Original Article

A study on stress-induced by anaesthesia and surgery training using live animals in a veterinary school

Krittee Dejyong[1*] Jutapoln Sunghan[1] Nantarat Chutijiratthitikan[1] Chonnakarn Jatchavala[2]

Abstract

The veterinary curriculum includes an anaesthesia and surgery course that utilizes live animal laboratories and euthanasia, potentially causing stress in students. We used a validated psychology questionnaire to assess their stress levels, confirming that live animal laboratories induce significant stress. Forty-two fourth year veterinary students, including individuals who identified as male, female and LGBTQ, and who held religious beliefs in Buddhism and Islam, volunteered to participate in the study. The study had received ethical approval from the Human Research Ethics Committee at Prince of Songkla University. After a series of stress questionnaires before and after Veterinary surgery and anaesthesia class, female and Islamic students reported more stress in both carcass and live animal laboratories, while Buddhism students and male students reported less stress, respectively (p<0.05). The live animal laboratory induced more stress and excitement, resulting in shorter sleeping durations on the night before compared to the carcass laboratory. Additionally, tangible stress was reflected in shorter sleeping times on the night before the live animal laboratory compared to the carcass laboratories. Overall, this study provides evidence to improve veterinary education by addressing the stress associated with live animal surgery and anaesthesia training. Future research should expand the study population and investigate the lifelong stress experienced by veterinary surgeons and anaesthesiologists.

Keywords: Stress, anaesthesia and surgery, live animals, veterinary school

[1]*Department of Veterinary Science, Faculty of Veterinary Science, Prince of Songkla University, Thailand.*
[2]*Department of Psychiatry, Faculty of Medicine, Prince of Songkla University, Thailand.*
****Correspondence**: krittee.d@psu.ac.th* (K. Dejyong)
Received January 24, 2023
Accepted May 15, 2023
https://doi.org/10.14456/tjvm.2023.12

Introduction

Upon completion of veterinary surgery and anaesthesia courses, students are expected to acquire knowledge and competency in performing fundamental clinical surgeries and anaesthesia procedures. However, the use of live animals for surgical and anaesthesia training in veterinary schools is a common practice. However, ever since the first animal welfare act UK, established in 1986 (Garner, 2006), benefits of using live animals for research and teaching have been of concern. This brought about live animal replacement reduction and refinement used in veterinary school teaching by alternative methods. Although, computer simulators, ex-vivo models and cadavers have been developed to replace animal models (Bauer and Seim III, 1992, Silva *et.al*, 2007), the specificity of biology and the physiology of living tissues and vital body systems are important for vital experience learning; for example, hemostasis, tissue trauma, level of consciousness and pain. These bring about real-operation situations; including, emergency threatening and team cooperation drives and pressure to student responsibility on the animal's life. Although this causes a lot of stress, management skills increase.

A study on veterinary students showed a significant increase in the anxiety of surgical course students compared with non-surgical course students; however, training reduced anxiety for real live animal surgery in clinics (Langebæk *et.al*, 2012). Surgical training environments were also related to the negative and positive emotions of students (Langebæk *et.al*, 2012), which are attributed to personal self-confidence and learning attitudes in the future. However, the roles of responsibility between the operation teammates, including the surgeon, the surgeons' assistant, an anaesthesiologist and the nurse may have influence on individual stress and learning outcomes. Causes of stress in surgery and anaesthesia practice may come from bloody visuals, pain imagination and the margin between being alive and dead during anaesthesia. A study in human hospitals has shown anaesthesia took a key response in peri-operative mortality at 28.8%, while the other causes were 5.2% (Arbous *et.al*, 2001). Another study in a veterinary hospital showed the mortality rate of anaesthetized animals was from 0.12% to 17.33%, depending on the illness (Bille *et.al*, 2012). Moreover, stress in veterinary medicine studies seems to be noticed when live animals are used in surgery and anaesthesia training due to euthanasia (Scotney *et.al*, 2015). Euthanasia was found to be the key to stress induction as well as shared conscience and depression among teammates, because it contrasts to the kindness of veterinary ethical feelings (Morris, 2012). Moreover, the effect of euthanasia on veterinary students has a relationship with suicide, because of the experience of "fearlessness about death" and this association increases with the times of euthanasia (Witte *et.al*, 2013).

Stress questionnaires are commonly used as standard tools for evaluating mental health, particularly in medical students (Backović *et.al*, 2012, Wahed and Hassan, 2017). Nevertheless, it is important to note that the questionnaire used in this study may contain some individual bias. Therefore, it may be valuable to incorporate objective stress indicators, such as heart rate variability, which is linked to neurohormonal status, to supplement the subjective questionnaire data (Thayer *et.al*, 2012). There is another stress measurement, cortisol, that could have been used, but its measurement can be affected by various factors, such as underlying diseases and sex (Hamer and Steptoe, 2012).

This study aimed to investigate the relationship between acquired competency and stress associated with live animal usage during surgery and anaesthesia co-training at a veterinary school. Additionally, it aimed to examine the impact of various factors, including gender and religion, on the competency and stress experienced during live animal surgery and anaesthesia co-training. The findings of this study can potentially inform and enhance the teaching and learning practices in surgery and anaesthesia in veterinary schools or other related disciplines that require live laboratory animals, particularly from a mental health perspective.

Materials and Methods

The Doctor of Veterinary Medicine (DVM) degree curriculum at the Faculty of Veterinary Science, Prince of Songkla University, Songkhla, Thailand, consists of 3 years of pre-clinical study, 2 years of clinical study, and a final year of clinical rotation. As part of their 4th and 5th year clinical skill training, 42 volunteers, aged 21-23 years and of all genders, who were enrolled in basic surgery and anaesthesia courses from 2019-2021, were recruited for this study. All volunteers were provided with information about the study and a consent form to sign, which was approved by the human research ethics committee of Prince of Songkla University, Songkhla, Thailand.

Ovariohysterectomies (OVH) and healthy patient anaesthesia are fundamental surgery and anaesthesia courses for veterinary students. The competency and stress of students were evaluated during two surgery and anaesthesia laboratory sessions, which included cadaveric OVH surgery in the 11th week and live animal OVH surgery in the 14th week of the course schedule. The study was conducted in accordance with the ethics of animal use approved by the Institutional Animal Care and Use Committee (Reference numbers 10/2019 and 24/2019, Project codes 2561-05-079 and 2561-05-092, respectively). The competency of students was evaluated by laboratory instructors using a competency evaluation form (see Supplementary table 1), which is routinely used for surgery and anaesthesia laboratory assessments at the Faculty of Veterinary Science, Prince of Songkla University. The evaluation was conducted after obtaining ethical approval and ensuring the welfare of animals used in the laboratory. Stress was assessed using a questionnaire (0-25), adapted from the Stress questionnaire; International Stress Management Association, Monmouthshire, UK (UK, 2013) and last night's sleeping duration on Monday (Areni *et.al*, 2011) at the 1st, 11st,and 14th week of courses. The volunteer's name was concealed from the recorders and interpreters by an investigator, who was assigned to gather the questionnaires from volunteers. Therefore, the samples were divided into

three groups; including a control group (1st week), a Cadaveric group (11st week) and a Live animal group (14th week).

Questionnaire: The stress questionnaire (Supplementary table 2) was tested for validity by three experts, by calculation of the Item-Objective Congruence Index (IOC) (Rovinelli and Hambleton, 1976) and back translation test (Brislin, 1970, Yu *et.al*, 2004). IOC = ΣR/N; (R = score from an expert, N = number of experts) and back translated. This consisted of: R = +1: question meets the objective, R = 0: uncertain that the question meets the objective, and R = -1: question does not meet the objective. An IOC between 0.50 and 1.00 indicates good validity and an IOC below 0.5 indicates poor validity.

The original questionnaire was forward translated to Thai and then grammar-checked for style and comprehensibility. After this, the translated questionnaire was back translated to English by experts and, finally, the back-translated version was compared with the original version. Agreement on back translation was evaluated by adaptation of the coding scheme of AlGhamdi and AlShammari (2007) (AlGhamdi and AlShammari, 2007), which is codes: 1 = satisfactory agreement between back translation and original, 2 = almost satisfactory agreement, but with one or two words being uncertain, and 3 = doubtful translation. The code in each question was evaluated by three assessors, then arranged into 4 categories: category 1: code 1 marked by all assessors; category 2: code 2/3 marked by all assessors; category 3: code 2/3 marked by two assessors; and category 4: code 2/3 marked by one assessor. For category 1, the question was certified, for categories 2, the question was revised and for categories 3 and 4, the question was refined.

After establishing good validity and reliability (r) of the questionnaire, it was administered to a sample of twenty-two first-year veterinary students to assess its test-retest reliability, which was evaluated based on the percentage of agreement between the test and retest results. The questionnaire included measures of heart rate and sleeping time, with heart rate being measured through individual auscultation using stethoscopes.

Sample size: The sample of volunteers (n) was calculated using Slovin's Formula (Slovin, 1960): $n = N/(1+Ne^2)$; [population (N) =75, error (e). Calculation of sample sizes was performed for hypothesis testing, with GPower 3.1.9.4 (Heinrich Heine University Düsseldorf, Dusseldorf, Germany) (Faul *et.al*, 2007). The sample size was 67 students, with a further 10% for estimated data loss, giving a total sample size of 74. Determining, based on significance level (α) at 0.05, Testing power (1-β) at 0.8 and Statistical assumptions, was: H_0: $\rho = 0$, H_1: $\rho > 0$. The researcher estimated that the two variables had correlations of approximately 0.3.

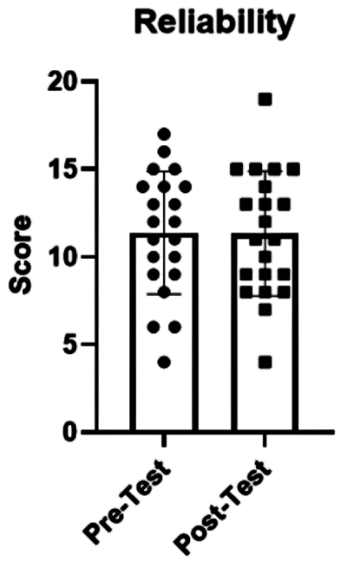

Figure 1 The difference between the pre-test and the post-test. The pre-test and post-test results demonstrated high reliability, with a total of 25 questions answered by 21 students. The data was analyzed using descriptive statistics and reported as Mean±SD.

Supplementary Table 1 Competency on surgery and anaesthesia of veterinary student assessment

This questionnaire is a part of "A study on stress-induced by anaesthesia and surgery training using live animals in a veterinary school" under the Human Research Ethics Committee (HSc-HREC), 62-23-1-1., Prince of Songkla University, Songkhla, Thailand.

Student: Forename: …… Surname: ……..Student ID: ……
Assessor: Forename: …… Surname: ……..
Training model: □ Cadaver □ Live animal (porcine) □ Live animal (canine)
Training and assessment Date: ……/……/…….. Time start …….. Time finish ……...
Role: □ Surgeon □ Surgeon Assistant □ Anesthesiologist □ Nurse
Animal Euthanasia: □ No □ Yes; if yes, who performed euthanasia: □ the student □ another teammate

---

Surgeon

No	Assessment	Yes	No	Comment
1	Hold scalpel correctly			
2	Hold forceps correctly and choose appropriate forceps for tissues			
3	Hold scissors correctly and choose appropriate scissors for tissues			
4	Appropriate suture and size for ligation (multifilament absorbable suture)			
5	Appropriate needle (Taper or round point needle)			
6	Appropriate ovarian apparatus ligation (double transfix and circumferential ligations)			
7	Appropriate cervix ligation (double transfix and circumferential ligations)			
8	Appropriate Linea alba closure with correct surgeon knot and distance (inter-stitches distance = 5 mm and edge distance = 2-3 mm			
9	Appropriate suture pattern for subcutaneous closure (Subcuticular pattern)			
10	Appropriate suture pattern and knot for skin closure			
The "Yes" answer score = 1 (one). The "No" answer score = 0 (zero).				Total

Anaesthesiologist

No	Assessment	Yes	No	Comment
1	Appropriate animal status assessment by American Society of Anaesthesia (ASA)			
2	Choose an appropriate reservoir bag 0-4.5 kg = ½ L, 4.6-9 kg = 1 L , and 9.1-27.2 kg= 2 L			
3	Appropriate sedative, analgesic, anaesthetic calculation			
4	Secure animal restraint (animal and human safe)			
5	Appropriate IV catheterization under aseptic technique			
6	Appropriate anaesthesia depth assessment for endotracheal intubation (anaesthesia depth at stage 3 plane 2; no swallowing reflex, relax Jaw tone, no withdrawal reflex and downward of eyeball position)			
7	Appropriate endotracheal intubation under aseptic technique			
8	Fluid rate adjustment correctly (sec/drop)			
9	Monitor equipment has been set up and interpreted correctly			
10	Anaesthesia record correctly and easy for interpretation			
The "Yes" answer score = 1 (one). The "No" answer score = 0 (zero).				Total

Interpretation

4 points or less: Inadequate competency.

5 - 7 points: Adequate competency

8 points or more: Good competency

Supplementary Table 2 Stress of Veterinary student questionnaire

This questionnaire is a part of "A study on stress-induced by anaesthesia and surgery training using live animals in a veterinary school" under Human Ethical Review Committee no: HSc-HREC 62-23-1-1, Prince of Songkla University, Songkhla, Thailand.

Instructions:
1) Please read the questions in each section very carefully
2) Answer all questions by marking in the box that matches you whether yes or no, and answer yes, even if only partially with you
3) Please be honest and constructive in your comments

Forename:…… Surname: …….. Student ID: ……
Gender: □ Male □ Female □ LGBT Age:…… Religious: □ Buddhism □ Islam □ Christianity □ Other……
Place of Birth…….. Living status □ alone □ friends □ family
Pet □ No □ Yes please specify….Species/Number…… Responsible □ you □ family member(s)
Previous pet(s) dead □ No □ Yes Species/Number……
Killing or Euthanasia experience □ No □ Yes Species/Number……
Average grade accumulation (GPAX):
□ 0-2.5 □ >2.5-3.0 □ >3.0-3.25 □ >3.25-3.5 □ >3.5-3.75 □ >3.75-4.00

Sleeping time on the last night…….hours Sleeping quality □ Very bad □ Bad □ Fair □ Good □ Excellent
Have you eaten during 0-6 hours before doing this questionnaire? □ No □ Yes
Did you drink alcoholic beverages during 0-12 hours before doing this questionnaire? □No □ Yes
Did you use sedative drugs or tranquilliser during 0-12 hours before doing this questionnaire? □No □ Yes
---
Training model □ Cadaver □ Live animal (porcine) □ Live animal (canine) □ Other……
Training Date: ……/……/…….. Date of answer ……/……/……..
Role: □ Surgeon □ Surgeon Assistant □ Anesthesiologist □ Nurse
Animal Euthanasia: □ No □ Yes; if yes, who performed euthanasia: □ I did □ Teammate
---

No.	Questions	Yes	No
1	I frequently read or think about today study at night		
2	If there is enough time, I can practice training more efficiently.		
3	I deny or ignore problems in the hope that they will go away		
4	I tend to do things by myself instead of consulting my teammates to make sure that I will achieve perfect results.		
5	I underestimate how long it takes to do training		
6	I feel that I have set a high goal in learning / using life. Until difficult to achieve the goal		
7	I feel lost in confidence		
8	I often have feelings of guilt. If I relax too much and not preparing to read the book before the practice		
9	I found myself thinking about any problems, even when I should relax		
10	Although I have slept enough, I feel tired when I woke up		
11	I usually just nod or trying to end the conversation when they speak slowly or tend to not understand		
12	I have a tendency to eat, talk, walk, ride , or drive quickly		
13	My appetite has changed either wanting to eat more or decrease or eat spicy food		
14	I feel irritated or angry if the car or traffic in front seems to be going too slowly/ I become very frustrated at having to wait in a queue		
15	If something or someone really annoys me I will bottle up my feelings		
16	When I play sport or games, I really try to win whoever I play/I feel to be a winner in every circumstance		
17	I found that I had mood swings difficult decision making, less concentration or decreased ability to remember.		
18	I find fault and criticize others rather than praising, even if it is deserved		
19	When others say, I will make it seem like listening even though I was thinking about other things in that moment		
20	My sexual interest decreases or there may be a change in the menstrual cycle.		
21	I find myself grinding my teeth or frequent thirsty		
22	Increase in muscular aches and pains especially in the neck, head, lower back, shoulders		
23	I am unable to perform tasks as well as I used to, my judgment is clouded or not as good as it was		
24	I find I have a greater dependency on alcohol, caffeine, or nicotine		
25	I find that I don't have time for many interests / hobbies outside of study		
The "Yes" answer score = 1 (one). The "No" answer score = 0 (zero).			Total

Please give any comments related to your mental or emotion during this period.

Overall stress (please circle the number that represent you stress level)

Relax ←----------									----------→Extremely stress
1	2	3	4	5	6	7	8	9	10

Interpretation

4 points or less: Least likely to suffer from stress-related illness.

5 - 13 points: More likely to experience stress related ill health either mental, physical or both. The student would benefit from stress management / counselling or advice to help in the identified areas.

14 points or more: The most prone to stress showing a great many traits or characteristics that are creating un-healthy behaviours. This means that student is also more likely to experience stress & stress-related illness e.g. diabetes, irritable bowel, migraine, back and neck pain, high blood pressure, heart disease/strokes, mental ill health (depression, anxiety & stress). It is important to seek stress-therapist help or stress management counselling

Inclusion and exclusion criteria: The voluntary research program was offered to 4th year veterinary students enrolled in 145-446 Veterinary Anaesthesiology and 145-555 Companion Animal Surgery at the Faculty of Veterinary Medicine, Prince of Songkla University. Participants with serious health problems, drug addiction, alcoholism, sedative or tranquilizer use, a history of mental illness or who considered it inconvenient to provide information or did not wish to voluntarily participate were excluded from the study.

Statistical analysis: We estimated the relationship between competency and stress and conducted hypothesis testing using either the Spearman Rank Correlation Coefficient or the Kendall's Tau Rank Correlation Coefficient. To assess the impact of euthanasia experience, gender, religion and pet ownership on stress, we conducted a Two-Way ANOVA and used Tukey's multiple comparisons test with a 95% confidence level and a 5% margin of error. We performed all statistical analyses using Prism 7 GraphPad software (GraphPad Software, California, US).

Ethical proof: The ethical approval for this study was obtained from the Human Research Ethics Committee (HSc-HREC) at Prince of Songkla University, Thailand, and the project code assigned was HSc-HREC 62-23-1-1, indicating that all procedures and protocols used in the study were conducted in accordance with the relevant ethical guidelines and standards.

Results

Validity and reliability of the questionnaire: To establish the validity of the questionnaire, three experts were asked to evaluate 25 questions using the Item-Objective Congruence Index (IOC) and a back translation test. The IOC (Mean±SD; Max, Min) was 1.0±0.00; 1; 1 for 16 questions, 0.7±0.58; 0, 1 for 2 questions (15, 18), and 0.3±0.58; 0, 1 for 4 questions (4, 6, 11, 14). However, all 4 questions were refined and approved by the assessors before the back-translation assessment. All back translations from the Thai version were approved for the English version.

The preliminary validation of the questionnaire was conducted on the first day of the semester with 21 first-year veterinary students at the Faculty of Veterinary Science, Prince of Songkla University. Participants were aged between 18-20 years and consisted of 11 females, 8 males and 2 LGBTQ+ individuals. Religious status was as follows: 11 Buddhism, 3 Islamic and 3 other. 15 participants had pets, while 6 did not and 6 had experience of animal euthanasia. The average sleeping duration (Mean±SD; Min, Max) before the test was 6.17±1.18 hours; 4 to 8 hours, the average heart rate (Mean±SD; Min, Max) was 80.8±9.05; 67 to 90 beats per minute (bpm), and the average respiratory rate (Mean±SD; Min, Max) was 35.5±16.57; 13 to 67 bpm. None of the participants had taken anti-anxiety or tranquilizer drugs but a few students (1-2) had consumed alcohol within 12 hours before the laboratory in each group. Additionally, 10-12 students had not had breakfast before the laboratory in each group.

The reliability was assessed by comparing between the test before and one hour after the class (Biswas *et.al*, 2019). The mean difference between pre-test and post-test scores on each question (n=25) for the 21 participating students was 3±3.13 (Mean±SD). Statistical analysis showed no significant difference between the pre-test and post-test scores (p= 0.8966) and a high correlation coefficient (r) of 0.8897 (p<0.0001) was observed (refer to Figure 1).

General information: The study included 42 participants, consisting of 12 males and 30 females. Among them, 34 identified as Buddhist and 8 as Muslim. 19 participants reported previous euthanasia experience, while 22 had no experience. In terms of pet ownership, 33 participants reported having pets, such as dogs, cats or exotic animals, while 19 did not. None of the participants reported taking anti-anxiety or tranquilizer medication. However, a few students (1-2 per group) reported drinking alcohol within 12 hours before the laboratory and 10-12 students in each group did not consume breakfast before the laboratory.

Stress questionnaire scoring: The average score on the stress questionnaire was 11.17±3.981, 11.40±4.747 and 11.62±5.481 in the control group, the carcass group and the live animal group, respectively (Figure 2). The overall scores did not differ significantly among the groups. However, in the carcass group, female students had significantly higher stress scores than male students (12.30±4.130 vs 8.93±5.594, p=0.0316) and in the live animal group, female students also had significantly higher stress scores than male students (12.70±4.728 vs 8.68±6.473, p=0.0052) (Figure 2).
Furthermore, in both the carcass and live animal groups, Islamic students had significantly higher stress scores than Buddhism students (carcass group: 11.37±4.654 vs 5.71±4.838, p<0.0001; live animal group: 11.62±5.600 vs 5.68±5.538, p<0.0001) (Figure 2). However, there were no significant differences between students with and without euthanasia experience, or between students with and without pets (p>0.05) (Figure 2).

Sleep duration: The effect of stress on students' sleeping duration was also assessed in this study. The results showed that the students who were going to perform live animal laboratory had a significantly shorter sleeping duration than those who were going to perform carcass laboratory, with mean±SD values of 6.14±1.555 hours and 6.86±1.013 hours, respectively (p=0.0404) (Figure 3). The minimum and maximum sleeping duration in the control group, the carcass group and the live animal group were 3:9, 5:8.5, and 2:8 hours, respectively. However, there was no significant difference between the three groups regarding gender, religion, euthanasia experiences and pet ownership (p>0.05) (Figure 3).

Physiological change in heart rate: The effect of stress on the sympathetic nervous system was assessed by measuring heart rate (beats per minute, bpm) in all groups. The results showed that the heart rate in the carcass group was significantly higher than that in the live animal group, with values of 85.90±11.371 and

80.98±13.411 bpm, respectively (p=0.0221) (see Figure 4). However, there was no significant difference observed between the three groups when comparing the effect of stress based on gender, religion, euthanasia experiences and pet ownership (p>0.05) (see Figure 4).

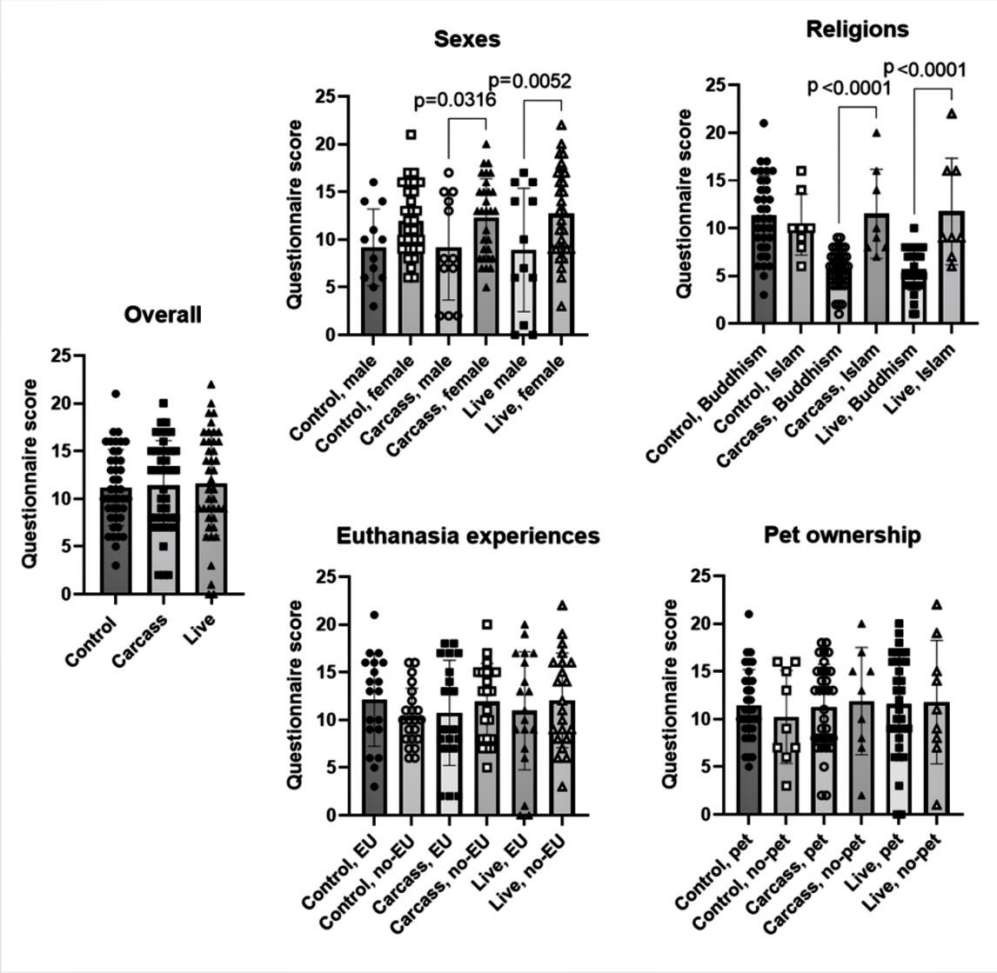

Figure 2 The Stress questionnaire scoring. The stress scores (0-25) of the control group, carcass group and live animal group were compared based on different factors such as gender, religion, euthanasia experience and pet ownership. Although there was no significant difference in the overall stress score between the groups, a significant difference was found between the carcass and live animal groups in relation to gender and religion. Specifically, female students and Islamic students in the live animal group reported significantly higher stress scores compared to male students and Buddhism students (p<0.05). The data is presented as Mean±SD.

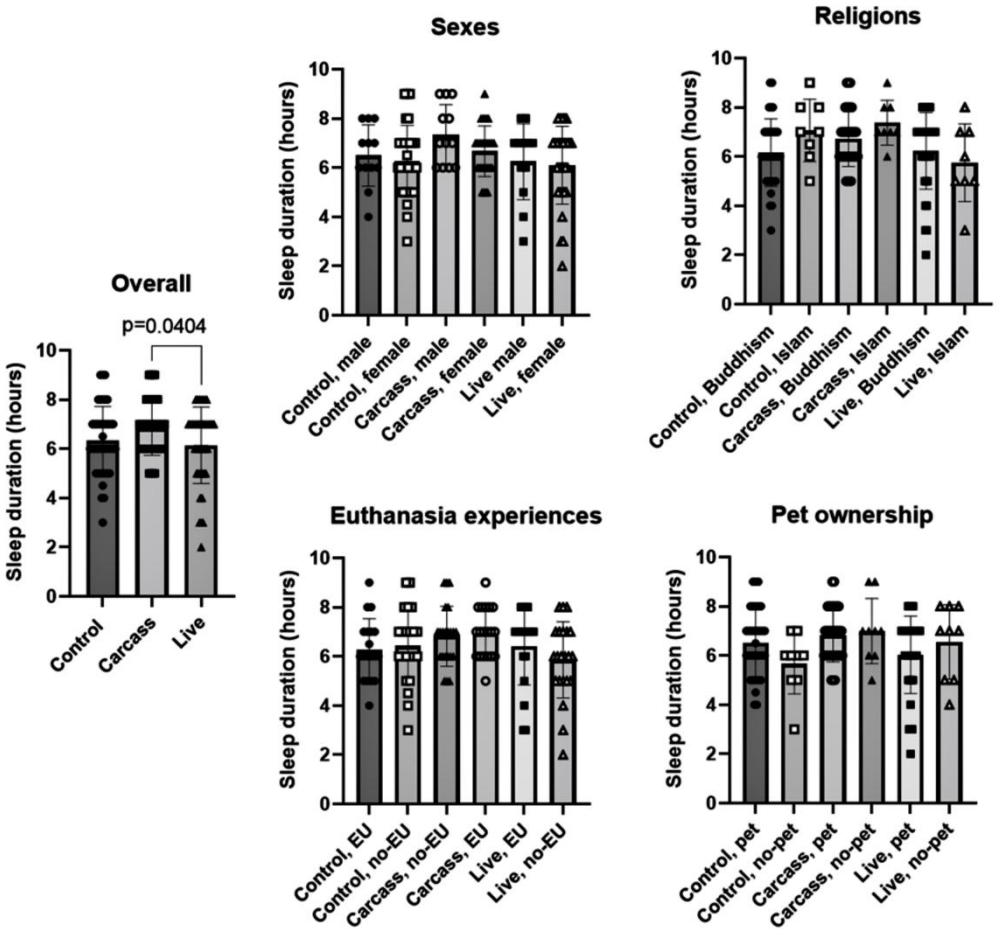

Figure 3 The sleeping duration. The duration of sleeping was compared among the control group, the carcass groups and the live animal group based on the following factors: gender, religion, euthanasia experiences and pet ownership. Significant differences were observed between the carcass group and the live animal group in terms of overall sleeping duration. However, no significant difference was observed between the groups based on gender, religion, euthanasia experiences and pet ownership. The results are presented as Mean±SD.

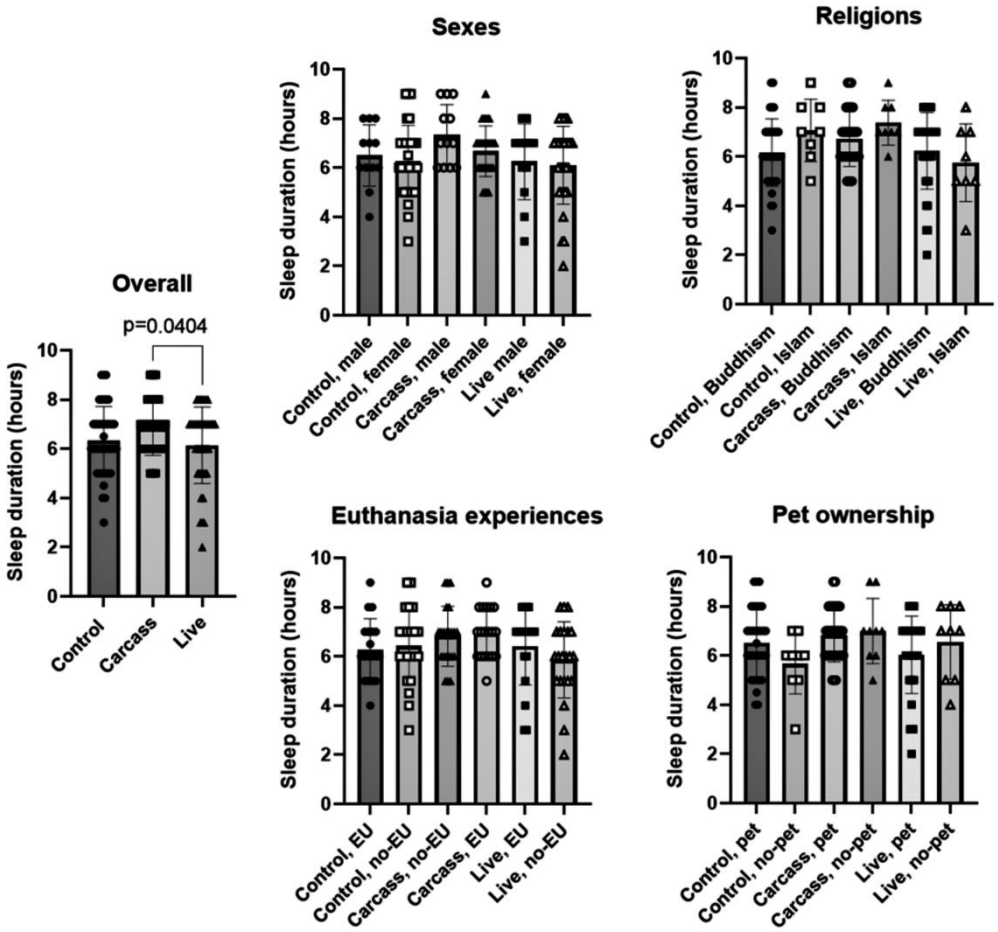

Figure 4 The heart rate. The impact of stress on heart rate was analyzed by comparing the control group, the carcass group and the live animal group across various factors including gender, religion, euthanasia experiences and pet ownership. The results showed a significant difference between the carcass group and the live animal group in terms of heart rate. However, there was no significant difference observed between the groups based on gender, religion, euthanasia experiences or pet ownership. The data is presented as Mean±SD.

Discussion

Although, no difference in stress levels between the groups of the carcass groups and the live animal groups was shown in this study, there was a significant difference between the carcass and the live animal group in Buddhism students and Islamic students. This may indicate disturbances in the core beliefs of each religion, from the surgery and anaesthesia co-training (Kovacic, 2020). "Pàõàtipàtà veramaõã" (Kovacic, 2020), which means to abstain from killing in general, is one of the main concepts of practice in ordinary life for Buddhists. However, the Muslim perspective mentions; "Tayyibat," or permission to kill an animal not for food (Makrooh), but must kill in a; "good and pure method in both physical and moral senses"(Rahman, 2017). It is essential to re-consider the students' perspectives on the procedure of the; "Tayibat" and "Makrooh," in the co-training of living animals for study in veterinary practice, so as to reduce Muslim students' feelings of stress and guilt. However, we did not analyse the genders as related to the religions, due to the small number of the Islamic male population and no other populations from other religions.

Stress is gender dependent; wherein, females tended to be easily stressed, with higher stress levels when compared to males (Bonner and Brimhall, 2022). Additionally, they reacted to the stress in different ways; the females responded with increased subjective feelings, whereas, the males showed more pronounced neural activity in stress (Kuhn *et.al*, 2022). However, this study-categorized gender as only "Male" or "Female". In this study there were only 2 students identifying as LGBTQ and although psychological pattern may be an essential factor in our research, there was too small a population number to make a statistical comparison. even though we emphasize the LGBTQ option in the questionnaire, which may be because the research was during the LGBTQ Social Changes in Thailand and a greater population is needed in any future study (Jatchavala and Udomratn, 2019). Moreover, we did not classify coping patterns and other stressors, such as romantic relationships, which may relate to the results as confounding factors (Braithwaite and Holt-Lunstad, 2017).

Sleep duration and quality are reported in many professionals' resilience; especially in the same area of this veterinary school (Jatchavala and Pitanupong, 2019), which is related to protecting their stress levels as well as many other psychological difficulties, such as burnout syndrome (Pitanupong and Jatchavala, 2018). Brief psychoeducation on the topic of "sleep hygiene;" such as, the effects of caffeine and alcohol,

avoiding disturbances and appropriate duration of sleep may be beneficial for the whole mental health status of the students (Otsuka *et.al*, 2020). However, the heart rate in overall factors in the live animal group was lower than that in the carcass group. This was the opposite of other studies in which, stress caused a physiological increase in the heart rates in both males and females (Kuhn *et.al*, 2022, Taelman *et.al*, 2009). However, cardiac function was required to be compared individually, depending on each student's physical status and might be confounded due to the continuous stress factors throughout the course. More subjective analysis of sleeping and stress is available, such as, salivary cortisol (Kuhn *et.al*, 2022) and serum melatonin(Andersen *et.al*, 2014) level measurements. The results showed no significant differences in euthanasia experience and pet ownership between the live animal and carcass groups. It is possible that these factors are influenced by assorted variables, such as the species of the euthanized animal (e.g. food animal) and the level of attachment to their pet(s).

In conclusion, this study provides important insights to improve veterinary education, especially with regard to the stress associated with live animal usage in surgery and anaesthesia training. The findings suggest that reducing the number of animal laboratories and increasing the use of simulators could be a more effective option. However, it is important to note that none of the studies compared the effectiveness of both methods on student learning. Further research is needed to investigate the potential impact of reducing live animal usage on student competency in surgery and anaesthesia (Sanders *et.al*, 2008). The questionnaire used in this study was a reliable tool that can be applied to larger population studies. Results indicated that female students and Islamic students experienced more stress in both carcass and live animal laboratories, while Buddhism students and male students reported less stress, respectively (p<0.05). Live animal laboratories induced more stress and excitement, resulting in shorter sleeping duration on the night before compared to carcass laboratories. Future studies should aim to expand the study population to include under-represented groups, such as LGBTQ students and other genders related to religion. Additionally, lifelong studies on stress experienced by professional veterinarians, especially veterinary surgeons and anaesthesiologists, would be an interesting research topic to explore.

Conflict of interest statement: The author(s) declare no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

Availability of data: The data that supports the findings of this study is openly available in [repository name e.g "figshare"] at http://doi.org/[doi], reference number [reference number].

Acknowledgements

Financial support: This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors.

References

AlGhamdi KM and AlShammari SA 2007, Arabic version of Skindex- 16: translation and cultural adaptation, with assessment of reliability and validity. Int. J. Dermatol; 46: 247-252.

Andersen L, Werner M, Rosenberg J and Gögenur I 2014, A systematic review of peri- operative melatonin. Anaesthesia; 69: 1163-1171.

Arbous MS, Grobbee D, Van Kleef J, De Lange J, Spoormans H, Touw P, Werner F and Meursing AEE 2001, Mortality associated with anaesthesia: a qualitative analysis to identify risk factors. Anaesthesia; 56: 1141-1153.

Areni CS, Burger M and Zlatevska N 2011, Factors affecting the extent of Monday blues: Evidence from a meta-analysis. Psychol Rep; 109: 723-733.

Backović DV, Ilić Živojinović J, Maksimović J and Maksimović M 2012, Gender differences in academic stress and burnout among medical students in final years of education. Psychiatria danubina; 24: 175-181.

Bauer MS and Seim III H 1992, Alternative methods to teach veterinary surgery. Humane innovations and alternatives (USA).

Bille C, Auvigne V, Libermann S, Bomassi E, Durieux P and Rattez E 2012, Risk of anaesthetic mortality in dogs and cats: an observational cohort study of 3546 cases. Vet Anaesth Analg; 39: 59-68.

Biswas A, Sen S and Ray K 2019, Reliability assessment of pre-post test questionnaire on the impact of a daylong clinical pharmacology workshop among medical professionals. Asian J Med Sci; 10: 93-97.

Bonner HS and Brimhall A 2022, Gender Differences in Law Enforcement Officer Stress and Coping Strategies. Police Quarterly; 25: 59-89.

Braithwaite S and Holt-Lunstad J 2017, Romantic relationships and mental health. Curr Opin Psychol; 13: 120-125.

Brislin RW 1970, Back-translation for cross-cultural research. J Cross Cult Psychol; 1: 185-216.

Faul F, Erdfelder E, Lang A-G and Buchner A 2007, G* Power 3: A flexible statistical power analysis program for the social, behavioral, and biomedical sciences. Behav Res Methods; 39: 175-191.

Garner R 2006, Animal welfare: A political defense. J Animal L & Ethics; 1: 161.

Hamer M and Steptoe A 2012, Cortisol responses to mental stress and incident hypertension in healthy men and women. J Clin Endocrinol Metab; 97: E29-E34.

Jatchavala C and Pitanupong J 2019, Resilience in medical doctors within the areas of the Southern Thailand Insurgency. Siriraj Med J; 71: 228-233.

Jatchavala C and Udomratn P 2019. Women and Gender, Diversity and Mental Health. Diversity in Global Mental Health. Springer, pp.31-40.

Kovacic M.2020. The Buddhist Ethics of Killing: metaphysics, phenomenology, ethics. University of Melbourne,

Kuhn L, Noack H, Wagels L, Prothmann A, Schulik A, Aydin E, Nieratschker V, Derntl B and Habel U 2022, Sex-dependent multimodal response profiles to psychosocial stress. Cerebral Cortex.

Langebæk R, Eika B, Jensen AL, Tanggaard L, Toft N and Berendt M 2012, Anxiety in veterinary surgical students: a quantitative study. J Vet Med Educ; 39: 331-340.

Langebæk R, Eika B, Tanggaard L, Jensen AL and Berendt M 2012, Emotions in veterinary surgical students: a qualitative study. J Vet Med Educ; 39: 312-321.

Morris P 2012. Blue juice: euthanasia in veterinary medicine. Temple University Press.

Otsuka Y, Kaneita Y, Itani O and Tokiya M 2020, A school-based sleep hygiene education program for adolescents in Japan: a large-scale comparative intervention study. Sleep Biol Rhythms; 18: 27-36.

Pitanupong J and Jatchavala C 2018, A study on the comparison of burnout syndrome, among medical doctors in the restive areas and non-restive areas of the South Thailand Insurgency. J Health Sci Med Res; 36: 277-289.

Rahman SA 2017, Religion and animal welfare—An islamic perspective. Animals; 7: 11.

Rovinelli RJ and Hambleton RK 1976, On the use of content specialists in the assessment of criterion-referenced test item validity.

Sanders CW, Sadoski M, van Walsum K, Bramson R, Wiprud R and Fossum TW 2008, Learning basic surgical skills with mental imagery: using the simulation centre in the mind. Med Educ; 42: 607-612.

Scotney RL, McLaughlin D and Keates HLJJotAVMA 2015, A systematic review of the effects of euthanasia and occupational stress in personnel working with animals in animal shelters, veterinary clinics, and biomedical research facilities. 247: 1121-1130.

Silva RMGd, Matera JM and Ribeiro AACM 2007, New alternative methods to teach surgical techniques for veterinary medicine students despite the absence of living animals. Is that an academic paradox? Anatomia, Histologia, Embryologia; 36: 220-224.

Slovin E 1960, Slovin's formula for sampling technique. Retrieved on February; 13: 2013.

Taelman J, Vandeput S, Spaepen A and Huffel SV. Influence of mental stress on heart rate and heart rate variability. In: 4th European conference of the international federation for medical and biological engineering 2009, pp.1366-1369. Springer.

Thayer JF, Åhs F, Fredrikson M, Sollers III JJ and Wager TD 2012, A meta-analysis of heart rate variability and neuroimaging studies: implications for heart rate variability as a marker of stress and health. Neurosci Biobehav Rev; 36: 747-756.

UK ISMA (2013).Stress Questionnaire, http://isma.org.uk/wp-content/uploads/2013/08/Stress-Questionnaire.pdf

Wahed WYA and Hassan SK 2017, Prevalence and associated factors of stress, anxiety and depression among medical Fayoum University students. Alexandria J Med; 53: 77-84.

Witte TK, Correia CJ, Angarano DJS and Behavior LT 2013, Experience with euthanasia is associated with fearlessness about death in veterinary students. 43: 125-138.

Yu DS, Lee DT and Woo J 2004, Issues and challenges of instrument translation. West J Nurs Res; 26: 307-320.