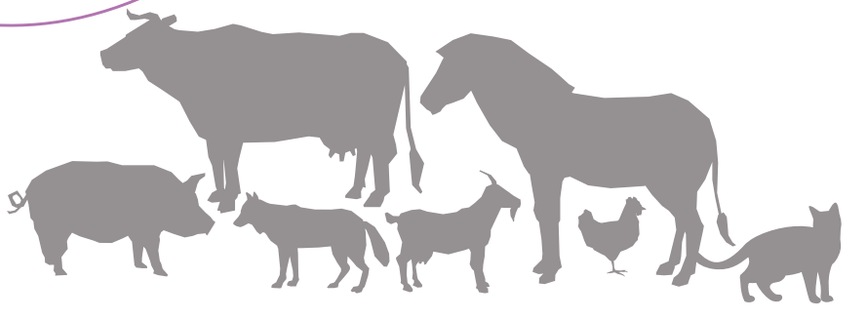
JURNAL VETERINAR MALAYSIA

MALAYSIAN VETERINARY MEDICAL ASSOCIATION

ISSN 0128-2506

Jurnal VETERINAR MALAYSIA



Aim and Scope

The **Jurnal Veterinar Malaysia** is a peer-reviewed journal that publishes original research work, reviews, case reports, short communications and letters to the editor from any veterinary-related fields. The journal aims to publish scientific-based evidence research articles that promote the advancement of veterinary medicine and the health and wellbeing of animals. The copyright of papers accepted for publication is that of the Malaysian Veterinary Medical Association and **Jurnal Veterinar Malaysia** is published twice a year.

VOL. 33 NO. 1-2 (DEC)2021 &
VOL. 34 NO. 1 (JUNE) 2022

Jurnal VETERINAR MALAYSIA

CASE REPORTS

1
Double-chambered right ventricle, membranous ventricular septal defect, and pulmonic stenosis in a British Shorthair
Z.P. Leong,M.M. Noordin and W.W. Lee

5
Heartworm extraction in a 5-year-old mongrel bitch presented with right-sided heart failure
Q.T. Nah, Z.P. Leong, W.W. Lee and T. Thivya

JOURNAL ARTICLES

11
Knowledge and attitudes of Malaysian and UK veterinarians on pain and analgesia in cats
S.S. Zaini,C. Phillips, J.R.D.Mackay and F. Langford

24
Inhibitory effectsof Tongkat Ali (*Eurycoma longifolia*) extracton lighting stress-induced sleep disorders in pigs
Y. Kuroki,F. Tsuda, F. Tawara and S. Tsugumari

SHORT COMMUNICATIONS

29
Preliminary study on the skin thickness of adult Malayan tapir
Y.Donny

33
***In vitro* viral inhibitory effect of cat litter on feline coronavirus infection**
N.J.S. Abu Sopian, S.S. Arshad and F. Mustaffa-Kamal

38
Guidelines For Authors

ISSN 0128-2506

Jurnal Veterinar Malaysia (JVM) is the official journal of the Malaysian Veterinary Medical Association (MAVMA). It was published formally as Kajian Veterinar and the Malaysian Veterinary Journal.

ISSN 0128-2506

https://www.mavma.org.my/jvm/issues

EDITORIAL OFFICE
c/o Faculty of Veterinary Medicine
Universiti Putra Malaysia
43400 UPM Serdang, Selangor Darul Ehsan
Malaysia

Tel. :+603-9769 3466
Fax :+603-8947 1971
E-mail : journal@mavma.org.my

J. Vet. Malaysia (2021) 33 (1-2):1-4

Case report

DOUBLE-CHAMBERED RIGHT VENTRICLE, MEMBRANOUS VENTRICULAR SEPTAL DEFECT, AND PULMONIC STENOSIS IN A BRITISH SHORTHAIR

Z.P. LEONG[1*], M.M. NOORDIN[2] and W.W. LEE[3]

[1]Leong Vetcardio Services, 56000, Kuala Lumpur
[2]Faculty of Veterinary Medicine, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor
[3]Lee Veterinary Clinic, 58200 Kuala Lumpur

SUMMARY

A five-month-old British shorthair kitten previously diagnosed with hypertrophic cardiomyopathy and feline infectious peritonitis was presented for cardiology examination. The findings of right cardiomegaly on chest radiography and electrocardiogram did not support a diagnosis of hypertrophic cardiomyopathy. On echocardiogram, muscular bands dividing the right ventricular chamber into two sub- chambers, narrowing of the pulmonary valve annulus, and a left-to-right shunt through amembranous ventricular septal defect were seen. Therefore, a diagnosis of double-chambered right ventricle, membranous ventricular septal defect, and pulmonic stenosis was made. Despite medications, the kitten eventually developed Eisenmenger syndromeand hind-limb paralysis, and was euthanized. Post-mortem examination for the heart and liver confirmed the above diagnoses.

Keywords: congenital heart disease, double-chambered right ventricle, Eisenmenger syndrome, ventricular septal defect

INTRODUCTION

Double-chambered right ventricle (DCRV) is a rare congenital heart disease in which the anomalous fibromuscular bundles divide the right ventricle into a proximal high-pressure and a distal normal pressure chamber (Brockman *et al*., 2009). In cats, it has been reported by MacLean *et al*. (2002), Koffas *et al*. (2007), Brockman *et al*. (2009),and Mizuno *et al*. (2010). By contrast, literature on concomitant DCRV and ventricular septal defect in cats is scarce. Since there is only one report which reviewed the ante- and post-mortem findings in a cat with concurrent double caudal vena cava (Dirven *et al*., 2010), the aim of the present case report is to elucidate clinical presentation and disease progression, and post-mortem findings in a British shorthair kitten with concomitant DCRV, pulmonic stenosis, and ventricular septal defect.

CASE REPORT

A five-month-old British shorthair weighing 0.85 kg was presented due to an increased breathing effort. It had a history of ascites and was previously diagnosed with hypertrophic cardiomyopathy (HCM) via echocardiography and feline infectious peritonitis (FIP) by the preceding veterinarians. According to the owner, the diagnosis of FIP was based on the positive ImmunoComb® feline coronavirus antibody test (Biogal Galed Labs, Israel) although the Rivalta's test was negative and the plasma globulin level was normal. Since then, the cat was treated with clopidrogel (Plavix® 75 mg, 18.75 mg/cat, PO, SID), and benazepril (Fortekor™ 5 mg, 0.25 mg/kg, PO, SID) and diltiazem (Herbesser® 30 mg, 9.4 mg/kg, PO, SID). Besides, it had been receiving a course of antiviral treatment (GS-441524) for the treatment of FIP. From the above history, further diagnostic workups were aimed at investigating the cause of ascites and confirming the heart disease. The top differentials for the ascites in the kitten included FIP, right heart disease, and hypoproteinemia.

Upon physical examination, the cat was bright and alert with flaccid abdomen. There was no neurologic deficit or jaundice observed. Systolic left and right murmurs grade IV/VI were auscultated. Chest radiography revealed right cardiomegaly (vertebral heart score of 11), apex elevation, and dilated caudal vena cava (Figure 1a, 1b). Besides, electrocardiogram showed sinus rhythm with a heart rate of 188 bpm, in addition to right ventricular enlargement pattern indicated by deep S waves in leads I and II (Figure 1c). The above findings were untypical for HCM, and an echocardiogram was pursed under sedation with pethidine (CCM, 3 mg/kg, IM) due to the lack of patient cooperation. On right parasternal long-axis view (Figure 2a, 2b) of echocardiography, the left atrium (LA) was dilated [LA: aorta (Ao): 2.5; reference range < 1.6] whereas the interventricular septal (4.4-4.9 mm) and left ventricular (LV) free walls (4.6 mm) during diastole were mildlythickened. Besides, the right atrium (RA) was severely dilated and the right ventricular (RV) wall showed marked concentric hypertrophy (7.0 mm). Further, a hyperechoic band which extended from the RV apex to just distal to the right ventricular outflow tract (RVOT) was also seen. On color Doppler (Figure 2d), there was a left-to-right shunt through membranous ventricular defect, and presence of tricuspid regurgitation and turbulence at the RVOT. The velocity across the stenotic RVOT was 3.27 m/s, giving rise to a pressure gradient of 42.9 mmHg. Moreover, ultrasonography of the liver showed dilated hepatic veins.

*Corresponding author: Dr. Leong Zi Ping (Z.P. Leong) ; Email: leong0908@yahoo.com

Editorial history:
Paper received: January 2021
Accepted for publication: December 2021
Published: December 2021

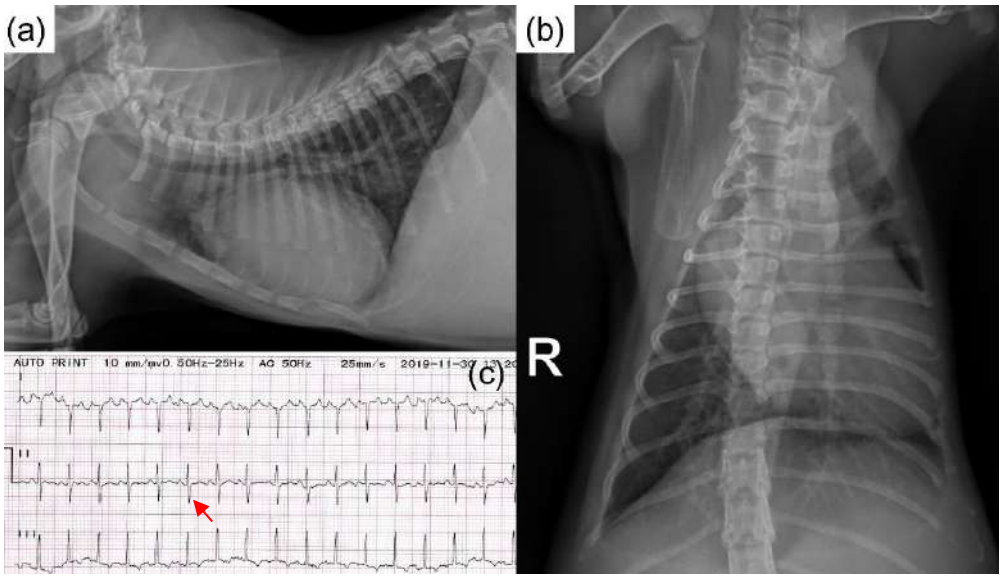


Figure 1. (a) Right lateral chest radiography showing severe cardiomegaly and dilated vena cava (asterisk). (b) The cardiac silhouette occupied almost the entire thoracic region on dorsoventral view. (c) Electrocardiogram showing sinus rhythm and deep S waves on leads I and II (red arrows).

Based on the above echocardiographic findings, a diagnosis of doubled-chambered RV, membranous ventricular septal defect, and pulmonic stenosis was made. These findings also led us to believe that the previous ascites was a manifestation of right congestive heart failure but not FIP, given the lack of consistent clinical signs such as jaundice, neurological deficits, and protein-laden effusion but the presence of hepatic venous congestion. Recommendations were given to replace diltiazem with atenolol (Ternolol 50 mg, 1 mg/kg, PO, SID) while maintaining the benazepril and clopidrogel medications. While the FIP treatment was not recommended, the owner decided to complete the course of treatment. Since then, the cat was more active and showed less breathing effort for about 2 weeks. Although the cat did not exhibit clinical signs of deterioration, the revisit 28 days later showed LV hypertrophy on the echocardiogram. The interventricular septal and LV free walls during diastole were 6.2 mm and 7.3 mm, respectively.

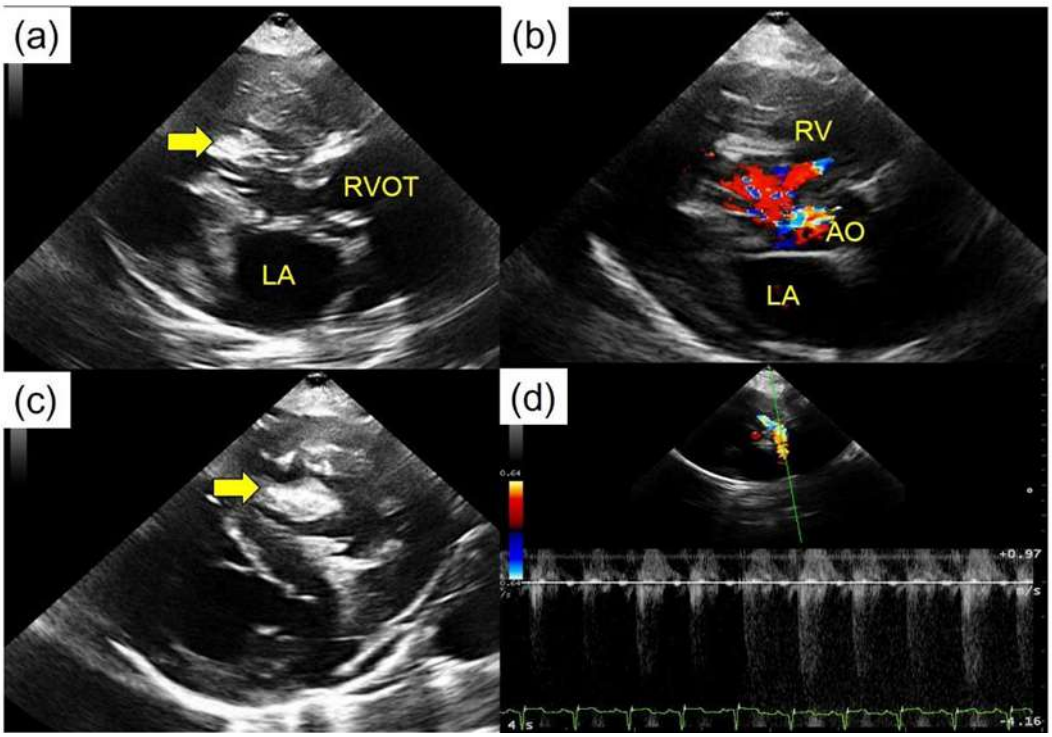


Figure 2. Echocardiogram showing (a) the presence of hyperechoic band in the right ventricle (RV) which inserts below the right ventricular outflow tract (RVOT). Note the severe RV hypertrophy. (b) The color Doppler showing communication between the left ventricle and RV through membranous ventricular septal defect. (c) The short axis view demonstrating the hyperechoic band and the RV hypertrophy. (d) On Doppler study, there was a high pressure gradient (3.27 m/s) across the stenotic RVOT, indicating pulmonic stenosis.

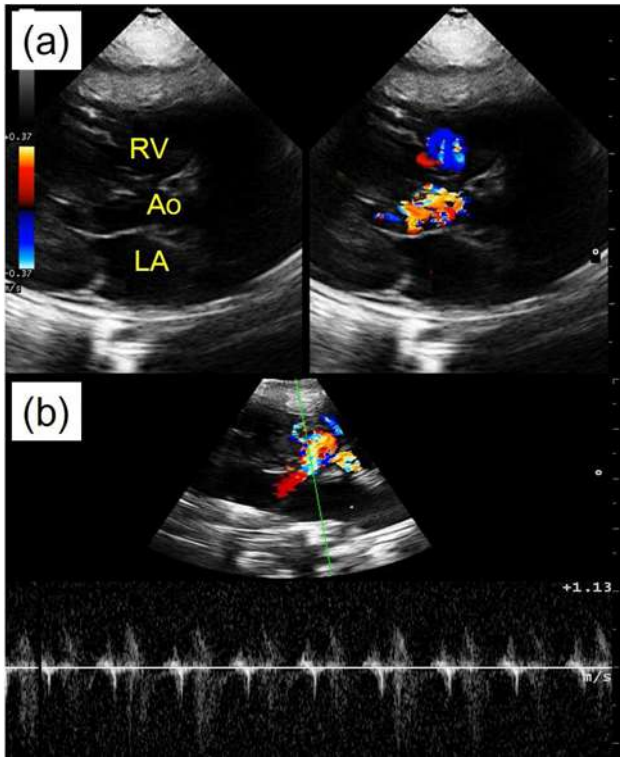


Figure 3. On day 28, the (a) B-mode and (b) Doppler studies showing (a) right-to-left shunt from right ventricle (RV) to left ventricle across the ventricular septum defect, indicating development of Eisenmenger syndrome. Ao: Aorta.

Further blood shunt from the RV to LV across the VSD was observed during systole, indicating the occurrence of Eisenmenger syndrome (Figure 3). The atenolol dosage was adjusted to the increased body weight (1.4 kg). On day 52, the cat showed high sleeping respiratory rates and distended abdomen. Serum biochemistry showed hypoglycemia [2.65 mmol/L; Reference range (RR): 4.11-8.84 mmol/L], mildly decreased creatinine (68 µmol/L; RR: 71-212 µmol/L), and markedly elevated alanine aminotransferase (>1000 U/L; RR: 12-130 U/L). The increase in alanine aminotransferase level was likely caused by hepatic congestion and hypoxia due to the right heart failure. Further, the normal globulin (47 g/L; RR: 28-51 g/L) and albumin (27 g/L; RR: 22-40 g/L) levels also indicated that the FIP and hypoproteinemia were less likely to cause the ascites. To control the ascites, furosemide (Lasix 40 mg, 1 mg/kg, PO, BID) was prescribed, which resolved the ascites for about 50 days. On day 148, it developed bilateral hind-limb paralysis and the owner opted for euthanasia.

Post-mortem limited to the heart and liver was performed with the owner's consent. Grossly, the RA and RV were markedly enlarged. Two muscular bands extended from the RV to about 5 mm distal the pulmonary valves were found (Figure 4a). On cross-section (Figure 4b), there was severe hypertrophy of the interventricular septum (10 mm), and LV (14 mm) and RV (11 mm) walls. The RV wall cross-section had multifocal whitish areas indicating myocardial infarction. A communicating hole between LV and RV was also identified (Figure 4c).

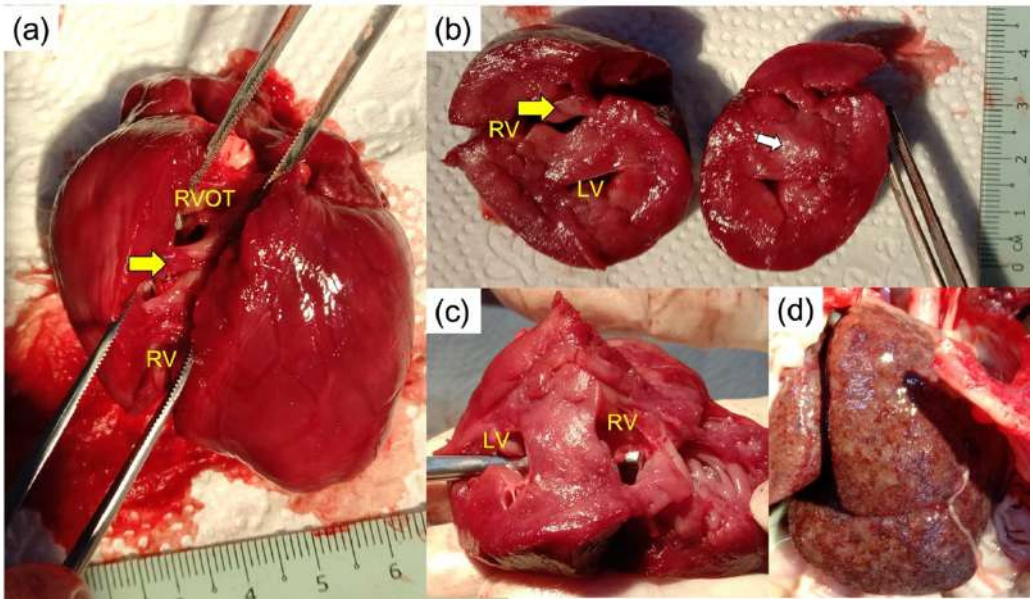


Figure 4. Post-mortem examination showing (a) presence of two muscular bands which extended from the right ventricular (RV) apex to distal to the right ventricular outflow tract (RVOT). (b) On cross-section of the heart, there was hypertrophy of the LV and RV. The muscular band (yellow arrow) and multiple whitish areas (white arrow) suggesting infarction were also observed. (c) A connecting hole from the LV to RV was documented. (d) The liver showed cirrhosis and nodular gene ration.

The liver also showed mottled appearance and cirrhosis with nodular degeneration (Figure 4d) suggestive of chronic passive congestion, and there was severe purulent ascites. The post-mortem results confirmed that the echocardiographic diagnosis of right congestive heart failure and Eisenmenger syndrome were due to DCRV and VSD. However, without performing a full-body post-mortem examination, histopathology, and immunochemistry, the FIP was not ruled out.

DISCUSSION

The present case represents Malaysia's first reported case of double-chambered right ventricle (DCRV), membranous ventricular septal defect (VSD), and pulmonic stenosis in a cat and provides insightful information about ante- and post-mortem findings of the rare feline congenital heart disease which subsequently developed Eisenmenger syndrome and hind-limb paralysis. While Koffas *et al*. (2007) discovered membranous VSD incidentally in a cat from nine cats diagnosed with DCRV, their study focused more on the clinical characteristics of DCRV so to that of the concurrent DCRV and VSD remains obscure. On the other hand, Koie *et al*. (2000) reported the concurrent DCRV and VSD in a female pug which died due to encephalitis.

In humans, the DCRV is often associated with other cardiac anomalies such as VSD, pulmonic stenosis, and tetralogy of Fallot (Chang *et al*., 1996; Fellows *et al*., 1977). Of these, VSD is present in up to 90% of cases and occurs as a result of improper expansion of the bulboventricular junction and incomplete fusion of the bulbar and endocardial cushion elements that normally close the superior portion of the ventricular septum (Galiuto *et al*., 1996). Further, the anomalous muscle bundles of the DCRV proposed to be due to localized aberrant overgrowth of the trabeculated myocardium (Hindle *et al*., 1968).

In cats, dilation of the obstructed RVOT with a balloon catheter (MacLean *et al*., 2002) and an arthroscopic grasping instrument (Mizuno *et al*., 2010) were attempted with disappointing outcomes. Indeed, the thick muscular band in the present case did not appear to be easily mended via an interventional therapy. On the other hand, Koffas *et al*. (2007) and Brockman et al. (2008) reported successful treatment of the DCRV via partial ventriculotomy and incised patch graft technique. However, there is no report on the treatment of the concurrent DCRV and VSD in cats. Indeed, the incised patch graft technique may relieve the high pressure within the RVOT, but the cat weighed only 1.4 kg, which was a little too small to allow an open-chest surgery. Furthermore, repairing the VSD under total venous inflow occlusion was deemed impossible without cardiopulmonary bypass and cardiopledgia.

The cardiogenic cause of ascites in the kitten was supported by central venous congestion, indicated by dilated caudal vena cava on the chest radiographs, right atriomegaly on echocardiography, dilated hepatic veins on liver ultrasounds, and mottled appearance of the liver on necropsy. By contrast, ascites due to feline infectious peritonitis (FIP) was questionable given the lack of consistent clinical signs and laboratory results. Although the cat showed positive antibody titer for feline coronavirus, as demonstrated by the preceding veterinarian, hyperglobulinemia and positive Rivalta's test were not observed, making the diagnosis of FIP highly unlikely. Further, the cause of hind-limb paralysis remained unclear, although we speculated that it could be caused by reduced tissue perfusion and hypoxia due to Eisenmenger syndrome as well as arterial thromboembolism given the severe bi-atrial enlargement.

CONCLUSION

While feline infectious peritonitis represents the top differential in kittens or young cats which show cavitary effusion, congenital heart defects such as double-chambered right ventricle (DCRV) with concurrent ventricular septal defect and pulmonic stenosis should also be suspected. In the context of making the diagnosis, the skill to evaluate the clinical presentation and interpret the laboratory and diagnostic imaging results is indispensable. Unfortunately, treatment outcome for DCRV is often disappointing.

ACKNOWLEDGEMENTS

The author thanks Professor Dr. Fujii Yoko from Azabu Univerisity, Japan and staff of Lee Veterinary Clinic for their assistance.

REFERENCES

Brockman, D.J., Borer, K.E., Baines, S.J., Hughes, D., Fuentes, V.L. (2009). Partial right ventriculectomy using the incised patch technique to treat double chambered right ventricle and chylothorax in a cat. Veterinary Surgery. 38: 631-635.

Chang, R.Y., Kuo, C.H., Rim, R.S., Chou, Y.S., Tsai, C.H. (1996). Transesophageal echocardiographic image of double-chambered right ventricle. Journal of the American Society of Echocardiography. 9: 347-352.

Dirven, M.J.M., Szatmári, V., Cornelissen, J.M.M., van den Ingh TSGAM. (2010). Double-chambered right ventricle (DCRV), ventricular septal defect, and double caudal vena cava in a cat. Tijdschr Diergeneeskd. 135: 180-188.

Fellows, K.E., Martin, E.C., Rosenthal, A. (1977). Angiocardiography of obstructing muscular bands of the right ventricle. American Journal of Roentgenology. 128: 249-256.

Galiuto, L., O'Leary, P.W., Seward, J.B. (1996). Double-chambered right ventricle: electrocardiographic features. Journal of the American Society of Echocardiography. 9: 300-305.

Hindle, W.V., Engle, M.A., Hagstrom, J.W.C. (1968). Anomalous right ventricular muscles. American Journal of Cardiology. 21: 487-495.

Koffas, H., Fuentes, V.L., Boswood, A., Connolly, D.J., Brockman, D.J., Bonagura, J.D., Meurs, K.M., Koplitz, S., Baumwart, R. (2007). Double chambered right ventricle in 9 cats. Journal of Veterinary Internal Medicine. 21: 76-80.

Koie, H., Kurotobi, E.N., Sakai, T. (2000). Double-chambered right ventricle in a dog. Journal of Veterinary Medical Science. 62: 651-653.

MacLean, H.N., Abbott, J.A., Pyle, R.L. (2002). Balloon dilation of double-chambered right ventricle in a cat. Journal of Veterinary Internal Medicine. 16: 478-484.

Mizuno, M., Uechi, M., Stoneham, A., Rush, J.E., Ebisawa, T., Yamano, S., Hori, Y., Yoshioka, K., Keating, J.H., Brown, D.J. (2010) Double-chambered right ventricle in two cats. Advances in Animal Cardiology. 43: 27-34.

J. Vet. Malaysia (2022) 34 (1): 5-10

Case Report

HEARTWORM EXTRACTION IN A 5-YEAR-OLD MONGREL BITCH PRESENTED WITH RIGHT-SIDED HEART FAILURE

Q.T. NAH[1], Z.P. LEONG[2*], W.W. LEE[1] and T. THIVYA[1]

[1]Lee Veterinary Clinic, 58200, Kuala Lumpur
[2]Leong VetCardio Services, 56000, Kuala Lumpur

SUMMARY

A 5-year-old female mongrel dog was presented due to the complaint of dyspnoea and ascites. Diagnostic work-ups consisting of thoracic and abdominal radiography, abdominocentesis and fluid analysis, haematology, serum biochemistry, heartworm antigen test, electrocardiography, and echocardiography revealed right-sided congestive heart failure due to caval syndrome, atrial tachycardia, and pulmonary hypertension. After a few days of stabilization, jugular catheterization was performed under general anaesthesia and seven heartworms were isolated. The dog also received heartworm treatment in accordance to the guidelines by American Heartworm Society and other medications to control the right-sided heart failure.

Keywords: Caval syndrome, heartworm disease, jugular catheterization, pulmonary hypertension, right-sided congestive heart failure.

INTRODUCTION

Canine heartworm (*Dirofilaria immitis)* is a vector-borne, parasitic filarial nematode which resides in the pulmonary arteries. In caval syndrome, the altered right heart hemodynamic (Kitagawa, *et. al.,* 1987) and the heavy worm burden result in retrograde migration of the worms to the right ventricles, right atrium, and vena cava (Jones, 2015). Subsequently, the heartworms partially or completely occlude the closure of tricuspid valves, causing life-threatening right-sided heart failure (Jones, 2015). Given the grave prognosis in the affected dogs and the lack of expertise, many veterinarians in Malaysia do not consider heartworm retrieval as a treatment option. This case report describes successful medical and surgical management of caval syndrome and the associated comorbidities in a dog.

CASE REPORT

A 5-year-old, female mongrel dog weighing 22.5 kg was presented due to severe abdominal distension, inappetance, and reduced activity level. The dog was not vaccinated or dewormed, and fed with home-cooked leftovers. On physical examination, the dog was alert but emaciated (body condition score: 2/5), tachypneic (respiratory rate: 60 breaths per minute), and recumbent. Auscultation revealed irregular rapid heart rate (200 beats per minute) with grade IV/VI bilateral systolic murmurs. Fluid waves were also palpable during abdominal palpation.

*Corresponding author: Dr. Leong Zi Ping (Z.P. Leong) ; Email: leong0908@yahoo.com

Editorial history:
Paper received: January 2021
Accepted for publication: April 2022
Published: June 2022

Right lateral chest radiography (Figure 1a) showed cardiomegaly (vertebral heart score: 11.5), pulmonary vessel enlargement, and broncho-interstitial patterns. On the dorsoventral view (Figure 1b), there were enlarged pulmonary trunk and patchy pulmonary infiltrates. The abdominal radiography (Figure 1c) showed a loss of serosal details due to ascites.

The vertebral heart score was 11.5. The lung fields showed mixed bronchial, interstitial, and vascular patterns (Figure 1b). Dorsoventral chest radiography showing an enlarged cardiac silhouette. The pulmonary trunk and the right heart were enlarged. Tortuous pulmonary vessels were noticeable on the left thoracic region (arrows). There were also bronchial and interstitial patterns (Figure 1c). Right lateral abdominal radiography showing ascites, marked by a loss of the serosal details and a distended abdomen.

A total of 4.7 L of odourless, clear straw-coloured modified transudate (specific gravity: 1.028; protein concentration: 3.6 g/dL) was aspirated from abdominocentesis, and the re-measured body weight was 18 kg. Haematology (Table 1) showed moderate non-regenerative microcytic normochromic anaemia, mild thrombocytosis, and mild neutrophilic leucocytosis while serum biochemistry did not reveal remarkable findings, therefore ascites due to hypoproteinaemia and liver disease was ruled out. The dog also tested positive for heartworm antigen but negative for antibodies against *Anaplasma phagocytophilum*, *Anaplasma platys*, *Ehrlichia canis*, *Ehrlichia ewingii*, and *Borrelia burgdorferi* using a Snap 4Dx Plus test (IDEXX).

Electrocardiography (Figure 2) showed a gradual decrease and increase in heart rate of 219 bpm. There were presence of ectopic P waves and reduced QRS amplitudes (R wave: 0.7 mV), which indicate atrial tachycardia. Although mean electrical axis was within normal limit (82°), right ventricular enlargement was suspected given the presence of S waves in leads I, II, III, and aVF.

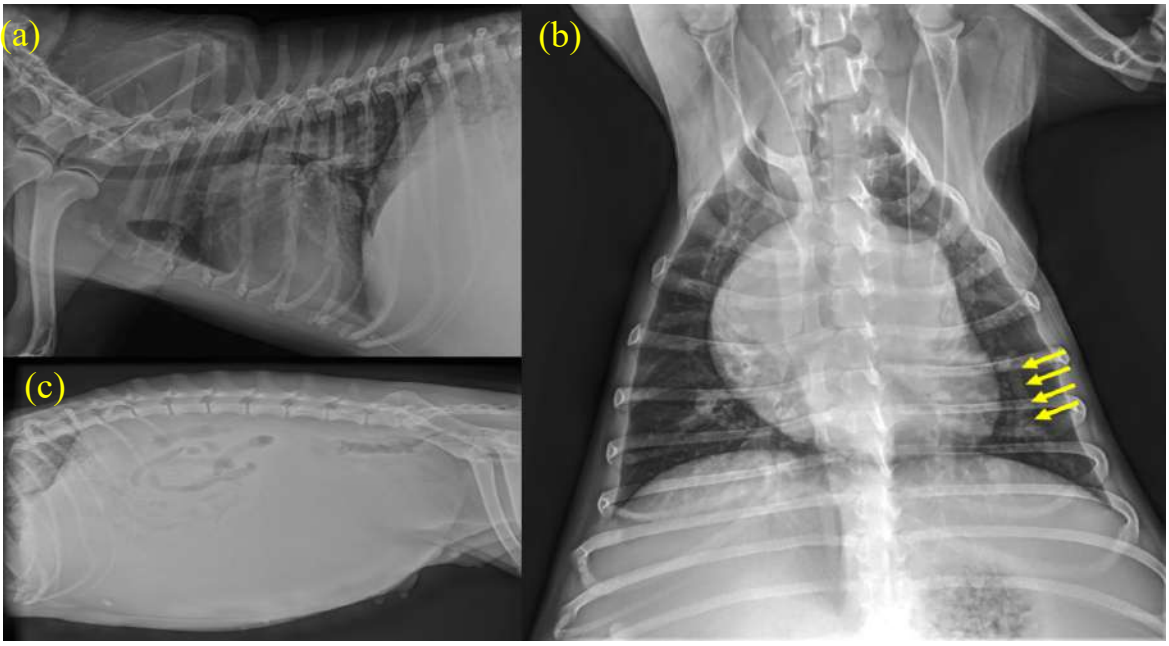


Figure 1. (a) Right lateral chest radiography showing an enlarged cardiac silhouette. The vertebral heart score was 11.5. The lung fields showed mixed bronchial, interstitial, and vascular patterns. (b) Dorsoventral chest radiography showing an enlarged cardiac silhouette. The pulmonary trunk and the right heart were enlarged. Tortuous pulmonary vessels were noticeable on the left thoracic region (arrows). There were also bronchial and interstitial patterns. (c) Right lateral abdominal radiography showing ascites, marked by a loss of the serosal details and a distended abdomen.

Table 1. Haematology and serum biochemistry results

Parameter (Unit)	Value	Reference
Red blood cell ($\times 10^{12}$/L)	**4.19**	5.65-8.87
Packed cell volume (%)	**22.8**	37.3-61.7
Haemoglobin (g/dL)	**8.6**	13.1-20.5
Mean corpuscular volume (fL)	**54.4**	61.6-73.5
Mean corpuscular haemoglobin concentration (g/dL)	**20.5**	21.2-25.9
Reticulocytes (K/µL)	73.3	10.0-110.0
White cell count ($\times 10^{9}$/L)	**20.14**	5.05-16.76
Neutrophils ($\times 10^{9}$/L)	**16.17**	2.95-11.64
Lymphocytes ($\times 10^{9}$/L)	2.61	1.05-5.10
Monocytes ($\times 10^{9}$/L)	1.12	0.16-1.12
Eosinophils ($\times 10^{9}$/L)	0.21	0.06-1.23
Platelet (K/µL)	**485**	148-484
Plateletcrit (%)	**0.48**	0.14-0.46
Glucose (mmol/L)	6.97	4.11-7.95
Urea (mmol/L)	4.1	2.5-9.6
Creatinine (µmol/L)	61	44-159
Total protein (g/L)	62	52-82
Albumin (g/L)	25	23-40
Globulin (g/L)	37	25-45
Alanine transferase (U/L)	29	10-125
Alkaline phosphatase (U/L)	**16**	23-212
Total bilirubin (µmol/L)	4	0-15
Cholesterol (mmol/L)	3.43	2.84-8.26

Note: Highlighted values represent abnormal values

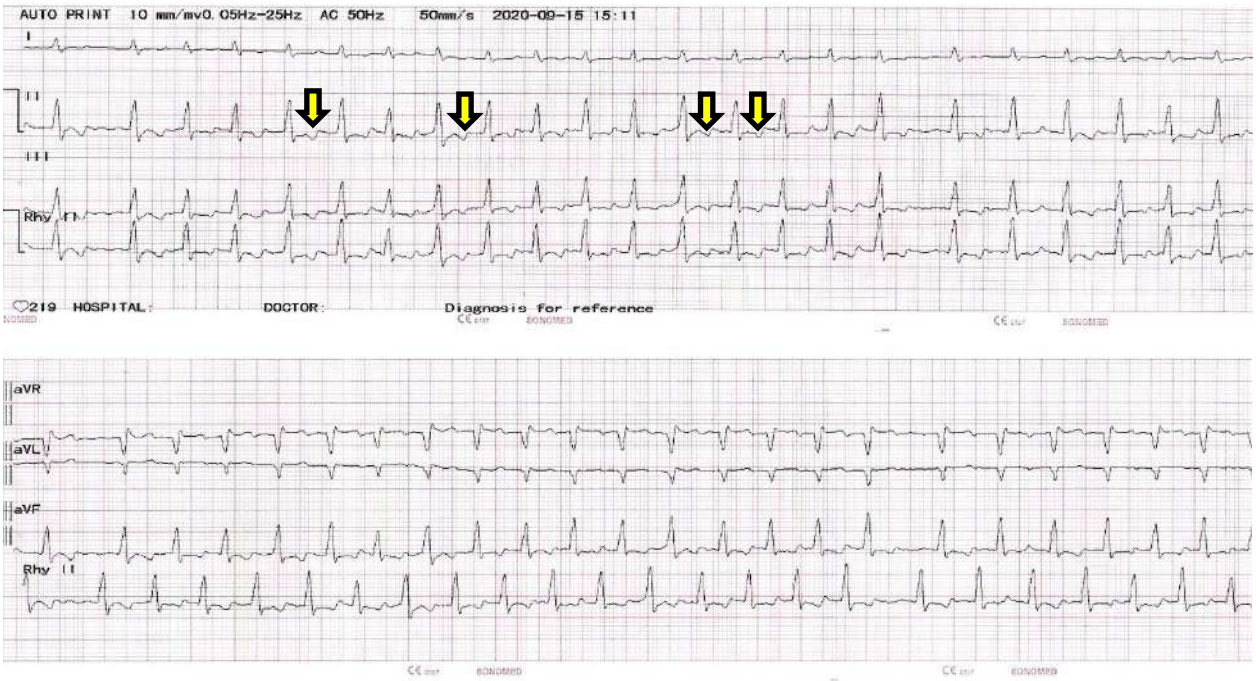


Figure 2. Electrocardiography showing a high ventricular rate of 219 bpm. Some P waves superimposed on the T waves (arrows). Right ventricular enlargement was indicated by the presence of S waves in the leads I, II, III, and aVF.

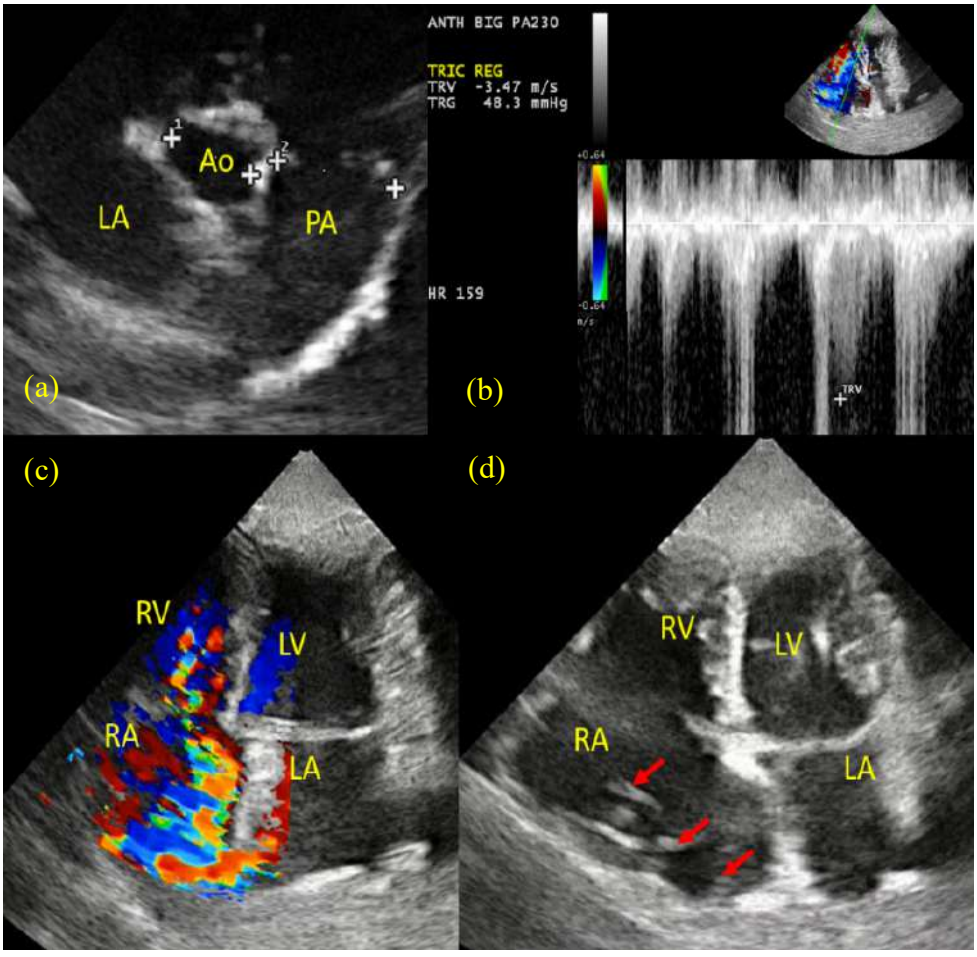


Figure 3. Findings of echocardiography; (a) the pulmonary artery (PA) was dilated compared to the aortic root (Ao). (b) Tricuspid regurgitation velocity was measured 3.47 m/s, giving rise to trans-tricuspid pressure gradient of 48.3 mmHg (c) Colour Doppler showed severe tricuspid regurgitation into the dilated right atrium (RA). (d) Multiple heartworms appeared as hyperechoic lines (red arrows) in the right atrium.

On the echocardiography (Figure 3), dilatations of the left (left atrium to aorta ratio: 1.76) and right atria and pulmonary artery (pulmonary artery to aorta ratio: 1.41) were observed (Figure 3a). Septal flattening and paradoxical movement, as well as mitral, tricuspid (3.47 m/s), and pulmonic (2.53 m/s) regurgitations were also demonstrated (Figure 3b, 3c). These findings suggested that the dog had a high probability of pulmonary hypertension (systolic pulmonary arterial pressure: 48.2 mmHg; diastolic pulmonary arterial pressure: 25.6 mmHg), in accordance to American College Veterinary Internal Medicine (ACVIM) consensus statement guidelines. Moreover, multiple hyperechoic parallel lines resembling heartworms were also noticed in the right atrium, right ventricle, and pulmonary artery, confirming a diagnosis of caval syndrome (Figure 3d).

The dog was stabilised and treated with furosemide (Rasitol 40 mg, 1.3 mg/kg, PO, BID) to reduce the ascites, sildenafil citrate (IQNYDE 100 mg, 1.4 mg/kg, PO, BID) to treat the pulmonary hypertension, and diltiazem hydrochloride (Herbesser 30 mg, 1.7 mg/kg, PO, BID) to reduce the ventricular rates caused by atrial tachycardia. As for the heartworm disease, ivermectin and pyrantel (Heartgard, 1 tab, PO), doxycycline (200 mg, 5 mg/kg, PO, BID), and prednisolone (5 mg, 0.5 mg/kg, PO, BID) were given.

Three days later, heartworm extraction procedure was carried out. After pre-oxygenated at 5 L/min for 5 minutes, the dog was pre-medicated with fentanyl (Talgesic, 5 µg/kg, IV) and midazolam (DOMI, 0.2 mg/kg, IV). The dog was also given enrofloxacin (Baytril®, 5 mg/kg, intravenously) as a prophylactic antibiotic, enoxaparin (Clexane®, 0.8 mg/kg, SC) as an anti-coagulant to prevent thromboembolism, and chlorpheniramine maleate (Pirimat, 0.5 mg/kg, IV) to prevent histamine release and hence anaphylaxis due to the manipulation of the heartworms. After induced with propofol (Troypofol®, 6 mg/kg, IV), the right jugular area was surgically prepped. Skin and underlying tissue was carefully incised to isolate the right jugular vein. The proximal part was ligated with 2/0 nylon, followed by a nick incision distal to the proximal ligation. An angiography catheter (KDL, 5 Fr) with 4 nylon sutures tied on it was inserted via the incised vein (Figure 4a, 4b) into the right heart.

Echocardiography was performed simultaneously to visualize the heart chambers and the catheter (Figure 5a). Then, the catheter was twisted to entangle worms and pulled out slowly. The process was repeated until no heartworms were visible in the right atrium and right ventricle on the echocardiography (Figure 5b). The process successfully removed a total of 7 worms. Next, another angiography catheter was inserted into the right ventricle to measure right ventricular pressures (systolic: 48.3 mmHg; diastolic: 10 mmHg; mean: 22.7 mmHg). After that, the right jugular vein was ligated distally, followed by routine closure and bandage of surgical site. The dog recovered from anaesthesia uneventfully. Post-operatively, the dog received anti-inflammatory dexamethasone (Decan, 0.25 mg/kg, IV) and analgesic morphine (0.05 mg/kg, SC). Finally, a total of 150 mL of whole blood was also transfused to replace the blood loss from the procedure.

The dog continued to receive the adulticide treatment (3-dose melarsomine protocol) in accordance to American Heartworm Society (AHS) guidelines. Additionally, medications which included pimobendan (Cardisure® 10 mg, 0.3 mg/kg, PO, BID), sildenafil citrate (IQNYDE 100 mg, 1.3 mg/kg, PO, BID), diltiazem (Herbesser® 30 mg, 1.5 mg/kg, PO, BID) were also prescribed. The furosemide dosage was tapered to 0.6 mg/kg, PO, BID before discontinued.

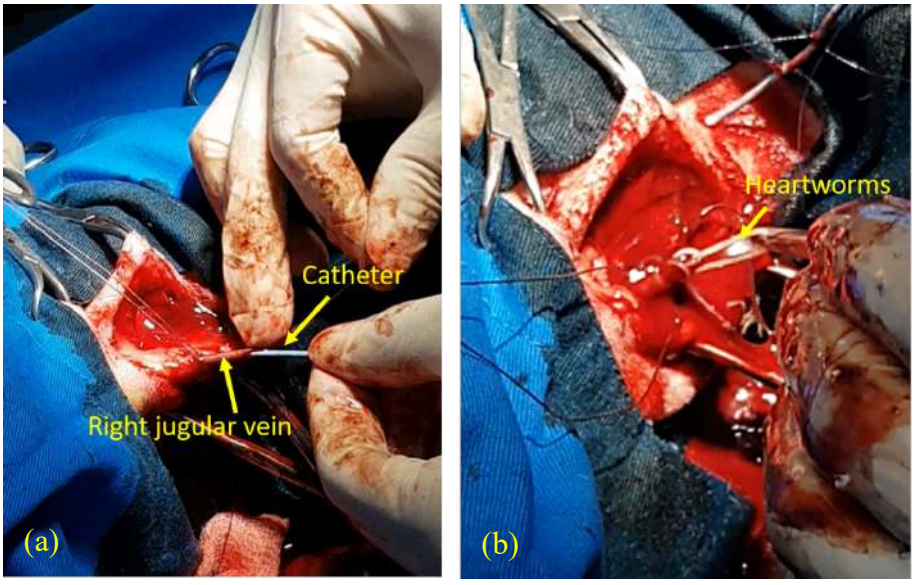


Figure 4. (a) A 5 Fr angiography catheter was inserted via the exteriorized right jugular vein into the right atrium. (b) The catheter was twisted to snare and remove heartworms.

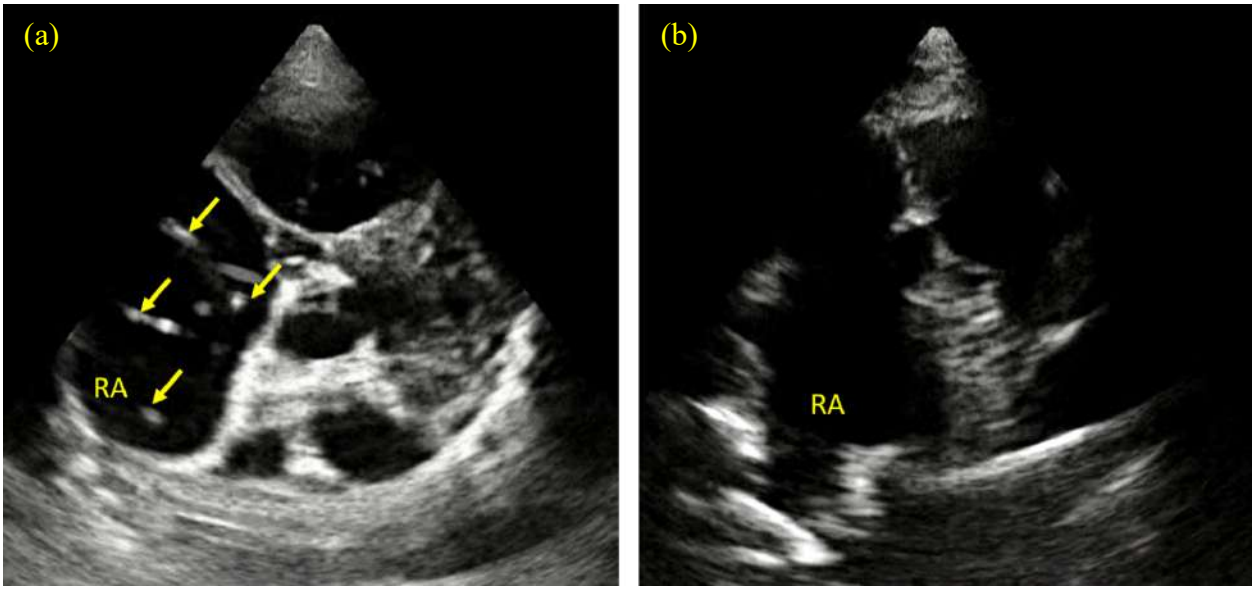


Figure 5. Echocardiography showing (a) presence of heartworms in the right atrium before the procedure. (b) After the extraction, no heartworms were visible on the echocardiography.

Several follow-up examinations showed that the dog was bright and alert with significantly reduced ascites. On day 94, the melarsomine (Immiticide®, Merial) treatment was completed. The dog's ventricular rates were maintained satisfactorily around 157 bpm (Figure 6a). However, the echocardiography (Figure 6b, 6c) revealed an aggravated pulmonary hypertension (systolic: 70.8 mmHg; diastolic: 29.8 mmHg), so the sildenafil dosage was increased (IQNYDE 100 mg, 3 mg/kg, PO, BID). Besides, the owner was educated to provide timely heartworm preventive medicine to the dog. At present, the dog is active and eating well.

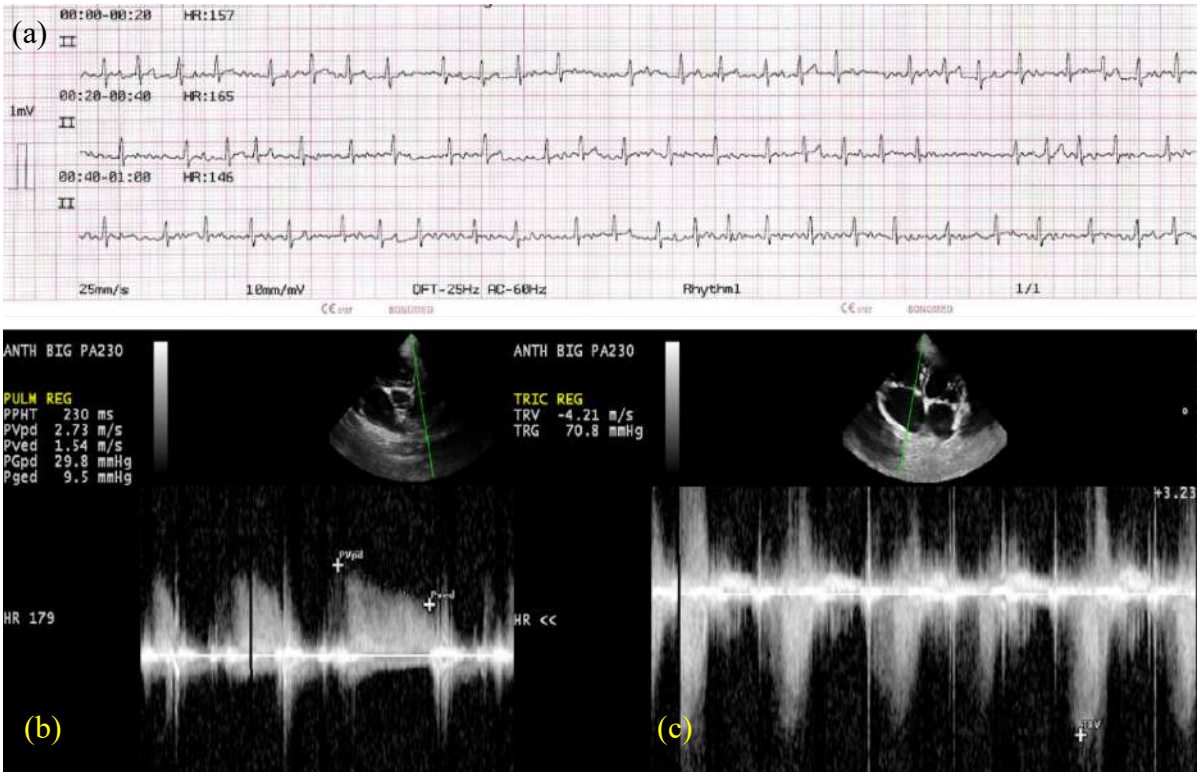


Figure 6. On day 94, the dog had (a) an average heart rate of 157 bpm despite the presence of ectopic P waves on the electrocardiography, but worsened pulmonary hypertension given increased (b) diastolic pulmonary arterial pressure of 29.8 mmHg and (c) systolic pulmonary arterial pressure of 70.8 mmHg.

DISCUSSION

Caval syndrome occurs when there is a high worm burden of more than 40-60 heartworms on average, which consequently obstructs the right cardiac hemodynamic (Atkins, 2005). On the other hand, Kitagawa *et al.,* (1987) reported that the retrograde heartworm migration is caused by decreases in blood flow volume and velocity in the right heart, but not solely due to the high worm burdens. To the best of our knowledge, we reported Malaysia's first case of successful heartworm extraction in a mongrel dog with caval syndrome which presented right-sided congestive heart failure, pulmonary hypertension, and atrial tachycardia, the procedure was performed successfully without complications. In the present case, the low number of heartworms in the dog led us believe that atrial tachycardia and pulmonary hypertension might play a role in reducing the right cardiac output and causing the heartworm migration from the pulmonary arteries. In addition, the lower worm load resulted in milder physical obstruction to the erythrocytes, and this might explain the absence of haemoglobulinemia and haemoglobulinuria.

Given the poor prognosis of the dog if heartworm extraction was not pursued, jugular catheterization was performed to remove the heartworms from the right atrium and right ventricle immediately. A snare was made from an angiography catheter in favour of its rigid property and ability to measurement right cardiac haemodynamic, although the use of red rubber feeding tube, basket retrieval device, and alligator forceps is also recommended (Jones, 2016).

Interestingly, the invasive measurement of the right ventricular pressure corresponded to the echocardiographic estimation of systolic pulmonary artery pressure and confirmed the diagnosis of pulmonary hypertension. In the present case, the pulmonary hypertension was classified as group 5 due to dirofilariasis, based on the proposed classification of pulmonary hypertension in the dogs by the ACVIM consensus statement guidelines. After the surgical extraction, the dog also received appropriate medical treatment in accordance to the AHS guideline to eliminate the possible remaining adult worms and microfilariae.

Despite the worsened pulmonary hypertension which was believed to be due to pulmonary thromboembolism from the dead worms (Hirano *et al.,* 1992), the dog showed resolved ascites and an increased exercise level at the present, implying favourable outcomes to the current treatments with diltiazem, sildenafil, and pimobendan. However, given the significant cardiopulmonary remodeling marked by severe right cardiomegaly and pulmonary hypertension, the dog has a fair-poor prognosis in which regular veterinary revisits are warranted to monitor the disease progression.

CONCLUSION

Canine caval syndrome may be presented with right-sided congestive heart failure in the absence of haemoglobulinemia and haemoglobulinuria, and is diagnosed by echocardiography and heartworm antigen test. Heartworm extraction should be considered as a life-saving treatment option regardless of the compromised hemodynamic in the affected dogs. Post-operative medical treatments should also be given to treat or control other comorbidities.

ACKNOWLEDGEMENTS

The authors thank all staff members of Lee Veterinary Clinic for the nursing care rendered to the dog during the hospitalization.

REFERENCES

Atkins, C. (2005). Canine heartworm disease. In: Ettinger SJ, Feldman EC (Eds.) Textbook of Veterinary Internal Medicine (6th ed., pp. 1118–1136). Elsevier Saunders.

American Heartworm Society (2020). Current canine guidelines for the prevention, diagnosis, and management of heartworm *(Dirofilaria immitis)* infection in dogs https://www.heartwormsociety.org/veterinary-resources/american-heartworm-society-guidelines

Hirano, Y., Kitagawa, H., and Sasaki, Y. (1992). Relationship between pulmonary arterial pressure and pulmonary thromboembolism associated with dead worms in canine heartworm disease. Journal of Veterinary Medical Science 54: 897-904.

Jones, S.L. (2015). Canine Caval Syndrome Series. Part 1: Understanding development of caval syndrome. Today's Veterinary Practice 63-67.

Jones, S.L. (2016). Canine Caval Syndrome Series. Part 3: Management of caval syndrome. Today's Veterinary Practice 55-61.

Kitagawa, H., Sasaki, Y., Sukigara, T., and Ishihara, K. (1987). Clinical studies on canine dirofilarial hemoglobinuria: Changes in right heart hemodynamics inducing heartworm migration from pulmonary artery. Japan Journal of Veterinary Science 49: 485-489.

J. Vet. Malaysia (2022) 34 (1): 11 - 23

Journal Article

KNOWLEDGE AND ATTITUDES OF MALAYSIAN AND UK VETERINARIANS ON PAIN AND ANALGESIA IN CATS

S.S. ZAINI [1, 3*], C. PHILLIPS[1], J.R.D. MACKAY[1] and F. LANGFORD[2]

[1]Royal (Dick) School of Veterinary Studies. The University of Edinburgh. Easter Bush Campus, Midlothian. EH25 9RG. United Kingdom
[2]Animal and Veterinary Sciences, Scotland's Rural College, West Mains Road, Edinburgh. EH9 3JG, United Kingdom
[3]Faculty of Veterinary Medicine, Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia

SUMMARY

Knowing that animals can feel pain does not necessarily mean that veterinarians will be able to manage pain. This study aimed to assess Malaysian and British veterinarians for their knowledge and attitude towards pain in cats. An online questionnaire (SurveyMonkey®) was developed to assess knowledge of- and attitudes towards cat pain and distributed to all registered veterinary surgeons in Malaysia and the UK. A total of 171 UK veterinarians and 265 Malaysian veterinarians completed the questionnaire. In general, demographics were similar between countries. The majority of respondents were female in Malaysia and UK. There were more recent graduates in the Malaysian sample and Malaysians were also more likely to be working in an urban environment than veterinarians working in the UK. Although both countries showed positive attitudes and knowledge of cat pain, pain assessment, and the use of analgesia in cats, however several differences were observed. The most important differences included (i) Malaysian veterinarians thought that they had insufficient knowledge of pain, (ii) UK veterinarians emphasized both behaviour and physiology in assessing cat pain where Malaysian veterinarians focused on physiological indicators, (iii) Malaysian veterinarians were more concerned about cost, record keeping and side effects from using Opioids and NSAIDs than veterinarian in the UK. Education on behaviour of cats suffering from pain and pain management in cats (e.g. pain assessment and pain relief) are important subject areas to be emphasized in the Malaysian veterinary curricula.

Keywords: attitudes, cat pain, knowledge, Malaysia, veterinarians

INTRODUCTION

Pain in humans can be defined as a multidimensional experience; it is not only what you perceive but also how it makes you feel emotionally (Reid *et al.*, 2013) and the same has been shown to be true in non-human mammals (i.e. pain in animals is *"an aversive sensory experience that elicits protective motor actions, results in learned avoidance and may modify species specific traits of behaviour, including social behaviour"* (Molony and Kent, 1997). The multidimensional nature of pain can cause emotional, psychological and physical experiences which can be observed through the behavioural changes of the animals experiencing painful stimuli. This significantly impacts an animal's welfare (Rutherford, 2002).

Historically there have been difficulties in recognising pain in cats, which is thought to be due to a lack of understanding of how cats exhibit pain-related behaviours (Merola and Mills, 2016). Another reason why pain recognition is considered challenging in cats is that they are considered a 'stoic' species. Alongside stoicism, there have been few pain scales that have been validated for cats (Steagall and Monteiro, 2019). Even this scale have been validated, but the use of scale might have some limitations, for example, certain demeanours (e.g. shy or fearful cat) may present high pain scores and it can be difficult to distinguish whether they are truly painful or whether their behaviour affects pain assessment (Steagall and Monteiro, 2019), and there may also be cultural or regional contexts (Phillips *et al.,* 2012; Reed and Upjohn, 2018) which affect the management of pain in cats such as there have been observed differences between the way analgesia for painful conditions in cats has been administered in Australia, New Zealand and the UK (Farnworth *et al.,* 2014). Comparative studies like this one are often limited in recruitment, making comparisons between populations difficult; however, it is notable that differences were observed between three economically wealthy English-speaking countries (namely, Australia, New Zealand and the UK). In upper-middle-income countries, these differences could be compounded by factors such as cultural differences (Reed and Upjohn, 2018), variations in education (Sugano, 2014), the inability of non-English speakers to access English speaking resources (e.g.: pain scales) and inadequate access to e-resources for continuing professional development (Murrell *et al.,* 2008).

A 'One World Health and Welfare' approach must consider the context of animal welfare education within each country to improve animal welfare. This is because different countries may provide different veterinary education curricula that may, in turn, affect the way veterinary students and graduates perceive animal welfare (Sugano, 2014).

Hitherto, most studies have been reported in English speaking countries. There is insufficient data from

*Corresponding author: Dr Syamira Syazuana Binti Zaini (SS Zaini);
Email: syamirasz@upm.edu.my;

Editorial history:
Paper received: May 2021
Accepted for publication: April 2022
Published: June 2022

Malaysia. Thus, it is important to explore the Malaysian veterinarians' perspective regarding cat welfare. Currently, there is insufficient information to make a meaningful comparison between Malaysian and UK veterinarians on this subject. Malaysia aims to achieve a standard of animal welfare that is as good as that found in countries such as the UK or New Zealand. The UK provides the best comparator for measuring animal welfare in Malaysia, because the UK is often regarded as having the highest level of animal welfare (Nizam and Tahman, 2019). Therefore, in this study, we will explore the differences between attitudes and knowledge regarding pain recognition in cats between Malaysia and the UK.

This study characterized the Malaysian veterinary population's: (i) Knowledge of cat pain, (ii) Attitudes towards cat pain, (iii) Ability to recognize cat pain, (iv) Current clinical practices relating to cat pain, and (v) To relate (i) – (iv) to demographic data and to compare and contrast these findings with veterinarians in the UK, and finally (vi) to understand how veterinarians would like to receive education on topics related to cat pain.

MATERIALS AND METHODS

Ethical approval, consent and data management

The study was approved by the Human Ethics Research Committee (HERC) on 25th January 2017, Royal (Dick) School of Veterinary Studies, University of Edinburgh: HERC approved project number HERC_56_16. The project also has ethical approval from the Malaysian Medical Research and Ethics Committee (MREC): approved project number nmrr-17-672-35076. All the answers were kept anonymously and were not shared with third parties. They were kept securely and stored on a secure server which was held in relation to the UK Data Protection Act in the United Kingdom.

Participant recruitment

UK

The Royal College of Veterinary Surgeons (RCVS) list of registered surgeons was used to recruit UK participants. A link to the questionnaire was emailed to every registered email address accredited by RCVS (n = 2100) on 13th of March 2017. A reminder email was sent out two weeks after the initial email. The questionnaire also was published on the Facebook pages of both the Royal (Dick) School of Veterinary Studies and the Vet Times.

Malaysia

There were a total of 1354 registered veterinarians with Malaysian Veterinary Council (MVC). 832 veterinarians were approached via email. Paper questionnaires were sent to 450 veterinarians who did not have email addresses. The author was unable to contact the remaining veterinarians (n = 72) due to lack of contact details (e.g. no available email address or practice address). The questionnaire also was released via social media (e.g. Facebook) by approaching a veterinarians' social media group discussion. All the methods of questionnaire distribution used different URL links to identify the mode of participant recruitment.

Questionnaire development

There were five sections in the questionnaire. The first section was 'Veterinary demographics' which focused on gender, year of graduation, school of graduation, area of practice, number of veterinarians and veterinary nurse/ assistant in the clinical practice and field of practice. For the second section, 17 Likert-like scale statements relating to 'Knowledge and attitude to cat pain' were developed. In section three, there were 29 statements developed to investigate the ability of veterinarians to recognize and assess pain in cats which include how useful each pain indicator (physiological and/ or behavioural) was to assess pain in cats. 27 statements were developed in the fourth section which dealt more specifically with analgesia use in cats. Section 5 collected opinions regarding preferable educational resources for 'Continuing education' in cat pain. The questionnaire can be made available upon request.

There were two versions of questionnaire (English and Bahasa Malaysia). The Bahasa Malaysia questionnaire was written by the researcher and then back-translated by other Malaysian veterinarians in English to ensure the similarity between the two versions. Both questionnaires were created in SurveyMonkey® (San Mateo, California, USA), the software package which we used as our model for developing, distributing and collecting data from respondents.

Pilot questionnaire

All the questions were pilot tested by a small group of UK and Malaysian veterinarians. Five veterinarians in the Royal (Dick) School of Veterinary Studies and the Hospital for Small Animal at the University of Edinburgh and six Malaysian veterinarians were involved in the pilot test. Two reminders were sent out after the second and the fourth weeks after the initial pilot questionnaire opened. This applied to both forms of questionnaire distribution (e.g. via email and social media). All the comments and feedback from veterinarians were incorporated into an amended questionnaire.

Statistical analysis

Descriptive analysis was used for each question where the response rate was calculated. Participants were included in the response rate if they completed 50% or more of their questionnaire. To examine differences between countries in the Likert-like scale questions, the analysis was run separately and tested with each univariate demographic by using the non-parametric statistical test (e.g. the Kruskal Wallis and Wilcoxson ranked tests). The test is considered to be significant if the *P*-value is less than 0.05. Statistical analysis was carried out in R studio, using R version number 3.4.3 (R Core Team, 2017). The following packages were used, *Likert* (Bryer *et al.,* 2016) and tidyverse (Hadley, 2017).

Table 1. Demographic details of respondents for UK and Malaysia.

Demographics	% UK (n)	% Malaysia (n)
****Gender**		
Men	17.0% (29)	29.5% (77)
Women	81.3% (139)	68.7% (180)
Prefer not to disclose	1.8% (3)	1.9% (3)
*****Year of graduation**		
Before 1960	0% (0)	0.4% (1)
1961-1970	0.6% (1)	0.4% (1)
1971-1980	2.3% (4)	1.9% (5)
1981-1990	9.9% (17)	6.1% (16)
1991-2000	13.5% (23)	9.5% (25)
2001-2010	39.2% (67)	30.5% (80)
2011-2016	34.5% (59)	51.1% (134)
School		
	UK: 85.4% (146)	UPM: 86.6% (227)
	Non-UK: 14.0% (24)	UMK: 5.0% (13)
	Other: 0.6% (1)	Other: 8.4% (22)
Area		
	Village/rural: 14.6% (25)	Rural: 4.3% (11)
	Small city/town: 40.9% (70)	Urban: 62.6% (161)
	Sub-urban city: 13.5% (23)	Mixed rural and urban: 29.6% (76)
	Urban city: 20.5% (35)	Not sure: 2.3% (6)
	Other: 10.5% (18)	Other: 1.2% (3)
****Field of practice**		
Small animal	71.9% (123)	54.6% (143)
Large animal	1.8% (3)	5.0% (13)
Educators	6.4% (11)	3.8% (10)
Mixed practice	9.4% (16)	19.1% (50)
Other	4.1% (7)	13.7% (36)
Small animal & educator	6.4% (11)	3.8% (10)
*****No. of veterinarians in a practice**		
1-3	25.7% (44)	51.9% (136)
4-6	34.5% (59)	16.8% (44)
7-10	15.8% (27)	12.6% (33)
More than 10	24.0% (41)	18.7% (49)
*****No. of veterinary nurses in a practice**		
No veterinary nurse	4.7% (8)	18.1% (47)
1-3	27.5% (47)	42.9% (111)
4-6	28.7% (49)	13.1% (34)
7-10	11.7% (20)	7.3% (19)
More than 10	27.5% (47)	18.5% (48)

The table shows the percentage (number of participants) in each demographics questions by country.
Note: UPM = University Putra Malaysia, UMK = University Malaysia Kelantan, * P <0.05, ** P< 0.01, *** P <0.001.

RESULTS

Of the 204 UK respondents, 171 (83.38%) were accordingly usable, and of the 341 Malaysian respondents, 265 (77.7%) were usable. Detailed demographic data is available in Table 1. For the UK responses, 81.3% respondents were women (n = 139). Most (39.2%, n = 67) graduated between the years 2001-2010, and 85.4% (n = 146) graduated from veterinary schools located in the United Kingdom. The majority of those who responded to this questionnaire practised in a small city or town (40.9%, n = 70). Over half of those questioned reported that they were small animal practitioners (71.9%, n = 123) and only 1.8% (n = 3) were large animal practitioners. Most practices were reported to have between 4-6 veterinarians (34.5%, n = 59) and 4-6 veterinary nurses (28.7%, n = 49) working in the practice.

For the Malaysian respondents, the majority of responses also came from female veterinarians (68.7%, n = 180). However, Malaysian respondents tended to be more recently qualified than UK respondents (from 2011 vs 2001 to 2010). The majority of Malaysian respondents practised in an urban area (62.6%, n = 161), the majority of whom working as small animal practitioners (54.6%, n = 143). When asked about the number of veterinarians and veterinary nurses in the clinical practice where they worked, 51.6 % (n = 136) reported there were 1-3 veterinarians and 42.9 % (n = 111) reported there were 1-3 veterinary nurses/ assistant veterinary officer (AVO) in their clinical practice.

Differences between knowledge, attitudes and capabilities of UK and Malaysian veterinarians towards cat pain

The application of knowledge in cat pain

This section of the questionnaire explored veterinarians' knowledge of cat pain, consisting of (i) their level of knowledge and skill in recognizing cat pain, their ability to manage cat pain and (ii) their ability to assess cat pain and their methods of assessing it. More details in Figure 1.

Respondents were also asked about the usefulness of sources for continuing education, and from where respondents would seek more information about cat

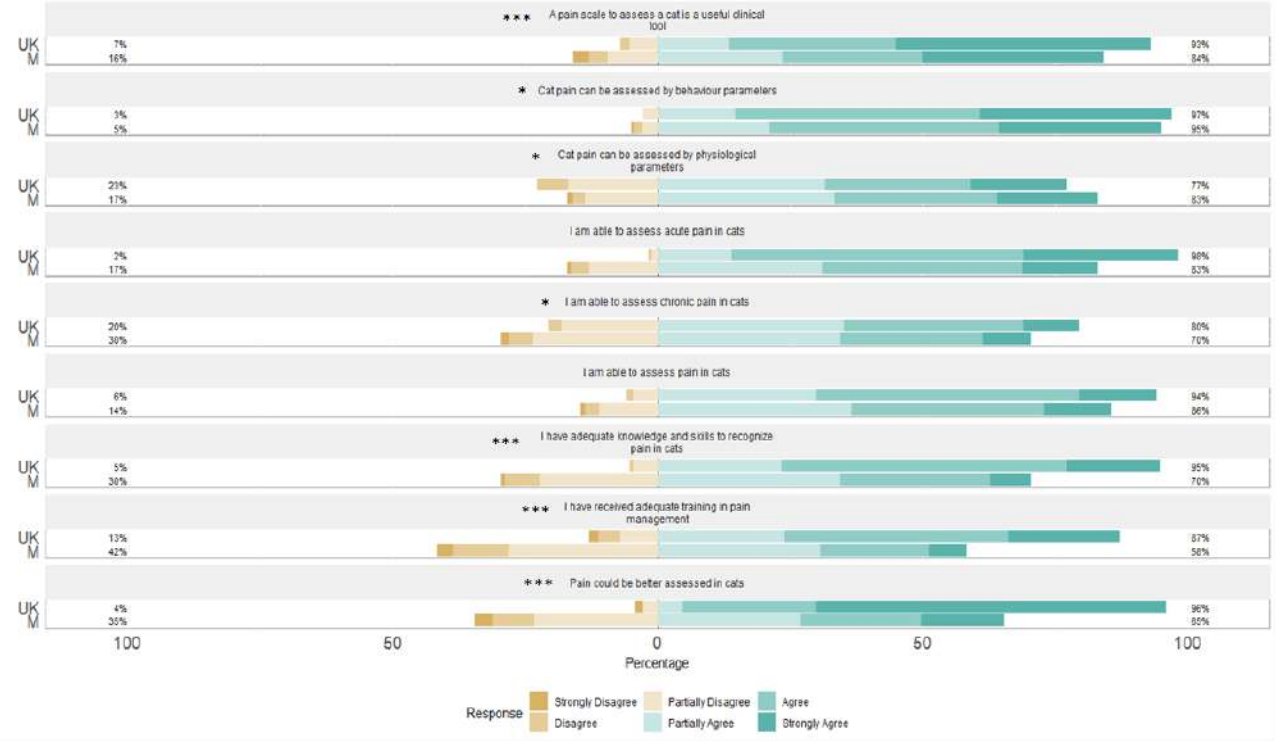


Figure 1. Proportion of veterinarians' difference between responses and country. Note: * P < 0.05, ** P < 0.01, *** P < 0.001

Table 2. Median, mean and standard deviation (SD) of veterinarians' difference between responses and country.

Attitudes to cat pain	UK		Malaysia		*P*-value
	Median score	Mean ± SD	Median score	Mean ± SD	
A degree of pain is required to stop cats being too active after surgery.	1.00	1.55 ± 1.085	4.00	3.85 ± 1794	*P* < 0.001
Surgery does not usually result in sufficient pain to warrant analgesic therapy.	1.00	1.11 ± 0.514	2.00	2.83 ± 1.710	*P* < 0.001
Analgesia is always beneficial.	6.00	5.49 ± 1.020	5.00	5.02 ± 1.130	*P* < 0.001
Pain prevention is better than pain relief.	6.00	5.66 ± 0.841	5.00	4.94 ± 1.220	*P* < 0.001
The client will not pay for analgesia.	1.00	1.65 ± 1.030	2.00	2.34 ± 1.250	*P* < 0.001
I have sufficient knowledge of pain relief in cats.	5.00	4.58 ± 0.987	4.00	3.94 ± 1.080	P < 0.001
6 answer options: 1 = Strongly disagree, 2 = Disagree, 3 = Partial disagree, 4 = Partial agree, 5 = Agree, 6 = Strongly agree					

Wilcoxon Signed-Rank test showed the difference between the UK and Malaysian veterinarians' responses towards six attitudes statements.

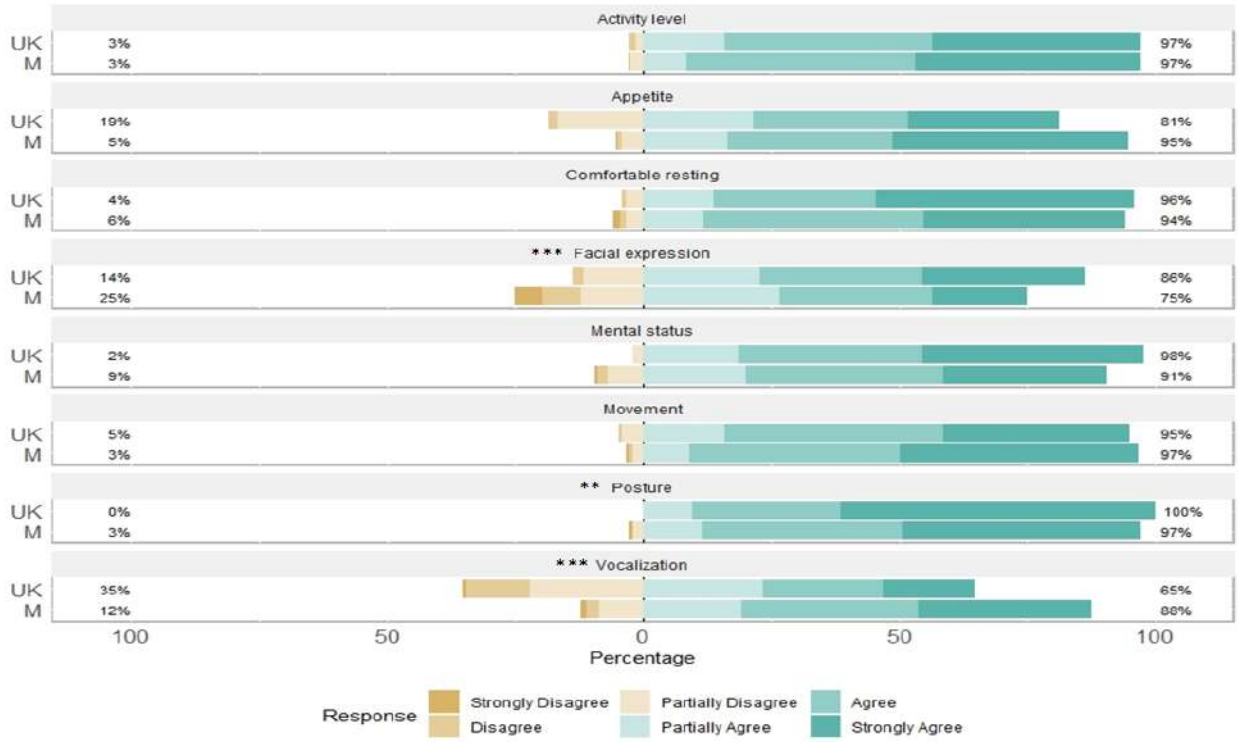


Figure 2. Prevalence of respondents towards cat pain indicators and country. Note: ** P < 0.01, *** P < 0.001.

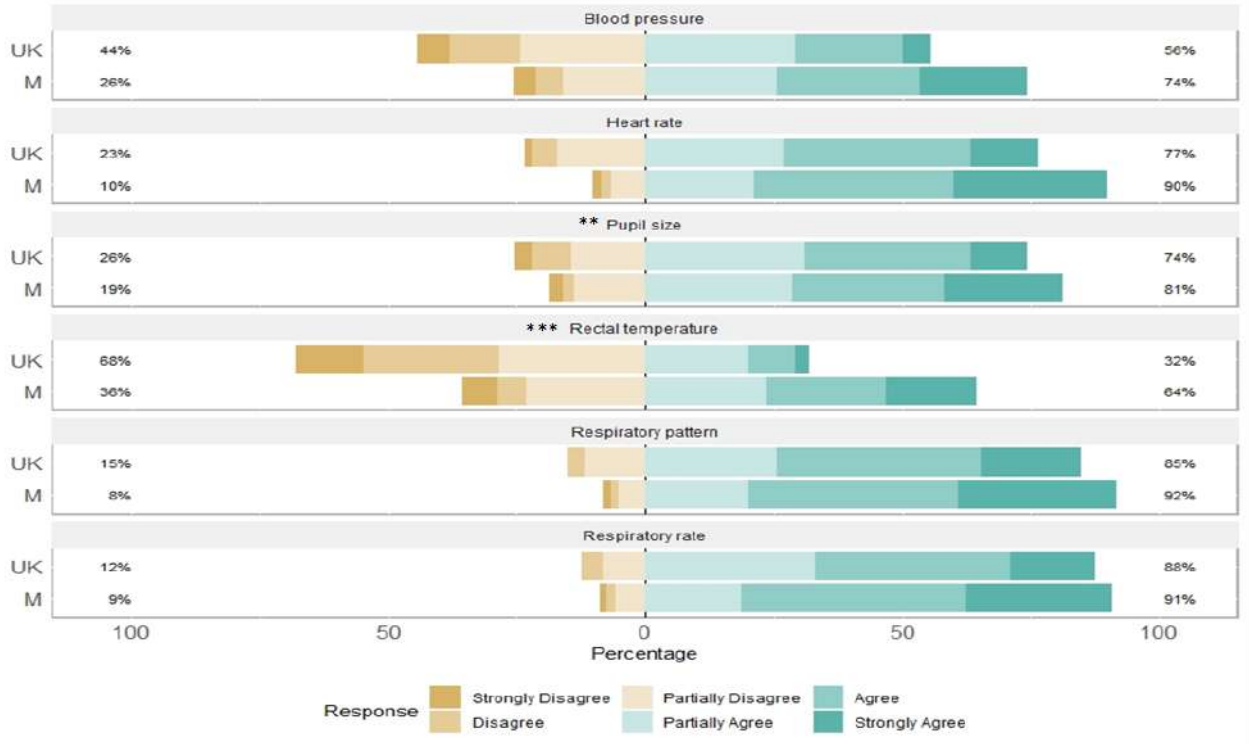


Figure 3. Prevalence of respondents towards cat pain indicators and country. Note: ** P < 0.01, *** P < 0.001.

pain. Veterinarians from both countries chose clinical experience most often as their most useful source for continuing education (UK: 44.7%, n = 72, Malaysian: 60.5%, n = 141), followed by articles (41.6%, n = 67) and formal education (40.4%, n = 65) for UK and seminar/workshop (50.9%, n = 118) and conferences (45.1%, n = 105) for Malaysia. However, there is only one significant difference between countries: concerning whether clinical experience is a useful source for updating and maintaining skill in managing cat pain, Malaysians were more likely to strongly agree that it is, than UK vets (W = 21212, $P = 0.018$ r = .11).

Attitudes to cat pain

Overall, respondents from the UK and Malaysia strongly agreed with the statement *'cats experience pain'* (UK: 65.5%, n = 112, Malaysia: 71.4% n = 187) and [cats] *'have the same physiological apparatus like humans do'* (UK: 68.4%, n = 117 and Malaysia: 43.5%, n = 114), and there was no significant difference between countries in their likelihood to agree with these statements. However, there were significant differences for six attitudes statements (Table 2).

Useful indicators for assessing cat pain

The majority of UK respondents agreed that posture (61.4%, n = 89) was an extremely useful indicator for assessing pain in cats, followed by comfortable resting (50.3%, n = 73) and mental changes (43.4%, n = 63). For Malaysian veterinarians, the greatest number of veterinarians rated posture (46.7%, n = 114), movement (46.7%, n = 114) and appetite (46.1%, n = 112) as useful indicators to assess cat pain (see Figure 2 and 3).

Further analysis showed that there were five significant differences in terms of the usefulness of cat pain indicators between veterinarians from the UK and Malaysia. More number of respondents from UK than Malaysian respondents scored "extremely useful" for facial expression (W = 13782, $P < 0.001$, r = .18) and posture (W = 15013, $P < 0.01$, r = .14) as cat pain indicators. In contrast, more number of respondents from Malaysian than UK respondents agreed that vocalization (W = 23516, $P < 0.001$, r = .27), rectal temperature (W = 25314, $P < 0.001$, r = .35) and pupil size (W = 20404, $P = 0.01$, r = .13) were useful indicators of cat pain.

Pain score

Medical conditions

There was little difference identified between both countries on the perception of the painfulness of each medical conditions (Figure 4). However, most Malaysian veterinarians considered (a) Lower Urinary Tract Infection (LUTI) (W = 27626, $P < 0.001$, r = .29) and (b) chronic Degenerative Joint Disease (DJD) (W = 27656, $P < 0.001$, r = .29) as "severely painful" compared to UK veterinarians who scored both conditions as "moderately painful".

Both countries scored (c) carnassial tooth abscess and (d) constipation as "moderately painful", however, this was shown by higher proportion of Malaysian veterinarians than UK veterinarians ((c) carnassial tooth abscess, W = 24358, $P < 0.002$, r = .15, and (d) constipation, W = 27772, $P < 0.001$, r = .29). Because UK veterinarians more split in their responses between moderately and severely painful for these conditions.

Surgical procedures

Similar findings were reported for the surgical procedures as to the medical conditions above. Overall, the responses were broadly similar between the two countries (Figure 5). However, most UK veterinarians were more likely than Malaysians to consider surgical procedures (e.g. abdominal exploratory-laparotomy (W = 16451, $P < 0.001$, r = .17), diaphragmatic hernia repair and (W = 17524, $P = 0.008$, r = .13), ovariohysterectomy (flank approach) (W = 17331, $P = 0.007$, r = .13)) as "severely painful". UK veterinarians also were more likely to consider castration as "moderately painful" than Malaysian veterinarians (W = 18059, $P = 0.027$, r = .11).

Cat pain management

The decision to prescribe analgesia in cats

About 70% of UK veterinarians said their decision to provide analgesia for cat patients was strongly impacted by contributions from veterinary nurse colleagues, compared to 28% of Malaysian veterinarians, and this was significantly different (W = 9263, $P < 0.001$, r = .47).

Rating the importance of the administration of analgesia in cats

The next question asked the respondents to rate the importance of providing analgesia for cat pain for a list of medical conditions and surgical procedures on a 6-point Likert scale. Overall, veterinarians from both countries showed a similar response with a slightly different level of agreement towards the question. Most of them scored "important" in regard to providing analgesia for conditions such as acute necrotizing pancreatitis (W = 9158.5, $P < 0.001$, r = .50), carnassial tooth abscess (W = 11416, $p < 0.001$, r = .35), lower urinary tract infection (W = 11892, $P < 0.001$, r = .33) and chronic Degenerative Joint Disease (W = 14994, $P < 0.001$, r = .19). UK veterinarians tended to rate the provision of analgesia more strongly (they scored "extremely important to provide analgesia") than Malaysian veterinarians (they only scored "important to provide analgesia"). Interestingly, there is a different response to the question of whether analgesia should be administered for constipation. While many of the UK veterinarians scored "important to provide analgesia" for constipation, Malaysian veterinarians scored "partial" or, "not important" to provide it (W = 11674, $P < 0.001$, r = .32).

The availability and duration of analgesia postoperatively

The availability and duration of analgesia when prescribed after surgical procedures was reported by the UK and Malaysian veterinarians. Overall, most respondents from both countries reported that they prescribed analgesia for all surgical procedures. However, UK veterinarians preferred to prescribed analgesia for a slightly longer time than Malaysians for post-operative Ovariohysterectomy (OVH), castration, abdominal exploratory laparotomy and diaphragmatic hernia repair.

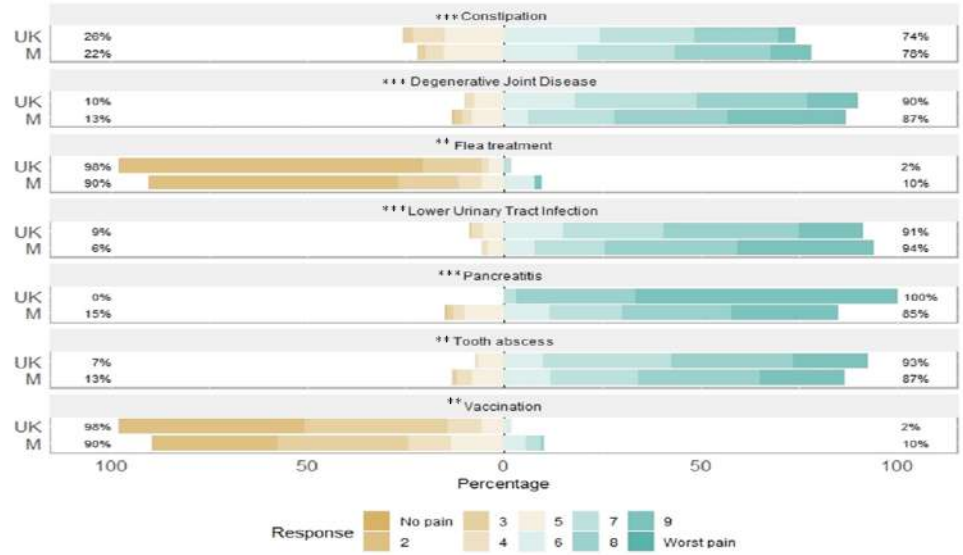


Figure 4. Average pain scoring for medical conditions by country.
Note: ** P < 0.01, *** P < 0.001.
Pain score chart:
1: No pain,
2-4: Mild pain,
5-7: Moderate pain,
8-10 (Worst pain): Severe pain.

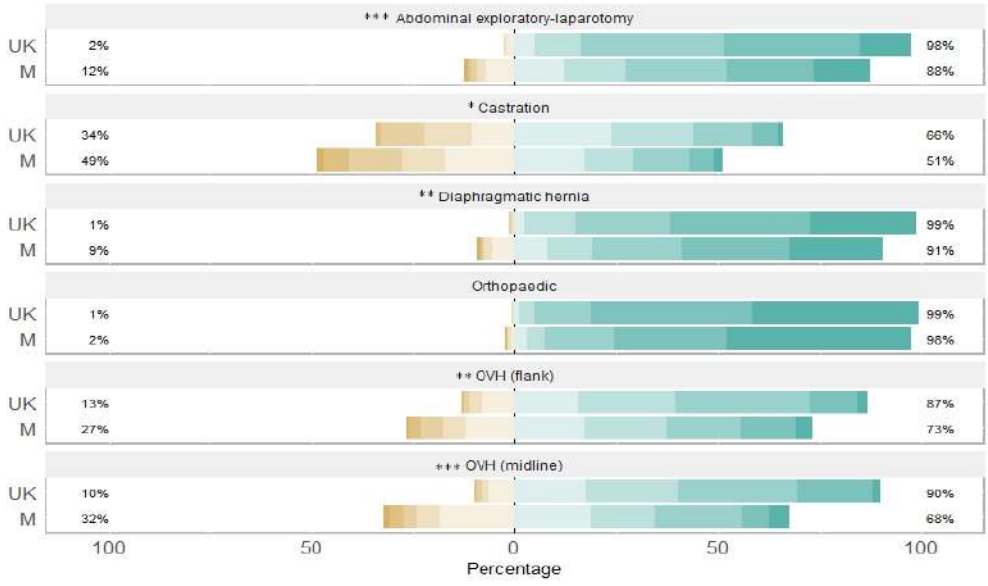


Figure 5. Average pain scoring for surgical conditions by country.
Note: * P < 0.05, ** P < 0.01, *** P < 0.001.
Pain score chart:
1: No pain,
2-4: Mild pain,
5-7: Moderate pain ,
8-10 (Worst pain): Severe pain.

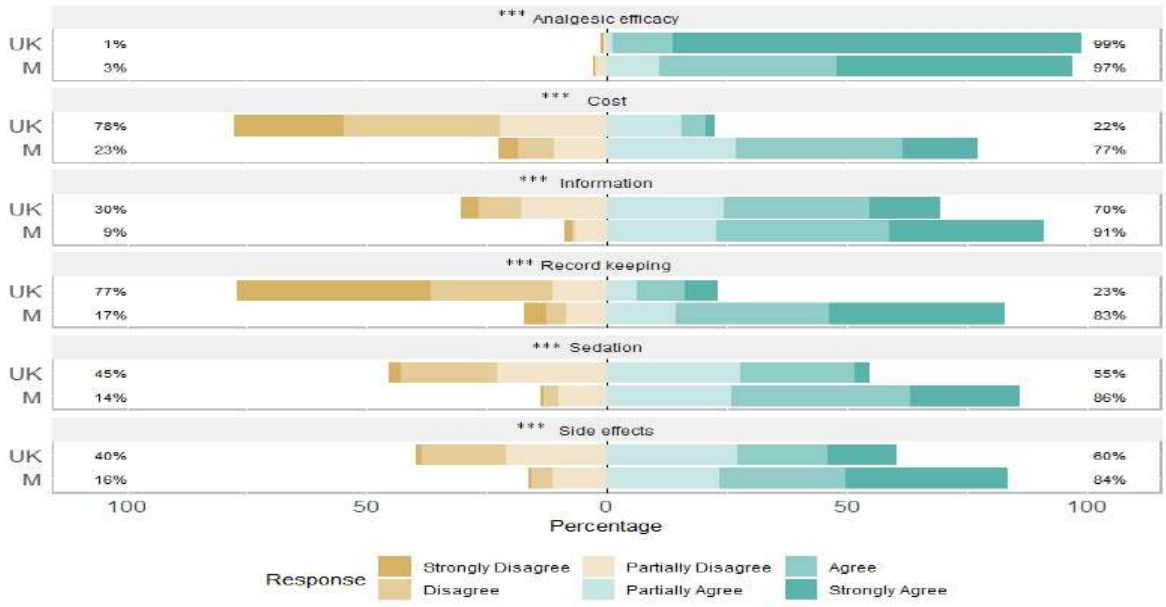


Figure 6. Proportion of veterinarians regarding factors influencing the choice of drug (Opioid) in cat pain
Note: ***$P < 0.001$

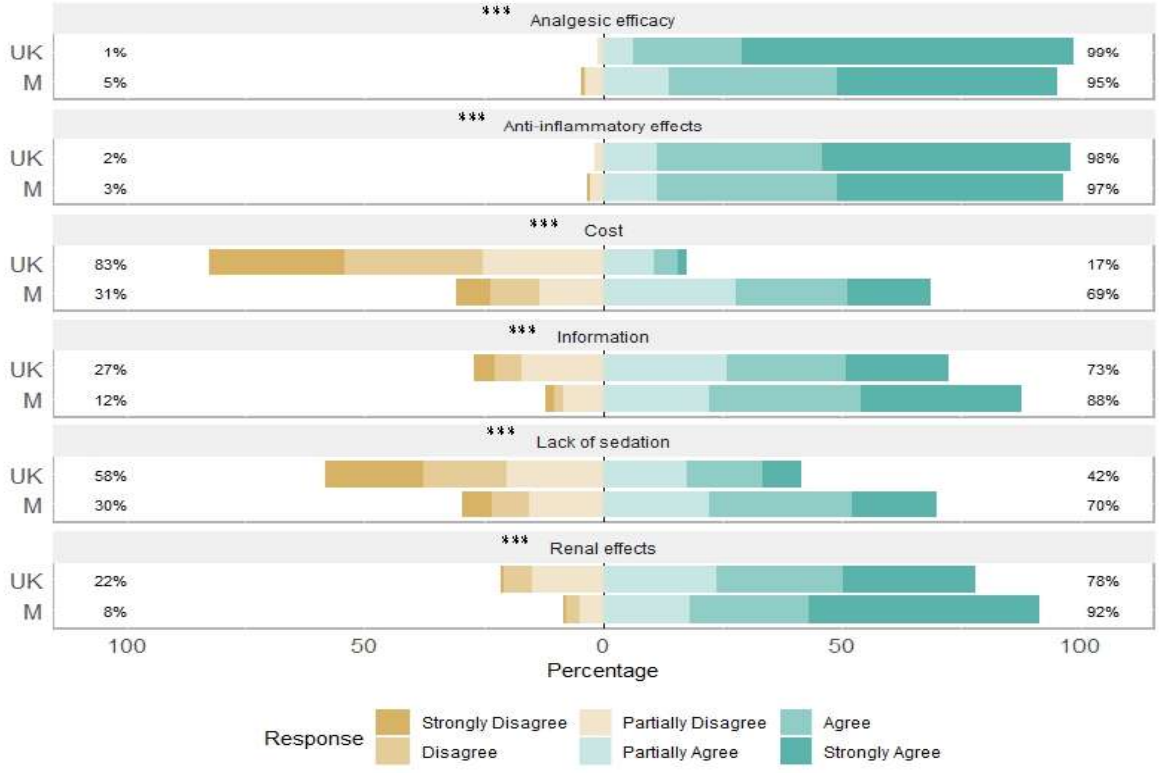


Figure 7. Proportion of veterinarians concerning factors influencing the choice of drug (NSAIDs) in cat pain.
Note: ***P < 0.001

Factors influencing the use of Opioid and NSAIDs in cats

Malaysian respondents agreed more strongly than those from the UK with most statements about factors contributing to their choice of Opioid and NSAIDs in cat pain: the cost, the need for record-keeping, side effects, sedative effects, and the availability of relevant information. UK respondents were more willing to use both types of drugs owing to their efficacy as an anti-inflammatory and analgesic (Figure 6 and 7).

DISCUSSION

This study characterises the attitudes and knowledge of Malaysian and UK veterinarians towards cat pain, in 2017. To our knowledge, this is the first study to examine the attitudes of veterinarians with regard to cat pain in Malaysia.

The majority of participants who responded from both countries were women, which is similar to the previous studies (Dohoo and Dohoo, 1996; Hewson *et al.,* 2006; Beswick *et al.*, 2016). However, Malaysian respondents were twice as likely to be male than those from the UK, perhaps because the majority of Malaysian veterinarians are male. More Malaysian respondents graduated in the 2010s, and practice in urban areas, than UK respondents who were more likely to graduate in the 2000s and practice in small cities or towns. There may be an implication that internet availability is more limited in non-urban Malaysian areas, but it is not possible to be certain of this. From this study, we can see that most of the Malaysian participants are considered to be "young" veterinarians because they had less than 10 years' experience in practice.

There are a number of potential limitations to this study's conclusions. It was not possible to calculate the exact response rate of both countries because the participation in the questionnaire was voluntary. In addition, the questionnaires were distributed through many sources such as by email, social media (e.g.: Facebook, Twitter), website and post. In both countries, the respondents may be biased merely by taking an interest in the subject matter of the questionnaire and consequently they took the time to complete the questionnaire (Hunt *et al.,* 2015). In this study, we can see that for both countries the majority of respondents were small animal practitioners, which is not surprising considering the study was aimed at the small animal practitioner community in both countries. There were small number of mixed practice veterinarians answering the survey and this could lead to some of their answers being less knowledgeable than those that focus on small animals as for example, they may have less awareness of the management of pain in cats. However, because of the very small subset (UK, n= 16 (9.4%), Malaysia, n= 50 (19.1%)) of mixed practice veterinarians in this survey, this effect was not analysed systematically.

There may also be cultural differences between the countries which affected the questionnaire responses. For example, responses from UK veterinarians were more often to the extremes of the Likert scales ("strongly agree" or "strongly disagree") whereas Malaysian respondents tended to express less strong agreement, or disagreement and hence be found around the middle of the scale. A potential explanation could be the cultural differences seen in Asian and Western societies, between individualism and collectivism (Lee *et al.,* 2002). Individualism emphasizes qualities such as uniqueness and difference (Markus and Kitayama, 1991; Lee *et al.*, 2002). This often characterizes cultures such as Western societies, which promote the individual's freedom to express personal opinions free from undue societal expectation (Lee *et al.,* 2002). Thus, UK responses potentially evidenced cultural individualism by tending to select extreme answers. In Asian societies notable for a collectivist culture, there is an expectation of harmonious interpersonal relationships which are particularly highly valued (Lee *et al.,* 2002) and one way to achieve this is to make sure one's answer to a question is unlikely to be too different from another's. We might see this as a tendency to be less extreme in answering questions. This may explain why we see our Malaysian respondents tending to provide less extreme answers. Even though there was a potential culturally-affected difference in responses between Malaysian and the UK, it is possible to generalize the results for the purposes of the present study. This is due to the coherence of the generally positive trend of responses between countries.

Overall, veterinarians in both countries reported positive attitudes towards the management of cat pain. However, when asked to what degree they agreed with the statement *'A degree of pain is required to stop cats being too active after surgery',* about half of the Malaysian respondents "partially agreed" with the statement. These findings are in line with previous research in the UK, 1999 (Capner *et al.,* 1999; Raekallio *et al.,* 2003). Historically, pain was believed to be beneficial for animals, in order to restrict movement after surgery and thus prevent further injury (Epstein *et al.,* 2015). However, advances in knowledge of the physiology and mechanism of pain in animals (Kongara *et al.,* 2016), means that animal pain is now described in terms similar to the scientific description of human pain (Williams *et al.,* 2005), particularly with regard to its development, conduction and modulation. Animals (for example, dogs and cats) share similar neural pathways to humans in regards to pain (Partridge and Rossmeisl, 2019), so a strong case can be made that animals also feel pain (Epstein *et al.,* 2015). There was a noticeable change in UK veterinarians' attitudes towards the use of analgesia to treat cat pain compared to findings conducted during 1996-1997 in the UK (Lascelles *et al.*, 1999). Changes in attitudes towards cat pain and analgesia among UK veterinarians with regard to knowledge and awareness

of animal pain and pain management could be due to significant improvement in veterinary education and practices in the UK (Farnworth *et al.*, 2014). In order to enrich Malaysian veterinarians' attitudes and knowledge with regards to animal pain and management of pain, UK and Malaysian veterinary communities could undertake knowledge transfer activities between these two countries; exchanging views, training and sharing of best practices with regards to pain perception, handling and other topics. Malaysian veterinarians' reported that they found some types of continuing education resources "useful", so these could be further developed, for example, the development of new forums for discussion, webinars, online discussions and workshops would all enhance the educational resources available to Malaysian veterinarians on cat pain. These also have been indicated as one feature of OIE's Global strategy for the further improvement of animal welfare worldwide (World Organisation for Animal Health, 2017).

Malaysian veterinarians were less likely to "strongly agree" that 'pain could be better assessed in cats', than UK veterinarians. Bearing in mind the tendency for Malaysians to avoid the extremes of scales, this is still an interesting finding. It may be that Malaysian veterinarians are already competent at assessing pain in cats and so their response here is accurate. In this study, Malaysian veterinarians reported preferring to assess cat pain by using physiological and behavioural parameters equally, whereas, from the UK veterinarians responses, most of them preferred behavioural indicators rather than physiological indicators to assess cat pain. It is now accepted that pain assessment in cats is not only based on physiological parameters but behavioural parameters (Epstein *et al.,* 2015), and in clinical settings, this can be challenging as cats may experience multiple negative emotions (e.g. stress, fear and anxiety) at once, because they find the clinical environment stressful (Väisänen, 2007) which later may affect the use of physiological parameters for assessing pain in cats. Therefore, a greater understanding of pain behaviours among veterinarians and Malaysian veterinarians, in particular, would be helpful in the assessment of pain in cats. The increased confidence of Malaysian veterinarians in their methods of assessing pain may, in fact, be based on a misunderstanding of the relative importance of physiological and behavioural measurements of pain.

As presented, pain is multidimensional experiences that involve not only physical sensation but also psychological experiences. Findings showed that most UK veterinarians agreed that physical (e.g.: posture) and psychological (e.g.: comfortable rest and mental changes) were useful indicators for assessing pain in cats. But, fewer Malaysian veterinarians chose psychological as the useful signs of cat pain. This could compromise the cat patients' welfare. To improve, veterinarians are required to understand feline normal behaviours. However, a study conducted by Kogan and colleagues reported that veterinarians had insufficient feline behavioural knowledge and this could be due to a few reasons (Kogan *et al.,* 2020). For example, a shortage of professionals and training in this area caused veterinarians to have less confidence to assess and manage cat behavioural health issues (Juarbe-Díaz, 2008; Calder *et al.,* 2017; Kogan *et al.,* 2020). At the time of writing, there are no formal feline behavioural health courses in the Malaysian veterinary curriculum. There are specialist courses on feline (and canine) medicine, but the extent to which behaviour is taught or even mentioned in not clear. As an understanding of behavioural health would improve all aspects of cat welfare, it is recommended. In a personal communication (U. Kaka, personal communication, December 21, 2021), the awareness and teaching about the use of pain scoring tools (e.g.: The Glasgow Feline Composite Measure Pain Scale (CMPS-Feline) and Feline Grimace scale) have been introduced into the curriculum for the years 4 and 5 at DVM since February 2020. However, there is no information about the efficacy of the use of pain scoring tools among students, so future studies could investigate this- i.e. in terms of their understanding to use these tools and in particular, apply in practice

Urinary Tract Infection (UTI) and chronic Degenerative Joint Disease (DJD) were listed as more severely painful for cats by Malaysian veterinarians than by UK veterinarians. This can be related to the types of case presentation in clinical practice in Malaysia and the UK. Most of the cases received by the UK veterinarians could be early-stage cases and so could still be manageable. In the Malaysian cases, a number of them are presented in the late stage by clients (Lyn *et al.,* 2012). Lyn *et al.* (2012) in a retrospective study, reported that the common clinical disease in cats in University of Veterinary Hospital Malaysia was kidney disease (33%) which included Urinary Tract Infection (UTI). The highest frequency of the disease occurred in geriatric cats. As the cost of treating this disease is prohibitive, owners of these cats usually only ought treatment when the cats were very unwell (Lyn *et al.,* 2012). However, veterinarians from both countries took the view that these cats require analgesia, which is a positive movement in better providing good welfare and management of pain in cats.

Many cat gonadectomy patients are discharged without receiving sufficient analgesic interventions (Farnworth *et al.,* 2014). Although the provision of analgesia on discharge was not directly questioned in this study, participants were questioned in both countries about the duration of analgesia prescribed for different types of surgical procedures in cats, within a set of specified time periods. Most participants prescribed analgesia for 24 hours or less post-ovariohysterectomy. According to WSAVA Global Pain Council's Pain Management Protocol for castration and ovariohysterectomy, post-surgery treatment of these cases may require analgesia for up

to three days after surgery (Mathews *et al.,* 2014). One experimental study looking at the requirement of analgesia for OVH and castration in cats and dogs proved that the number of female patients receiving rescue analgesia was greater than the number of males patients receiving it (Quarterone *et al.,* 2017). In the experiment, the animals received meloxicam (0.1 mg/kg body weight (BW), orally) and acepromazine (0.05 mg/kg BW, intramuscular (IM)) 1-2 hour prior surgery (Quarterone *et al.,* 2017). Then fentanyl (2 µg/kg BW, IV) was given as intraoperative analgesia (Quarterone *et al.,* 2017). Rescue analgesia is the administration of extra analgesia post-operative surgery based on the results of pain assessment. This difference in males and females receiving rescue analgesia could be due to the type of surgical procedures influenced by the degree of surgical trauma. For example, for castration, involves the removal of testicles which are external to the body. OVH involves the exploration internally, the excision of a number of muscles and tissues hence the pain endured by the female cats may be more than male cats. In the study, pain scoring tool such as the UNESP-Botucatu Multidimensional Composite Pain Scale (UBMCPS) for cats was used to evaluate the pain experienced by cat patients.

In this study, we asked about what influences the respondents in their choice of Opioid and NSAIDs in pain management in cats. Malaysian veterinarians were more concerned about the cost, side effects and record-keeping requirements of Opioid and NSAIDs for use in cats. But, for UK veterinarians, they were more concerned about the efficacy of both drugs as anti-inflammatory and as an analgesic. There are similarities between the attitudes expressed by Malaysian veterinarians in this study and those described by Brazilian (Lorena *et al.,* 2014) and South African veterinarians (Joubert, 2001). For example, the side effects of NSAID on the renal function. However, the side effect was reported very rarely in healthy cat patient (Lascelless *et al.,* 2007) if they are used correctly (Gurney, 2012).

Malaysian veterinarians reported that they were usually the sole decision-makers for providing analgesia to cats; however, in the UK such decisions were made collaboratively, where veterinarians took contributions from other veterinarians, colleagues and importantly, veterinary nurses who have received formal training before their employment within the veterinary practice. This training equips veterinary nurses with skills and knowledge which is a valuable resource for veterinarians in their decision making about analgesia for cats. In Malaysia, veterinary nurses/AVO do not receive such formal training, which may account for the difference between decision making in the UK and Malaysia, as Malaysian veterinary nurses are not formally trained.

Overall, participants rated all listed resources in the questionnaire as quite useful for future continuing education. Clinical experiences were reported as being beneficial for facilitating continuing education in cat pain management by veterinarians in both countries. Similar findings were reported in previous studies in both the UK and New Zealand (Lascelles *et al.,* 1999; Williams *et al.,* 2005; Beswick *et al.,* 2016). Therefore, in order to develop an understanding of pain in cats, as well as the skill to recognize it, newly graduated veterinarians are encouraged to work with those veterinarians who already have expertise in this field of practice. However, the requisite length of experience for developing such expertise could not be specified as this could vary between individual practitioners.

Together with attendance at seminars and conferences, Malaysian veterinarians rated online tools as "useful" for obtaining information about cat pain management. On the basis of this finding, it is clear that the internet is an effective medium for providing educational interventions to Malaysian veterinarians.

CONCLUSION

In summary, the results from this present study revealed that UK and Malaysia veterinarians' attitudes towards cat pain do not significantly differ in many key aspects. Both countries also showed positive responses to the use of analgesia in cats.

There were some key differences suggesting that Malaysian veterinarians had less understanding of pain relief, fewer worries about the effects of analgesia, and a lack of understanding of the importance of cat behaviour for pain assessment than UK veterinarians.

Therefore, the most important result of this study is not the overall differences between countries, but the need for continued focus in both the UK and Malaysia on education and training of current and future veterinarians with regard to cat behaviour, the recognition and management of pain in cats and how this impacts cat welfare.

ACKNOWLEDGEMENTS

The research study is supported by the Ministry of Higher Education, Malaysia is associated with Universiti Putra Malaysia. The authors wish to thank Malaysian Veterinary Council, Malaysian Small Animal Veterinary Association and Royal College of Veterinary Surgeons for their valuable help in recruiting Malaysian and UK participants for the research study

CONFLICT OF INTEREST

None of the authors of this paper has any financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

REFERENCES

Beswick, A., Dewey, C., Johnson, R., Dowsett-Cooper, J. and Niel, L. (2016): Survey of Ontario veterinarians' knowledge and attitudes on pain in dogs and cats in 2012. The Canadian Veterinary Journal, 57(12), p.1274.

Bryer, J., Speerschneider, K. and Bryer, M.J. (2016): Package 'likert'. Analysis and Visualization Likert Items. CRAN. Available at: https://cran.r-project.org/web/packages/likert/likert.pdf.

Calder, C.D., Albright, J.D. and Koch, C. (2017): Evaluating graduating veterinary students' perception of preparedness in clinical veterinary behavior for "Day-1" of practice and the factors which influence that perception: A questionnaire-based survey. Journal of Veterinary Behavior, 20, pp.116-120.

Capner, A., Lascelles, B.D.X. and Waterman-Pearson, A.E. (1999): Current British veterinary attitudes to perioperative analgesia for dogs. Veterinary Record, 145(4), pp.95-99.

Dohoo, S.E. and Dohoo, I.R. (1996): Postoperative use of analgesics in dogs and cats by Canadian veterinarians. The Canadian Veterinary Journal, 37(9), p.546.

Epstein, M.E., Rodan, I., Griffenhagen, G., Kadrlik, J., Petty, M.C., Robertson, S.A. and Simpson, W. (2015): 2015 AAHA/AAFP pain management guidelines for dogs and cats. Journal of Feline Medicine and Surgery, 17(3), pp.251-272. doi: 10.1177/1098612X15572062.

Farnworth, M.J., Adams, N.J., Keown, A.J., Waran, N.K. and Stafford, K.J. (2014): Veterinary provision of analgesia for domestic cats (Felis catus) undergoing gonadectomy: a comparison of samples from New Zealand, Australia and the United Kingdom. New Zealand Veterinary Journal, 62(3), pp.117-122. doi: 10.1080/00480169.2013.852447.

Gurney, M.A. (2012): Pharmacological options for intra-operative and early postoperative analgesia: an update. Journal of Small Animal Practice, 53(7), pp.377-386.

Hadley W. (2017): tidyverse: Easily Install and Load the 'Tidyverse'. R package version 1.2.1. Available at: https://CRAN.R-project.org/package=tidyverse

Hewson, C.J., Dohoo, I.R. and Lemke, K.A. (2006):. Perioperative use of analgesics in dogs and cats by Canadian veterinarians in 2001. The Canadian Veterinary Journal, 47(4), p.352.

Hunt, J.R., Knowles, T.G., Lascelles, B.D.X. and Murrell, J.C. (2015): Prescription of perioperative analgesics by UK small animal veterinary surgeons in 2013. Veterinary Record. doi: 10.1136/vr.102834.

Joubert, K.E. (2001): The use of analgesic drugs by South African veterinarians: continuing education. Journal of the South African Veterinary Association, 72(1), pp.57-60.

Juarbe-Díaz, S.V. (2008). Behavioral medicine opportunities in North American colleges of veterinary medicine: a status report. Journal of Veterinary Behavior, 3(1), pp.4-11.

Kogan, L.R., Hellyer, P.W., Rishniw, M. and Schoenfeld-Tacher, R. (2020): Veterinary behavior: Assessment of veterinarians' training, experience, and comfort level with cases. Journal of veterinary medical education, 47(2), pp.158-169.

Kongara, K., Squance, H.E., Topham, I.A. and Bridges, J.P. (2016): Attitudes and perceptions of veterinary paraprofessionals in New Zealand to postoperative pain in dogs and cats. New Zealand Veterinary Journal, 64(2), pp.112-116. doi: 10.1080/00480169.2015.1111172.

Lascelles, B.D.X., Capner, C.A. and Waterman-Pearson, A.E. (1999): Current British veterinary attitudes to perioperative analgesia for cats and small mammals. Veterinary Record, 145(21), pp.601-604. doi: 10.1136/vr.145.21.601.

Lascelles, B.D.X., Court, M.H., Hardie, E.M. and Robertson, S.A. (2007): Nonsteroidal anti-inflammatory drugs in cats: a review. Veterinary anaesthesia and analgesia, 34(4), pp.228-250.

Lee, J.W., Jones, P.S., Mineyama, Y. and Zhang, X.E. (2002): Cultural differences in responses to a Likert scale. Research in nursing & health, 25(4), pp.295-306.

Lorena, S.E., Luna, S.P., Lascelles, B.D.X. and Corrente, J.E. (2014). Current attitudes regarding the use of perioperative analgesics in dogs and cats by Brazilian veterinarians. Veterinary anaesthesia and analgesia, 41(1), pp.82-89.

Lyn L. M., Abdullah R., Dhaliwal G. K. and Omar M. A. (2012): 'Disease prevalence and clinicopathological changes in senior and geriatric cats presented to university veterinary hospital, Universiti Putra Malaysia', Abdullah R, Omar MA, Mutalib ARA, Rajion MA, Bahaman AR, Aziz SA, editors. 7th Proceedings of the Seminar in Veterinary Sciences, Universiti Putra Malaysia, Malaysia, 27 February – 02 March.

Markus, H.R. and Kitayama, S. (1991): Culture and the self: Implications for cognition, emotion, and motivation. Psychological review, 98(2), p.224.

Mathews, K., Kronen, P.W., Lascelles, D., Nolan, A., Robertson, S., Steagall, P.V., Wright, B. and Yamashita, K., 2014. Guidelines for recognition, assessment and treatment of pain: WSAVA Global Pain Council members and co-authors of this document. Journal of Small Animal Practice, 55(6), pp.E10-E68.

Merola, I and Mills, D. S. (2016): 'Systematic review of the behavioural assessment of pain in cats', Journal of Feline Medicine and Surgery. School of Life Sciences, University of Lincoln, Lincoln, United Kingdom: SAGE Publications Ltd, 18(2), pp. 60–76. doi: 10.1177/1098612X15578725.

Molony, V. and Kent, J.E., 1997. Assessment of acute pain in farm animals using behavioral and physiological measurements. Journal of animal science, 75(1), pp.266-272.

Murrell, J., Psatha, E.P., Scott, E.M., Reid, J. and Hellebrekers, L.J. (2008): Application of a modified form of the Glasgow pain scale in a veterinary teaching centre in the Netherlands. Veterinary Record, 162(13), pp.403-408. doi: 10.1136/vr.162.13.403.

Nizam, Q.N.H. & Tahman S.A. (2019): Animal welfare in Asia: specific flaws and strengths, future trends and objectives [PDF file], In: Hild S. & Schweitzer L. (Eds), Animal Welfare: From Science to Law, pp.109-118.

Partridge, B. and Rossmeisl Jr, J.H. (2019): Companion animal models of neurological disease. Journal of neuroscience methods, p.108484. doi: 10.1016/j.jneumeth.2019.108484.

Phillips, C.J.C., Izmirli, S., Aldavood, S.J., Alonso, M., Choe, B.I., Hanlon, A., Handziska, A., Illmann, G., Keeling, L., Kennedy, M. and Lee, G.H. (2012): Students' attitudes to animal welfare and rights in Europe and Asia. Animal Welfare-The UFAW Journal, 21(1), p.87. doi: 10.7120/096272812799129466.

Quarterone, C., Luna, S.P.L., Crosignani, N., De Oliveira, F.A., Lopes, C., da Maia Lima, A.F. and de Araújo Aguiar, A.J., 2017. Ovariohysterectomy requires more post-operative analgesia than orchiectomy in dogs and cats. The Canadian Veterinary Journal, 58(11), p.1191.

R Core Team (2017): R: A language and environment for statistical computing. R Found. Stat. Comput. Vienna, Austria. URL http://www. R-project. org/., page R Foundation for Statistical Computing.

Raekallio, M., Heinonen, K.M., Kuussaari, J. and Vainio, O. (2003): Pain alleviation in animals: attitudes and practices of Finnish veterinarians. The Veterinary Journal, 165(2), pp.131-135.

Reed, K. and Upjohn, M.M. (2018): Better Lives for Dogs: incorporating Human Behaviour change into a theory of change to improve canine Welfare Worldwide. Frontiers in veterinary science, 5, p.93.

Reid, J., Scott, M., Nolan, A. and Wiseman-Orr, L. (2013): Pain assessment in animals. *In Practice,* 35(2), pp.51-56. doi: 10.1136/inp.f631.

Rutherford, K.M.D. (2002): Assessing pain in animals. Animal Welfare, 11(1), pp.31-53.

Steagall, P.V. and Monteiro, B.P., 2019. Acute pain in cats: Recent advances in clinical assessment. Journal of feline medicine and surgery, 21(1), pp.25-34.

Sugano, T. (2014): Evaluation of computer aided learning on pain recognition with an investigation of veterinary students' perception of pain in animals. Dissertation. University of Edinburgh.

Väisänen, M.M., Tuomikoski-Alin, S.K., Brodbelt, D.C. and Vainio, O.M. (2008): Opinions of Finnish small animal

owners about surgery and pain management in small animals. Journal of Small Animal Practice, 49(12), pp.626-632. doi: 10.1111/j.1748-5827.2008.00626.x.

Williams, V.M., Lascelles, B.D.X. and Robson, M.C. (2005): Current attitudes to, and use of, peri-operative analgesia in dogs and cats by veterinarians in New Zealand. New Zealand Veterinary Journal, 53(3), pp.193-202.

World Organisation for Animal Health. (2017). *OIE Global Animal Welfare Strategy*. World Organisation for Animal Health.

J. Vet. Malaysia (2022) 34 (1): 24 – 28

Journal Article

INHIBITORY EFFECTS OF TONGKAT ALI (*Eurycoma longifolia*) EXTRACT ON LIGHTING STRESS-INDUCED SLEEP DISORDERS IN PIGS

Y. KUROKI[1,2*], F. TSUDA[3], F. TAWARA[3] and S. TSUMAGARI[3]

[1]D-LAB, Japan Tobacco Inc., 4-1-1, Toranomon, Minato-ku, Tokyo 105-6927, Japan
[2]Delightex Pte. Ltd. 230 Victoria Street, #15-01 Bugis Junction Towers, 188024, Singapore
[3]Veqta Co., Ltd., Kitahama-muneta Bldg., 1-9-10, Kitahama, Chuo-ku, Osaka 541-0041, Japan

SUMMARY

Tongkat Ali (*Eurycoma longifolia*), traditionally used as an herbal medicine in Southeast Asia, is known to have a variety of effects, including stress reduction. The authors divided 5-month-old pigs (three males and three females) into four groups consisting of a control group, which was not administered Tongkat Ali extract (TAE), and three groups administered TAE at 0.25%, 0.50%, or 0.75% of their feed, respectively, and observed the pigs for a period of eight weeks under conditions of disturbed sleep due to prolonged illumination. In terms of observation items, the pigs' vitality, appetite, fecal characteristics, respiration, emaciation, anxiety, restlessness, body temperature, body weight, and salivary amylase were measured as clinical symptom scores, and the intestinal microbiota in their feces was analyzed using next-generation sequencing (NGS). Although members of the control group showed anxiety and restless behaviors; such stress behaviors were clearly inhibited in the groups administered with TAE. Groups of pigs administered with TAE at the ratios of 0.25% and 0.50% showed a decrease in salivary amylase concentration ($p<0.05$) whereas the results from the control and 0.75% group were not significant. Analyses of intestinal bacteria using NGS showed that *Lachnospiraceae*, a family of bacteria known for producing butyric acid, increased significantly in the group administered with TAE at 0.50%, whereas it was not changed in the other groups. Based on the above findings, administration of TAE to fattening pigs under high levels of lighting-induced stress is expected to be effective and administration of TAE at 0.25% of the feed intake in particular, has been shown to effectively inhibit behaviors associated with stress.

Keywords: pig, illumination, induced-sleep disorder, stress behavior, Tongkat Ali (Eurycoma longifolia) extract

INTRODUCTION

Tongkat Ali, an ingredient in herbal medicine, is a plant belonging to the family Simaroubaceae and known by the scientific name *Eurycoma longifolia*. It grows naturally in lowland forests in Malaysia, Indonesia, and other areas in Southeast Asia. The roots, stems, and leaves of Tongkat Ali are used locally as ingredients in traditional folk medicine. The most well-researched effects of Tongkat Ali extract (TAE) are its applications in treating sexual dysfunction and male infertility (Mohd Tanbi *et al.,* 2010). It has been reported that the sperm counts of rats administered with standardized methanol extract of Tongkat Ali in doses of 200 mg/kg increased by 99.2% in comparison with that of controls ($p<0.01$) (Chan *et al*., 2009). It is also reported that administering TAE to estrogen-treated rats for 14 consecutive days increased spermatogenesis and sperm count, and that there exist latent drug actions that reverse the effects of estrogen in male rats (Wahab *et al*., 2010).

In addition, when testing the anxiety conditions of mice, administering TAE was found to be as effective as the sedative diazepam, which was used as a control (Ang *et al.,* 1999). Administering TAE to people under moderate stress was found to decrease cortisol and to increase testosterone in saliva (Talbott *et al.,* 2013). It has been reported that sleep deprivation induces hypertension, cardiovascular disease, and diabetes in humans because it increases sympathetic nervous system activity. It has been reported that sleep deprivation and intestinal bacteria are closely related to the onset of stress and disease in living organisms (Spiegel *et al.,* 1999, Nagai *et al.,* 2010). Although sleep duration in advanced nations such as the Netherlands, the United Kingdom, and New Zealand exceeds 7.5 hours per day, it is the longer in comparison to Japan and South Korea, at less than 6.5 hours per day (Tozer, 2018). The length and quality of human sleep is a major issue in modern society, and people are eager to achieve deep sleep as soon as they go to bed. Sleep problems and dysbiosis of the gut microbiome can lead to metabolic disorders in human beings (Neroni *et al.,* 2021). Intermittent hypoxia-exposed mice showed a higher abundance of *Firmicutes* and a smaller abundance of *Bacteroidetes* and *Proteobacteria* phyla than controls (Moreno-Indias *et al.,* 2015).

Pigs were used as a model of obstructive sleep apnea-hypopnea syndrome for humans (Kim *et al.,* 2019, Liu *et al.*, 2019). Exposure to 20 hours of illumination per day at 300 lux increases body temperature and spontaneous movements (Takeishi *et al.,* 2018), which is thought to cause induced-sleep disorders. TAE, an herbal medicine, is reported to have anxiolytic effects on rats and humans, and it is expected to be effective in treating sleep disorders, but there are no reports of such effects. In this study, the authors created an induced-sleep-disorder model in which

*Corresponding author: Dr. Yutaka Kuroki; Phone No.+65 8556 4376;
Email: yutaka@delightexplorers.com;

Editorial history:
Paper received: December 2021
Accepted for publication: June 2022
Published: June 2022

commercial pigs were subjected to prolonged periods of high illumination and reported the results of a comparative study on the effects of TAE administration in terms of intestinal microbiota, stress marker (salivary amylase), and clinical symptoms, including stress behaviors.

MATERIALS AND METHODS

Test animals and test conditions

A total of 24 healthy, 5-month-old LWD crossbred pigs were used as test animals. The test period consisted of a one-week acclimatization period and an eight-week test period. The test pigs were fed standard feed for testing, namely SDS No. 4 (Feed One Co., Ltd., Yokohama, Japan), twice per day at 8:00 a.m. and 4:00 p.m., and the amount of feed was limited to 2% of their body weight. The pigs had free access to drinking water using a waterer. The TAE (Physta, Biotropics Malaysia Berhad, Malaysia), extracted with high pressure water (Sambandan et al., 2006), was blended into the standard pig feed for testing in ratios of 0 %, 0.25 %, 0.50 %, and 0.75 %, and fed to the pigs in accordance with their body weight, the mean values of which at the start were 73.8 kg, 71.5 kg, 71.9 kg and 72.7 kg, respectively. Six pigs (three castrated males and three females) were assigned to each group, and the animals were housed individually in an open pigsty (4 m^2).

This experimental test was conducted from September to December 2020, with mean temperatures ranging from 30°C to 15°C.

Induction of sleep-disorders in pigs

A 450W floodlight (Toa Tsusho Company Limited, Osaka, Japan) was used as lighting to induce sleep disorder. During the one-week acclimation period, all four groups were exposed to light at 300 lux for 12 hours a day (Light: 6:00 a.m. to 6:00 p.m.; Dark: 6:00 p.m. to 6:00 a.m.). Once the test period commenced, sleep disorder was induced in the pigs in all four groups by exposing them to light at 300 lux for 20 hours a day (Light: 6:00 a.m. to 2:00 a.m.; Dark: 2:00 a.m. to 6:00 a.m.) (Takeishi et al., 2018).

Observation items and sampling

Clinical symptom scores were recorded daily, with a score of 0 (normal) and scores of 1, 2, 3 as abnormal. Anxiety was defined as nervous behaviours, such as being startled when touched or making abnormal noises. Restlessness was defined as behaviours such as pacing around the barn for no reason. Rectal body temperature was measured at 4 pm-5 pm according to the feeding time every four days for a total of 15 times, and body weight was measured once a week for a total of nine times. Saliva, for use in measuring amylase concentration measurement, and faeces, for use in analysing intestinal microbiota, were collected in tubes containing guanidine hydrocholoride twice in total, at the beginning and end of the test period (Ribeiro *et al*., 2018). The saliva was collected by centrifuging it out of ropes given to the pigs for chewing and individually measured using a salivary amylase monitor (Nipro Corporation, Osaka, Japan). The fecal matter was collected in a fixative, and the intestinal microbiota was analysed using a next-generation sequencer (MiSeq, Illumina, Inc., San Diego, USA).

Statistical analysis

The sums of clinical scores from day 0 through day 56 (daily) were analysed using the Steel-Dwass test; body temperature using the Tukey-Kramer test; and the rate of decrease in salivary amylase concentration using Fisher's exact test. Bacteria showing characteristic time-series variations before and after usage of TAE were identified using the Wilcoxon test. In addition, this test was conducted at a testing facility in Fukuchiyama City, Kyoto Prefecture, and in accordance with the animal testing regulations (ET209017) set forth by Kyodoken Institute.

RESULTS

The pigs in the control group began exhibiting anxious and restless behaviors from around the 15th day after the commencement of testing, and these behaviors were found to last until the final day of testing (56 days after the commencement of administration of TAE). There was no difference in anxious and restless behaviors pertaining to gender difference. In contrast, in the 0.25% TAE group, anxious and restless behaviors were barely observed during the eight-week test period. In the 0.50% TAE group, similar to the 0.25% group, few abnormal clinical symptoms were observed, but a temporary decrease in appetite was observed due to the bitter taste of the herbal medicine. In the 0.75% TAE group, three out of six pigs showed a decrease in appetite due to the bitter taste of the herbal medicine, showing a decrease in appetite of two males and one female. One of these pigs was excluded from the test on the 26th day after the commencement of testing due to a loss of vitality resulting from an extreme loss of appetite. The average total anxiety and restlessness

Table 1. Comparisons of seven clinical scores by level of TAE administration in induced-sleep-disordered pigs

	Control	0.25%	0.50%	0.75%
Vitality	0.0	0.0	2.0	1.5
Appetite	0.0 [a]	0.0 [a]	7.5 [b]	28.5 [c]
Fecal characteristics	0.0	0.0	0.3	0.0
Respiration	1.0	0.8	0.8	1.3
Emaciation	0.0	0.0	0.0	0.0
Anxiety	11.0 [d]	0.0 [e]	0.0 [e]	0.2 [e]
Restlessness	12.3 [f]	0.0 [g]	0.2 [g]	0.2 [g]
Total	24.5	0.8	10.8	22.6

Note: A score of 0 is considered normal and scores of 1, 2, 3 are considered abnormal (worse). Each value is shown as the sum of clinical scores from day 0 through day 56. P values obtained by Steel-Dwass test. Symbols with different letters (a,b,c),(d,e),(f,g) indicate mutual significant differences at $p < 0.05$.

scores in the control group were significantly higher than those in the 0.25%, 0.50%, and 0.75% TAE groups ($p < 0.05$). In addition, the 0.75% TAE group showed significantly higher (worse) appetite scores than the control group, 0.25% TAE group, and 0.50% TAE group ($p < 0.05$) (Table 1).

Comparisons of rectal temperature trends showed no obvious difference between the groups during the experimental period, but on the 40th and 44th days, the 0.25% TAE group and 0.75% TAE group showed temporarily lower temperatures than other groups. The salivary amylase concentrations were compared before (day 0) and after (day 56) the commencement of the test (Figure 1).

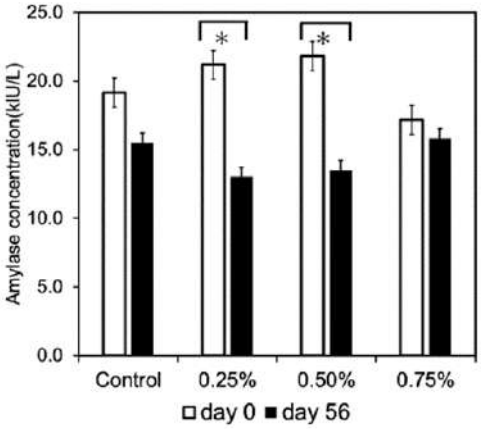


Figure 1. Comparisons of salivary amylase concentration on day 0 (open) and day 56 (solid) by level of TAE administration in induced-sleep-disordered pigs. Amylase concentration showed mean ± standard error. There was a significant difference in salivary amylase levels between day 0 and day 56 in the 0.25% TAE and 0.5%TAE groups, respectively (* $p < 0.05$).

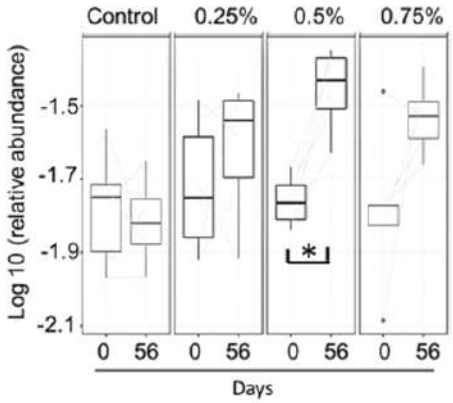


Figure 2. Comparisons of *Lachnospiraceae* in intestinal microbiota before (day 0) and after (day 56) by level of TAE administration in induced-sleep-disordered pigs There was a significant difference in amount of change of *Lachnospiraceae* between day 0 and day 56 in the 0.5 % TAE group (*$p < 0.05$), but not in the other groups.

There was a significant difference in salivary amylase levels between day 0 and day 56 in the 0.25% TAE and 0.5% TAE groups, respectively ($p<0.05$), but there was no difference in the levels between day 0 and day 56 in the control and 0.75% groups.

Analyses of changes in intestinal microbiota before (day 0) and after (day 56) administration of TAE showed significant differences in *Lachnospiraceae* family bacteria between the control group and the 0.50% TAE group on day 56 ($p<0.05$) (Figure 2).

DISCUSSION

In this test, anxious and restless behaviors were strongly inhibited in the groups administered TAE at ratios of 0.25 % and 0.50 % of their feed in comparison to the control group, and this is thought to be due to inhibitory effects on the sympathetic nervous system. In addition, salivary amylase levels decreased in the 0.25 % and 0.50 % TAE groups, which is thought to be due to a decrease in catecholamine. Salivary amylase is regulated by the sympathetic-adrenal medullary system and direct nerve action, and it increases with unpleasant stimuli. Thus, the rate of decrease in salivary amylase was compared between each group because a decrease in salivary amylase is an indicator of stress reduction (Fuentes *et al*., 2011, Xiao-jun *et al.*, 2016).

Pigs are considered to suffer no physiological problems under a minimum of eight hours of illumination at 40 lux. However, exposure to 20 hours of illumination per day at 300 lux increases body temperature and spontaneous movements (Neroni *et al*., 2021), which is thought to cause induced-sleep disorders. As significant anxious and restless behaviors in control group were observed under the prolonged and continuous lighting conditions of this test, it is highly probable that the induced-sleep disorders were due to sympathetic hyperactivity with shortened sleep. The authors observed decreases in body temperature of 1°C or more in mice administered TAE compared to non-administered mice (Kuroki *et al*., 2021). In addition, when a preliminary test was conducted from January to March, when mean temperatures were low (<10°C), body temperatures increased in the control group but decreased roughly one month after administration of TAE, and a significant difference of up to 1.5°C was observed between the administered and non-administered groups (unpublished). However, in this study, comparisons of rectal temperature trends showed no obvious difference between the groups during experimental period. The reason for the difference in the degree of decreases in body temperature between this test and the preliminary test is thought to be due to the weather temperatures during the test periods. In other words, inhibition effects on body temperature in the pigs given TAE was clearly found in winter, but such effects by TAE would be contradicted superficially in summer.

Stress in pigs includes social stress, such as cramped housing conditions, and environmental stress, such as heat, cold, sleep disorders, etc. It has been reported that stress caused by cramped housing conditions accompanying the increasing scale and consolidation of pig farming operations adversely affects pigs' ability to gain weight, which could result in economic losses on farms (Brumm,

1996, Johnston *et al*., 2017). Pigs are particularly susceptible to heat stress due to an absence of sweat glands, and reductions in milk production by sows during the hot season reduces piglet growth (Guo *et al*., 2018). There are reports that housing fattening pigs under high temperatures, such as 30°C, reduces feed intake and increases haptoglobin levels, which indicates inflammation, in comparison to fattening pigs housed under moderate temperatures, such as 23°C (Serviento *et al.,* 2020). The TAE used in this test has the potential to reduce heat stress in sows.

On the other hand, there was no influence on vitality and emaciation in the pigs during the experimental period, despite the decreased appetite in the 0.75% TAE group and the increased anxiety and restlessness in the control group. In addition, respiration rates and fecal characteristics in the pigs used in this study also showed no significant change. Therefore, stress levels caused by 20 hrs-lighting at 300 lux might be enough to maintain wakefulness in pigs.

A significant increase on day 56 in *Lachnospiraceae*, a family of bacteria known for producing butyric acid (Biddle *et al.,* 2013), was observed in the 0.50% TAE group. The *Lachnospiraceae* family has been reported to activate regulatory T cells (Portune *et al.,* 2017) and form tight junctions in intestinal mucosal cells (Braniste *et al.,* 2014) *Lachnospiraceae* bacterium 28-4, and *Lachnospiraceae phytofermentans* were enriched in pigs with low residual feed intake and high feed efficiency (Jiang *et al.,* 2021). These effects are due to butyric acid produced by the butyric acid-producing bacteria, and the increase in butyric acid-producing bacteria is expected to improve immunity and prevent harmful bacteria from invading the body.

TAE contains a bitter quassinoid as an active ingredient, and a significant decrease in appetite was observed in the 0.75% TAE group, and some individuals in the 0.50% TAE group also showed a decrease in appetite. As decreases in appetite among pigs affects their ability to gain weight, it is thought that the 0.75% and 0.50% doses of TAE were excessive. On the other hand, when administering 0.25% doses of TAE, no decrease in appetite was observed, and anxiety and restlessness were sufficiently inhibited. Therefore, the optimal TAE dosage for administration to pigs is thought to be 0.25% of the volume of feed.

In conclusion, administration of TAE to fattening pigs under high levels of stress is expected to be effective, as administration of TAE at 0.25% of the feed intake has been shown to effectively inhibit stress behaviors.

ACKNOWLEDGEMENTS

We would like to thank Mr. Nobuo Nakanishi of Kyotodoubutsukensa Institute for his cooperation and advice in conducting this test.

CONFLICT OF INTEREST

The authors declare no conflicts of interest associated with this manuscript.

REFERENCES

Ang, H.H. and Cheang, H.S. (1999). Studies on the anxiolytic activity of *Eurycoma longifolia* Jack roots in mice. Japanese Journal of Pharmacology. 79: 497–500

Biddle, A., Stewart, L., Blanchard, J. and Leschine, S. (2013). Untangling the genetic basis of fibrolytic specialization by *Lachnospiraceae* and *Ruminococcaceae* in diverse gut communities. Diversity. 5: 627–640.

Braniste, V., Al-Asmakh, M., Kowal, C., Anuar, F., Abbaspour, A., Tóth, M., Korecka, A., Bakocevic, N., Guan, N.L. and Kundu, P. (2014). The gut microbiota influences blood-brain barrier permeability in mice. Science Translational Medicine 6: 263

Brumm, M.C. (1996). Effect of space allowance on barrow performance to 136 kilograms body weight: NCR-89 Committee on Management of Swine. Journal of Animal Science. 74: 745-749.

Chan, K.L., Low, B.S., The, C.H. and Das, P.K. (2009). The effect of *Eurycoma longifolia* on sperm quality of male rats. Natural Product Communications. 4: 1331–1336.

Fuentes, M., Tecles, F., Gutiérrez, A., Otal, J., Martínez-Subiela, S. and Cerón J.J. (2011). Validation of an automated method for salivary alpha-amylase measurements in pigs (*Sus scrofa domesticus*) and its application as a stress biomarker. Journal of Veterinary Diagnostic Investigation. 23: 282-287

Guo, Z., Lv, L., Liu, D. and Fu, B. (2018). Effects of heat stress on piglet production/performance parameter. Tropical Animal Health and Production. 50: 1203−1208.

Jiang, H., Fang , S., Yang, H. and Chen, C. (2021). Identification of the relationship between the gut microbiome and feed efficiency in a commercial pig cohort. Journal of Animal Science. 99:3

Johnston, L.J., Rozeboom, D.W., Goodband, R.D., Moeller,S.J., Shannon, M.C. and Schieck, S.J. (2017). Effect of floor space allowances on growth performance of finishing pigs marketed at 138 kilograms. Journal of Animal Science. 95: 4917–4925.

Kim, L.J., Freire, C., Curado, T.F., Jun, J.C. and Polotsky, V.Y. (2019). The role of animal models in developing pharmacotherapy for obstructive sleep apnea, Journal of Clinical Medicine. 8: 2049-2063.

Kuroki, Y. and Miyazaki, K. (2021). Core body temperature reducing agent, food products, and sleep improving agent. Japan Patent No. 6,516,259,B2. Japan Patent and Trademark Office.

Liu, Y., Gao, L., Lv, W., Lin, L., Yi Wang, Y., Fan Jiang, F. and Feng, F. (2019). Pathologic and hemodynamic changes of common carotid artery in obstructive sleep apnea hypopnea syndrome in a porcine model. Turkish Journal of Medical Sciences. 49: 939-944.

Mohd Tanbi, M.I.B. and Kamarul Imran, M. (2010). *Eurycoma longifolia Jack* in managing idiopathic male infertility. Asian Journal of Andrology. 12: 376-380.

Moreno-Indias, I., Marta Torres, M., Montserrat, J.M., Sanchez-Alcoholado, L., Cardona,F., Tinahones, F.J., Gozal, D., Poroyko, V.A., Navajas, D., Queipo-Ortuño, M.I. and Farré, R. (2015). Intermittent hypoxia alters gut microbiota diversity in a mouse model of sleep apnoea, European Respiratory Journal. 45: 1055-1065

Nagai, M., Hoshide, S. and Kario, K. (2010). Sleep duration as a risk factor for cardiovascular disease—a review of the recent literature. Current Cardiology Reviews. 6: 54– 61.

Neroni, B., Evangelisti, M., Radocchia, G., Nardo, G.D., Pantanella, F., Villa, M.P. and Schippa, S. (2021): Relationship between sleep disorders and gut dysbiosis: what affects what?. Sleep Medicine. 87: 1-7

Portune, K.J., Benítez-Páez, A., Del Pulgar, E.M.G., Cerrudo, V. and Sanz, Y. (2017). Gut microbiota, diet, and obesity-related disorders—The good, the bad, and the future challenges. Molecular Nutrition and Food Research. 61:1

Ribeiro, R.M., Souza-Basqueira, M., Oliveira , L.C., Salles , F.C., Pereira , N.B. and Sabino, E.C. (2018). An alternative storage method for characterization of the intestinal microbiota through next generation sequencing. Revista do Institute Medicina Tropical de Sao Paulo. 60:e77

Tozer, J. (2018). Which countries get the most sleep? 1 843 Magazine. https://www.economist.com

Sambandan, T.G., Rha, C., Kadir, A.A., Aminudim, N. and Saad, J.M., (2006). U.S. Patent No. 7,132,117 B2. Washington, DC: U.S. Patent and Trademark Office.

Serviento, A.M., Labussière, E., Castex, M. and Renaudeau, D. (2020). Effect of heat stress and feeding management on growth performance and physiological responses of finishing pigs. Journal of Animal Science. 98: 12.

Spiegel, K., Leproult, R. and Van Cauter, E. (1999). Impact of sleep debt on metabolic and endocrine function. Lancet. 354(9188): 1435–1439.

Takeishi, K., Kawaguchi, H., Akioka, K., Noguchi, M., Arimura, E., Abe, M., Ushikai, M., Okita, S., Tanimoto, A. and Horiuchi, M. (2018). Effects of dietary and lighting conditions on diurnal locomotor activity and body temperature in microminipigs. In Vivo. 32: 55-62.

Talbott, S.M., Talbott, J.A., George, A. and Pugh, M. (2013). Effects of Tongkat ali on stress hormones and psychological mood state in moderately stressed subjects. Journal of the International Society of Sports Nutrition 10: 28

Wahab, N.A., Mokhtar, N.M., Halim, W.N.H.A. and Das, S. (2010). The effect of *Eurycoma longifolia Jack* on spermatogenesis in estrogen-treated rats. Clinics (Sao Paulo). 65: 93–98.

Xiao-jun , Z.. Zhan-bin, W., Wei-guang , B., Qian-kun, G., Xi-qing, W., Xian-hong, G., HAO Yue , H., Yan-jun, C. (2016). Effect of different modes of farrowing pens on reproductive performance and stress level of sows. Acta Veterinaria et Zootechnica Sinica 47: 2027-2036.

Short communication

PRELIMINARY STUDY ON THE SKIN THICKNESS OF ADULT MALAYAN TAPIR

Y. DONNY

Ex-situ Conservation Division, Department of Wildlife and National Parks (DWNP), Peninsular Malaysia, KM 10 Jalan Cheras, 56100 Kuala Lumpur, Malaysia

SUMMARY

The thickness of skin from nine selected parts of the body of 22 (9 males:13 females) Malayan tapirs (*Tapirus indicus)* was measured and recorded. A 5cm x 5 cm skin samples from the dorsal & lateral neck, lateral facial, medial and lateral foreleg and hind leg, dorso-lateral part of the hindquarter skin (pelvis area) and dorsal back (above last thoracic vertebrae) was obtained and measured using a digital calliper at the precision of ±0.02 mm. The skin at the dorsal neck is the thickest (21.36 mm) followed by the lateral neck (11.13 mm) and lateral hindleg (10.80 mm), while the lateral facial skin (6.05 mm) is the thinnest followed by the medial hindleg (6.13 mm) and medial foreleg (6.85 mm). The comparison of skin thickness between females and males was statistically not significant. The information gathered from this study may be important for the management of tapir in captivity.

Keywords: Malayan tapir, Tapirus indicus, skin thickness

INTRODUCTION

Tapiridae is a family of four species which include the Malayan tapir (*Tapirus indicus*), Brazilian tapir (*Tapirus terrestris*), Baird's tapir (*Tapirus bairdii*) and Mountain tapir (*Tapirus pinchaque*). Among the four species, the Malayan tapir is the largest and distinguished by its black and white body coloration. The adult has a body weight ranging from 295-430 kg (Quse and Fernandes-Santos, 2014). The females are often larger than the males by 25 kg to 100 kg (Gearty, 2012).

The Malayan tapir is the only tapir species native to Southeast Asia, and they are distributed from the southern part of Myanmar and Thailand, Peninsular Malaysia and the Island of Sumatera, Indonesia (Lynam *et al*., 2008). The Malayan tapir habitat comprises of swamp peat area to the lowland forest (Brooks, 1997). However, they also reported being seen at an elevated area at 2400 meters above sea level (Holden *et al*., 2003). The type of habitat where the Malayan tapir lives require a 'tough' body and strong skin to go through the thick and dense forest. The tapirs have thick skin (Pollock and Ramsey, 2003; Lilia *et al*., 2010), the skin around the neck is between 20-30 mm thick and very hard (Meijaard and Strien, 2003), and this allows them to withstand the thorny and dense bushes. However, at the point of this article written, there are no published reports specifically on the skin thickness of Malayan tapirs. Therefore, this preliminary study was conducted to document the thickness of different parts of the skin of adult Malayan tapir. The information gained from this study could benefit the management of the species in captivity.

*Corresponding author: Dr. Donny Yawah (Y. Donny); Email: donny.yawah@gmail.com

Editorial history:
Paper received: October 2021
Accepted for publication: Feb 2022
Published: June 2022

MATERIALS AND METHODS

Twenty-two (n=22) dead adult Malayan tapir were involved in this study. All tapirs were roadkill's; died due to traumatic injury due to collision with the vehicle. The skin samples from the dead Malayan tapir were collected within 24 hours after death. A 5 cm X 5 cm skin sample was obtained from different parts of the body as illustrated in Figure 1; dorsal and lateral neck, lateral facial, medial and lateral foreleg and hind leg (just above the hook and knee joint), dorso-lateral part of the hindquarter skin (pelvis area) and dorsal back (above last thoracic vertebrae). The skin sample was incised using a sharp scalpel blade. The subcutaneous or muscle tissues attached to the skin were removed before measurement. The skin samples were either directly measured or chilled (5^{o}C) until measured. A digital calliper (Absolute™, Mitutoyo Corp., Japan) with a precision of ±0.02 mm was used to measure the thickness of each skin sample. All collected data were documented in Microsoft Excel, and the comparison of skin thickness between female and male were analysed using IBM™ SPSS (Version 26).

RESULTS AND DISCUSSION

The overall average skin thickness of different parts of the body of 22 adult Malayan tapirs was shown in Table 1. The skin at the dorsal neck was the thickest (21.36 mm) followed by the lateral neck (11.13 mm) and lateral hindleg (10.80 mm). The skin structure at these parts was denser and less flexible. Meanwhile, the thinnest part of the tapir skin was at the lateral facial skin (6.05 mm) followed by the medial hindleg (6.13 mm) and medial foreleg (6.85 mm). The skin at this part was softer and more flexible. The Mann Whitney U test revealed no significant difference in skin thickness between the female and male Malayan tapir ($P>0.05$).

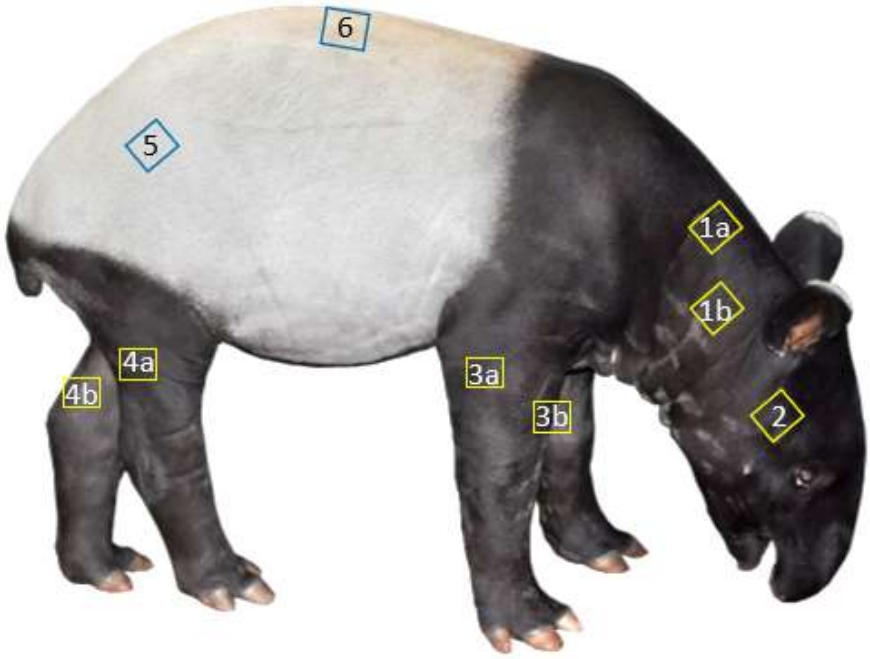


Figure 1. Skin sampling at dorsal neck (1a), lateral neck (1b), lateral facial (2), lateral foreleg (3a), medial foreleg (3b), lateral hindleg (4a), medial hind leg (4b), dorso-lateral part of the hindquarter (pelvis) (5) and dorsal back (6).

In general, skin is the outer tissue covering the body of a vertebrate animal that is made up of three layers, the epidermis, dermis and hypodermis with three main functions: protection, regulation, and sensation (Yousef *et al*., 2017). In Malayan tapir, except for the toes (hoof) and eyes, the skin covers the whole body. The skin of the dorsal and lateral parts of the body is thick and hard. Meanwhile, the skin at the inguinal area, medial part of both limbs and ventral parts of the abdomen and thoracic area are thinner, more elastic and softer. Although the female Malayan tapir is heavier than the male, there are no significant differences in skin thickness between male and female.

In this study, the overall average skin thickness of the different parts of the body of Malayan tapirs is 10.12 mm. This is indeed considerably thicker than the skin of horses, 1.2 mm to 7 mm (Wakuri *et al*., 1995). In the wild habitat, the thick skin protects when the Malayan tapir moves through the dense undergrowth (Lekagul & McNeely, 1988). Meanwhile, Sanborn and Watkins (1950), presumed that the thick skin of the dorsal and lateral part of the neck is for protection from predator bites. Although no published report of wild Malayan tapirs being prey of predators, the Malayan tapirs could still become the victim of predators such as tigers and panthers. This study confirmed that the skin at the dorsal part of the neck is the thickest as the average thickness is 21.36 mm. Thus, it may give added protection as it is presumably the skin is not easily torn by the predator bite. However, the thick skin may impede detection of early lesions under the skin, such as accumulation of pus or other body fluid, because changes may not be observable at the affected area during a physical examination and delay treatment.

The neck region is the best area to deliver drugs intramuscularly for sedation. Therefore, at least an inch 18G needle is needed to deliver the drugs intramuscularly. Although intramuscular administration could also be done at the gluteus muscle, the tapir is sensitive when medication is administrated at this region may be due to pain when pierced with a needle. Meanwhile, due to the thickness and stiffness of the skin, subcutaneous fluid or medication could not be given through most of the body parts. The best area to give subcutaneous fluid or medication is at the ventral area of the body especially at the skin flap between the ventral abdomen and limbs but with a limited amount. In addition, the findings indicated the most suitable site for microchip implantation under the skin, which is behind the ears, as the skin at this area is not too thick and has less risk of microchips migration. The information on the skin is also important for the veterinarian especially during a surgical procedure as the thickness information may help the surgeon to decide on the suture type and size. Furthermore, diagnostic test such as tuberculin skin test can be decided to be conducted at the most appropriate part of the body.

The information gathered from this study could improve the health and husbandry management of this species in captivity. However, this is only primary work; therefore, further study involving more samples should be done. It is also recommended to add histological analysis of skin from different body parts in future study.

Table 1. The skin thickness from nine parts of the female and male Malayan tapir's body

Female

Tapir No.	Skin measurement (mm)								
	Dorsal neck	Lateral Neck	Lateral Facial	Lateral Foreleg	Medial foreleg	Lateral hindleg	Medial hindleg	Pelvis	Dorsal back
1	17.00	8.00	6.50	NT	6.00	7.00	10.50	6.00	9.00
2	28.56	14.55	7.41	NT	6.49	NT	5.09	8.30	11.55
3	25.71	10.80	7.50	NT	6.40	NT	5.51	8.58	9.25
4	20.50	NT	3.74	8.30	5.61	8.66	6.11	6.40	NT
5	22.30	NT	7.20	10.39	6.18	10.55	5.54	8.26	NT
6	25.02	13.34	NT	NT	NT	13.06	NT	11.14	10.48
7	21.40	9.40	5.70	11.00	8.70	11.40	4.00	7.70	10.40
8	23.77	10.17	5.38	NT	4.86	12.00	4.53	8.14	NT
9	20.57	9.18	6.60	NT	6.44	10.83	5.67	9.23	NT
10	19.90	10.35	5.86	7.06	6.74	7.39	4.90	9.81	14.20
11	22.14	NT	7.90	10.80	6.32	10.90	NT	7.14	10.71
12	19.00	9.60	5.60	10.30	9.00	10.00	7.30	7.60	9.00
13	22.00	14.30	7.30	11.67	6.67	12.00	6.30	10.30	9.60
No of cases	13	10	12	7	12	11	11	13	9
Average	**22.14**	**10.97**	**6.39**	**9.93**	**6.62**	**10.34**	**5.95**	**8.35**	**10.47**

Male

No.	Skin measurement (mm)								
	Dorsal neck	Lateral Neck	Lateral Facial	Lateral Foreleg	Medial foreleg	Lateral hindleg	Medial hindleg	Pelvis	Dorsal back
14	24.84	11.87	4.41	NT	9.50	NT	7.43	7.35	10.27
15	20.62	8.15	7.06	NT	7.67	NT	9.11	7.62	8.44
16	NT	11.70	6.04	NT	6.12	11.66	5.52	NT	11.20
17	14.90	11.60	6.40	11.70	6.50	10.20	7.60	8.20	8.40
18	22.33	9.51	4.84	10.52	8.25	10.86	7.67	8.64	8.82
19	19.87	10.59	5.62	10.08	NT	10.48	3.44	6.69	NT
20	22.14	12.98	5.79	8.23	4.32	NT	4.12	11.75	NT
21	20.60	12.30	5.00	11.00	5.30	12.00	3.60	11.00	10.60
22	19.33	13.00	6.30	10.60	9.00	12.33	8.33	11.00	8.33
No of case	8	9	9	6	8	6	9	8	7
Average	**20.58**	**11.30**	**5.72**	**10.36**	**7.08**	**11.26**	**6.31**	**9.03**	**9.44**

Overall average (Male & female)	**21.36**	**11.13**	**6.05**	**10.14**	**6.85**	**10.80**	**6.13**	**8.69**	**9.95**

Note: NT = not taken

ACKNOWLEDGEMENTS

I would like to thank all the wildlife veterinarians and staff from the Department of Wildlife and National Parks, especially Dr. Zubaidah binti Kamarudin, Dr. Norsharina binti Arshad, Dr Nurfadnida binti Jaafar and Jawahir binti Jaafar for their help in collecting the skin samples. Special thanks to the Director-General of the Department of Wildlife and National Parks for allowing me to conduct this study.

CONFLICT OF INTEREST

No conflict of interest to declare.

REFERENCES

Gearty, W. (2012). *Tapirus indicus (On-Line)*, Animal Diversity Web. Retrieved October 8, 2021 from http://animaldiversity.org/accounts/Tapirus_indicus/

Holden, J., Yanuar, A., & Martyr, D. (2003). The Asian Tapir in Kerinci Seblat National Park, Sumatra: Evidence collected through photo-trapping. Oryx. 37(1): 34-40

Brooks, Daniel M.; Bodmer, Richard E.; Matola, Sharon (compilers). (1997). Tapirs - Status Survey and Conservation Action Plan. (English, Spanish, Portuguese.) IUCN/SSC Tapir Specialist Group. IUCN, Gland, Switzerland and Cambridge, UK. viii + 164 pp.

Lekagul, B. & McNeely, J. (1988). Mammals of Thailand. Second Edition. Thailand: Darnsutha Press.

Lilia, K., Rosnina, Y., Abd Wahid, H., Zahari, Z. Z., and Abraham, M. (2010). Gross anatomy and ultrasonographic images of the reproductive system of the Malayan Tapir (Tapirus indicus). Anatomia, histologia, embryologia. 39: 569-575.

Meijaard, E., and Strien, N. V. (2003). The Asian Tapir (Tapirus indicus). In Briefing Book, Malay Tapir Conservation Workshop. Krau Wildlife Reserve, Malaysia.

Pollock, C. G. and Ramsay, E. C. (2003). Serial immobilization of a Brazilian tapir (Tapirus terrestris) with oral detomidine and oral carfentanil. Journal of Zoo and Wildlife Medicine, 34: 408-410.

Quse, V. and Fernandes-Santos, R.C. (2014). Tapir Veterinary Manual (2nd Edition). IUCN/SSC Tapir Specialist Group (TSG). 155p. https://tapirs.org/wp-content/uploads/2017/03/Tapir-Veterinary-Manual-2014.pdf

Sanborn, C. C. and Watkins, A. R. (1950). Notes on the Malay Tapir and other game animals in Siam. Journal of Mammalogy. 31: 430-433.

Wakuri, H., Mutoh, K., Ichikawa, H., and Liu, B. (1995). Microscopic anatomy of the equine skin with special reference to the dermis. Okajimas folia anatomica Japonica. 72: 177-183.

Yousef, H., Alhajj, M., and Sharma, S. (2017). Anatomy, skin (integument), epidermis. StatPearls Publishing. https://www.ncbi.nlm.nih.gov/books/NBK470464/.

Short Communication

IN VITRO VIRAL INHIBITORY EFFECT OF CAT LITTER ON FELINE CORONAVIRUS INFECTION

N.J.S. ABU-SOPIAN, S.S. ARSHAD and F. MUSTAFFA-KAMAL*

Faculty of Veterinary Medicine, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

SUMMARY

Feline enteric coronavirus (FECV) generally causes asymptomatic infection to mild diarrhoea in multi-cat household, but sporadically may develop into the highly fatal feline infectious peritonitis (FIP). The route of transmission of FECV is via faecal-oral route through sharing of litter boxes. The aim of this study was to investigate the viral inhibitory effect of commercial cat litters available in Malaysia against Feline Coronavirus (FCoV) *in vitro*. A total of six types of cat litters were chosen based on their availability in 10 pet shops surveyed within Klang Valley, Malaysia. To determine the viral inhibitory effect of the cat litters such as wood pellet, kenaf, bentonite, silica, tofu or non-clumping clay, these cat litters were mixed with FIPV 79-1146 strain, incubated for 2 hours at 37°C with 5% CO2 and the supernatants were filtered and inoculated into 24-well plates of Crandell Rees feline kidney (CRFK) cells. Cytopathic effects (CPE) were observed after 48 hours. Median tissue culture infectious dose (TCID50) was used to determine the viral titre using Spearman-Karber method. Statistical analysis using Kruskal-Wallis test comparing the different cat litters revealed a significant viral inhibitory effect against FCoV of which significant results were observed among wood pellet, kenaf and bentonite versus FCoV-untreated controls. However, only wood pellet managed to completely inhibit virus infection in cell culture, while the other five cat litters reduced virus load to varying extent from 2-fold to 6-fold reduction. In conclusion, wood pellet, kenaf and bentonite successfully inhibited FIP virus growth in CRFK cells presumably by adsorbing virus and binding the virions. Future in vivo study needs to be done to test the inhibitory effects of the cat litters in natural settings.

Keywords: cat litter, feline coronavirus, viral inhibitory effect

INTRODUCTION

Feline coronavirus (FCoV) is an enveloped single-stranded RNA virus in the *Coronaviridae* family. FCoV manifests in two biotypes; feline enteric coronavirus (FECV), which is ubiquitous in catteries and multi-cat households and the mutated form, feline infectious peritonitis virus (FIPV) (Pedersen, 2009). FECV generally causes asymptomatic infection to a mild gastroenteritis but mutation of FECV leads to a FIPV biotype that causes a highly fatal and systemic disease. FECV transmission occurs by faecal-oral route through the sharing of cat litter especially in multi-cat environment (Pedersen, 2009).
Currently, there is only one commercially available intranasal vaccine to aid in the prevention of FCoV for kittens of more than 16 weeks old; however, the efficacy of this vaccine is questionable (Pedersen, 2009). In addition, maternally derived FCoV antibodies in kittens decline after 60 days, which leaves a window for infection before vaccination can be performed. Therefore, preventing infection of FCoV at the primary level can avoid the potential occurrence of FIP. There was a recent evidence that Fuller's earth-based cat litters can reduce occurrence of infection or re-infection of FCoV, thus decreasing the chances of FIP to develop (Addie *et al*., 2020). However, this type of cat litter is not commonly found in pet stores in Malaysia.

*Corresponding author: Dr. Farina Mustaffa-Kamal;
Email: farina@upm.edu.my

Editorial history:
Paper received: June 2021
Accepted for publication: June 2022
Published: June 2022

In Malaysia, prevalence of FCoV is as high as 84% in a study conducted within two catteries (Sharif *et al.*, 2009). In another study, 95% of sampled cats were positive by RT-PCR with majority were type I isolates (Amer *et al*., 2012). Fuller's earth-based cat litter or aluminium magnesium silicate was shown able to inhibit FCoV infection in cell culture (Addie *et al*., 2020). However, composition of cat litters varies according to different regions depending on availability of raw materials. Whether the cat litters available in Malaysia would have the same viral inhibitory effect are still unknown.

Therefore, identifying cat litters that are available in Malaysia that have similar antiviral properties could aid in reducing transmission of FeCV. Hence, the aim of the study was to investigate the *in vitr*o viral inhibitory effect of commercially available cat litters following FCoV infection in Malaysia.

MATERIALS AND METHODS

Selection of Cat Litters

Ten pet shops in Klang Valley, Selangor were surveyed for the types of cat litters sold. Six different types of cat litters were chosen primarily according to their materials. Availability of each type of cat litters in the pet shops were also recorded to determine the common commercial cat litters found in Malaysia.

Cell Culture Preparation

Crandell-Reese feline kidney (CRFK) cells of passage 54 at more than 90% confluency were sub-cultured into T75 flasks (SPL Life Sciences, Korea). Cells were grown in minimum essential media (MEM) (Gibco, United States) supplemented with 15% of heat-inactivated fetal bovine serum (FBS) (Gibco, United States) and 1% of Penicillin-Streptomycin containing 10,000U Penicillin and 5mg Streptomycin (HiMedia, India) (Addie et al., 2020). After removing the used media, the cells were thoroughly rinsed twice with 10 mL Phosphate Buffer Saline (PBS, pH 7.2) to remove traces of serum that may inhibit the activity of trypsin. Next, 2 mL of 0.5% trypsin-EDTA (Gibco, United States) were added and incubated at 37ºC for 2 min to 3 min. The flask was removed from incubator and swirled gently to aid in detachment of the cells. Then, 2 mL of pre-warmed media as described above were added into the flask (Addie *et al*., 2020). Subsequently, the media containing detached cells in the flask were transferred to a 15 mL centrifuge tube (SPL Life Sciences, Korea) and centrifuged at 15,000 rpm for 5 min. Afterwards, supernatant was removed and 4 mL of growth media was added and resuspended using pipette (Eppendorf, Germany) to break the clumping of cells. The cells were then seeded into two T75 flasks at 2 mL with concentration of $2x10^6$ cells/mL per flask with additional 8 mL of growth medium and incubated at 37ºC with 5% CO_2 (Eppendorf, Germany) for 2-3 days up until confluency.

CRFK cells that reached more than 90% confluency were used to subculture into four 24-well plates (BioFil, India). Cell suspension with 4 mL volume were divided equally into two 50ml centrifuge tubes (Thermo Fisher Scientific, United States) and added with 22 mL of growth medium for subsequent resuspension. Finally, 500 uL of the cells were pipetted into each well of 24-well plate and incubated at 37°C with 5% CO_2.

Virus Propagation

CRFK cells in T25 flask at 80-90% confluency was rinsed with 5 mL PBS twice. The cells were then inoculated with 0.1 mL of FIPV 79-1146 strain and incubated for 1 hour. After incubation, 5 mL of growth medium was added and flask was incubated at 37°C with 5% CO_2 (Thermo Fisher Scientific, United States) until cytopathic effect (CPE) was observed. Next, the flask was subjected to freeze-thaw cycle for three times to break the cells and for allowing the virus to be released into the supernatant. Then, the content was pipetted into a 15 mL centrifuge tube. The tube was centrifuged at 3000 rpm for 5 min and the virus supernatant was kept in -20°C until further used. The FIPV 79-1146 virus propagation in CRFK cells was repeated several times until the virus stock was adequate for the subsequent analyses.

Median Tissue Culture Infectious Dose

Median tissue culture infectious dose (TCID50) method was used for virus titer quantification. CRFK cells were grown into 24-well plate at $0.05x10^6$ cells per well and incubated at 37°C with 5% CO_2 for 2 days and a series of 10-fold dilution of FIPV 79-1146 were inoculated after the CRFK cells reached 90% confluency. The cells were monitored daily for CPE. $TCID_{50}$/mL value was calculated using Spearman-Karber method (Spearman, 1908; Karber, 1931)

Sample Processing and Residual Virus Titration

A total of 4 mL of FIPV 79-1146 strain at 3.75×10^7 $TCID_{50}$/mL were mixed with 1 gram (g) of cat litter sample in a 15 mL centrifuge tube and rotated (Stuart Scientific, United Kingdom) at room temperature for 2 hrs. Then, the tube was spun at 3000 rpm for 10 min (Kubota, Japan) and supernatant was transferred into 1.5 mL centrifuge tube (Eppendorf, Germany). Next, the tube was spun at 14,000 rpm for 5 min and the virus supernatant was filtered through a 0.45 µM filter (Sartorius Stedim, France) (Addie *et al*., 2020). A total of 100 uL virus supernatant was subjected to a 10-fold serial dilution and the dilution series was made in triplicates. Then, TCID50 method of viral quantification was performed as described earlier to determine the viral titre upon treatment with the cat litters. The plates were incubated at 37°C with 5% CO_2 and CPE was examined and recorded for 48 hours.

As a positive control, 1 mL of FIPV at 3.75×10^7 $TCID_{50}$/mL without cat litter was added into 15 mL centrifuge tube and treated as above. As for negative control, 4 mL of MEM without FIPV was mixed with 1 g cat litter and treated as above to check for possible cytotoxic effects of the litter towards the cells. Cells that remained healthy at the end of observation period were deemed to be free from cytotoxic effect that may be induced by the cat litters.

Statistical Analysis

Statistical analysis with non-parametric Kruskal-Wallis test with post-hoc analysis of Dunn's multiple comparisons were carried out using GraphPad Prism (Version 8.4.3, USA) to compare the effect of viral inhibition among the different cat litters with p-value <0.05 considered as significant.

RESULTS

Identification of Common Commercial Cat Litters

A survey of 10 pet stores in Klang Valley, Selangor revealed six common types of cat litters which were wood pellet, kenaf, bentonite, tofu, silica, and non-clumping clay. Silica was the most common cat litter where it was found in nine out of ten stores, followed by bentonite and wood pellet at eight out of ten stores (Figure 1). The least common cat litter was non-clumping clay whereby only two stores carried that litter type.

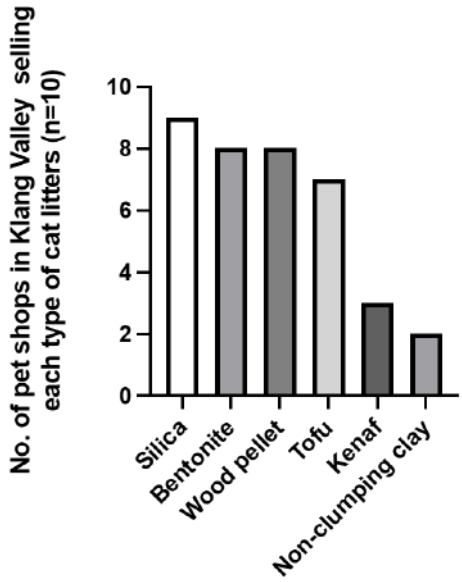


Figure 1. Number of pet shops (n=10) carrying a particular litter type in descending order. *In Vitro*

Effect of Cat Litter Against FCoV

The effects of cat litter on FCoV infectivity were calculated and summarized in Table 1. None of the six cat litters cause cytotoxicity to CRFK cells. Infected cells were shown to have CPE characterized by cell ballooning, cell clumping and detachment from wells.

All six cat litters reduced virus titre up to a varying extent. Only wood pellet litter managed to fully inhibit FCoV infection significantly in cell culture compared to control (Table 1). Non-clumping clay and tofu litter were able to reduce virus titre by 2-fold compared to control. Silica and bentonite reduced FCoV load by 4-fold and 5-fold, respectively. Subsequently, kenaf almost fully inhibited FCoV infectivity demonstrated by 6-fold reduction of viral titre. There were significant inhibition of viral activity among the six cat litters tested ($p= 0.0077$, $p<0.05$), with wood pellet, kenaf and bentonite showed significant result when compared to virus control (Figure 2). However, comparison between non-clumping clay, tofu and silica litters showed no significant result on virus titre when compared to controls ($p>0.05$).

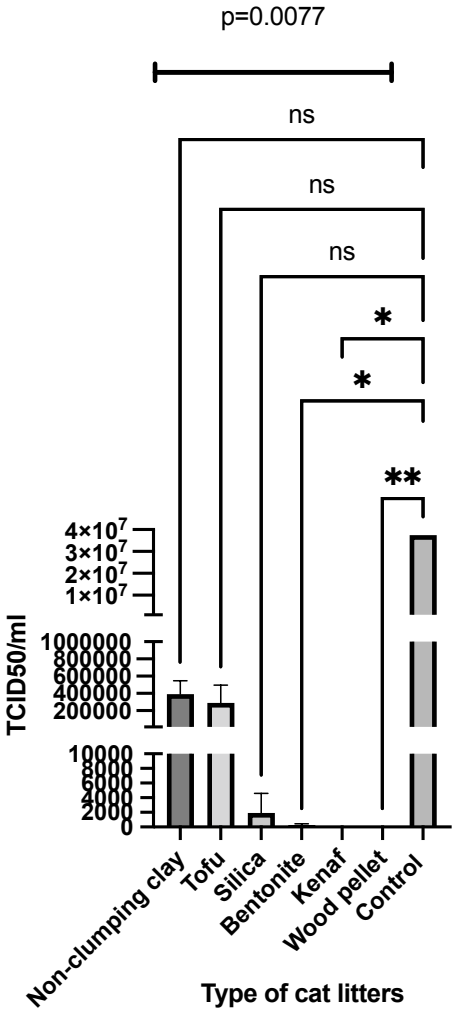


Figure 2. Feline coronavirus (FCoV) titer (TCID50) in six cat litters (n=6) compared to control (uninfected). Post-hoc analysis of Kruskal-Wallis test (p=0.007) comparing the mean of each litter with control of which * indicates p<0.05, ** indicates p<0.001.

Table 1. Reduction of FCoV titre for cell culture infection in decreasing effect

Cat Litters	Cytotoxicity	$TCID_{50}$/mL Before	$TCID_{50}$/mL After	Fold Reduction
Wood pellet	No	5×10^7	0	Complete reduction
Kenaf	No	5×10^7	0.9 x 10	6 – fold
Bentonite	No	5×10^7	1.7×10^2	5 – fold
Silica	No	5×10^7	1.9×10^3	4 – fold
Tofu	No	5×10^7	2.9×10^5	2 – fold
Non-clumping clay	No	5×10^7	3.9×10^5	2 – fold

DISCUSSION

A variety of cat litters made from different types of materials can be found in the market today to meet pets' and owners' demands. Each type of litter comes with its own advantages and disadvantages in terms of cost, dustiness, tracking ability, clumping ability and ease of disposal (Adelson and Pardilla, 2021). For example, cats appeared to have preferences over clumping litters such as tofu and bentonites compared to unclumping litters such as crystal or wood pellet; however, in general, tofu litter is expensive compared to crystal or wood pellet litters (Nielson, 2001; Adelson and Pardilla, 2021).

Based on the results from this *in vitro* study, all six cat litters have viral inhibitory properties with a varying extent. Three cat litters were identified to be potentially used as litters that have viral inhibitory effects based on the significant result of the viral titers which were the wood pellet, kenaf and bentonite. However, only wood pellet was able to completely reduce viral titer to zero. This is the ideal situation because even with low viral load or infectious dose, there is possibility of successful viral replication within individual cats especially those with no protective immunity (Pedersen, 2009). The exact mechanism of wood pellet inhibitory effect on viral infection in cell culture is not presently known. However, a study by Lupini *et al.* (2009), showed that chestnut and quebracho wood extracts have *in vitro* antiviral activity against avian reovirus (ARV) and avian metapneumovirus (AMPV). The antiviral activity was presumed to be due to an interaction of tannins extracted from the plant and viral proteins that leads to inhibition of viral attachment and penetration of cell membrane. Tannins are a class of polyphenolic compounds that can bind to and precipitate proteins from a solution (Combs, 2016). The wood pellet litter used in the study was made from pine wood (Pinus spp.) where condensed tannins can be found (Filgueira *et al.,* 2017; Li and Maplesden, 1998). Further research needs to be conducted to determine whether the tannins are the active compounds responsible for viral inhibitory effect of wood pellet on FCoV. On the other hand, kenaf (Hibiscus cannabinus) was almost able to completely reduce viral titre load (6-fold reduction). As previously stated, the mechanism of this plant against FCoV infection in cell culture is unknown, although there was evidence of phytotoxic and fungitoxic properties of kenaf essential oil (Kobaisy *et al*., 2001).

Bentonite, the third cat litter with significant viral titre reduction by 5-fold, presumably reduced cell culture infection through good adsorbent property. A study by Clark *et al*. has proven an excellent capability (>90% adsorption) of sodium bentonite to adsorb bovine coronavirus (Clark *et al*., 1998). According to the study, the virions have larger diameters than the pores of the adsorbent substance particles causing the virions to be attached to the external surface of the particles. In other words, the material can only bind the virus together but infectivity of the virus remains. Addie *et al*. (2020) also stated that Fuller's earth-based cat litters which has similar properties to bentonite, possibly prevented cell culture infection by binding the virus rather than actually killing it.

Another observation made throughout the study is the high liquid absorption property of wood pellet and kenaf. Absorption refers to the phenomenon where molecules of a substance enter the bulk or volume of the absorbent, in contrast with adsorption which is a process of adhesion of molecules onto the surface of solid particle. There are two hypotheses derived from this where the first one being wood pellet and kenaf presumably have the ability to absorb fluids from the virus leading the virus particles to dry up and become inactivated. The other theory is the cat litters absorb the liquid in the supernatant, leading to ease of adsorption of virion particles onto the litter material, and forms a tight cat litter-to-virion complex and sediments in the centrifuge tube. This allows for less free-flowing virions in the supernatant, where viral titre reduction is observed after inoculation.

In summary, wood pellet, kenaf and bentonite have its own chemical or physical properties that may contribute to the inhibition of virus in cell culture.

CONCLUSION

Overall, all six cat litters (wood pellet, kenaf, bentonite, silica, tofu, non-clumping clay) were able to reduce FCoV titer in CRFK cell culture. Only wood pellet cat litter was able to completely prevent virus infection in cell culture but it is not as cost-effective. Although kenaf and bentonite cat litters also have significant viral titer reduction, virions were still present whereby even a single virus particle could still infect a cat.

ACKNOWLEDGEMENTS

The authors wish to acknowledge all the staff of Veterinary Virology Laboratory, Faculty of Veterinary Medicine, Universiti Putra Malaysia for their help in completing this research project.

CONFLICT OF INTEREST

None of the authors of this paper has financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

REFERENCES

Addie, D., Houe, L., Maitland, K., Passantino, G., & Decaro, N. (2020). Effect of cat litters on feline coronavirus infection of cell culture and cats. *Journal of Feline Medicine and Surgery*, *22*: 350–357.

Adelson, K.I., & Pardilla, A. (2021, Dec). The best cat litter: According to Vets. The Strategist. https://nymag.com/strategist/article/best-cat-litter.html#multiple

Amer, A., Siti Suri, A., Abdul Rahman, O. (2012). Isolation and molecular characterization of type I and type II feline coronavirus in Malaysia. *Journal of Virology.* 9:278.

Clark, K. J., Sarr, A. B., Grant, P. G., Phillips, T. D., & Woode, G. N. (1998). In vitro studies on the use of clay, clay minerals and charcoal to adsorb bovine rotavirus and bovine coronavirus. *Veterinary Microbiology*, *63*(2–4), 137–146.

Combs, C.A. (2016). Biochemistry Research Trends: tannins: biochemistry. Food Sources and Nutritional Properties. Nova Science Publishers, Inc., New York.

Filgueira, D., Moldes, D., Fuentealba, C., & García, D. E. (2017). Condensed tannins from pine bark: A novel wood surface modifier assisted by laccase. *Industrial Crops and Products*, *103*, 185–194.

Kärber, G. (1931). Beitrag zur kollektiven Behandlung pharmakologischer. *Beitrag Zur Kollektiven Behandlung Pharmakologischer Reihenversuche.*, *7*, 1–4.

Kobaisy, M., Tellez, M. R., Webber, C. L., Dayan, F. E., Schrader, K. K., & Wedge, D. E. (2001). Phytotoxic and fungitoxic activities of the essential oil of kenaf (Hibiscus cannabinus L.) Leaves and its composition. *Journal of Agricultural and Food Chemistry*, *49*(8), 3768–3771.

Li, J., & Maplesden, F. (1998). Commercial production of tannins from radiata pine bark for wood adhesives. *Transactions of the Institution of Professional Engineers New Zealand. Electrical, Mechanical, and Chemical Engineering Section*, *25*(1), 46–51.

Lupini, C., Cecchinato, M., Scagliarini, A., Graziani, R., & Catelli, E. (2009). In vitro antiviral activity of chestnut and quebracho woods extracts against avian reovirus and metapneumovirus. *Research in Veterinary Science*, *87*(3), 482–487.

Neilson, J.C., 2001. Pearl vs. Clumping: Litter Preference in a Population of Shelter Cats. American Veterinary Society of Animal Behavior, Boston, pp. 14.

Pedersen, N.C. (2009). A review of feline infectious peritonitis virus infection: 1963-2008. *Journal of Feline Medicine and Surgery*, *11*(4), 225-58

Sharif, S., Arshad, S. S., Hair-Bejo, M., Omar, A. R., Zeenathul, N. A., & Hafidz, M. A. (2009). Prevalence of feline coronavirus in two cat populations in Malaysia. *Journal of Feline Medicine and Surgery*, *11*(12), 1031–1034.

Spearman, C. (1908). The method of 'right and wrong cases' ('constant stimuli') without Gauss's formulae. *British Journal of Psychology, 1904-1920*, *2*(3), 227–242

Please read the guidelines and follow these instruction carefully as by doing so will ensure that the publication of your manuscript is as rapid and as efficient as possible. The Editorial Board reserves the right to return the manuscripts that are not prepared in accordance with these guidelines Your co-operation is greatly appreciated!

INSTRUCTIONS TO AUTHORS

Jurnal Veterinar Malaysia is a peer-reviewed journal that publishes original research work, reviews, case reports, short communications and letters to the editor from any veterinary related fields. The copyright of papers accepted for publication is that of the Veterinary Association Malaysia and the journal is published twice a year. *Jurnal Veterinar Malaysia* is published in English.

Submission of manuscripts

Submission of manuscripts to the *Jurnal Veterinar Malaysia* is via submission system accessible at www.mavma.org.my under manuscript submission tab. An acknowledgment of receiving submission of manuscripts will be provided. Authors must submit articles in **WORD** format (saves as .doc or .docx) and not as PDF files. PDF proofs will be automatically generated from uploaded files and these are used for subsequent reviewing. Queries concerning the submission process or journal procedures should be sent by e-mail to journal@mavma.org.my.

The Corresponding Author, who is normally the Author submitting the paper, will be asked to confirm that the article is original and is not being considered for peer-reviewed publication elsewhere. Submission also implies that all of the Authors have approved the paper for release and are in agreement with its content. The Corresponding Author will also be required to confirm that all Authors have made substantial contributions to (1) the conception and design of the study or acquisition of data or analysis and interpretation of data, (2) drafting the article or revising it critically for important intellectual content, and (3) final approval of the version to be submitted. Contributors who do not meet these criteria for authorship should be listed in an Acknowledgements section. Upon acceptance of the article by *Jurnal Veterinar Malaysia*, the Author(s) will be asked to transfer the copyright of the article to the Publisher. This transfer will ensure the widest possible dissemination of information.

Plagiarism

Plagiarism is globally recognised as a serious academic offence. The Corresponding Author will be asked to tick a box to confirm acceptance of these guidelines before approval of the PDF file and completion of the submission process.

Animal welfare

Where animals have been used in a study, the institutional ethical or animal welfare Authority under which the work was conducted must be stated, along with the specific authorisation reference number and the date of approval (if applicable). The *Jurnal Veterinar Malaysia* will reject any paper where there is reason to believe that animals have been subjected to unnecessary or avoidable pain or distress.

Conflict of interest

At the end of the text, under a subheading "Conflict of interest" statement, all Authors must disclose any financial and personal relationships with other people or organisations that could influence (bias) their work. Examples of potential conflicts of interest include employment, consultancies, stock ownership, honoraria, paid expert testimony, patent applications or registrations. Conflicts of interest may also exist where a commercial company donates (or provides funding for a study that uses) drugs, equipment, test kits, vaccines, reagents or other products manufactured or marketed by that company. If no conflicts of interest exist, this should be stated as "None of the authors of this paper has any financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper".

Role of the funding source

All sources of funding should be declared in an Acknowledgements section at the end of the text. Authors should declare the role of study sponsors, if any, in the study design, in the collection, analysis and interpretation of data, in the writing of the manuscript and in the decision to submit the manuscript for publication.

PREPARING THE MANUSCRIPT

Title page

A title page must be included at the start of the article. This should give the paper's title, names of Author(s), the name(s) and address(es) of the institution(s) where the work was done and other Authors' addresses where these differ. If the article is a Review or Short Communication, this should be clearly indicated at the left top on the title page. Insert a page break at the bottom of the title page before the Abstract.

Except where all Authors come from the same department, each Author should be identified using a number superscript ([1,2,3] etc.), and the Corresponding Author should be designated by an asterisk (*) as follows, example:

HEMATOLOGICAL PROFILES OF THE MALAYSIAN BEAR (*Helarctos Malayanus*) KEPT IN CAPTIVITY

C.A. Azlan[1*], A. Siti Suri[1], H. Latiffah[1], A.R. Bahaman[1], R. Mat Naim[2] and L. Kevin[3]

[1]Faculty of Veterinary Medicine, Universiti Putra Malaysia (UPM), UPM, Serdang, Selangor, Malaysia.
[2]Zoo Negara, Hulu Kelang, Ampang, Selangor, Malaysia.
[3]Zoo Taiping, Jalan Pekeliling, Taman Tasik Taiping, Taiping, Perak.

The contact details of the corresponding Author [telephone number, fax number (if available), and E-mail address] should then be given using the following format:
* Corresponding author: Dr Azlan Che Mat (C.A. Azlan);
Phone No : +603 123 4567; Fax No: +603 234 5678;
Email: c_azlan@putra.upm.edu.my

If it is desirable to indicate that more than one author contributed equally to the work, the numeric superscript ([a]) should be placed after the names. Then below the corresponding author details you should state:
[a] These authors contributed equally to the work. It is not usually acceptable for all authors to be acknowledged as equal contributors to a study.

Format and layout

Authors submitting papers that are suitable for consideration but do not comply fully with this guide will be asked to amend the text and re-submit. Articles must be written in the English language. UK English is preferred. Please use the spelling as printed by The Oxford English Dictionary. *Jurnal Veterinar Malaysia* will not edit submitted manuscripts for style or language; therefore Authors are advised to write clearly and simply, and to have their article checked by English proficient writers before submission.

- Use double-spacing except for Title page and References, which should be in single spacing. Time New Roman, font size 12 point, is preferred for all text (including Tables, Figures, References and all symbols). A smaller font size maybe used in Tables if space is limiting, and line spacing may also be adjusted in Tables where necessary. Formatting must not be customised since this impedes editing.
- Continuous line numbers are required throughout the text.
- Bacteria, viruses, genes, mutations, genotypes, and alleles should be indicated in italics as appropriate.

1. FULL PAPER (Original Articles)

Manuscripts of a full paper should be arranged as follows: (1) Title page; (2) Summary and up to five Keywords in alphabetical order to be supplied below the Summary; (3) the main text must be sub-divided into Introduction, Materials and methods, Results, Discussion, and Conclusions; (4) Conflict of interest statement; (5) Acknowledgements;

(6) References; (7) Tables; (8) Figure legends; (9) Figures (uploaded as separate files). The sections should not be numbered. The text should not exceed 5,000 words in length or about 6 pages of the Journal including tables, illustrations and references.

SUMMARY. The summary should not exceed 250 words (with no sub-headings), which should emphasise objectives/brief background, the experimental procedure, results and conclusions.

KEYWORDS. Please include 3 to 5 keywords representing the main content of the article.

INTRODUCTION. This should describe briefly the background and any previously published literatures related to the work. It should indicate the justification and objective(s) of the work being presented.

MATERIALS AND METHODS. An account of the animals or samples as well as the experimental design, methods and techniques used should be described. Detailed descriptions that have been cited in appropriate references should be avoided.

RESULTS. These should be concise and the data should be logically analysed and presented. Tables and figures illustrating the same data should be avoided.

DISCUSSION. This section evaluates and interprets the findings and should not repeat the results. It also compares the result of present study with those of earlier studies and should include the author's conclusion.

ACKNOWLEDGEMENTS. Only persons who have made substantial contributions to the study should be acknowledged.

REFERENCES. Only pertinent references should be cited. Authors are strongly advised to use reference management software such as EndNote. However, references should be checked carefully for accuracy and corrected manually to ensure the format matches exactly the *Jurnal Veterinar Malaysia* style described below. In the text, the name(s) of author(s) should be as follows: Lim and Yusof (1985) or (Lim and Yusof, 1985); Saroja (1985a); Saroja (1985b); papers with more than two authors should be referred to by the first author followed by *et al*. The list of references should be single spaced arranged in alphabetical order and further sorted chronologically. All authors' name should be included. Titles of journal should be abbreviated according to the ISI Journal Title Abbreviations. References to books and monograph should include the name(s) of author(s) or editor(s) followed by the date of publication in brackets, the title of the chapter or article, the full title of the book, the edition (if any), page referred to, the publisher and place of publication. The following are examples for guidance.

Book
Turner, H.N. and Yong, S.S.Y. (1990). Quantitative Genetics in Sheep Breeding. Cornell University Press, Ithaca.

Serial Article
Moore, L.J. and Rutter, J.M. (1989). Attachment of *Moraxella bovis* to calf corneal cells and inhibition by antiserum. Australian Veterinary Journal. 66: 39-42.

Paper in Edited Book
Roberts, S.J. (1986). Abortion and other gestational diseases in mares. In: Current Therapy in Theriogenology. Morrow, D. A. (Ed.) 2nd. ed., W.B. Saunders Co., Philadelphia. pp. 705-710.

Conference Proceedings
Seetha, D.Y., Dahlia, W. and Sivagurunathan, S. (1991). Acetylcysteine treatment in a cat with acetaminophen toxicosis. In Proceedings of the 7th Veterinary Association Malaysia Scientific Congress, Seremban, Veterinary Association Malaysia. pp. 83-84.

Unpublished Materials (e.g. thesis, reports, etc.)
Ali, A.H. (1980). Studies on the prevention of neonatal calf diarrhoea, MVM Thesis, Glasgow.

Please note:

- Insert a page break only after the Title page, after the Abstract with Keywords, after the References section, between each Table, and before the Legends to figures.
- The Results and Discussion sections must be distinct and not combined.
- Avoid sub-headings in the Discussion section.
- References must not be included within the Conclusions section.

2. CLINICAL PAPERS AND CASE REPORTS

These are either short or full papers that follow the overall arrangements described above. However, they may include the following:

i. Introduction: explaining why the case is being reported, with reference where necessary to previous reports.
ii. An account of the history and clinical examination of the case and laboratory workup sufficiently complete to enable a similar case to be recognised.
iii. The diagnosis and, where applicable, a differential diagnosis.
iv. An account of the treatment and the subsequent course of the condition.
v. An account of the findings at post mortem examination where appropriate.
vi. A discussion.

3. SHORT COMMUNICATIONS

Short communications are short papers and should follow the requirements for full manuscripts, but the text must not exceed 2,000 words or 3 printed pages of the Journal (including tables, figures and plates). The paper should not be divided into conventional sections. They describe either the results of complete experiments but are less comprehensive than full length articles or short clinical reports. Headings for the Summary, Keywords, Acknowledgements, Conflict of interest statement and References should be included, but there should be no other headings or subheadings in the main text. There should be no more than 10 references in a Short Communication. A Summary of not more than 125 words is required and up to five Keywords should be supplied below it.

4. REVIEW PAPERS

Review papers are accepted only if they conform to the following criteria:

i. The author or co-author(s) must have publication (in refereed journals) in the reviewed area.
ii. Provide new knowledge or a high-calibre synthesis or important knowledge.
iii. Critical review of a specific area of scientific research.
iv. Preferably pertinent to the Malaysian context.

Review papers may be commissioned or proposed. Authors wishing to submit a review article are advised to contact the Editor at journal@mavma.org.my. Reviews are about 4000 words in length and may cover any relevant aspect of veterinary science or comparative medicine. Reviews should follow the layout for Original Articles, but with the main text subdivided as appropriate to the subject matter, starting with a Summary and Introduction and incorporating Conclusions and a Conflict of interest statement. Sections should not be numbered.

5. LETTER TO EDITOR

The purpose of "Letters to Editor" are:

i. to report *preliminary accounts* of research findings for publication, and
ii. to discuss or expand on scientific points made in articles recently published in the Journal.

Letters are limited to ONE (1) printed page. Acceptability of letters will be decided by the Editorial Board.

Abbreviations and symbols
Only standard abbreviations and symbols should be used. Abbreviations in the title and at the beginning of a sentence should be avoided. The full term for which an abbreviation stands should precede its first use in the text, e.g. agar gel precipitation tests (AGPT). Standard units of measurement are exempted. Whenever possible, measurements should be in the International System of Units.

Manufacturers

Manufacturers and suppliers should be indicated within the text after the name of the product. For example: 'diazepam (Valium, Roche)' or 'using an infusion pump (Medfusion 2010, Medex)'. Addresses/locations of manufacturers is not necessary and the use of ® or ™ should be avoided. Note: proprietary names must not appear in the title or summary. Dosage of drugs should also be given, e.g. 6 x 106 i.u. procaine penicillin (Bipen; Gist-Brocades).

Tables

Tables should be few, simple and typed with double spacing on separate sheets. Each table should have a title (above the table) that summarizes the whole table, numbered and cited in sequence using Arabic numerals (i.e. Table 1, 2, 3 etc.), and place after the reference list. Complex tables are not acceptable and should be summarized rather than tabulated. Footnotes to tables should be indicated by [a, b] etc. and typed at the bottom of the relevant table. Information in tables should not be duplicated in figures and vice versa. The tables should be placed at the end of the main text after the References but before the Figure Legends, with one table per page.

Figure and illustrations

This should be provided after tables (if present). The Editors will reject Figures of an unacceptable standard or ask the Authors to replace them. Figure should be numbered in sequence (using Arabic numerals - i.e. Figure 1, 2, 3 etc), have short title of figure and figure legend. Do not write the legends on the figures. Scale bars must be provided on all photomicrographs and electron micrographs.

In preparing Figures, Authors should note the following:

- Use uniform lettering and sizing of the original artwork.
- Use only the following fonts in your figures: Times New Roman, Arial, Courier, Helvetica, Symbol.
- Number the figures according to their sequence in the text.
- Provide all figures as a separate files (Figure 1, Figure 2 etc).
- Do not included in the main manuscript.

Artwork formats

Regardless of the application used, when your electronic artwork is finalised, please 'save as' or convert the images to one of the following formats (Note the resolution requirements for line drawings, half-tones, and line/half-tone combinations given below):

EPS : Vector drawings. Embed the font or save the text as 'graphics'.
TIFF : Colour or greyscale photographs (half-tones): Always use a minimum of 300 dpi.
TIFF : Bitmapped line drawings: Use a minimum of 1,000 dpi.
TIFF : Combinations of bitmapped line/half-tone (colour or greyscale): A minimum of 500 dpi is required.

DOC, XLS or PPT: If the electronic artwork is created in any of these Microsoft Office applications, please supply 'as is'.

All Figures will be published in colour on-line, but colour will only be used in the hard copy of the *Jurnal Veterinar Malaysia* where it is considered to be essential to the presentation of the paper (colour reproduction charges may apply).

The Editorial Board reserves the right to reject any manuscript

Proofs

The corresponding Author will be advised by the Editor when the paper has been accepted for publication. One set of page proofs in PDF format will be sent by e-mail to the corresponding Author.

The *Jurnal Veterinar Malaysia* will do everything possible to publish an accepted article quickly and accurately. Therefore, it is important to ensure that all author corrections are sent back to in one communication; Authors should check proofs very carefully before replying, since inclusion of any subsequent corrections cannot be guaranteed. Proof reading is the responsibility of the authors, but final Editor's correction may also be incorporated at proof stage. Once the final corrections have been made, *Jurnal Veterinar Malaysia* will aim to publish the paper electronically within 6-8 weeks. The hard copy version containing the paper may follow later, normally within 12 months of acceptance.

Offprints

The Corresponding author will, at no cost, be provided with a PDF file of the article via e-mail. The PDF file is a watermarked version of the published article and includes a cover sheet with the journal cover image and a disclaimer outlining the terms and conditions of use.

SUBMIT TO JVM

BENEFITS:

1. ***No article processing fee for local corresponding author***
2. ***Open access dissemination of your article***
3. ***Peer-reviewed by an international board***
4. ***Indexed by MyJurnal***

http://jvm.opussoft.net

MALAYSIAN VETERINARY MEDICAL ASSOCIATION
JURNAL VETERINAR MALAYSIA


PERSATUAN PERUBATAN VETERINAR MALAYSIA
MALAYSIAN VETERINARY MEDICAL ASSOCIATION