

Stress burden for dogs in a simple soundproof cage: Heart rate variability and behavioural analyses

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Abstract: Dog barking, which reaches around 100 dB, often becomes a noise issue in urban environments. One potential solution, a simple soundproof cage, has recently been marketed. To our knowledge, no study has been conducted to investigate what sort of stress burden is imposed on dogs kept in cages, and it may raise animal welfare concerns. The purpose of this study was, therefore, to reveal whether staying in a soundproof cage caused stress for the dogs or not. Ten healthy domestic dogs (5 males, 5 females) of small body size were evaluated. The heart rate variability (HRV), behaviour, and internal and external temperature during confinement in a soundproof cage for each dog were analysed. The HRV analyses revealed no significant differences for any variables between confinement and non-confinement (HR, $P > 0.999$ 9; rMSSD, $P = 0.359$ 4; SDNN, $P = 0.359$ 4; LF, $P = 0.652$ 3; HF, $P = 0.128$ 9; LF/HF, $P = 0.222$ 7). Overall, in our behavioural analysis, there were no significant differences between confinement and non-confinement ($P = 0.105$ 5). In conclusion, the HRV and behavioural analyses did not indicate an increased stress burden on the dogs during confinement in the soundproof cage compared with non-confinement. The inner temperature of the cage was not elevated either. Nevertheless, the stress imposed on dogs caused by a soundproof cage should always be considered on an individual basis, and the time spent in such a cage should always be kept to a minimum.

Keywords: dog vocalisation; grooming; inner and outer temperature; licking; shaking

Dogs are increasingly being kept entirely indoors. In particular, the keeping of dogs in urban apartment blocks is becoming more and more widespread (Baranyiova et al. 2005; Gracia et al. 2008; Domingues et al. 2015). The sound level for a dog's bark is around 100 dB, but can often reach up to 120 dB (Salesa et al. 1997). Generally, sound levels above 85 dB can cause hearing loss in humans (Fink 2017). Dog barking can often become a noise issue in urban environments (Carter 2016). The

welfare of dogs with behavioural problems is still a multi-disciplinary issue in many countries (Haupt et al. 2007).

A soundproof cage is a dog housing unit which reduces the level of a dog's barking noise into the surrounding environment, and it has recently been marketed internationally in countries including Japan and the USA, as a possible way of resolving conflicts with neighbours concerning the nuisance of dogs barking. According to the companies

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that market them, the soundproof cage is relatively affordable and, to a certain extent, it provides a noise cancelling effect. In addition, the design takes ventilation into account through air outlets. To the best of our knowledge, no study has been conducted on the level of stress burden imposed on dogs housed in the cage, and the ready availability of such a cage for use, without validation, is likely to raise animal welfare concerns. In the light of the animal welfare issues faced by dogs in many countries (Houpt et al. 2007), the amount of stress that such a cage may impose on dogs should be examined. If this cage is validated as a short-term non-stressful housing unit for dogs, it could offer a solution to the noise issue caused by dogs barking.

We speculated that for dogs that are familiar with resting in a cage, staying in this type of housing unit may not cause any major stress, but it may be stressful for dogs that are unfamiliar with this type of confinement.

Accordingly, in this study, we aimed to investigate whether dogs placed in a simple soundproof kennel will experience physical or psychological stress, by measuring the heart rate variability (HRV) and performing behavioural analyses in real time. HRV is a periodic variation in the heart rate, and it can be used as an index for the evaluation of the autonomic nervous function of the heart (Berntson et al. 1997; Cygankiewicz and Zareba 2013). The temperature variations inside the kennel were also measured.

MATERIAL AND METHODS

Study animals and experiment period

All the dogs for the study were selected from dogs that came to the Department of Rescue Animal Medicine in the Satooya Animal Clinic (Saitama, Japan) (Ethical approval No. 18-011). Healthy dogs were chosen from dogs which visited the clinic for pet boarding or temporary dog custody within the clinic.

We evaluated ten small dogs (five males and five females; No. D1 to D10) which were used to living in a cage environment. The dog breeds included in the study consisted of three Chihuahuas, one Miniature Pinscher, one Miniature Schnauzer, one Yorkshire Terrier, one Border Collie, and two mixed breeds. Social isolation may be a cause of increased stress-

related behaviour (Cozzi et al. 2016), therefore, subjects with no reported separation anxiety were selected.

Each dog underwent an individual analysis period over four consecutive days, between August and September 2018. Each 24-hour block in the analysis period was randomly selected for either a “confinement” or “non-confinement” setting, and the HRV analyses were performed. In this study, “confinement” refers to a setting where a dog is kept in a wired cage which is further covered with a honeycomb cardboard, and “non-confinement” refers to the setting where a dog is simply kept in a wired cage without the cover (Figure 1).

In each setting, the dogs were subject to the same 24-hour schedule for 4 days. Water was provided *ad libitum* in the cage. An acclimatisation period was set as the first 24 h for each dog for each setting, which was followed by 24 h of the analysis period. Then a switch was made to the other setting, followed by an acclimatisation period and analysis period. Each dog was tested continuously for the duration of 96 hours. Thirty minutes of free time out of the cage twice a day, in the morning and evening, were set, so that a continuous period of 11.5 h in the cage were followed by 30 min of exercise which was repeated over four days. Each dog spent 30 min each morning and evening before meals in an air-conditioned dog run of about 10 square metres. In the run, the dogs were free to join the other dogs.

The study area was located within the boarding room, where the cage was placed, so that the dogs within the cage could be observed through a transparent acrylic panel, and the cage was surrounded by other dogs and staff.

During the analysis period, the dogs defecated mainly at the dog run in the morning and evening, and if the dogs urinated or defecated in the cage, the urine and faeces were immediately removed by the staff.

Simple soundproof cage

The study was conducted one case at a time, and the room was not used for anything else for the duration of the study. The cage used was a readily available, cardboard-type animal housing unit with padding (Danbocchi Wan, Vibe Inc., Tokyo, Japan) (Figure 2).

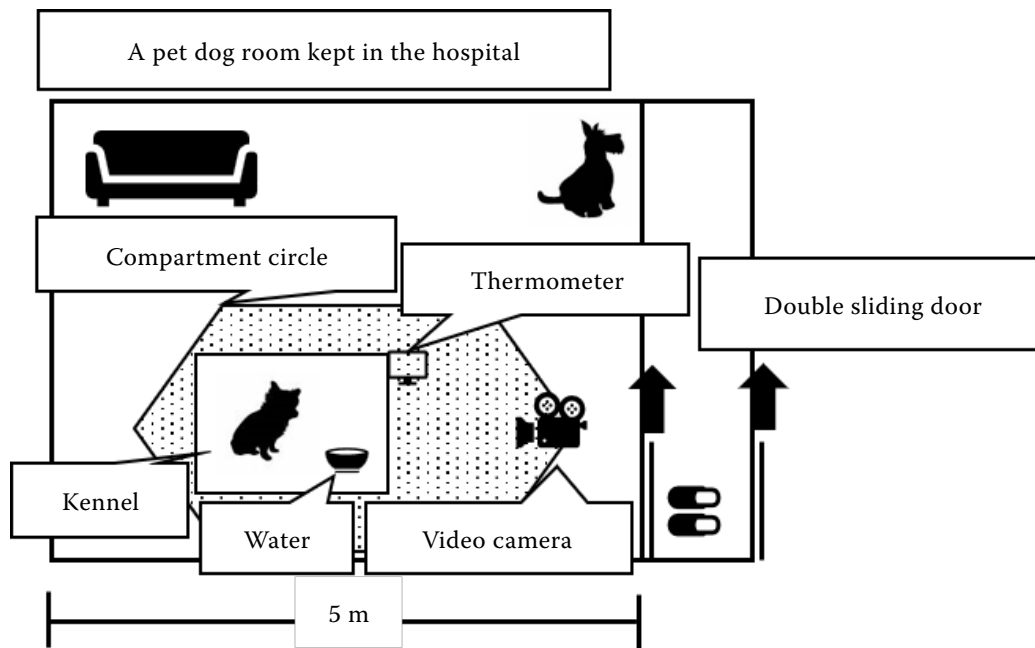


Figure 1. A diagram of the room and the experimental set-up

The study area was set in a part of a pet hotel room within the animal hospital. Dogs staying at the pet hotel were able to walk freely outside of the compartment circle. Because the study dogs were in the cage (designated as the kennel in the drawing), there was no direct contact between the dogs



Figure 2. A simple soundproof cage

(A) No cardboard cover. (B) With a cardboard cover. A standard dog cage was covered; the internal dimensions were $W730 \times D560 \times H560$ (mm), and the external dimensions were $W800 \times D630 \times H595$ (mm). The front of the cage was covered with a transparent acrylic panel, through which the tested dog could see outside the soundproof cage. In the photo on the right, we can see that the dog inside the covered cage can see us through the acrylic panel

The internal dimensions of the cage were $W730 \times D560 \times H560$ (mm), and the external dimensions were $W800 \times D630 \times H595$ (mm). According to the product manual, the cage is made of a special honeycomb cardboard, and the partition board can attenuate sound, reducing it from 90 dB to about 60 dB.

Stress measurement

Measurements were made in real time using a Holter electrocardiography (ECG) system (RAC-5203; Nihon Kohden Co., Ltd., Tokyo, Japan), and the HRV was subject to a power spectral density

analysis using MemCalc/Chiram3 software (GMS Co., Ltd., Tokyo, Japan).

Analysis variables

There were three variables for the Holter ECG, with the first being the heart rate (HR). The second set of variables were the HRV variables in a time-series analysis [root mean square successive difference – rMSSD (ms) and standard deviation of the NN intervals – SDNN (ms)], which is an autonomic nervous system index (Pomeranz et al. 1985). The third set of variables were the HRV frequency analysis (cyclic) variables [low frequency (LF) spectra (ms.ms), high frequency (HF) spectra (ms.ms), and LF/HF]. The mean values were calculated for each of the above variables and analysed statistically to compare the “confinement” with the “non-confinement” setting. Other factors measured included the air temperatures inside and outside the cage, which were measured continuously for 96 h for all the dogs (wireless thermometer RT-100; Custom Co., Ltd., Tokyo, Japan). The temperature was recorded 24 h a day for 4 days, with the temperature displayed hourly.

The data were analysed statistically using the Wilcoxon matched pairs signed rank test.

Holter ECG (5-lead attachment)

During the preparation prior to the acclimatisation period, the hair was shaved from the ventral side of the thoracic region of each dog using an electric hair clipper. Electrodes were positioned onto the skin and then fixed with flexible adhesive tape. NASA- and CM5-lead ECGs were used for the HRV analyses. The Holter ECG recording device was renewed every 24 h, at the same time as the switch between the settings with and without the cage.

Behavioural recording and analysis

A video camera was installed to record footage of the dogs' behaviours. The recording medium was changed every 48 h, and the behaviour was continuously recorded for 96 hours. Each dog's behaviour was analysed based on the report by Kurachi et al. (2017). The recording unit used recorded every 2-hour period, in all conditions. The first 24 h

of the study, in both settings, was used as the acclimatisation period. This meant that a total of 48 h, the first 24 h in each setting with and without the cage, were excluded from the 96 h total. The animal behaviour analysis was conducted based on the remaining 48 h following the acclimatisation period, excluding when the video screen was shifted and when the dog left the cage to walk or eat.

Behavioural analysis

The footage captured by the video camera was analysed to see if any of the four stress behaviours stated below were observed (Beerda et al. 1998); the aim was to determine the frequency of each behaviour and not the duration of the stress behaviour. For example, if a dog barked once or if it barked three times in a row, this was counted as one occurrence of the stress behaviour. No software was used for the behavioural analysis.

The ethogram consisted of barking (making barking noises), licking (licking around the nose or mouth), grooming (licking the trunk or limbs), and body shaking (full-body trembling and shivering; a motion made when a dog shakes water off its wet body).

The data were analysed statistically using the Wilcoxon signed-rank test to determine if there were any significant differences in the stress behaviours expressed in the dogs confined in the cage compared with that of the non-confinement.

RESULTS

Heart rate variability (HRV)

The analysed data is comprised of nine dogs because data could not be obtained from one male dog (No. D1) and, therefore, it was excluded from the study. The dogs had body weights ranging from 1.7 kg to 3.4 kg (median: 2.1 kg) and their age ranged from 2 months to 8 years (median: 12 months) (Table 1). The HRV analyses revealed no significant differences for any variable between the “confinement” and “non-confinement” settings. The results for the HR (bpm; median without = 101.7, with = 99.70, $P > 0.999$ 9), rMSSD (median of differences = –2.850, $P = 0.359$ 4), SDNN (median of differences = –6.350, $P = 0.359$ 4), LF (medi-

Table 1. Study dogs – ten healthy small dogs that frequently barked were selected following a physical examination (five males and five females)

No.	M/F	Breed	Age (months)	Body weight (kg)
Dog 1	M	mix breed	24	3.0
Dog 2	F	mix breed	12	3.1
Dog 3	M	Miniature Pinscher	12	2.1
Dog 4	F	Chihuahua	60	2.0
Dog 5	M	mix breed	12	1.9
Dog 6	M	Chihuahua	7	2.2
Dog 7	F	Chihuahua	96	1.9
Dog 8	F	Miniature Schnauzer	3.5	1.7
Dog 9	F	Yorkshire Terrier	12	2.4
Dog 10	M	Border Collie	2	3.4
Median	–	–	12	2.1

F = female; M = male

an of differences = -303.1 , $P = 0.6523$), HF (median of differences = -906.6 , $P = 0.1289$), and LF/HF (median of differences = -0.02000 , $P = 0.2227$) are presented in Figures 3A, 3B, 3C, 3D, 3E, and 3F, respectively.

Internal vs external temperature in the simple soundproof cage

The difference between the internal (24.55°C) and external (24.35°C) temperatures in the cage did not exceed 2°C (SD, 1.9478 ; $P = 0.1844$). We made one notable observation concerning the air environment in the kennel during the inspection at the end of the study period, which was that a relatively large quantity of animal hair was found on the mesh that served as an air outlet.

Behavioural analysis

The total stress behaviours were tabulated from the observations for each dog (10 dogs; No. D1 to D10) during the recording period, with the acclimatisation period, the time with the camera slippage during recording, the prolonged periods of absence from the cage, and the handling time were excluded from the total recording time (Table 2). As the acclimatisation period, a total of 48h was excluded of the first 24h with and without cage of total 96-hour period. The observation time for each dog ranged from 18 h to 46 hours. The Wilcoxon

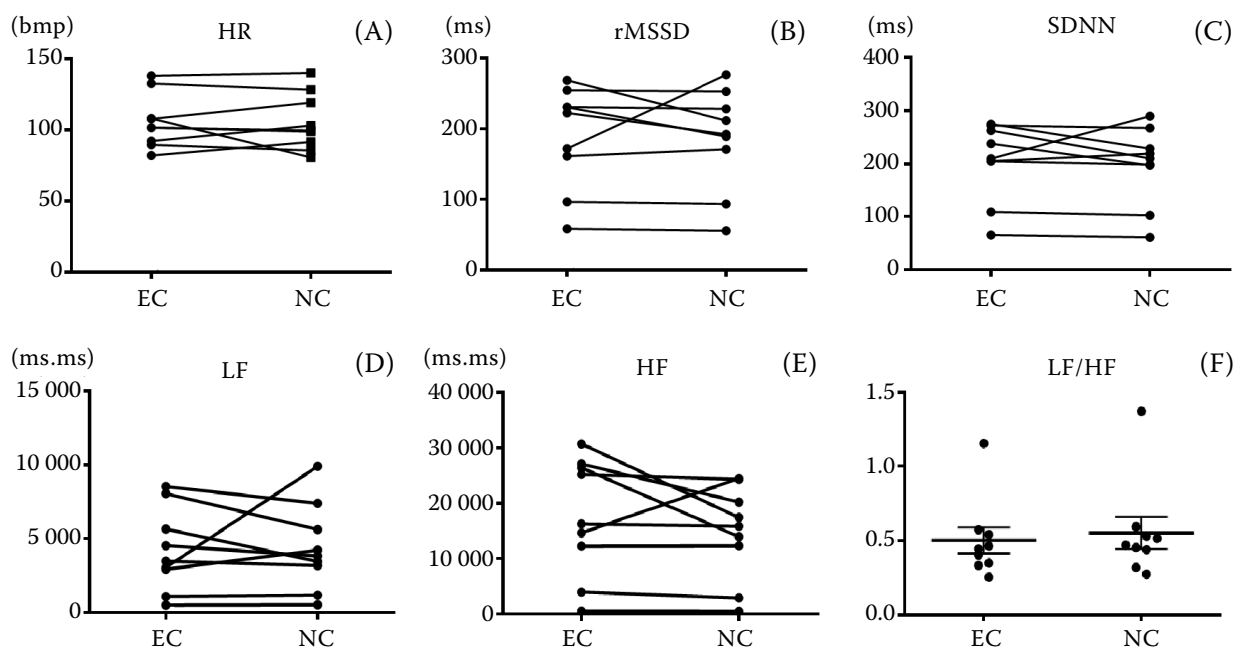


Figure 3. Heart rate variability (HRV) analyses

No significant differences were observed with or without a kennel (cardboard). (A) HR (bpm; $P > 0.9999$); (B) rMSSD ($P = 0.3594$); (C) SDNN ($P = 0.3594$); (D) LF ($P = 0.6523$); (E) HF ($P = 0.1289$); (F) LF/HF ($P = 0.2227$); cardboard (+), EC = experimental group; cardboard (–), NC = no cardboard group; HF = high frequency; HR = heart rate; LF = low frequency; rMSSD = root mean square successive difference; SDNN = standard deviation of the NN intervals

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Table 2. Stress behaviours – stress behaviour following the acclimatisation period: barking (making barking noises), licking (licking around the nose or mouth), grooming (licking the trunk or limbs), and body shaking; Wilcoxon signed-rank test results

Dog No.	Rec time* (hours)	Stress behaviour	Individual stress behaviours (frequency in rec time)		Total stress behaviours (frequency in rec time)	
			(+)	(–)	(+)	(–)
Dog 1	42	barking	26	66	248	400
		licking	92	193		
		grooming	55	67		
		body shaking	75	74		
Dog 2	44	barking	26	29	447	288
		licking	188	129		
		grooming	35	40		
		body shaking	198	90		
Dog 3	40	barking	12	87	379	486
		licking	140	132		
		grooming	41	61		
		body shaking	186	206		
Dog 4	18	barking	18	12	216	271
		licking	59	147		
		grooming	19	7		
		body shaking	120	105		
Dog 5	40	barking	31	202	346	459
		licking	151	86		
		grooming	38	38		
		body shaking	126	133		
Dog 6	42	barking	19	52	379	385
		licking	127	91		
		grooming	14	37		
		body shaking	219	205		
Dog 7	44	barking	0	14	353	445
		licking	243	335		
		grooming	7	11		
		body shaking	103	85		
Dog 8	46	barking	44	60	372	417
		licking	75	101		
		grooming	28	35		
		body shaking	225	221		
Dog 9	40	barking	21	164	265	439
		licking	81	126		
		grooming	39	31		
		body shaking	124	118		

Table 2 to be continued

Dog No.	Rec time* (hours)	Stress behaviour	Individual stress behaviours (frequency in rec time)		Total stress behaviours (frequency in rec time)	
			(+)	(–)	(+)	(–)
Dog 10	44	barking	183	135	349	337
		licking	113	140		
		grooming	9	25		
		body shaking	44	37		
Mean	40	barking	23.5	63.0	335.4	392.7
		licking	120.0	130.5		
		grooming	31.5	36.0		
		body shaking	125.0	111.5		
Standard deviation (SD)	7.589 47	barking	49.566 12	61.588 07	67.122 57	68.962 38
		licking	53.653 42	68.892 67		
		grooming	14.981 66	17.926 52		
		body shaking	58.794 56	59.835 11		
P-value	–	barking		0.065 8	0.105 5	
		licking		0.493 5		
		grooming		0.591 2		
		body shaking		0.578 7		

*The remaining time from the total recording time excluding the acclimatisation period and the time when the camera was shifted; (+) presence of the simple soundproof kennel; (–) absence of the kennel

singed-rank test revealed no significant differences between the “confinement” and “non-confinement” settings (barking, $P = 0.065\ 8$; licking, $P = 0.493\ 5$; grooming, $P = 0.591\ 2$; body shaking, $P = 0.578\ 7$; total stress behaviours, $P = 0.105\ 5$).

If a dog started eating food immediately after being offered food, it had an appetite (appetite = 1), and if it did not start eating immediately, it had no appetite (appetite = 0). The dogs’ appetites after the habituation period were 1.0 (median = 1.0) with or without (median = 1.0) the cage ($n = 10$, $P = 0.210\ 5$). Although there were no statistically significant differences, three dogs showed a loss of appetite in the cage. These dogs ate without problems once they left the cage.

DISCUSSION

Overall, none of the HRV variables showed any significant difference in the study, however, variations in the individual dogs were observed between the “confinement” and “non-confinement” settings.

Animal No. D2 showed a decreased SDNN with a decreased rMSSD and an elevated HR. These findings resemble the response to distress reported in a previous study (Gacsi et al. 2013), thus, it is possible that No. D2 perceived the cage to be a stressful environment. Furthermore, depressed parasympathetic nervous activity is a good indicator of a negative emotional state (Michels et al. 2013; Katayama et al. 2016). In this study, two dogs (No. D2 and D5) showed a reduction in the rMSSD and HF – both of which are indicators of parasympathetic nervous activity. Conversely, four dogs (No. D3, D4, D7, and D9) showed elevated indicators of parasympathetic nervous activity. These findings suggest the possibility of large inter-individual differences in the stress burden experienced in a cage.

Problematic dog barking can be dealt with in several ways. Treatment of dog barking using medication is effective (Podberscek et al. 1999). However, daily medication may represent a burden for both the dog and owner (Song et al. 2016). It is critical that puppies and young dogs are given appropriate training from a young age to prevent later barking

caused by separation anxiety and other factors (Hall 2017). However, owners of smaller dogs, which are more common in urban areas, are much less likely to be interested in training them than owners of larger dogs (Baranyiova et al. 2009). This lack of interest exacerbates the problem of dog barking and should raise the alarm over the importance of early training, even for small dogs. Training takes time, and it is better to start at the early age of dogs.

This study focused on small dogs which are frequently kept in apartment buildings. HRV is mainly mediated by the activities of the cardiac vagus and sympathetic nerves (Pomeranz et al. 1985). HR, which elevates during periods of anxiety and excitation and decreases during times of rest, showed no significant differences between the “confinement” and “non-confinement” settings. Other indicators (HF, LF, rMSSD, SDNN, LF/HF) also showed no significant differences between the “confinement” and “non-confinement” setting. Specifically, these HR components were: HF, which is mediated by the cardiac vagus nerve and respiratory fluctuations; LF, which is mainly mediated by the cardiac sympathetic nerve, although it is affected by the cardiac vagus nerve; LF/HF, which is an indicator of the cardiac sympathetic nerve; rMSSD, an indicator of parasympathetic nervous activity; and SDNN, an indicator of autonomic nervous system activity (Pomeranz et al. 1985). These are indicators of stress, but none of the results of this study showed a significant difference between the dogs in the cage with and without the cover. In other words, there was no evidence that the cage was a stressor for the dogs. On the other hand, parasympathetic hyperactivity was also absent when the dogs were in the covered cage that could block out external noise.

The results of the behavioural analysis were consistent with those for HRV, showing no significant increase or decrease in stress behaviours due to confinement in the cage. However, three dogs (No. D5, D7, and D9) showed a loss of appetite during confinement. None of these dogs showed any health abnormalities and started to eat again as soon as they exited the cage. It may be due to the attenuation of the external sound and poor visibility caused by covering the cage with a simple soundproof structure.

An elevation in the atmospheric temperature is a stressor for dogs (Hales and Bligh 1969). To ascertain the risk of temperature elevation within the confined space of a cage, we evaluated the dogs dur-

ing the summer period (August and September), when the external temperature is at its highest level, and a consequent increase in the cage’s internal temperature is also likely (Honjo et al. 2018). We observed no differences exceeding 2 °C between the internal and external temperatures. However, since the dogs examined in this study were mainly small dogs, a further study may be necessary for larger dogs. It may be necessary to clean the ventilation openings more frequently for large dogs than for small dogs. One of the limitations of this study was that it did not include other stress behaviours that could have been recorded, such as digging on the cage floor, howling or whining, and elimination in the cage. It would be desirable to record these stress behaviours in further studies.

There was no increase in the stress burden on the dogs observed during confinement in a soundproof cage compared with those in non-confinement. It may be that use of this cage could be expanded in the future as a portable cage, allowing, for example, owners to evacuate together with their dogs in the event of a natural disaster or other emergency. However, the result of the study also suggested that there are individual differences in response to stress. When using a cage, the appetite and other responses of each individual should be taken into consideration. In addition, it is important to emphasise that the use of these cages should be limited to situations where the purpose is to improve the safety and well-being of the dogs and should be of the shortest duration possible, having animal welfare issues in mind.

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Conflict of interest

The authors declare no conflict of interest.

REFERENCES

- Baranyiova E, Holub A, Tyrlik M, Janackova B, Ernstova M. The influence of urbanization on the behaviour of dogs in the Czech Republic. *Acta Vet Brno*. 2005;74(3):401-9.

<https://doi.org/10.17221/238/2020-VETMED>

- Baranyiova E, Holub A, Tyrlik M. Body size and behaviour traits of dogs in Czech households. *Acta Vet Brno*. 2009; 78(1):107-14.
- Beerda B, Schilder MB, Van Hooff JA, De Vries HW, Mol JA. Behavioural, saliva cortisol and heart rate responses to different types of stimuli in dogs. *Appl Anim Behav Sci*. 1998;58(34):365-81.
- Berntson GG, Bigger JT Jr, Eckberg DL, Grossman P, Kaufmann PG, Malik M, Nagaraja HN, Porges SW, Saul JP, Stone PH, van der Molen MW. Heart rate variability: Origins, methods, and interpretive caveats. *Psychophysiology*. 1997 Nov;34(6):623-48.
- Carter SB. Establishing a framework to understand the regulation and control of dogs in urban environments: A case study of Melbourne, Australia. *Springerplus*. 2016 Jul 27;5(1):1190.
- Cozzi A, Mariti C, Ogi A, Sighieri C, Gazzano A. Behavioral modification in sheltered dogs. *Dog Behav*. 2016;2(3):1-12.
- Cygankiewicz I, Zareba W. Heart rate variability. *Handb Clin Neurol*. 2013;117:379-93.
- Domingues LR, Cesar JA, Fassa AG, Domingues MR. Guarda responsavel de animais de estimacao na area urbana do municipio de Pelotas, RS, Brasil [Responsible pet animal guardianship in the urban area of the municipality of Pelotas in the state of Rio Grande do Sul, Brazil]. *Cien Saude Colet*. 2015 Jan;20(1):185-92. Portuguese.
- Fink DJ. What is a safe noise level for the public? *Am J Public Health*. 2017 Jan;107(1):44-5.
- Gacsi M, Maros K, Sernkvist S, Farago T, Miklosi A. Human analogue safe haven effect of the owner: Behavioural and heart rate response to stressful social stimuli in dogs. *PLoS One*. 2013;8(3):e58475.
- Gracia MJ, Calvete C, Estrada R, Castillo JA, Peribanez MA, Lucientes J. Fleas parasitizing domestic dogs in Spain. *Vet Parasitol*. 2008 Feb 14;151(2-4):312-9.
- Hales JR, Bligh J. Respiratory responses of the conscious dog to severe heat stress. *Experientia*. 1969 Aug;25(8): 818-9.
- Hall NJ. Persistence and resistance to extinction in the domestic dog: Basic research and applications to canine training. *Behav Processes*. 2017 Aug;141(Pt 1):67-74.
- Honjo K, Shiraki H, Ashina S. Dynamic linear modeling of monthly electricity demand in Japan: Time variation of electricity conservation effect. *PLoS One*. 2018 Apr 30;13(4):e0196331.
- Haupt AK, Goodwin D, Uchida Y, Baranyiova E, Fatjo J, Kakuma Y. Proceedings of a workshop to identify dog welfare issues in the US, Japan, Czech Republic, Spain and the UK. *Appl Anim Behav Sci*. 2007;106(4):221-33.
- Katayama M, Kubo T, Mogi K, Ikeda K, Nagasawa M, Kikusui T. Heart rate variability predicts the emotional state in dogs. *Behav Processes*. 2016 Jul;128:108-12.
- Kurachi T, Irimajiri M, Mizuta Y, Satoh T. Dogs predisposed to anxiety disorders and related factors in Japan. *Appl Anim Behav Sci*. 2017;196(6):69-75.
- Michels N, Sioen I, Clays E, De Buyzere M, Ahrens W, Huybrechts I, Vanaelst B, De Henauw S. Children's heart rate variability as stress indicator: Association with reported stress and cortisol. *Biol Psychol*. 2013 Oct;94(2):433-40.
- Podberscek AL, Hsu Y, Serpell JA. Evaluation of clomipramine as an adjunct to behavioural therapy in the treatment of separation-related problems in dogs. *Vet Rec*. 1999 Sep 25;145(13):365-9.
- Pomeranz B, Macaulay RJ, Caudill MA, Kutz I, Adam D, Gordon D, Kilborn KM, Barger AC, Shannon DC, Cohen RJ, et al. Assessment of autonomic function in humans by heart rate spectral analysis. *Am J Physiol*. 1985 Jan; 248(1 Pt 2):H151-3.
- Salesa G, Peyvandi RHA, Milligan S, Shield B. Noise in dog kenneling: Is barking a welfare problem for dogs. *Appl Anim Behav Sci*. 1997;52(3-4):321-9.
- Song Y, Peressin K, Wong PY, Page SW, Garg S. Key considerations in designing oral drug delivery systems for dogs. *J Pharm Sci*. 2016 May;105(5):1576-85.

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