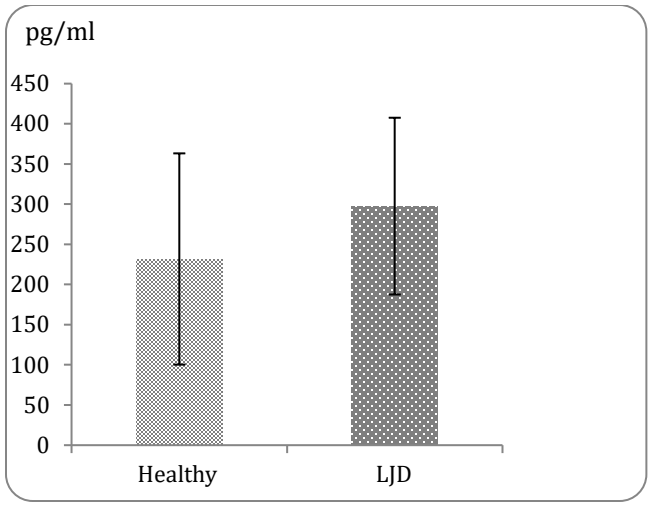

Figure 4. BAP/d-ROMs ratios in kangaroos with and without lumpy jaw disease (LJD).

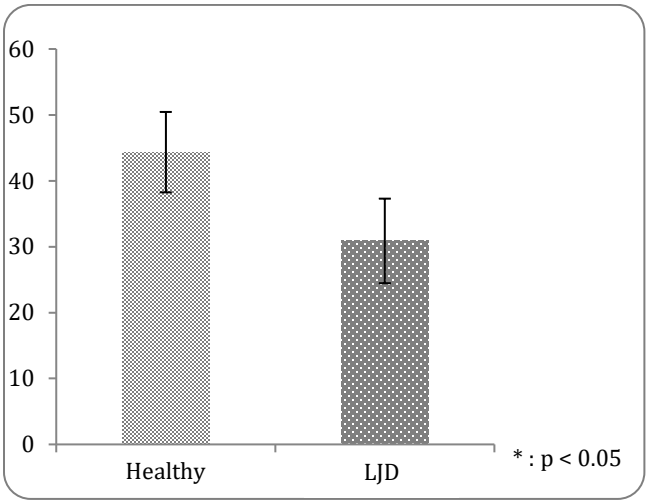

Figure 5. Salivary cortisol levels in kangaroos with and without lumpy jaw disease (LJD).

It is possible that the tumors and inflammations that form in the oral cavity and elsewhere due to LJD caused the levels of active oxygen and free radicals to rise, increased the occurrence of severe inflammatory reactions, and elevated the level of oxidative stress. However, no significant difference was observed in the BAP levels between the healthy and LJD groups in this study, the LJD group tended to have lower levels, suggesting that the antioxidant activity was also reduced in the LJD animals. The antioxidant activity can change based on a variety of factors; a lower resistance due to the presence of disease or other factors is a major contributor, as are insufficient vitamin intake, dietary content, and exercise (Byers and Perry, 1992; Puterman *et al.*, 2010). A variety of external factors may have contributed to the lower BAP levels in the LJD group. For instance, if a tumor in the oral cavity prevents an animal from feeding properly, a discrepancy in the intake of external antioxidants may arise. Additionally, the isolation of severe cases of LJD may have an effect. The latent antioxidant activity expressed as BAP/d-ROMs was significantly lower in the LJD group. This indicates a disruption of the homeostatic mechanisms that protect the oxidative balance. The blood cortisol levels were significantly higher in the LJD group. A significant difference was not observed in salivary cortisol levels, though the levels did tend to be higher in the LJD group. Cortisol is an adrenocortical hormone of the glucocorticoid class. An essential hormone in living things, cortisol is involved in the metabolism of glucose, proteins, lipids, and electrolytes, as well as bone metabolism and the immune mechanism. As cortisol responds acutely to stress, it is often used to evaluate stress (Hellhammer *et al.*, 2009). Consistent with our previous findings, the significantly higher blood cortisol levels in the LJD group may indicate a significantly higher stress response compared to the healthy group. This may be due to factors such as the inability to feed without pain or inflammation of the affected areas. However, cortisol levels fluctuate throughout the day and are greatly affected by factors such as the amount of stress that is experienced at the time samples are collected (McKenzie and Deane, 2003; McKenzie and Deane, 2005; McKenzie *et al.*, 2004). It is possible that different sampling times and durations, as well as differences in individual stress levels during sampling, greatly impacted the results. In the future, when describing such measurements, factors that could influence the evaluation of the subjects' state should also be examined in detail. Many aspects of LJD are still being investigated, including its causes and treatments. Our findings regarding changes in the levels of stress-related substances may be useful for elucidating the onset mechanism or other aspects of this disease. More multifaceted investigations should be performed, such as surveys of the differences between wild and captive animals, including differences related to the various rearing environments in zoos. The relationship between LJD severity and fluctuations in the levels of each substance, correlations with the course leading to disease onset, and correlations with the clinical symptoms and behavioral changes should also be investigated.

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