UNIVERSITI PUTRA MALAYSIA

ASSESSMENT OF THE AGE, WEIGHT, AND INTEROBSERVER AGREEMENT ON PELVIC LIMB REFLEXES IN HEALTHY DOGS IN SELANGOR

CHUAH TIONG WAN

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A project paper submitted to the

Faculty of Veterinary Medicine, Universiti Putra Malaysia

In partial fulfillment of the requirement for the

DEGREE OF DOCTOR OF VETERINARY MEDICINE

Universiti Putra Malaysia

Serdang, Selangor Darul Ehsan.

December 2022

CERTIFICATION

It is hereby certified that we have read this project paper entitled "Assessment of the age, weight, and interobserver agreement on pelvic limb reflexes in healthy dogs in Selangor.", by Chuah Tiong Wan and in our opinion is satisfactory in terms of scope, quality and presentation as partial fulfillment of the requirement for the course VPD 4999- Final Year Project.

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DR. INTAN NUR FATIHA SHAFIE

DVM (UPM), PhD (GLASGOW)

Senior Lecturer

Faculty of Veterinary Medicine

Universiti Putra Malaysia

(Supervisor)

_____

ASSOCIATE PROFESSOR DR. LAU SENG FONG

DVM (UPM), PhD (NETHERLAND)

Associate Professor

Faculty of Veterinary Medicine

Universiti Putra Malaysia

(Co-Supervisor)

ACKNOWLEDGEMENT

Throughout this final year project, it was a challenging but rewarding journey for me. Along the journey, there are many people who lend me their hand to support me when I needed them. I would like to give my deepest appreciation to them. Without them, my final year project will not be completed.

First and foremost, I would like to express my heartfelt appreciation to my supervisor Dr Intan Nur Shafie. I am grateful that Dr Intan has accepted me and become my supervisor for my final year project despite her busy schedule. Her knowledge in veterinary neurology, patience,passione and motivation has guided me to complete my research. Her professional comments have given me a lot of inspiration. Besides my supervisor, I would like to express my gratitude to my Co-supervisor Associate professor Dr Lau Seng Fong, she has been very supportive throughout my research. Even though Dr Lau has a packed schedule, she always provided her help whenever we need her. I could not have imagined having a better supervisor and cp-supervisor for my research project.

Also, I am sincerely grateful to Nur Dinah Nur Hardy, my FYP mate. She always shares her point of view and data unreservedly to support my project. In addition, she will always be there to lend me her hand whenever I face obstacles in conducting my FYP.

Furthermore, a wholehearted thanks to Tan Ee Ling. She was not involved in the project, and she has her own final year project to be carried out. Nevertheless, she used

her limited time to assist me when my projects required manpower. I am tremendously thankful for her selfless act.

Finally, I am pleased for my friends and family for their unlimited support physically and spiritually throughout my studies.

List of Abbreviations

CP	Conscious proprioception
CLUTD	Canine lower urinary tract disease
EMG	Electromyography
FYP	Final Year Project
IACUC	Institutional animal care and use committee
k	Interobserver agreement
LMN	Lower motor neuron
NWR-T	Nociceptive withdrawal reflex threshold
TRT	Total reflex time
T_c	Gastrocnemius soleus H reflex latency
UMN	Upper motor neuron

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ABSTRAK

Abstrak daripada kertas kerja projek yang dikemukakan kepada Fakulti Perubatan Veterinar untuk memenuhi sebahagian daripada keseluruhan kursus VPD 4999-Projek Ilmiah Tahun Akhir.

PENILAIAN FAKTOR UMUR, BERAT BADAN DAN PERJANJIAN ANTARA PEMERHATI TERHADAP REFLEKSI TANGGUH PELVIK DALAM ANJING SIHAT DI SELANGOR

Oleh

Chuah Tiong Wan

2022

Penyelia: Dr Intan Nurfatiha Shafie

Penyelia Bersama:Associate professor Dr Lau Seng Fong

Pemeriksaan refleks anggota pelvis seperti patellar, sciatic, gastrocnemius, dan refleks penarikan digunakan untuk menentukan pengaruh umur dan berat pada refleks penggredan. Pada masa ini, masih boleh dipertikaikan sama ada umur dan berat badan mempengaruhi refleks dan refleks yang mana lebih dipercayai. Kajian ini bertujuan untuk mengenal pasti faktor-faktor yang mempengaruhi refleks untuk memberi pengamal gambaran yang lebih

jelas tentang pemeriksaan neurologi. Refleks 25 ekor anjing jantan dan 5 ekor anjing sihat betina berlainan baka dengan umur median 6 (1-14) tahun dan berat median 19.76 (3.3 - 41.23) kg telah diperiksa oleh dua pelajar di bawah pengawasan doktor haiwan berpengalaman. Peperiksaan itu dirakam video dan dinilai oleh empat pemerhati dengan tahap kepakaran yang berbeza. Refleks anjing dinilai berdasarkan kumpulan umur dan berat yang berbeza. Bagi kumpulan umur, anjing (n=30) dibahagikan kepada dua subkumpulan, iaitu <7 tahun (n=12) dan ≥7 tahun (n=18). Bagi kumpulan berat, kumpulan anjing yang sama telah ditugaskan kepada dua subkumpulan iaitu <15 kg (n=10) dan ≥15 kg (n=20). Kedua-dua kaki belakang dinilai dalam setiap kumpulan. Korelasi pangkat Spearman menunjukkan bahawa umur tidak mempunyai pengaruh ke atas refleks anggota pelvis manakala berat badan tidak mempunyai korelasi dengan refleks anggota pelvis kecuali refleks sciatic. Pekali Fleiss Kappa (k) telah dikira untuk persetujuan antara pemerhati pada empat refleks. Nilai k (0.422) adalah yang tertinggi (sederhana) untuk refleks pengeluaran. Refleks sciatic menunjukkan kedua tertinggi (adil) dengan nilai k (0.294) diikuti refleks patellar (0.146). Refleks gastrocnemius mempunyai perjanjian antara pemerhati terendah (lemah) dengan nilai k (-0.011)

Kata kunci: Refleks anggota pelvis; umur; Berat badan; Perjanjian antara pemerhati; Anjing yang sihat

ABSTRACT

An abstract of the project paper presented to Faculty of Veterinary Medicine in partial fulfilment of the course VPD 4999- Final Year Project.

ASSESSMENT OF THE AGE, WEIGHT, AND INTEROBSERVER AGREEMENT ON PELVIC LIMB REFLEXES IN HEALTHY DOGS IN SELANGOR

By

Chuah Tiong Wan

2022

Supervisor: Dr.Intan Nurfatiha Shafie

Co supervisor: Associate professor Dr Lau Seng Fong

Pelvic limb reflexes examinations such as patellar, sciatic, gastrocnemius, and withdrawal reflexes are used to determine the influence of age and weight on grading reflexes. Currently, it is still debatable whether age and weight influence reflexes and which reflexes are more reliable. This study aims to identify factors that influence reflexes to give practitioners a clearer picture of neurological examinations. The reflexes of 25 male and 5 female healthy dogs of distinct breeds with a median age of 6 (1-14) years and a median weight of 19.76 (3.3 -41.23) kg were examined by two students under the supervision of experienced vets. The examination was videotaped and evaluated by four observers with

different levels of expertise. The dogs' reflexes were evaluated based on different age and weight groups. For the age group, the dogs (n=30) were divided into two subgroups, which were < 7 years (n=12) and ≥7 years (n=18). For the weight group, the same group of dogs was assigned into two subgroups which were < 15 kg (n=10) and ≥15 kg (n=20). Both hindlimbs were assessed in each group. Spearman rank correlation shown that age has no influence on pelvic limb reflexes while weight has no correlation with pelvic limb reflexes but sciatic reflex. Fleiss Kappa coefficient (*k*)was calculated for interobserver agreement on four reflexes. *k* value (0.422) was the highest (moderate) for withdrawal reflexes. Sciatic reflex showed the second highest (fair) with a *k* value of (0.294) followed by patellar reflex (0.146). The gastrocnemius reflex had the lowest interobserver agreement (poor) with a *k* value of (-0.011)

Keywords: Pelvic limb reflexes; Age; Weight; Interobserver agreements; Healthy dog

1.0 Introduction

The neurological examination is often defined as a complex procedure distinct from the routine physical examination (Michael et al., 2011). The neurologic examination may be utilized to pinpoint a lesion's area and provides some information about the disease's severity (Snow, 2012). Fundamentals of neuroanatomy, neurophysiology, and the terminology used in clinical neurology are important in neurological examination and lesion localization.

The neurologic examination includes observations of mental status, posture and gait, musculoskeletal palpation, conscious proprioception (CP), and cranial nerve function (Snow, 2012). These are conducted during the hands-off and hands-on examinations. The hands-off examination is carried out before the hands-on examination. The hands-off examination included mental status, postural, and gait while the hands-on included musculoskeletal palpation, conscious proprioception, and cranial nerve function.

If there is any abnormality in posture and gait, spinal reflex evaluation should be conducted. The spinal reflex examination is done to determine if the neurological condition is of the upper motor neuron (UMN) or lower motor neuron (LMN) kind. As a result, the examiner can narrow down the lesion's location to spinal cord segments or peripheral nerves (Simon & Natasha, 2012).

According to Simon and Natasha (2012), LMN cell bodies are placed within the grey matter of the lumbosacral intumescence (L4-S3) of the pelvic limb and cervicothoracic intumescence (C6-T2) of the thoracic limb. Lesion at the lumbosacral

intumescence could lead to loss of lumbosacral segmented reflex and decreased muscle tone and mass. On the other hand, due to UMN release of the inhibitory modulatory effect on LMN, lesion cranial to lumbosacral intumescence will lead to normal to increase segmented spinal reflex.

According to Snow (2012), foreleg reflexes are less consistent and more difficult to elicit than hindleg reflexes. A few types of tests are used to evaluate the spinal response of the hindlimb. Snow (2012) stated that patellar reflex, gastrocnemius reflex, direct sciatic reflex, withdrawal reflex, and cranial tibial reflex could be used in pelvic limb spinal reflex examination.

This study aims to determine the outcome of age, weight, and interobserver agreement on pelvic limb reflexes in non-neurological dogs in Selangor. Information regarding factors affecting the hindlimb spinal reflex stated above is still limited. Successful identifying factors influencing the degree of spinal reflex can provide clinicians with a better understanding and higher accuracy of neurological examination.

2. 0 Literature review

2.1 Neurological examinations and spinal reflexes

Neurological examination is the single most important step to diagnose neurological disease and allocate the lesion location (Nick, 2001). The neurological examination is a crucial part of the overall diagnostic process and serves as the starting point for other diagnostic tests such as advanced imaging, cerebrospinal fluid analysis, and electrodiagnostic (Abdelhakiem *et al*., 2015). The result of the neurological examination is required to combine with patient signalment and history to provide a differential diagnosis (Laurent & Mark, 2013). Spinal nerve reflexes are part of the neurological evaluation other than cranial nerve examination, postural and gait, sensorium, postural reaction, and muscle tone (Alexander, 2020). Normal or exaggeration in spinal reflexes has been referred to as a UMN lesion while the decrease in spinal reflexes and acute muscle atopy have been referred to as an LMN lesion (Thomas, 2016). Joan (2012) stated that a lesion that disrupts the descending motor pathways from the supraspinal neurons that converge on the LMN pool is referred to as UMN weakness while a lesion of the ventral spinal cords' grey matter that affects the LMN's axon, which travels to the muscle through the spinal nerve roots and peripheral nerves, is known as LMN weakness. Lesions located at C1-C5 will lead to a UMN sign on the hindleg while lesions located at C6-T2 will lead to an LMN sign on the hindleg (Snow, 2012). There are two parts of the thoracolumbar region of the spinal cord: the cranial is T3-L3 which is the pelvic limb's UMN while the caudal part is L4-S3 which is the pelvic limb's LMN (Garosi, 2014). A systematic and logical manner is important and if failure to proceed in such a way will result in a chaotic and

incompetent investigation and may have detrimental consequences for both the patient and its owner (Rob, 2008).

2.2 Pelvic limb reflexes

2.21 Patellar reflexes

Patella reflexes, also known as quadriceps reflexes are induced by tapping the quadricep femoris muscles' tendon which is located distal to the patella (Etsuro, 2015). A normal reflex is an extension of the stifle joint (Cherly, 2006). The patellar reflex is the most reliable tendon reflex used in neurological examination (Garosi, 2004). According to Etsuro (2015), the patella reflex is a monosynaptic reflex that will involve sensory and motor neuron while tapping the patellar tendon and the extrafusal and intrafusal muscle stretches. Evans and de Lahunta (2013) stated that the patella is a sesamoid bone that articulates with the trochlea of the femur and is intercalated in the tendon of insertion of the quadriceps. The rectus femoris (cranial), vastus lateralis (lateral), vastus medialis (medial), and a fourth belly, the vastus intermedius, make up the quadriceps muscle (Evans & de Lahunta, 2013). According to Evans and de Lahunta (2017), rectus femoris originates from the ilium while vasti muscles originated from the proximal femur, all of them inserted into the tibial tuberosity. These muscles are innervated by the femoral nerve and are responsible for stifle joint extension and hip flexion (Evans & De Lahunta,2017). The femoral nerve appears from the most cranial portion of the lumbosacral plexus (L4-L5) and innervates the quadriceps muscle (Nicholas,1995). Depressed or loss of patellar reflex means that the lesions within the LMN's reflex arc whereas a normal or hyperreflexia patellar reflex refers to the lesions located at the cranial to the spinal cord segment that has

the UMN's reflex arc (Schatzberg *et al*., 2012). Injury in the femoral nerve may be unable to support a dog's weight due to the stifle joint not extending and sensation and knee-jerk response could be lost (Esturo, 2015).

2.22 Sciatic nerve reflexes

Cheryl (2006) explained that putting a finger in the sciatic notch and tapping it against the sciatic nerve. By hitting an index finger that has been positioned in the depression between the greater trochanter of the femur and the ischial tuberosity with the reflex hammer, the sciatic nerve is directly activated (Snow, 2012). The hip, stifle joint, and hock briefly extending suggests a proper sciatic nerve response (L6–S2). The sciatic nerve innervates the hamstring group which is responsible for stifle joint flexion and hip joint extension together with gluteal muscle which is supplied by gluteal nerves (Nicholas, 1995). The Hamstring group consists of the semitendinosus muscle, semimembranosus muscle, and biceps femoris muscle which they located on the caudal side of the femur (Evans & de Lahunta,2013). Evans and de Lahunta (2017) stated that the origin of the hamstring group is at the ischiatic tuberosity and inserted in different locations each. Biceps femoris is inserted in tuber calcanei; semitendinosus muscle is inserted in the tibia's distocranial margin; the semimembranosus is inserted in the medial condyle of the tibia, and the distal medial lip of the caudal rough surface of the femur. Sciatic and common peroneal nerve injuries may impact locomotion, but the animal can still support weight due to the femoral nerve's role in stabilizing the stifle joint (Etsuro, 2015).

2.23 Gastrocnemius reflex

The tibial branch of the sciatic nerve, which emerges from the L7-Sl spinal cord segments, innervates the gastrocnemius muscle (Oliver et al., 1997). By hitting the common calcanean tendon between the muscle belly and the insertion on the calcaneus, the gastrocnemius reflex is activated (Snow, 2012). A short extension of the hocks shows a healthy gastrocnemius muscle response (Cheryl, 2006). The muscles that extend the hock and flex the digits are supplied by the tibial nerve. Most animals' caudal surface of the limb and the plantar surface of the foot both receive cutaneous sensory innervation from it (Michael et al.,2011). Evans and de Lahunta (2013) stated that all those tissues adhering to the tuber calcanei of the calcaneus make up the common calcanean tendon, often known as the Achilles tendon (*tendo calcaneus communis*). Although the tendons of the mm. biceps femoris, semitendinosus, and gracilis also contribute to its creation, the tendons of the mm. flexor digitorum superficialis and gastrocnemius are its primary constituents. Dropped hock joint during walks or support weight, atrophied gastrocnemius muscle, and plantar aspect of foot loss of sensation are signs of pure tibial nerve injury (Michael et al.,2011).

2.24 Withdrawal reflex

The withdrawal reflex , also known as the flexor reflex is classified as a polysynaptic reflex that involves sensory, motor, and interneurons (Etsuro, 2015). The withdrawal reflex (L6-S1) in the hind leg primarily serves as a sciatic nerve test (Snow, 2012). The sensory and motor reflex pathways that mediate flexion of the limb in response to direct stimulation of toe pads are tested by the flexor reflex (Etsuro, 2015). Cheryl (2006) explained that a typical withdrawal reaction is shown when the toe is compressed with fingers or forceps and if the animal looks about, yells, or growls, pain is present. Michael *et al.* (2011) stated that the normal response is complete flexion of the hip, stifle, and hock, which is the entire limb. According to Joan *et al.* (2011), the superficial peroneal nerve on the dorsal surface and the tibial nerve on the plantar surface are the primary branches of the sciatic nerve which forms the sensory nerves from the digits of the pelvic limbs. Micheal *et al*. (2011) explained that flexor muscle contraction activated by the interneurons through sciatic motor neurons and relaxation of extensor muscles activated by inhibitory interneurons through extensor motor neurons allows complete flexion of the limb and withdrawal of the limb from a noxious stimulus such as pain. Also, Marc *et al.* (2011) stated that spinal cord lesions will lead to bilateral depression of reflex while peripheral nerve lesions will lead to unilateral absence of withdrawal reflex.

2.25 Cranial tibial reflex

The cranial tibial reflex is mediated by the spinal cord L6 to S2 which is the peroneal nerve and innervated the cranial tibial muscles. By tapping on the proximal part of the cranial tibial muscles, reflex flexion of the tarsus can be noticed. This flexion shows that cranial tibial reflex has been elicited. However, the cranial tibial reflex is unreliable and difficult to elicit compared to other pelvic reflexes such as patellar and withdrawal. Hyperextended hock and knuckled paw are known as signs of cranial tibial reflex dysfunction (Laurent & Mark, 2014).

2.3 Grading system

For animals with compressive spinal cord lesions, the severity of the neurological lesions is used as a prognostic indicator (Snow, 2012). The severity of the neurological disease has been described by a grading scheme using a scale from 0 to 5, where 5 is the most severe. Grade one is present of spinal pain without neurologic deficits; grade two is ambulatory with loss of conscious proprioception; grade three is non-ambulatory paresis with loss of conscious proprioception; grade four is the retention of deep pain perception with complete paralysis; grade five is loss of deep pain perception with complete paralysis (Forterre *et al*., 2010).

According to Sharp and Simon (2005), myotatic reflex score is used for reflex grading. The scoring is from 0 to +4 which include absent (0), reduce (+1), normal (+2), increased (+3), and clonus (+4). In spinal reflex, absence (0) or reduction (+1) of reflex refers to a lesion of L6-S1 segments; a normal (+2) reflex means that normal spinal cord segments and nerves; acute lesions of the descending pathway may lead to increased (+3) reflex; sustained flexion of left and right pelvic limbs is seen in clonus (+4) reflex (Michael *et al.*, 2011).

2.4 Factors that affect the pelvic limb reflexes

Numerous research looked at the usefulness of neurological tests for identifying spinal cord lesions. In one of these experiments, cervical disc herniation in dogs was detected using the withdrawal reflex. The study's findings indicated that the withdrawal reflex was unreliable for identifying lesion sites (Forterre *et al*., 2008). Nevertheless, Bierrun *et al*. (2011) stated that nociceptive withdrawal reflex threshold (NWR-T) in human is a reliable tool to assess the spinal nociceptive pathways sensitivity in chronic pain patient. The effectiveness of the patellar reflex in neurolocalizing thoracolumbar lesions was investigated retrospectively. The findings demonstrated that in dogs with single and multiple thoracolumbar lesions, the patellar reflex was around 87% accurate in identifying whether the lesions were in the upper motor neurons (UMNs) or the lower motor neurons (Murakami *et al*., 2014). Also, De Lahunta and Glass (2009) in veterinary field and Litvan *et al.* (1996) in human field agreed that patellar reflex has the highest reliability.

We believe that older animal might have some age-related alteration. Duncan *et al.* (1975) stated that there is myelin ballooning, demyelination and remyelination observed in the lumbar spinal nerves of dogs as the age-related changes. Adey *et al*. (1997) reported that there are skeletal muscle changes such as changed in muscle fibre proportions, ultrastructural aberrations and atrophy observed in old men. This could be applied on older animal if these changes present in dog. These skeletal muscle and spinal alterations might lead to decrease in reflex grading.

In a human study comparing total reflex time of patellar reflex of two different weight group, weightlifter (heavier in weight) has longer total reflex time compared to long-distance runner (Kamen *et al*., 1981). However, there are limited findings on the influence of weight on animal spinal reflexes.

Other non-neurological factors such as the length of the femur, the kind of muscle fibers in the rectus femoris (Larsson, 1978), physical activity level (Clarkson, 1978), have been observed to affect the reflex in humans. Nonetheless, these factors will not be discussed in this study.

3.0 Materials and method.

This research has been approved by the institutional animal care and use committee (IACUC). Conventional sampling has been used as the study design. A total of 30 dogs of distinct breeds with a median age of 6 (1-14) years and a median weight of 19.76 (3.3-41.23) kg were involved. 25 of them are male while 5 dogs are female. These dogs are sampled from the statutory body (Bomba Unit) and Volunteer's pets. Breeds of dogs including Springer spaniels (40%), toy poodles (7%), Border collies (4%), German Pointer (3%), Labrador (23%), Cockle Spaniel (3%), Local (10%), Malinois (3%) and mixed local (7%). Except for poodles and local breeds, other breeds of dogs are sampled from the statutory body. Including marks were a normal neurological examination and no history of neurological disorders. However, there are 7 dogs that have non-neurological issues such as hip issues, heart diseases, long-term CLUTD, aural hematoma, and autoimmune skin disease. Samples are grouped according to age and weight group. There are twelve dogs aged more than 7 while 18 dogs aged less than 7. There are also twenty dogs weighing more than 15 kg while 10 dogs weighing less than 15 kg. Physical and neurological examinations were done on all dogs prior to the reflex test by vets. The examination is done by a year 4 veterinary student who has done multiple pieces of training and the entire process was supervised by an experienced neurologist. The whole reflex test process is video recorded under the same regulated circumstances which are the same room, lateral recumbency position, same camera (Canon Powershot sx620HS), and similar camera position (5 cm approximately dorsal from body and paws, mid-abdomen included). The reflex tests such as patellar reflex, withdrawal reflex, gastrocnemius reflex and sciatic

reflex were done by the student on both hindlimbs using a reflex hammer known as the Taylor percussion hammer. One examination took about three minutes. The dogs are restrained by trained volunteers and watched by the owners. Grading of the reflexes was done two weeks after the test. The grading was done by four observers which consisted of a neurologist, an experienced vet, and two years four veterinary students. The examiner was included and evaluated the video together with other observers. Observers graded the examination footage blindly without knowing the dogs' names and in a randomized numbering. Each observer evaluated and scored the level of reflex using a universal scoring scale (0= absent; 1= reduced; 2=normal; 3= exaggerated; 4=clonus). One dog is excluded as its' reflex video was used as a test run. There are fifty-eight videos of one reflex for each dog on both hindlimbs observed. Hence a total of two hundred thirty-two videos were observed and graded.

3.1 Data analysis:

The normality test by Shapiro-Wilk was used to analyse whether the reflex grades for both the weight group and age group are normally distributed or non-normally distributed. Spearman rank correlation was used to evaluate whether age and weight correlate with pelvic limb reflexes. Fleiss Kappa Coefficient was used to determine the interobserver agreement on withdrawal, sciatic, patellar, and gastrocnemius reflexes. All the statistical analyses were performed using IBM SPSS Statistics for Windows, version 26 (IBM Corp.N. Y., USA). Differences at $P \leq 0.05$ are considered significant at 95% confidence level.

4.0 RESULTS

Although Dafkin *et al.* (2013) stated that accuracy of reflex assessment has no correlation with the level of observers' expertise, study of Giebels et al. (2014) has shown the higher observer's level of expertise, the higher the interobserver agreement for reflex-presence and reflex-briskness-evaluation. Hence, the pelvic limb reflexes gradings of the neurologist were used as the only data to be analysed.

4.1 Normality test

Using the normality test by Shapiro-Wilk, we can determine whether our data is normally distributed or non-normally distributed. The results have shown that the reflex grades of both weight and age groups are non-normally distributed. The p-values of both age and weight groups on four different reflexes are less than 0.05 ($p < 0.05$). If p-values more than 0.05 show that the data are non-normally distributed. Hence, non-parametric tests were chosen to analyse the data.

4.2 Spearman rank Correlation

Spearman rank correlation was used to evaluate whether age and weight correlate with pelvic limb reflexes. According to Ratner (2009), the correlation coefficient is used to measure the strength of two linear variables. The correlation coefficient can be any value in the interval between +1 and -1. The value of 0 shows no linear relationship; +1 indicates a perfect positive linear relationship; -1 indicates a perfect negative linear relationship. Values between 0 and 0.3 show a weak positive relationship; values between 0.3 to 0.7 shows a moderative positive relationship; Values between 0.7 to 1.0 indicates a strong positive relationship.

Spearman's rho	Patellar	Gastrocnemius	Sciatic	Withdrawal
Age (r^2linear)	0.023	0.078	0.016	0.027
Weight(r^2linear)	0.003	0.009	0.537	0.002

Figure 1: Spearman rank correlation between reflexes gradings (patellar, gastrocnemius, sciatic, withdrawal) and both groups (age and weight).

From figure 1, we could interpret that patellar reflex grading has a weak positive relationship with both age (0.023) and weight group (0.003). Moreover, gastrocnemius reflex grading has a weak positive relationship with both ages (0.078) and weight (0.009) groups. For Sciatic nerve gradings, it does not have a strong relationship with the age group (0.016) but it does have a moderately strong relationship with the weight group (0.537). Finally, we observed no significant relationship between withdrawal reflex grading with both age (0.027) and weight group (0.002)

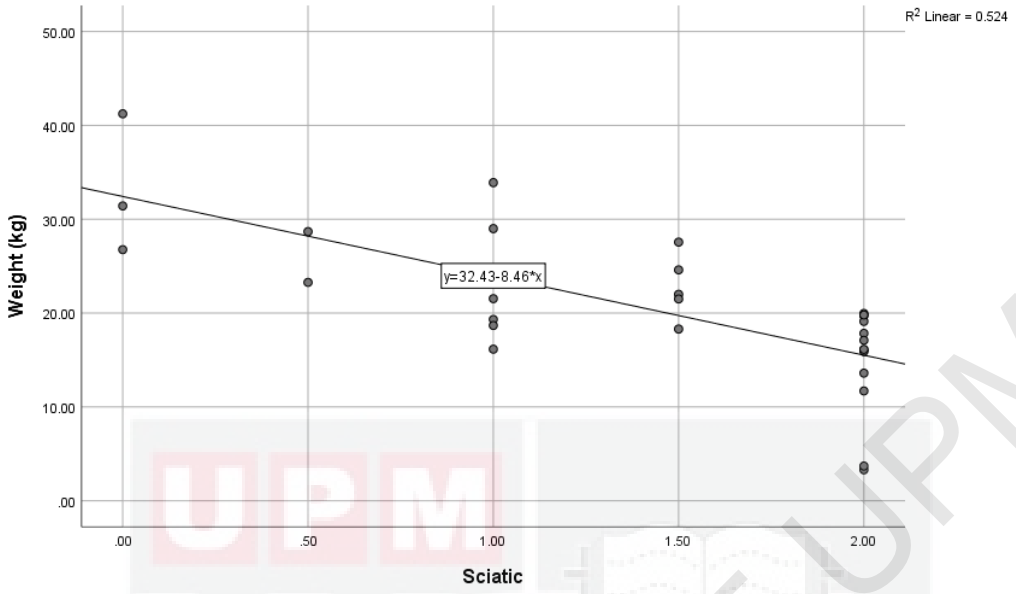

Figure 2: Linear regression of correlation between the sciatic nerve reflex grading and the weight

In this graph, we can interpret that sciatic nerve reflex grading has a negative correlation with weight. When the weight of the animal decreases, the sciatic nerve grading will be increased.

4.3 Interobserver agreement.

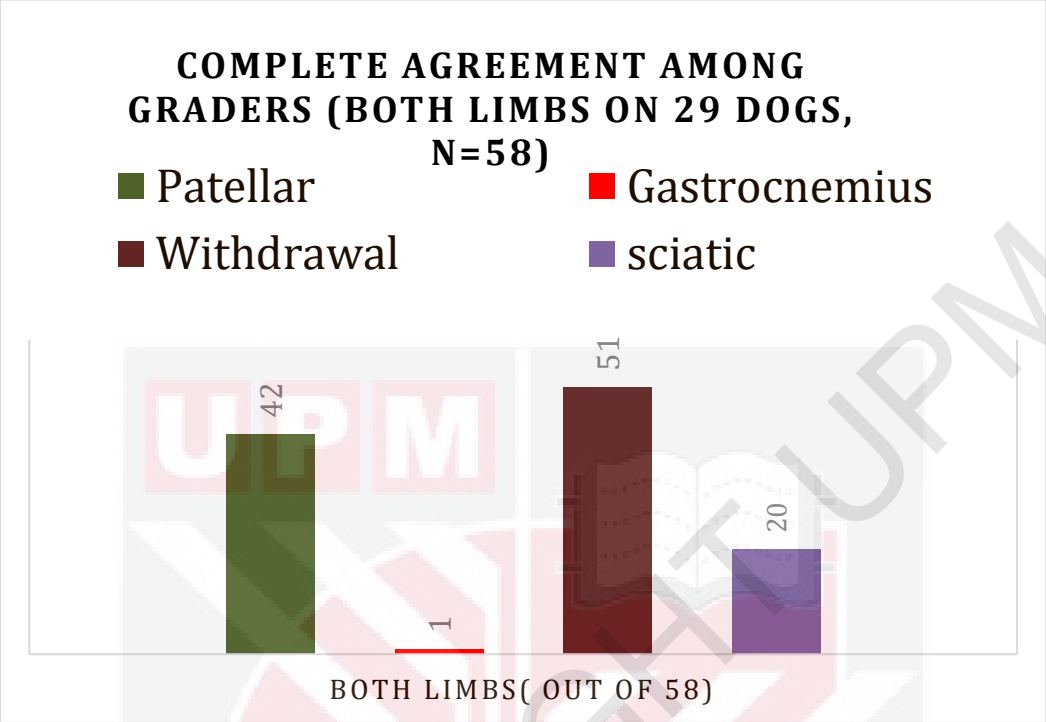

Figure 3: Table of agreement. Complete agreement among graders for equal scoring by all four observers on four reflexes based on grading on both hindlimbs on 29 dogs (N=58).

Figure 3 shows that the complete agreement on each hindlimb reflexes grading among graders. The total number of common agreements on each reflex is calculated based on the total number of same grades given by all observers on each pelvic limb reflex (left and right legs) of twenty-nine dogs. Hence, there will be a total fifty-eight marks if reach 100 percent perfect agreement.

Gastrocnemius reflex has only scored one out of fifty-eight marks, which mean all graders have only reached one complete agreement on a dog right hindlimb. Sciatic nerve reflex has second least common agreement which scored 20 out of 58. Patellar reflex has second

highest scores which the reflex has reached forty-one common agreements among all graders. Withdrawal reflex is considered the reflex that is easiest to reach complete agreement among graders as it has fifty-one out of 58 scores. It means every withdrawal reflex done will have 88 percent chances that all grader will has the same opinion on grading.

Noticeably, both withdrawal and patellar reflexes are rated as the easiest reflexes to elicit among graders in a brief survey after done gradings of all dogs' reflexes. On the other hand, gastrocnemius reflex was rated as the hardest reflex to elicit in the survey.

Fliess kappa coefficient test was chosen to determine whether the subjective review matched with the statistical test. Fliess kappa coefficient (Fleiss *et al*., 2003) is a measure of inter-rater agreement that assess the degree of consensus among two or more raters. Altman (1999) has suggested a classification to evaluate the strength of agreements based on the value of Cohen's kappa coefficient. Value of Fleiss' kappa (κ) less than 0.20 is indicated as poor; 0.21 to 0.40 indicates fair agreement; 0.41 to 0.60 indicates moderate agreement; 0.61 to 0.80 indicates good; 0.81 to 1.00 indicates an almost perfect agreement.

Reflex	Gastrocnemius	Withdrawal	Sciatic	Patellar
Fleiss' kappa,κ	-0.011	0.422	0.294	0.146
Interpretation	Poor agreement	Moderate agreement	Fair agreement	Poor agreement

Figure 4: Interobserver agreement among graders on four pelvic limb reflexes. Fleiss' kappa,κ values represent the level of agreement among different raters.

This table shows the gastrocnemius reflex has the lowest kappa value(κ)of -0.011, which is interpreted as poor agreement. This matched with the subjective feedback among graders mentioned above. Patellar reflex has κ value of 0.146, which is second low value and interpreted as poor agreement. This does not fit with the subjective opinion of graders. Sciatic nerve reflex has fair interobserver agreement which its κ value is second highest (0.294). Among other pelvic limb reflexes, withdrawal reflex has achieved moderate agreement with κ value of 0.422. This matched with the subjective feedback from the brief survey among graders.

5.0 Discussion

Our data show that none of pelvic limb reflexes has influenced by the age. Sciatic nerve reflex grading increase when weight decrease. Weight has no influence on other reflexes. Withdrawal reflex has the highest interobserver agreement followed by sciatic reflex, patellar reflex and gastrocnemius has the lowest interobserver agreement.

Canine pelvic limb reflexes in our study can be evaluated dependable by our trained observers. However, results of different studies should be compared as they are vary in terms of task characteristics, examiners' competency, methodology or the scoring scale used (Koran, 1975). We have fixed the positioning of the subject and classified them into age and weight group as these factors might have influenced on the reflex-activity. Breed might have interference on the pelvic limb reflexes however in our study the breed factor is not analysed due to there are insufficient number of dogs in each breed statistically.

However, there are still factors that might influenced on the neurological examination of the veterinary patients such as anxiety and temperature. According to Dick (2002), deep tendon reflexes in human is difficult to be elicited from an anxious patient as they have contracting muscle. Denys (1991) stated that biologic and neurophysiologic process influenced by temperature, and it might be led to detected falsely or unable to detect existing abnormality. Also, electromyography (EMG) is used in most of the human and few animal studies that identified influencing factors of the reflex-activity. Nonetheless, this process requires general anaesthesia in veterinary field due to EMG has high liable to factors such as adjacent muscle activity and examined subject alertness (Giebels *et al*.,2014). It is not suitable to perform in routine practice. Thus, we did not include this as

the aim of our study is to identify the factor that affect pelvic limb reflex to provide practitioner a clearer picture during their practice.

According to Levine et al. (2002), they reported that there is an age-dependent decrease in patellar reflex grade or an increase in total reflex time (TRT) prolongation among neurologically normal dogs. This report matched our alternative hypothesis. However, our result did not obtain the similar conclusion as our findings show that patellar reflex has neither related to age nor weight in terms of grading. The reason behind it might be because they have a higher number of dogs (86) compared to this study (29). David et al. (2008) stated that precision increases with the tested sample sized. Also, the previous report has categorized dog into two age groups (more than 10 and less than 10). In the other hand, we categorised our sample into two age groups (more than 7 and less than 7). This showed that they have grouped larger ages dog into their aged dog categories compare to us. In human study, the gastrocnemius-soleus H reflex latency (T_c) was not correlated with age (Shahram *et al*.,2004). However, there is limited information whether gastrocnemius reflex is non-age dependent reported in animal. Other reflexes such as sciatic reflex and withdrawal have no similar research done on animal to support my results which these reflexes are not age dependent.

Levine *et al*. (2002) also reported that weight has no relation on patellar reflex. This correlates with our findings. From our results, sciatic nerve reflex is weight dependent decrease in grading. Nevertheless, there are no data reported. We think the reason behind might be weight determine the size of the dog. If the dog is smaller in size, it is easier to hit on the sciatic nerve as our finger can fit in the groove of greater trochanter of femur and ischial tuberosity well. Moreover, the amplitude to hit on the sciatic nerve will be smaller

compared to a heavier dog to create same degree of reflex grade. However, this statement is based on personal experience rather than a systemic investigation.

Interobserver agreement are affected by three factors. These factors are the examiners, the subject to be examined and the examination itself (Clinical disagreement, 1980). Examination procedure is uniform for every observer. Also, there are same subject and a standardized scoring scale scoring-scale for the graders. The scoring scales used is 'myotatic reflex scales' which is commonly used in veterinary field and able to increase reflex evaluation comparability among graders. A few studies used video analysis for interobserver agreement calculation although it does not represent clinical settings. Hence, evaluating using a same video set up so that the results of different graders are comparable (Giebels *et al.*,2014). In clinical studies, the perfect agreement is extremely rare (Koran,1975). A 'moderate' interobserver agreement was scored by a lot of medical studies using Kappa analysis to evaluate reflex.

Withdrawal reflex was rated as the easiest reflex to be grade and it has proven statistically. When the reflex stimulated, there is a complete flexion of the hip, stifle, and hock, which is the entire limb. Thus, this response is more easily to be visualised and graded compared to other reflexes. Sciatic reflex has higher interobserver agreement (k) value which is 0.294 compared to patellar reflex (0.146). However, this does not match with the subjective opinion of observers that patellar reflex is rated as one of the easiest reflexes be graded among the graders. It might be due to different graders have different experiences. In this case, our graders come with different level of expertise. There is only one grader specialized in veterinary neurology. Different in experiences will have different opinion even though it is an obvious response. According to Michael *et al*. (2011), gastrocnemius

reflex is difficult to elicit and quantify. Gastrocnemius reflex stimulates extension of hock and contraction of the gastrocnemius muscle belly. This response is not as obvious as other reflexes such as patellar and withdrawal reflexes. Hence, it is rated as most difficult pelvic limb reflex to be graded and statistically shown that it has poor interobserver agreement.

6.0 CONCLUSION

Our result has shown that there are no pelvic limb reflexes influenced by age factor. Dogs in our study shows no significant difference on reflexes grading in different age groups. Weight has no influence on pelvic limb reflexes except for the sciatic nerve reflex. In our findings, the sciatic nerve reflex has negative correlation with weight. Moreover, withdrawal reflex has the highest interobserver agreement among four graders with distinct experience in veterinary field. Sciatic nerve reflex has the second highest interobserver agreement followed by patellar reflex. Gastrocnemius reflex has the lowest interobserver agreement among the pelvic limb reflexes and rated as the hardest reflex to be graded. Hence, successfully identifying the factors that contribute to hindlimb reflex grading could provide the veterinarian a better diagnosis and lead to an effective treatment plan.

7.0 RECOMMENDATION

I. Nervous dog should be given more time and patient while doing the reflex test as nervous dog's muscle has a more contracted muscle and this would affect its reflex grading.

II. The reflex tests should be done on a room with colder temperature as this could reduce the anxiety of dogs.

III. The degree of video recording and video clarity are the main issue of grading thru video. Interobserver agreement or grade on the spot could provide a more accurate result.

IV. Grading bias may exist as not every grader has sufficient experience in veterinary neurology field. We should increase more numbers of expert clinicians.

V. The method we collect sample is convenient sampling. This method may not represent the true population. We should involve samples from diverse backgrounds.

8.0 REFERENCES.

Afkin C, Green A, Kerr S, McKinon W. The patellar reflex: does activity of quadriceps femoris muscles reflect leg movement? Neurol Res. 2012; 34: 623-626.

Abdelhakiem, M., Asai, Y., Kamishina, H., Katayama, M., & Uzuka (2015). The accuracy of the patellar reflex for localization of the site of a single level thoracolumbar disc herniation in dogs. *TURKISH JOURNAL OF VETERINARY AND ANIMAL SCIENCES*, *39*, 589–593. https://doi.org/10.3906/vet-1503-66

Altman, D. G. (1999). Practical statistics for medical research. New York: Chapman & Hall/CRC Pres

Aminoff MJ, editor. Aminoff's Electrodiagnosis in Clinical Neurology. 6th ed. London: W.B. Saunders; 2012 Stam J, van Crevel H. Reliability of the clinical and electromyographic examination of tendon reflexes. J Neurol. 1990; 237: 427-431.

Balagopal P, Rooyackers OE, Adey DB, et al. Effects of aging on in vivo synthesis of skeletal muscle myosin heavy-chain and sarcoplasmic protein in humans. Am J Physiol 1997;273:790–800

Borin A, Mello LE, Neiva FC, Testa JR, Cruz OL. Experimental video analysis of eye blink reflex in a primate model. Otol Neurotol. 2012; 33: 1625-1629

Biurrun Manresa, J.A., Neziri, A.Y., Curatolo, M. et al. Test–retest reliability of the nociceptive withdrawal reflex and electrical pain thresholds after single and repeated stimulation in patients with chronic low back pain. Eur J Appl Physiol 111, 83–92 (2011). https://doi.org/10.1007/s00421-010-1634-0

Clarkson PM. The relationship of age and level of physical activity with the fractionated components of patellar reflex time. J Gerontol 1978; 33: 650–656.

Cheryl Chrisman, L. (2016). The Neurologic Examination. https://cdn.brief.vet/webfiles/CB+%26+VTB/Articles/ProP_Neurologic+Examination.pdf

Dafkin C, Green A, Kerr S, Veliotes D, McKinon W. The accuracy of subjective clinical assessments of the patellar reflex. Muscle Nerve. 2013; 47: 81-88.

Dewey, C. W., & Costa, D. C. R. (2016). *Practical guide to canine and feline neurology*. Wiley-Blackwell.

Dewey CW, Ducoté JM. A Practical Guide to Canine & Feline Neurology. Dewey CW, editor. Second ed. Ames (Iowa): Wiley Blackwell; 2008.

Dick JP. The deep tendon and the abdominal reflexes. J Neurol Neurosurg Psychiatry. 2003; 74: 150-153.

Denys EH. AAEM minimonograph #14: The influence of temperature in clinical neurophysiology. Muscle Nerve. 1991; 14: 795-811.

Essex MJ, Goldsmith HH, Smider NA, Dolski I, Sutton SK, Davidson RJ. Comparison of video- and EMG-based evaluations of the magnitude of children's emotion-modulated startle response. Behav Res Methods Instrum Comput. 2003; 35: 590-598.

Fleiss, J. L., Levin, B., & Paik, M. C. (2003). Statistical methods for rates and proportions (3rd ed.). Hoboken, NJ: Wiley.

Forterre F, Konar M, Tomek A, et al. Accuracy of the withdrawal reflex for localisation of the site of cervical disk herniation in dogs: 35 cases (2004-2007) .JAm Vet Med Assoc, 2008;232:559-563.

Garosi L. Lesion localization and differential diagnosis. In: Platt SR, Olby NJ, editors. BSAVA Manual of Canine and Feline Neurology. 3rd ed. Gloucester, UK: British Small Animal Veterinary Association; 2004. pp. 24–34

FRCVS, B. V. S. P. D. D. M., Wheeler, S. J., MRCVS, B. V. M. P. D., & Sharp, N. J. H. (2005). *Small animal spinal disorders (second edition)*. Mosby.

Hayes GM, Granger N, Langley-Hobbs SJ, Jeffery ND. Abnormal reflex activation of hamstring muscles in dogs with cranial cruciate ligament rupture. Vet J. 2013; 196: 345-350.

Jean Biau, D., Kernéis, S., & Porcher, R. (2008, June 20). Statistics in Brief: The Importance of Sample Size in the Planning and Interpretation of Medical Research.

Kamen G, Kroll W, Zigon ST. Exercise effects upon reflex time components in weight lifters and distance runners. Med Sci Sports Exerc. 1981;13(3):198-204. PMID: 7253874

Koran LM. The reliability of clinical methods, data and judgments (second of two parts). N Engl J Med. 1975; 293: 695-701.

Kreder HJ, Hanel DP, McKee M, Jupiter J, McGillivary G, Swiontkowski MF. Consistency of AO fracture classification for the distal radius. J Bone Joint Surg Br. 1996; 78: 726-731.

Laerd Statistics (2019). Fleiss' kappa using SPSS Statistics. Statistical tutorials and software guides. Retrieved November 18, 2022, from https://statistics.laerd.com/spss-tuorials/fleiss-kappa-in-spss-statistics.php

Larsson L. Morphological and functional characteristics of the ageing skeletal muscle in man. Acta Physiol Scand 1978;457(Suppl):1–36

Lorenz, M. D., Coates, J. R., & Kent, M. (2011). *Handbook of Veterinary Neurology (fifth edition)*. W.B. Saunders.

Litvan I, Mangone CA, Werden W, Bueri JA, Estol CJ, Garcea DO, Rey RC, Sica RE, Hallett M, Bartko JJ. Reliability of the NINDS Myotatic Reflex Scale. Neurology. 1996 Oct;47(4):969-72. doi: 10.1212/wnl.47.4.969. PMID: 8857728

Murakami T, Feeney DA, Willey JL, Carlin BP. Evaluation of the accuracy of neurologic data, survey radiographic results, or both for localization of the site of thoracolumbar intervertebral disk herniation in dogs. Am J Vet Res 2014; 75: 251–259.

Oliver JE Jr, Lorenz MD, Kornegay JN, Handbook of veterinary perception neurology. 3rd edn. WB Saunders Co, Philadelphia, 1997:3-46.14.Loderstedt, S. (2014). Evaluation of the biceps tendon reflex in dogs. *Journal of Veterinary Medicine and Research*.

Ratner, B. The correlation coefficient: Its values range between +1/−1, or do they?. J Target Meas Anal Mark 17, 139–142 (2009). https://doi.org/10.1057/jt.2009.5

Platt, S. R., & Olby, N. J. (2013). *Bsava Manual of Canine and Feline Neurology*. British Small Animal Veterinary Association.

Sadeghi, S., Ghavanini, M., Ashraf, A. et al. Effects of age and leg length upon central loop of the Gastrocnemius-soleus H-reflex latency. BMC Neurol 4, 11 (2004).

Snow, L. (2012) *Neurologic examination - a practitioner's guide.* Australian Veterinary Practitioner, 42 (2). pp. 248-255.

Schubert,T. (2016). Physical and Neurologic examinations. MSD veterinary manual.

Schatzberg SJ, Kent M, Platt SR. Neurologic examination and neuroanatomic diagnosis. In: Tobias KM, Johnston SA, editors. Veterinary Surgery: Small Animal. 1st ed. Volume I. St. Louis, MO, USA: Saunders; 2012. pp. 325–339.

Uemura, E. E. (2015). *Fundamentals of canine neuroanatomy and neurophysiology*. John Wiley and Sons, Inc.

Vroomen PC, de Krom MC, Knottnerus JA. Consistency of history taking and physical examination in patients with suspected lumbar nerve root involvement. Spine (Phila Pa 1976). 2000; 25: 91-96.

Vogel HP. Influence of additional information on interrater reliability in the neurologic examination. Neurology. 1992; 42: 2076-2081.

[No authors listed]. Clinical disagreement: I. How often it occurs and why. Can Med Assoc J. 1980; 123: 499-504