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Stress assessment using hair cortisol of kangaroos affected by the lumpy jaw disease

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ABSTRACT. The aim of this study was to objectively assess stress of kangaroos affected by lumpy jaw disease (LJD) using plasma and hair cortisol concentrations. The plasma and hair samples were collected from kangaroos with LJD and healthy controls. Collected hair samples were extracted with methanol after washing with isopropanol, following which they were processed with the cortisol enzyme immunoassay kit. The plasma cortisol concentration of LJD animals tended to be higher than that of the control. Ventral hair cortisol, but not dorsal hair, of LJD animals was significantly higher than that of the control. In conclusion, stress in kangaroos infected with LJD could be assessed by measuring ventral hair cortisol.

KEY WORDS: hair cortisol, kangaroo, lumpy jaw disease, objectively assessment, stress

Lumpy jaw disease (LJD) is an oral pyogranulomatous osteomyelitis generally contracted by humans [15], sheep [10] and macropods [1], and is presumably caused by infection with *Fusobacterium necrophorum*, *Corynebacterium pyogenes*, *Dichelobacter nodosus* and *Actinomyces* [1, 12]. The clinical symptoms of LJD in macropods include swelling of the infection site, discharge of green pus, inappetence and loss of vitality, and individuals with LJD may die from starvation due to being unable to eat because of pain. LJD affects animals in both natural and captive environments [1, 4]; however, the relationship between LJD and stress is emphasized under a captive environment [5, 14]. There have been attempts to identify the pathogen, to investigate the clinical symptoms and to survey therapeutic methods; however, many unknown aspects of the disease remain.

Blood cortisol is commonly used in many animals including kangaroos [14] as an objective index of stress, because it is secreted immediately under various types of stress. However, this index requires frequent collection of blood, resulting in the drawing of blood itself contributing to stress. Therefore, there is increasing focus on the effectiveness of stress assessment using cortisol in feces, urine and saliva, which are more easily collected [17, 19, 20, 22]. Furthermore, due to the influence of circadian rhythm, timing of the collection needs to be considered. Recently, however, it was reported that cortisol can be detected in hair [9]. Because cortisol in hair is mainly supplied from the capillary around the hair follicle [2, 21], cortisol in the hair reflects the blood cortisol concentration of the period when the hair was produced. Therefore, chronic stress can be assessed by measuring cortisol content in hair [16].

Although the relationship between LJD and stress has been pointed out in macropods in captivity [5, 14], the mechanism responsible remains incompletely understood. It is hypothesized that the evaluation of long term stress can become one of the important factors in the prevention and treatment of LJD. Therefore, in the present study, we aimed to objectively assess stress in kangaroos affected by LJD using cortisol concentrations in plasma and hair.

All procedures were reviewed and approved by the Institutional Animal Care and Use Committee of the School of Veterinary Medicine, Rakuno Gakuen University (Japan). Thirty seven eastern gray kangaroos (*Macropus giganteus*) with LJD of 6.7 ± 3.1 years (mean ± standard deviation of the mean) in age and with a body weight of 23.9 ± 10.1 kg were examined in the present study. The definitive diagnosis of LJD was made based on clinical findings, such as facial swelling, weight loss, excessive salivation and flicking of the tongue [12]. Twenty five eastern gray kangaroos of 6.5 ± 3.1 years in age and with a body weight of 27.2 ± 11.7 kg were used as the control group. The health statuses of the control animals were determined on the basis of physical examinations by zoo veterinarians. All animals were kept at Hibiki Animal World (Fukuoka, Japan) and consumed concentrated pellets (ZC Pellets, Oriental Yeast Co., Ltd., Tokyo, Japan) for herbivores in accordance with the manufacturer's guidelines and had *ad libitum* access to hay (timothy and alfalfa), vegetables (including carrots, cabbage and potatoes), apples and water.

Used kangaroos were tame to breeding staffs very much. All samplings were performed from 11:00 to 16:00 of the day within

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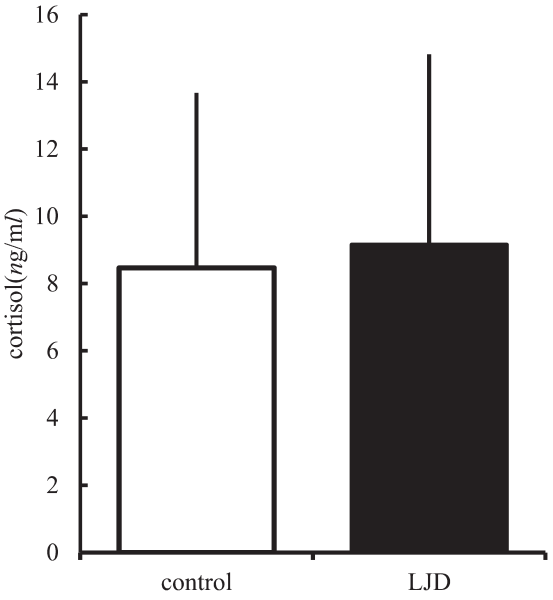

Fig. 1. Plasma cortisol concentration in kangaroos with lumpy jaw disease (LJD) and the healthy controls. The data are expressed as the mean ± standard deviation (SD).

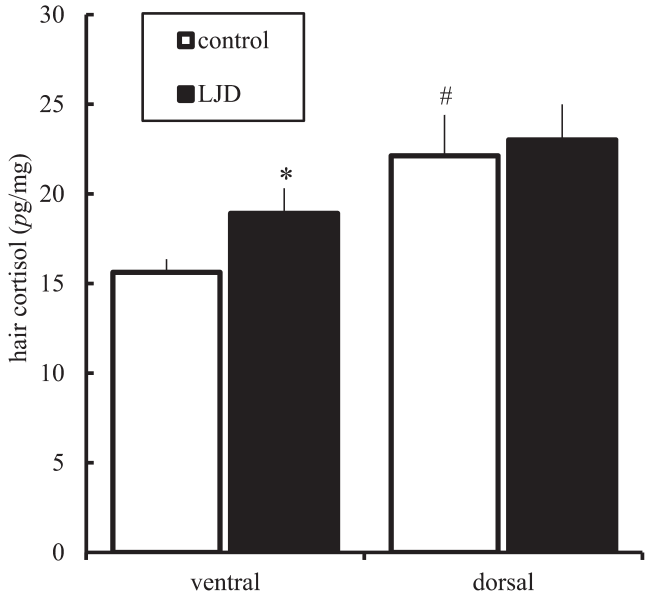

Fig. 2. The cortisol concentrations of the ventral (hypogastric) and the dorsal (lumbar) hairs in the control and lumpy jaw disease (LJD) animals. The data are expressed as the mean ± standard deviation (SD). * indicates significant difference to the control ($P<0.05$, *t*-test). # indecates significant difference to ventral ($P<0.05$, *t*-test).

five min after a breeding staff captured a kangaroo. For each plasma sample, 5 m*l* of whole blood was collected via jugular venipuncture into heparinized tubes and then centrifuged for 10 min at 3,000 × g at 4°C within 1 hr of collection. Collected plasma samples were immediately stored at −30°C until use. Hair samples of approximately 1 g each were collected on the dorsal (lumbar) and the ventral (hypogastric) regions. The hair samples were collected by severing the hair roots using scissors. The collected hair was stored under room temperature and shading until analysis.

The method of washing the hair and extracting hair cortisol was adopted from Yamanashi *et al*. [27]. Hair was placed in a 15 m*l* tube with isopropanol and shaken gently for 5 min. After washing, samples were placed in a clean hood for approximately 5 days. For each hair sample, a dried sample of 40 mg was placed into a 2 m*l* tube and crushed by Micro Smash MS-100 (TOMY SEIKO, Tokyo, Japan: 5,000 rpm for 150 sec). A volume of 1 m*l* of methanol was added to each tube and incubated at room temperature for 24 hr while being shaken gently using a rotator. After extraction, the samples were centrifuged for 10 min, after which, the supernatant was decanted into a clean 10 m*l* tube. The extracted samples were then dried at 38°C under nitrogen gas. The samples were reconstituted with 0.3 m*l* phosphate-buffered saline before the assay.

The cortisol levels of collected plasma samples and extracted hair samples were measured with a cortisol enzyme immunoassay kit (Arbor Assays, Ann Arbor, MI, U.S.A.).

Normally distributed data were reported as the mean ± standard deviation (SD), and non-normally distributed data were expressed as the median and ranges. For normal and non-normally distributed data, the Student's *t*-test and the Mann-Whitney *U*-test were employed for comparison between groups after ANOVA as the *F* test.

The plasma cortisol concentration tended to be higher in LJD animals than in controls (Fig. 1). The hair cortisol concentration in the ventral hair of LJD animals was significantly higher than that of the control (Fig. 2); however, a significant difference was not reflected in the dorsal hair. In addition, dorsal hair cortisol concentration was significantly higher than that ventral hair cortisol concentration in the control.

In the present study, the plasma cortisol concentration between the control and LJD animals was not significantly different. Most of the LJD animals used in the present study were kangaroos for which there were LJD treatment histories, and they had shown low disease severities during the period of the current study. Therefore, it is suggested that this is the reason why the difference in the plasma cortisol concentration between control and LJD animals during the period of the present study was not significant.

Furthermore, ventral hair cortisol of LJD animals was significantly higher than that of the controls. Recently, it was reported that chronic stress increases hair cortisol of human and nonhuman primates [8, 25, 26] and that hair cortisol is an effective biomarker for the assessment chronic stress. Blood cortisol diffused in both sebum and sweat, and these may deposit in hair from the outside [7, 13]. In addition, it was demonstrated the existence of a functional HPA-like system in human hair follicles [11]. However, a major route of incorporation is thought to be from the capillary around the hair follicle [2, 21]. Therefore, the significantly higher hair cortisol concentration of LJD animals than that of the control was probably due to long term accumulation, despite LJD not significantly affecting the plasma cortisol concentration in cases of low disease severity. But, it is necessary to investigate whether

blood cortisol of how much term is reflected to hair, because the cycle of the hair was not demonstrated in kangaroos.

In the present study, dorsal hair cortisol was significantly higher than that of ventral hair in the control. In addition, ventral hair cortisol in LJD animals was significantly higher than that in the control; however, this significant difference was not reflected for dorsal hair. In cattle and dogs, it is reported that various factors, such as hair color and position, affect hair cortisol [3, 6, 9]. It is thought that the difference between mechanism in association with the melanocytic development and differentiation [23, 24] and hair growth rate [18] affects hair cortisol as a difference of color and position. Hair color of kangaroos differs according to its position (dorsal: brown and ventral: light brown); this may explain the difference in the hair cortisol in the current experiment.

In conclusion, stress in kangaroos infected with LJD could be assessed by measuring ventral hair cortisol, even in cases of low disease severity where the plasma cortisol concentration does not significantly increase.

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REFERENCES

1. Antiabong, J. F., Boardman, W., Moore, R. B., Brown, M. H. and Ball, A. S. 2013. The oral microbial community of gingivitis and lumpy jaw in captive macropods. *Res. Vet. Sci.* **95**: 996–1005. [Medline] [CrossRef]
2. Barroso, M., Gallardo, E., Vieira, D. N., López-Rivadulla, M. and Queiroz, J. A. 2011. Hair: a complementary source of bioanalytical information in forensic toxicology. *Bioanalysis* **3**: 67–79. [Medline] [CrossRef]
3. Bennett, A. and Hayssen, V. 2010. Measuring cortisol in hair and saliva from dogs: coat color and pigment differences. *Domest. Anim. Endocrinol.* **39**: 171–180. [Medline] [CrossRef]
4. Blanden, D. R., Lewis, P. R. and Ferrier, G. R. 1987. Vaccination against lumpy jaw and measurement of antibody response in wallabies (Macropus eugenii). *Vet. Rec.* **121**: 60–62. [Medline] [CrossRef]
5. Bradley, A. J., McDonald, I. R. and Lee, A. K. 1980. Stress and mortality in a small marsupial (Antechinus stuartii, Macleay). *Gen. Comp. Endocrinol.* **40**: 188–200. [Medline] [CrossRef]
6. Burnett, T. A., Madureira, A. M., Silper, B. F., Nadalin, A., Tahmasbi, A., Veira, D. M. and Cerri, R. L. 2014. Short communication: Factors affecting hair cortisol concentrations in lactating dairy cows. *J. Dairy Sci.* **97**: 7685–7690. [Medline] [CrossRef]
7. Cook, T. J. and Spector, A. R. 1964. Excretion of Intravenously Administered Radioactive Hydrocortisone in Skin Surface Lipids. *J. Invest. Dermatol.* **43**: 413–414. [Medline] [CrossRef]
8. Fairbanks, L. A., Jorgensen, M. J., Bailey, J. N., Breidenthal, S. E., Grzywa, R. and Laudenslager, M. L. 2011. Heritability and genetic correlation of hair cortisol in vervet monkeys in low and higher stress environments. *Psychoneuroendocrinology* **36**: 1201–1208. [Medline] [CrossRef]
9. González-de-la-Vara, M. R., Valdez, R. A., Lemus-Ramirez, V., Vázquez-Chagoyán, J. C., Villa-Godoy, A. and Romano, M. C. 2011. Effects of adrenocorticotropic hormone challenge and age on hair cortisol concentrations in dairy cattle. *Can. J. Vet. Res.* **75**: 216–221. [Medline]
10. Hoefs, M. and Bunch, T. D. 2001. Lumpy jaw in wild sheep and its evolutionary implications. *J. Wildl. Dis.* **37**: 39–48. [Medline] [CrossRef]
11. Ito, N., Ito, T., Kromminga, A., Bettermann, A., Takigawa, M., Kees, F., Straub, R. H. and Paus, R. 2005. Human hair follicles display a functional equivalent of the hypothalamic-pituitary-adrenal axis and synthesize cortisol. *FASEB J.* **19**: 1332–1334. [Medline]
12. Jackson, S. 2003. Australian Mammals: Biology and Captive Management. CSIRO Publishing, Melbourne.
13. Jenkins, M. E., Rivarola, M. A., Brusilow, S. W. and Migeon, C. J. 1969. Excretion of 4-14C-cortisol and 1,2-3H-D-aldosterone in human thermal sweat. *J. Clin. Endocrinol. Metab.* **29**: 1102–1106. [Medline] [CrossRef]
14. King, J. M. and Bradshaw, S. D. 2010. Stress in an Island kangaroo? The Barrow Island euro, Macropus robustus isabellinus. *Gen. Comp. Endocrinol.* **167**: 60–67. [Medline] [CrossRef]
15. Lerner, P. I. 1988. The lumpy jaw. Cervicofacial actinomycosis. *Infect. Dis. Clin. North Am.* **2**: 203–220. [Medline]
16. Meyer, J. S. and Novak, M. A. 2012. Minireview: Hair cortisol: a novel biomarker of hypothalamic-pituitary-adrenocortical activity. *Endocrinology* **153**: 4120–4127. [Medline] [CrossRef]
17. Möstl, E. and Palme, R. 2002. Hormones as indicators of stress. *Domest. Anim. Endocrinol.* **23**: 67–74. [Medline] [CrossRef]
18. Moya, D., Schwartzkopf-Genswein, K. S. and Veira, D. M. 2013. Standardization of a non-invasive methodology to measure cortisol in hair of beef cattle. *Livest. Sci.* **158**: 138–144. [CrossRef]
19. Nagel, C., Trenk, L., Aurich, C., Ille, N., Pichler, M., Drillich, M., Pohl, W. and Aurich, J. 2016. Sympathoadrenal balance and physiological stress response in cattle at spontaneous and PGF2α-induced calving. *Theriogenology* **85**: 979–985. [Medline] [CrossRef]
20. Palme, R., Fischer, P., Schildorfer, H. and Ismail, M. N. 1996. Excretion of infused 14C-steroid hormones via faeces and urine in domestic livestock. *Anim. Reprod. Sci.* **43**: 43–63. [CrossRef]
21. Pragst, F. and Balikova, M. A. 2006. State of the art in hair analysis for detection of drug and alcohol abuse. *Clin. Chim. Acta* **370**: 17–49. [Medline] [CrossRef]
22. Remer, T., Maser-Gluth, C. and Wudy, S. A. 2008. Glucocorticoid measurements in health and disease--metabolic implications and the potential of 24-h urine analyses. *Mini Rev. Med. Chem.* **8**: 153–170. [Medline] [CrossRef]
23. Roulin, A., Almasi, B., Rossi-Pedruzzi, A., Ducrest, A. L., Wakamatsu, K., Miksik, I., Blount, J. D., Jenni-Eiermann, S. and Jenni, L. 2008. Corticosterone mediates the condition-dependent component of melanin-based coloration. *Anim. Behav.* **75**: 1351–1358. [CrossRef]
24. Slominski, A., Tobin, D. J., Shibahara, S. and Wortsman, J. 2004. Melanin pigmentation in mammalian skin and its hormonal regulation. *Physiol. Rev.* **84**: 1155–1228. [Medline] [CrossRef]
25. Stalder, T., Kirschbaum, C., Heinze, K., Steudte, S., Foley, P., Tietze, A. and Dettenborn, L. 2010. Use of hair cortisol analysis to detect hypercortisolism during active drinking phases in alcohol-dependent individuals. *Biol. Psychol.* **85**: 357–360. [Medline] [CrossRef]
26. Van Uum, S. H., Sauvé, B., Fraser, L. A., Morley-Forster, P., Paul, T. L. and Koren, G. 2008. Elevated content of cortisol in hair of patients with severe chronic pain: a novel biomarker for stress. *Stress* **11**: 483–488. [Medline] [CrossRef]
27. Yamanashi, Y., Morimura, N., Mori, Y., Hayashi, M. and Suzuki, J. 2013. Cortisol analysis of hair of captive chimpanzees (Pan troglodytes). *Gen. Comp. Endocrinol.* **194**: 55–63. [Medline] [CrossRef]