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PROGRAMA DE PÓS-GRADUAÇÃO EM SAÚDE, BEM-ESTAR ANIMAL E
PRODUÇÃO ANIMAL SUSTENTÁVEL NA FRONTEIRA SUL

LUCIANA VELASQUES CERVO

MONITORAMENTO DO BEM-ESTAR DE CÃES PARTICIPANTES DO PROJETO
REABILITAÇÃO EM ITAJAÍ, SANTA CATARINA, BRASIL

REALEZA

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Dissertação apresentada ao Programa de Pós-graduação em Saúde, Bem-estar animal e Produção animal sustentável na Fronteira Sul da Universidade Federal da Fronteira Sul, como requisito parcial para a obtenção de grau de Mestre em Saúde, Bem-estar animal e Produção Animal Sustentável.

Orientadora: Profa. Dra. Dalila Moter Benvegnú

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RESUMO

O conceito de Bem-estar animal remete às questões de adaptação dos indivíduos e pode ser acessado pela mensuração de quanto as necessidades dos animais estão sendo satisfeitas. Este estudo foi constituído por manuscrito de revisão sistemática da literatura a respeito das particularidades dos estudos sobre bem-estar de cães e também por manuscrito sobre avaliação de bem-estar dos cães terapeutas do ReabilitaCão. O primeiro manuscrito envolveu um levantamento de dados da literatura, utilizando critérios PRISMA. Após a definição de termos descritores foram selecionados 44 artigos científicos publicados no Pubmed, de janeiro de 1990 a janeiro de 2021; escritos em língua inglesa ou portuguesa, com livre acesso eletrônico. O termo descritor mais citado nos artigos foi *roaming owned dogs* (cães domiciliados soltos). Utilizamos a estratégia PICO para responder quais os principais aspectos dos estudos sobre bem-estar de cães. Verificou-se que a maioria das pesquisas sobre bem-estar de cães utilizam medidas positivas, de interação e resposta comportamental, para avaliação de comportamento de cães domiciliados. O segundo manuscrito visou a mensuração do bem-estar animal de 14 cães terapeutas vítimas de maus tratos, resgatados no município de Itajaí, Santa Catarina, Brasil, sendo que dentre esses: 5 cães receberam 1 avaliação e 9 cães receberam 2 avaliações, com 3 meses de diferença entre elas, totalizando 23 avaliações (n=23). Após tratamento Veterinário, castração e vacinação os cães são encaminhados para um canil adaptado para recebê-los no Complexo Penitenciário do Vale do Itajaí, onde participam do ReabilitaCão, que se trata de um projeto de cinoterapia destinado ao auxílio na ressocialização dos apenados participantes, que por sua vez têm previsão de redução de pena. Este estudo teve por objetivo monitorar o bem-estar dos cães terapeutas do ReabilitaCão. Para avaliação fisiológica dos cães foi realizada a pesagem, inspeção das mucosas, a aferição de temperatura retal, da frequência respiratória e cardíaca dos animais. Além disso, foi realizada uma coleta de 6mL de sangue por meio de punção da veia jugular ou cefálica dos cães, destinada à hemograma e dosagem de cortisol, lactato e glicemia em jejum. Ainda, foram realizadas análises comportamentais e ultrassonografia da glândula adrenal dos cães. As análises estatísticas foram realizadas com auxílio do Programa PSPP (versão 1.2.0-g0fb4db). Variáveis numéricas foram analisadas via Teste -T pareado, para comparação no decorrer do tempo, e associadas por meio do Teste de Correlação de Pearson. Os resultados encontrados foram comprimento 15,9±1,72 mm para a adrenal esquerda (AE) e comprimento 15,14±01,36 mm para a adrenal direita (AD) (n=11). Além disso, encontramos valores médios de cortisol sérico de 2,181±1,35 mcg/dL (n=22); de glicemia de 73,434±13,86 mg/dL (n=23); e para lactato de 3,331±1,21 mmol/L (n=19). Eosinofilia foi a alteração mais frequente encontrada em 47,83% (11) dos cães (n=23). Na avaliação comportamental 56,52% (13) dos cães apresentaram comportamento categorizado como agressivo e medroso (n=23). Conclui-se que os cães terapeutas do ReabilitaCão apresentam boa condição de bem-estar determinada pela avaliação ultrassonográfica da glândula adrenal associada com a dosagem de cortisol. No entanto, a avaliação comportamental e a dosagem de glicose sugerem estresse crônico dos animais.

Palavras-chave: Canino, Estresse, Etologia, Penitenciária, Pet terapia.

ABSTRACT

The concept of Animal Welfare refers to the adaptation issues of individuals and can be accessed by measuring of the animals' needs are being satisfied. This study consisted of a systematic literature review manuscript about the particularities of studies on dog welfare and also a manuscript on the welfare assessment of ReabilitaCão therapist dogs. The first manuscript was a survey of literature data, using PRISMA criteria. After defining descriptor terms, 44 scientific articles published in Pubmed from January 1990 to January 2021 were selected; written in English or Portuguese, with free electronic access. The descriptor term most cited in the articles was *roaming owned dogs*. We used the PICO strategy to answer the main aspects of dog welfare studies. It was found that most research on dog welfare uses positive measures such as interaction and behavioral responses to assess the behavior of domiciled dogs. The second manuscript aimed to measure the animal welfare of 14 therapist dogs victims of relinquishment, rescued in the city of Itajaí, Santa Catarina, Brazil, and among these 5 received 1 evaluation and 9 received 2 evaluations with 3 months of difference between than, totaling 23 evaluations. After Veterinary treatment, castration and vaccination, the dogs are sent to an adapted kennel to receive them at the Vale do Itajaí Penitentiary Complex. Inside the Penitentiary, where participate in the ReabilitaCão, which is a dog therapy project aimed at helping the resocialization of the inmates, who in turn are expected to reduce their sentences. This monitoring aims to analyze the well-being of dogs of ReabilitaCão. For the physiological evaluation of the dogs, weighing, inspection of the mucous membranes, rectal temperature, respiratory and heart rate of the animals were performed. In addition, a collection of 6mL of blood was performed by the puncture of the jugular or cephalic vein of the dogs, intended for the hemogram and measurement of cortisol, lactate, and fasting glucose. Furthermore, behavioral analyzes and ultrasound of the adrenal gland of the dogs were conducted. Statistical analyzes were performed using the PSPP Program (version 1.2.0-g0fb4db). Numerical variables were analyzed via the paired T-Test for comparison purposes over time and associated using Pearson's Correlation test. The results found for left adrenals (LA) were a mean length of 15.9 ± 1.72 mm; and, for right adrenals (RA) (n=11), a mean length of 15.14 ± 1.36 mm. Besides, we found a mean of serum cortisol of 2.181 ± 1.35 mcg/dL (n=22); glycemia was 73.434 ± 13.86 mg/dL (n=23); and a mean lactate of 3.331 ± 1.21 mmol/L (n=19). Eosinophilia was the most frequent alteration in blood count, found in 47.83% (11) of dogs (n=23). Regarding behavioral assessment, 56.52% (n=13) of dogs showed behaviors classified as aggressive and fearful (n=23). We concluded that the therapy dogs sheltered in the ReabilitaCão Project show good well-being conditions, determined by an ultrasound exam of adrenal glands associated with serum cortisol and lactate measurements. However, behavioral evaluation and fasting glycemia results indicate the chronic stress response of these animals.

Keywords: Canine, Stress, Ethology, Prison, Pet therapy.

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1 INTRODUÇÃO

Conforme levantamento realizado pelo Instituto Pet Brasil (2019) “a população pet no Brasil chega a cerca de 140 milhões de animais, sendo que desses em torno de 3,9 milhões encontram-se em situação de vulnerabilidade, ou seja, sob tutela de famílias também em situação de vulnerabilidade social”. E ainda, “por volta de 172 mil animais estão em situação de abandono sob tutela de ONG’s e grupos de proteção de animais” (INSTITUTO PET BRASIL, 2019).

Diante desta realidade, onde existe um grande número de animais errantes, semierrantes e em situação de vulnerabilidade em nosso país, podemos verificar que algumas Prefeituras têm se organizado para oferecer “serviços de atendimento Médico Veterinário apenas para animais errantes e semierrantes e castrações para animais de proprietários que recebam benefícios sociais provenientes do Governo Federal, Estadual ou Municipal, devidamente comprovados” (JARAGUÁ DO SUL, 2014).

No entanto, na maioria dos municípios não há nenhuma estrutura para abrigar os animais de rua que são reconhecidamente responsabilidade do Estado, como podemos verificar no Artigo 23 da Constituição da República Federativa do Brasil, “é competência comum da União, dos Estados, do Distrito Federal e dos Municípios: [...] VI - proteger o meio ambiente e combater a poluição em qualquer de suas formas; VII - preservar as florestas, a fauna e a flora” (BRASIL, Constituição 1988).

Atualmente, esta tarefa tem sido desempenhada pelo terceiro setor, ou seja, instituições sem fins lucrativos, que geram bens e serviços de caráter público, como ONGs, e em parceria com a iniciativa privada, principalmente, com clínicas veterinárias, buscando arduamente, por meio de campanhas de arrecadação de recursos da sociedade, realizar castrações, imunizações e identificação eletrônica de animais errantes e semierrantes, além, de abrigar temporariamente em seus lares esses animais até que ocorra uma possível adoção.

Desta maneira, “continua a acumular-se evidência de benefício mútuo na relação entre humanos e os seus animais, tendo sido identificada uma necessidade de criar um conjunto de diretrizes aceitas universalmente para o bem-estar dos animais de companhia” (RYAN et al., 2018).

De acordo com Faraco (2008):

[...] a preocupação de vários pesquisadores tem sido elucidar os mecanismos de ação que explicariam o papel positivo dos animais para as pessoas. Para tanto, são

sugeridos diversos mecanismos, sendo que, na maior parte, são enfatizados os supostos atributos intrínsecos dos animais, bem como seu valor como instrumentos vivos para promoção de mudanças positivas no autoconceito e comportamento de pessoas. Estas modificações se apoiariam no desenvolvimento de várias habilidades e no exercício de responsabilidades.

O ReabilitaCão (ANEXO I), projeto único em Santa Catarina, idealizado pela agente penitenciária Bruna Longen e desenvolvido no Complexo Penitenciário do Vale do Itajaí/SC, mantido pela Justiça Estadual e Federal, tem ações realizadas com comprometimento e responsabilidade para com a vida dos seres humanos e dos animais. O Complexo Penitenciário do Vale do Itajaí possui uma equipe técnica multidisciplinar de classificação responsável pela avaliação dos detentos internos do regime semi-aberto e sua seleção para este projeto de ressocialização e redução penal.

“O objetivo do ReabilitaCão é oferecer a oportunidade aos internos de criar empatia e ensinar práticas de cuidados de animais resgatados, além de preparar os cães para torná-los passíveis para adoção” (SECRETARIA DE ESTADO DA ADMINISTRAÇÃO PRISIONAL E SOCIOEDUCATIVA, 2020). Para isso os detentos fazem o adestramento básico dos cães com treinamento de obediência: senta, deita, fica, vem, “caixa”, controle e andar na guia, visando socialização e ambientação.

Nos Estados Unidos, mais de 290 instituições correcionais em todos os 50 Estados implementaram programas de treinamento de cães (COOKE; FARRINGTON, 2016). Tais projetos sociais buscam a reabilitação de apenados e cães errantes e semi-errantes mediante a contribuição da interação humano-animal como é o caso do *Hill's Pet Shelter Program* e do *Pawsitive Change*.

O monitoramento do bem-estar dos cães envolvidos em projetos de cinoterapia, especialmente, aqueles que permanecem em canis, é fundamental e precisa ser realizado periodicamente por meio de avaliações comportamentais e fisiológicas. Podemos conceituar o “bem-estar de um indivíduo como seu estado em relação às suas tentativas de adaptar-se ao seu ambiente” (BROOM, 1986).

Há indicadores clínicos utilizados na avaliação do bem-estar animal, como os fisiológicos (frequência respiratória, cardíaca, temperatura, condição corporal, pressão arterial), os bioquímicos (enzimas e hormônios relacionados com o estresse), os imunológicos relacionados com a enfermidade, as lesões e a dor. Os indicadores comportamentais são obtidos mediante registros observacionais e inventários comportamentais, como catálogos e etogramas (CEUA/USP, 2015).

A avaliação comportamental pode ser considerada subjetiva, porém, é vital para manutenção de um projeto envolvendo cães terapeutas. Essa pode ser realizada por meio de

observação comportamental e com diferentes métodos de amostragem. Segundo Caldéron (2010), “a avaliação do bem-estar animal é fundamental para a proposição de ações corretivas ou de implementação de programas de saúde e de promoção da qualidade de vida dos animais”.

No método de amostragem animal focal para observação comportamental proposto por Altmann; Lang (1974) todas as ocorrências de (inter) ações especificadas de um indivíduo, ou grupo específico de indivíduos, são registradas durante cada período de amostra, e (ii) um registro é feito da duração de cada período de amostragem e, para cada indivíduo focal, a qualidade de tempo durante a amostra que está realmente à vista. Uma vez escolhido, um indivíduo focal é seguido na medida do possível durante cada um dos seus períodos de amostra. Além disso, sob algumas condições, é possível registrar todas as ocorrências de certas classes de comportamentos em todos os membros do grupo durante cada período de observação (ALTMANN; LANG, 1974).

Ainda, de acordo com Del-Claro (2010):

[...] a amostragem do animal focal consiste na técnica utilizada na observação de um único indivíduo ou grupo de indivíduos (grupo focal), que permite realizar censos de comportamento dos indivíduos em intervalos regulares de tempo; também permite que, em um determinado grupo de animais, o pesquisador mude de indivíduo focal a cada minuto, por exemplo.

Polgár et al. (2019) explica que “o desenvolvimento de métodos ideais para avaliar o bem-estar dos cães em canis é claramente um esforço difícil, embora valioso”. O autor sugere também medidas fisiológicas, comportamentais e cognitivas relacionadas ao estresse crônico e agudo em cães de canis (ANEXO II).

A respeito do estresse Nardi; Roza (2016) trazem que:

[...] é diretamente relacionado à liberação de adrenalina e o efeito simpatomimético. O efeito adrenérgico promove eventos cardiovasculares de aumento do fluxo sanguíneo na microcirculação muscular e na vasoconstrição de outros vasos sanguíneos que, nesse processo, desviam leucócitos do pool marginal vascular para o pool circulante, sendo este pool o local de coleta de sangue para a realização do hemograma. Esse evento pode resultar no leucograma em contagens leucocitárias elevadas em até o dobro dos valores leucocitários totais. Essas elevações são refletidas principalmente nos neutrófilos, mas também podem ser identificadas em linfócitos. Na linhagem neutrofilica, não é observado desvio a esquerda, pois, a migração de neutrófilos para o pool circulante é de neutrófilos maduros (segmentados).

Segundo Ryan et al. (2018):

[...] um baixo nível de bem-estar, como consequência de um fator de stress negativo, resultará em: aumento da frequência cardíaca; aumento da temperatura corporal; aumento da frequência respiratória; aumento da glicemia; alteração dos níveis de atividade (aumento ou diminuição); sudação das almofadas plantares; e arfar. E, por conseguinte, níveis elevados de stress negativo ao longo de um maior período de

tempo conduzem a: perda de peso; aumento da porcentagem de gordura corporal e diminuição da massa muscular; diminuição da função imune, com aumento do rácio neutrófilos/linfócitos; diminuição da função reprodutiva; e disfunção cognitiva.

Neste estudo, utilizaremos também outros indicadores: a **glicemia** aumentada em situação de estresse agudo devido à mobilização da reserva de glicogênio do fígado para a corrente sanguínea. Do mesmo modo, o estresse agudo aumenta o consumo de oxigênio, levando a diminuição desse elemento químico, o que dispara o mecanismo de respiração celular anaeróbica aumentando os níveis do **lactato** sérico.

Os glicocorticoides e as catecolaminas são considerados os principais indicadores de estresse. O estresse fisiológico propicia a liberação do hormônio adrenocortical pela pituitária e de cortisol pela adrenal, que ocorre frente à inúmeros processos sistêmicos, tais como: insuficiência renal, cetoacidose diabética, desidratação, resposta inflamatória e dor ocasionada por trauma. No sangue circulante, podem se observar várias alterações, tais como saída de neutrófilos do pool marginal para o circulante, bem como egressão de neutrófilos maduros do pool de estoque da medula óssea para o pool circulante. A neutrofilia é esperada sem desvio à esquerda, haja vista que toda essa migração neutrofílica ocorre com células maduras. Os esteroides podem provocar linfopenia por apoptose linfocitária e interferência na recirculação dos linfócitos, confinando-os por mais tempo em tecidos linfoides. Eosinopenia e monocitose também podem estar presentes no leucograma. Essas alterações podem ocorrer em diversos processos patológicos, porém, são mais comuns em cães com hiperadrenocorticismo ou Síndrome de Cushing e aqueles tratados com administração de corticosteroides (NARDI; ROZA, 2016).

“A ultrassonografia abdominal é usada para avaliar o tamanho e a forma das adrenais e para pesquisar anormalidades adicionais no abdômen” (NELSON; COUTO, 2020).

Hiperadrenocorticismo (Síndrome de Cushing) de ocorrência espontânea está associado com a produção endógena excessiva de hormônios esteroides (principalmente glicocorticoides e, ocasionalmente, mineralocorticoides ou hormônios sexuais). A doença é ocasionada por um tumor de adrenal hiperfuncional (15 a 20% dos casos) ou por tumor de pituitária, ou hipófise (80 a 85% dos casos) (MEDLEAU; HNILICA, 2009).

A respeito da adrenal de estresse Tochetto et al. (2018) encontraram prevalência de 5,3% desta alteração e também que:

[...]macroscopicamente, cães com adrenal de estresse tinham córtex irregular. “A morfologia dessa lesão, observada nos cães deste estudo, é idêntica ao padrão descrito para adrenais de estresse em humanos, porém não foram encontrados estudos que descrevam essa lesão em animais, talvez isso se deve a inexistência de pesquisas abrangentes sobre adrenais, em que o foco não seja neoplasias e alterações relacionadas às síndromes clínicas.

Diante do exposto, justifica-se a importância do presente estudo, o qual buscou investigar informações a respeito da condição clínica e de bem-estar dos cães que participam do projeto ReabilitaCão no Complexo Penitenciário do Vale do Itajaí.

2 OBJETIVOS

2.1 OBJETIVO GERAL

Monitorar o bem-estar de cães participantes do Projeto ReabilitaCão em Itajaí, Santa Catarina, Brasil.

2.2 OBJETIVOS ESPECÍFICOS

- Redigir artigo de revisão da literatura abordando o perfil dos estudos relacionados com bem-estar animal;

Em relação ao Projeto ReabilitaCão realizado na Penitenciária Estadual de Itajaí, Santa Catarina, Brasil:

- Avaliar transversalmente parâmetros fisiológicos de cães para monitoramento do bem-estar animal;
- Realizar exames de imagem de cães ao início do estudo com intuito de avaliar a glândula adrenal;
- Analisar transversalmente o comportamento de cães para monitoramento do bem-estar animal;
- Efetuar análises sanguíneas transversais de cães para mensurar variáveis relacionadas com estresse.

3 HIPÓTESE

Evolução positiva da condição clínica e de bem-estar dos cães terapeutas participantes do projeto ReabilitaCão.

4 MATERIAL E MÉTODOS

Este estudo foi protocolado sob nº 8163240321 na Comissão de Ética no Uso de Animais da Universidade Federal da Fronteira Sul (CEUA/UFS) e aprovado na reunião de

06/08/2021. Além disso, foi protocolado sob CAAE 46567121.4.0000.5564 no Comitê de Ética em Pesquisa da Universidade Federal da Fronteira Sul (CEP/UFFS) e aprovado pelo parecer 4.818.627 de 30/06/2021.

4.1 TIPO DE ESTUDO

Trata-se de um estudo descritivo e quantitativo transversal que visa conhecer o estado de bem-estar de cães do ReabilitaCão, por meio de avaliações fisiológicas, bioquímicas, de imagem e comportamentais.

4.2 PARTICIPANTES E LOCAL DO ESTUDO

O projeto foi realizado com a participação dos cães do Canil vinculado ao Projeto ReabilitaCão, do Complexo Penitenciário do Vale do Itajaí, que fica localizado na Estrada Geral, Rua João Thomaz Pinto, s/n - Canhanduba, Itajaí, Santa Catarina, Brasil.

O canil possui área interna composta por baias individuais cobertas, onde os cães podem acessar livremente para repousar, especialmente a noite. E também, uma área externa cercada com brinquedos, obstáculos semelhantes aos de um circuito de *agility* e piscina, neste espaço podem exercitar-se e interagir com os outros cães sob acompanhamento dos detentos.

Os cães passaram por exames de imagem no início do projeto. Tais exames foram realizados por Médica Veterinária autônoma terceirizada.

4.3 DELINEAMENTO EXPERIMENTAL

Foi realizada avaliação de bem-estar de 14 cães, sendo que dentre esses: 5 cães receberam uma avaliação e 9 cães receberam duas avaliações, com três meses de diferença entre elas, tendo como n amostral 23 cães.

Os dados da avaliação fisiológica foram comparados com os valores de referência e analisados estatisticamente para verificar se há diferença significativa.

4.3.1 PROCEDIMENTO COM OS CÃES

Primeiramente, foi realizada a contenção física do paciente, dependendo da situação foi utilizada também focinheira, por segurança. Em seguida, foi realizada a pesagem, inspeção das

mucosas, aferição de temperatura retal, frequência respiratória e cardíaca. Além disso, neste momento os cães também foram fotografados somente para registro em prontuário. Após, foi realizada a colheita das amostras de sangue através de punção da veia jugular ou cefálica destinadas à hemograma e análises bioquímicas séricas. No início do projeto foi realizado, exames de imagem dos cães, através de estudos ultrassonográfico da glândula adrenal. Para isso, a Médica Veterinária ultrassonografista dirigiu-se até o canil com o aparelho de ultrassom. Durante os exames de imagem, o animal recebeu a contenção física para posicionamento adequado, em alguns casos foi necessário o uso de focinheira, e no momento da ultrassonografia foi realizada tricotomia da região abdominal e aplicação de gel na região do abdômen.

4.3.1.1 Avaliação comportamental

A análise do comportamento dos cães foi realizada por meio dos métodos de observação animal focal do comportamento dos cães no momento do exame físico, interagindo com as pesquisadoras e com os detentos que auxiliaram na contenção dos cães, e nas posturas corporais do cachorro (HOUPY, 2011) (AnexoV).

Utilizamos ainda a “escala de agressão canina: como o cão reage ao estresse e ameaça” (HORWITZ, et al., 2009) (Anexo VI).

O seu comportamento foi categorizado em 4 classes referente ao comportamento de medo: agressivo, medroso, inquieto e dócil.

4.3.1.2 Avaliação de parâmetros fisiológicos

A avaliação fisiológica dos cães foi composta por pesagem, aferição da temperatura retal, coloração de mucosas, frequência cardíaca e respiratória, além de exames laboratoriais (Anexo III).

A temperatura retal foi aferida por meio de termômetro digital. Foi considerado intervalo de referência valores entre 37,5 a 39,2°C. A coloração das mucosas foi avaliada pela inspeção de mucosa da cavidade oral e mucosa ocular, sendo valor de referência: mucosas normocoradas. Já as frequências cardíacas e respiratórias foram mensuradas por observação e auscultação com estetoscópio. Foram considerados como intervalo de referência para frequência cardíaca 60 a 180 batimentos por minuto e para frequência respiratória 10 a 30 movimentos por minuto (TILLEY; SMITH, 2008).

Foi realizada a identificação de situação de estresse agudo dos cães, onde devido à liberação de catecolaminas, detectamos no exame clínico: aumento de frequência cardíaca e frequência respiratória; aumento de temperatura corporal; mucosas hipocrômicas; pelagem arrepiada; e pupilas dilatadas.

Também foi identificada situação de estresse crônico que pode ocorrer ganho de peso, pois, o animal fica ansioso e ingere mais alimento ou também pode diminuir esta ingestão ocorrendo perda de peso. Portanto, o peso corporal foi considerado um indicador de estresse.

Por outro lado, o animal que manifesta obesidade também, pode estar manifestando possível quadro de ansiedade e estresse crônico.

Também foram verificadas e registradas a incidência de patologias relacionadas ao estresse, por exemplo, dermatopatias. Neste caso, o diagnóstico de determinadas patologias nos cães foi um indicador de estresse.

4.3.1.3 Técnicas e instrumentos para coleta sanguínea

Após jejum de 12 horas foi realizada uma coleta de 6mL de sangue dos cães por meio de punção da veia jugular ou cefálica dos cães, no período da manhã. Desta amostra de sangue, parte foi acondicionada em tubos contendo EDTA, outra parte em tubo sem anticoagulante e uma terceira parte ainda foi mantida na própria seringa de coleta. O primeiro e o segundo tubos foram encaminhados em caixa térmica refrigerada até o laboratório terceirizado (Laboratório Lenzi®) onde foram realizados hemograma e dosagem de cortisol sérico. A partir da amostra de sangue total mantida na seringa de coleta foram realizados testes de lactato e glicemia.

Por ocasião deste projeto são considerados os intervalos de referência LACVeT, 2018 (Anexo IV) para parâmetros laboratoriais.

4.3.1.3.1 Hemograma

O hemograma fornece o **eritrograma** que consiste na determinação do número de eritrócitos por μL de sangue, concentração de hemoglobina (g/dL), taxa de hematócrito (%), volume corpuscular médio (VCM), concentração de hemoglobina celular média (CHCM), hemoglobina corpuscular média (HCM) e avaliação morfológica dos eritrócitos nos esfregaços sanguíneos. O eritrograma pode nos trazer como resultado a policitemia relativa que ocorre por diminuição da quantidade de plasma e redistribuição eritrocitária. Algumas alterações relacionadas com a ocorrência de policitemia são desidratação ou desvio de fluido sistêmico e

contração esplênica. A contração esplênica também pode induzir policitemia relativa, e esse fato pode acompanhar animais que realizarem quantidade elevada de exercício ou por ação da epinefrina em animais estressados, que apresentem sinais de estresse durante a coleta das amostras de sangue (NARDI; ROZA, 2015).

Por meio do hemograma, foi determinado também o **leucograma**, o qual também permite detectar estresse, quando marcado por leucocitose com neutrofilia, monocitose, eosinopenia e linfopenia.

4.3.1.4 Análises bioquímicas

A partir do soro sanguíneo dos cães foi realizada a dosagem do **cortisol sérico** pelo Método de Quimioluminescência.

Para a mensuração do **lactato no sangue** total foi utilizado o analisador *Lactato Detect TD-4261 Eco Diagnóstica*. De acordo com a bula do equipamento, esse aparelho faz a medição do lactato utilizando um biossensor amperométrico que mede a intensidade do sinal elétrico gerado pela reação de lactato na amostra de sangue com o sistema de enzimas metabolizadoras implantado nas tiras de teste.

Para a determinação da **glicemia em jejum** foi usado o *Sistema para Monitorização de Glicemia Descarpac Plus*. Segundo a bula do equipamento, nesse sistema a tira reagente contém um eletrodo que mede os níveis de glicose na corrente sanguínea, sendo que a glicose da amostra se mistura com o reagente presente na tira (Glicose desidrogenase) gerando uma corrente elétrica dependente da quantidade de glicose presente.

4.3.1.5 Exames de imagem

Foram realizados, exames de imagem dos cães, através de estudo ultrassonográfico no início do projeto para avaliação da glândula adrenal.

Para o estudo ultrassonográfico das glândulas adrenais dos cães foi aplicada a técnica de ultrassonografia em modo B, com transdutor de alta frequência, visando avaliar posicionamento, tamanho, formato, ecogenicidade e diâmetro.

Inicialmente, foi definido como critério de exclusão dos cães deste estudo, os animais que, eventualmente, apresentassem hiperplasia da glândula adrenal identificada na ultrassonografia abdominal, devido ao fato de que essa pode resultar em hipercortisolismo.

Na data agendada para realização do exame de imagem dois cães encontravam-se em pós-operatório de ovariohisterectomia e de mastectomia total, respectivamente, e por este motivo não foi realizada ultrassonografia desses animais.

4.4 ANÁLISE DOS DADOS

Os dados da avaliação fisiológica e das análises sanguíneas foram comparados com os valores de referência.

As informações coletadas pela avaliação comportamental foram analisadas de maneira descritiva, qualificando os resultados em quatro categorias: agressivo, medroso, inquieto e dócil.

As análises estatísticas foram realizadas com auxílio do Programa PSPP (versão 1.2.0-g0fb4db). Os resultados foram expressos na forma de média±desvio padrão da média, mediana e intervalo interquartil. Variáveis numéricas foram analisadas via Teste -T pareado para fins de comparação no decorrer do tempo. Para análises de associação entre as variáveis foi utilizado o Teste de Correlação de Pearson, sendo o valor-p menor que 0,05.

Heron José de Santana Gordilho

Editor Responsável

Revista Brasileira de Direito Animal

09 de março de 2022

Prezado Professor Heron,

Venho por meio desta, manifestar interesse em submeter o manuscrito intitulado “Profile of studies on welfare of dogs: a systematic review of the literature” na seção Ética Animal, da Revista Brasileira de Direito Animal, e-ISSN: 2317-4552 e ISSN Impresso: 1809-9092.

Respeitosamente,

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PROFILE OF STUDIES ON WELFARE OF DOGS: A SYSTEMATIC REVIEW OF THE LITERATURE

PERFIL DOS ESTUDOS SOBRE BEM-ESTAR DE CÃES: REVISÃO SISTEMÁTICA DE LITERATURA

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ABSTRACT

This systematic review of the literature sought to investigate the characteristics of studies related to the welfare of dogs. The main question was: what are the main aspects of studies on dog welfare? A survey of the literature data was carried out using PRISMA criteria. It searched for scientific articles published in the electronic database PubMed between January 1990 and January 2021, written in English or Portuguese, and offered free electronic access. We searched for the following descriptor terms: *apartment dogs, confined dogs, dog welfare, dog well-being, free-ranging dogs, household confined dogs, indoor dogs, movement restrictions of dogs, roaming owned dogs, sheltered dogs, and spatial restriction of dogs*. Using the PICO (Population, Intervention, Comparators, Outcome) strategy including four components: studies on dogs (population); welfare assessment (intervention); studies that used positive behavior measures, such as interaction and behavioral responses, to assess dog welfare (comparator); peculiarities of studies referring to the welfare condition of dogs (result). 44 articles published on PubMed were reviewed. Studies related to dog behavioral assessment prevailed, with 50% (22). The studied population involved 85,492 animals, of which 75% belonged to the following categories: domiciled (16); free-ranging (9); and shelter (8). The descriptor term most cited in the articles was roaming owned dogs. In this study, we found that most research on dog welfare uses positive measures to assess the behavior of domiciled dogs. We conclude that the behavioral assessment of dog welfare is fundamental to improving the relationship between humans and dogs and preventing abandonment.

KEYWORDS: Ethology; Dog; Comfort; Temperament.

RESUMO

Esta revisão sistemática da literatura buscou investigar as características dos estudos relacionados com o bem-estar de cães. A questão principal era: quais os principais aspectos dos estudos sobre bem-estar de cães? Foi realizado um levantamento de dados da literatura, utilizando critérios PRISMA, mediante busca de artigos científicos no: PubMed; de janeiro de 1990 a janeiro de 2021; escritos em inglês ou português; com livre acesso eletrônico. Buscamos pelos seguintes termos descritores: *cães de apartamento, cães confinados, bem-estar de cães, conforto de cães, cães soltos, cães domiciliados confinados, cães de ambiente interno, restrição de movimento de cães, cães domiciliados com acesso à rua, cães de abrigo e restrição espacial de cães*. Utilizamos a estratégia PICO (População, Intervenção, Comparação, Desfecho), incluindo 4 componentes: estudos sobre cães (população); estudos sobre avaliação de bem-estar (intervenção); estudos utilizando medidas de comportamento positivas, de interação e resposta comportamental, avaliando bem-estar de cães (comparação); peculiaridades dos estudos de bem-estar de cães (resultado). Revisamos 44 artigos publicados no PubMed. Os estudos relacionados com avaliação comportamental de cães prevaleceram totalizando 50% (22). A população estudada totalizou 85,492 animais, desses 75% pertenciam as seguintes categorias: domiciliados (16); errantes (9); e de abrigo (8). O termo descritor mais citado foi *cães domiciliados com acesso à rua*. Verificou-se que a maioria das pesquisas sobre bem-estar de cães utilizam medidas positivas para avaliação de comportamento de cães domiciliados. Conclui-se que a avaliação comportamental de bem-estar de cães é fundamental para melhoria do relacionamento do ser humano com os cães e prevenção do abandono.

PALAVRAS-CHAVE: Etologia; Cachorro; Conforto; Temperamento.

SUMMARY: 1 Introduction 2 Materials and methods 2.2 PICO strategy 2.2.1 Participants 2.2.2 Interventions 2.2.3 Comparators 2.2.4 Outcomes 3 Results 4 Discussion 5 Conclusion 6 References **Attachment I** Figure 1 Article selection flowchart **Attachment II** Table 1 Articles selected and included in the systematic review **Attachment III** Table 2 Characterization of the studied dog population **Attachment IV** Table 3 Parameters evaluated in selected studies

1. Introduction

Well-being assessment is a complex variable that depends on hematological, immunological, biochemical, and behavioral parameters, among others. Shelter Quality is a protocol often used to evaluate the welfare of shelter dogs, based on “four welfare principles: good feeding, good housing, good health, and appropriate Behaviour” (BARNARD et al., 2016).

Dogs are affected by experiences lived throughout their lives, in their relationships with humans and other animals. From the evolution of the concept of the five liberties, the Farm Animal Welfare Council (COUNCIL, 2009) formulated the concept of animal welfare as "a life worth living." This concept aims to minimize negative experiences and maximize those which are positive from the point of view of the animal. For this, we luckily have several tools to assess if the animals are in fact living this way and laws to guarantee their right to life.

Recently, the Lei Sansão (Samson Law) was sanctioned (Law no. 14.064 amended Law no. 9.605), which, despite its speciesism, still elevates dogs and cats to the level of pet and “increased the penalties provided for by law to the committed crime of animal mistreatment in the case of dogs or cats. The penalty for the described conducts will be imprisonment from two to five years, fine, and prohibition of custody” (BRAZIL, 2020). This constitutes a legal strategy already used in several countries to definitively prevent the entry of dogs and cats into the meat production chain.

Space deprivation can certainly be included among the limiting situations for the comfort that one hopes to offer animals. However, free access to the outdoors is also life-threatening. Therefore, domiciled and loose dogs are expected to be the group with the best quality of life. In the British Animal Welfare Act (ANIMAL WELFARE ACT, 2006), we find the concept of “Duty of Care”, i.e., “a person commits an offence if he does not take such steps as are reasonable in all the circumstances to ensure that the needs of an animal for which he is responsible are met to the extent required by good practice.”

Thus, these needs are “a suitable environment, a suitable diet, to be able to exhibit normal behaviour patterns, to be housed with, or apart from, other animals, and to be protected from pain, suffering, injury and disease (ANIMAL WELFARE ACT, 2006).”

There is a trend toward assessing dog welfare via measures that reflect positive response behaviors, indicators of status positive emotional. According to Vignors; Lawrence (2019), “These measures include animal autonomy, play, positive affect, positive human-animal relationships, social interaction, and appropriate genetic selection.” Positive well-being measures are more aligned with preventing behavioral disorders and, consequently, dog abandonment to the detriment of behavioral responses indicating responses to negative experiences, such as fear, stress, and pain.

According to Rioja-Lang et al. (2020), “prioritised welfare issues included lack of knowledge of welfare needs, social behaviour issues, problem behaviours, inappropriate diet and environment, lack of veterinary care, consequences from breeding decisions, poor pain management, delayed euthanasia and chronic ill health.”

Salman et al. (2000) listed “the top 10 reasons for relinquishment due behavioral of dogs: bites, aggressive toward people, escapes, destructive inside, destructive outside, disobedient, problems between new pet and other pets, aggressive toward animals, soils house, and vocalizes a lot.”

Regarding the C-BARQ (Canine Behavioral Assessment & Research Questionnaire) “the questionnaire may prove helpful in screening companion and working dogs for behavior problems and, if widely adopted, may also promote greater consensus among behavioral practitioners regarding the classification of problem behaviors (HSU; SERPELL, 2003).”

This is a descriptive study via a systematic review of the literature using PRISMA criteria (Preferred Reporting Items for Systematic reviews and Meta-Analyses) (MOHER et al., 2015), which will seek to identify the main characteristics of studies analyzing dog well-being.

2. Materials and methods

A survey of literature data was carried out by selecting scientific articles published on the PubMed database between January 1990 to January 2021 which were written in English or Portuguese and offered free electronic access.

We searched for the following descriptor terms: apartment dogs, confined dogs, dog welfare, dog well-being, free-ranging dogs, household confined dogs, indoor dogs, movement restrictions of dogs, roaming owned dogs, sheltered dogs, and spatial restriction of dogs.

Initially, the studies were separated by title. Then, by abstract. Finally, the selected studies were fully read. Studies were chosen as eligible only after these steps.

2.2 PICO strategy

2.2.1 Participants

Studies involving dogs which were purebred; mixed-breed; female; male; of any age; in variable body condition; spayed or not; neutered or not; owned confined, owned free-roaming, free-ranging, and shelter dogs were included.

2.2.2 Interventions

Studies assessing the welfare of dogs in any type of confinement via clinical, physiological, hormonal, and immunological parameters were selected, as well as studies measuring behavior by ethograms.

2.2.3 Comparators

This systematic review sought to compare studies which used measures of positive behavior for evaluating dog welfare, such as interaction and behavioral responses.

2.2.4 Outcomes

The peculiarities of dog welfare studies using positive behavioral measures regarding the preference of dogs for human proximity and canine behavior are shown in our results.

3 Results

Our results evinced the peculiarities of the studies assessing dog welfare. Initially, we found 87 articles containing the search terms descriptors (Figure 1) on the PubMed database.

However, we included 44 articles in this systematic review, as follows: confined dogs (6), dog welfare (1), dog well-being (1), free-ranging dogs (7), household confined dogs (2), indoor dogs (7), movement restrictions of dogs (3), roaming owned dogs (10), sheltered dogs (4) and spatial restriction of dogs (3) (Table 1). The most common descriptor term in the studies was roaming owned dogs with a 22.72% frequency.

Regarding the year of publication of the studies included in this review, we found that 65.91% (29) were published in the years 2017, 2018, 2019, and 2020. This finding shows the growing interest in animal welfare in recent years.

The countries of origin of the selected studies with the highest frequency were: United States (7); India (6); Australia (4); United Kingdom (4); and France (4), representing 54.54% (24) of the studies included in this research.

The n sampling of dogs in the studies included in this systematic review totaled 85,492 animals (Table 2). Only 10 articles provided information about the sex of the studied dogs. Of these, 30,581 were females and only 6,505 were males.

Regarding the group of dogs studied in the articles included in this review: 75% belonged to the following categories: domiciled (16); strays (9); and shelter dogs (8) (Table 2).

Concerning the assessed parameters (Table 3), most studies — 50% (22) — are related to dog behavioral assessment; and the rest, to the following main indicators: vaccination, neutering and access to the outdoors (2); vaccine response (5); survival and fecundity (4); canine diseases (6); attacks (3); and stress (2).

Regarding the diagnostic tools used in the reviewed studies, we found the following: assessment of human-animal interaction (14); rabies vaccination and serology (7); assessment of behavioral problems (6); questionnaires (5); retrospective studies (3); cortisol measurement (2); neutering (2); bacteriological examination (1); demographic study (1); estimation model (1); GPS monitoring (1); and parasitological exam (1).

Regarding behavioral parameters, 54.55% (12) of studies were related to positive behavior measures, such as interaction and behavioral responses, whereas 45.45% (10) of the behavioral studies emphasized negative measures of behavior such as disorders and stress.

Among the studies that used positive behavior measures, seven (58.33%) evinced the preference of dogs due to human proximity and five (41,67%) were conducted only with canine behavior.

4. Discussion

Dogs prefer to stay near humans, “dogs tend to build trust based on affection, not food (BHATTACHARJEE et al., 2017).” “The preference of dogs for denning close to humans is a behavioural adaptation (MAJUMDER et al., 2016).”

However, free-ranging dogs show a different behavior than that of the shelter and pet dogs since “only adults adjusted their behaviour based on the reliability of the human experimenter after being rewarded; free-ranging dogs show a tendency to respond to human pointing gestures (BHATTACHARJEE et al., 2019).” Thus, Brubaker et al. (2019) studied canine gazing behavior and found that “Free-ranging dogs responded to the human’s change in attentional state by looking significantly less at the human in the inattentive condition compared to the attentive condition.”

Pet and shelter dogs “gazed significantly more at the human in the both the inattentive and attentive conditions compared to the free-ranging dogs and also spent more time in the proximity of the experimenter (BRUBAKER et al., 2019).”

According to Serpell (2017), “pet dogs pay special attention to humans and especially to their owners with whom they usually have a more interactive relationship.” Regarding indoor pet dogs, Duranton et al. (2017) showed that “dogs visibly synchronized their location with their owner (staying in close proximity and moving to the same area), as well as their activity and temporal changes in activity (moving when their owner moved, standing still when their

owner stood still, and gazing in the same direction as their owner).” On outdoor pet dogs, Duranton et al. (2018) “found that dogs visibly synchronized both their location (staying in close proximity) and their activity (moving when their owner moved, and at the same pace, and standing still when their owner stood still) with those of their owners.” “The shelter dogs synchronized their locomotor activity with their caregiver less strongly than pet dogs in a previous study. Shelter dogs also maintained greater distances to their caregivers than pet dogs with their owners (DURANTON et al., 2019).”

Reviewing studies that emphasize canine behavior, Paul; Bhadra (2018) “revealed the presence of widespread allo-parenting by both adult males and females. Free-ranging dogs are not cooperative breeders like wolves but are rather communal breeders.”

Concerning persistence in an impossible task, Lazzaroni et al. (2019), “found that pet dogs and captive dogs were more manipulative and persistent than free-ranging dogs,” i.e., there are differences among pet, captive, and free-ranging dogs in relation to the behavior to obtain food.

Regarding obesity lifestyle management, German (2010) recommends that “both dogs and cats can also be encouraged to ‘work’ for their food by moving the food bowl between rooms before feeding or using feeding toys.” Sum et al., 2019 concluded that “feeding toys may be helpful during weight loss programs.”

“Beagle dogs exhibited diurnal patterns of locomotor activity that varied as a function of age, cognitive status, and housing environment (SIWAK et al., 2003),” whereas Maher et al. (2019) “identified that location, season and hour of day were significantly associated with the roaming activities of free-roaming domestic dogs.” And recommend that “when planning rabies control strategies movement controls should be targeted to the early evening.”

5. Conclusion

There is a growing interest in monitoring dog well-being, especially in developed countries. This study found that most studies on dog welfare uses tools to assess the behavior of owned dogs, following the trend of using positive welfare indicators.

With the advances in better understanding canine behavior we become able to use tools and strategies to improve our relationship with dogs, preventing undesirable behaviors that often lead to their abandonment.

Dogs prefer proximity to humans and are even able to synchronize their behavior with their owners or caregivers. The history of the relationship between humans and dogs started centuries ago, motivated by mutual benefit. This relationship, nowadays, requires commitment from humans, as well as respect for our friendship with dogs.

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ATTACHMENT I

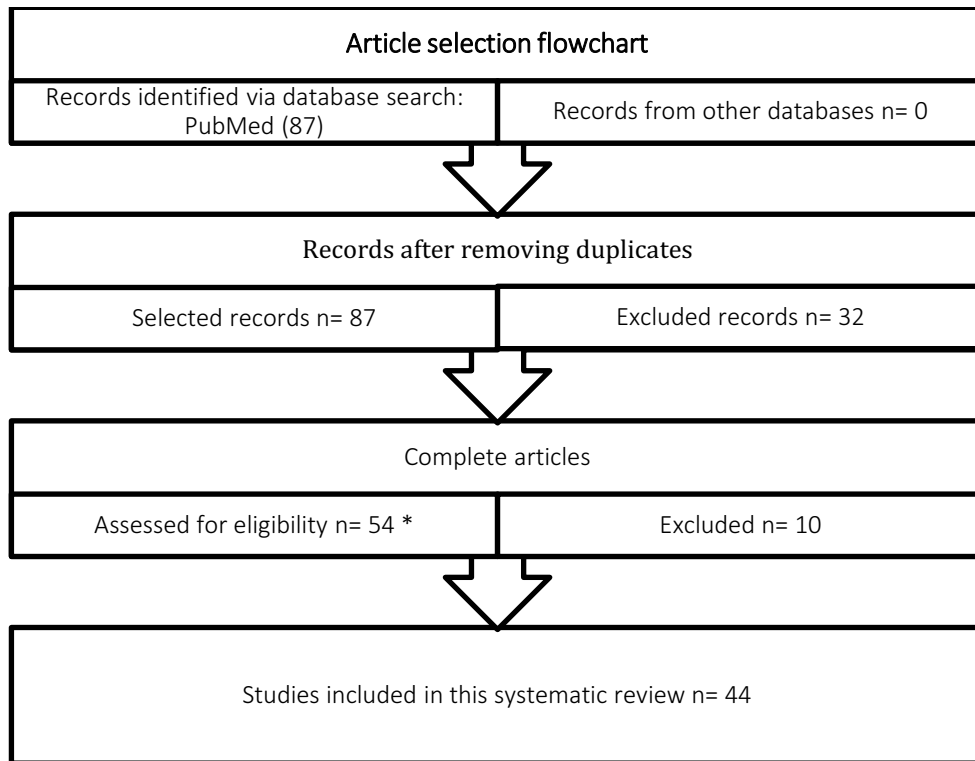


Figure 1 Article selection flowchart

**One of the articles identified was neither a duplicate nor a complete article.*

ATTACHMENT II

Table 1. Articles selected and included in the systematic review

	Title	Author	Year	Country
<i>Descriptor term: confined dogs</i>				
1	Care of Dogs and Attitudes of Dog Owners in Port-au-Prince, the Republic of Haiti	Fielding, W. J. et al.	2012	Haiti
2	Prevalence of antibody against rabies among confined, free-roaming and stray dogs in a transit city of Nigeria.	Olugasa, B. O. et al.	2011	Nigeria
3	Companion and free-ranging Bali dogs: Environmental links with personality traits in an endemic dog population of South East Asia	Corrieri, L. et al.	2018	Bali
4	Escape rates and biting histories of dogs confined to their owner's property through the use of various containment methods	Starinsky, N. S. et al.	2017	United States
5	Elimination behavior of shelter dogs housed in double compartment kennels	Wagner, D. et al.	2014	United States
6	Fecundity and longevity of roaming dogs in Jaipur, India	Reece, J. F. et al.	2008	India
<i>Descriptor term: dog welfare</i>				
7	Health-related welfare prioritisation of canine disorders using electronic health records in primary care practice in the UK	Summers, J. F. et al.	2019	United Kingdom
<i>Descriptor term: dog well-being</i>				
8	One Health Solutions to Obesity in People and Their Pets	Bartges, J. et al.	2017	United Kingdom
<i>Descriptor term: free-ranging dogs</i>				
9	Assessing reproductive patterns and disorders in free-ranging dogs in Jodhpur, India to optimize a population control program	Totton, S. C. et al.	2010	India
10	Free-ranging dogs prefer petting over food in repeated interactions with unfamiliar humans	Bhattacharjee, D. et al.	2017	India
11	What influences the home range size of free-roaming domestic dogs?	Dürr, S. et al.	2017	Australia
12	Denning habits of free-ranging dogs reveal preference for human proximity	Majumder, S. S. et al.	2016	India
13	The great Indian joint families of free-ranging dogs	Paul, M.; Bhadra, A.	2018	India
14	The role of life experience in affecting persistence: A comparative study between free-ranging dogs, pet dogs and captive pack dogs	Lazzaroni, M et al.	2019	Austria
15	Free-ranging dogs show age related plasticity in their ability to follow human pointing	Bhattacharjee, D. et al.	2019	India
<i>Descriptor term: household confined dogs</i>				
16	Clinical features and outcome in dogs and cats with obsessive-compulsive disorder: 126 Cases (1989-2000)	Overall, K. L.; Dunham, A. E.	2002	United States
17	The influence of poverty and rabies knowledge on healthcare seeking behaviors and dog ownership, Cameroon	Costa, G. B. et al.	2018	Cameroon
<i>Descriptor term: indoor dogs</i>				

18	Increases in heart rate and serum cortisol concentrations in healthy dogs are positively correlated with an indoor waiting-room environment	Perego, R. et al.	2014	Italy
19	Interspecific behavioural synchronization: Dogs exhibit locomotor synchrony with humans	Duranton, C. et al.	2017	France
20	Impact of feeding method on overall activity of indoor, client-owned dogs	Sum, D. K. et al.	2019	United States
21	Pet dogs synchronize their walking pace with that of their owners in open outdoor areas	Duranton, C. et al.	2018	France
22	Locomotor activity rhythms in dogs vary with age and cognitive status	Siwak, C. et al.	2003	United States
23	Association between indoor air pollution and respiratory disease in companion dogs and cats	Lin, C. H. et al.	2018	Taiwan
24	Behavioural risks in male dogs with minimal lifetime exposure to gonadal hormones may complicate population-control benefits of desexing	McGreevy, P. D. et al.	2018	United States
Descriptor term: <i>movement restriction of dogs</i>				
25	Short term consequences of preventing visitor access to kennels on noise and the behaviour and physiology of dogs housed in a rescue shelter	Hewison, L. F. et al.	2014	United Kingdom
26	Parasitologic examination and associated risk factors of domestic dogs at the domestic-wildlife interface in the Iberá wetlands Ecoregion, Argentina	Natalini, B. et al.	2020	Argentina
27	Investigation of the temporal roaming behaviour of free-roaming domestic dogs in Indigenous communities in northern Australia to inform rabies incursion preparedness	Maher, E. K. et al.	2019	Australia
Descriptor term: <i>roaming owned dogs</i>				
28	Census and vaccination coverage of owned dog populations in four resource-limited rural communities, Mpumalanga province, South Africa	Conan, A. et al.	2017	South Africa
29	Survey of <i>Campylobacter</i> spp. in owned and unowned dogs and cats in Northern Italy	Giacomelli, M. et al.	2015	Italy
30	Evaluation of community-based dog welfare and rabies project in Sanur, a sub-district of the Indonesian island province of Bali	Utami, N. W. A. et al.	2019	Indonesia
31	The demography of free-roaming dog populations and applications to disease and population control	Morters, M. K. et al.	2014	United Kingdom
32	Dog demographics and husbandry practices related with rabies in Cameroon	Bouli, F. P. N. O. et al.	2020	Cameroon
33	Fatal dog attacks in Canada, 1990-2007	Raghavan, M.	2008	Canada
34	Epidemiology and surveillance of human animal-bite injuries and rabies post-exposure prophylaxis, in selected counties in Kenya, 2011-2016	Ngugi, J. N. et al.	2018	Kenya
35	Rabies vaccination of 6-week-old puppies born to immunized mothers: A randomized controlled trial in a high-mortality population of owned, free-roaming dogs	Arega, S. et al.	2020	South Africa
36	Demographic studies of owned dogs in the Northern Peninsula Area, Australia, to inform population and disease management strategies	Hudson, E. G. et al.	2018	Australia
37	Understanding Dog Bites: The Important Role of Human Behavior	Reese, L. A.; Vertalka, J. J.	2020	United States
Descriptor term: <i>sheltered dogs</i>				

38	The effects of human attentional state on canine gazing behaviour: a comparison of free-ranging, shelter, and pet dogs	Brubaker, L. et al.	2019	Germany
39	Do behaviour assessments in a shelter predict the behaviour of dogs post-adoption?	Clay, L. et al.	2020	Australia
40	Relationship between sociability toward humans and physiological stress in dogs	Shin, Y. J.; Shin, N. S.	2017	South Korea
41	When Walking in an Outside Area, Shelter Dogs (<i>Canis familiaris</i>) Synchronize Activity With Their Caregivers but Do Not Remain as Close to Them as Do Pet Dogs	Duranton, C. et al.	2019	France
<i>Descriptor term: spatial restriction of dogs</i>				
42	Exploring anhedonia in kennelled dogs: Could coping styles affect hedonic preferences for sweet and umami flavours?	Luna, D. et al.	2020	Chile
43	Chronic Stress in Dogs Subjected to Social and Spatial Restriction . I. Behavioral Responses	Beerda, B. et al.	1999	Netherlands
44	Chronic Stress in Dogs Subjected to Social and Spatial Restriction. II. Hormonal and Immunological Responses	Beerda, B. et al.	1999	Netherlands

ATTACHMENT III

Table 2 Characterization of the studied dog population

N sampling		Group of dogs
Descriptor term: <i>confined dogs</i>		
1	1290 residents and 1804 dogs	Owned dog
2	190 dogs	116 Confined, 61 free-roaming, and 13 stray
3	105 dogs	15 Companion; 60 free-ranging; 30 puppies (excluded)
4	974 owners and 1053 dogs	Owned dogs
5	579 dogs	Shelter dogs
6	25.000 females caught for spaying	Roaming dogs
Descriptor term: <i>dog welfare</i>		
7	557 dogs	Dogs
Descriptor term: <i>dog well-being</i>		
8	36 dogs e 92 human participants	36 PP (human participants and companion dogs) 56 PO (people alone)
Descriptor term: <i>free-ranging dogs</i>		
9	5400 dogs; females	Free-ranging dogs
10	103 dogs	Adult free-ranging dogs
11	135 dogs	Free-roaming domestic dogs
12	148 dogs	Free-ranging dogs
13	23 litters	Free-ranging dogs; 84 puppies, and 50 adults
14	72 tested; 43 females and 29 males	35 Free-ranging dogs, 25 pet dogs and 16 captive pack dogs
15	209 dogs	Free-ranging dogs - 83 adults, 58 juveniles, and 68 puppies
Descriptor term: <i>household confined dogs</i>		
16	126 cases; obsessive-compulsive disorder	103 dogs and 23 cats
17	208 households; 141 dogs	Owned dogs
Descriptor term: <i>indoor dogs</i>		
18	24 dogs	Owned dogs
19	48 dogs; 24 females, and 24 males	Owned dogs
20	24 dogs; 8 females, and 16 males	Owned dogs
21	36 dogs; 18 females, and 18 males	Pet dogs
22	79 dogs; <i>Beagle</i> breed; 42 females, and 37 males	Shelter dogs;
23	230 dogs and 118 cats	Companion dogs and cats
24	6235 castrated male dogs	Owned dogs
Descriptor term: <i>movement restriction of dogs</i>		
25	15 dogs	Shelter dogs
26	51 dogs	Domestic dogs
27	132 dogs; 8 communities	Free-roaming domestic dogs in Indigenous communities

Descriptor term: <i>roaming owned dogs</i>		
28	942 dogs; 2969 households	Owned dogs
29	171 dogs and 102 cats	Household pets; shelter housed dogs; dogs from breeding kennels
30	2098 dogs	Owned dogs
31	3240 dogs	Owned dogs
32	707 dogs; 2500 households	Owned dogs
33	28 fatalities from dog-bite injuries	Owned dogs
34	6720 dogs; 7307 bite records	Owned free-roaming dogs (78% of bites)
35	346 dogs	Owned free-roaming dogs
36	813 dogs	Free-roaming dogs
37	478 bite records	Roaming dogs (both feral and owned)
Descriptor term: <i>sheltered dogs</i>		
38	72 dogs; 36 females, and 36 males	Free-ranging, shelter, and pet dogs
39	123 dogs	Shelter dogs
40	37 dogs	21 Companion dogs and 16 shelter dogs
41	30 dogs	Shelter dogs
Descriptor term: <i>spatial restriction of dogs</i>		
42	14 dogs; <i>Beagle</i> breed	Shelter dogs
43	15 dogs; <i>Beagle</i> breed; five females and 10 males	Shelter dogs
44	15 dogs; <i>Beagle</i> breed; five females and 10 males	Shelter dogs

ATTACHMENT IV

Table 3 Parameters evaluated in selected studies

Parameters evaluated
Descriptor term: <i>confined dogs</i>
Confinement, neutering, and vaccinations for rabies
Antibodies against rabies virus in sera
Behavioral observations: <i>Dog Personality Questionnaire (Jones, 2008)</i>
Survey of dog owners about escape rates for confined dogs and history of biting
Elimination behavior of shelter dogs
Estimates of fecundity and longevity in roaming dogs
Descriptor term: <i>dog welfare</i>
Retrospective study about eight common canine disease
Descriptor term: <i>dog well-being</i>
Assessment of the effectiveness of a combined people and pet weight loss programme
Descriptor term: <i>free-ranging dogs</i>
Assessment of reproductive patterns and disorders during neutering
Immediate social reward and long-term food and social rewards
Roaming monitoring in free-roaming domestic dogs using global positioning system (GPS); identification of roaming predictors (the sex and reproductive status of the dogs)
Denning habits and preference for human proximity
Behavioral interactions
Persistence when presented with a novel object containing food that could not be accessed
Responsiveness
Descriptor term: <i>household confined dogs</i>
Obsessive-compulsive disorder (OCD)
Dog confinement, history of rabies vaccination, and history of biting
Descriptor term: <i>indoor dogs</i>
Effect of waiting rooms environment on serum cortisol, glucose, and heart rate
Behavioral synchronization between owners and indoor dogs
Activity time using food dispensing toys versus bowls
Behavioral synchronization between owners and outdoor dogs
Locomotor activity that varied as a function of age, cognitive status, and housing environment
Questionnaires to pet owners regarding air pollution and respiratory diseases
Reported behavior to the online Canine Behavioral Assessment and Research Questionnaire (<i>C-BARQ</i>)
Descriptor term: <i>movement restriction of dogs</i>
Visitor access to kennels
Intestinal parasites, richness, and dog movement restriction
Roaming activities and risk factors (age, sex, location, season, and time of day)
Descriptor term: <i>roaming owned dogs</i>
Information on number, sex, age, and rabies vaccination status
Prevalence, species distribution, and risk factors for <i>Campylobacter</i> sp.
Evaluation of dog welfare and rabies control Project: rabies vaccination coverage, body condition, sterilization, and confinement practices

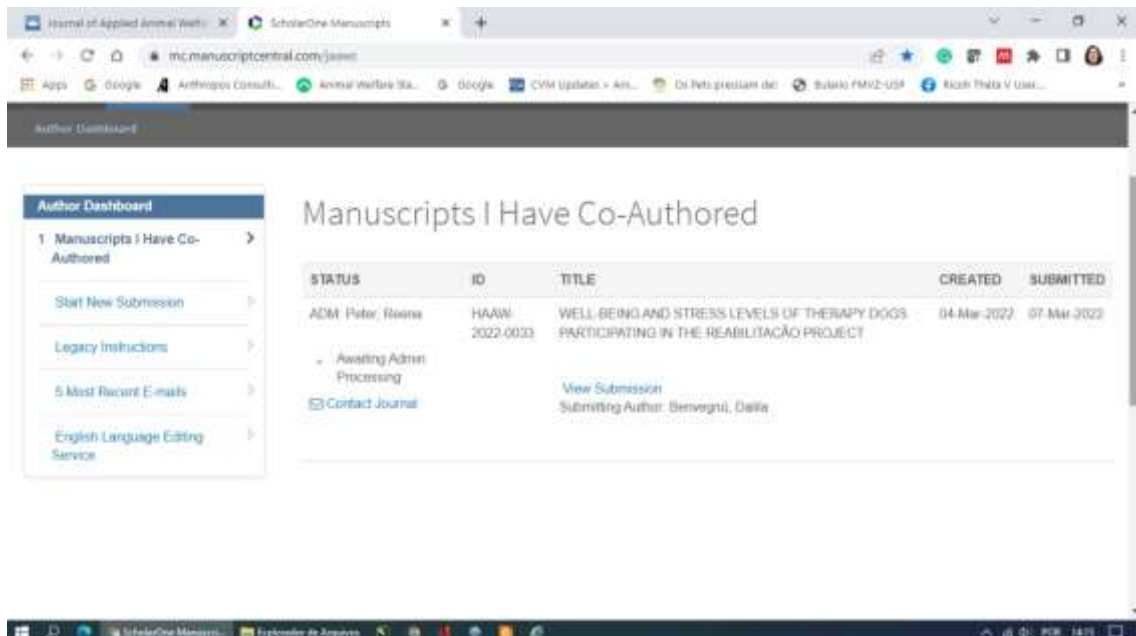
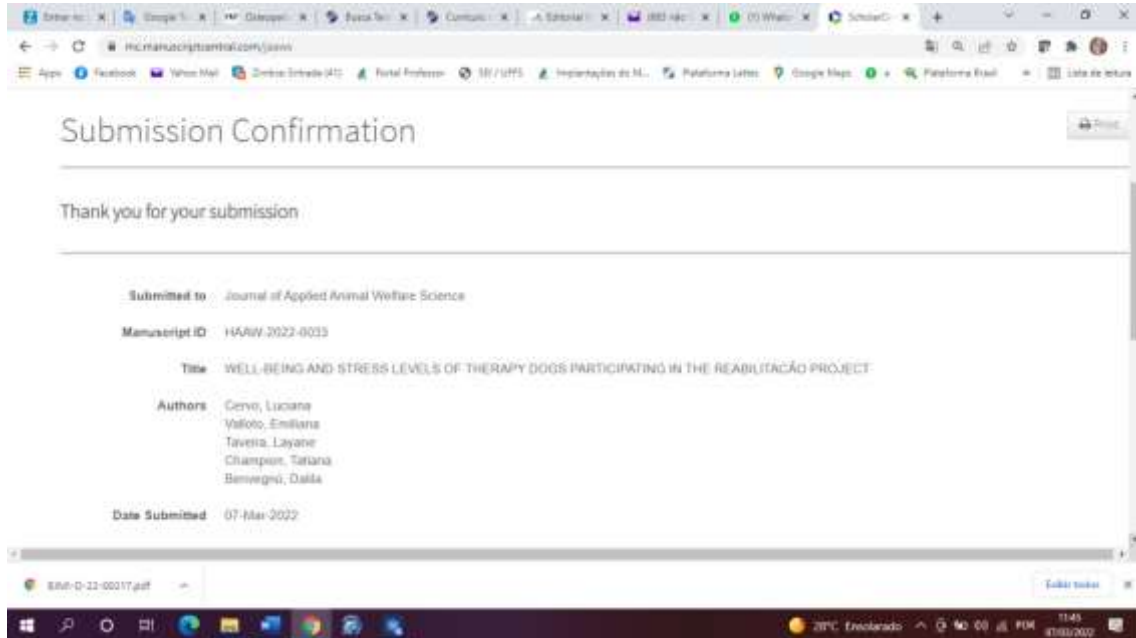
Demography study to disease and population control
Vaccine status against rabies
Predominant factors to dog-bite injuries owned, known dogs; residential location; children's unsupervised access to area with dogs; and rural/remote areas
Human animal-bites and post-exposure rabies prophylaxis
Effect of rabies vaccination of six-week-old puppies born to immunized mothers
Survey to estimate dog demographic information and investigate owners' dog management behaviors
Abandonment contribute to dog bite risk human
Descriptor term: <i>sheltered dogs</i>
Response of dogs to human attentional state; gazing behavior
Behavior assessment of friendliness/sociality, fear, and anxiety before and post-adoption
Relation between sociability and physiological stress
Behavioral synchronization between caregivers and outdoor dogs
Descriptor term: <i>spatial restriction of dogs</i>
Acceptability and preference for sucrose and monosodium glutamate; anhedonia assessment in domestic dogs
Behavioral and physiological measurements of dogs in chronic stress subjected to social and spatial restriction
Hormonal and immunological responses of dogs in chronic stress subjected to social and spatial restriction; catecholamines, cortisol, and leucocytes



**WELL-BEING AND STRESS LEVELS OF THERAPY DOGS
PARTICIPATING IN THE REABILITACÃO PROJECT**

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WELL-BEING AND STRESS LEVELS OF THERAPY DOGS PARTICIPATING IN THE REABILITAÇÃO PROJECT

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ABSTRACT

This study aimed to measure animal well-being in 14 rescued therapy dogs in the city of Itajaí, Santa Catarina, Brazil. After treatment, neutering, and vaccination, dogs are sheltered in ReabilitaCão Project at the Vale do Itajaí Penitentiary Complex, aimed at assisting the resocialization of convicts and providing for sentence reductions. Thus, this study analyzes the effects of pet therapy on the promotion of well-being in ReabilitaCão dogs via adrenal Ultrasound measurements; behavioral assessment; biochemical tests of cortisol, glucose, and lactate; and blood count. In this study, 14 mixed-breed therapy dogs, 8 were females and 6 males, with a mean weight of 13.32 ± 4.90 kg and mean age of 3 years and 10 months. Adrenal Ultrasound was performed by measuring length of left and right adrenals. Blood samples were also collected to assess blood count; serum cortisol; lactate; and glycemia. The results found for left adrenals were a mean length of 15.9 ± 1.72 mm; and for right adrenals ($n=11$), a mean length of 15.14 ± 1.36 mm. We found a mean cortisol of 2.181 ± 1.35 mcg/dL ($n=22$), glycemia 73.434 ± 13.86 mg/dL ($n=23$), and lactate of 3.331 ± 1.21 mmol/L ($n=19$). Eosinophilia was the most frequent alteration in blood count, found in 47.83% ($n=11$) of dogs ($n=23$). Regarding behavioral assessment, 56.52% ($n=13$) of dogs showed behaviors classified as aggressive and fearful ($n=23$). We concluded that the therapy dogs from ReabilitaCão show good well-being conditions, determined by an adrenal Ultrasound, associated with cortisol and lactate measurements. However, behavioral evaluation and glycemia results indicate the chronic stress response of these animals.

KEYWORDS: adrenal ultrasound; canine; stress; pet therapy; penitentiary.

INTRODUCTION

Implemented at the Complexo Penitenciário do Vale do Itajaí (Vale do Itajaí Penitentiary Complex) in the state of Santa Catarina, Brazil, the ReabilitaCão project [a play on the words “reabilitação” (rehabilitation) and “cão” (dog)] it’s a unique project in Santa Catarina, idealized by the prison agent Bruna Longen, maintained by the State and Federal Courts, which has achieved positive outcomes for the entire prison system. It is conducted with commitment and responsibility for the lives of humans and animals. In the process, “inmates train dogs while learning skills that assist their rehabilitation” (HUMBY; BARCLAY, 2018).

The Itajaí Valley Penitentiary Complex has a multidisciplinary technical classification team responsible for evaluating the semi-open regime inmates and their selection for this re-socialization and penal reduction project.

According to the Secretaria de Estado da Administração Prisional e Socioeducativa (State Secretary of Prisional and Socioeducational Administration) (2020), the “ReabilitaCão project aims to offer inmates the opportunity to create empathy and teach care practices for rescued animals, preparing them to be eligible for adoption.” For this, the inmates do the basic training of the dogs with obedience training: sit, lie down, stay, come, "box", control, and walk on the lead, aiming at socialization and adaptation.

Humby e Barclay (2018) conceptualized that “prison dog programs are a rehabilitative tool, which involves a dog being paired with one or more specially selected inmates who train, socialize, and care for the dog for a specified period of time (...).”

Moreover, “the introduction of behaviour therapy programmes to rescue shelters may produce great benefits, serving to reduce the incidence of dogs that are re-homed with undesirable behaviours, and again promoting more dog–human interactions” (WELLS, 2004).

It is important to monitor animal well-being in animal-assisted therapy projects, especially in those in which dogs are kept in shelters. Well-being parameters must be periodically evaluated by behavioral and physiological assessments. “The welfare of an individual is its state as regards its attempts to cope with its environment” (BROOM, 1986).

Polgár et al. (2019) explain that “developing optimal methods to assess the welfare of dogs in kennels is clearly a difficult, though worthwhile, endeavour.” The authors also suggest the evaluation of physiological, behavioral, and cognitive measurements that indicate chronic or acute stress in kenneled dogs.

From Beerda et al. (1999), “as in situations of acute stress, chronic stress may lead to an increased central stimulation of the HPA axis, resulting in increased cortisol (the primary glucocorticoid in dogs) secretion.”

“The synergistic nature of the hormonal interactions suggests that stress hyperglycemia cannot be ascribed to a single hormone, but is a consequence of the combined elevations of several hormones” (EIGLER et al., 1979).

“Hyperlactataemia is probably indicative of a stress response, with increased metabolic rate and sympathetic nervous system activation inducing a state of accelerated glycolysis and modified bioenergetic supply” (GARCIA-ALVAREZ et al., 2014).

Dogs usually send signals to communicate with us before they bite. Because of this, “the canine Ladder of Aggression: how a dog reacts to stress or threat” (HORWITZ et al., 2009) was developed. Such reactions occur under the influence of catecholamines in acute stress responses, in which we observe the occurrence of physiological leukocytosis, characterized by eosinophilia. However, in chronic stress responses, which are mediated by glucocorticoids, we find stress leukogram patterns where eosinopenia occurs.

“The assessment of the leukogram and its patterns is an excellent method to detect not only inflammation, severity of a disease or the response to a treatment, but also the occurrence of physical stresses or painful events” (CAFAZZO, 2014).

“Ultrasonography has become an important modality for the evaluation of the adrenal glands in small animals, because both normal and abnormal adrenal glands can be imaged, the procedure can be performed quickly and without anesthesia, and ultrasonographic equipment has become more available to practitioners” (BARTHEZ et al., 1998). According to Soulsby (2014), “it is the preferred imaging modality in evaluating adrenal glands in veterinary medicine.”

Environmental sensory stimuli act on the central nervous system, influencing the hypothalamus to produce hypothalamic hormones that control the anterior pituitary. This gland produces the adrenocorticotrophic hormone (ACTH), which acts in the adrenal cortex and stimulates the production of cortisol, which, in turn, acts in many tissues.

Thermal comfort is one sensory stimulus that directly influences adrenal gland activity. However, phenotypic (breed) and behavioral characteristics can determine the size of the adrenal gland, making it difficult to standardize reference values for adrenal gland measurements.

This study provided information about the well-being of therapy dogs participating in the ReabilitaCão project in the Complexo Penitenciário do Vale do Itajaí via behavior assessment; clinical examination; blood count; serum

glucose, lactate, and cortisol assessments, and ultrasound evaluation of adrenal glands.

MATERIAL AND METHODS

This study was conducted with the approval of the Ethics Committee on Animal Use at the Universidade Federal da Fronteira Sul (CEUA/UFFS), Campus Realeza, Brazil, on August 6, 2021, under opinion no. 8163240321, and with the approval of the Research Ethics Committee at the Universidade Federal da Fronteira Sul (CEP/UFFS) on June 30, 2021, under opinion no. 4.818.627 (protocol nº CAAE 46567121.4.0000.55).

This study included 14 therapy dogs from the kennel linked to the ReabilitaCão project from the Complexo Penitenciário do Vale do Itajaí, located in Santa Catarina, Brazil.

The kennel has an internal area composed of individual covered stalls with concrete floors, where dogs can freely access to rest and sleep, especially at night. And also, an outdoor area with a grass floor surrounded by toys, obstacles made of wood similar to those of an agility circuit, and a little swimming pool, in this space they can exercise and interact with other dogs under the monitoring of inmates.

Included dogs were mixed-breed, with a mean weight of 13.32 ± 4.90 kg, mean age of approximately 3 years and 10 months ± 2 years and 4 months. In total, our sample consisted of eight females and six males ($n=14$). Among these, 5 dogs received one evaluation and 9 dogs received two evaluations, with a three-month difference between them, with a sample size of 23 dogs.

Behavior assessment was performed right before the physical examination, there was interaction with the researchers and with the inmates who helped to restrain the dogs. It was based on the “focal animal sampling method” proposed by Altmann; Lang (1974), performed during the physical examination, which used references from “body postures of the dog” (HOUPY, 2011). “The canine Ladder of Aggression: how a dog reacts to stress or threat” (HORWITZ et al., 2009) was also used. Thus, their behavior was classified into four classes: aggressive, fearful, aroused, and neutral.

The animals were physically restrained, using a muzzle for safety, and photographed (only for medical records). Then, physical examinations were performed, which included: weighing with a model digital scale BAL 160 Cadence Electronic Scale®; inspection of the oral mucosa; measurement of rectal temperature using a digital clinical thermometer G-Tech model TH 400®; heart rate check using a model Stethoscope Rappaport Premium®; and measurement of respiratory rate by observing and counting respiratory movements.

Then, blood samples (6 mL) were collected via puncture of the jugular or the cephalic vein, intended for hemogram and biochemical analyses of serum cortisol, glucose, and lactate.

An imaging examination was performed via an ultrasound study of adrenal glands. During imaging examination, the animals were physically restrained to ensure proper positioning. Occasionally, it was necessary to use a muzzle and, for the ultrasound, the abdominal region was shaved and gel, applied to the area.

The exclusion criteria for the dogs in this study were those animals that eventually presented adrenal gland hyperplasia identified on abdominal ultrasound, due to the fact that this can result in hypercortisolism.

For the ultrasound study of the adrenal glands of dogs, the B-mode ultrasound technique was applied with a high-frequency transducer to assess positioning, size, shape, echogenicity, and diameter of adrenal glands. The device used was the Mindray Z50®. The length, caudal pole, and cranial pole of the left and right adrenal gland were measured.

Soon after blood collection and ultrasound examination, the fasting dogs returned to the kennel in which they lived and received food and water.

From the blood serum of the dogs, the biochemical parameter serum cortisol was estimated by the chemiluminescence method, with a reference value between 0.5 and 5.5 mcg/dL. Serum cortisol was measured in the morning, respecting the circadian cycle of dogs.

The analyzer Lactato Detect TD-4261 Eco Diagnóstica® was used to measure blood lactate levels. A reference value of 0.5 to 2.5 mmol/L was considered. The Descarpack Plus® Blood Glucose Monitoring System was used to determine fasting blood glucose, with reference values between 65 and 118 mg/dL.

Physiological assessment and blood analysis data were compared with reference values in Tilley; Smith (2008) and LACVeT (2018).

Statistical analyses were performed using the PSPP Program (version 1.2.0-g0fb4db). Results were expressed as mean±standard deviation of the mean, median, and interquartile range. Numerical variables were analyzed via the paired t-test for comparison over time. To analyze the association between variables, Pearson's Correlation test was used with a p-value set at lower than 0.05.

RESULTS AND DISCUSSION

This study included 14 therapy dogs from the kennel linked to the ReabilitaCão project from the Complexo Penitenciário do Vale do Itajaí, located in Santa Catarina, Brazil. Among these, 5 dogs received one evaluation because they are adopted between the visitations and 9 dogs received two evaluations, with a three-month difference between them, with a sample size of 23 dogs.

The dogs in this study were housed for a mean period of 157.217 ± 76.400 days (n=23).

Regarding behavioral assessment, we found that 56.52% (n=13) of the dogs showed behavior categorized as aggressive and fearful, whereas 43.48% (n=10) showed aroused and neutral behavior (total n=23). Among the nine dogs that remained in the shelter between our first and second visits, we found that 66.66% (n=6) showed behavior categorized as aggressive and fearful, and 33.33% (n=3), aroused and neutral behavior, remaining the same between the first and second assessments.

One possible explanation for these results could be the fact of the short time between the visits, three months may not be enough to change Behaviour despite the training received from inmates and because dogs have no behavior history known since they are rescued from relinquishment.

The results of the behavioral assessment of ReabilitaCão dogs suggest that the animals are in a situation of chronic stress. According to Beerda et al. (1997) "(...) increases in frequencies of vocalizations and behavioural elements associated with fear and submission (snout licking, paw lifting and a lowered posture) may occur in dogs that experience stress."

The average body weight found for the study dogs was 14.283±4.702 kg (6.1–21.7) (n=23). There was a difference between the body weight of the dogs that remained in the shelter between the first and second assessments: 14.789±4.320 kg (7.9–20.9) vs. 15.778±4.209 kg (9.2–21.7), p=0.006 (n=9).

In our study, two dogs maintained the same weight between the first and second assessments. Meanwhile, seven dogs gained weight during this period, with an average weight gain of 9.87% (n=7).

In their study, Ricci et al (2007) found that “the dogs had been keeping in the shelter for an average of 14 months and more than 60% were overweight or obese(...) Weight gain occurs when an animal cumulates a positive energy balance for an extended period.”

Physical examination analyzed mean rectal temperature in degrees Celsius (°C), mean heart rate in beats per minute, and mean respiratory rate in respiratory movements per minute (Table 1). Results are within the reference values shown in the literature (TILLEY; SMITH, 2008). Furthermore, p-values (Table 1) in the paired t-test were higher than the 0.05 significance level. Therefore, we found no significant difference between the means of the analyzed variables.

Table 1. Results* of the physical examination of dogs sheltered in the ReabilitaCão Project

Physical examination under the full evaluation (n=23)					
Mean body temperature (°C)		Mean heart rate (bpm)		Mean respiratory rate (bpm)	
38.7°C±0.32		95±19,00		38±12.21	
Physical examination in sequential evaluations (n=9)					
Mean body temperature (°C)		Mean heart rate (bpm)		Mean respiratory rate (bpm)	
1st evaluation	2nd evaluation	1st evaluation	2nd evaluation	1st evaluation	2nd evaluation
38.5°C±0.39	38.8°C±0.32	92±19.97	93±17.33	32±11.07	42±13.70
p=0.071		p=0.914		p=0.949	

*Results shown as mean±standard deviation.

Perego et al. (2014) assessed “serum cortisol and glucose concentrations, and heart rates in 24 dogs after 20 minutes in a waiting room (group A) or in an outdoor garden (group B).” The mean heart rate found for group B (93.2 ± 24.4 bpm) is similar to those found in our second evaluation of ReabilitaCão therapy dogs (93 ± 17.3333 bpm).

Based on these results, we can observe that, although we found no significant differences in mean respiratory rates, the values found in our second assessment were higher than in our first assessment. This may be explained by the variation in ambient temperature between physical examinations.

According to the website Climatempo (2022), the minimum temperatures in the city of Itajaí in August and September, in which our first assessments were carried out, remains “between 14 and 15°C and the maximum temperature at 20°C”; whereas in December, we find higher temperatures with “a minimum temperature of 20°C and a maximum temperature of 26°C” (CLIMATEMPO, 2022).

This result agrees with that found by Davis et al. (2017): In general, dogs responded to an increase in ambient temperature by increasing their respiratory rate. This is so the body can dissipate heat and maintain homeostasis.

Likewise, we can observe that, despite no significant differences and the fact that our results are within reference values, our second evaluation found an increase of 0.3°C in the rectal temperature of the dogs studied.

From Carter; Hall (2018), “as temperature, humidity and wind speed all influence body temperature, it is important to consider the thermal impact of the combined effect of all three, when investigating environmental impact on body temperature.”

Still regarding physical examination, we found that 26.1% (n=6) of the dogs showed pale oral mucosa staining, whereas 73.9% (n=17), normochromic oral mucosa (total n=23). This evaluation was repeated in nine dogs, and we found that, in the first evaluation, 44.44% (n=4) of the dogs showed pale oral mucosa staining and 55.56% (n=5), normochromic oral mucosa. In the second evaluation, 100% (n=9) showed normochromic oral mucosa staining.

Given the above, it is suggested that ReabilitaCão dogs had a subtle decrease in fearful behavior with a lower “fight-or-flight response,” evidenced by the normochromic mucosal staining in the second evaluation.

Regarding the “fight-or-flight or acute stress response” Chmelíková et al. (2020) state that, among other changes, “catecholamine (epinephrine and norepinephrine) release results in glycolysis, vasoconstriction in many networks of minute blood vessels, and vasodilatation of blood vessels in the skeletal muscles and liver”. Thus, “white or pale mucous membranes may indicate vasoconstriction or anemia, both of which may lead to decreased oxygen delivery to the tissues” (LATIMER-JONES, 2020). Therefore, as dogs remain in shelters for longer, an adaptation occurs, decreasing acute stress reactions.

Also, reestablished normochromic coloring in mucous membranes may indicate that ReabilitaCão dogs showed recovery from verminous diseases since in those that showed eosinophilia, as shown below, the use of a vermicide (Pyrantel Pamoate and Praziquantel) was prescribed. According to the results of Qadir et al. (2011), “a significant increase ($P<0.01$) in eosinophils and decrease in lymphocytes were also observed in parasitized compared to non-parasitized animals.”

In the hemogram evaluation, we verified the occurrence of eosinophilia, the most frequent alteration in blood count, found in 47.83% ($n=11$) of the dogs. In our sample, 34.78% (8) showed no significant changes in blood count; 8.70% ($n=2$) had anemia; 4.35% ($n=1$), neutropenia; and 4.35% ($n=1$), lymphocytosis (total $n=23$).

When comparing the first and second blood tests ($n=9$), we found eosinophilia in 55.56% ($n=5$) of the dogs in the first analysis, with a decrease to 33.33% ($n=3$) in the second evaluation. In the first analysis, 33.33% ($n=3$) showed no significant changes in blood count, with an increase to 44.44% (4) in the second examination. Regarding anemia, 11.11% ($n=1$) of the dogs showed anemia in both evaluations, and the same occurred to lymphocytosis, 11.11% ($n=1$).

According to Budziak et al. (2016), who also found eosinophilia in their study with shelter dogs, this can be explained “mainly by the intense parasitism since, as they are shelter animals and without a clinical history, there is not enough information to prove the other causes described.”

The mean found for plasma cortisol in ReabilitaCão dogs was 2.181 ± 1.353 mcg/dL ($n=22$) (Table 2), though the sample from one animal was insufficient to measure cortisol. All dogs presented cortisol values within reference values: 0.5–

6.0 µg/dL or 13.8–165.5 nmol/L (LACVeT, 2018). Between the first and second collections, it was possible to repeat the measurement of serum cortisol in eight of the dogs which remained in the shelter.

Table 2. Results* of biochemical tests of cortisol, glucose, and lactate in dogs sheltered in the ReabilitaCão Project.

Biochemical tests under full evaluation					
Mean Cortisol (mcg/dL)		Mean Glucose (mg/dL)		Mean Lactate (mmol/L)	
n=22		n=23		n=19	
2.181±1.35 mcg/dL		73.434±13.86 mg/dL		3.331±1.21	
Biochemical tests in sequential evaluation					
Mean Cortisol (mcg/dL)		Mean Glucose (mg/dL)		Mean Lactate (mmol/L)	
n=8		n=9		n=6	
1st evaluation	2nd evaluation	1st evaluation	2nd evaluation	1st evaluation	2nd evaluation
2.525±1.74	1.475±0.88	78.444±9.62	62.333±10.44	2.816±1.32	3.150±1.31
p=0.087		p=0.003		p=0.622	

*Results shown as mean±standard deviation.

We performed a paired t-test with the cortisol variables of the first and second blood collection in ReabilitaCão dogs (n=8). The p-value found was 0.087, a value greater than the significance level of 0.05. Therefore, we found no significant difference between the average plasma cortisol of the kenneled dogs, although the average value of the second collection was lower than the first.

This decrease could be explained by adaptation to simple acts, stressors, and common everyday situations since they are adjustments of the organism to maintain balance, also called homeostasis. Stress mediators have a dual activity because the organism can react differently to a homotypic or heterotypic stressor, and habituation to certain stressors can occur. This ambiguous activity of mediators in the face of stressors reminds us of “Allostasis: A New Paradigm to Explain Arousal Pathology” by Sterling and Eyer (1988).

According to Beerda et al. (1999), “due to physiological adaptations and stressor-specific responses undisturbed levels of cortisol may not exclude chronic stress, but increased cortisol levels are a strong indication for it.”.

Regarding the “plasma cortisol” welfare measurement, Polgár et al. (2019) state that feasibility requires skill to obtain and it’s expensive to analyze; indicate acute stress; as it fails to provide a clear valence, only indicating arousal”; concerning potential confounds in the sampling procedure, as well as diurnal patterns, temperature, and activity levels which may confound results.

The average fasting glycemia in ReabilitaCão dogs was 73.434 ± 13.855 mg/dL (n=23). Between the first and second collections, it was possible to repeat the glucose assessment in nine dogs which remained in the shelter. These showed mean blood glucose levels of 78.444 ± 9.619 mg/dL in the first collection and of 62.333 ± 10.440 mg/dL in the second.

A paired t-test was performed for the fasting glucose variables collected during the first and second blood collection in ReabilitaCão dogs (n=9). The p-value found was 0.003, i.e., a value lower than the significance level of 0.05. Therefore, we observed a significant difference between blood glucose averages, resulting in a lower blood glucose value in the second collection.

We found a difference between fasting blood glucose means in the first and second blood collection of the dogs, and the main interference factor to be considered is the period of stay in the shelter.

Therefore, we performed the Pearson’s correlation test using the number of days for the sheltered animal as variable “x” and blood glucose as variable “y,” which resulted in an r value equal to -0.77261. Thus, it was possible to verify a strong negative correlation, i.e., as the length of stay in the shelter increased, the fasting blood glucose of the dogs decreased, which may characterize a low glycogen reserve, available in the form of glucose, in the liver, possibly due to the chronic stress of confinement, which makes the body less responsive to stress and its main mediating hormone cortisol. In case of acute stress, we would possibly find results suggesting hyperglycemia.

From Chen et al. (2020), “(...) chronic stress induces the imbalance of glucose homeostasis”. In our study, the second evaluation showed lower mean cortisol levels, suggesting an adaptation process and explaining the decrease in blood glucose in the second evaluation. According to Harvey and Ferrier (2012), “cortisol, adrenaline, and glucagon are catabolic signals that promote the breakdown of proteins, triacylglycerols, and glycogen.”

In their study, which evaluated the cortisol levels of sheltered dogs, Hennessy et al. (1997) found that “groups of dogs confined in the shelter for their 1st, 2nd, or 3rd day had higher cortisol levels than a group maintained in the shelter for more than 9 days.”

Regarding the average found for fasting lactate in ReabilitaCão dogs, we obtained a value of 3.331 ± 1.205 (1.3–4.6) mmol/L ($n=19$), with four samples showing error 46 in the performance of the test. According to the instruction manual of the Lactato Detect TD-4261® instrument, this error 46 happens when hematocrit values exceed the detection limit. However, the hematocrit values of these samples were 44; 54.2; 41.9; and 40%, all within the reference values for dogs.

The average lactate found in ReabilitaCão dogs is above the reference range considered (0.5 to 2.5 mmol/L, LACVeT, 2018). This result is similar to the results found in dogs trained for agility competitions during their recovery period, i.e., five minutes after exercise.

In their study on dogs, Rovira et al. (2007) found the following “resting lactate (LA) values in dogs trained for agility competitions 2.4 ± 0.71 (1.4–3.8) mmol/L” and “significant increases in LA (4.56 ± 0.36 mmol/L) concentrations were observed after exercise”. They observed that, five minutes post-exercise, lactate values were 3.4 mmol/L.

Between the first and second collections, it was possible to repeat the lactate assessment of six dogs which remained in the shelter. They showed mean lactate levels equal to 2.816 ± 1.322 mmol/L in the first collection and equal to 3.150 ± 1.305 mmol/L in the second collection.

Although there was no difference between the lactate averages in the first and second blood collections, we observed an increase in serum lactate in the second collection; a result more akin to the results found by Rovira et al. (2007) in dogs trained for agility competitions post-exercise: “the duration and the speed of agility competitions do not result in a strong physical stress for trained dogs”.

Given the above, we can suggest that ReabilitaCão dogs were in a stress situation similar to that of dogs trained for agility. Moreover, the structure of the ReabilitaCão project has an area intended for training and exercise with therapy dogs, activities which are regularly developed under the inmates’ supervision.

The result found for the average weight of ReabilitaCão dogs is 14.283 ± 4.702 kg (6.1–21.7). The average weight for the dogs evaluated for serum lactate was 14.253 ± 4.374 kg (6.1–21.7 kg). In dogs which had their serum lactate reassessed, we found a difference between the results of the first (13.650 ± 3.683 kg) and second collections (14.667 ± 3.878 kg), $p=0.037$ ($n=6$).

Therefore, we performed the Pearson's correlation test using the serum lactate in the first collection as variable "x" and the body weight of dogs in the first collection as variable "y", which resulted in a value of r equal to 0.80202.

Likewise, we performed the Pearson's correlation test using the serum lactate in the second collection 2 as variable "x" and the body weight of dogs in the second collection 2 as variable "y", which resulted in a value of r equal to 0.63548.

Therefore, it was possible to verify a strong positive correlation between serum lactate and body weight in the first collection and a moderate positive correlation in the second collection. These results suggest that the increase in body weight between the first and second evaluations justifies the higher levels of serum lactate in the second collection.

This correlation agrees with the results found by Franco et al. (2016). In this study, the mean lactate value found for dogs whose body weight ranged from 8.1 to 26 kg was 3.11 ± 0.53 mmol/L (2.0–3.9). According to the authors, "when the serum lactate values were related to the measured clinical parameters, a significant correlation of the values with heart rate and body weight could be identified, suggesting the interference of the clinical parameter and the variation of body weight in the serum lactate values canine."

In our study, however, we found a strong negative correlation evidenced by the value of r equal to -0.79054 in the Pearson's correlation test which used serum lactate from the first collection as variable "x" and heart rate of dogs at the time of the first evaluation as variable "y".

The results found for the ultrasound evaluation are shown as median and interquartile range (25% and 75%), mean and standard deviation (Table 3). Except for the measurement of left adrenal length, the measurements found are equivalent to those found by Melián, C et al. (2020) in clinically healthy dogs falling under the weight category > 10–20 kg, $n=22$ (Table 3).

Table 3. Ultrasound adrenal glands measurements in dogs sheltered in the ReabilitaCão Project, (mean weight 14.90±4.27 kg). Data are shown as median interquartile range (25–75th percentile), mean±standard deviation (n=11)

Ultrasound adrenal glands measurements under full evaluation (n=11)		
Left Adrenal Gland		
Length (mm)	Cranial Pole Width (mm)	Caudal Pole Width (mm)
Median and interquartile range (25–75th percentile)		
15.9 (14.6-16.4)	4.8 (4.3-5.1)	4.7 (4.3-4.95)
* 21.50 (19.40–24.40)	* 4.50 (4.10–4.90)	* 5.00 (4.50–5.50)
Mean±standard deviation		
15.93±1.72	4.70±0.47	4.65±0.50
14.21–17.65	4.23–5.17	4.15–5.16
**16.80±6.20	** 3.80±0.80	** 4.40±0.70
Right Adrenal Gland		
Length (mm)	Cranial Pole Width (mm)	Caudal Pole Width (mm)
Median and interquartile range (25–75th percentile)		
15.0 (14.15-15.45)	4.9 (4.3-5.1)	4.8 (4.25-5.0)
* 16.90 (13.40–19.40)	* 5.30 (4.60–6.20)	* 4.70 (4.20 - 5.70)
Mean±standard deviation		
15.14±1.36	4.71±0.54	4.72±0.47
13.77–16.50	4.17–5.25	4.25–5.19
** 16.70±3.30	** 4.10±0.90	** 4.80±1.10

* Melián, C. et al. (2020); **Fernandez, S. et al. (2017)

Regarding Melián, C et al. (2020) “the study was performed in Las Palmas de Gran Canaria” being that “the >10–20 kg weight category of clinically healthy dogs included mixed-breed (11), French Bulldog (4), American Staffordshire Terrier (2), Staffordshire Bull Terrier (2), Border Collie (2), and Canarian Sight Hound (1).” Our study only included mixed-breed dogs. However, among these, one of them showed a similarity with the Schnauzer breed and another with the Pit Bull breed. This fact may explain the smaller results in the measurement of the length of the left adrenal gland in ReabilitaCão therapy dogs in Itajaí.

Another factor that might explain this difference in length of the left adrenal between the dogs of the two studies could be due to climate, which, in Itajaí, Santa Catarina, Brazil, is characterized as humid subtropical.

“The humid subtropical climate, predominant in Santa Catarina, provide pleasant temperatures, ranging from 13 to 25°C, with rains distributed throughout the year” (SANTA CATARINA, 2017).

“According to Thomas Whitmore’s research centre at Syracuse University Las Palmas is the city with the best climate in the world” (GONDA-SOROCZYŃSKA; OLCZYK, 2017).

According to the institution AVMA (2012), “Generally, for dogs and cats, the ambient temperature should be kept above (...) 10°C, and below (...) 26.6°C (...). Animals should be protected from extreme temperatures so as to maintain their health and render their environment comfortable.”

For Bhatnagar et al. (2006), “Exposure to stressful stimuli produces widespread physiological and behavioural effects in animals, including changes in hypothalamic-pituitary-adrenal (HPA) function (1), body temperature (2) and food intake and body weight gain (3).”

Eik-nes; Samuels (1958), in their study on stress in dogs, state that “during “stress,” the metabolism or “utilization” of adrenal steroids may be increased. Since this would result in a reduction of the concentration of adrenal steroids in the blood, this might constitute a stimulus for increased secretion of ACTH by the anterior pituitary gland.” “Cold or heat stresses produce marked accumulation of cortisol in the adrenal gland (...) in mice” (WON; LIN 1995). Thus, thermal discomfort can act as a sensory stimulus and cause changes in hypothalamic and pituitary hormonal responses and, consequently, alterations in adrenal gland dimensions.

The results of adrenal gland measurements in therapy dogs in this study are also similar to the results found for healthy dogs by Fernandez, S. et al. (2017) (Table 3). That study was conducted in Jaboticabal, Brazil, which has a tropical climate. However, ultrasonography was performed to evaluate the adrenal glands of 30 healthy adult dogs, aged from one to seven years, weighing from two to 15 kg, and of different breeds: French Bulldog (17), Pug (10), and Shih Tzu (3).

This research was conducted despite the limitations of time and security of the penitentiary. Our sample totaled 14 dogs but 5 of them were adopted during the study. Also, dogs have no medical or Behaviour history known because they come from relinquishment.

CONCLUSION

The ReabilitaCão dogs show good well-being, determined by physical examination and ultrasound evaluation of adrenal glands associated with the measurement of blood cortisol. However, behavioral assessment and glucose measurement suggest chronic stress in those animals.

Serum lactate measurements showed similar stress conditions between ReabilitaCão dogs and dogs trained for agility. However, their body weight increased significantly between our first and second assessments. The ReabilitaCão project has an area intended for training and exercise with therapy dogs and these activities are carried out regularly under the inmates' supervision.

Both the assessment of well-being and the diagnosis of endocrinopathies require an association between hematological variables and imaging tests so that we have access to the physiological and immunological stress responses of dogs.

Regarding support measures to optimize the well-being of ReabilitaCão dogs, it is suggested to provide veterinary medical support for weekly clinical and post-surgical follow-ups of the animals; periodic deworming, preferably repeating drug dosage after 15 days; immunization against canine flu; care with length of exposure to the sun, especially for those dogs with light coats; and care in the maintenance of body weight via diet and exercise.

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5 CONCLUSÃO

O estudo transversal do bem-estar dos cães do **ReabilitaCão**, por meio de avaliações comportamentais e fisiológicas, indicou **estresse crônico que as atividades de cinoterapia no Complexo Penitenciário do Vale do Itajaí desencadeiam nos cães.**

O estresse crônico dos animais foi evidenciado por avaliação comportamental, principalmente, com indícios de sinais de agressividade ou medo por parte de 56,52% dos cães e também pelo tempo de confinamento que possui uma forte correlação negativa com a glicemia dos cães.

O estudo forneceu embasamento para tomar decisões sobre melhorias no projeto, principalmente, com relação ao tratamento clínico daqueles animais que apresentaram alterações hematológicas significativas.

A pesquisa contribuiu para o aprendizado e ressocialização dos aprisionados. Tivemos a colaboração dos detentos para a realização do projeto o que possivelmente, trouxe benefícios para todos os envolvidos, fortalecendo a empatia, companheirismo, espírito de equipe e cooperação.

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ANEXO I

Quadro 1. Ficha de Identificação do Projeto ReabilitaCão

Ficha de Identificação do Projeto ReabilitaCão (INSTITUTO AMBIENTAL ECOSUL, 2020)
Nome: ReabilitaCão
Data de Implementação: 04/10/2019
Setor de Atuação: Execução Penal e Psicologia
Número de Participantes (remunerados): 1
Número de Participantes (voluntários): 12
Número de Pessoas Beneficiadas: 350
Resumo: Em avaliação realizada, a equipe técnica da Complexo Penitenciário do Vale do Itajaí concluiu pela necessidade de novas estratégias para retomada do apenado ao convívio social após o cumprimento da pena. O projeto ReabilitaCão atua em três linhas principais: O resgate, recuperação, bem-estar e recolocação em novos lares dos cães abandonados no entorno do Complexo Penitenciário do Vale do Itajaí; A ressocialização do apenado, despertando seu senso de responsabilidade, valores e empatia, através dos cuidados com os animais resgatados; A reinserção destes apenados no mercado de trabalho após o cumprimento da pena, por meio da capacitação profissional em cursos de banho e tosa e auxiliar de veterinário. O método escolhido foi a Cinoterapia (terapia assistida com cães), que vem sendo utilizado em outras unidades prisionais no Brasil e do exterior, como tratamento coadjuvante, auxiliando melhorias em todos os sentidos na saúde e personalidade dos apenados.
Problema: Alto índice de cães abandonados nas redondezas do Complexo Penitenciário do Vale do Itajaí em precárias condições de saúde e nutrição e a necessidade de ocupação, ressocialização e capacitação para o mercado de trabalho dos apenados através do convívio, cuidados, empatia e interação com animais.
Objetivos do Projeto: <ol style="list-style-type: none">1. Resgate, recuperação, bem-estar e recolocação em novos lares dos cães abandonados no entorno do Complexo Penitenciário do Vale do Itajaí;2. Ressocialização do apenado, despertando seu senso de responsabilidade, valores e empatia, através dos cuidados com os animais3. Reinserção deste apenados no mercado de trabalho após o cumprimento da pena, por meio da capacitação profissional em cursos de banho e tosa, adestramento e auxiliar de veterinário
Descrição: Estudos da equipe técnica do Complexo Penitenciário do Vale do Itajaí resultou na necessidade de novas estratégias para reintrodução do apenado ao convívio social, com técnicas e resultados diversos dos até então obtidos pelos métodos tradicionais aplicados em Unidades Prisionais. Baseados em modelos utilizados com sucesso em outros países, optou-se pela Cinoterapia, após a constatação da sensibilidade e interesse de alguns apenados pelos animais de companhia foi criado o projeto ReabilitaCão, estimulando o desenvolvimento de suas potencialidades intelectuais, sociais e emocionais através dos cuidados com animais, como facilitadores à sua reinclusão na sociedade após o cumprimento da pena. O Projeto recebe o apoio e parceria das Ongs. e ativistas independentes regionais. Os animais participantes são resgatados do abandono no entorno do Presídio e são castrados, vacinados, recebem assistência veterinária, alimentação e abrigo e tem garantido o seu bem-estar através

da tutela dos apenados. Após o que se convencionou denominar “Ciclo de Passagem”, participam de feiras de adoção realizadas pelas Ongs. Visando preservar os limites estabelecidos, a introdução de novos animais é proporcional àqueles resgatados, recuperados e adotados. Ao mesmo tempo são oferecidos cursos de auxiliar de veterinário e banho e tosa aos apenados previamente selecionados a partir de laudos psicológicos individuais elaborados por uma comissão técnica da Unidade Prisional. O Projeto suscitou também a formação de uma equipe multidisciplinar de servidores Policiais e Agentes Penais, Psicólogos Prisionais e Veterinários na área da Cinoterapia. A Direção do Complexo Penitenciário peticionou e o juizado da Vara de Execuções Penais da Comarca de Itajaí avaliou e após conhecer in loco o Projeto e ouvidas a Promotoria de Justiça e a Defensoria pública da Comarca, deferiu a remição de pena aos presos participantes, de acordo com o Art. 28 da Lei de Execução Penal.

Resultados:

32 (trinta e dois) cães resgatados em 10 (dez) meses de projeto;

21 (vinte e um) cães adotados por famílias responsáveis nas feiras de adoção;

32 (trinta e dois) cães a menos nas ruas, foram castrados, vacinados e nutridos.

Criação de uma modalidade diferenciada de reabilitação do apenado no Estado de Santa Catarina, buscando seu aprimoramento e contribuindo para seu retorno à sociedade.

Formação de uma equipe multidisciplinar de servidores Policiais e Agentes Penais, Psicólogos Prisionais e Veterinários na área da Cinoterapia.

Despertar o interesse no Projeto nas Unidades Prisionais de Florianópolis, Jaraguá do Sul, Palhoça e ao TJ do RS.

ANEXO II

Table 1
Practicality of Animal-Based Measures for Assessing Welfare of Kennel Dogs. A summary of the feasibility and confounds of physiological, behavioural, and cognitive measures, including inference to whether they indicate chronic (across multiple days) or acute (within a single day) stress, and whether their values (stress v eustress) can be determined.

Welfare Measure	Feasibility	Chronic v. Acute	Clear subject?	Potential Confounds	References
PHYSIOLOGICAL MEASURES					
Cortisol – urinary or fecal	Relatively easy to obtain. Expensive to analyse.	Acute	No – only indicates arousal	Diurnal patterns, temperature, activity levels may confound results	Beards et al., 2008; Stephens and Lodge, 2009; Menni et al., 2005; Rooney et al., 2007; Arcuri et al., 2008; 2009; Ryan et al., 2013; Vitale et al., 2011; Buckner et al., 2010
Cortisol – salivary	Relatively easy to obtain. Expensive to analyse.	Acute	No – only indicates arousal	Sampling procedure as well as diurnal patterns, temperature, activity levels may confound results	Stewart and Mitchell, 1992; Beards et al., 1996, 1998; Hill et al., 2008; Pini et al., 2014; Goh et al., 2010
Cortisol – plasma	Requires skill to obtain. Expensive to analyse.	Acute	No – only indicates arousal	Sampling procedure as well as diurnal patterns, temperature, activity levels may confound results	Beards et al., 1996; Clark et al., 1997; Hennessy et al., 1993, 2003a, 2003b, 2006, 2011; Dooly et al., 2007
Cortisol – hair/fur	Relatively easy to obtain. Expensive to analyse.	Chronic	No – only indicates arousal	Some evidence that hair colour and handling may influence results. Hair processing procedures vary in effectiveness of removing cortisol.	Reisner and Hesteva, 2003; Ryan et al., 2003; Krukowski et al., 2013; Buckner and Swanson, 2014; Vitale et al., 2014; Nicholson and Albonetti, 2009; Puster, 2009; Gray et al., 2007; Chomarov et al., 2017
Heart rate	Requires special equipment	Acute	No – only indicates arousal	Dogs body movement and posture may affect measure. Wearing the device may distress some dogs.	Marin et al., 2005; Wernsdorf et al., 2013; Gray et al., 2017; Wernsdorf et al., 2017
Heart rate variability	Requires special equipment	Acute	No – likely only indicates arousal	Dogs body movement and posture may affect measure. Wearing the device may distress some dogs.	Marin et al., 2005; Kawaguchi et al., 2010; Fujita et al., 2016; Wernsdorf et al., 2013; Gray et al., 2017; Wernsdorf et al., 2017
Temperature	Thermography requires special expensive equipment, discomfort may impair handling/handlers	Acute	No – only indicates arousal	Environment for thermometers may confound results. Thermographic results may be influenced by external temperature, moisture, discomfort and heat reflecting ability of environment. Some dogs may also show sensitive behaviour towards camera.	Cygan et al., 2009; Puri et al., 2014; Wernsdorf, 2014; Hill et al., 2014; Goh et al., 2014; Thomsen et al., 2013; Orange and Carter, 2013; Tikhonov et al., 2014; Wernsdorf and Müller, 2016; Rorer et al., 2016
Immune function (IgA/ antibody and WBC levels)	Requires skill to obtain. Expensive to analyse.	Chronic	Potentially – more research needed	Some studies have found significant links, and some individual variations. Some evidence of diurnal patterns. Breed and age need to be taken into account. Handling may confound results depending on sampling method.	Wernsdorf et al., 1995; Clark et al., 1995; Strass et al., 1999; Kikawa and Uchida, 2002; Glass et al., 2014; Dooly et al., 2016; Panagiotou, 2016
Weight change	Relatively easy to assess	Chronic	Potentially – more research needed	Changes in diet, exercise levels, or medications could confound results. Some physical ailments (e.g. worms) may influence weight without necessarily immediately impacting welfare.	Stress and Johnson, 1982; Rooney et al., 2008
Disease incidence	Relatively easy to assess	Chronic	Yes	Outbreak of disease or close proximity and poor cleaning procedures in kennels	Stebbins et al., 2004; Erba and Wernsdorf, 2009; Lerner and Clark, 2003
Oxytocin – urinary	Relatively easy to obtain. Expensive to analyse.	Acute	Yes	More research is needed	Stank et al., 2011; Pálinka et al., 2016
Flu mucus	Requires dry environment to assess	Acute	No	More research is needed. Could also be caused by warm temperatures	Waggoner, 2007
Pupillary dilation	Difficult to assess without confounding	Acute	No – only indicates arousal	Lighting and evaluation need to be taken into account. More research is needed. Could also be caused by allergies at physical irritants. Breed differences in facial morphology may also confound results.	Waggoner, 2007; Puri et al., 2014
Eye mucus	Relatively easy to assess	Acute	Unclear	More research is needed. Effects of diet/dieting/medication also need to be considered.	Puri et al., 2014
Facial consistency	Easy to assess	Acute	No – only indicates arousal	More research is needed. Effects of diet/dieting/medication also need to be considered.	Waggoner, 2007; Puri et al., 2014
Excessive shedding of skin or hair / skin dryness	Relatively easy to assess	Chronic	Unclear	More research is needed. Effects of diet/dieting/medication also need to be considered.	Waggoner, 2007; Puri et al., 2014
BEHAVIOURAL MEASURES					
Aggression & fear related behaviours	Easy to assess	Acute	Yes	Individual differences in behaviours established.	Beards et al., 1997, 1998a; Stephens and Lodge, 2009; Hill et al., 2008; Rooney et al., 2008, 2009, 2010; Fitzwill et al., 2014
Response to strangers	Easy to assess	Acute	Yes	Ferrous experiences/early socialisation may influence response.	Hilborn, 1996; Ariani and Trösel, 2014; Goolley et al., 2014
Response to novel object	Easy to assess	Acute	Yes	Ferrous experiences/early socialisation may influence response. Some indication of breed differences.	Wankler, 1971; King et al., 2002; Beckman et al., 2007; Lay et al., 2007

(continued on next page)

Table 1 (continued)

Welfare Measure	Feasibility	Chronic v. Acute	Clear subject?	Potential Confounds	References
Abnormal repetitive behaviour	Easy to assess	Chronic	Yes	Behaviours can persist even after stimuli has been removed from enriched environment. Behaviours may persist because they are rewarded. Behaviours may serve as coping mechanisms.	Hilborn et al., 1992; Beards et al., 1998a; Stank and Latham, 2004; Stephens and Lodge, 2009; Tschö and Mills, 2007; Hennessy et al., 2007; Rooney et al., 2008; Waggoner, 2010; Denton et al., 2014; Panagiotou et al., 2016, 2018; Beards et al., 1998a; Frasure et al., 2008 as cited in Rooney et al., 2009; Liu et al., 2010
Coprophagia	Easy to assess	Chronic	Unclear	More research is needed. Possible breed differences.	Herrn et al., 1992; Clark et al., 1997; Stephens and Lodge, 2009
Excessive barking	Easy to assess	Both	Unclear	More research is needed. Possible breed differences. Behaviours may persist because they are rewarded.	Clark et al., 1997; Stephens and Lodge, 2009; Hill et al., 2008; Fikles and Cohen, 2014
Excessive drinking	Easy to assess	Both	Unclear	More research is needed. Temperature or previous experiences may confound.	Haley et al., 2005; Hill et al., 2009; Rautava et al., 2012; Jones et al., 2015; Puri et al., 2016; Chivers and Horrocks et al., 2016
Activity/Resting	May require specialised equipment	Both	Unclear	Difficult to distinguish between rest and boredom/learned helplessness/depression states	Haley et al., 2005; Hill et al., 2009; Rautava et al., 2012; Jones et al., 2015; Puri et al., 2016; Chivers and Horrocks et al., 2016
Play	Spontaneous play needs video recording. Enriched play relatively easy to assess.	Both	Yes	Ferrous experiences/early socialisation may influence willingness to play. Possible breed differences. Spontaneous behaviours are more relevant than elicited ones.	Hilborn, 1998; Rooney et al., 2008; Waggoner et al., 2009; Hilborn et al., 2008; Hilborn et al., 2010; Waggoner et al., 2010; Hill and Spinka, 2011; Trösel et al., 2013; Johnson et al., 2013; Wernsdorf et al., 2013; Gray et al., 2014; Buckner et al., 2015; Turner, 2011; Abbey-Bishop et al., 2017
COGNITIVE MEASURES					
Cognitive bias	Requires training/time	Chronic	Yes	Differences in excitability and motivation may influence results	Frederf et al., 2004; Hill et al., 2009, 2010; Bascara et al., 2011; Dunbar et al., 2013; Grammatik-Garabito et al., 2016
Learning ability	Requires training	Both	Unclear	Individuals may be differently motivated by different rewards. Different training styles may be more/less effective.	Glass et al., 2009; Hilborn et al., 2010; Rooney and Dunbar, 2011; Hill et al., 2009

Figura 1. Resumo das medidas fisiológicas, comportamentais e cognitivas relacionadas ao estresse crônico e agudo em cães de canis (POLGÁR et al., 2019).

ANEXO III

Quadro 2. Ficha de Monitoramento de Bem-Estar Animal

Ficha de Monitoramento de Bem-Estar Animal			
Identificação do animal			
Nome do Animal:		Idade:	
Sexo:		Cor da Pelagem:	
Raça:		Microchip:	
Questionário de avaliação comportamental dos cães participantes do ReabilitaCão			
Avaliação fisiológica dos cães participantes do ReabilitaCão			
Cortisol Sérico mcg/dL		Lactato mmol/L	Glicose mg/dL
Hemograma: Eritrograma		Hemácias em milhões/mm ³	
		Hemoglobina em g/dL	
		Hematócrito em %	
		Volume Corpuscular Médio em fL	
		Concentração de hemoglobina corpuscular média em %	
Hemograma: Leucograma		Leucócitos Totais / μ L	
		Bastonetes % e / μ L	
		Segmentados % e / μ L	
		Eosinófilos % e / μ L	
		Basófilos % e / μ L	
		Monócitos % e / μ L	
Hemograma: Plaquetas		Plaquetas 10 ⁵ / μ L	
Peso (kg)		Frequência Cardíaca bpm	
Temperatura Retal (°C)		Frequência Respiratória mpm	
Coloração de mucosas			
Uso de medicamento			
Observações			Data:

ANEXO IV

Quadro 3. Intervalos de referência dos parâmetros clínicos avaliados

Intervalos de referência			
Cortisol Sérico 0,5 a 6,0 mcg/dL		Lactato 0,5 a 2,5 mmol/L	Glicose 65 a 118 mg/dL
Hemograma: Eritrograma		Hemácias em milhões/mm ³	
		5,5 a 8,5	
		Hemoglobina em g/dL	
		12 a 18	
		Hematócrito em %	
37 a 55		Volume Corpuscular Médio em fL	
60 a 77		Concentração de hemoglobina corpuscular média em %	
32 a 36		Leucócitos Totais / μ L	
6.000 a 17.000		Bastonetes % e / μ L	
0 a 3		0 a 300	
Segmentados % e / μ L		60 a 77	
3.000 a 11.500		Eosinófilos % e / μ L	
2 a 10		100 a 1.250	
Basófilos % e / μ L		0	
raros		Monócitos % e / μ L	
3 a 10		150 a 1.350	
Linfócitos % e / μ L		12 a 30	
1.000 a 4.800		Hemograma: Plaquetas	
Plaquetas 10 ⁵ / μ L		2 a 5	
Peso (kg)	variável	Frequência Cardíaca bpm	
60 a 180		Temperatura Retal (°C)	
37,5 a 39,2		Frequência Respiratória movimentos respiratórios/minuto	
10 a 30		Coloração de mucosas	
Normo-coradas		Observações	

ANEXO V

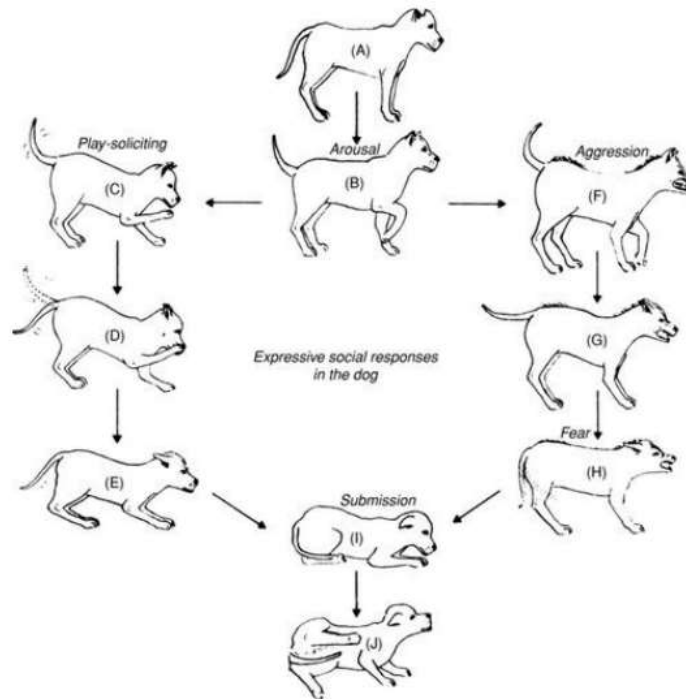


Figura 2. Posturas corporais do cachorro (HOUP, 2011): (A, B) Neutro, alerta, posições atentas; (C) solicitação de brincadeira; (D, E) ativo e passivo saudação submissa - observe abanar a cauda e mudar a posição das orelhas e na distribuição do peso nos membros anteriores e posteriores; (F-H) mudança gradual de postura agressiva para postura ambivalente entre agressiva e defensiva de medo; (I) submissão passiva; e (J) rolagem e apresentação da região inguinal-genital (SAUNDERS, W.B. Co., 1975 citado por HOUP, 2011).

ANEXO VI

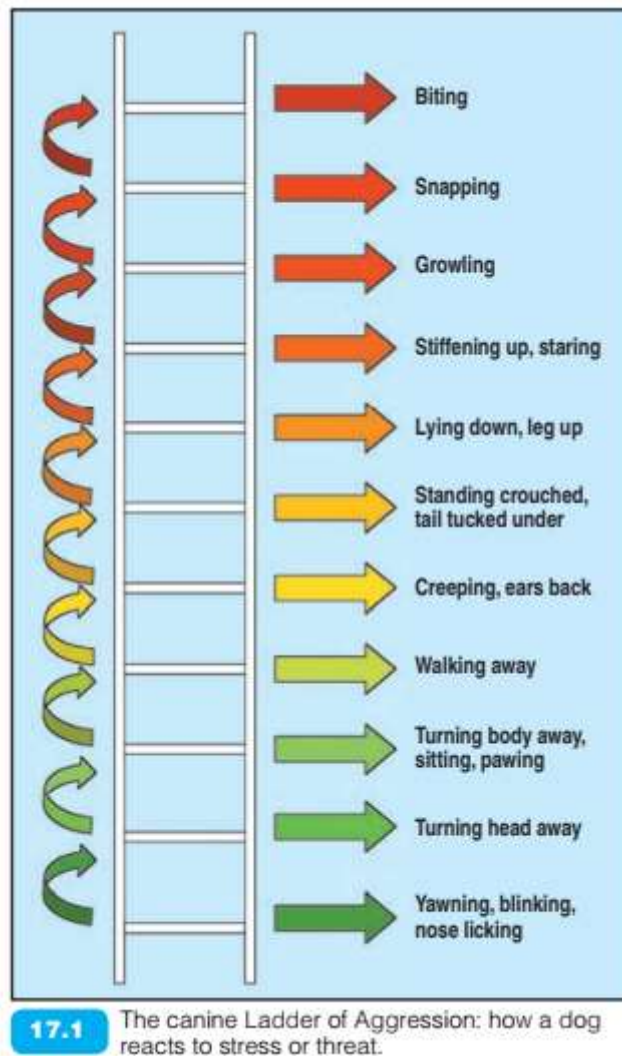


Figura 3. A escala de agressão canina: como o cão reage ao estresse e ameaça (HORWITZ, et al., 2009).

ANEXO VII

UNIVERSIDADE FEDERAL DA FRONTEIRA SUL
PRÓ-REITORIA DE PESQUISA E PÓS-GRADUAÇÃO
COMISSÃO DE ÉTICA EM PESQUISA

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

Título da Pesquisa:	MONITORAMENTO DO BEM-ESTAR ANIMAL E HUMANO DE CÃES E DETENTOS PARTICIPANTES DO PROJETO REABILITAÇÃO EM ITAJAÍ, SANTA CATARINA, BRASIL
CAAE	46567121.4.0000.5564
Parecer de aprovação CEP/UFS	4.818.627
Data de aprovação	30/06/2021
Nome do pesquisador responsável:	Luciana Velasques Cervo
Nome dos demais participantes:	Dalila Moter Benvegnú

Natureza da pesquisa: O Sr (a) está sendo convidada a autorizar a participação nesta pesquisa que tem como finalidade monitoramento do bem-estar animal e humano, através da avaliação comportamental e fisiológica de cães e de humanos que participam do ReabilitaCão.

Identificação dos participantes: cães e detentos participantes do Projeto ReabilitaCão do Complexo Penitenciário do Vale do Itajaí – SC.

Objetivo da pesquisa: Monitorar o bem-estar animal e humano de cães e indivíduos privados de liberdade participantes do Projeto ReabilitaCão em Itajaí, Santa Catarina, Brasil.

Justificativa do estudo: Esta pesquisa contribuirá com o conhecimento a respeito da aplicação da cinoterapia na ressocialização de apenados no Brasil.

Envolvimento na pesquisa: ao participar deste estudo o Sr (a) permitirá que a pesquisadora realize coleta de sangue e registro de suas respostas ao questionário de monitoramento de bem-estar. O Sr (a) tem liberdade de se recusar a participar e ainda se recusar a continuar participando em qualquer fase da pesquisa, sem qualquer prejuízo para o Sr (a). Sempre que quiser poderá pedir mais informações sobre a pesquisa através do telefone da pesquisadora do projeto e, se necessário através do e-mail da Comissão de Ética em Pesquisa (CEP).

Sobre os dados necessários: Os dados coletados serão referentes ao monitoramento do bem-estar dos cães e dos detentos.

Sobre o questionário comportamental: As perguntas contidas no questionário envolvem informações socioeconômicas, de histórico e sobre a visão do detento a respeito de bem-estar mental, benefícios psicológicos, efeitos percebidos e mudanças positivas relacionadas à cinoterapia com respostas do tipo: discordo plenamente, discordo parcialmente, não discordo nem concordo, concordo parcialmente e concordo plenamente.

Riscos e desconforto: A participação nesta pesquisa não traz complicações legais. A coleta de sangue é um procedimento invasivo, que não causa grande dor e desconforto ao paciente, além de ser por um período muito curto de tempo. Porém, poderá ocasionar dor local e hematomas. Para minimizar os possíveis riscos da coleta de sangue, serão aplicados os seguintes cuidados: coleta em local limpo, posicionamento adequado do detento, posicionamento adequado do torniquete, antissepsia local, pressionar o local após a coleta por 1 a 2 minutos e aplicar curativo oclusivo no local da punção. Será orientado ao detento para não dobrar o braço, não carregar peso e não dobrar a manga da roupa por 1 hora após a coleta. No momento da aplicação do questionário os detentos poderão sentir algum tipo de constrangimento ao responder determinadas perguntas, porém, a entrevista será individual e confidencial e, eventualmente, poderão responder não informado. Os participantes do projeto poderão ser identificados e ter seus dados vazados. Para minimizar este risco, os questionários serão aplicados em papel impresso e somente a pesquisadora terá acesso ao seu conteúdo para tabulação dos dados. A tabulação de dados e análise estatística será realizada em notebook particular da pesquisadora que possui senhas complexas, firewall e antivírus, programas atualizados e backup realizado regularmente. Em caso de vazamento de dados, serão comunicados a este respeito a penitenciária, os participantes e também as autoridades responsáveis por investigar crimes de vazamento de dados mediante registro de boletim de ocorrência eletrônico, conforme a Lei Geral de Proteção de Dados. Os procedimentos adotados nesta pesquisa obedecem aos Princípios Éticos segundo a Comissão Nacional de Ética em Pesquisa (CONEP).

Medidas de sigilo e confidencialidade: todas as informações coletadas neste estudo são estritamente confidenciais. Somente os pesquisadores terão conhecimento dos dados. Para identificação dos indivíduos serão utilizadas somente as letras iniciais do nome dos detentos. A publicação da pesquisa preservará o anonimato dos participantes.

Benefícios: Esperamos que este estudo traga informações importantes sobre a saúde e bem-estar dos cães e dos detentos, de forma que o conhecimento que será construído a partir desta pesquisa possa trazer mais informações sobre a cinoterapia, terapia com cães. Os animais participantes de cinoterapia e os detentos precisam ter suas condições de saúde e bem-estar monitoradas. Existe um grande número de cães errantes, semierrantes e em situação de vulnerabilidade em nosso país, abandonados à própria sorte, sendo inclusive vítimas de maus tratos nas ruas, estes encontram um lar no ReabilitaCão. Associado a este quadro temos o presidiário, um ser humano que está privado de sua liberdade momentaneamente e que futuramente, após cumprir sua pena, será reintegrado ao convívio social. É amplamente reconhecido que os animais são aliados da saúde humana, diminuindo estresse, depressão e doenças crônicas. A cinoterapia trata-se de ferramenta eficaz na ressocialização dos detentos, no entanto, exige monitoramento constante da condição de bem-estar e saúde dos cães, bem como, o acompanhamento multidisciplinar do detento. Existe grande número de cães errantes, semierrantes e em situação de vulnerabilidade em nosso país. No município de Itajaí, uma alternativa de abrigo para estes animais é o canil do ReabilitaCão, onde os mesmos recebem a função de cães terapeutas. O projeto oferece a oportunidade aos detentos de criar empatia e aprendizado de cuidados de cães resgatados. O monitoramento do bem-estar dos cães envolvidos em projetos de cinoterapia é fundamental e precisa ser realizado periodicamente por meio de avaliações comportamentais e

fisiológicas.

Devolutiva: A devolutiva dos resultados será realizada por meio de uma apresentação da pesquisa, tipo seminário, para os detentos e para a equipe da penitenciária e também fornecimento de cópia da dissertação à penitenciária e do artigo publicado em periódico científico.

Pagamento: o Sr (a) não terá nenhum tipo de despesa para participar desta pesquisa, bem como nada será pago por sua participação.

Após estes esclarecimentos, solicitamos o seu consentimento de forma livre para participar desta pesquisa. Portanto preencha, por favor, os itens que se seguem:

Consentimento Livre e Esclarecido

Tendo em vista os itens acima apresentados, eu, de forma livre e esclarecida, manifesto meu consentimento em participar da pesquisa.

Nome do Detento:	
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Nome e Assinatura do Detento

Nome e Assinatura do Pesquisador

Data: ____/____/____

CONTATOS

Pesquisador: Luciana Velasques Cervo

Telefone: 4792000-2926

Endereço: Rua Padre Alberto Romuald Jakobs, 395, sala 06, Bairro Vila Lenzi, Jaraguá do Sul – SC Cep 89252-452

Email: lucianavelasques@gmail.com

CEP/UFFS:

Telefone: (49) 2049-3745

Endereço: Rodovia SC 484, km 02, Fronteira Sul, Bloco da Biblioteca, sala 310, 3º andar, Bairro Área Rural, Chapecó – SC Cep 89815-899

Email: cep.uffs@uffs.edu.br

ANEXO VIII

UNIVERSIDADE FEDERAL DA FRONTEIRA SUL
PRÓ-REITORIA DE PESQUISA E PÓS-GRADUAÇÃO
COMISSÃO DE ÉTICA NO USO DE ANIMAIS

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

Título da Pesquisa:	MONITORAMENTO DO BEM-ESTAR ANIMAL E HUMANO POR MEIO DE AVALIAÇÃO COMPORTAMENTAL E FISIOLÓGICA DE CÃES E DETENTOS PARTICIPANTES DO PROJETO REABILITACÃO EM ITAJAÍ, SANTA CATARINA, BRASIL
Nome do pesquisador responsável:	Luciana Velasques Cervo
Nome dos demais participantes:	Dalila Moter Benvegnú
CEUA nº	8163240321
Data da aprovação	06/08/2021

Natureza da pesquisa: A Sra. está sendo convidada a autorizar a participação de seus animais nesta pesquisa que tem como finalidade monitoramento do bem-estar animal e humano, através da avaliação comportamental e fisiológica cães e humanos que participam do ReabilitaCão.

Identificação dos animais: cães participantes do Projeto ReabilitaCão do Complexo Penitenciário do Vale do Itajaí – SC.

Envolvimento na pesquisa: ao participar deste estudo a Sra. permitirá que a pesquisadora examine, colete sangue, observe e registre o comportamento dos animais, transporte os cães até a Clínica Veterinária da UNISOCIESC para exames de radiografia e ultrassonografia. A Sra. tem liberdade de se recusar a participar e ainda se recusar a continuar participando em qualquer fase da pesquisa, sem qualquer prejuízo para o seu animal. Sempre que quiser poderá pedir mais informações sobre a pesquisa através do telefone da pesquisadora do projeto e, se necessário através do telefone da Comissão de Ética no Uso de Animais (CEUA).

Sobre os dados necessários: Os dados coletados serão referentes ao monitoramento da saúde e bem-estar dos cães.

Riscos e desconforto: a participação nesta pesquisa não traz complicações legais. Procedimentos não invasivos, realizados rotineiramente, que não causam grande dor e desconforto ao paciente, além de ser por um período muito curto de tempo. Os procedimentos adotados nesta pesquisa obedecem aos Princípios Éticos na Experimentação Animal segundo o Colégio Brasileiro de Experimentação Animal (COBEA), Lei Federal 11794, de 08 de outubro de 2008 e à Lei Estadual 11977, de 25 de agosto de 2008.

Confidencialidade: todas as informações coletadas neste estudo são estritamente confidenciais. Somente os pesquisadores terão conhecimento dos dados.

Benefícios: esperamos que este estudo traga informações importantes sobre a saúde e bem-estar dos cães, de forma que o conhecimento que será construído a partir desta pesquisa possa trazer mais informações sobre a cinoterapia, o pesquisador se compromete a divulgar os resultados obtidos.

Pagamento: a Sra. não terá nenhum tipo de despesa para participar desta pesquisa, bem como nada será pago por sua participação.

Após estes esclarecimentos, solicitamos o seu consentimento de forma livre para participar desta pesquisa. Portanto preencha, por favor, os itens que se seguem:

Consentimento Livre e Esclarecido

Tendo em vista os itens acima apresentados, eu, de forma livre e esclarecida, manifesto meu consentimento em participar da pesquisa.

Nome da coordenadora geral do projeto ReabilitaCão			
Número de documento:	()	CPF	Inserir número:
	()	RG	Inserir número:

Nome e Assinatura **da coordenadora geral do projeto ReabilitaCão**

Nome e Assinatura do Pesquisador

Data: ____/____/____

TELEFONES

Pesquisador: 4792000-2926

Orientador: (46) 99974-7739

CEUA/UFGS: (46) 3543-8394