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INTRA-ARTICULAR CHARACTERISTICS OF DOGS WITH MEDIAL PATELLAR LUXATION

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A Thesis Submitted in Partial Fulfillment of the Requirements
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Thesis Title	INTRA-ARTICULAR CHARACTERISTICS OF DOGS WITH MEDIAL PATELLAR LUXATION
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เศรษฐพงศ์ จารุภัทรากร : ลักษณะภายในข้อต่อของสุนัขที่เป็นโรคสะบ้าเคลื่อนเข้าทางด้านใน (INTRA-ARTICULAR CHARACTERISTICS OF DOGS WITH MEDIAL PATELLAR LUXATION) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: ผศ. สพ.ญ. ดร. ชาลิกา หวังดี, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม: รศ. น.สพ. ดร. วิจิตร บรรลุนารา, หน้า.

การศึกษาหาความสัมพันธ์ของระดับสะบ้าเคลื่อนเข้าด้านในของสุนัขกับภาพถ่ายรังสีข้อเข่า ลักษณะของความเสื่อมภายในข้อเข่าของสุนัขที่มีสะบ้าเคลื่อนเข้าด้านใน และความสัมพันธ์กับการเปลี่ยนแปลงทางจุลพยาธิวิทยาและตัวบ่งชี้ทางชีวภาพ โดยแบ่งข้อเข่าจำนวน 38 ข้อ ออกเป็น 4 กลุ่ม ได้แก่ กลุ่มข้อเข่าปกติ (4 เข่า) และกลุ่มสะบ้าเคลื่อนเข้าด้านในระดับที่ 2 (13) 3 (12) และ 4 (9) สุนัขทุกตัวได้รับการประเมินตรวจและให้คะแนนการเดินกะเผลก ระดับของสะบ้าเคลื่อน คะแนนความเสื่อมข้อเข่าจากภาพถ่ายรังสี การตรวจทางจุลพยาธิวิทยาของเยื่อหุ้มข้อ การนับแยกเซลล์รวมทั้งการตรวจหาไซโตคายน์จากน้ำในข้อต่อ และการให้คะแนนการอักเสบของข้อเข่าในขณะทำการผ่าตัดแก้ไขสุนัขที่มีสะบ้าเคลื่อนเข้าด้านใน นำข้อมูลมาวิเคราะห์หาความสัมพันธ์และความแตกต่างระหว่างกลุ่มข้อเข่าปกติและกลุ่มสะบ้าเคลื่อนเข้าด้านใน จากการศึกษาพบความแตกต่างของคะแนนการกะเผลกระหว่างสุนัขปกติและสุนัขที่มีสะบ้าเคลื่อนเข้าด้านในระดับที่ 3 และ 4 พบความแตกต่างของคะแนนการตรวจทางจุลพยาธิวิทยาระหว่างสุนัขปกติและสุนัขที่มีสะบ้าเคลื่อนเข้าด้านในระดับที่ 4 และพบความแตกต่างของคะแนนการเสื่อมข้อเข่าหว่างกลุ่มสุนัขปกติและกลุ่มสุนัขที่มีสะบ้าเคลื่อนเข้าด้านในทุกระดับ พบความสัมพันธ์ของระดับของสะบ้าเคลื่อนกับการกะเผลก กับผลการตรวจทางจุลพยาธิวิทยา และกับคะแนนเยื่อหุ้มข้อ พบความสัมพันธ์ของการเกิดข้อเสื่อมและการเปลี่ยนแหลงของกระดูกอ่อนผิวข้อกับระยะเวลาที่เกิดสะบ้าเคลื่อนและอายุสุนัข และพบความสัมพันธ์ของคะแนนเยื่อหุ้มข้อกับการกะเผลก foam cell และการเปลี่ยนแปลงทางจุลพยาธิวิทยา พบการเปลี่ยนแปลงของกระดูกอ่อนผิวข้อมีความสัมพันธ์กับเม็ดเลือดขาวชนิดนิวโทรฟิลล์ นอกจากนี้ยังพบความสัมพันธ์ของภาพถ่ายรังสีกับการเกิดข้อเสื่อมและการเปลี่ยนแปลงของกระดูกอ่อนผิวข้อ แต่ความรุนแรงของข้อเสื่อมไม่สัมพันธ์กับระดับของสะบ้าเคลื่อน มีเพียง MCP-1 ชนิดเดียวที่ตรวจพบในน้ำในข้อเข่าของสุนัขที่เป็นสะบ้าเคลื่อนเข้าด้านในแต่ไม่พบความสัมพันธ์ของ MCP-1 กับพารามิเตอร์อื่นๆ การไม่พบไซโตคายน์ที่สัมพันธ์กับความรุนแรงของโรคข้อเสื่อมอาจเนื่องจากโรคสะบ้าเคลื่อนเข้าด้านในในสุนัขพันธุ์เล็กทำให้เกิดการเสื่อมของข้อได้ แต่ไม่รุนแรงเท่ากับในสุนัขพันธุ์ใหญ่และในสุนัขที่มีปัญหาเอ็นไขว้หน้าขาด การเปลี่ยนแปลงของกระดูกอ่อนผิวข้อและการเสื่อมของข้อเข่าจะเพิ่มขึ้นตามระยะเวลาของโรคและอายุของสุนัขที่เพิ่มขึ้น การผ่าตัดแก้ไขจึงควรทำให้เร็วที่สุดเพื่อป้องกันการพัฒนาของข้อเข่าเสื่อมที่รุนแรงขึ้น

ภาควิชา	ศัลยศาสตร์	ลายมือชื่อนิสิต .....
สาขาวิชา	ศัลยศาสตร์ทางสัตวแพทย์	ลายมือชื่อ อ.ที่ปรึกษาหลัก .....
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5875323231 : MAJOR VETERINARY SURGERY
KEYWORDS: PATELLAR LUXATION / OSTEOARTHRITIS / SYNOVIUM / ANALYSIS / BIOMARKER

SETHAPONG JAHRUPATRAKORN: INTRA-ARTICULAR CHARACTERISTICS OF DOGS WITH MEDIAL PATELLAR LUXATION. ADVISOR: ASST. PROF. CHALIKA WANGDEE, D.V.M., M.S., Ph.D., D.T.B.V.S., CO-ADVISOR: ASSOC. PROF. WIJIT BANLUNARA, D.V.M., M.S., Ph.D., D.T.B.V.P., pp.

The study investigated the relationship of degree of patellar luxation of dogs with joint radiography, intra-articular degenerative change of the stifle with medial patellar luxation (MPL), histopathologic change, and biomarker index. Thirty-eight stifles were divided into four groups: normal stifle joints (4), grades 2 (13), 3 (12), and 4 (9) MPL. Demographic data, lameness score, and degree of patellar luxation of all dogs were assessed and recorded. Radiographs were taken to assess osteophyte formation. Synovial tissue was collected for microscopic evaluation differential cell counts, and cytokines measurement. Stifle joints with MPL were scored during surgical correction. All observed data were analyzed for correlations and differences among the normal and MPL stifle groups. There were significant differences in the lameness score between the normal and grades 3 and 4 MPL groups, the histopathologic score between normal and grade 4 MPL groups, the osteoarthritis (OA) score between normal and all grades of MPL. Correlations were found between degree of patellar luxation and the lameness, histopathologic, and synovial scores. Correlations of OA and the cartilage score were found with duration of luxation as well as age at surgery. Correlations were found between the synovial score and the lameness score, foam cell, and histopathologic score. The cartilage score was significantly correlated with number of neutrophils. In addition, correlations were found between the radiographic score and the OA and cartilage scores. In conclusion, MPL in small breed dogs can cause stifle OA. Radiographic score was correlated with cartilage score and OA score but the severity of OA was not related to the severity of PL. Only MCP-1 was found in synovial fluid but correlation of MCP-1 with other parameters was not found. The reason was that other cytokines relating to the severity of OA could not be detected in this study. MPL in small breed dogs might cause less OA than that in the large breed dogs and dogs with cranial cruciate ligament rupture. Severity of cartilage erosion and OA are related with duration of disease and age of dogs. Therefore, surgical correction should be performed as soon as possible in order to prevent further development of degenerative joint disease.

Department:	Veterinary Surgery	Student's Signature	.....
Field of Study:	Veterinary Surgery	Advisor's Signature	.....
Academic Year:	2017	Co-Advisor's Signature	.....

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CHAPTER I

INTRODUCTION

Importance and rationale

Osteoarthritis (OA) is the most common form of arthritis in dogs and it has been estimated approximately 20% of adult dogs affected (Johnston, 1997). OA can be classified as primary in origin or secondary from joint disease, and it can be idiopathic. Most dogs can be identified as secondary OA and stifle joint is one of the most affected joint occurrence following medial patellar luxation (MPL) which is a highly affected in small-breed dogs (Alam et al., 2011b). MPL presented highly prevalence in Pomeranian dogs in Thailand of 75% (Wangdee et al., 2017). The clinical signs of osteoarthritis can be characterized by inactivity stiffness, lameness, pain, exercise intolerance, limited range of motion, joint pain on manipulation that dramatically reduce quality of patient life (Frost-Christensen et al., 2008; Baltzer et al., 2009; Innes, 2012a).

Osteoarthritis can be clinically diagnosed by several methods, including history taking of previous disease, physical examination, radiography of joint, and synovial fluid analysis. Survey radiography is a standard tool used for clinical diagnosis, monitor on disease progression and assessment of treatment (Innes et al., 2004; De Bruin et al., 2007a). However, it is low sensitivity and some studies showed unrelation between clinical the improvement and radiographic finding. The surgical treatment of MPL showed significantly improvement of limb use and lameness score whereas radiographic score of OA increased significantly and it was progressive for a continuous period (Roy et al., 1992). The arthroscopic investigation in canine unilateral CCLR found unaffected joint presenting some degrees of cruciate damage and obvious synovitis whereas radiography revealed mild joint effusion in the cranial compartment (Little et al., 2014).

Osteoarthritis is a silent and slow progressive disease so it is difficult to diagnosis in early stage. The early treatment is benefit to prevent or delay further cartilage disruption. Therefore, early detection of OA is important in clinical practice. Histological evaluation is a powerful tool to diagnose OA and it has been used as a gold standard for pathological diagnosis, disease presenting, progression of the disease, and severity of OA (Cook et al., 2010). Histological changes usually occur in the early stage of disease progression before patients presenting clinical signs ae well as changing in imaging modalities and in gross lesions were detected. In an experimental model of induced OA by cruciate transection, microscopic changes in cartilage, bone, synovium and menisci showed abnormality at 1-2 weeks after operation whereas macroscopic changes were obviously seen after 2 weeks of induction (McDevitt et al., 1977). Most studies of OA in MPL based on diseases induced in experimental animals and focused on the pathogenesis of OA and biomarker in order to detect the early degenerative changes or diagnose OA (McDevitt et al., 1977; Matyas et al., 2004; Frost-Christensen et al., 2008; Alam et al., 2011a; Alam et al., 2011b). As the authors knowledge, there are only two clinical studies reporting the relationship between clinical and pathological parameters of dogs with the severity of medial patellar luxation. One study of surgical correction in congenital MPL showed the most prevalent location of cartilage erosion occurring on disto-medial part of patellar articular surface and the correlation of cartilage erosion was not found with grade of luxation, age, and gender except in grade IV MPL that correlated with and the percentage of cartilage erosion. In addition, increasing in cartilage erosion positively related to body weight (Daems et al., 2009). The other study of surgical correction in MPL dogs showed that 39% of dogs demonstrated cartilage lesions predominantly on the medial part of femoral trochlea and patella. Furthermore, the risk factors found that age, sex and degree of PL were related to cartilage erosion (Chomdej et al., 2016).

Less information of the correlation among pathological parameters, clinical parameters and histopathologic changes in severity of MPL. The study of medial patellar luxation in dogs may provide informative data in order to gain more knowledge

and to prevent secondary OA causing from this disease as well as to improve the treatment outcome.

The first hypothesis is severity of demographic disease and radiography having correlations with intra-articular structural changes both in gross and in histological features. The second hypothesis is different severities of stifle osteoarthritis presenting the different levels of biomarkers.

The aims of this study are to investigate the relationship on demographic diseases, joint radiography and degenerative changes in intra-articular structures of stifle with medial patellar luxation in dogs and to evaluate cellular changes on histopathologic feature as well as biomarker index. We hypothesized that (1) the severities of demographic disease and radiography have correlation with intra-articular structural changes in gross and histological features (2) different severities of stifle osteoarthritis in medial patellar luxation present the different levels of biomarkers. Clinical relevance, we might use these parameters in order to precisely evaluate the severity of osteoarthritis and to assess the efficacy of medical treatment or supplement use in osteoarthritic patient.

CHAPTER II

LITERATURE REVIEW

2.1 Osteoarthritis

Osteoarthritis (OA) is defined as abnormality in production and destruction articular cartilage, alterations of subchondral bone structure and metabolism, increase in periarticular osteophytosis and in variable degrees of synovitis (Fujita et al., 2006; Frost-Christensen et al., 2008; Alam et al., 2011b). OA is the most common form of arthritis in dogs and cats and in human as well, and it has been estimated approximately 20% of adult dogs affected (Johnston, 1997). The clinical signs of OA can be characterized by inactivity stiffness, lameness, exercise intolerance, limited range of motion (ROM), and pain. This resulting in improper function of the affected joint can reduce quality of life (Frost-Christensen et al., 2008; Baltzer et al., 2009; Innes, 2012a). OA can be classified as primary or idiopathic and secondary. Secondary OA can be divided into 2 categories as abnormal load on regular joint structures such as obesity or regular load on abnormal joint structures including hip dysplasia, elbow incongruency, patellar luxation (PL), cranial cruciate ligament rupture (CrCLR), osteochondrosis, or traumatic events. (Klocke et al., 2005). Most dogs can be identified as secondary OA from abnormality of joint structure.

2.2 Medial patellar luxation

Patellar luxation is one of the most common stifle joint problem seen in veterinary medicine especially in dogs. The direction of luxation can occur in medial or lateral or bidirectional. The luxation occurs primarily as congenital or developmental, with traumatic event less commonly seen (Hulse, 1981; Gibbons et al., 2006; Kowaleski et al., 2012). Medial patellar luxation (MPL) has a high prevalence affecting in small-breed dogs especially in Pomeranian dogs of 75% and small-breed dogs have a risk approximately 10-12 times that of large-breed dogs. (Hulse, 1981; Hayes et al., 1994; Gibbons et al., 2006; Kowaleski et al., 2012; Soontornvipart et al.,

2013). Lateral patellar luxation (LPL) is mostly found in large-breed dogs although MPL is diagnosed more frequently (Hayes et al., 1994; Gibbons et al., 2006). The predisposed breeds of PL are Toy Poodle, Yorkshire Terriers, Chihuahuas, Pomeranians, Boston Terriers and large breed dogs including Siberian Husky, Labrador Retrievers, Golden Retrievers, Alaskan Malamute (Hulse, 1981).

2.2.1 Anatomy and function of the stifle joint

The stifle joint is a compound joint classified as complex condylar synovial joint and it is composed of soft tissue and bony structures. Bony structures consist of distal femoral condyle, proximal tibia and the patella (an ossification in the tendon of insertion of the quadriceps muscle group or sesamoid bone). Soft tissue structures of the stifle joint are not only synovial membrane, infra-patella fat pad, and meniscus but also the ligaments of the stifle joint comprising patellar ligament, medial collateral ligament, lateral collateral ligament, cranial cruciate ligament and caudal cruciate ligament. Three main articulations of the stifle joint are femorotibial joint, patellofemoral joint and tibiofibular joint (Innes, 2012a; Kowaleski et al., 2012). The femorotibial joint is a roller-like condyle of the femur articulating with expanded condyles of the tibia by menisci interposition between two bones. It acts as primary weight-bearing articulation of the stifle joint. The patellofemoral joint is a saddle joint consisting of patellar articular surface and femoral trochlear groove. The proper articulation of these joints provides nutrition and exchanges metabolic waste for the articular cartilage. If abnormal articulation occurs to the joint, it could be resulting in degenerative changes of the articular cartilage. Primary motion of the stifle joint is rotation and flexion-extension, and the proper movement of the stifle joint requires quadriceps (extensor) mechanism to extend the stifle joint. Quadriceps mechanism of the stifle joint is composed of quadriceps muscle group (rectus femoris, vastus lateralis, vastus intermedius and vastus medialis), patella, trochlear groove, straight patellar ligament and tibial tuberosity. The proper alignment of these structures is necessary for stability as well as effectiveness of quadriceps mechanism of the stifle joint. The patella is embedded in the quadriceps tendon with inner articular convex which allows

fully articulation to the femoral trochlea and it encloses with parapatellar fibrocartilages. When the stifle flexes and extends, patellar is gliding on the femoral trochlear groove, and medial and lateral parapatellar fibrocartilages ride on the medial and lateral trochlear ridges enhancing stability to the patella (Hulse, 1981; Roush, 1993; Kowaleski et al., 2012). The patella has biomechanical function as a lever arm to enhance the quadriceps efficiency of stifle extensor mechanism and to provide rotary and anterior stability to the joint (Roush, 1993).

2.2.3 Pathophysiology of medial patellar luxation

The pathophysiology of canine medial patellar luxation has been extensively reviewed but it still remains controversial. However, anatomic abnormality of entire pelvic limb has been accepted as one of reasonable pathogenesis of patellar luxation (Hulse, 1981; Roush, 1993). The luxation can occur due to congenital abnormality, developmental disorders or acquire (traumatic) disease. The musculoskeletal abnormality of the femur or the tibia or both can lead to luxation of the patella. The severity of musculoskeletal pathology relies on duration and degree of patellar luxation mostly affecting in immature animals. The alteration of skeletal deformity occurs in immature animals with open epiphyseal plate which have the ability to grow leading to more severe condition. The severity of deformity depends on age at develop luxation and degree of patellar luxation. The abnormal development is explained by Hueter-Volkmann law that the increased compressive force on an active physis retards growth, while decreased compressive force accelerates growth (Simon, 1978) (figures 1-2). Therefore, the medial displacement of quadriceps muscle group increases pressure on the medial side of the distal femoral epiphysis and decreases pressure on the opposite side. This abnormal stress directly loads on epiphyseal plate of the distal femur and the proximal tibia along the axis and perpendicular to the growth plate resulting in distal femoral varus deformity, external torsion of distal femur, proximal tibial valgus deformity, external torsion of distal tibia and internal deviation of tibial tuberosity (figures 2-3). Mature dogs with medial patellar luxation generally develop degenerative joint disease due to lack of proper patellofemoral articulation instead of

skeletal deformity (Simon, 1978; Hulse, 1981; Roush, 1993). In mature dogs with traumatic fracture of the femur or the tibia, medial patellar luxation can occur secondary as a complication of malunion and/or malalignment after fracture healing.

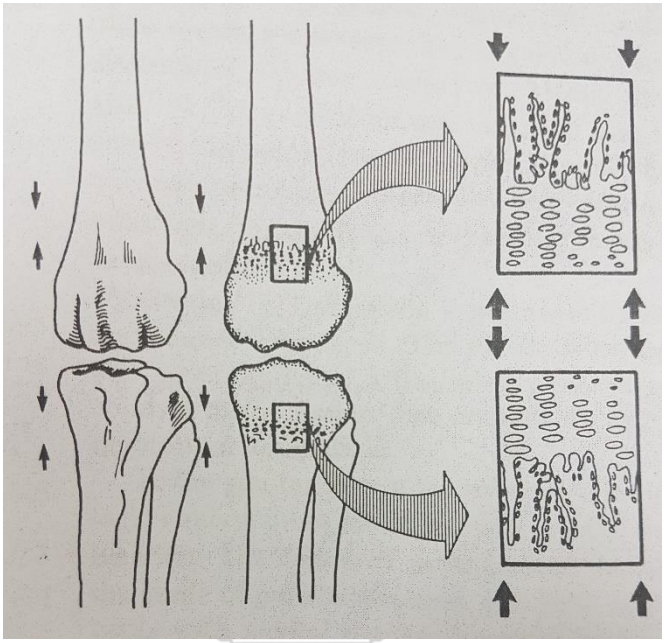


Figure 1 Diagram of normal forces loaded on the growth plate. (From Hulse DA: pathophysiology and management of medial patellar luxation in the dog. Philadelphia: Lea & Febiger, 1981.)

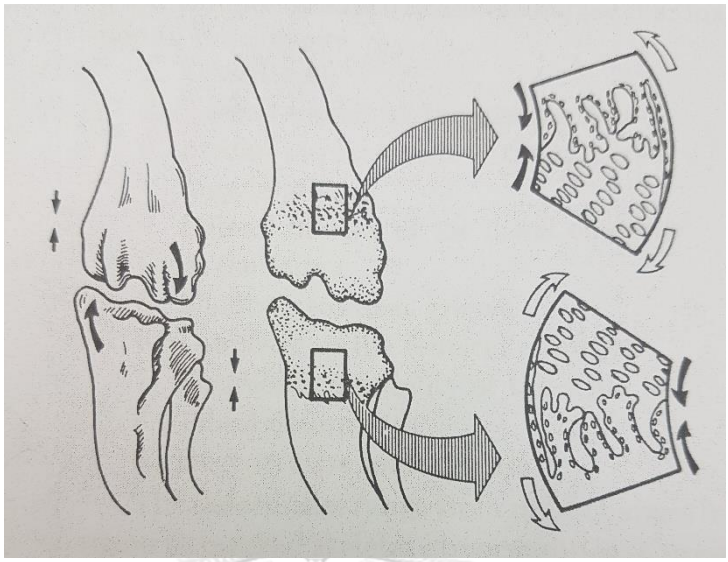


Figure 2 Diagram of abnormal forces from medial displacement of quadriceps muscle group loaded on the growth plate, resulting in distal femoral varus deformity and/or tibial valgus deformity. The magnification of growth plate in the box show the abnormal forces affected the growth rate. (From Hulse DA: pathophysiology and management of medial patellar luxation in the dog. Philadelphia: Lea & Febiger, 1981.)

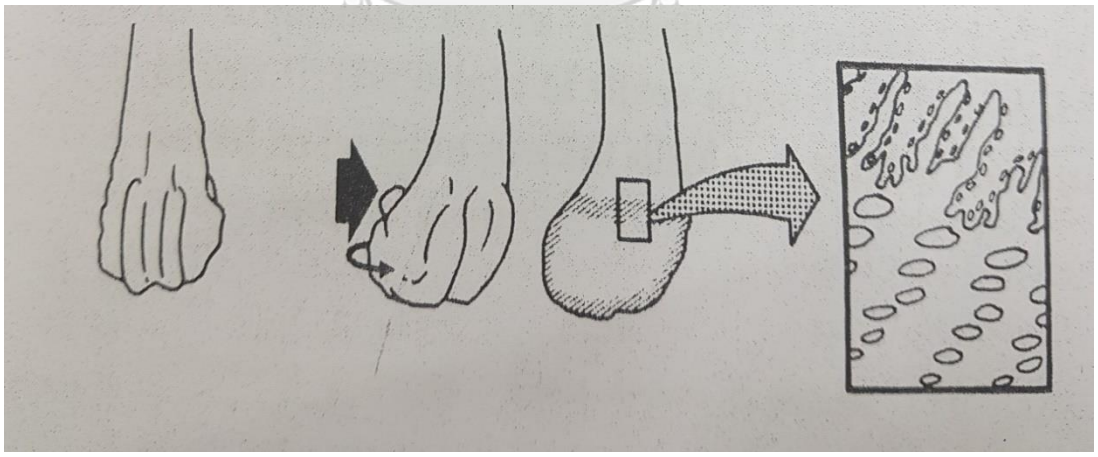


Figure 3 Diagram of abnormal forces from medial displacement of quadriceps muscle group loaded perpendicular to the growth plate, resulting in external torsional deformity. (From Hulse DA: pathophysiology and management of medial patellar luxation in the dog. Philadelphia: Lea & Febiger, 1981.)

Medial patellar luxation can be classified into four grades according to Singleton (1969) (figure 4):

Grade 1: In extension and flexion of the stifle joint, patella normally glide in the trochlear groove but manual luxation of the patella can be achieved when the pressure releases the patella spontaneously reduces to the normal position.

Grade 2: The patella may luxate during extend or flex the stifle joint and it may spontaneously reduce to the trochlear groove.

Grade 3: The patella is outside to the medial trochlear ridge most of the time and it may be reduced by manipulation, but re-luxation of the patella occurs after release the pressure. Medial rotation of the tibial tuberosity and Medial displacement of quadriceps muscle are usually seen in most dogs.

Grade 4: The patella is permanently luxated and cannot be manually reduced. The femoral trochlear groove is shallow, absent, or hypoplastic which combines with hypoplastic of the patella. The patella generally articulates to the new groove of the medial femoral condyle. The angular and torsional deformities of the femur and the tibia are marked.

2.2.4 Clinical signs and diagnosis of medial patellar luxation

Clinical signs involved with medial patellar luxation vary from asymptomatic, mild, moderate or severe weight-bearing. Dogs may walk normally or intermittently or consistently with vary degree of hind limb lameness. In grade 1 luxation, dogs are typically no clinical sign and luxation usually incidental diagnoses on routine physical examination. In grade 2 luxation, dogs typically show intermittent lameness associated with medial luxation of the patella and lameness may occur as a result of luxation and reduction of patella during movement of the stifle joint. When patella luxates, dogs may immediately skip and hold the leg without discomfort with flex and extend the joint leading to patellar reposition and the dogs can walk normally. This Intermittent lameness can be called “skipping lameness”. In grade 3 luxation, dogs typically walk with stifle semi-flex and internal tibial rotation at the level of stifle joint

because the patella luxates most of the time during ambulation. This posture refers as a “crouched” gait. Lameness is probably associated with the degree of cartilage erosion on the patella surface and on medial trochlear ridge of the femur, and degree of synovial membrane inflammation. In grade 4 patellar luxation, the dog may develop a crablike posture due to permanently luxation of the patella which cannot be manually reduced.

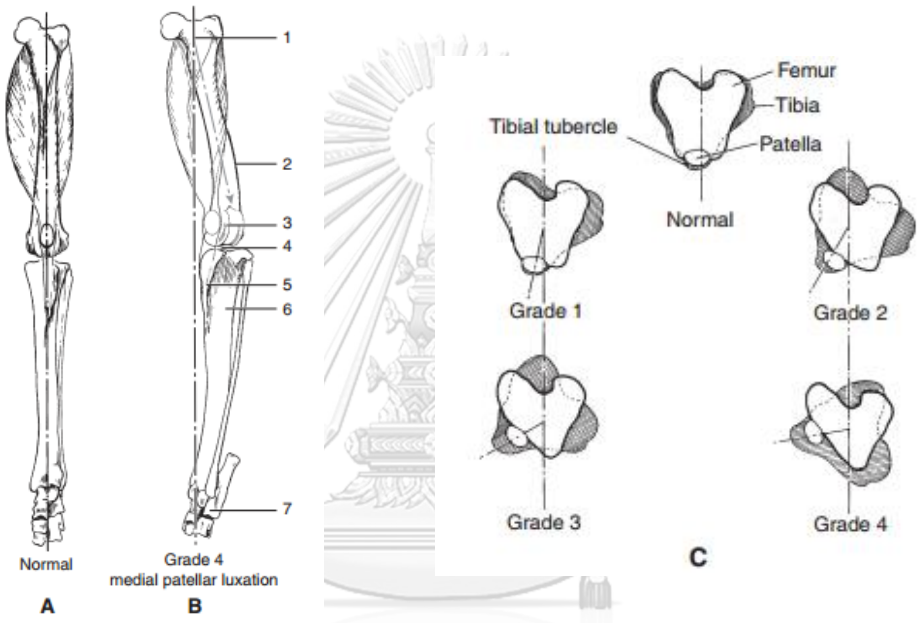


Figure 4 Diagrams of normal and severe form of medial patellar luxation concurrent with skeletal deformities of pelvic limbs. Cranial view of left normal pelvic limb (A). The quadriceps mechanism and patella are aligned in the appropriate position; dashed line connecting the center of the proximal femur to the center of the distal tibia. Grade 4 medial patellar luxation accompany with deformities (B): 1, Coxa vara; 2, Distal femoral varus or genu varum; 3, Shallow trochlear groove with rarely develop medial ridge; 4, Hypoplastic medial femoral condyle; 5, Internal torsion of tibial tuberosity associated with internal rotation of the tibia at the stifle joint; 6, Proximal tibial varus; 7, Internal torsion of foot notwithstanding external torsion of the distal tibia. The cross section of region of trochlear sulcus of the femur is dark outline and shade in the proximal tibial cross section show in normal stifle and in grade 1 through 4 medial

patellar luxation (C). Note that position of the tibia relative to the femur changes from internal rotation of the tibia at the stifle joint and position of the patella seated to femoral trochlear in each grade with deformity of medial trochlear ridge. (From Piermattei DL, Flo GL, DeCamp C: The stifle joint. In Brinker, Piermattei, and Flo's handbook of small animal orthopedics and fracture repair, ed4, St Louis, 2006, Saunders/Elsevier.)

The surgical model of osteoarthritis induced with MPL in dogs observed osteoarthritic lesion found in the stifle joint as well as the lesion found on cranial cruciate ligament with disclosed thickening and defibrillation of the ligament fibers progressively at the time, and partial tear of cranial cruciate ligament was found in two OA-induced stifles. The authors suggested that medial displacement of quadriceps muscle group caused angular and rotational deformities of the distal femur and the proximal tibia and loss of function to against cranial drawer motions which created abnormal stress on the cranial cruciate ligament leading to injure and rupture the ligament (Alam et al., 2011a).

Medial patellar luxation can be diagnosed from physical examination but radiography is useful for observing bony deformity, pelvic conformation, and degenerative changes of the stifle joint. Palpation and manipulation of the affected joint is the gold standard for diagnosis and grading patellar luxation as well as to screen present skeletal deformity of pelvic limb and to rule out concomitant cranial cruciate ligament disease or others pathologic features that possibly cause hind limb lameness. Gait evaluation at walk or trot and posture evaluation are performed to define severity and characterize lameness as well as to assess bony deformity. The physical examination can be performed in standing position or in lateral recumbency with the side to be examined facing upward.

The palpation is used to evaluate position of the patella during stifle flexion and extension, to observe the femoropatellar instability and the ability to reduce patella to the femoral trochlear groove, and to exam alignment of quadriceps mechanism and stifle range of motion.

2.2.5 Treatment

Canine medial patellar luxation can be treated either conservatively or surgically. Treatment options usually depend on condition of the patient, degree of patellar luxation, age of patients, severity of skeletal deformities and clinical lameness. Conservative treatment is recommended in asymptomatic dog with grades 1 or 2 MPL or in some old dogs. Surgical treatment is recommended in grades 3 and 4 MPL especially immature dogs to reduce progression of skeletal deformity and osteoarthritis, and to avoid irreversible quadriceps contracture. Surgery is indicated in lame dogs associated with grade 2 MPL. The aim of surgical correction of patellar luxation is to re-align the quadriceps mechanism and to stabilize the patella with in the trochlear groove in order to re-establish normal anatomy and limb function.

The surgical techniques of medial patellar luxation are categorized into soft tissue and bone reconstructions (Kowaleski et al., 2012; Fossum, 2013).

1. Soft tissue reconstruction is aim to relieved tension on the extensor mechanical or contracted fibrous tissue of the medial side and to tighten the extended tissue on the lateral side for medial patellar luxation. The techniques include medial desmotomy release, imbrication of lateral retinaculum, tibial antirotation suture ligaments, lateral reinforcement, tibial derotation, and rectus femoris transposition.

2. Bone reconstruction used to correct medial patellar luxation including trochleoplasty technique for deepening and widening the trochlear groove, tibial tuberosity transposition for realignment quadriceps mechanism due to medial deviation of tibial crest, and corrective osteotomies for correcting skeletal deformities such as distal femoral varus, femoral torsion, proximal tibial valgus and tibial torsion (Piermattei and Flo, 2006; Kowaleski et al., 2012).

In general, the trochleoplasty is typically done first following with re-alignment of quadriceps mechanism and soft tissue reconstruction if a femoral osteotomy is desired, this procedure should be done before trochleoplasty procedure (Kowaleski et al., 2012).

Soft tissue reconstructive surgery alone should be avoided due to high frequency of patellar relaxation that requires revision surgery (Arthurs and Langley-Hobbs, 2006). According to previous studies in complication associated with surgical correction of MPL, the result showed that femoral recession trochleoplasty and tibial tuberosity transposition techniques was favorable with low frequency of patellar re-luxation while retinacular/capsular release resulted in high frequency of major complications (Arthurs and Langley-Hobbs, 2006; Cashmore et al., 2014). Therefore, the combination of bone reconstruction is preferable for correcting MPL to reduce risk of major complications.

2.2.6 Outcomes

Surgical correction shows good outcomes especially in grades 2 and 3 MPL by improve limb use, decrease lameness, and provide patellofemoral stability. In grade 4 MPL, the outcome depends on degree of skeletal deformity presented. Althrough the surgical outcome is usually good in most cases of uncomplicated patellar luxation, the pathologic features of osteoarthritis at the affected stifle may decrease outcome and prolong lameness (Roush, 1993). Previous study showed progressive OA found in both conservative and surgical treatments of MPL (Roy et al., 1992).

Normal alignment of quadriceps mechanism after surgical correction provides normal limb function and eliminates pain in spite of OA still progression. The surgical treatment of patellar luxation should be considered in early of life to minimize the development of skeletal abnormality and to prevent irreversible osteoarthritis. During the stifle joint movement, the forces act to the articular cartilage then pass through subchondral bone causing pumping mechanism which provides nutrition and exchanges the waste product occurring from cartilage metabolism at the joint (Hulse,

1981; Innes, 2012a). The pumping mechanism in dogs with MPL does not properly occur leading to degenerative changes of cartilage but this process can be reversible if the quadriceps mechanism has been restored to create the normal pumping mechanism through the joint by early treatment. If patella continuously luxates for a long period of time until the degenerative process of cartilage reaches to the irreversible stage, the degenerative joint disease will consequently occur.

2.3 Anatomy of the joint

The normal joint is composed of synovial membrane, articular cartilage, and subchondral bone.

The normal synovial tissue can be characterized into three main types including areolar, adipose and fibrous synovium based on the contents of subintimal structure. The subintimal layer in some areas is comprised of perimysium, periosteum or cartilage. The areolar synovium is special tissue due to crimped into fold and vanish when stretched. The normal synovial membrane is relatively acellular structure composed of a distinct 1-2 cell layer of intimal and subintimal lining layers. The subintimal lining layer is consisted of scattered fibroblasts, adipose cells, blood vessels and some residence lymphocytes and macrophages. In addition, subintima also contains lymphatic vessels, venules and nerve fiber. The synovial intima cells include synoviocyte type A and B cells. The synoviocyte type A has cell surface markers indicting that it originates from macrophage lineage, and synoviocyte type B is fibroblast lineage which 70-90% of intimal cells belong to type B cells. The type B cell is capable to produce and secrete synovial fluid that includes collagens, lubricin, fibronectin and hyaluronan. The normal function of synovial membrane is to allow movement of adjacent tissue by maintaining intact non-adherent tissue surface, lubricate cartilage, control synovial fluid volume, provide nutrition, and remove waste product from chondrocyte metabolism via synovial fluid. The normal synovial tissue can produce a low volume of inflammatory cytokines such as interleukin-1 (IL-1), interleukin-6 (IL-6), and tumor necrosis factor alpha (TNF-α) when compared with synovitis. Moreover, the anti-inflammatory cytokine such as IL-1 receptor antagonist (a

naturally IL-1 inhibitor) has high concentration when compared with inflammatory cytokine in normal synovial tissue. The normal condition of the cytokines helps to preserve homeostasis in the normal joint (Smith, 2011).

The articular cartilage is composed of extracellular matrix (ECM) with approximately 70% of water and the remaining constituent is organic extracellular matrix. Organic ECM contains proteoglycan aggrecan, type II collagen and other collagens, and non-collagenous proteins but the main component is type II collagen (figure 5). Non-calcified distinct of the articular cartilage can be divided into 3 zones according to matrix composition and cellular tabulation including superficial zone, intermediate zone and radial zone. Zone of calcified cartilage (ZCC) locates next to the radial zone which is separated by tidemark. Articular cartilage attaches to subchondral bone by interdigitating of ZCC (Oegema et al., 1997). Generally, articular cartilage is avascular, aneural, and alymphatic but in aging, vessel and nerve can invade from subchondral bone so these structures will be visible in the articular cartilage. A main function of cartilage is the absorption and distribution of mechanical load. The orientation of collagen network and high content of proteoglycan aggrecans in ECM provide tensile strength and compressive elasticity to the articular cartilage. In addition, structure and composition of proteoglycan aggrecans play an important role in its ability to entrap water and nutrition between ECM and synovial fluid via pumping mechanism.

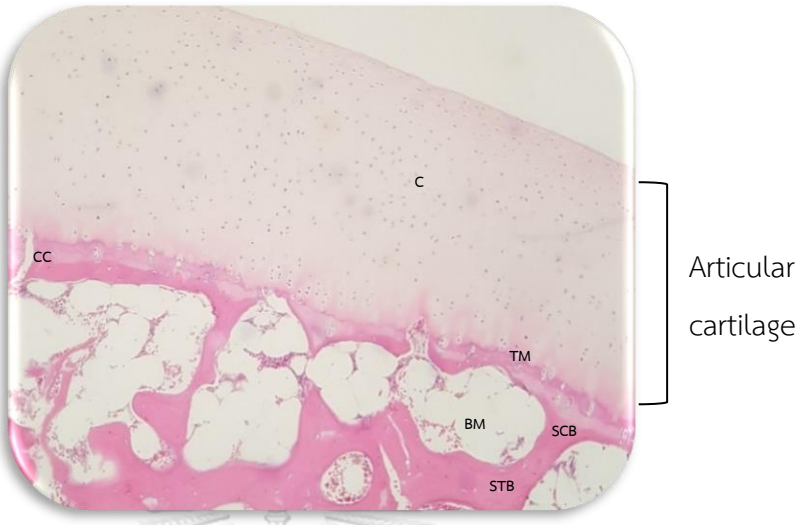


Figure 5 The articular surface of the femoral condylar ridge in normal dog staining with Hematoxylin & Eosin (H&E). Histologically, the main structural compositions of articular cartilage include chondrocytes, tidemark (separating articular cartilage and calcified cartilage), subchondral cortical bone and subchondral trabecular bone. C, Chondrocyte; CC, Calcified cartilage; TM, Tidemark; SCB, Subchondral cortical bone; STB, Subchondral trabecular bone; BM, Bone marrow.

The proteoglycan aggrecans synthesized and secreted by chondrocyte are composed of aggrecan, hyaluronic acid (HA) and link protein (LP). The proteoglycan monomer comprises first (G1) and second (G2) globular domain. One side of the proteoglycan attaches to HA and the other side attaches to core protein, which also binds with chondroitin (CS), and keratan sulphate (KS) chain (figure 6). The relative proportion of CS and KS in ECM of cartilage and collagen network plays an important role in maintenance osmotic or swelling pressure of cartilage (Maroudas, 1979; Poole et al., 1994).

Subchondral bone is a plate-like layer of thin cortical bone adjacent to cancellous bone situated underneath articular cartilage. Bone structure and ECM are composed with osteoblastic cells which synthesize new osteoid matrix until calcified to new bone, and osteoclastic cells which decalcify and resorb bone in response to local biomechanical stimuli, soluble mediators and systemic hormonal factors (Martel-

Pelletier et al., 2016). The subchondral bone plate has arterial, venous vessels and nerves that they are visible as tiny branches in the ZCC and it has ability to remodeling and plays a major role in articular cartilage metabolism. The important function of subchondral bone plate and trabecular structure is provided support and absorb most mechanical forces from weight-bearing which transmits across the articular cartilage (Madry et al., 2010; Martel-Pelletier et al., 2016).

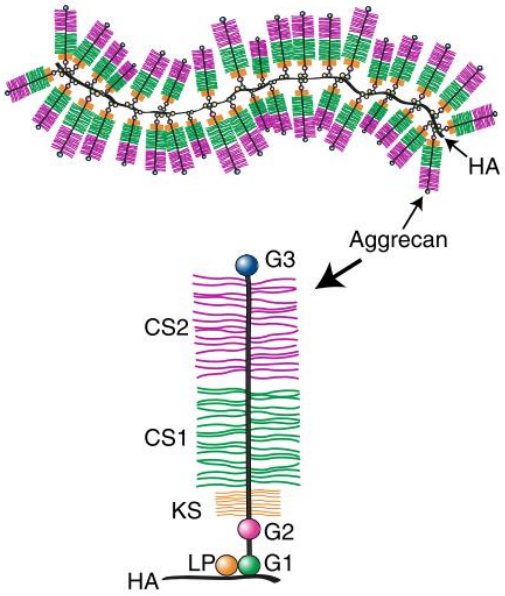


Figure 6 The structure of proteoglycan aggrecans composed of a central hyaluronan (HA) with aggrecan and link protein. The domains of aggrecan core protein are indicated as G1, G2 and G3 globular regions, keratan sulphate-rich domain (KS), and chondroitin sulphate 1 and 2-rich domain. (From (Roughley and Mort) : the role of aggrecan in normal and osteoarthritic cartilage, 2014.)

2.3.1 Pathophysiology of OA

The pathophysiology of OA associates with alterations of the tissue adjacent the joint. The major pathological changes are articular cartilage damage and degeneration, extensive metabolic change and sclerotic of subchondral bone, osteophyte or enthesophyte formation and synovitis (Innes, 2012b). Several studies have been focused on OA research including animal model of OA induced by cranial cruciate transection and by medial patellar luxation, and clinically spontaneous CCLR and medial patellar luxation (McDevitt et al., 1977; Matyas et al., 1999; Matyas et al., 2004; Garner et al., 2011; BENNETT and BAUER, 1937; Alam et al., 2011a; Alam et al., 2011b; Faldyna et al., 2004; Lemburg et al., 2004; Muir et al., 2005; Fujita et al., 2006; de Bruin et al., 2007; Maccoux et al., 2007; Frost-Christensen et al., 2008; Garner et al., 2011; Muir et al., 2011; Little et al., 2014; Gibbons et al., 2006).

Synovitis is synovial inflammation characterized by the infiltration of inflammatory cells mainly mononuclear cells predominantly with T lymphocytes, hypertrophy and hyperplasia from proliferation of synovial lining cells (figure 7) (Innes, 2012b). Previous studies reported that lymphocytes, plasma cells, fibroblast, macrophages and dendritic cell increased when compared to normal synovial tissue (Klocke et al., 2005; Bleedorn et al., 2011). Increasing macrophage density detected by immunohistochemistry of CD11 and CD18 antibodies staining illustrated that the osteoarthritic change of the stifle with CCLR may induce by macrophage (Klocke et al., 2005). Increased proportion of $CD4^+$ and $CD8^+$ T lymphocytes and $CD3^+CD4^-CD8^-$ T lymphocytes has been investigated in synovial tissue of dogs with CrCLR compared to healthy Beagles suggested that $CD3^+CD4^-CD8^-$ T lymphocytes was related to development of stifle synovitis and arthritis over time (Bleedorn et al., 2011). An experimental model of osteoarthritis by transected cranial cruciate ligament showed abnormal cartilage, bone, synovium and menisci in 1-2 weeks after operation whereas macroscopic changes were obviously seen in more than 2 weeks (McDevitt et al. (1977).

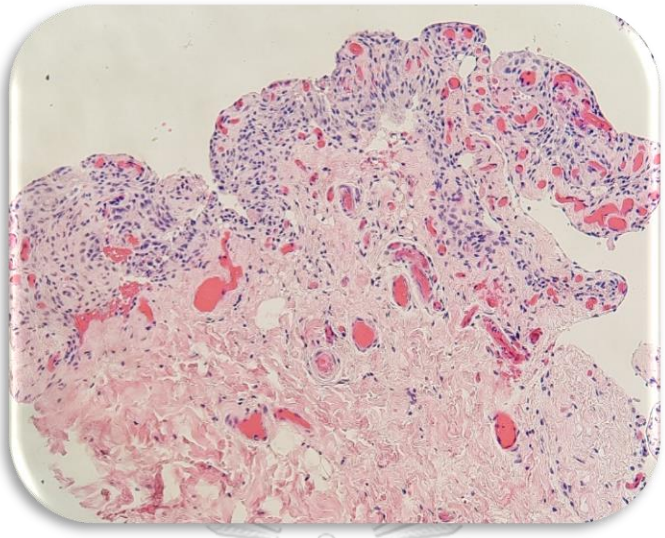


Figure 7 The synovial membrane of the stifle joint from MPL dogs reveal 1-2 cells thickness of intimal layer with moderate lymphocytic cells infiltration in synovial intimal and subintimal layer with moderate increase in vascularity and some area had perivascular mononuclear cells infiltration. (H&E stain. Original magnification 400X).

In human, the synovial tissue of OA presented the most abundant of macrophages and T cells, lower numbers of mast cells, B cells and plasma cells, and less numbers of natural killer cells and dendritic cells while none of neutrophil was seen. Another study found infiltration of several inflammatory cells including 65% of macrophages in the lining layer, 22% of T cells outstandingly in the sub-lining layer and some in the deep layer, 5% of B cells and < 1% of plasma cells in the synovium (Pessler et al. (2008). Helper-T (Th) cell population can be differentiated as Th1, Th2 and Th17 along with regulatory cells (Th3 and Tr1) and Th1 cell was seen more frequent than Th17 cells (de Lange-Brokaar et al., 2012).

Pro-inflammatory cytokines are synthesized by inflammatory cells in the synovium, synoviocytes, and chondrocytes, for instance, interferon-gamma (IFN-γ), tumor necrosis factor-alpha (TNF-α), IL-1β, IL-6, and IL-8 which are potentiate the cascade of biological active substances such as matrix metalloproteinases (MMPs: MMP-1, MMP-2, MMP-3, etc.), cathepsins, and tartate-resistance acid phosphatase

(TRAP) resulting in synovitis and intraarticular cartilage destruction (Fujita et al., 2006; Alam et al., 2011b; Bleedorn et al., 2011). The pro-inflammatory cytokines in synovial fluid and gene expression in immune system in synovial fluid and synovial tissue can be used as biological markers for osteoarthritic change. In recent reports, mRNA expression of pelleted cells in synovial fluid was determined and the result showed cathepsin-K, MMP-9, TRAP, and invariant chain(li) significantly increased in the stifle with CrCLR compared to healthy dogs and other forms of arthritis (Muir et al., 2007). In addition, synovial fluid of dogs with CCLR and MPL found mRNA expression of IL-8, IL-10, and IFN-γ significantly different between arthropathic and normal joints (de Bruin et al., 2007b).

IFN-γ and TNF-α are important effectors of Th1 lymphocytes and activated macrophages, respectively. These cytokines play an important role in development and continuation of synovitis (Little et al., 2014). The study of OA in canine MPL found significantly increased in TRAP and MMP-2 level and significantly decreased in TIMP-2 in synovial fluid at 3 months and in serum at 6 months after induction. In addition, these sustainably elevated in the entire period of experiment except MMP-2 significantly increased only at 3 months (Alam et al., 2011b). In cartilage matrix breakdown of synovitis, collagenolytic activity in pathologic chondrocytes and synovium correlated with degree of synovitis evaluating from histological grading (Pelletier et al., 1985).

The secondary OA induced by MPL was successful tool as experimental model used in dogs and rabbits. Induced patellar displacement in rabbits resulted in articular-cartilage hypertrophy, degeneration, atrophy and erosion with subsequent eburnation of the subchondral bone similar to degenerative joint disease (Bennett and Bauer, 1937). In congenital medial patellar luxation, cartilage erosion was frequently seen on the disto-medial side of patellar surface in all grades of MPL but large defect significantly found in grade 4 MPL and heavy dogs (Daems et al., 2009). In dogs with OA induced by MPL, clinical signs were found at 3 months and radiographic changes were detected at 6 months after surgery. Macroscopic lesions of OA indicated cartilage changes with increase in opacity and thickening than that in normal joint which include

partial thickness of cartilage erosion at 6 months and osteophyte formation at 12 months as well as CCL fibrous thickening and defibrillation were inspected. Histological appearance revealed focal disruption, fissure and fibrillation on articular cartilage and infiltration of mononuclear reactive cells markedly increased in the synovial and CCL tissue (Alam et al., 2011a).

CHAPTER III

MATERIALS AND METHODS

3.1 Animals

Dogs presented at the Small Animal Teaching Hospital, Faculty of Veterinary Science. Chulalongkorn University were evaluated for the patellar instability according to orthopedic examination (Piemattei et al., 2006). General physical and neurological examination were also observed. Small breed dogs with age from 6 months to 6 years old and weight from 1.5 to 5 kilograms without sex predilection were included in this study. The owners signed in the consent form to allow their dogs to involve this study. Animal age, sex, weight and breed were recorded. Each stifle was recorded for duration and grade of patellar luxation. Lameness score was given before surgical correction as described by Hazewinkel et al. (2008) (table 1). The dogs (n=38 stifles) were divided into 2 groups.

Table 1 Lameness scoring system (Hazewinkel et al., 2008)

Lameness score	Description
0	No lameness
1	Mild lameness; normal at a walk with mild lameness at a trot
2	Moderate lameness; consistent lameness at a walk with pronounced lameness at a trot
3	Severe lameness; toe-touching to some weight bearing at a walk and non-weight bearing trot
4	Non-weight bearing lameness

Group 1: 33 dogs (n = 34 stifles) with MPL were recruited into this group. Stifle joints with history of surgery, lateral patellar luxation (LPL), bidirectional patellar luxation (BPL) and MPL concurrent with CCLR were excluded from this study. The severities of patellar luxation were classified into 4 grades according to Singleton (1969) canine patellar luxation classification system (table 2). Grades 2, 3 and 4 MPL were 12, 13 and 9 stifle joints, respectively.

Table 2 Grading system of medial patellar luxation [modified from Singleton (1969)]

Grade	Description
1	The patella typically sits in the normal position. Manual pressure at the patella in the medial direction can luxate it to the medial side and when pressure is released, it spontaneously reduces to the normal position.
2	The patella is in or out of the trochlear groove during stifle joint flexion, extension, or internal and external rotation of the limb and spontaneously reduces to the normal position.
3	The patella permanently luxates to the medial side of femoral trochlear groove, can be manually reduced into the trochlear groove and re-luxates when pressure is released.
4	The patella permanently luxates to the medial side of the femoral trochlear groove and cannot be reduced to the trochlear groove.

Group 2: Normal stifle joints (n = 4 stifles) used as control dogs were recruited from dogs which died of others disease unrelated to this study and had normal stifle joints diagnosed from survey radiography and medical record. Dogs with displacement of patellar bone or osteoarthritis in radiography, including osteophyte formation, joint effusion, and subchondral bone cyst formation, and the stifle joints with degenerative changes from gross examination were excluded.

3.2 Radiography

All stifle joints were examined on the radiographs for osteophyte formation. Two standard orthogonal radiographs (FCR CAPSULA V VIEW workstation®) of the stifle joint were done under general anesthesia before surgery. In cranio-caudal radiographic view, the x-ray beam was centered at the stifle joint including the distal third of femur and the proximal third of tibia. In medio-lateral radiographic view, the x-ray beam was centered just behind the femoral condyles and covered between distal third of femur and the proximal third of tibia. The radiographs were accepted when the femoral condyles superimpose (De Bruin et al., 2007a). Four locations on the cranio-caudal radiographic view including medial and lateral femoral condyles, and medial and lateral edges of the tibial plateau and six locations on the medio-lateral radiographic view including proximal and distal poles of patella, proximal and distal trochlear ridges, and cranial and caudal edges of the tibial plateau were scored (figures 8-9). Osteophyte formation were scored from 0 (absent) to 3 (severe) (table 3) (Frost-Christensen et al., 2008; Wessely et al., 2017). Total osteophyte score was used for statistical analysis (maximum score 30).

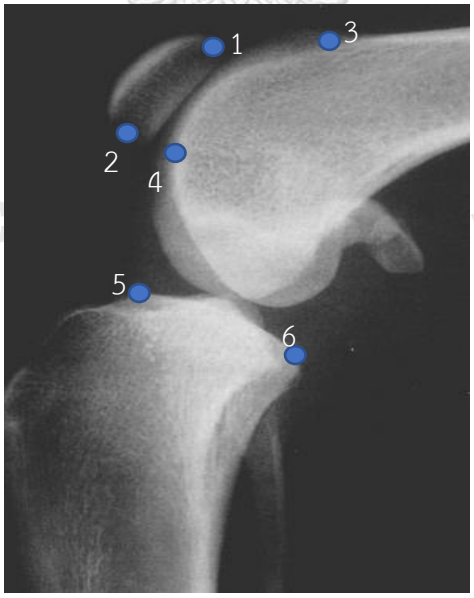


Figure 8 The assessment points of the stifle joint in medio-lateral radiographic view. (1 = proximal pole of patella, 2 = distal pole of patella, 3 = proximal trochlear ridge, 4 = distal trochlear ridge, 5 = cranial aspect of tibial plateau, 6 = caudal aspect of tibial plateau).

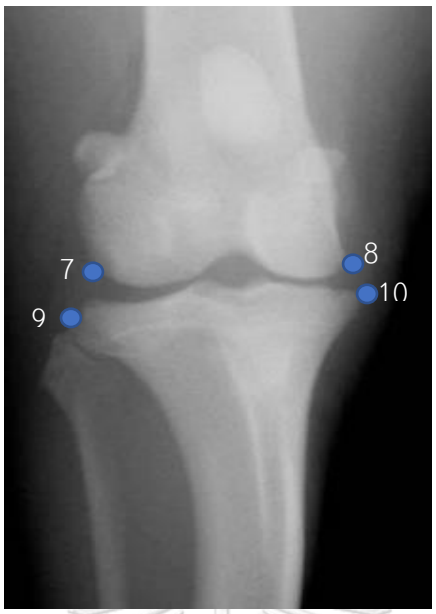


Figure 9 The assessment points of the stifle joint in cranio-caudal radiographic view. (7 = lateral femoral condyle, 8 = medial femoral condyle, 9 = lateral tibial condyle, 10 = medial tibial condyle).

Table 3 The radiographic scores of osteophyte formation (Frost-Christensen et al., 2008; Wessely et al., 2017).

Score	Severity	Radiographic changes
0	Absent	No evidence of osteophyte formation or subchondral sclerosis
1	Mild	Mild osteophyte formation with/without subchondral sclerosis
2	Moderate	Moderate osteophyte formation with/without subchondral sclerosis
3	Severe	Severe osteophyte formation with/without subchondral sclerosis

3.3 Anesthetic protocols and postoperative care

Blood from all dogs was collected for complete blood counts (CBC) and standard blood chemistry profile including alanine amino transferase (ALT), alkaline phosphatase (ALP), blood urea nitrogen (BUN), creatinine (Cr), total protein (TP), and

albumin (Alb) to assess health status before surgery. Food and water must be withheld for 12 and 6 hours, respectively before premedication. Acepromazine maleate (0.02 mg/kg) and morphine sulphate (0.5 mg/kg) were intramuscularly administrated as premedication. Propofol (4-6 mg/kg) was intravenously administered to induce general anesthesia that was maintained with Isoflurane in 100% oxygen. Prophylactic cefazolin (25mg/kg) was administered intravenously. Epidural nerve block was performed using a combination of bupivacaine (1mg/kg) and morphine (0.1 mg/kg). Cephalexin sodium (25 mg/kg) and Carprofen (2.2 mg/kg) were given twice a day for 5 and 10 days as postoperative antibiotics and as postoperative pain control and anti-inflammation, respectively.

3.4 Fluid and tissue samples

3.4.1 Synovial fluid samples and biomarker assay

Synovial fluid was collected by arthrocentesis with 23 gauge needle 1 inch length and 3 ml syringe at lateral to the stifle joint and parallel to the patellar ligament, when the joint in slightly extension (figure 10) before routinely cranio-lateral approach to the joint as described by Johnson (2014). The sample was contained in 1.5 ml Eppendorf without anticoagulant. One drop of the sample was used for fresh smear on a glass slide, air dried and fixed with 90% methanol for 1 minute then air dried again. Giemsa solution was prepared from giemsa stock 1 ml diluted with distilled water 9 ml. After that, slides were stained with giemsa solution for 25 minutes and washed out with clean water. The number of differential cell populations was manually counted at the mid of synovial fluid smear using a serpentine pattern until 100 cells counted (figure 11). The number of cells in each counted field were recorded and all counted field was reported as percentage (Dusick et al., 2014). Sample was

centrifuged (KUBOTA 1300®) at 15,000 rpm, 4 °c for 10 minutes (de Bruin et al., 2007b) to separate pellet and supernatant. Supernatants were preserved in -80°C until assay.

Figure 10 Stifle arthrocentesis, the needle is introduced lateral to the patella and advanced in a medial and proximal direction toward the medial condyle of the femur.

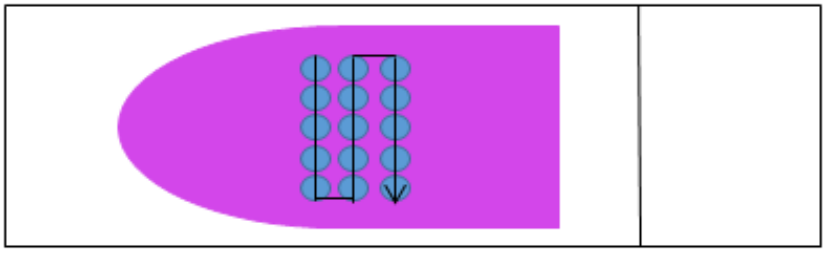


Figure 11 The serpentine pattern for differential cell count of synovial fluid smear. [Modified from Dusick et al. (2014)].

The supernatant was analyzed for cytokine expression by using a magnetic-bead Luminex (mLuminex) cytokine 8-plex including TNF-α, IFN-ɣ, IL-6, IL-8, IL-10, IL-18, MCP-1 and GM-CSF. Protocol was followed according to the manufacturer's instructions of panel kit. Canine cytokine panel standard was prepared by reconstituting canine cytokine panel standard with 250 µl of deionized water. The

cytokine panel was mixed by inverting the vial and vortex for 5-10 seconds and used as standard 7. Then standards 6, 5, 4, 3, 2, and 1 were prepared by adding 150 µL of assay buffer to each of six polypropylene microfuge tubes. Performed 4-fold serial dilutions by adding 50 µL of the standard 7 to the standard 6, and transferred 50 µL of the standard 6 to the standard 5, the standard 6 to the standard 5, the standard 5 to the standard 4, the standard 4 to the standard 3, the standard 3 to the standard 2, and the standard 2 to the standard 1. Every step of 4-fold serial dilutions made well mix. The standard 0 (background) was assay buffer (figure 12). After serial dilution, the tubes should have the following concentrations for constructing standard curves. The synovial fluid sample was thawed in room temperature (25 °C) and centrifuged at 10,000 g for 10 minutes. The supernatant of each sample was incubated with hyaluronidase 2 mg/ml in working concentration (Merck, USA) 1:1 volume mixture at 37 °C for 60 minutes (Alam et al., 2011b; Garner et al., 2011; Orita et al., 2011; Jayadev et al., 2012). The samples were centrifuged at 1,000 g for 5 minutes and the supernatant was used for the assay. The immune assay procedure was performed by adding 200 µL of assay buffer into each well of 96-well plate. The plate was sealed and mixed on shaker for 10 minutes at room temperature (20-25°C). The assay buffer was removed by attracting the plate with handheld magnet and then added 25 µL of each standard or control into the appropriate wells. Each well was added 25 µL of assay buffer. 25 µL of appropriate matrix solution was added to the background, standard, and control wells. 25 µL of prepared synovial fluid samples was added into the appropriate wells. Duplication of each sample was running in the same plate. 25 µL of the Mixed Beads was added to each well. The plate was sealed with a plate sealer and wrapped with foil and incubated with agitation on a plate shaker for 2 hours at room temperature (20-25 °C). The well contents were gently removed and the plate was washed 3 times by using wash buffer. 25 µL of detection antibodies was added into each well. Then, the plate was sealed and covered with foil and incubated with agitation on a plate shaker for 1 hour at room temperature (20-25°C). 25 µL of

Streptavidin-Phycoerythrin was added to each well containing the 25 µL of detection antibodies. The plate well contents were gently removed and washed plate 3 times by using wash buffer. Then 150 µL of drive fluid was added to each well. Finally, the beads were resuspended on a plate shaker for 5 minutes at room temperature and the plate was run on Luminex Magpix. The unit of cytokine level was reported in picogram/milliliter.

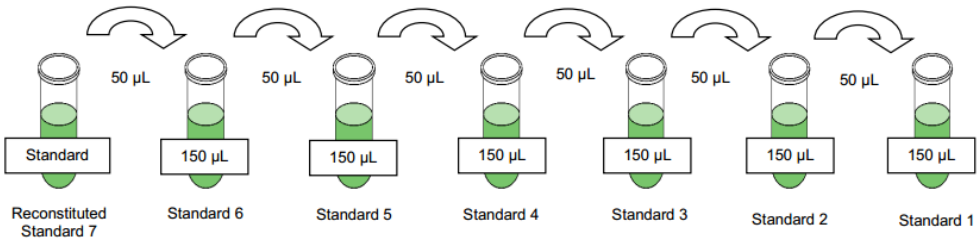


Figure 12 The process of 4-fold dilution of canine cytokine panel standard used as a standard curve for calculating cytokine concentration (from manufacturer's instruction manual, 2013).

3.4.2 Synovial membrane samples and histology

Synovial membrane was collected at the lateral and proximal edges of incision arthrotomy in all groups and fixed with 10% buffered formalin for 24 hours. Then the synovial membrane was histologically processed and embedded in paraffin, cut into 3 µm thick sections, and stained with hematoxylin and eosin (H&E) as described by LEE (1986). The tissue sections were deparaffinized in xylene 3 times for 10 minutes each, then hydrated tissue by used absolute and 95% ethyl alcohol 2 times for 5 minutes in each concentration and wash out alcohol with running tap water 5 minutes, then stain with Harris's hematoxylin for 6-10 minutes and wash in running tap water again. The exceed Harris's hematoxylin was eliminated by dipping 1% acid alcohol (1% conc. HCl in 70% alcohol) for 3 times and immediately wash acid out in running tap water, then neutralize tissue in saturated lithium carbonate for 5 seconds and running tap

water. Slides were incubated in distilled water and 95% ethyl alcohol for 1 minute, respectively and then stained in Eosin for 2-3 minutes. The exceed eosin color was removed by soak in 95% ethanol for 30 seconds, 2 times and absolute ethanol for 1 minute, 3 times respectively. The sections were incubated in xylene 3 times for 3, 5, and 10 minutes respectively. In the final process, complete H&E staining slides were mounted with glass slides by mounting medium.

Table 4 Microscopic grading of synovial changes (modified from Cook et al. (2010))

Severity of histopathological criteria	severity
Lining cells characteristics	
A: 1-2 layers of cells	0
B: 3-6 layers of cells	1
C: >6 layers of cells	2
Lining characteristics	
A: No villous hyperplasia	0
B: short villi	1
C: Finger-like hyperplasia	2
Cell infiltration characteristics	
A: No cellular infiltration	0
B: Mild inflammatory cell infiltrates	1
C: Moderate inflammatory cell infiltrates without lymphoid follicles	2
D: Severe, diffuse inflammatory cell infiltrates including lymphoid follicles	3
Vascularity	
A: < 5 cross sectionals of capillaries	0
B: 5-29 cross sectionals of capillaries	1
C: 30-50 cross sectionals of capillaries	2
D: >50 cross sectionals of capillaries	3

Severity scores are given as; 0 = normal, 1= mild, 2 = moderate, 3= severe.

When complete histological grading from table 4 in each category, total score from 3-5 randomly fields was reported as mean total severity of synovial changes (a maximum of 10) and the total score was used for statistical analysis.

Histological assessment based on The OsteoArthritis Research Society International (OARSI) (Cook et al., 2010). The samples were blinded and evaluated by a single pathologist. Synovial membrane with H&E stain, synovial cells characteristics, lining characteristics, cellular infiltration characteristics and vascularity were graded using 40x objective lens from criteria as following the criteria show in table 4.

3.5 Macroscopic morphology

The stifle joint was examined the gross pathologic features. Five locations of femoral condyle including medial and lateral trochlear ridges, lateral, middle and medial portions of proximal femoral condyles as well as lesions on patellar articular surface (figure 13) were photographed with high-resolution image during surgical arthrotomy. The gross lesions including the location of synovial membrane changes, cartilage hypertrophy, cartilage erosion and osteophyte formation were evaluated by single observer to avoid unaware of the source of the photographs (SJ). The affected stifle joint was scored as described in table 5.

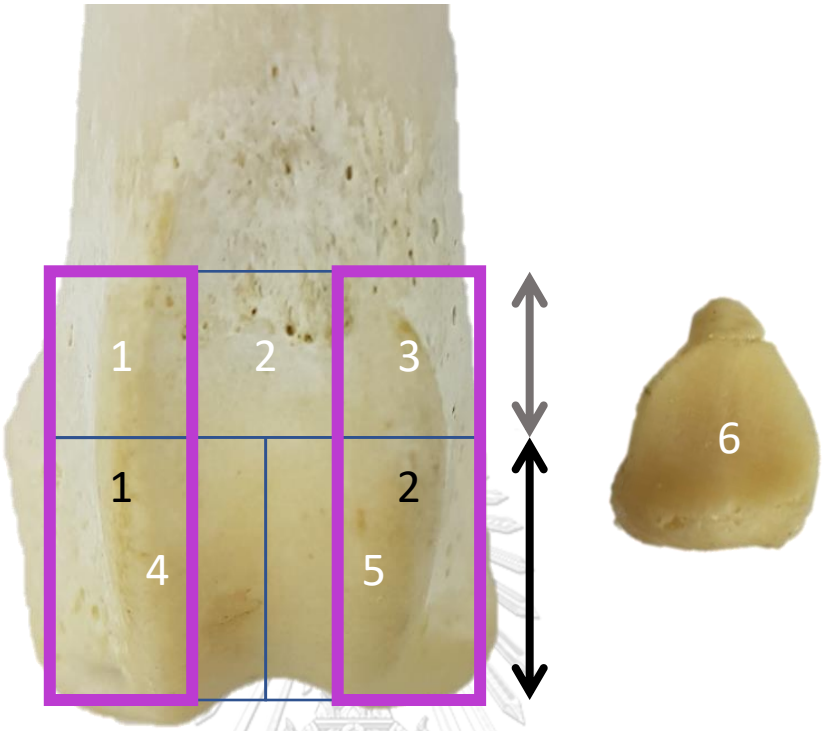


Figure 13 Schematic representation of right femoral condyle and patella articular surface. Each box represents anatomic region. Blue boxes are used for cartilage score and purple boxes for synovial score and osteophyte formation score. (1= proximo-lateral articular surface, 2= proximo-middle articular surface, 3= proximo-medial articular surface, 4= lateral articular surface, 5= medial articular surface, 6= patellar articular surface).

Table 5 Macroscopic morphology grading system

Macroscopic morphology	grading
Synovial membrane characteristics	
Angiogenesis	
A: None	0
B: Slightly	1
C: Strong	2
Articular cartilage characteristic	
A: Smooth surface - Outbridge 0	0
B: Slightly fibrillated/roughened surface – Outbridge 1	1
C: Fibrillated surface with focal partial thickness lesions – Outbridge 2	2
D: Deep lesions with surrounding damage – Outbridge 3	3
E: Large areas of severe damage – Outbridge 4	4
Osteophyte formation characteristics[a]	
A: Normal	0
B: Solitary growth	1
C: Confluent growth	2

Note: Gross morphology grading system (Matyas et al., 2004; Mastbergen et al., 2006; Cook et al., 2010).

[a] Development along the rim of the femoral trochlea.

Severity scores are given as; 0 = normal, 1= mild, 2 = moderate, 3 = severe, 4 = most severe.

3.6 Data presentation and statistical analysis

Sex and breed were reported as descriptive statistic. The Kolmogorov-Smirnov test with Dallal-Wilkinson-Lilliefor P value (KS test) was used to determine the parametric variables for normal distribution. Age, weight, lameness score, duration of disease, severity of patellar luxation, radiographic score, differential cell counts, histopathologic score, MCP-1, and synovial, cartilage, osteophyte and OA scores were reported as mean ± SD or median (range). The Kruskal-Wallis test was used to analyze the non-parametric variables and the parametric variables that were not normally distributed, and the differences were determined by Dunn's correction for multiple comparison test. One-way analysis of variance (ANOVA) was used to analyze parametric variables and the differences were determined by Tukey's correction for multiple comparison test.

The correlation of pathological parameters (gross morphology grading, histologic grading, synovial fluid smear and synovial fluid biomarker) and clinical parameter (body weight, lameness score, degree of patellar luxation, age at surgery, duration of lameness and radiographic score) of normal and MPL groups were assessed by spearman's rank test or Pearson's correlation. The Pearson's correlation was used to analyze normally distributed parametric variables. Statistical analysis used the statistical package Graphpad Prism 6 program (PISM® ver. 6.01, GraphPad, Inc). The results were considered statistically significant at a p-value < 0.05.

CHAPTER IV

RESULTS

4.1 Animals

Thirty-seven dogs (38 stifle joints) were recruited and categorized into normal and MPL groups. The normal group comprised 4 healthy stifle joints (2 left hind limbs and 2 right hind limbs) from 4 mixed breed dogs including 2 males and 2 females which met the inclusion criteria. The median age was 5.7 months (range 6 to 24 months) and the median body weight was 3.7 kilograms (range 3.5 to 4.5 kilograms). The MPL group consisted of 34 stifle joints (33 dogs) including 18 and 16 stifle joints of the left and right hind limbs, respectively. Luxation was bilateral in 32 dogs (96.97%) and unilateral in 1 dog (3.03%). Only one stifle joint was used for analysis except a dog with bilateral MPL grade 4. The severities of medial patellar luxation in these 34 stifle joints were grades 2, 3, and 4 in 12, 13 and 9 stifle joints, respectively. The dogs were 21 (63.63%), 8 (24.24%), 1 (3.03%), and 3 (9.1%)Pomeranians, Chihuahuas, Yorkshire terriers and mixed breed, respectively. Of 33 dogs, 21 (63.64%) were female and 12 (36.36%) were male. The means (±SD) of body weight, age, duration of disease and lameness score, and medians (range) of the lameness score of the dogs with normal and each grade of MPL stifle groups are shown in table 6. Medians of body weight (range) of dogs with grades 2, 3 and 4 MPL were 2.8 (1.6-5.1), 3.5 (2.2-4.9), and 2.6 (2.3-3.8) kilograms, respectively. Medians of age (range) of dogs with grades 2, 3, and 4 MPL were 12 (2.2-50), 27.5 (8-96), and 21 (8-67) months, respectively. Medians of the duration of disease (range) with grades 2, 3, and 4 MPL were 3 (0.67-9), 2.5 (1-6), and 1 (0.5-5) months, respectively. The age and body weight of dogs were not significantly different among the normal and MPL groups. There were significant differences in duration of disease between the normal and grades 2 and 3 MPL groups (p = 0.002) and in lameness score between normal group and grades 3 and 4 MPL (p = 0.001).

Table 6 Means (±SD) of body weight, age at surgery, duration of disease and medians (range) of lameness score of dogs with normal and each grade of MPL stifle groups.

Demographic data	Normal	MPL II	MPL III	MPL IV	*p*-value
Body weight (kg)	3.9±0.53	3.15±1.13	3.33±0.87	2.82±0.48	0.08*
Age at surgery (month)	15±7.75	25.31±21.12	37.33±27.59	21.67±18.05	0.31*
Duration of disease (month)	0^{a}	$3.36±2.4^{b}$	$3.08±1.93^{b}$	$2.02±1.71^{ab}$	0.002**
Lameness score	0^{b}	1^{ab} (0-2)	2^{a} (0-3)	2^{a} (2-3)	0.001**

^{a,b}Values with different superscript letters in the same row are significantly different ($p < 0.05$).

**p*-value obtained from one-way ANOVA.

***p*-value obtained from Kruskal-wallis test.

Correlation was found between degree of patellar luxation and lameness score ($r_s = 0.59$, $p < 0.001$). The correlations of the demographic data (body weight, age at surgery, and duration of disease) with degree of patellar luxation and lameness score were not found (table 7).

Table 7 Correlation of demographic data (body weight, age at surgery, duration of disease) with degree of patellar luxation and lameness score of dogs with normal and MPL stifle groups.

	Body weight		Age at surgery		Duration of disease		Lameness score	
	r_s	*p*-value	r_s	*p*-value	r_s	*p*-value	r_s	*p*-value
Degree of PL	-0.26	0.13	0.24	0.15	0.13	0.44	0.59	<0.001
Lameness score	-0.22	0.19	0.12	0.47	0.02	0.92		

PL, patellar luxation.

4.2 Radiography

Radiography of stifle joints was performed under generalize anesthesia in 2 orthogonal views (medio-lateral view and cranio-caudal view). Medians (range) of the radiographic score in normal, grades 2, 3, and 4 MPL stifle group are shown in figure

14. The radiographic score was not significantly different among the normal and all grades of MPL groups ($p = 0.45$). In cranio-caudal view, radiographs illustrated osteophyte formation mostly on the lateral femoral condyle (LFC) in all grades of MPL [20.59% (7/34)] and on the medial femoral condyle (MFC) [2.94% (1/34)]. However, osteophyte formation was not found on the lateral (LTC) and medial tibial condyles (MTC). In medio-lateral view, radiographs revealed osteophyte formation on the distal pole of the patella (DPa), proximal trochlear sulcus (PTS), distal trochlear sulcus (DTS), cranial tibial plateau (CrTP) and caudal tibial plateau (CdTP). Osteophyte formation was not found on the proximal pole of the patella (PPa). The frequency of osteophyte formation are shown in table 8 and the radiographs on medio-lateral and cranio-caudal views of a dog with medial patellar luxation are shown in figure 15.

Table 8 The frequency of osteophyte formation in each grade of MPL on cranio-caudal and medio-lateral views.

	Cranio-caudal view (n= 34)				Medio-lateral view (n= 34)					
	LFC	MFC	LTC	MTC	PPa	DPa	PTS	DTS	CrTP	CdTP
MPL II	15.38% (2/13)	0% (0/13)	0% (0/13)	0% (0/13)	0% (0/13)	30.77% (4/13)	0% (0/13)	7.69% (1/13)	0% (0/13)	0% (0/13)
MPL III	25.00% (3/12)	8.00% (1/12)	0% (0/12)	0% (0/12)	0% (0/12)	0% (0/12)	8.00% (1/12)	0% (0/12)	8.00% (1/12)	8.00% (1/12)
MPL IV	22.22% (2/9)	0% (0/9)	0% (0/9)	0% (0/9)	0% (0/9)	0% (0/9)	0% (0/9)	0% (0/9)	11.11% (1/9)	11.11% (1/9)
Total	20.59% (7/34)	2.94% (1/34)	0% (0/34)	0% (0/34)	0% (0/34)	11.76% (4/34)	2.94% (5/34)	2.94% (1/34)	5.88% (2/34)	5.88% (2/34)

LFC, lateral femoral condyle; MFC, medial femoral condyle; LTC, lateral tibial condyle; MTC, medial tibial condyle; PPa, proximal pole of patella; DPa, distal pole of patella; PTS, proximal trochlear sulcus; DTS, distal trochlear sulcus; CrTP, cranial tibial plateau, and CdTP, caudal tibial plateau.

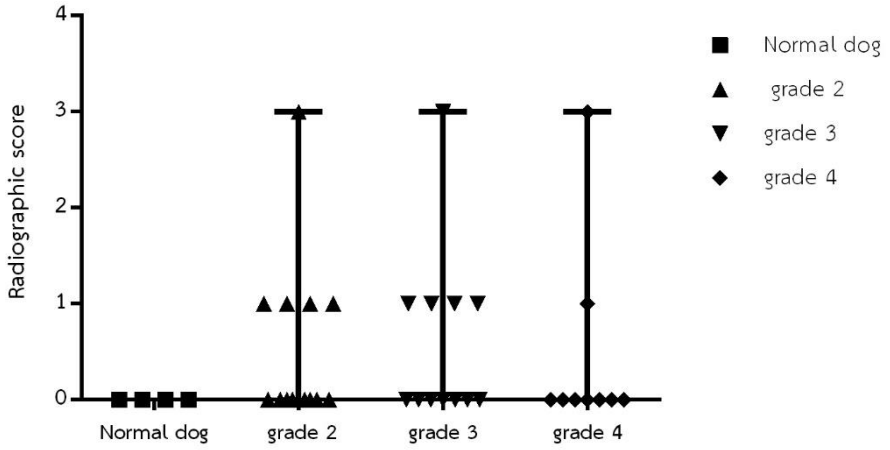


Figure 14 Radiographic score of normal, grades 2, 3, and 4 MPL stifle groups with median and range.

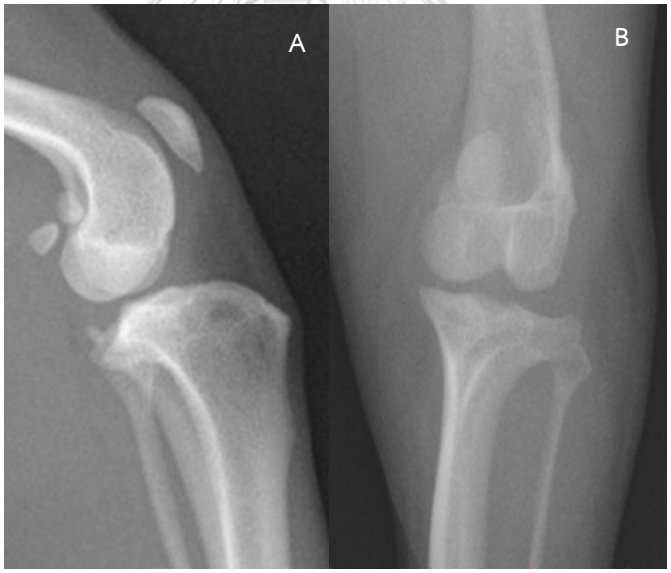


Figure 15 Radiographs of a dog with medial patellar luxation. In medio-lateral view (A), mild osteophyte formation is presented (score = 1) at the distal pole of the patella. In cranio-caudal view (B), mild osteophyte formation (score = 1) is presented on the lateral femoral condyles.

The correlations of radiographic score with demographic data, degree of patellar luxation and lameness score were not found (table 9). The correlations of radiographic score with differential cell counts and MCP-1 are shown in table 11.

Table 9 The correlation of radiographic score with demographic data (body weight, age at surgery, duration of disease), degree of patellar luxation, and lameness score between of normal and MPL groups.

	Body weight		Age at surgery		Degree of PL		D.O.D.		Lameness score	
	r_s	*p*-value	r_s	*p*-value	r_s	*p*-value	r_s	*p*-value	r_s	*p*-value
Radio score	-0.21	0.21	0.26	0.11	0.05	0.77	0.12	0.49	-0.04	0.83

PL, patellar luxation; Radio, radiographic; D.O.D., duration of disease.

4.3 Fluid and tissue samples

In fresh smear of synovial fluid, the percentages of differential cell count were not significantly different between normal and each grade of MPL stifle groups. The percentages of differential cell count including monocyte, foam cell, lymphocyte, neutrophil, synoviocyte, and monocyte chemoattractant protein-1 (MCP-1) are presented as mean±SD (table 10). The MCP-1 was mainly cytokine and was found in normal and MPL stifles. In addition, granulocyte-monocyte colony stimulating factor (GM-CSF), interleukin-6 (IL-6), and interleukin-8 (IL-8) were presented only in a few stifle joints that could not be used for analysis. The remaining chemokines and cytokines (TNF-alpha, IFN-gamma, IL-10, IL-18) were below the limit of detection. Cells in synovial fluid are shown in figure 16.

Table 10 Comparison of monocyte, foam cell, lymphocyte, neutrophil and synoviocyte count from fresh smear of synovial fluid and MCP-1 of normal and each grade of MPL stifle groups.

	Normal (n=4)	MPL 2 (n=13)	MPL 3 (n=12)	MPL 4(n=9)	*p*-value
Monocyte (%)	44.83±11.57	49.31±14.64	55.75±14.81	40.89±9.93	0.12*
Foam cell (%)	8.17±4.36	11.08±10.89	12.92±7.85	17.78±11.30	0.21**
Lymphocyte (%)	25.5±15.92	22.31±13.10	18.17±10.63	18±7.55	0.76*
Neutrophils (%)	0.83±0.75	2.77±3.22	*0.92*±1.00	2.67±3.46	0.17**
Synoviocyte (%)	22.00±11.44	14.54±6.97	11.00±10.39	20.67±7.35	0.08*
MCP-1 (pg/ml)	158.20±102.70	64.11±51.73 (n=8)	114.70±88.20 (n=8)	112.80±75.15 (n=8)	0.27*
HP score	0^{a}	1^{ab} (0-3)	1^{ab} (0-3)	2^{b} (0-3)	0.04**

[a,b] Values with different superscript letters in the same row are represent significantly different ($p < 0.05$). MCP-1, monocyte chemoattractant protein-1; HP, histopathologic.

**p*-value obtained from one-way ANOVA.

***p*-value obtained from kuskal-wallis test.

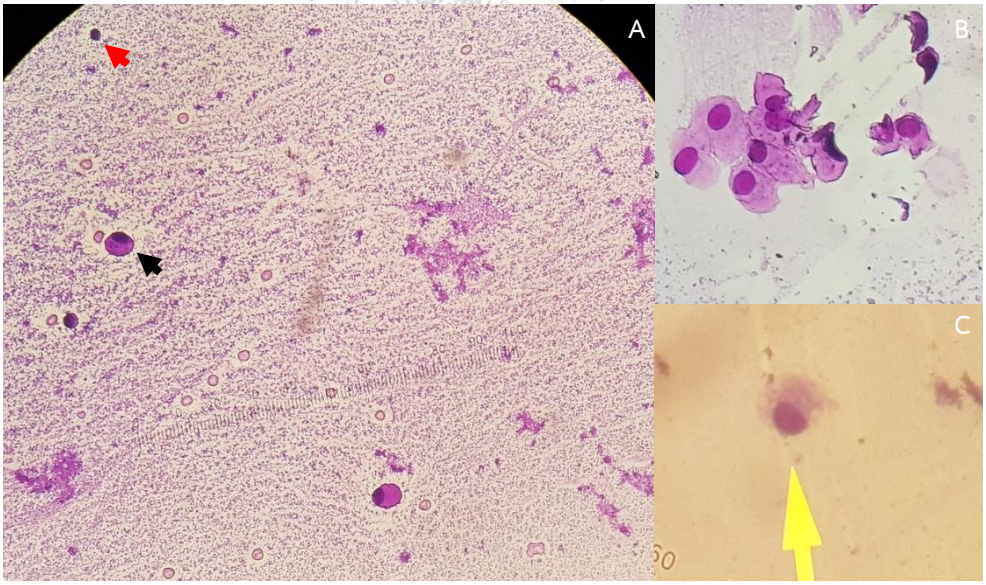


Figure 16 Fresh smear of synovial fluid from a dog with medial patellar luxation. The microscopic finding presents fluid protein observed as pink granular material with the background of RBC, lymphocyte (red arrow) and, macrophage (black arrow) (A). (Giemsa stain. Original magnification 100X). The cluster of synoviocytes or intimal cells with a

few large mononuclear cells (B) and vacuolated mononuclear cell or foam cell (C). (Giemsa stain. Original magnification 400X).

There was no significant difference of MCP-1 between normal group and each grade of MPL. The correlations of differential cell count, MCP-1 and demographic data are shown in table 11. There were correlations between number of foam cells and degree of patellar luxation (r_s = 0.35, p = 0.03), and between synoviocyte and duration of disease (r_s = -0.39, p = 0.01). Histopathologic features of synovial membrane were assessed and scored according to the criteria described in materials and methods. In normal group, synovial membrane presented 1-2 cell layers of synovial intima and residence lymphocyte in synovial intima and subintimal layers. No evidence of inflammatory cell infiltration and villous hyperplasia was found in synovial membrane. In MPL group, most synovial membrane had mostly 1-2 cell layers and some presented 3 cell layers in synovial intima layer without villous hyperplasia. Vascularity mildly increased in some joints of MPL group. Mononuclear cells (lymphocytes were predominated than plasma cells) were mainly inflammatory cells that infiltrated in synovial intima and subintima layers of MPL group. Grades 2 and 3 MPL had normal the mononuclear infiltration of 53.85% (7/13) and 33.33% (4/12), respectively and mildly increased infiltration of 46.15% (6/13) and 66.67% (8/12), respectively. Grade 4 MPL had equally normal, mild and moderate degrees of the mononuclear infiltration of 33.33 % (3/9). The histopathologic score was significantly different between the normal and grade 4 MPL groups (p = 0.04) but was not significantly different among grades of MPL.

Histopathologic score correlated only with degree of patellar luxation (r_s = 0.44, p <0. 01) . The correlations of histopathologic score with demographic data and differential count percentage are shown in table 11. Histologic specimen of synovial membrane are shown in figure 17-19.

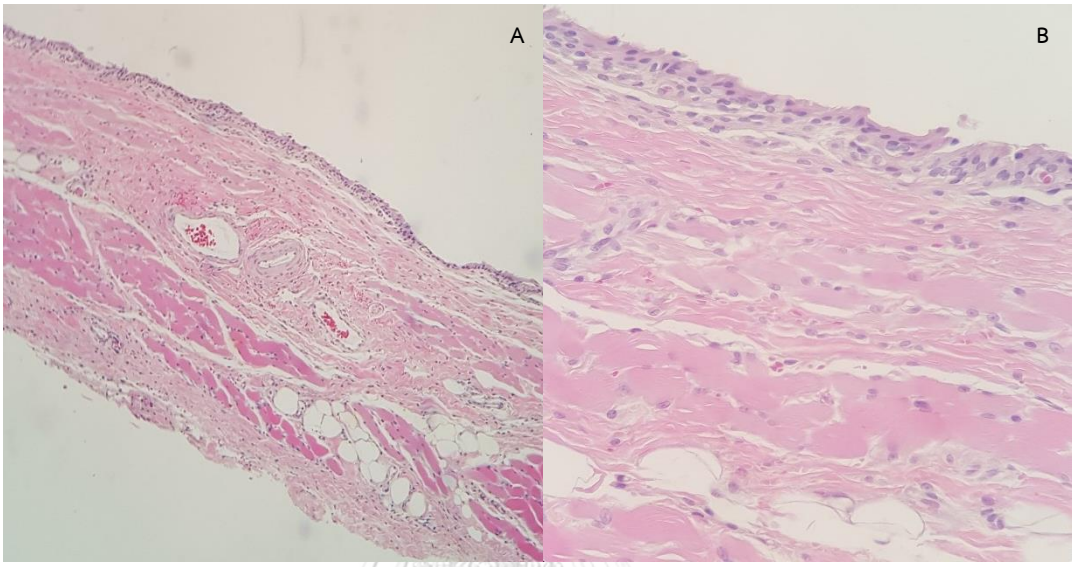


Figure 17 The synovial membrane of a normal dog reveal 1-2 intimal layer with some residence lymphocytes in subintimal layer which is normally found. Total microscopic grading score is 0. (H&E stain. Original magnification 100X (A) and 400X (B)).

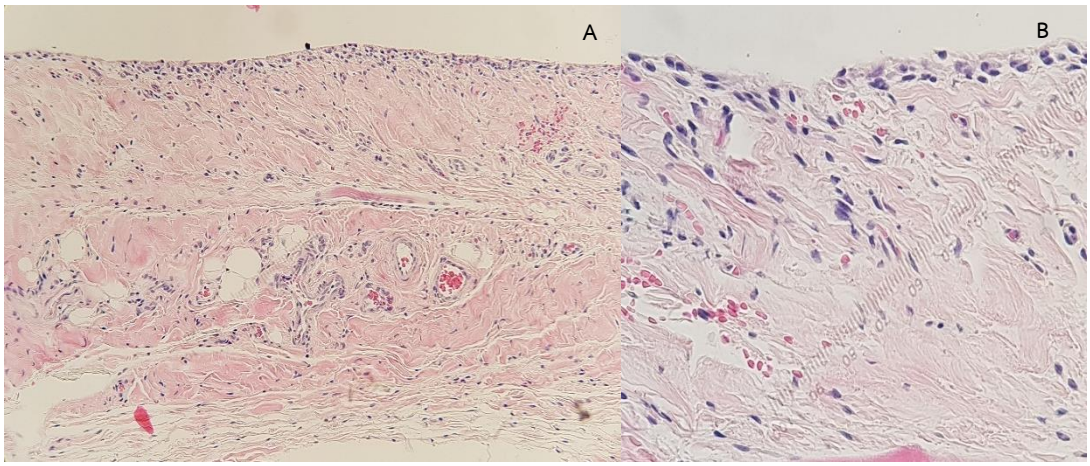


Figure 18 The synovial membrane of a dog with grade 2 medial patellar luxation reveal 1-2 intimal layer with mild lymphocytic cells infiltration in synovial intimal and subintimal layer and mild increase in vascularity. Total microscopic grading score is 2. (H&E stain. Original magnification 100X (A) and 400X (B)).

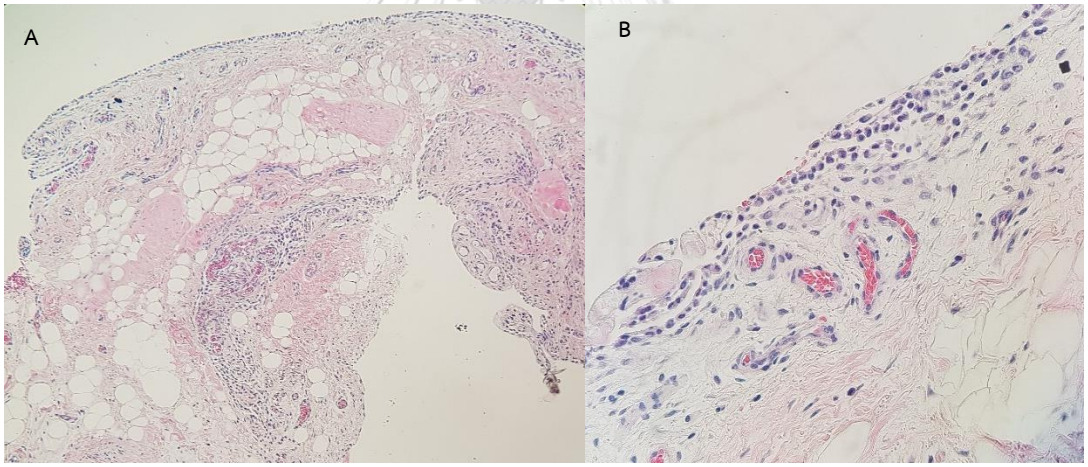


Figure 19 The synovial membrane of a dog with grade 4 medial patellar luxation reveal 1-2 intimal layer with moderate lymphocytic cells infiltration in synovial intimal and subintimal layer without increase vascularity. Total microscopic grading score is 2. (H&E stain. Original magnification 100X (A) and 400X (B)).

Table 11 The correlation of differential count of synovial fluid smear, radiographic score, histopathologic score and demographic data (body weight, age at surgery), duration of disease (D.O.D.), lameness score and degree of patellar luxation of normal and MPL groups.

	Body weight		Age at surgery		D.O.D.		Lameness score		Degree of PL		Radio score		HP score		MCP-1	
	r_s	*p*-value	r_s	*p*-value	r_s	*p*-value	r_s	*p*-value	r_s	*p*-value	r_s	*p*-value	r_s	*p*-value	r_s	*p*-value
Monocyte	0.16	0.35	0.04	0.82	0.24	0.14	-0.11	0.49	-0.14	0.39	0.16	0.34	-0.32	0.05	0.09	0.66
Foam cell	-0.25	0.14	-0.1	0.56	0.10	0.56	0.21	0.21	0.35	0.03	0.10	0.56	0.11	0.52	-0.10	0.61
Lymphocyte	0.29	0.08	-0.14	0.41	-0.04	0.81	-0.12	0.46	-0.08	0.64	-0.27	0.10	0.30	0.07	-0.04	0.84
Neutrophil	-0.12	0.48	-0.07	0.68	0.22	0.19	0.13	0.45	-0.04	0.83	0.16	0.34	-0.20	0.24	0.16	0.42
Synoviocyte	-0.25	0.12	0.002	0.99	-0.39	0.01	0.23	0.16	0.09	0.60	-0.15	0.39	-0.03	0.86	-0.01[p]	0.95[p]
MCP-1	0.27	0.17	0.26	0.18	-0.08	0.67	-0.23	0.23	0.04	0.85	-0.33	0.09	-0.01	0.96		
HP score	-0.21	0.21	-0.11	0.51	0.24	0.15	0.17	0.30	0.44	<0.01	-0.02	0.90			-0.01	0.96

[p]correlation coefficiency and *p*-value obtained from pearson's correlation. D.O.D., duration of disease.

4.4 Macroscopic morphology

Macroscopically, osteoarthritis score (OA score) was evaluated from characteristic of synovial membrane (synovial score), articular cartilage (cartilage score) and osteophyte formation (osteophyte score). There was significant difference in OA score between the normal and each grade of MPL (p = 0.01) groups. The significant differences of synovial score were found between the normal group and grades 3 and 4 MPL and between grades 2 and 4 MPL (p = 0.0002). Cartilage score was significantly different between the normal group and grade 2 MPL (p = 0.005) while osteophyte score was significantly different between the normal group and grade 3 MPL (p = 0.02). Median and range of OA, synovial, cartilage, and osteophyte scores of the normal and MPL groups are shown in table 12. The most frequency distribution of synovial, cartilage and osteophyte scores were on the medial femoral trochlear ridge. All normal stifle joints had normal synovial membrane, and white and shiny articular cartilage without osteophyte formation.

Table 12 Median (range) of synovial, cartilage, osteophyte and OA scores of the normal and MPL groups.

	Normal	MPL II	MPL III	MPL IV
Synovial score	0^{c}	3^{ac} (0-5)	3^{ab} (2-4)	5^{b} (2-6)
Cartilage score	0^{b}	7^{a} (0-12)	3.5^{ab} (1-12)	4^{ab} (0-9)
Osteophyte score	0^{b}	2^{ab} (0-6)	3.5^{a} (0-6)	2^{ab} (0-4)
OA score	0^{b}	12^{a} (3-17)	10^{a} (4-22)	11^{a} (6-16)

^{a,b,c} Values with different superscript letter in the same row are significantly different (p < 0.05).

The gross extent of synovial membrane inflammation, cartilage erosion and osteophyte formation varied from absent to severe changes. Locations of cartilage erosion were mostly on the medial femoral trochlear ridge (73.53%) and patellar articular surface (70.59%). Patellar erosion was found mostly on the latero-distal part but less on the proximal and medial parts. Articular cartilage erosion was severe in grades 2 and 4 MPL. Surface of the proximal aspect of the trochlear ridge appeared

smooth but had white and opaque cartilage more than other areas in some dogs with MPL. In addition, osteophyte formation was found mostly on the medial femoral trochlear ridge. In grades 2 and 3 MPL, the osteophyte formation was seen on the proximal trochlear sulcus. The frequency distribution of OA, synovial, cartilage and osteophyte scores are shown in table 13. Photographs of the gross lesion from normal dogs and dogs with MPL are shown in figure 20-22.

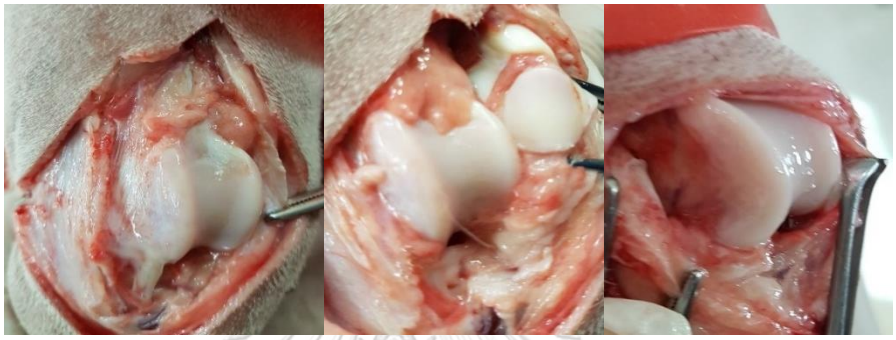


Figure 20 Right stifle joint of a normal dog. There is normal appearance of synovial membrane, white and shiny normal cartilage of both femoral condyles and patellar articular surface.

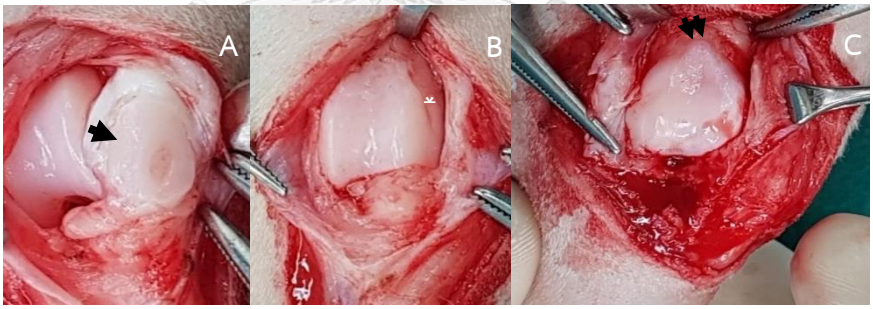


Figure 21 Intra-operative photos of cartilage surface of the patella and the femoral condyle. The patellar surface shows a large area of full thickness cartilage erosion at the disto-lateral part of patella and a large area of cartilage fibrillation (arrow) at medial part of patella (A). The proximo-medial part of femoral trochlear ridge presents a full thickness cartilage erosion (asterisk) exposing the subchondral bone (B). The proximal part of the femoral trochlea has an osteophyte formation (double arrows) with moderate synovitis (C).

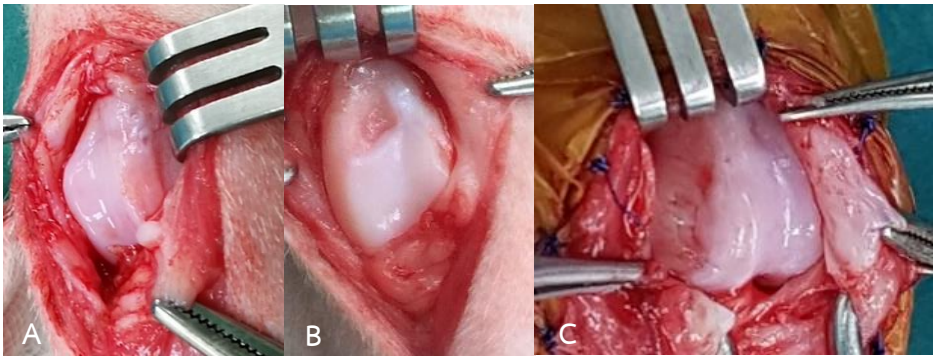


Figure 22 Intra-operative photos of the cartilage surface of the femoral condyle. The medial femoral trochlear ridge presents a large area of full thickness cartilage erosion exposing the subchondral bone (A). The proximal part of femoral trochlear groove shows a large area of full thickness cartilage erosion (B). The large area of depression and damage to the medial femoral condyle occurs from permanent luxation of patella to the medial femoral condyle (C).

Table 13 Frequency distribution of synovial, cartilage, and osteophyte scores on macroscopic morphologic observation of stifle joints in each grade of MPL and total distribution in MPL group.

	Osteoarthritis score (OA score)											
	Synovial score			Cartilage score						Osteophyte score		
	Lateral	Medial	patella	PL	MP	PM	Lat.	Med.	Pat.	Proximal	Lateral	medial
MPL II	61.54% (8/13)	84.62 (11/13)	76.92% (10/13)	7.69% (1/13)	46.15% (6/13)	61.54% (8/13)	0.00% (0/13)	76.92% (10/13)	92.30% (12/13)	38.46% (5/13)	38.46% (5/13)	53.85% (7/13)
MPL III	91.67% *(11/12)*	100% *(12/12)*	100% *(12/12)*	16.67% *(2/12)*	75% *(9/12)*	16.67% *(2/12)*	8.33% *(1/12)*	66.67% *(8/12)*	66.67% *(8/12)*	66.67% *(8/12)*	58.33% *(7/12)*	66.67% *(8/12)*
MPL IV	88.89% *(8/9)*	100% *(9/9)*	100% *(9/9)*	22.22% *(2/9)*	22.22% *(2/9)*	0% *(0/9)*	0% *(0/9)*	77.78% *(7/9)*	44.44% *(4/9)*	11.11% *(1/9)*	22.22% *(2/9)*	55.56% *(5/9)*
Total	79.41% *(27/34)*	97.06% *(33/34)*	91.18% *(31/34)*	14.71% *(5/34)*	50.00% *(17/34)*	14.71% *(5/34)*	2.94% *(1/34)*	73.53% *(25/34)*	70.59% *(24/34)*	41.18% *(14/34)*	41.18% *(14/34)*	58.82% *(20/34)*

PL, proximo-lateral part of articular surface; MP, proximo-middle part of articular cartilage; PM, proximo-medial part of articular cartilage; Lat, lateral; Med, medial and Pat, patella.

The synovial score was significantly correlated with degree of patellar luxation (r_s = 0.72, p < 0.0001), lameness score (r_s = 0.42, p = 0.01), foam cell number (r_s = 0.35, p = 0.03), and histopathologic score (r_s = 0.42, p = 0.0001). The cartilage score was correlated with duration of disease (r_s = 0.38, p = 0.02), age at surgery (r_s = 0.43, p = 0.01) radiographic score (r_s = 0.40, p = 0.01), and neutrophil number (r_s = 0.37, p = 0.02). Furthermore, OA score was correlated with duration of disease (r_s = 0.37, p = 0.02), age at surgery (r_s = 0.39, p = 0.02), radiographic score (r_s = 0.42, p = 0.01), and synovial score (r_s = 0.39, p = 0.02). Correlations of OA score, synovium score, cartilage score, osteophyte score with demographic data's, differential cell counts, MCP-1, radiographic score and histopathologic score are shown in table 14.

Table 14 Correlations of synovial score, cartilage score, osteophyte score, OA score with duration of disease (D.O.D.), body weight, degree of patellar luxation, lameness score, age at surgery, radiographic score, differential count percentage, MCP-1 and histopathologic score of the normal and MPL groups.

	Synovial score		Cartilage score		Osteophyte score		OA score	
	r_s	p-value	r_s	p-value	r_s	p-value	r_s	p-value
D.O.D (mth)	0.28	0.09	0.38	0.02	0.28	0.09	0.37	0.02
BW. (kg)	-0.32	0.05	-0.14	0.41	0.25	0.14	-0.10	0.57
Degree of PL	0.72	<0.01	0.05	0.75	0.2	0.23	0.24	0.15
Lame score	0.42	0.01	0.08	0.62	0.15	0.37	0.18	0.28
Age at surgery	0.06	0.71	0.43	<0.01	0.15	0.35	0.41	0.01
Radio score	0.17	0.30	0.40	0.01	0.23	0.17	0.33	0.04
Monocyte	-0.20	0.22	-0.11	0.51	0.27	0.1	-0.07	0.66
Foam cell	0.35	0.03	0.08	0.62	-0.11	0.51	0.14	0.40
lymphocyte	-0.12	0.49	0.004	0.98	0.06	0.74	0.01	0.97
Neutrophil	0.12	0.47	0.37	0.02	-0.03	0.84	0.30	0.07
synoviocyte	-0.04	0.83	0.04	0.82	-0.10	0.58	0.02	0.88
MCP-1	-0.05	0.79	-0.38	0.05	-0.14	0.46	-0.32	0.10
HP score	0.58	<0.01	0.12	0.49	0.04	0.81	0.22	0.19

OA, osteoarthritis; D.O.D., duration of disease; BW, body weight; PL, patellar luxation; MCP-1, monocyte chemoattractant protein-1; HP, histopathologic; radio, radiographic; Lame, lameness.

CHAPTER V

Discussion

Medial patellar luxation is one of the most common orthopedic disease in dogs leading to gradually develop secondary OA due to patellar instability and abnormal extensor mechanism (Bennett and Bauer, 1937; Hulse, 1981; Roush, 1993; Alam et al., 2011a; Alam et al., 2011b). The significant difference in body weight was not found between the normal and MPL groups. The body weight was not correlated with OA score (synovial, cartilage and osteophyte scores). In contrast with the finding of Daems et al. (2009), body weight had positive correlation with the percentage of cartilage erosion on patellar articular surface in congenital MPL in various breeds (small to giant breeds). In human study, elevated body weight increased the risk of knee OA progression because of the excess load from increased weight (Felson et al., 2004). In small breed dogs, the body weight is less likely to cause lameness and OA progression.

The duration of disease positively correlated with OA score and cartilage score. Cartilage destruction and osteoarthritis progressively develop over the time which is consistent with other reports (Bennett and Bauer, 1937; Alam et al., 2011a). The duration of disease was the period from the first visiting to the date of surgery. However medial patellar luxation often develops in early of life and most owners do not recognize this abnormality until they present signs of lame or are diagnosed for MPL. Therefore, the duration of disease does not indicate real period of disease occurrence but it might present duration of lameness which showed some correlation with OA and cartilage scores. To achieve the accurate duration of disease, patellar luxation screening should be routinely performed. However, lameness is clinical sign of OA, this correlation may represent a coincidence between duration of lameness and progression of OA. In addition, duration of disease negatively correlated with synoviocyte. Normally, the synoviocytes are found in synovial tissue but the relation between duration of disease and the number of synoviocyte was not reported. Moreover, morphology of the synoviocyte is similar to monocyte and makes it difficult

to distinguish. Therefore, staining with sudan black should be used to identify monocyte (Villanueva and Schumacher, 1987). Synovitis is an early stage of OA which may have proliferation of synoviocyte in the synovial fluid only in the early stage.

The degree of PL moderately correlated with lameness, and histopathologic scores, and highly correlated with synovial score. The finding of correlation between the degree of PL and lameness score is similar to previous studies (Gibbons et al., 2006; Wangdee et al., 2013). The correlation also found between lameness score and synovial score. There was significant difference in lameness score between the normal and grades 3 and 4 MPL groups. In addition, the correlation was found between the synovial and histopathologic scores. MPL causes joint instability from abnormal patellofemoral articulation, and malalignment of quadriceps mechanism reducing range of motion of the stifle joint causing joint stiffness, synovitis, and secondary osteoarthritis (Hulse, 1981; Alam et al., 2011a). The more severe PL is, the more impairment quadriceps mechanism occurs causing more joint inflammation representing in macroscopic and microscopic changes of synovial tissue.

Synovial fluid analysis is one of the useful tools for diagnosis of joint diseases both in dogs and human including rheumatoid arthritis (RA), crystal-induced arthritis (gout arthritis), septic arthritis, and OA. However, the diagnosis should be made from history, clinical signs, joint radiography and physical examination (Villanueva and Schumacher, 1987; Gibson et al., 1999).

The foam cell is vacuolated or activated monocyte which is activated by foreign materials such as chemical, microorganism, and dead cells, afterward it recruits augmentative macrophages in response to inflammatory signals (Murray and Wynn, 2011). Normally, foam cells are found over 10% of the differential cell counts in osteoarthritis. Increase in the percentage of foam cells was related to the severity of patellar luxation and synovial score. The severity of patellar luxation leads to synovial inflammation resulting in degenerative process and activation of local immune response seen as elevation of foam cells.

There was no significant difference in differential cell counts among the normal and all grades of MPL groups similar to a previous report (de Bruin et al., 2005). The previous study indicated direct smear of synovial fluid was more sensitive and specific

for identifying inflammatory arthritis but it was inaccurate method for estimating and differentiating cell count in the normal and degenerative groups. In addition, it cannot discriminate between immune-mediated and infective disease, or between normal joints and joints with osteoarthritis (Gibson et al. (1999). Osteoarthritis is classified to non-inflammatory arthropathy, so cytology of synovial fluid is normal to slightly increase in total nucleated cell count predominantly with large mononuclear cells (Villanueva and Schumacher, 1987; Fernandes, 2008). There are several methods to analyze synovial fluid including total nucleated cell count, mucin clot test, and total protein concentration but a small volume of synovial fluid from our patients is not enough for these analyses.

In osteoarthritis, neutrophil is absent or increases but it is usually within the reference limits (Fernandes, 2008) (Pedersen, 1978). The percentage of neutrophils was positively correlated with cartilage score; however, it is in normal range. This correlation could be a co-incidental finding with severity of cartilage lesion or it could be due to severity of cartilage lesion affecting on to the inflammation, resulting in increased migration of neutrophil into the inflammatory site. Only MCP-1 expressed in the synovial fluid in all joints but there was no significant difference in MCP-1 among the normal and all grades of MPL groups, while GM-CSF, IL-6, and IL-8 are found only in a few joints with low concentration which cannot be used for calculation. Previous study in animal model of OA found that IL-6 was expressed in high level in the early stage of OA and also found high correlation between the concentration of IL-6 and the stimulation of cartilage proteoglycan biosynthesis. The result suggested that IL-6 may play a role in mediating response to cartilage injury (Venn et al., 1993). In human, the concentration of IL-6 was significantly lower in Kellgren and Lawrence grades (KL grade) 3 and 4 than in KL grades 1 and 2 and IL-6 was negatively correlated with KL grading. IL-6 had an important role in OA progression and IL-6 activity increased in the earlier phases of OA preventing cartilage destruction (Orita et al., 2011). In addition, mRNA expressions of IL-8, IL-10 and IFN-ɣ were found in 100 %, 62.5 % and 25 %, respectively, of medium to large breed dogs with MPL which were different from normal dogs (de Bruin et al., 2007b). IL-8 is one of cytokine using for detecting OA with high sensitivity and specificity (Garner et al., 2011). The contrast might be due to the different severity

of OA between small and large breed dogs. The effect of excess load in the large breed dogs leads to progression of OA (Daems et al., 2009; Chomdej et al., 2016). Monocyte chemoattractant protein-1 (MCP-1) is one of the C-C chemokine family which is produced by various cell types including monocytes, endothelial cells, fibroblasts, keratinocytes, mesangial cells and synovial cells. The primary biologic function of MCP-1 is to promote the migration of monocytes and lymphocytic T cells (Carr et al., 1994; Yuan et al., 2001). The comparative induced OA by cranial cruciate ligament transection, total meniscectomy and creation of full-thickness grooves in the cartilage of the weight-bearing portion of the medial femoral condyle found that MCP-1 was significantly increased only in cranial cruciate transection at 12 weeks after surgery compared with baseline and non-operated joints. In addition, MCP1 may be used as diagnostic biomarker of OA with high sensitivity and specificity and used in evaluating treatment efficacy (Garner et al., 2011). Although recent study showed that MCP-1 had high sensitivity and specificity to detect OA, it cannot differentiate OA from the normal joint in this study. It might be because of small sample size in this present study or less severe or in different stage of OA of MPL in small breed dogs. Increase in the sample size or reorganize stage of OA may found more cytokines in the synovial fluid. No villous hyperplasia and hypertrophy found in synovial intima of synovial membrane both in most dogs with MPL and normal dogs. Mild mononuclear cell infiltration in the subintimal layer of most dogs with MPL but was seen less in normal dogs similar to a previous report (Frost-Christensen et al., 2008).

The radiographic score was not significantly different among the normal and all grades of MPL groups. The correlations were found between radiographic score and cartilage score, and between radiographic score and OA score. The early stage of degenerative changes in osteoarthritis of dogs suffering from MPL is difficult to detect from radiography while macroscopic changes can be observed. At the later stages, progression of OA can be seen on radiographic changes as well as on macroscopic changes including subchondral sclerosis and osteophyte formation which agree with previous reports of OA induced by cranial cruciate ligament transection and MPL (Matyas et al., 2004; Alam et al., 2011a). Although, survey radiography of joint has low

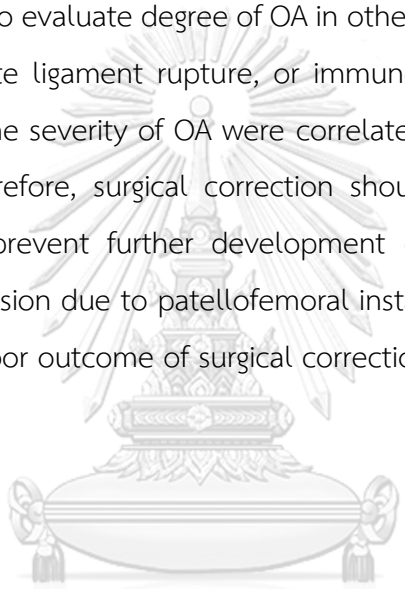
sensitivity to detect an early stage of OA but it is still a standard tool for diagnosing and monitoring OA (Innes et al., 2004).

The present study found positive correlation between OA score and age at surgery, and between cartilage score and age at surgery similar to other reports (Roy et al., 1992; Chomdej et al., 2016). In contrast with the finding of Daems et al. (2009), age at surgery did not correlate with severity of OA. We found that OA score was the same in all grades of MPL. However, synovial score in grade 4 MPL was more severe than in grades 2 and 3 MPL, but cartilage in grade 2 MPL had more erosion than in other grades. Osteophyte formation in grade 3 MPL was more than in grades 2 and 4 MPL. The possibility is cause and progressive OA in differences severity of MPL dogs occurring due to mal-tracking of patellofemoral joint during stifle movement as well as MPL results in joint instability from medial deviation of the patella from the femoral trochlear groove leading to synovitis and osteoarthritis. The more severe PL is, the more impairment quadriceps mechanism and the misarticulating of patellofemoral joint compress and stress to synovial tissue causing more joint inflammation. In grade 2 MPL dogs, the consequence of repetitive luxation of the patella out of the trochlear groove from normal articulation and stress across the articular cartilage surface caused damage and erosion of the cartilage (Bennett and Bauer, 1937; Hulse, 1981; Daems et al., 2009; Alam et al., 2011a; Chomdej et al., 2016). The continue progression of PL for a period of time will cause more cartilage erosion followed by osteophyte formation which could be seen more in grade 3 MPL. Osteophyte can develop from periosteum and synovium as well; however, the trigger of this process is unknown (Bennett and Bauer, 1937; McDevitt et al., 1977).

In this study, the most area of cartilage erosion on the patella was on the latero-distal part while the less areas were on the proximal and medial parts of the patella. In contrast with the findings of others, the most area of patellar erosion were on the distal and medial parts (Daems et al., 2009), and on the lateral and medial parts (Chomdej et al., 2016). The most area of femoral trochlear erosion in this study was on the medial trochlear ridge which was in agreement with previous report (Chomdej et al., 2016). During locomotion of the hind limb, patella glides within the trochlear sulcus upward to medial trochlear ridge, and luxates from the trochlear ridge

during joint extension. The greatest force occurs during lateral surface of the patella contact to medial trochlear ridge when luxated or reduced patella to the medial trochlear ridge.

In conclusion, all grades MPL in small breed dogs can cause stifle OA. The severity of OA is not related to the severity of PL. Radiographic score was correlated with cartilage score and OA score. Furthermore, only MCP-1 found in synovial fluid but no correlations of MCP-1 with other parameters were found. MPL in small breed dogs is less aggressive than that in large breed dogs and in dogs with CrCLR. The comparative study should be done to evaluate degree of OA in other joint diseases such as patellar luxation, cranial cruciate ligament rupture, or immune mediated joint disease. The cartilage erosion and the severity of OA were correlated with the duration of disease and age of dogs. Therefore, surgical correction should be performed as soon as possible in order to prevent further development of degenerative joint disease. Preexisting cartilage erosion due to patellofemoral instability could cause progression of OA leading to the poor outcome of surgical correction.



REFERENCES

Alam M, Lee H, Kim M and Kim N 2011a. Surgical model of osteoarthritis secondary to medial patellar luxation in dogs. Vet Med - Czech. 56(3): 123-130.

Alam MR, Ji JR, Kim MS and Kim NS 2011b. Biomarkers for identifying the early phases of osteoarthritis secondary to medial patellar luxation in dogs. J Vet Sci. 12(3): 273-280.

Arthurs GI and Langley-Hobbs SJ 2006. Complications Associated with Corrective Surgery for Patellar Luxation in 109 Dogs. Vet Surg. 35(6): 559-566.

Baltzer AW, Moser C, Jansen SA and Krauspe R 2009. Autologous conditioned serum (Orthokine) is an effective treatment for knee osteoarthritis. Osteoarthr. Cartil. 17(2): 152-160.

Bennett GA and Bauer W 1937. Joint changes resulting from patellar displacement and their relation to degenerative joint disease. J Bone Joint Surg Br. 19(3): 667-682.

Bleedorn JA, Greuel EN, Manley PA, Schaefer SL, Markel MD, Holzman G and Muir P 2011. Synovitis in dogs with stable stifle joints and incipient cranial cruciate ligament rupture: a cross-sectional study. Vet Surg. 40(5): 531-543.

Carr MW, Roth SJ, Luther E, Rose SS and Springer TA 1994. Monocyte chemoattractant protein 1 acts as a T-lymphocyte chemoattractant. Proc. Natl. Acad. Sci. U.S.A. 91(9): 3652-3656.

Cashmore RG, Havlicek M, Perkins NR, James DR, Fearnside SM, Marchevsky AM and Black AP 2014. Major complications and risk factors associated with surgical correction of congenital medial patellar luxation in 124 dogs. Vet Comp Orthop Traumatol. 27(04): 263-270.

Chomdej S, Ongchai S and Nganvongpanit K 2016. Prevalence of cartilage erosion in canine patellar luxation and gene expression in affected joints.

Cook JL, Kuroki K, Visco D, Pelletier JP, Schulz L and Lafeber FP 2010. The OARSI histopathology initiative - recommendations for histological assessments of osteoarthritis in the dog. Osteoarthr. Cartil. 18 Suppl 3: S66-79.

Daems R, Janssens LA and Beosier YM 2009. Grossly apparent cartilage erosion of the patellar articular surface in dogs with congenital medial patellar luxation. Vet Comp Orthop Traumatol. 22(3): 222-224.

De Bruin T, De Rooster H, Bosmans T, Duchateau L, Van Bree H and Gielen I 2007a. Radiographic assessment off the progression of osteoarthrosis in the contralateral stifle joint off dogs with a ruptured cranial cruciate ligament. Vet Rec. 161(22): 745-750.

de Bruin T, de Rooster H, van Bree H and Cox E 2005. Interleukin-8 mRNA expression in synovial fluid of canine stifle joints with osteoarthritis. Vet. Immunol. Immunopathol. 108(3–4): 387-397.

de Bruin T, de Rooster H, van Bree H, Duchateau L and Cox E 2007b. Cytokine mRNA expression in synovial fluid of affected and contralateral stifle joints and the left shoulder joint in dogs with unilateral disease of the stifle joint. Am J Vet Res. 68(9): 953-961.

de Lange-Brokaar BJE, Ioan-Facsinay A, van Osch GJVM, Zuurmond AM, Schoones J, Toes REM, Huizinga TWJ and Kloppenburg M 2012. Synovial inflammation, immune cells and their cytokines in osteoarthritis: a review. Osteoarthr. Cartil. 20(12): 1484-1499.

Dusick A, Young KM and Muir P 2014. Relationship between automated total nucleated cell count and enumeration of cells on direct smears of canine synovial fluid. Vet. J. 202(3): 550-554.

Felson DT, Goggins J, Niu J, Zhang Y and Hunter DJ 2004. The effect of body weight on progression of knee osteoarthritis is dependent on alignment. Arthritis Rheumatol. 50(12): 3904-3909.

Fernandes PJ 2008. Synovial fluid analysis. In: Diagnostic cytology and hematology of the dog and cat. ed. RDT Rick L. Cowell, James H. Meinkoth, Dennis B. DeNicola (ed). 195-214.

Fossum TW 2013. Diseases of the joints. In: Small animal surgery. fourth ed. TW Fossum, CW Dewey, CV Horn, AL Johnson, MC M., RM G., KS Schulz, and WM D. (ed). 1353-1362.

Frost-Christensen LN, Mastbergen SC, Vianen ME, Hartog A, DeGroot J, Voorhout G, van Wees AM, Lafeber FP and Hazewinkel HA 2008. Degeneration, inflammation, regeneration, and pain/disability in dogs following destabilization or articular cartilage grooving of the stifle joint. Osteoarthr. Cartil. 16(11): 1327-1335.

Fujita Y, Hara Y, Nezu Y, Schulz KS and Tagawa M 2006. Proinflammatory Cytokine Activities, Matrix Metalloproteinase-3 Activity, and Sulfated Glycosaminoglycan Content in Synovial Fluid of Dogs with Naturally Acquired Cranial Cruciate Ligament Rupture. Vet Surg. 35(4): 369-376.

Garner BC, Stoker AM, Kuroki K, Evans R, Cook CR and Cook JL 2011. Using animal models in osteoarthritis biomarker research. The journal of knee surgery. 24(4): 251-264.

Gibbons SE, Macias C, Tonzing MA, Pinchbeck GL and McKee WM 2006. Patellar luxation in 70 large breed dogs. J Small Anim Pract. 47(1): 3-9.

Gibson NR, Carmichael S, Li A, Reid SW, Normand EH, Owen MR and Bennett D 1999. Value of direct smears of synovial fluid in the diagnosis of canine joint disease. Vet Rec. 144(17): 463-465.

Hayes AG, Boudrieau RJ and Hungerford LL 1994. Frequency and distribution of medial and lateral patellar luxation in dogs: 124 cases (1982-1992). J Am Vet Med Assoc. 205(5): 716-720.

Hazewinkel HAW, van den Brom WE, Theyse LFH, Pollmeier M and Hanson PD 2008. Comparison of the effects of firocoxib, carprofen and vedaprofen in a sodium urate crystal induced synovitis model of arthritis in dogs. Res Vet Sci. 84(1): 74-79.

Hulse DA 1981. Pathophysiology and management of medial patellar luxation in the dog. Veterinary medicine, small animal clinician : VM, SAC. 76(1): 43-51.

Innes JF 2012a. Arthritis. In: Veterinary Surgery Small Animal. ed. D Karen M. Tobias, MS, DACVS and V Spencer A. Johnston, DACVS (ed). 1078-1111.

Innes JF 2012b. Arthritis. In: Veterinary Surgery Small Animal. ed. KM Tobias and SA Johnston (ed). W.B. sauders. 1078-1111.

Innes JF, Costello M, Barr FJ, Rudorf H and Barr AR 2004. Radiographic progression of osteoarthritis of the canine stifle joint: a prospective study. Vet Radiol Ultrasound. 45(2): 143-148.

Jayadev C, Rout R, Price A, Hulley P and Mahoney D 2012. Hyaluronidase treatment of synovial fluid to improve assay precision for biomarker research using multiplex immunoassay platforms. J Immunol Methods. 386(1): 22-30.

Johnson KA 2014. Piermattei's Atlas of Sugical Approaches to the Bone and Joints of the Dog and Cat. fifth ed. In. v.

Johnston SA 1997. Osteoarthritis. Joint anatomy, physiology, and pathobiology. Vet Clin North Am Small Anim Pract. 27(4): 699-723.

Klocke NW, Snyder PW, Widmer WR, Zhong W, McCabe GP and Breur GJ 2005. Detection of synovial macrophages in the joint capsule of dogs with naturally occurring rupture of the cranial cruciate ligament. Am J Vet Res. 66(3): 493-499.

Kowaleski MP, Boudrieau RJ and Pozzi A 2012. Stifle joint. In: Veterinary Surgery Small Animal. ed. (ed). W.B. Saunders. 906-998.

LEE GL 1986. Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology. third ed. In: Blakiston Division, McGraw-Hill.

Little JP, Bleedorn JA, Sutherland BJ, Sullivan R, Kalscheur VL, Ramaker MA, Schaefer SL, Hao Z and Muir P 2014. Arthroscopic assessment of stifle synovitis in dogs with cranial cruciate ligament rupture. PloS one. 9(6): e97329-e97329.

Madry H, van Dijk CN and Mueller-Gerbl M 2010. The basic science of the subchondral bone. Knee Surg Sports Traumatol Arthrosc. 18(4): 419-433.

Maroudas A 1979. Physicochemical properties of articular cartilage. Adult articular cartilage. 215-290.

Martel-Pelletier J, Barr AJ, Cicuttini FM, Conaghan PG, Cooper C, Goldring MB, Goldring SR, Jones G, Teichtahl AJ and Pelletier J-P 2016. Osteoarthritis. Nat Rev Dis Primers. 2: 16072.

Mastbergen SC, Marijnissen AC, Vianen ME, Zoer B, van Roermund PM, Bijlsma JW and Lafeber FP 2006. Inhibition of COX-2 by celecoxib in the canine groove model of osteoarthritis. Rheumatology (Oxford, England). 45(4): 405-413.

Matyas JR, Atley L, Ionescu M, Eyre DR and Poole AR 2004. Analysis of cartilage biomarkers in the early phases of canine experimental osteoarthritis. Arthritis Rheum. 50(2): 543-552.

McDevitt C, Gilbertson E and Muir H 1977. An experimental model of osteoarthritis; early morphological and biochemical changes. J Bone Joint Surg Br. 59(1): 24-35.

Muir P, Schaefer SL, Manley PA, Svaren JP, Oldenhoff WE and Hao Z 2007. Expression of immune response genes in the stifle joint of dogs with oligoarthritis and degenerative cranial cruciate ligament rupture. Vet Immunol Immunopathol. 119(3–4): 214-221.

Murray PJ and Wynn TA 2011. Protective and pathogenic functions of macrophage subsets. Nat Rev Immunol. 11: 723.

Oegema TR, Carpenter RJ, Hofmeister F and Thompson RC 1997. The interaction of the zone of calcified cartilage and subchondral bone in osteoarthritis. Micros Res Tech. 37(4): 324-332.

Orita S, Koshi T, Mitsuka T, Miyagi M, Inoue G, Arai G, Ishikawa T, Hanaoka E, Yamashita K, Yamashita M, Eguchi Y, Toyone T, Takahashi K and Ohtori S 2011. Associations between proinflammatory cytokines in the synovial fluid and radiographic grading and pain-related scores in 47 consecutive patients with osteoarthritis of the knee. BMC Musculoskelet Disord. 12: 144.

Pedersen NC 1978. Synovial fluid collection and analysis. Vet Clin North Am. 8(3): 495-499.

Pelletier JP, Martel-Pelletier J, Ghandur-Mnaymneh L, Howell DS and Woessner JF, Jr. 1985. Role of synovial membrane inflammation in cartilage matrix breakdown in the Pond-Nuki dog model of osteoarthritis. Arthritis Rheum. 28(5): 554-561.

Pessler F, Chen LX, Dai L, Gomez-Vaquero C, Diaz-Torne C, Paessler ME, Scanzello C, Cakir N, Einhorn E and Schumacher HR 2008. A histomorphometric analysis of synovial biopsies from individuals with Gulf War Veterans' Illness and joint

pain compared to normal and osteoarthritis synovium. Clinical rheumatology. 27(9): 1127-1134.

Piemattei D, Flo G and DeCamp C 2006. Handbook of Small Animal Orthopedics Fracture Repair. In: Orthopedic Examination and Diagnostic Tools. ed. (ed). SAUDERS, ELSEVIER. 3-24.

Piermattei D and Flo G 2006. Chapter 18 - The Stifle Joint In: Brinker, Piermattei, and Flo's Handbook of Small Animal Orthopedics and Fracture Repair (Fourth Edition). ed. GL Flo, CE DeCamp, I by, and FD Giddings (ed). Saint Louis: W.B. Saunders. 562-632.

Poole AR, Ionescu M, Swan A and Dieppe PA 1994. Changes in cartilage metabolism in arthritis are reflected by altered serum and synovial fluid levels of the cartilage proteoglycan aggrecan. Implications for pathogenesis. J Clin Invest. 94(1): 25-33.

Roughley PJ and Mort JS 2014. The role of aggrecan in normal and osteoarthritic cartilage. J Exp Orthop. 1: 8.

Roush JK 1993. Canine Patellar Luxation. Vet Clin North Am Small Anim Pract. 23(4): 855-868.

Roy RG, Wallace LJ, Johnston GR and Wickstrom SL 1992. A retrospective evaluation of stifle osteoarthritis in dogs with bilateral medial patellar luxation and unilateral surgical repair. Vet Surg. 21(6): 475-479.

Simon MR 1978. The effect of dynamic loading on the growth of epiphyseal cartilage in the rat. Cells Tissues Organs. 102(2): 176-183.

Singleton WB 1969. The Surgical Correction of Stifle Deformities in the Dog. J Small Anim Pract. 10(2): 59-69.

Smith MD 2011. The normal synovium. The open rheumatology journal. 5: 100-106.

Soontornvipart K, Wangdee C, Kalpravidh M, Brahmasa A, Sarikaputi M, Temwichitr J, Lavrijsen IC, Theyse LF, Leegwater PA and Hazewinkel HA 2013. Incidence and genetic aspects of patellar luxation in Pomeranian dogs in Thailand. Veterinary journal (London, England : 1997). 196(1): 122-125.

Venn G, Nietfeld JJ, Duits AJ, Brennan FM, Arner E, Covington M, Billingham ME and Hardingham TE 1993. Elevated synovial fluid levels of interleukin-6 and tumor

necrosis factor associated with early experimental canine osteoarthritis. Arthritis Rheum. 36(6): 819-826.

Villanueva TG and Schumacher HR 1987. Cytologic examination of synovial fluid. Diagn Cytopathol. 3(2): 141-147.

Wangdee C, Leegwater PAJ, Heuven HCM, van Steenbeek FG, Techakumphu M and Hazewinkel HAW 2017. Population genetic analysis and genome-wide association study of patellar luxation in a Thai population of Pomeranian dogs. Research in Veterinary Science. 111: 9-13.

Wangdee C, Theyse LFH, Techakumphu M, Soontornvipart K and Hazewinkel HAW 2013. Evaluation of surgical treatment of medial patellar luxation in Pomeranian dogs. Vet Comp Orthop Traumatol. 26(06): 435-439.

Wessely M, Bruhschwein A and Schnabl-Feichter E 2017. Evaluation of Intra- and Inter-observer Measurement Variability of a Radiographic Stifle Osteoarthritis Scoring System in Dogs. Vet Comp Orthop Traumatol. 30(6): 377-384.

Yuan GH, Masuko-Hongo K, Sakata M, Tsuruha JI, Onuma H, Nakamura H, Aoki H, Kato T and Nishioka K 2001. The role of C-C chemokines and their receptors in osteoarthritis. Arthritis Rheumatol. 44(5): 1056-1070.

APPENDIX

VITA

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