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Studies on the Assessment of Hair Cortisol Concentration in Dogs by Radioimmunoassay (RIA)

C.N. Galdhar[1], Shubham Vyas[1], R.V. Gaikwad[1], J.R. Kawade[1], P.M. Sapkal[1]

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ABSTRACT

Background: Hair cortisol Concentration (HCC) is often used as a measure of chronic stress responses in humans. The use of Radioimmunoassay (RIA) and HCC to assess stress in hospitalized dogs has been poorly reported in the literature in India and Central East Asia. This paper presents a modified protocol for measuring HCC in dog hair samples for stress assessment using RIA.

Methods: Estimation of HCC concentrations of healthy (n=20) and hospitalized (n=20) dogs was done using RIA. Hair samples were collected from a dog's nape region and weighed to measure HCC concentration. The study used RIA-enabled dissevered spiking and detectable concentration-based modifications to measure HCC. Five known cortisol concentration standards (*viz* 19.0 nmol/l, 47.9 nmol/l, 190 nmol/l, 238.66 nmol/l and 713.33 nmol/l) were used, with the first three readily available with a cortisol RIA kit. The samples were spiked with 50 µL of the standard and analysed in a gamma counter to take counts per minute (CPM). The final concentration of HCC was estimated in nmol/l and derived in pg/mg as per standard methods.

Result: Healthy dogs had a mean HCC of 6.24±0.84 pg/mg, while hospitalized dogs had 31.65±5.87 pg/mg. A significant deviation was observed in both groups. A box and whisker graph showed an HCC range of 0.36 to 13.78 pg/mg for healthy dogs and 8.70 to 65.25 pg/mg for hospitalized dogs. The interquartile range (25^{th} to 75^{th} percentile) was 3.55 to 9.24 pg/mg for healthy dogs and 14.43 to 46.22 for hospitalized dogs. The observed median for healthy dogs was 6.16 pg/mg and 22.62 pg/mg for hospitalized dogs. The study shows higher HCC in hospitalized sick dogs compared to clinically healthy ones, suggesting hair sample collection as a simple stress assessment method and RIA as a promising HCC detection technique.

Key words: Hair cortisol concentration (HCC), Healthy dogs, Hospitalized dogs, Radioimmunoassay (RIA).

INTRODUCTION

Serum cortisol measurement is an invasive procedure that can disrupt behavior and stress levels. Urinary cortisol is a widely used indicator of stress in dogs, as it is collected without significant stress. Naturally voided urine samples can study stress hormones in animals, but they only represent the average concentration in blood during urine formation. Cortisol measurements in serum, urine and saliva reflect systemic concentrations but cannot assess enduring levels. Non-invasive techniques are increasingly used to assess hypothalamic-pituitary-adrenal axis activity in humans. Measurement of hair cortisol offers the merit of a minimally invasive sampling procedure and simple storage (Corradini *et al*., 2013). During the last two decades, hair cortisol concentration (HCC) has proven to be a promising marker for the evaluation of increased hypothalamic-pituitary-adrenal axis activity caused by repeated or long-term stressful conditions (Heimburge *et al*., 2020).

Radioimmunoassay (RIA) is a sensitive *in vitro* method for the assessment of antigens *i.e*., hormones, minerals, vitamins, *etc.* from biological fluids. ^{125}I is a commonly used radioisotope among others for RIA due to its long half-life (t1/2 = 60 days). This method offers a convenient assay of large numbers of samples with excellent precision. The practice of RIA in the measurement of hormones for veterinary clinical use has been extensively studied in India (Dadke *et al*., 2018; Roopali *et al*., 2020; Galdhar *et al*., 2021; Jayabhaye *et al*., 2021; Galdhar *et al*., 2022 and Salutgi *et al*., 2023).

[1]Veterinary Nuclear Medicine Including Radio Isotope Laboratory, Department of Veterinary Clinical Medicine, Mumbai Veterinary College, Maharashtra Animal and Fishery Sciences University, Parel, Mumbai-400 012, Maharashtra, India.

Corresponding Author: C.N. Galdhar, Veterinary Nuclear Medicine Including Radio Isotope Laboratory, Department of Veterinary Clinical Medicine, Mumbai Veterinary College, Maharashtra Animal and Fishery Sciences University, Parel, Mumbai-400 012, Maharashtra, India. Email: chgaldhar@gmail.com

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Hair cortisol is often used as a measure of chronic stress responses in humans, nonhuman primates and companion animals across the globe (Corradini *et al*., 2013; Meyer *et al*., 2014; Vives *et al*., 2015). HCC is not yet widely used in India. The purpose of this paper is to report the development of RIA enabled novel protocol to measure HCC in hair samples from dogs for stress assessment.

MATERIALS AND METHODS

Statutory approval

The present study was initiated after permission from the Institutional Ethics Committee for Veterinary Clinical

Research (IEC-VCR) and Institutional Bio-safety Committee (IBSC) of Mumbai Veterinary College, Maharashtra Animal and Fishery Sciences University (MAFSU), Mumbai-India.

Selection of healthy and hospitalized dogs

A total of 20 non-descript clinically healthy dogs from the clinical setup of Mumbai Veterinary College, Parel-Mumbai, were ethically enrolled to measure HCC. They consisted of 14 males and 06 females, with a mean age of 5.63±0.39 years and mean body weight of 19.33±0.59 respectively. Health checks, anamnesis and laboratory tests (blood count, liver function test, kidney function test) were conducted to assess health, ensuring normal limits were met.

A total of 20 non-descript hospitalized dogs, admitted for various illnesses at the clinical setup of Mumbai Veterinary College, Parel-Mumbai, were ethically enrolled to measure HCC. They consisted of 13 males and 07 females, with a mean age of 4.65±0.57 years and mean body weight of 17.60±1.63 respectively.

Hair sample collection and hormone determinations by RIA

One gram hair sample was collected from the nape region of the dog by blunted surgical scissors and weighed 250 mg. The samples were then washed with isopropanol and dried at room temperature for 3-5 days. After powdering, 30 mg of powdered hair was transferred to 2 ml eppendorf tube and 1.5 ml High liquid pressure chromatography (HPLC) grade methanol was added. The samples were incubated for 16 hours and sedimented for 2 hours, then 1ml of supernatant was transferred into another 2 ml epindoff tube and complete drying was done by evaporation at room temperature for 24 hours. The dried samples were mixed with 200 µL of phosphate buffer saline (PBS of pH 7.5) and used as hair sample aliquots. Nist *et al.* (2020) used enzyme-linked immunoassay to assess HCC, as the concentration of cortisol is very small in hair samples and not detected by the assay. In the present study, marginal modifications were undertaken. In our study, we used RIA-enabled dissevered spiking and detectable concentration-based modifications to measure HCC. Five known cortisol concentration standards were used for the spiking of hair samples. The standard concentrations used were 19.0 nmol/l, 47.9 nmol/l, 190 nmol/l, 238.66 nmol/l and 713.33 nmol/l respectively. The first three standards *viz*. 19.0 nmol/L, 47.9 nmol/L and 190 nmol/L were readily available with a cortisol RIA kit, while the remaining two standards were created by dilution/serial dilution of known standards with zero calibrators. In our case, after pipetting 50 µL of standard and 50 µL of hair aliquot samples into the appropriate antibody-coated test tube, the samples were spiked with 50 µL of the known standard (random any one among-19.0 nmol/l, 47.9 nmol/l, 190 nmol/l, 238.66 nmol/l and 713.33 nmol/l) during the assay procedure. We maintained the manufacturer's protocol for the rest of the assay procedure. The samples were analysed in a gamma counter to take counts per minute (CPM) followed by extraction of HCC on the semi-log graph paper. Further, the known standards concentration was subtracted and the final concentration of hair samples was measured in nmol/l. Quality control parameters *viz.* magnitude of control samples and recovery percentage were studied to validate every assay. The HCC in pg/mg was derived as per the method and formula outlined by (Nist *et al*., 2020).

Statistical analysis

Mean and standard error for each parameter of collected data was calculated and analysed statistically for comparison as per the methods suggested by Snedecor and Cochran (2004). A nonparametric statistical analysis was also used for the analysis of the results.

RESULTS AND DISCUSSION

Estimation of HCC concentrations of healthy (n=20) and hospitalized (n=20) dogs was done using RIA by employing dissevered spiking and detectable concentration-based modifications. Assay passed all recommended quality control parameters *viz*. magnitude of control samples provided with kits and percent recovery. The standard curve of the assay was plotted and the HCC was interpolated from the standard curve. The Mean, interquartile range (*i.e*., 25th to 75th percentile) and median of HCC in healthy dogs and hospitalized dogs are presented in Table 1 and Fig 1.

The mean value of HCC of healthy dogs was recorded as 6.24±0.84 pg/mg and the hospitalized dog was 31.65±5.87 pg/mg, respectively (Table 1). A statistically significant ($p<0.05$) deviation was recorded in HCC in healthy and hospitalized dogs.

A box and whisker graph (Fig 1) was plotted for comparison of HCC between healthy and hospitalized dogs. The 'T' bar represents the data which is equal to the range. In the present study, the observed range for healthy dogs was 0.36 to 13.78 pg/mg and for hospitalized dogs was 8.70 to 65.25 pg/mg respectively. The box represents the middle half of the data. The present study reports the interquartile range (25th to 75th percentile) as 3.55 to 9.24 pg/mg for healthy dogs and 14.43 to 46.22 for hospitalized dogs, respectively. The Horizontal bar in the box is the median of the data. In the present study, the observed median for healthy dogs was 6.16 pg/mg and for hospitalized dogs was 22.62 pg/mg respectively.

Table 1: Alterations in HCC (pg/mg) in healthy and hospitalized dogs (n=20).

Parameter	Dogs	Mean±SE	t stat	t critical (5%)
HCC (pg/mg)	Healthy dogs (n=20)	6.24±0.84	4.29*	2.02
	Hospitalized dogs (n=20)	31.65±5.87		

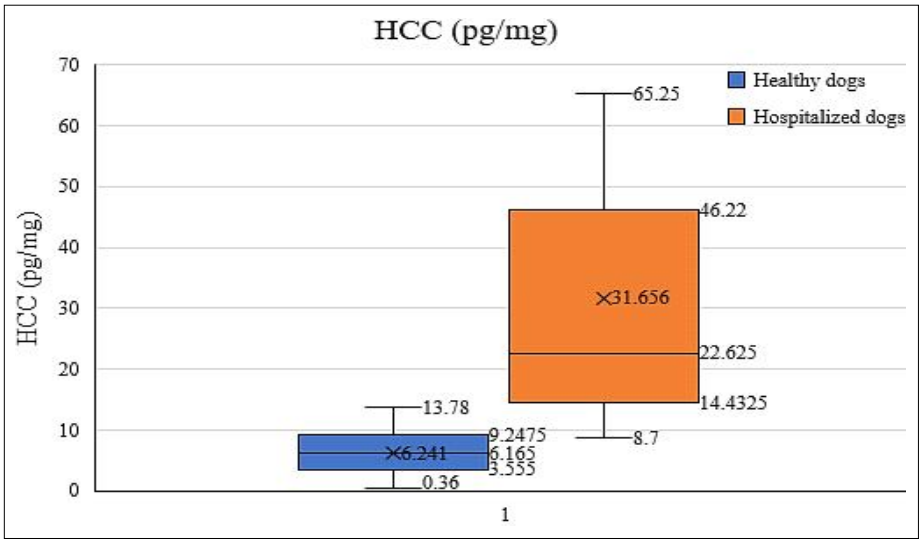

Fig 1: A box and whisker graph showing alterations in HCC (pg/mg) in healthy and hospitalized dogs (n=20).

The present study reports, a statistically significant ($p<0.05$) difference in the mean HCC of healthy and hospitalized dogs. These findings are in close agreement with Corradini *et al.* (2013) and Van der Laan *et al*. (2022). Corradini *et al.* (2013) evaluated HCC in the diagnosis of hypercortisolism in dogs. They mentioned that the evaluation of cortisol from hair samples offered the advantage of easy sample collection. Van der Laan *et al.* (2022) studied hair cortisol levels in dogs in shelters and after adoption. They found that HCC analysis is a reliable, feasible non-invasive method for evaluating cortisol levels in shelter dogs, particularly when comparing levels over longer periods.

The promising study by Packer *et al.* (2019), highlighted that hair cortisol is a promising indicator of chronic stress in dogs. They emphasized its responsiveness to various stressors, including behavior, disease, medication, lifestyle and the social environment. Ouschan *et al.* (2013) reported elevated hair cortisol levels in dogs with hypercortisolism, proposing hair cortisol analysis as a non-invasive diagnostic tool. Expanding on this, Park *et al.* (2016) identified increased hair cortisol in dogs with Canine Atopic Dermatitis, suggesting its potential for linking stress to specific health conditions. Furthermore, Bryan *et al.* (2013) advocated tracking hair cortisol over time to monitor gradual changes associated with disease progression, especially in conditions related to adrenal hypo- or hyperfunction in dogs.

Radioimmunoassay (RIA) is a gold standard method for detecting hormone concentrations and assessing antigens in biological fluids. It uses I^{125} radioisotope for its long half-life, offering convenient and accurate assays for large samples. The investigation utilized RIA-enabled dissevered spiking and detectable concentration-based modifications to measure HCC in dogs. This promising approach can be used to study stress in hospitalized and confined dogs, potentially defining stress. In the present investigation, we used RIA-enabled dissevered spiking and detectable concentration-based modifications to measure HCC. This offered a promising approach for the detection of HCC. This modified method may be widely used to study stress not only in hospitalized dogs but also to appraise stress-reducing measures in canine welfare.

CONCLUSION

The study demonstrates RIA-enabled measurement of HCC in healthy and hospitalized dogs, marking the first report from India and Central East Asia. We recommend that hair sample collection is an easy method for assessing stress and RIA offers a promising method for detecting HCC.

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Conflict of interest

The authors declare that there is no conflict of interest.

REFERENCES

Bryan, H.M., Adams, A.G., Invik, R.M., Wynne-Edwards, K.E. and Smits, J.E. (2013). Hair as a meaningful measure of baseline cortisol levels over time in dogs. Journal of the American Association for Laboratory Animal Science. 52(2): 189-196.

Corradini, S., Accorsi, P.A., Boari, A., Beghelli, V., Mattioli, M., Famigli Bergamini, P. and Fracassi, F. (2013). Evaluation of hair cortisol in the diagnosis of hypercortisolism in dogs. J. of Vet. Int. Med. 27: 1268-1272.

Dadke, A., Galdhar, C.N., Gaikwad, R.V. and Kadam, D.P. (2018). Studies on biochemical alterations in hypothyroid dogs. Ind. J. Vet. Med. 38: 35-38.

Galdhar, C., Chandra, S., Dadke, A., Gaikwad, R. and Sarode, A. (2021). Metabolic and hormonal changes in water buffaloes during postparturient peak lactation. Buffalo Bulletin. 40: 565-570.

Galdhar, C.N., Gaikwad, R.V., Msare, P.S., Guard, K.V., Vaidya, S., Bhojne, G.R., Waghmare, S.P., Jadhav, R.K., Swami, S.B., Borikar, S.T., Koujalagi, S. and Parkhe, A.G. (2022). Triidothyroxine (TT3), Total thyroxine (T4) and Free Thyroxine (FT4) reference range in healthy dogs by radioimmunoassay (RIA). Ind. J. of Ani. Resh. 1-5. doi: 10.18805/IJAR.B-4822.

Heimbürge, S., Kanitz, E., Tuchscherer, A. and Otten, W. (2020). Is it getting in the hair? -Cortisol concentrations in native, regrown and segmented hairs of cattle and pigs after repeated ACTH administrations. Gen. and Comp. Endo. 295: 1-9.

Jayabhaye, R.M., Galdhar, C.N., Dadke, A.R., Gaikwad, R.V., Banik, S. and Rajkhowa, S. (2021). Radio immune assay (RIA) enabled thyroid profile (TT3, TT4 and fT4) in pigs. Ind. J. Vet. Med. 41: 9-15.

Meyer, J., Novak, M., Hamel, A. and Rosenberg, K. (2014). Extraction and analysis of cortisol from human and monkey hair. J. of Vis. Exp. 83: 1-6.

Nist, M.D., Sullenbarger, B.A., Harrison, T.M. and Pickler, R.H. (2020). Protocol to measure hair cortisol in low mass samples from very preterm infants. Nur. Res. 69(4): 316-321.

Ouschan, C., Kuchar, A. and Mostl, E. (2013). Measurement of cortisol in dog hair: A noninvasive tool for the diagnosis of hypercortisolism. Veterinary Dermatology. 24(4): 428-431.

Park, S.H., Kim, S.A., Shin, N.S. and Hwang, C.Y. (2016). Elevated cortisol content in dog hair with atopic dermatitis. Japanese Journal of Veterinary Research. 64(2): 123-129.

Packer, R.M., Davies, A.M., Volk, H.A., Puckett, H.L., Hobbs, S.L. and Fowkes, R.C. (2019). What can we learn from the hair of the dog? Complex effects of endogenous and exogenous stressors on canine hair cortisol. Plos One. 14(5): e0216000. https://doi.org/10.1371/journal.pone.0216000.

Roopali, B., Roy, S., Roy, M. and Galdhar, C.N. (2020). Epidemiological study of canine hypothyroidism in Chhattisgarh state, India. Int J. Curr Microbiol Appl Sci. 9: 1432-1439.

Salutgi, P., Galdhar, C., Sonigra, R., Natu, K., Mumbarkar, N., Mathkar, S., Dalvie, A. and Gaikwad, R. (2023). Radio immune assay (RIA) enabled total triiodothyronine (TT3) and total thyroxine (TT4) in canine trypanosomiasis: First case report from Maharashtra (India). Iran. J. of Para. 18: 107-112.

Snedecor, G.M. and Cochran, W.C. (2004). Statistical Methods, 8th Edition. Iowa State University Press, Ames, Iowa.

Van der Laan, J.E., Vinke, C.M. and Arndt, S.S. (2022). Evaluation of hair cortisol as an indicator of long-term stress responses in dogs in an animal shelter and after subsequent adoption. Scientific Reports. 12: 5117. doi: 10.1038/s41598-022-09140-w.

Vives, A.H., De Angel, V., Papadopoulos, A., Strawbridge, R., Wise, T., Young, A.H., Arnone, D. and Cleare, A.J. (2015). The relationship between cortisol, stress and psychiatric illness: New insights using hair analysis. J. of Psy. Res. 70: 38-49.