

# Report 2014



A project of

NARILIS (Namur Research Institute for Life Sciences)



And



La Fondation Mont Godinne

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#### Preface

It is in April 2010 that we decided to start collaboration between our institutions (University of Namur and University of Louvain-La-Neuve [CHU Dinant-Mont Godinne]) and develop research projects to investigate musculoskeletal diseases by using an ovine model and modern imaging and laboratory techniques.

The project was initialled called "OASIS", stating for Osteo-ArthritiS In The Sheep. Since then, our team has expanded and other research topics have arisen relating both to humans and animals. They include for example osteo-arthritis, intervertebral disc disease, back pain, and tendon diseases. In consequence, OASIS states now for "Omnibus Animalibus Studla Sanitatis"...the study of health for all and with all animals, including man.

Besides numerous publications, the main satisfaction of those years comes from the team we have built. Young radiologist technicians, experienced radiologists, veterinary PhD students, master and bachelor degree students and technicians in morphology work with us in a friendly and constructive atmosphere. Today, we master numerous up-to-date research techniques and can offer to the scientific community and stakeholders efficient and competitive research services.

This would not have been possible without the trust and support of the senior management of the University of Namur and the CHU Dinant-Mont Godinne. La Fondation Mont Godinne has strongly supported us since the initiation of the project. We want also to acknowledge NARILIS (Namur Research Institute for Life Sciences) for their support. We take also this opportunity to thank our sponsors: Siemens Belgium, Guerbet Belgium and VTrade. Therefore, we are proud to submit this first report, demonstrating that it was worth to bet on this adventure.

We are now ready for more ambitious projects. Several major projects are planned with our collaborators. A culture of research has emerged in medical and veterinary students. We trust that this new generation will join us to strengthen the team, and share the wonderful enthusiasm and pleasure to work together that we both enjoy every day.



Jean-Michel Vandeweerd

Jean-François Nisolle

#### Our mission

To research on diseases

To improve human and animal health

and

To train undergraduates and postgraduates

To practice on the basis of the best available research

for

The benefit of the society and the profession



#### Research focus of OASIS



Our group is dedicated to the study of musculoskeletal diseases, mostly those occurring in man and horses. Important diseases, such as osteoarthritis (OA) and intervertebral disc degeneration (IVDD), are studied by using an ovine model. We have also a special interest for musculoskeletal diseases in sport horses and for the improvement of diagnostic and therapeutic techniques in this species. This research is extended to clinical aspects by accessing the clinical cases seen by our collaborators in private veterinary practice.

However we also study our ovine model for other research such as cardio-vascular diseases in man. In addition, we offer our expertise and services in health science to answer various research questions in animal and man.

Researching is only useful if relevant data are made available to the profession, and if the veterinarians are able to use appropriately the scientific information that is published. This means that undergraduates and postgraduates should be trained to the use of the best available scientific evidence to support their medical decisions. Our group is therefore dedicated to the development of education tools to help veterinary practitioners and to train students to research.

# 1. Diseases occurring in man

# 1.1. Osteoarthritis (OA)

OA is a disease of the synovial joints (diarthrodial joints), that causes pain and disability in human adults, and can lower the quality of life. For example, OA is the most common reason for total knee and total hip replacement. It is a chronic joint disease affecting 30-50% of adults over 65 years. Arthritis, including OA, has a total cost of approximately 2% of the gross domestic product in the USA.

OA is a degenerative process of the joint characterized by progressive degeneration of the articular cartilage, along with reduced joint function. Articular cartilage has biomechanical properties that are largely attributable to its extracellular matrix. In OA, the biosynthetic machinery of collagen and proteoglycans by chondrocytes is unable to keep up with the anabolic demands leading to a net depletion of the extracellular matrix. Early biochemical changes in cartilage include degradation of the collagen network, loss of proteoglycans and modification of water uptake and swelling pressure. As cartilage has a very limited capacity for healing, assessment of cartilage composition should be performed as early as possible when there is a risk to develop OA and should be part of the evaluation of treatments of OA in longitudinal studies in living patients.

Animal models are commonly used to study OA, including sheep. In 2010, our group decided to benefit from the facilities and pedigree flock of the Ovine Research Center of the University of Namur by developing the use of an ovine knee model for the study of OA.

Since then, OASIS has answered several research questions:

- What is the anatomy of the synovial cavity of the ovine knee? Publication [P] 1,6
- How can we inject the synovial cavity of the ovine knee? P2,6
- What is the computed tomographic (CT) anatomy of the ovine knee? P3,6
- What is the magnetic resonance imaging (MRI) anatomy of the ovine knee? P4,7

- What is the anatomy of menisci at MRI, CT and 3D segmentation? P5,7
- What is the prevalence of cartilage defects in a population of research sheep? P8,9
- How can we anesthetize research sheep? P10,11
- Can we assess cartilage defects of the ovine knee at baseline by computed tomography?<sup>P12</sup>
- How do the number of tidemarks and the thickness of calcified cartilage vary in the knee in a population of sheep without clinical signs of OA and of various ages?<sup>P13</sup>
- How does the biochemical composition of the cartilage vary in the knee in a population of sheep without clinical signs of OA and of various ages?<sup>P14</sup>
- What are the existing compositional imaging techniques that can be used to assess biochemical composition of articular cartilage?<sup>P15</sup>

Six ongoing projects are answering the following questions:

- Can we reproduce OA by an insult of the subchondral bone in an ovine model?
- What do we know about the effects of corticosteroids on articular cartilage?
- What is the effect of corticosteroids on the structure and biochemical content of cartilage in the ovine knee?
- What is the effect of corticosteroids on the structure and biochemical content of the ovine menisci?
- What is the effect of corticosteroids on the metabolites of cartilage in the ovine knee?
- What is the effect of corticosteroids on the metabolites of the ovine menisci?
- What can we expect from metabolomics in the in vitro study of cartilage?

# 1.2. Intervertebral disc diseases (IVDD)

Low back pain is a disabling condition with a major socioeconomic impact, concerning millions of individuals each year. Although the pathogenesis remains uncertain, IVDD is believed to be a major cause of low back pain and lumbar disc herniation. IVDD is a multifaceted, chronic process involving detrimental, progressive changes in disc composition, structure, and function. In human medicine, over the past few decades, MRI has largely replaced CT and CT myelography in the assessment of spinal pathology at clinical institutions where MRI is available. The use of biochemical MRI and parametric mapping techniques is becoming increasingly important.

Numerous in vivo animal models, both naturally occurring and experimentally induced, have been used to study IVDD, including ovine models. Experimental models can be induced surgically, physically, and chemically. It has been shown that lumbar ovine and human IVDs have similar gross anatomical features.

Despite sheep have been increasingly used as a large animal model for the human spine, there are aspects of this model that warrant further characterization. OASIS has answered several research questions:

- What is the magnetic resonance imaging of the lumbar spine in the sheep? P16,18
- How can we inject lumbar disks under CT guidance? P17,19

- Is there naturally occurring disc disease in the sheep? P20
- How can we inject the L6-S1 disk in the sheep? P21

Two other ongoing projects are answering the following questions:

- How can we characterize the biochemical and histological changes of the L6-S1 disk in naturally occurring IVDD in the sheep?
- How can metabolomics characterize the changes of the L6-S1 disk in vitro in naturally occurring IVDD in the sheep?

# 1.3. Cardio-vascular diseases

Atherosclerosis occurs commonly in man. Large animal surgeons are often involved in research on stents using animal models, such as sheep. However, little information is published regarding the angiographic anatomy of the iliac arteries in the ovine species. Two projects answered the following research questions:

- What is the anatomy of iliac arteries by contrast arteriography in the sheep and the salient features that would be relevant for the surgical placement of stents, in comparison to the characteristics in humans?
- What is the anatomy of coronary arteries by contrast arteriography in the sheep and the salient features that would be relevant for the surgical placement of stents, in comparison to the characteristics in humans? P23

# 1.4. Miscellaneous

In the past, we have answered several epidemiological questions referring to universal precautions and infectious diseases in man. <sup>P24, 26, 27</sup> We participated to studies referring to urodynamic studies in women, <sup>P25</sup> especially answering the question:

 Does fosfomycine trometamol prevent urinary tract infection after urodynamic studies? <sup>P28, 29</sup>

# 2. Diseases occurring in animals

# 2.1. Horses

# 2.1.1. Musculo-skelettal diseases

The OASIS group has a particular interest in equine orthopaedics. Nowadays, sport horses reach exorbitant values and owners expect an outstanding service. Diagnostic and therapeutic strategies and techniques are similar to those used for man.

# 2.1.1.1. Back pain

Back problems, including pain and reduced motion, have been identified in the horse and can influence significantly locomotion and performance. Lameness and back problems often occur simultaneously. Back pain can result from primary lesions of soft tissue or bone in the thoracolumbar spine. It can also secondarily result from lameness in one or more limbs. Conditions of the thoracolumbar vertebral column include impingement of spinous processes (kissing spines), spondylosis deformans, epaxial muscle inflammation, supraspinous ligament desmitis and dorsal articular process osteoarthritis. Treatment aims to treat the cause when it is identified or the symptoms of pain otherwise.

The OASIS group has an expertise in the management of back pain and has published several papers about its clinical approach, both nationally and internationally, before <sup>P31-36</sup> and after 2010. <sup>P37-39</sup>

Recently, the OASIS group answered the following research questions.

- Does desmotomy of the interspinous ligament improve back pain in kissing spines in the horse? P40
- Can cryosurgery of lumbar nerves be used for treatment of back pain in horses: a preliminary histological study? P41,42

One project is currently working on the following question:

• Can ultrasound guided cryosurgery be used for the treatment of back pain in horses?

# 2.1.1.2. Lameness

Lameness is common in horses and can be difficult to differentiate into different pathologies. Our group has an extensive experience in lameness investigation and has much communicated on the subject, especially about the use of modern imaging techniques such as MRI and CT before 2010 <sup>P43-49</sup> and after. <sup>P50</sup>

A number of clinical tests have been described. In the horse, nerve and joint blocks are commonly used to identify the origin of the lameness within the foot. Medical imaging is then used to attempt to correlate pain with lesion of anatomical structures of the foot. In the last decade, MRI has been used where conventional imaging modalities such as radiography and ultrasonography could not diagnose the source of foot pain. Low-field MRI in standing patients has become a common imaging technique in lameness investigation.

The OASIS group has answered the following research questions:

- How can we compare arthro CT and 3T MRI optimal sequences to identify cartilage defects in the fetlock joint? <sup>P51-53</sup>
- What is the correlation between responses to the "plank test" and lesions of anatomical structures in the foot identified using low-field MRI? <sup>P54</sup>
- What is the impact of section images and scans on the learning of anatomy? P166

# 2.1.1.3. Orthopaedic surgery

The OASIS group has an extensive experience in equine surgery and has worked to the improvement and the development of surgical techniques. Much work has been dedicated to minimal invasive techniques and procedures assisted by computed tomography or MRI before <sup>P55-67</sup> and after 2010. <sup>P67, 68, 70, 71</sup>

The OASIS group has also investigated recently the following research questions:

- What can we expect from CT to assist orthopaedic surgery? P69
- Can we perform neurectomy of peroneal and tibial nerves by cryosurgery to treat bone spavin?
- How can we inject the navicular bursa under ultrasonographic guidance?

• What are the histological characteristics of the synovial plica in the dorsal compartment of the fetlock joint in the horse? P74

The OASIS group has ongoing projects to answer the following question:

- What is the direction of the fibers in the suspensory ligament?
- What are the differences between radiography, 0.27 T MRI, CT and 3.0T MRI in assessing the navicular bone?
- What are the differences between radiography, 0.27 T MRI, CT and 3.0T MRI in assessing the the distal phalanx?

# 2.1.2. Soft tissue surgery in horses

Several members of the team are board certified large animal surgeons and work in private or university clinics. They have to manage soft tissue cases and to apply gold standard techniques. Improving surgical technique is part of their mission as Diplomates of The European College of Veterinary surgeons (ECVS).

They have published several times in that field before P75-84 and after 2010. P85-91

For example, the group answered the following questions:

- Can we use VAC (vacuum assisted) therapy to treat wounds? P86
- What's the use of biopsies of the pelvic flexure at surgery? P92
- How to interpret venous lactate in the horse with colics? P93, 94, 98
- What is the Computed Tomography (CT) and Magnetic Resonance Imaging (MRI) anatomy of the eye and orbit? P95-97

Another ongoing project will investigate the following question:

• What is the accuracy of remote monitoring (video camera) of hospitalized horses?

# 2.2. Other animals

The OASIS group conducted also several projects with research students to answer to the following research questions:

- What is the evidence about effects of gaz anesthesia in pigeons? P99
- What are cardiovascular and respiratory parameters in the awake pigeon and during anaesthesia at the individual's MAC of isoflurane while breathing spontaneously?

There are ongoing projects answering to:

- What is the difference between gaz anesthesia with sevoflurane and isoflurane in the pigeon?
- Is T2 mapping an accurate technique to assess the biochemical composition of menisci in dogs?
- Is metabolomics feasible to assess canine menisci in vitro and how can we interpret the results?
- What are the histological characteristics of mitral valves in Kavalier King Charles?
- What is the anatomy of arteries in the sheep?

# 3. Education to Evidence Based Practice and to Research

# 3.1. Education of postgraduates

Evidence based veterinary medicine (EBVM) has largely developed from the concepts promulgated in human medicine. Veterinary pioneers have tried to apply convincingly techniques that are only partially transferable from human to veterinary practice. Our group has investigated the following questions: "Is evidence-based medicine so evident in veterinary research and practice? "<sup>P130</sup> and "How do veterinary practitioners make decisions?" <sup>P131</sup>. We have identified several difficulties and needs.

Firstly, available studies in veterinary medicine may be poorly designed, executed, analysed, reported and inadequately peer-reviewed. There are indications that the veterinary literature lacks publications of randomized controlled studies, systematic reviews and meta-analyzes. In other words, the practice of EBVM necessitates that scientists produce and publish high standard research and strong levels of evidence. If quality is lacking, quantity is also required to evaluate consistency of results. This must be a goal of a research group.

Secondly, veterinarians need some skills to critically appraise the information that would be available. The language of research, statistics and scientific methodology is not necessarily understandable by practitioners. We should engage students, academics and all veterinary professionals in developing those skills that are necessary to practice EBVM. It is our role as a research group to train postgraduates and undergraduates to the use of the best available research information in making clinical decisions. Our group has played that role for 8 years. More than sixty EBVM papers and books have been published for postgraduate education P<sup>101-166</sup> and 13 papers about undergraduate education (not in the reference list).

There seems to be a consensus throughout the scientific community to develop strategies to improve the level of evidence of research studies and to standardize their reporting. While guidelines to report trials and observational studies were recently published, there is a need to standardize the conducting and publication of systematic reviews. We think that special attention should be paid to the summary (abstract) of those studies. A special effort should be made to write informative summaries that are easy to understand for practitioners. The OASIS group has produced several reviews, including three important systematic reviews to answer the following questions:

- What is the scientific evidence of efficacy of nutraceuticals to alleviate clinical signs of osteoarthritis? <sup>P147,148</sup>
- What is the scientific evidence of efficacy of nutraceuticals in hepatic failure? P156,157
- What is the scientific evidence about the effects of corticosteroids on articular cartilage? P165

#### 3.2. Research students

Given that expectations for faculty research have risen at the same time that higher education is facing demands for increased public accountability, the advantages of strengthening the connection between research and teaching seem clear, and several studies encourage a stronger connection. Different strategies are commonly proposed for strengthening the nexus: (1) bringing research into the classroom; (2) involving students in research projects.

Though it is easy to bring research into the classroom and use it to inform teaching, to be beneficial, the research must illuminate essential course content without distracting from it or confusing more than it clarifies.

Engaging students in research projects has probably more positive effects such as retention of students in academic programs, pursuit of graduate study, cognitive learning gains, acquisition of research knowledge and skills. Our group considers it is our duty to demonstrate to students that research is enjoyable and not out of reach. We have to help students develop critical and scientific thinking. We want also help them develop their curriculum vitae, notably by relevant communications or publications.

We started to engage students in our research projects in 2010. Purposely, we did not limit this action to students with higher academic level, but we opened it to all motivated undergraduates and postgraduates. Importantly, we did not limit research subjects to the OASIS' topics; we also accepted to work on subjects that were of particular interest for the students themselves. For example, one student in 2013 was willing to research on anesthesia in birds.<sup>P99, 100</sup> Another one researched on the impact of using MRI and CT scans in learning anatomy.<sup>P166</sup>

The engagement of students in research projects in the last four years is summarized in the table below.

Year	Bachelor	Master students
	students	
2010/2011	7	2
2011/2012	3	0
2012/2013	3	2
2013/2014	8	3
2014/2015	18	9

The quality of their work is excellent; this can be demonstrated by the communications (congresses) and papers that have been published on the basis of their work.

We are much respectful of their work and consider it is a mission to train them to communication. Thanks to our sponsors and donators, we give them the opportunity to take part to congresses. We hope that more donators will help us in the future in this important part of education.

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#### The team



#### Faculty

Professor Jean-Michel Vandeweerd Doctor in Veterinary Medicine, Master in Higher Education, PhD in Veterinary Sciences, Certificate in Equine Soft Tissue Surgery (RCVS), Diplomate of the European College of Veterinary Surgeons, Member Associate of The Académie Vétérinaire de France



Jean-Michel graduated at The University Faculties Notre-Dame de la Paix in Namur in 1986, then at the University of Liège in 1989. He holds a Certificate in Equine Surgery (Royal College of Veterinary Surgeons). His supervisor was Professor Barrie Edwards (University of Liverpool). He obtained a Master in Higher Education from the University of Liege. He is Diplomate of ECVS (European College of Veterinary Surgeons); he was trained under the supervision of Dr F.Desbrosse. He holds a certificate of master of experience and has a PhD in Veterinary Sciences. He trained in Health Science Research at the University of Maastricht. He is president of the commission Evidence Based Medicine of AVEF (Association Vétérinaire Equine Française). He is an associate member of the Académie Vétérinaire de France. He has worked in his own private practice in Belgium from 1990 till January 2003 (small animals and horses) and has been lecturer in equine orthopaedic surgery at the University of Liverpool from 2003 till 2007. He has been consultant at the equine clinic Desbrosse (France) since he returned to Belgium. He has also been involved in educational projects at the University of Liège from 2009 to 2012. He is now Professor of Animal Anatomy and EBM at the University of Namur. His research interests are musculoskeletal diseases, equine surgery and EBM.

Dr Fanny Hontoir Doctor in Veterinary Medicine



Fanny graduated at The University Faculties Notre-Dame de la Paix in Namur (Belgium) in 2004, then at the University of Liège (Belgium) in 2007. She holds a diploma in radioprotection (Ulg). She has been working in small animal practice in France from 2007 to 2010. She has been appointed as assistant in anatomy at the University of Liège from 2010 to 2011. She has a certificate of master of experience. She is now assistant in veterinary anatomy at the University of Namur. She is one of the locomotives of the OASIS project. She is currently doing her PhD under the supervision of JM Vandeweerd and Peter Clegg. The objective of her research is to evaluate and compare advanced imaging techniques to assess the structure and biochemical content in normal and diseased cartilage. She is also developing a new ovine model of osteoarthritis.

Professor Jean-François Nisolle Doctor in Medicine Medical imaging specialist



Jean-François graduated in Medicine from the Catholic University of Louvain in 1985. He obtained his complementary Masters' degree in Diagnostic Radiology in 1989. He is currently head of radiology clinics at the CHU Dinant-Godinne, Catholic University of Louvain. Appointed as clinical lecturer in 2012, he is medical imaging supervisor as well as vice-president of the medical imaging commission. He is mainly interested in the investigation of musculo-skelettal diseases, by using up-to-date technology such as radiography, ultrasonography, computed tomography, magnetic resonance and SPECT-CT. He is also invited Professor at the UNamur.

Professor Peter Clegg MA VetMB PhD CertES DipEVCS MRCVS



Peter qualified from the University of Cambridge in 1987 and spent four years working in equine practice. He then completed a Residency in Equine Surgery at the Royal Veterinary College in 1994 obtaining the RCVS Certificate in Equine Orthopaedics during this time. Between 1994 -1997 he undertook research at the University of Liverpool in the field of equine osteoarthritis for which he was awarded a PhD in 1997. Peter has worked at the University of Liverpool since 1997 as Lecturer and Senior Lecturer and subsequently was appointed as Chair in Equine Orthopaedics in 2005. In 2003, he was awarded a Wellcome Trust Research Leave Fellowship to undertake research into cartilage repair in conjunction with the University of Manchester. Peter is a Diplomate of the European College of Veterinary Surgeons and a RCVS Specialist in Equine Surgery. He performs veterinary duties at Chatsworth Three day event. His research interests are arthritis, cartilage and bone physiology and pathology, tendon injuries and epidemiology of orthopaedic injuries.

Professor Nathalie Kirschvink Doctor in Veterinary Medicine PhD



Nathalie graduated at the University of Liège in 1997. She has a PhD in veterinary sciences. She is Professor of Physiology at the University of Namur. She is also invited Professor at the University of Liège. She is Head of the Department of Veterinary Medicine and director of the Ovine Research Center at the UNamur. She is member of the Veterinary Comparative Respiratory Society , of the board of the Veterinary Comparative Respiratory Society , of the board of the Veterinary Comparative Respiratory Society , of the board of the Veterinary Comparative Respiratory Society , of the Belge de Physiologie et de Pharmacologie fondamentales et cliniques. She is Membre Administrateur at the FICOW (filière interprofessionnelle caprine et ovine wallonne) and at the Centre d'Economie Rurale (CER). Her research expertise includes respiratory physiology and pathology in domestic and laboratory animals, as well as in sport animals. She currently investigates emerging infectious diseases in cattle and sheep (Bluetongue, Schmallenberg, Q fever).

Professor Pascal GUSTIN Doctor in Veterinary Medicine PhD Diplomate of ECVPT Membre de l'Académie Belge de Médicine



Pascal is Diplomate of the European College of Veterinary Pharmacology and Toxicology. He obtained his PhD in 1998 and is member of the Royal Academy of Medicine in Belgium. He works now at the University of Liège as full professor in the Unit of Pharmacology-Pharmacotherapy-Toxicology, Département of Functional Sciences, Faculty of Veterinary Medicine, University of Liège. He is invited professor at the JiaoTong University of Shanghai (School of medicine). He is expert for the Federal Agency for Medicinal Products and Medical Devices (AFMPS) (Chairman of the Committee on the veterinary medicinal products), Federal Agency for the Safety of the Food Chain, and European Medicines Agency (EMA) (London). His research and field of expertise is the Fundamental and applied Pharmacology and Toxicology (biomedical sciences, veterinary medicine, respiratory system, osteo-arthritis, inflammation, pollution, veterinary medicinal products, food safety, biocides, preclinical and clinical trials).

Dr Pauline Meirlaan Doctor in Veterinary Medicine



Pauline graduated in 2012 at the University of Liege. She is interested in small animals and horses. Since October 2012, she is assistant in pharmacology at the Faculty of Veterinary medicine at the University of Liege. She is a PhD student, studying the following research question under the supervision of Jean-Michel Vandeweerd and Pascal Gustin: "What is the effect of triamcinolone hexacetonide on articular cartilage in an ovine model?"



Professor Charles Nicaise PhD

Charles graduated at the University of Brussels (Université Libre de Bruxelles, ULB) in biomedical sciences in 2002. He worked as Associate Researcher, Laboratory of Gastroenterology (ULB) between 2002 and 2005. He did his PhD at the Laboratory of Histology, Neuroanatomy and Neuropathology (ULB). He carried out a Post-doctoral Fellowship at the Jefferson Hospital for Neuroscience in 2011, and at the Department of Pathology (Erasme Hospital, & Center for Microscopy and Molecular Imaging, ULB) in 2012. He is Professor of Histology at the Department of Veterinary Sciences and Department of Medicine, University of Namur. Dr Marie Henin, Dr Francoise Kayser and Dr Cecile Abraham from CHU Dinant-Godinne (Catholic University of Louvain) recently joined the team.



The faculty also benefits from the expertise of our colleagues Professor Benoît Muylkens, Professor Claire Diederich, Professor Michel Jadot, Dr Caroline Canon, Dr Simon Tew, Professor Alexandra Dugdale, Dr Hélène Coulon, Dr Fabien Gabriel, Mr Hubert Meurisse,

# **Collaborators in private practice**

Dr Roland\_ Perrin Doctor in Veterinary Medicine, Diplomate of the European College of Veterinary Surgeons, Diplôme d'Etudes Supérieures Vétérinaire (DESV) de Chirurgie equine, Member of the Académie Vétérinaire Française

Roland graduated at the Ecole Nationale Vétérinaire de Lyon in 1982. He is Diplomate of the European College of Veterinary Surgeons since 1996. He obtained a Diplôme d'Etudes Supérieures Vétérinaire (DESV) de Chirurgie équine in 2007. He became a Member of the Académie Vétérinaire Française in 2011. He is owner and partner with Dr Laurent Brogniez of the « Clinique Vétérinaire équine Desbrosse » near Paris, one of the best equine clinics in France. He has been Regent at the ECVS Board (2006 -2009), President of the Public Relation Committee of ECVS (2006-2009) and President of the scientific committee of ECVS (2011). He is "FEI Vet". His interests are equine lameness, sport medicine in show jumpers and dressage horses, and equine surgery.

Dr Laurent BROGNIEZ Doctor in Veterinary Medicine

Laurent graduated at the Ecole vétérinaire of Lyon in 2003. He holds a diploma in radioprotection and is "Vétérinaire FEI". His interests are lameness, sport medicine in showjumpers and dressage horses, and medical imaging, especially MRI.

Dr Helene Guiteras graduated from the University of Namur in 2010 and from the University of Liege in 2013. She was appointed by the Equine Clinic Desbrosse in 2013. Dr Capucine Bailly graduated in 2007. She has been intern at the Cirale-ENVA in 2007. She has worked for the Clinique Desbrosse since 2009. Dr Pauline Cantet graduated from the Ecole Veterinaire de Nantes in 2011 and was appointed by the clinic in 2012. All have an interest in orthopaedics and are responsible of the emergency service at the clinic.





#### Scientific and technical assistants

We can rely upon a terrific team of scientific and technical assistants, all dedicated to and efficient in their work: our radiotechnologists in Mont-Godinne (Renaud Vanderstricht, Max Tallier, Quentin Delplace and Nicolas Desmet), our team at the laboratory of anatomy (Yves De Raeve, Vincent Simon and Robert Graffin), Laetitia Wiggers at the laboratory of biomolecular biology, and our collaborators at the Center of Ovine Research (Christine Baricalla, Mariane Raes, Benoit Bolkaerts, Nicolas Noel and Xavier Van De Sype).



From left to right: Christine Baricalla, Marianne Raes, Benoit Bolkaerts, Nicolas Noel, Xavier Van De Sype



From left to right: Robert Graffin, Vincent Simon (and Louis), Laetitia Wiggers, Yves De Raeve



Quentin Delplace, Renaud Vanderstricht, Nicolas Desmet et Max Tallier ,



ECVS congress Gent 2011 (from right to left; Research students: Céline Cintas, Claire Lambert, Charles Vande Catsyne, Mathieu Lamberts, Nicolas Herteman; Faculty: Dr Fanny Hontoir, Prof Jean-Michel Vandeweerd)

Céline Cintas graduated at the University of Namur in 2010, then at the University of Liège in 2013. She did an internship in equine medicine and surgery at the Vet School of Lyon in 2014. She worked as a research student with OASIS in 2010 answering the research question "What is the magnetic resonance imaging anatomy of the ovine stifle". She presented her results at the ECVS congress of Gent and was co-author of a publication in Vet Surgerv. Claire Lambert graduated at the University of Namur in 2012. She is finalizing her master in Veterinary Medicine at the University of Liège. She worked in 2011 on the research question "What is the anatomy of menisci at MRI, CT and 3D segmentation?" She presented her results at the ECVS congress of Gent. Charles-Andrew Vande Catsyne graduated at the University of Namur in 2011, then at the University of Liège in 2013. He is now doing a PhD entitled "Analyse du processus d'ossification endochondrale chez des souris déficientes pour la protéine SHIP2, un modèle potentiel pour l'opsismodysplasie humaine". He worked in 2011 on the research question "What is the magnetic resonance imaging anatomy of the ovine stifle?" He presented his results at the ECVS congress of Gent and was co-author of a publication in Vet Surgery. Mathieu Lamberts graduated at the University of Namur in 2011, then at the University of Liège in 2013. He worked in 2011 on the research question "How can we inject the synovial cavity of the ovine knee?" He presented his results at the ECVS congress of Gent and was co-author of a publication in The Veterinary Journal. **Nicolas Herteman** graduated at the University of Namur in 2011, then at the University of Liège in 2013. He is currently doing an internship at the university of St Hyacinthe. He has a research project on respiratory obstructive disease in horses under the supervision of Professor JP Lavoie. He worked in 2011 on the research question "What is the anatomy of the synovial cavity of the ovine knee?" He presented his results at the ECVS congress of Gent and was co-author of a publication in The Veterinary Journal. Marion Chabrier and Laurie Andre answered to the question "What is the computed tomographic (CT) anatomy of the ovine knee?" and presented their work at the ECVS congress of Gent in 2011. Laurane Lebrun and Camille Coisnon graduated from the University of Namur in 2011 and from the University of Liege in 2013. They were co-authors of one educational paper in "Pratique vétérinaire équine". Camille conducted a systematic review on nutraceuticals that was published in Le Point Veterinaire and Journal of Veterinary Internal Medicine.



ECVS congress Barcelona 2012: Alice Foulon, Marie Squélart and Fabienne Neveu.

Alice Foulon, Marie Squélart and Fabienne Neveu graduated from the University of Namur in 2012 and are now in their final years at the University of Liège. They are interested in small animal medicine. They answered the following research questions: "What is the magnetic resonance imaging of the lumbar spine in the sheep?" and "How can we inject lumbar disks under CT guidance?" They results were presented at the ECVS congress of Barcelona and published in Anatomia, Histologia, Embryologia and European Spine Journal.



Marie Goossens

**Marie Goossens** graduated from the University of Namur in 2013. With OASIS, she compared propofol and pentobarbital for the anesthesia of sheep. Her work was presented at the ECVS congress of Rome in 2013. It is in press in Sciences et Techniques de l'Animal de Laboratoire. **Camille Vézié** and **Audrey Masselot** graduated also from Namur in 2013 and participated to a literature review respectively about the use of tiludronate in horses and the use of nutraceuticals in dogs. Their work was published respectively in "Médicaments du système locomoteur - Prescription et médicaments en médecine équine, 2 ème edition" and in Companion Animal. **Dr François Primosig** wrote his master thesis (radiology specialist) under the supervision of JF NBisolle on intervertebral disc disease.



Romain Pirson and Amandine Matagne at the WCO in Sevilla

**Romain Pirson** studied biology at the University of Namur where he graduated with a Master in Biochemistry and Cellular and Molecular Biology in 2014. The main objective of his master thesis was to assess morphological changes of the subchondral bone plate (the cartilage-bone interface) in naturally occurring osteoarthritis in sheep. This study documented the variation of the number of tidemarks and the thickness of calcified cartilage and hyaline cartilage of the knee in a population of sheep without clinical signs of OA and with various ages. He then went to the University of Cambridge where he worked on murine Noroviruses and tried to characterize a minor capsid protein. **Amandine Matagne** graduated also in Biochemistry and Cellular and Molecular Biology in 2014. She studied the biochemical changes of articular cartilage occurring with age in the sheep. They presented their work at the WCO congress (World congress on osteoarthritis and osteoporosis) 2014 in Sevilla. **Philippe Ernotte** performed his master thesis (hospital physician) with Hubert Meurisse on imaging of ovine cartilage.



Helene Thibout

**Helene Thibout** investigated whether "We can improve learning of anatomy by using CT and MRI imaging? Her work was presented at the congress of AIPU in Mons in 2014. She is currently finishing her veterinary studies at the University of Liege. The review of **Camille Loiseau** about treatment of tendonitis with stem cells was published in "Médicaments du système locomoteur - Prescription et médicaments en médecine équine, 2 ème edition"



From left to right: Shana Labed, Fanny Lang, Benjamin Godart, Julie Botman, Nathalie Sternon, Clarisse D'Aout, Justine Dalla Valle

They called themselves "The Dream Team". They all obtained their bachelor degree in veterinary science from the University of Namur in 2014. Shana Labed and Fanny Lang assessed the correlation between the "plank test" and lesions of the foot identified by MRI in the horse. Julie Botman researched on the following questions: "What is the evidence about effects of gaz anesthesia in pigeons?" and "What are cardiovascular and respiratory parameters in the awake pigeon and during anaesthesia at the individual's MAC of isoflurane while breathing spontaneously?" She published her results in Le Point Veterinaire and Veterinary Anesthesia And Analgesia. She will compare Isoflurane and Sevoflurane in a future study. The work of Nathalie Sternon improved oxygenation of the sheep under anesthesia. She is coauthor of a publication in Sciences et Techniques de l'Animal de Laboratoire. Clarisse D'Aout studied "What is the Computed Tomography (CT) and Magnetic Resonance Imaging (MRI) anatomy of the eye and orbit". She communicated about her work at the ECVS congress of Copenhagen and at the AVEF congress of Pau; she published her work in Anatomia, Histologia, Embryologia. She is still involved in OASIS, preparing a manuscript about the anatomy of the eye. Justine Dalla Valle has researched on the question "Can cryosurgery of lumbar nerves be used for treatment of back pain in horses: a preliminary histological study?" Her results were also presented in Copenhagen and Pau. She is still involved in the project and has developed a surgical technique to freeze the nerves under ultrasound guidance. She will soon transfer the technique to clinical cases. **Benjamin Godart** conducted a very thorough research on the anatomy of arteries in sheep; he will finalise his work in 2015.



Besides Clarisse D'Aout, Justine Dalla Valle, Julie Botman and Benjamin Godart, other master students also take part to research in 2014-2015. Amelie Ballieu is in first year master in veterinary medicine; she is investigating with OASIS the histological characteristics of the synovial pad in the dorsal pouch of the metacarpo-phalangeal joint in the horse. Fabienne Neveu is currently finishing her veterinary studies and researches with us on techniques to inject the lumbarsacral disc in the sheep. She is also involved in several other projects ion intervertebral disc disease. Yang Zhao is student in Medicine at the Jiao Tong University of Shangai. She is taking part to our research on the effects of corticosteroids on articular cartilage. Two master students in biology of the university of Namur (BBMC), Giulia Grisanti and Florian Crokaert, will join us in January 2015 to investigate the effects of corticosteroids on the cartilage of menisci in the ovine knee.



Amélie Ballieu, Fabienne Neveu, Yang Zhao

Our team of research students for 2014-2015 include also 18 third year bachelor in veterinary science of the University of Namur.

Antoinette Terlinden is developing a surgical technique for cryosurgery of the tibial and peroneal nerves in the horse. Sarah Deremince is investigating the performance of blood lactate dosage in horses with colic to predict survival and treatment indication. Charlotte Foiret (pictured with Léopoldine) is studying the direction of fibers in the suspensory ligament in the hindlimb of horses. Elena Czaikowski is assessing whether remote monitoring of hospitalised horses with video cameras and iPhones is reliable.



**Morgane Joscht**, **Marion Martin**, **Soizic Gochard** and **Claire HouseIstein** are investigating the anatomy and arteriography of iliac and coronary arteries in the sheep. Their work will be very useful for future work in atherosclerosis research.



**Nicolas Lallemand** is assessing the feasibility of H-NMR metabolomics to assess metabolites concentrations in the intervertebral discs of sheep. **Khadija Nozri** is testing an injection technique of the lumbarsacral disc in the sheep. **Louise Vandermersch** is studying the prevalence of natural intervertebral disc degeneration (IVDD) by using CT and MRI T2 mapping. **Constance Dabrowski** is using biochemical techniques to investigate IVDD.



**Margaux Legrand** and **Madisson Hautem** are assessing respectively whether T2 mapping of menisci in the dog reflects biochemical content of the menisci and whether H-NMR metabolomics is a reliable technique to study biochemical contents of canine menisci.



**Roxane Tarer** and **Charlotte Peindaveine** (not pictured) are comparing assessments of the foot by veterinarians (by radiography and 0.27 T MRI) and by human radiologists (by CT and 3.0 T MRI). **Anne-Claire Diguet** is evaluating ex vivo a new ultrasound guided technique of injection of the navicular bursa in the horse. **Tracy Rock** is exploring histological changes of valvular disease in Cavalier King Charles.



# Techniques available


Our network has access to all the facilities that are necessary to conduct gold standard research.

Center of Ovine Research

- Pedigree flock of sheep (more than 400 ewes)
- Breeding farm
- Fully equipped large animal surgical theatre
- Laboratory
- Transport facilities

CHU Mont Godinne Dinant

- 3.0T and 1.5T MRI
- CT scans
- Ultrasonography and radiography

Unit of integrated veterinary research

- Gross anatomy laboratory
- Laboratory for conventional histology, immunohistology and electron microscopy
- Laboratory for biochemical investigation

Via our partners of the University of Liverpool

- Micro CT
- N-MRI

Via our partners in private practice

- Digital radiography
- Standing CT for horses
- 0.27T MRI
- Surgical facilities for horses

OASIS benefits also of the support by NARILIS.

The Namur Research Institute for Life Sciences (NARILIS) was created in 2010 as a partnership between the University of Namur (UNamur) and the CHU Dinant Godinne. NARILIS is a centre of excellence in multidisciplinary life science research, with a regional anchorage point around Namur in Belgium. NARILIS conducts research activities focused on human health, dealing with both fundamental and clinical research and especially favouring translational research from basic research to medical practice. NARILIS builds on multidisciplinary expertise, involving experts from multiple areas e.g. biologists, physicists, chemists, pharmacists, clinicians...NARILIS includes three research centres: the Namur Thrombosis and Hemostasis Center (NTHC), the Namur Nanosafety Center (NNC) and the Namur Medicine & Drug Innovation Center (NAMEDIC).

Shared resources provide NARILIS investigators access to several technological facilities: the biobank of CHU Dinant Godinne, a Proteomic and MassSpec facility at UNamur, a platform for *in vitro* radiobiology studies, a platform for analytical morphology at UNamur consisting of a confocal microscope and a BD Pathway855 system, a platform for multimodal imaging at CHU Dinant Godinne, a genotyping facility at UNamur and a Biosafety Level 3 laboratory at UNamur.

# **Publications of OASIS**



Communications are in italic. Papers are in bold.

# Focus 1. Diseases occurring in man

# <u>Osteoarthritis</u>

2011

- HERTEMAN N, LAMBERTS M, KIRSCHVINK N, MUYLKENS B, NISOLLE JF, MEURISSE H, CLEGG P, VANDEWEERD JM. – Anatomy of the synovial cavities of the ovine stifle – Proceedings of the 20<sup>th</sup> annual scientific meeting of the ECVS – Ghent – Belgium – 2011 – p 52.
- LAMBERTS M, HERTEMAN N, KIRSCHVINK N, MUYLKENS B, NISOLLE JF, MEURISSE H, CLEGG P, VANDEWEERD JM – Comparison of three techniques to inject the synovial cavities of the stifle in the sheep – Proceedings of the 20<sup>th</sup> annual scientific meeting of the ECVS – Ghent – Belgium – 2011 – p 54.
- CHABRIER M, ANDRÉ L, KIRSCHVINK N, MUYLKENS B, NISOLLE JF, MEURISSE H, CLEGG P, VANDEWEERD JM – Computed tomography arthrography (CTA) anatomy of the ovine stifle – Proceedings of the 20<sup>th</sup> annual scientific meeting of the ECVS – Ghent – Belgium – 2011 – p 47.
- LAMBERT C, KIRSCHVINK N, MUYLKENS B, NISOLLE JF, MEURISSE H, CLEGG P, VANDEWEERD JM – An anatomic study of ovine menisci by computed tomographic arthrography (CTA), 3D segmentation and magnetic resonnance imaging (MRI) – Proceedings of the 20<sup>th</sup> annual scientific meeting of the ECVS – Ghent – Belgium – 2011 – p 53.
- VANDECATSYNE C, CINTAS C, KIRSCHVINK N, MUYLKENS B, NISOLLE JF, MEURISSE H, CLEGG P, VANDEWEERD JM – Magnetic resonance imaging anatomy of the ovine stifle described from images acquired with high-field magnet (3 Tesla) – Proceedings of the 20<sup>th</sup> annual scientific meeting of the ECVS – Ghent – Belgium – 2011 – p 59.

- VANDEWEERD JM, KIRSCHVINK N, MUYLKENS B, HERTEMAN N, LAMBERTS M, DEPIEREUX E, CLEGG P, BONNET P, NISOLLE JF – A study of the anatomy and injection techniques of the ovine stifle by positive contrast arthrography, computed tomography arthrography and gross anatomical dissection – The Veterinary Journal, 2012,193(2):426-432.
- VANDEWEERD JM, KIRSCHVINK N, MUYLKENS B, CINTAS C, VANDE CATSYNE C, HONTOIR F, CLEGG P, COOMER R, NISOLLE JF – Magnetic resonance imaging (MRI) anatomy of the ovine stifle – Veterinary surgery, 2013, 42(5):551-558.

 PIERSON A, KIRSCHVINK N, MUYLKENS B, HONTOIR F, RAES M, CLEGG P, NISOLLE JF, BÉDUIN JM, ANTOINE N, PIRET J, GUSTIN P, VANDEWEERD JM – Prevalence of naturally occuring cartilage defects in the ovine stifle (knee) – Abstracts of the 2012 World Congress on Osteoarthritis – Barcelona – Spain – 2012 – pp S73-S74.

2013

- 9. VANDEWEERD JM, HONTOIR F, KIRSCHVINK N, CLEGG P, NISOLLE JF, ANTOINE N, GUSTIN P Prevalence of naturally occurring cartilage defects in the ovine knee Osteoarthritis Cartilage, 2013,21(8):1125-1131.
- GOOSSENS M, WANG X, HONTOIR F, NISOLLE JF, CLEGG P, KIRSCHVINK N, VANDEWEERD JM – Comparison of IV pentobarbital and IV propolation for induction and maintenance of anesthesia in sheep – Proceedings of the 22<sup>nd</sup> ECVS congress – Rome – Italy – 2013 – p. 139.

2014

- 11. VANDEWEERD JM, VERMEYLEN A, GOOSSENS M, STERNON N, HONTOIR F, NISOLLE JF, DUGDALE A Anesthésie chez le mouton de laboratoire Sciences et Techniques de l'Animal de Laboratoire In press, 2014.
- 12. HONTOIR F, NISOLLE JF, SIMON V, VANDERSTRICHT R, TALLIER M, KIRSCHVINK N, CLEGG P, VANDEWEERD JM – Accuracy of computed tomography arthrography to detect cartilage defects in the ovine knee – Proceedings of the OARSI (Osteoarthritis research society international) congress – Paris – France – 2014.
- PIRSON R, MATAGNE A, NISOLLE JF, HONTOIR F, MEIRLAEN P, GUSTIN P, KIRSCHVINK N, CLEGG P, VANDEWEERD JM – What does multiplication of tidemarks mean in the ovine femoro-tibial joint? – Proceedings of the WCO (World congress on osteoarthrtitis and osteoporosis) – Sevilla – Spain – 2014 – p 172.
- 14. MATAGNE A, PIRSON R, NISOLLE JF, HONTOIR F, MEIRLAEN P, GUSTIN P, KIRSCHVINK N, CLEGG P, TEW S, VANDEWEERD JM – Biochemical composition of cartilage with naturally occurring defects in the ovine femoro-tibial joint. – Proceedings of the WCO (World congress on osteoarthrtitis and osteoporosis) – Sevilla – Spain – 2014 – p 173.
- 15. HONTOIR F, CLEGG P, NISOLLE JF, TEW S, VANDEWEERD JM Magnetic resonance compositional imaging of articular cartilage: what can we expect in veterinary medicine? The Veterinary Journal In press,.

# Intervertebral disc disease

2012

 SQUÉLART M, NEVEU F, FOULON A, KIRSCHVINK N, MUYLKENS B, HONTOIR F, CLEGG P, NISOLLE JF, VANDEWEERD JM - MRI anatomy of the ovine lumbar spine - Proceedings of the 21th Scientific Meeting of the ECVS – Barcelona – Spain – 2012 – p. 68. 17. NEVEU F, FOULON A, SQUÉLART M, KIRSCHVINK N, MUYLKENS B, HONTOIR F, CLEGG P, NISOLLE JF, VANDEWEERD JM - Computed Tomography (CT) guided needle insertion into intervertebral discs in the ovine lumbar spine: a cadaveric study - Proceedings of the 21th Scientific Meeting of the ECVS – Barcelona – Spain – 2012 – p. 61.

2013

18. NISOLLE JF, NEVEU F, HONTOIR F, CLEGG P, KIRSCHVINK N, VANDEWEERD JM – CT-guided injection technique into intervertebral discs in the ovine lumbar spine – European Spine Journal – 2013, 22(12):2760-2765.

2014

19. NISOLLE JF, WANG XQ, SQUÉLART M, HONTOIR F, KIRSCHVINK N, CLEGG P, VANDEWEERD JM – Magnetic Resonance Imaging (MRI) Anatomy of the Ovine Lumbar Spine – Anatomia Histologia Embryologia – 2014, 43(3):203-209.

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- 20. NISOLLE JF, VANDERMEERSCH L, DABROWSKI C, KIRSCHVINK N, NEVEU F, MEIRLAAN P, MUYLKENS B, CLEGG P, VANDEWEERD JM C Intervertebral disc degeneration occurs naturally at the lumbar-sacral disc in the sheep – Submitted for the ECVS Congress 2015 in Berlin.
- 21. NOZRY K, NEVEU F, KIRSCHVINK N, VANDEWEERD JM, GUSTIN P, CLEGG P, NISOLLE JF CT-guided injection into the lumbar-sacral intervertebral disc in the ovine lumbar spine Submitted for the ECVS Congress 2015 in Berlin.

### Cardio-vascular diseases

# Submitted

- 22. JOSCHT M, MARTIN M, HENIN M, NISOLLE JF, KIRSCHVINK N, CLEGG P, MEIRLAEN P, GODART B, COULON H, NEVEU F, VANDEWEERD JM – Angiographic anatomy of iliac arteries in the sheep – Submitted for the ECVS Congress 2015 in Berlin.
- 23. GOCHARD S, HOESELSTEIN C, HENIN M, NISOLLE JF, NEVEU F, MEIRLAEN P, KIRSCHVINK N, CLEGG P, GODART B, COULON H, VANDEWEERD JM – Angiographic anatomy of coronary arteries in the sheep – Submitted for the ECVS Congress 2015 in Berlin.

# <u>Miscellaneous</u>

- 24. REDA A, VANDEWEERD JM, EGATA G, SYRE T Exposure of health care workers to body fluids in Ethiopia: attitude towards Universal Precautions J Hosp Infect 2009, 71(2):163-169.
- 25. HOUGARDY V, VANDEWEERD JM, PEATTEE A, REDA A The Impact Of Explicative Leaflets On Patient Satisfaction With Urodynamics: A Double-Blinded Randomized Controlled Trial Neurourol Urodyn. 2009, 28(5):374-379.
- 26. REDA A, FISSEHA S, MENGISTIE B, VANDEWEERD JM Standard Precautions: Occupational Exposure and Behavior of Health Care Workers in Ethiopia – PLoS ONE - 2010, 5(12).

 REDA A, SEYOUM B, YIMAM J, ANDUALEM G, FISEHA S, VANDEWEERD JM – Antibiotic susceptibility patterns of Salmonella and Shigella isolates in Harar, eastern Ethiopia - Journal of Infectious Diseases and Immunity – 2011, 3(8), 134-139.

# 2012

28. PUISSANT E, BOONEN M, XIAOQING W, VANDEWEERD JM, FLAMION B, JADOT M – A new factor of bone resorbtion? – Abstract Book of The Autumn meeting of The Belgian Society for Cell Biology and Development – Namur – Belgium – 2012

# 2013

29. HOUGARDY V, DE LANDSHEERE L, BÉLIARD A, VANDEWEERD JM, NISOLLE M – Efficacité de l'antibiothérapie (fosfomycin trometamol) pour prévenir les infections urinaires lors d'un examen urodynamique : un essai clinique contrôlé aléatoire - Actes du 36 ème Congrès de la SIFUD-PP – Nice – France – 2013.

# 2014

 HOUGARDY V, VANDEWEERD JM, DE LANDSHEERE L, PEATTIE A, BELIARD A, NISOLLE M – Effectiveness of prophylactic antibiotic intervention (fosfomycin trometamol) to prevent urinary tract infections (UTI) due to urodynamic studies (UDS) in women: a randomized controlled trial - Abstract book of United Kingdom Continence Society. Annual scientific – London – UK – 2014 – p. 48

# Focus 2. Diseases in animals

# Horses

# Back pain

- 31. DESBROSSE F, VANDEWEERD JM. Back pain and lameness: a clinical approach to assess their relationship - 9th World Equine Veterinary Association Congress – 2006 – Marrakech – Morocco.
- 32. DESBROSSE F, VANDEWEERD JM. Dorsalgies et boiteries chez le trotteur. Approche clinique de leur relation - Journée Européenne AVEF – 2006 – Roissy – France.
- 33. VANDEWEERD JM, DESBROSSE F Innervation and techniques for injection of lumbar spine in the horse – SIVE Congress – 2006 – Bologna – Italy.
- 34. VANDEWEERD JM, DESBROSSE F, CLEGG P, HOUGARDY V, BROCK L, WELCH A, CRIPPS P Innervation and nerve injections of the lumbar spine of the horse: a cadaveric study Equine Veterinary Journal 2007, 39(1):59-63.
- 35. DESBROSSE FG, PERRIN R, LAUNOIS T, VANDEWEERD JM, CLEGG PD -Endoscopic resection of dorsal spinous processes and interspinous ligament in ten horses - Veterinary Surgery – 2007, 36(2):149-155.
- 36. PERRIN R, LAUNOIS T, DESBROSSE G, VANDEWEERD JM, CLEGG P Chirurgie mini-invasive de rapprochement des apophyses epineuses – Pratique Vétérinaire Equine – 2008, 40 :143-146.

- 37. VANDEWEERD JM, DESBROSSE F. Approche diagnostique lors de dorsalgie chez le cheval Abstract-vet 2013, 2:30-34.
- 38. VANDEWEERD JM, DESBROSSE F. Approche thérapeutique des dorsalgies primaires du cheval Abstract-vet 2013, 3:31-33.
- 39. VANDEWEERD JM, DESBROSSE, F.- Stratégie thérapeutique et réhabilitation lors de dorsalgies chez le cheval Abstract-vet 2013, 4:35-37.
- 40. COOMER RP, MCKANE SA, SMITH N, VANDEWEERD JM A controlled study evaluating a novel surgical treatment for kissing spines in standing sedated horses Veterinary Surgery 2013, 41(7):890-897.

- 41. DALLA VALLE J, CANON C, NICAISE C, PERRIN R, LAUNOIS T, BROGNIEZ L, HONTOIR F, CLEGG P, VANDEWEERD JM – Can cryosurgery be used for treatment of back pain in horses? A preliminary histological study – Proceedings of the 23rd Annual Scientific Meeting of ECVS – Copenhagen – Denmark – 2014.
- 42. DALLA VALLE J, CANON C, NICAISE C, PERRIN R, LAUNOIS T, BROGNIEZ L, HONTOIR F, CLEGG P, HOUGARDY V, VANDEWEERD JM - Modifications histologiques des nerfs lombaires après cryochirurgie: une étude ex vivo – Proceeding du Congrès AVEF – Pau – France – 2014 – p.250, 251.

## <u>Lameness</u>

- 43. DESBROSSE F, VANDEWEERD JM. Classification of joint injuries and diseases Congress of Equine Practitioners – Moscow – Russia – 2008.
- 44. VANDEWEERD JM, PERRIN R, DESBROSSE F, LAUNOIS T, CLEGG P, BROGNIEZ L – A technique for computed tomography (CT) of the foot in the standing horse – Hippos-Canis-Felis Congress – Liège – Belgium – 2008.
- 45. VANDEWEERD JM, PERRIN R, DESBROSSE F, LAUNOIS T, CLEGG P, BROGNIEZ L - Standing CT To Prepare P3 Internal Fixation – Voorjaarsdagen Amsterdam – Amsterdam – The Netherlands – 2008.
- 46. DESBROSSE F, VANDEWEERD JM, PERRIN R, LAUNOIS T, CLEGG P, BROGNIEZ L – Lesions of the foot diagnosed with Computed Tomography (CT) – Congress of Valence – Valence – Spain – 2008.
- 47. DESBROSSE FG, VANDEWEERD JM, PERRIN R, LAUNOIS T, CLEGG PD A technique for Computed Tomographic (CT) of the foot in the standing horse Equine Veterinary Education 2008, 20(2):93-98.
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50. PERRIN R, VANDEWEERD JM – How has MRI changed our approach to the orthopaedic case: surgeon's point of view? – Proceedings of the 20<sup>th</sup> annual scientific meeting of the ECVS – Ghent – Belgium – 2011 – pp 133,134.

2012

- 51. HONTOIR F, DAMAN N, SIMON V, VANDERSTRICHT R, TALLIER M, NISOLLE JF., VANDEWEERD JM Computed tomographic arthrography to identify cartilage defects in the metacarpo (tarso)- phalangeal joint in the horse Proceedings of the Voorjaarsdagen Amsterdam 2012 p 293, 294.
- 52. HONTOIR F, SIMON V, TALLIER M, VANDERSTRICHT R, NISOLLE JF, VANDEWEERD JM – Spécificité et sensibilité de l'arthrographie par scanner (ACT) et de l'imagerie par résonance magnétique (IRM) 3 tesla pour identifier les lésions cartilagineuses de l'articulation métacarpo (tarso)-phalangienne chez le cheval – Actes des 40 èmes Journées annuelles de l'AVEF – Reims – France – 2012, pp. 221-222.

# 2014

53. HONTOIR F, NISOLLE JF, MEURISSE H, SIMON V, TALLIER M, VANDERSTRICHT R, ANTOINE N, PIRET J, CLEGG P, VANDEWEERD JM. – A comparison of 3T magnetic resonance imaging and computed tomography arthrography to identify structural cartilage defects of the fetlock joint in the horse – The Veterinary Journal –. 2014, 199(1):115-122.

## Submitted

54. BROGNIEZ L, PERRIN R, LABED S, LANG F, CLEGG P, VANDEWEERD JM – Associations between responses to hoof extension test and lesions identified by magnetic resonance imaging (MRI) – Submitted at the ECVS congress Berlin 2015.

# Orthopaedic surgery

- PERRIN R, VANDEWEERD JM, LAUNOIS T, BROGNIEZ L, DESBROSSE F Interventional surgery using computed tomography (CT) - Congress ECVS – Basel – Switzerland – 2008 – pp 293-295.
- 56. PERRIN R, VANDEWEERD JM., LAUNOIS T, BROGNIEZ L, DESBROSSE F Computed Tomography (CT) to assist orthopaedic surgery in the horse: clinical experience with a newly developed ambulatory scanner - Congress ECVS – Basel – Switzerland – 2008 – pp 186-189.
- 57. VANDEWEERD JM, PERRIN R, DESBROSSE F, LAUNOIS T, CLEGG P, BROGNIEZ L – Computed Tomography (CT) to assist internal fixation of the distal sesamoid bone in horses – Congress ESVOT– Munich – Germany – 2008.
- 58. VANDEWEERD JM, PERRIN R, DESBROSSE F, LAUNOIS T, CLEGG P, BROGNIEZ L - Computed Tomography (CT) in standing position to prepare surgery for screw insertion into the distal phalanx in horses: an ex-vivo study – Congress ACVS – USA – 2008.
- 59. VANDEWEERD JM, DESBROSSE FG, PERRIN R, LAUNOIS T, CLEGG PD, BROGNIEZ L Computed Tomography (CT) in standing position to identify

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## Focus 1 Diseases in man



# What is the anatomy of the synovial cavity of the ovine knee?

The study "Anatomy of the Synovial Cavities of The Stifle (Knee) in the Sheep" was presented at the ECVS congress of Gent in 2011 and published in The Veterinary Journal in 2012.

# Take home message

Communication between femoro-patellar, medial femoro-tibial and lateral femoro-tibial compartments occurred in all cases. This joint should be considered one synovial structure with three communicating compartments. Major recesses identified: (1) suprapatellar, (2) medial and lateral supracondylar, (3) subpopliteus recess, (4) tendinous recess.

# Introduction

The ovine knee (stifle) has frequently been used as the joint of choice to evaluate surgical and medical therapeutics of osteoarthritis (OA). Synovial cavities are accessed to administer therapeutics and to assess OA with invasive techniques like arthroscopy. Though the ovine stifle has been increasingly used as a model for the human knee, precise anatomical descriptions of the knee in sheep are lacking. The aim of this study was to improve anatomical knowledge of the stifle using positive-contrast arthrography, computed tomography arthrography (CTA) and gross anatomy.

# Material and methods

Specimens - Hind limbs (*n*= 24) from twelve Texel sheep, euthanatized for reasons other than hind limb lameness, were disarticulated at the level of the coxo-femoral joint and collected within 12 h of euthanasia .*Positive-contrast arthrography* - Injection of the stifle joint was performed with contrast medium. Latero-medial, cranio-caudal and cranio-medial caudo-lateral oblique radiographic projections (Iconos 200, Siemens, 60 KV) were taken of each limb to assess the presence of contrast material within the three compartments. *Computed tomography arthrography* - The limbs were subsequently examined by CTA (Emotion 6, Siemens). *Gross anatomy* - The synovial cavities were injected with 26 mL of a resin mixture until moderate distension of the joint was achieved. The position of anatomical structures was carefully noted in relation to the hard and colored molding of resin.

# Results

The stifle joint is a single entity with three communicating compartments. The femoro-patellar compartment (FPC) communicates with the medial femoro-tibial compartment along the abaxial aspect of the medial trochlear ridge (C1) and with the lateral femoro-tibial compartment along the abaxial aspect of the lateral trochlear ridge of the femur (C3).

Major recesses were identified: (1) the femoro-patellar compartment (FPC) bulged medially and laterally to the tibio-patellar ligament and presented a suprapatellar pouch cranially to the distal femur; (2) medial and lateral supracondylar recesses were identified caudally (MSCR, LSCR);(3) a double communicating tract (C2) joined the lateral femoro-tibial compartment and a recess under the tendon of the popliteus laterally and caudally (subpopliteus recess, SPR); (4) the lateral femoro-tibial compartment had a distal synovial recess (tendinous recess, TR) which extended distally on the lateral aspect of the proximal tibia, in the extensor groove, creating a sheath for the common tendon of the m peroneus tertius, m extensor longus digitorum and m extensor medialis (m extensor digiti III proprius). Figure 1 Medial view and lateral view of CT reconstruction of the stifle



Figure 2 Craniall view and caudal view of CT reconstruction of the stifle



#### Discussion

Injection of contrast and resin into the stifle joint was successful and allowed determination of synovial communications. This study confirmed that the stifle joint is a single entity with three communicating compartments. It confirmed also the presence of a distal tendon recess. However, other authors did not report the communicating tract between the cruciate ligaments, the latero-caudal recess under the popliteus tendon, the double communicating tract between the subpopliteal recess and the left femoro-tibial compartment, and the caracteristic anatomy of the proximal insertion of the tibialis cranialis and peroneus longus creating a sheath for the common tendon of the peroneus tertius, m extensor longus digitorum and m extensor medialis before their insertion on the latero-proximal aspect of the lateral trochlear ridge. This study demonstrates also the usefulness of CT arthrography in anatomic research. Our findings have also clinical implications. The tendinous recess may be an alternative site for synoviocentesis and injection in the living animal, as there would be less risk of damaging the articular cartilage. The recesses that were identified could be used as arthroscopic portals.

# How can we inject the synovial cavity of the ovine knee?

The study "Comparison Of Three Techniques To Inject The Synovial Cavities Of The Stifle In The Sheep" was presented at the ECVS congress of Gent in 2011 and published in The Veterinary Journal in 2012.

# Take home message

Three techniques can be used (retropatellar, paraligamentous, subtendinous). The subtendinous technique is as successful as the two conventional techniques and may be the site of choice for injection in the living sheep, as there would be less risk of damaging the articular cartilage if the animal moves.

# Introduction

Injections techniques of the ovine stifle are poorly described in the literature. Two sites have been reported for synoviocentesis in the bovine stifle. Either between the medial or lateral and intermediate patellar ligaments gives access to the femoro-patellar compartment; or, in the extensor groove of the tibia, cranial to the common tendon of the long digital extensor and peroneus tertius, provides access to the lateral femoro-tibial compartment. In human rheumatology, the knee joint is commonly injected laterally and caudally to the patella . The objectives of this study were to compare the three different injection techniques, and to assess whether they are easily performed.

# Material and methods

*Study Design* - Experimental ex vivo study. *Animals* - Cadaveric hindlimbs (n = 24) from 12 adult sheep. *Contrast study* - Injection of the stifle joint was performed with three different techniques: retropatellar (RPT), paraligamentous (PLT), subtendinous (STT) (Figure 1). One of each paired limb was randomly assigned to a technique. The injections were carried out by one experienced veterinary surgeon. The flux of the contrast medium was assessed by fluoroscopy and radiography. *Resin and dissection study* - One of each paired limb was randomly assigned to a technique. The synovial cavities were injected with a resin mixture. The injections were carried out by one inexperienced bachelor student. The limb was dissected to assess the penetration of resin in the compartments of the stifle joint.

# Results

In all limbs, on radiographs, contrast material filled the three compartments (femoro-patellar, medial and lateral femoro-tibial) in a well-defined manner, indicating complete anatomical communication. Dissections and gross anatomy confirmed the presence of resin in all compartments when the resin had been successfully injected intra-articularly.

There was no significant difference between the three techniques for each injector. PLT was significantly more successful when performed by an experienced clinician. The injections were successful respectively for RPT, PLT and STT in 91, 100 and 91 % of cases when performed by an experienced clinician and 83, 66 and 75 % by an inexperienced bachelor student.



**Figure 1.** Three techniques of injection. In the retropatellar technique (RPT), a 21G 1 <sup>1/2</sup> in needle was inserted horizontally under the lateral aspect of the patella, which was manually displaced laterally, at mid-distance between its base and apex. In the paraligamentous technique (PLT), the needle was inserted along the medial aspect of the patellar ligament at mid-distance between its distal and proximal insertions. In the subtendinous technique (STT), the needle was orientated proximo caudally and penetrated the synovial cavity, along the common tendon of peroneus tertius - extensor longus digitorum - extensor digiti III proprius, in the extensor groove, 2 cm below the level of the tibial plateau.

# Discussion

To our knowledge this is the first detailed description of injection techniques of the stifle in the sheep. The three techniques can be used to inject the joint. They are easy to perform as they were used successfully by an inexperienced bachelor student. STT is as successful as the two conventional techniques. With this technique, the needle is inserted along the bone till its tip reaches the tibial plateau without penetrating the lateral femoro-tibial compartment. This may be the site of choice for injection in the living sheep, as there would be less risk of damaging the articular cartilage if the animal moves.

# What is the computed tomographic anatomy of the ovine knee?

The study "Comparison Of Three Techniques To Inject The Synovial Cavities Of The Stifle In The Sheep" was presented at the ECVS congress of Gent in 2011 and published in The Veterinary Journal in 2012.

## Take home message

CTA was a useful technique to image the ovine stifle. One major advantage when working with CTA is the short examination time.

# Introduction

Computed tomography arthrography (CTA) is an imaging technique that can provide indirect information about the cartilage and soft tissues. It involves the intra-articular injection of contrast. It is a routine procedure in human orthopedic imaging. Unlike magnetic resonnance imaging (MRI), it uses ionizing radiation. However time to acquire scans is very short, in the range of 60 seconds, which might be an advantage when working with anesthetized animals. CTA of the stifle has been described in humans and dogs. However, there is no detailed anatomic description of the ovine stifle obtained with CTA. The objectives of this investigation were: (1) to describe normal CTA anatomy of the ovine stifle; (2) to provide reference images for CTA scans.

### Material and Methods

Twenty four limbs harvested from 12 adult sheep cadavers were evaluated. 10 ml radiodense contrast solution (7,5 ml Hexabrix 320 [meglumine and sodium ioxaglate] mixed with 2.5 ml saline) was injected with minimal pressure into the joint. The limbs were examined by computed tomography (Emotion6 Siemens). Acquisition protocol was: 130Kv, 80 mAs, pitch 0.4, collimation 0.63mm and rotation time of the tube 0.6 sec. Images of 0.63 mm were reconstructed with an increment of 0.3mm. From this isotropic volume of images, coronal, sagittal and transversal slices were obtained (1 mm thickness and 1 mm inter-slice gap).

# Results

The legend lists all the structures that can be assessed by CTA. An example of reference image is shown in Figure 1.

# Discussion

Arthrography was easy to perform. General anesthesia would be required for CTA in the living patient. The CTA procedures can be completed quickly with a very short period necessitating immobilization of the animal (1 minute) which is much less than reported time for MRI (magnetic resonance imaging). Multiplanar reconstructions were helpful for complete evaluation of relevant soft tissue structures of the ovine stifle. In comparison with MR imaging, it is more invasive because of the intra-articular injection of contrast material and uses ionizing radiations. The validity of CTA should be evaluated and compared to MRI in identifying cartilage defects in the ovine stifle.

**Legend.** 1 Femur, a Lateral condyle, b Medial condyle, 2 Tibia, 3 Patella, 4 Lateral meniscus, a Cranial horn of the lateral meniscus, b Caudal horn of the lateral meniscus, c Ligamentous insertion of the cranial horn, d Ligamentous insertion of the caudal horn, 5 Medial meniscus, a Cranial horn of the medial meniscus, b Caudal horn of the medial meniscus, c Ligamentous insertion of the caudal horn, d Ligamentous insertion of the caudal horn, 6 Cranial cruciate ligament, a Caudo-lateral bundle of the cranial cruciate ligament, b Cranio-medial bundle of the cranial cruciate ligament, 7 Caudal cruciate ligament, 8 Menisco-femoral ligament, 9 Femoro-patellar compartment, a Supra-patellar recess, 10 Lateral femoro-tibial compartment, 11 Medial femoro-tibial compartment, 12 Tendinous recess of the lateral femoro-tibial compartment, 13 Subpopliteus recess of the lateral femoro-tibial compartment, 14 Lateral supracondylar recess, 15 Medial supracondylar recess, 16 Combined tendon of the peroneus tertius - extensor longus digitorum - extensor digiti III proprius, 17 Patellar ligament, 18 Tendon of gastrocnemius, 20 Tendon of quadriceps femoris, 21 Communication between the femoro-patellar compartment and the lateral femoro-tibial compartment, 22 Communication between the femoro-patellar compartment and the collateral femoro-tibial compartment, 23 Adipous pad, 24 Synovial fold, 25 Lateral collateral ligament, 26 Medial collateral ligament, 27 Tibialis cranialis, 28 Communication between the femoro-tibial compartments through the cruciate ligaments

**Figure 1.** Reference images showing anatomic features of cruciate ligaments in sheep, including the communication tract between the medial and lateral femoro-tibial compartments. Transversal reformations of spiral CT arthrography data in a proximal plane (A) and distal plane (B). Coronal reformations in a caudal plane (C) and median plane (D). Sagittal reformations in two different planes, one more lateral to highlight the cranial cruciate ligament (E) and one more medial to highlight the caudal cruciate ligament (F).



# What is the magnetic resonance anatomy of the ovine knee?

The study "Magnetic resonance imaging anatomy of the ovine stifle described from images acquired with high-field magnet (3 Tesla)" was presented at the ECVS congress of Gent in 2011 and published in Veterinary Surgery in 2013.

### Take home message

MRI was a useful technique to image the ovine stifle. 3 Tesla systems should be a useful tool to assist in longitudinal studies with an ovine stifle model. However, the cartilage seems thin in several anatomical areas and the ability of MRI to adequately identify defects should be assessed.

### Introduction

In human medicine, magnetic resonance imaging (MRI) is considered the 'gold standard' imaging procedure to assess the knee joint. Though the ovine knee (stifle) has frequently been used as the joint of choice to evaluate surgical and medical therapeutics of osteoarthritis (OA), there is little information regarding 3 Tesla MRI evaluation of the ovine stifle. The objectives of this investigation were: (1) to describe normal MRI anatomy of the ovine stifle; (2) to provide reference images for MRI scans.

### Material and methods

Stifles from 12 cadavers underwent MRI examination. Several sequences were used: T1W, IW FS FSE, isotropic 3D sequence (DESS). Parameters were altered several times in order to improve the quality of images. Two types of coils 15 channel-knee and 4 channel-shoulder) and two positions in the gantry were tested on two other euthanized animals. Frozen sections of four stifles were obtained in the three planes (sagittal, coronal and transverse) for a thorough anatomical interpretation of MRI images.

# Results

Best images were obtained with the sequences of table 1. The knee coil was adequate to obtain images but, with intact animals, the stifle could not be entirely and satisfactorily positioned in the coil. On the contrary, it was convenient to use the shoulder coil positioned ventrally to the stifle of the animal in sternal recumbency with the hind limb in extension. There was good differentiation of anatomic structures, including soft tissues and cartilage, though cartilage was thin as demonstrated by measures taken in the median plane of each femoral and tibial condyle (0.7mm SD [0.1mm]) on PD FS images.

In all stifles, the craniomedial bundle of the cranial cruciate ligament inserted caudally to the cranial attachment of the medial meniscus. No transverse intermeniscal ligament was identified in 80% of stifles, whereas a few small ligamentous fibers were seen crossing from 1 cranial horn to the other in 20% of stifles. There was good differentiation of menisci, ligaments, and synovial cavities on MRI images. Two bundles were identified in all cranial cruciate ligaments on MRI. Sensitivity and specificity of 3T MRI for detection of transverse intermeniscal ligament were 42% and 84%, respectively. Examples of reference images are shown in Figure 1.

# Table 1. Acquisition parameters

Table 1 MRI Sequence	es
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	IW FS FSE sagittal	IW FS FSE dorsal	IW FS FSE transverse
TE (echo time; milliseconds)	37	37	35
TR (repetition time; milliseconds)	3200	3000	4400
Acquisition time (minutes)	3.26	2.11	3.06
FOV (cm)	16	16	16
Volume pixel	$0.4 \times 0.4 \times 2$	$0.5 \times 0.5 \times 2$	$0.5 \times 0.5 \times 2$
Matrix	384 × 384	320 × 320	320 × 320
Slice thickness (mm)	2	2	2

Figure 1. Example of reference image.



Figure 1 Sagittal IW FS FSE sequence in a parasagittal plane through the extensor groove situated at the lateral aspect of the proximal tibia. 1a lateral femoral condyle; 2 tibia ; 3 patella ; 4a cranial hom of lateral meniscus; 4b caudal hom of lateral meniscus ; 9 suprapatellar recess; 10 femoropatellar compartment; 11 subpopliteus recess; 12 subtendinous recess; 14 lateral supracondylar recess; 16 common tendon of the *m peroneus tertius, m extensor longus digitorum* and *m extensor medialis (m extensor digiti iii proprius)*; 18 tendon of the *m popliteus*.

#### Discussion

To our knowledge, this is the first descriptive study to provide a set of MRI images of the ovine stifle with a 3 Tesla system. It demonstrates that it is feasible but that positioning and the choice of the coil is essential to apply the technique in living animals. In addition, it is expected that the animal should remain anesthetized for 50 minutes to acquire sequences that enable the study of soft tissues, subchondral bone and cartilage on both hindlimbs. This anatomic study confirmed that the cranial cruciate ligament is made of two bundles, as described by Allen et al. However, it was not possible to identify the inter-meniscal ligaments that this author reported.

It was not our purpose to assess the efficacy of 3T MRI sequences to detect cartilage, our images illustrate the narrow thickness of ovine stifle cartilage, which may limit assessment of cartilage morphology and volume even with a 3T magnet. To see early morphologic degenerative changes in cartilage, imaging with a resolution of 0.2–0.4 mm is required.33 This may have implications for research and necessitate the development of sequences with high resolution.

# What is the anatomy of menisci at MRI, CT and 3D segmentation?

The study "An anatomic study of ovine menisci by computed tomographic arthrography (CTA), 3D segmentation, magnetic resonance imaging (MRI) and gross anatomy" was presented at the ECVS congress of Gent in 2011 and published in Veterinary Surgery in 2013.

# Take home message

MRI, CTA and 3D segmentation were useful to investigate the anatomy of ovine menisci. Segmentation was not difficult and might provide a useful 3D representation of the meniscus before surgical intervention.

# Introduction

Meniscal pathology has been reported in several species including man, dog and horse. However, there is limited information about the shape, ligamentous insertions and imaging anatomy of ovine menisci. The objective of this study was to document the anatomy of ovine menisci by computed tomography arthrography (CTA), 3D segmentation, magnetic resonance imaging (MRI), and gross anatomy.

# Material and methods

Stifles from 12 cadavers were examined by CTA (computed tomographic arthrography), 3D segmentation (computerized 3Disolation of menisci from surrounding tissue in CT images) and 3T MRI (magnetic resonance imaging). Dissection was performed afterwards and the menisci were observed and photographed. In two other pairs of frozen limbs transverse sections were obtained, were photographed and compared to the corresponding magnetic resonance image for identification and confirmation of each anatomic structure.

# Results

In 100 % of cases, on a dorso-ventral view, the contour of the medial meniscus (MM) could be superposed to the arm of a C. In 79 % of cases, the contour of the lateral meniscus (LM) was similar to the curvature of a zero [0] and of a C in 21 % of cases. This was visible on 3D segmentation images (Figure 1) and specimens. Mean time to isolate menisci and surrounding structures was 11 minutes. An example of MRI anatomy of menisci is shown in Figure 2.

Figure 1. The O and C shape by gross anatomy and 3D segmentation









*Figure 2. Example of MRI anatomy.* Transverse IW FS FSE sequence obtained at the level of menisci (A) and dorsal IW FS FSE sequence obtained through the caudal part of the tibial plateau (B). 1a lateral femoral condyle; 1b medial femoral condyle 2 tibia ; 4 lateral meniscus; 4a cranial horn of lateral meniscus; 4b caudal horn of lateral meniscus ; 4c cranial lateral meniscotibial ligament; 5 medial meniscus; 5a cranial horn of medial meniscus; 5b caudal horn of medial meniscus; 5c cranial medial meniscotibial ligament; 5d caudal medial meniscotibial ligament; 6 cranial cruciate ligament; 6a caudolateral bundle of cranial cruciate ligament; 7 caudal cruciate ligament; 8 meniscofemoral ligament; 14 lateral supra- condylar recess; 15 medial supra- condylar recess; 16 common tendon of the m peroneus tertius, m extensor longus digitorum and m extensor medialis (m extensordigiti iii proprius); 18 tendon of the m popliteus; 21 infra- patellar fat pad; 22 lateral collateral ligament.



# Discussion

They provided accurate and complementary information to previous anatomic studies based on dissections. In our study, there were no ligaments between menisci. The menisci were easily delineated and it is likely that tears would be identified easily in clinical cases or meniscal tear models.

# What is the prevalence of cartilage defects in a population of research sheep?

The study "**Prevalence of naturally occurring cartilage defects in the ovine knee**" was presented at the OARSI congress in Barcelona in 2012, and published in Osteoarthritis and Cartilage in 2013.

# Take home message

Our data seem to indicate that naturally occurring OA exists in ageing sheep, at least subclinically. It might be useful to take into account prevalent cartilage defects at baseline in studies using ovine models.

### Introduction

Articular cartilage (AC) defects of the knee are common in humans and are encountered in approximately 60% of knee arthroscopies. The extent of pathology ranges from small focal defects to widespread damage to the articular surface. In animals, there is limited information about the prevalence and impact of AC defects. Palmar osteochondral disease (POD) and AC defects in the metacarpo- phalangeal joint are prevalent in Thoroughbred racehorses. In sheep, microscopic lesions of osteochondrosis were reported to be frequent in fast-growing lambs although few defects progressed to gross lesions. Spontaneous non infectious osteoarthritis (OA) has not been reported in this species. Proteoglycan loss, cartilage matrix atrophy and erosion of the cartilage consistent with OA change occur spontaneously in the unprotected region of the medial tibial plateau in goats as young as 2 years of age.

As ovine models of knee OA are an essential modality for exploring the pathophysiology and therapy of the disease, information about the prevalence of AC abnormalities in this joint would be useful.

The objective of this study was to determine the prevalence, anatomical location and severity of naturally occurring cartilage defects in the knee within a population of adult ewes enrolled for research.

# Materials and methods

Articular cartilage (AC) of the distal femur, proximal tibia and patella was assessed using OARSI recommendations for macroscopic and microscopic scoring of ovine cartilage. Synovial fluid analysis and histology of the synovial membrane were performed. All limbs were examined by computed tomography.

# Results

Twenty eight sheep (n = 28; 43 %) presented at least one score 2 or score 3 lesion. Twenty two (n = 22; 34 %) sheep were macroscopically normal. Most frequent localisations of lesions were: axial aspect of the central third of the medial tibial condyle (32,7% of the lesions), middle third of the medial femoral condyle (29,4%), middle third of the articular surface of the patella (9,8%), and axial aspect of the central third of the lateral tibial condyle (9,8%). Grade of macroscopic lesions was significantly (H(3) = 29.31, p 0.000) affected by age. Macroscopic score correlated well with histological changes that can be found in osteoarthritis (OA) (r 0.83; p 0.000). Neither clinical signs of OA, nor cytological and histological signs of inflammation were identified, while imaging abnormalities were very rare.

Figure 1. Examples of score 0 (yellow arrow), 1 (red arrow) and 2 (black arrow) defects.



# Discussion

The population of sheep in the "Centre de Recherche du Mouton" includes 450 ewes producing about 1000 lambs a year. Besides research in reproduction, retired animals are used for orthopaedic research and teaching. Retirement is usually due to non-orthopaedic diseases (such as mastitis, metritis, loss of fertility) and occurs between 5 and 11 years. In this study, younger animals were also used so that a larger range of ages was considered to assess the prevalence of cartilage defects, and the effect of age. This study demonstrated that score 2 and 3 cartilage defects were prevalent in 43 % of animals in this population of sheep, and they were correlated with histological changes that can be found in OA. However, no clinical signs of osteoarthritis were present, such as joint effusion and lameness. Synovial fluid and synovium analysis did not reveal any sign of inflammation. In addition, changes like osteophytes and SB sclerosis were nearly never identified by CT. The clinical significance of those AC defects remains to investigate.

In research, there is a perceived advantage in using naturally occurring models of OA in that they are more like human OA with slower onset and progression. Osteoarthritis is a disease of ageing, but age alone does not cause osteoarthritis; rather, the vulnerabilities of the joint that occur as part of ageing make the joint susceptible to disease. Thus, to obtain the most meaningful insights into human OA, it might be interesting to consider the use of old animals (after 8 year old for example) as well as to evaluate whether exercise could exacerbate the development of subclinical and clinical OA in adults between 4 and 8 year old. As macroscopic and histological scorings of AC abnormalities are essential outcome measures in research studies using ovine induced models, our results suggest that, in a population of crossed-bred Texels used for research, it might be useful to take into account prevalent cartilage defects at baseline.

# How can we anesthetize research sheep?

The study "**Comparison of IV pentobarbital and IV propofol for induction and maintenance of anesthesia in sheep**" was presented at the ECVS congress of Rome in 2013 and is in press in STAL (Sciences et Techniques de l'Animal de Laboratoire).

# Take home message

This study indicates that both protocols **(IV pentobarbital and IV propofol)** are safe. Vital parameters (heart rate, respiratory rate) remain stable and within normal limits. An additional advantage of pentobarbital is its costs. We can therefore likely recommend the use of pentobarbital for induction and maintenance of anesthesia in sheep both in research and field anesthesia.

# Introduction.

The present study was designed and conducted to compare the efficacy, cardio-vascular and respiratory effects of propofol and pentobarbital to induce and maintain anesthesia in the sheep. By answering that question, it will be possible to advise practitioners and researchers working with sheep.

# Material and methods.

In a cross-over study, six ewes aged 5 to 6 years old were anesthetized with IV pentobarbital or IV propofol. After sedation with 150µg/kg xylazine and 150µg/kg diazepam, pentobarbital was used at a dose of 3mg/kg for induction and 10mg/kg/h for maintenance, while propofol was used respectively at a dose of 2mg/kg and 20mg/kg/h. After induction, sheep were insufflated with 6L/min oxygen and several parameters were recorded every 5 min including heart rate, respiratory rate, SpO2 (pulse oxymetry) and depth of anesthesia. Rectal temperature was recorded every 10 min.

# **Results.**

Several sheep necessitated a supplementary dose (mean total induction doses were 4.8 mg/kg for pentobarbital and 2.8 mg/kg for propofol). The respiratory rate with pentobarbital (mean 27) was not significantly different from the one with propofol (mean 28). Heart rate with propofol (mean 99) was statistically higher than pentobarbital (mean 68) (t 5.2; p < 0.001). An increase of heart rate and respiratory rate occurred after 30 minutes with propofol. Respiratory movements were more briskly with propofol. Quality of anesthesia was significantly better with pentobarbital (W = 21, N = 6, p = 0.005). SpO2 remained stable (between 90 and 100 %) with no significant difference between both protocols. Rectal temperature was not statistically different between pentobarbital (mean 38.8°C) and propofol (mean 38.6°C). Bloating did not occur and slight regurgitation was noticed in all animals. One sheep developed an apnea of 1 minute at induction with propofol. Recovery was longer with pentobarbital. No sheep had other adverse events.

Steps	Time	Pentobarbital	Propofol		
Premedication	T 0 minute	Xylazine IV 150µg /kg	Xylazine IV 150µg /kg		
	T 5 minutes	Diazepam IV 150µg /kg	Diazepam IV 150µg /kg		
Induction	T 10 minutes	Pentobarbital 3 mg/kg	Propofol 2 mg/kg		
	T 13 minutes	Incremental dose if no effect (hypnosis,	Incremental dose if no effect		
		muscular relaxation)	(hypnosis, muscular relaxation)		
Intubation and oxygenation (6L/min)					
Maintenance	T 75 minutes	10 mg/kg/h in 0.9 NaCl	20 mg/kg/h in 0.9 NaCl		

Table 1. Anesthetic protocols used in this study (IV = intravenously)

*Figure 1. Induction of anesthesia.* After induction with either PFL or PBL, the sheep was placed in sitting position and the neck was extended. A laryngoscope (Kawe, Germany) was used to visualize the glottis, and a cuffed 8.0 endotracheal tube was inserted, inflated with 15ml air and secured. It was connected to an ambu-bag and 100 % oxygen was administered.





## Discussion

This study indicates that both protocols are safe. Vital parameters (heart rate, respiratory rate) remain stable and within normal limits. In combination with 100 % O2 insufflations, both drugs ensure satisfactory SpO2. The dose of propofol used in this study at the beginning of maintenance (20 mg/kg/h) was not sufficient to insure a satisfactory depth of anesthesia necessitating increasing the rate to 30 mg/kg/h. The anesthesia was of good quality and all parameters were stable when pentobarbital was used. Propofol at the above doses seemed to be associated with lighter anesthetic state. One animal developed an apnea after induction with propofol. Apnea is common (Correia et al, 1996) and has been suggested to be attributed to the rapid rate of administration (Pablo et al, 1997). Injection was probably too fast in our case. Interestingly, movements of the thorax were less briskly with pentobarbital. Considering the context of this study (investigation of an ovine model of OA) and the need to identify the best anesthetic protocol to immobilize those research animals during MRI or CT, pentobarbital is probably the drug of choice due to the quality of anesthesia and the quieter respiratory movements. An additional advantage of pentobarbital is its costs: mean cost of the procedure was 2.8 euros for pentobarbital and 31.5 for propofol. We can therefore likely recommend the use of pentobarbital for induction and maintenance of anesthesia in sheep both in research and field anesthesia. However, our study was limited to six individuals and our results should be confirmed in a larger number of animals.

# Can we assess cartilage defects of the ovine knee at baseline by computed tomography arthrography?

The study "Accuracy of computed tomography arthrography (CTA) for assessment of articular cartilage defects in ovine stifles" was presented at the OARSI (OsteoArthritis Research Society International) congress of Paris in 2014.

# Take home message

CTA is an acceptable method to non-invasively detect naturally occurring cartilage defects in the ovine knee. This technique can be applied on living animal. It could be used as a tool to assess cartilage morphology at baseline in clinical trials.

# Introduction

Articular cartilage (AC) defects can be prevalent in stifles of sheep used for research. They also develop in this joint in induced models of osteoarthritis. Though assessment of AC defects by imaging modalities is useful in longitudinal studies, it can be limited by the small thickness of the ovine stifle cartilage. Computed tomography (CT) arthrography (CTA), in man, has been reported to be an accurate technique to identify AC defects joints with thin cartilage such as hip or ankle. The objective of this study was to assess the accuracy of CTA to evaluate naturally occurring cartilage defects of the ovine stifle joint.

# Material and methods

**Animal and imaging.** Hindlimbs (n=28) were collected from crossed Texel ewes. Knees were injected with contrast medium (6ml of Hexabrix® in 14 mL saline) and were flexed and extended 100 times to provide a homogeneous coating of the articular surfaces. The limbs were subsequently examined with an Emotion 6 (Siemens). Acquisition protocol was: 130KV, 80mAs, pitch 0.4, collimation 0.63mm and rotation time of the tube 0.6s. Images of 0.63mm were reconstructed with an increment of 0.3mm. CTA was also performed In two living sheep (4 hindlimbs) under anaesthesia to test the feasibility of the technique.

**Assessment.** After imaging, hindlimbs were dissected and the articular surfaces were examined by gross observation. Samples were harvested from all sites with macroscopic lesions and from randomly selected macroscopically intact areas to perform histological assessment. CT images were analyzed blindly by two observers. Repetition of the blinded assessment allowed determining intra-observer reproducibility. Imaging scoring is summarized in Table 1.

**Statistics.** Spearman's rank order test was used to assess correlation between macroscopic and histological scoring. Sensitivity, specificity, positive predictive value and negative predictive value were calculated by using gross anatomy and histopathology as gold standard (only sites where histopathology confirmed the macroscopic classification of defects were used; i.e. where a score 2 defect assessed macroscopically was a partial defect not involving the SB at microscopy, while a score 3 defect was a full thickness defect). Inter-observer and intra-observer agreement were assessed by using Kappa statistics.
# Results

105 histological samples were processed and analyzed. There was substantial agreement between macroscopic examination and histology (Spearman correlation coefficient 0.75; P < 0.0001). CTA sensitivity and specificity were respectively 86.12% +/-1.20 and 94.44%+/-0.56 to detect over-all cartilage defects (no defect versus defect). There was substantial agreement between scores at macroscopic examination and those at CTA (Spearman correlation coefficient 0.83; P < 0.0001). Inter- and intra-rater agreement was good (Kappa value: 0.67 and 0.93, respectively). In living subjects, two difficulties were encountered: breathing movements and limb positioning. However, there was no significant difference between specificities and sensitivities of CTA in living animals and cadavers.

# Figure 1. Examples of cartilage defects



# Discussion

The technique seems accurate to identify cartilage defects at baseline. However, results should be confirmed when the entire body is used and especially in living animals. In lateral recumbency, despite the forced extension of the limb, the pelvis remained in the acquisition field, affecting the quality of image since the radiation has to go through pelvis, soft tissues and knee before reaching detectors.





# How do the number of tidemarks and the thickness of CC vary in the knee in a population of sheep without clinical signs of OA and of various ages?

The study "Age-related changes of the cartilage-bone interface in the ovine knee" was presented at the Worl congress on osteoarthritis and osteoporosis congress in Sevilla in 2014. It has been submitted and is currently under review.

## Take home message

This study shows that (1) CC and HC thickness vary with anatomical localization in the knee; (2) the number of tidemarks vary with age, (3) there is no correlation between the number of tidemarks and other histological changes that are characteristic of OA (OARSI histological score), (4) CC and HC thickness do not vary significantly in this population of healthy animals between 0 and 8 years It might be more relevant to consider other criteria rather than duplication of tidemarks in developing histological scoring methodologies.

## Introduction

As the ovine knee is commonly used to study OA, it is important to characterize and understand the changes of its cartilage-bone interface. The objectives of this study were to document the variation of the number of tidemarks and the thickness of calcified cartilage (CC) and hyaline cartilage (HC) of the knee in a population of sheep without clinical signs of OA and with various ages.

## Material and methods

3 to 4 mm thick osteochondral slabs were cut at the centre of the medial tibial condyle (TCM), medial femoral condyle (FCM), lateral tibial condyle (TCL) and lateral femoral condyle (FCL) which we have identified as predilection sites for cartilage erosions. Tibial slabs were centered on the intercondylar eminence. Femoral slabs were obtained in the center of the middle third of the circonference of the condyle (black arrow). Rectangles (Figure 1) illustrate the histological slides that were obtained, each abaxial (Ab) and axial (Ax) part being assessed separately at microscopy. Samples were stained with toluidine blue. Slices were examined by two investigators working in consensus and cartilage was scored using the OARSI criteria for histological evaluation of articular cartilage in sheep. A Modified OARSI Score of OA was also calculated by summing the different subscores except that referring to the qualitative assessment of the tidemark (Modified OARSI Score = structure + cell cloning + chondrocyte density + inter-territorial toluidine blue + extension). Number of tidemarks and CC and HC thicknesses were assessed at 6 different equidistant sites (left third, middle third, and right third of the axial and abaxial part of the sample). The OARSI score for histopathology of articular cartilage in sheep, number of tidemarks and thicknesses were assessed in the eight subregions (TCLAb, TCLAx, TCMAb, TCMAx, FCLAb, FCLAx, FCMAb, FCMAx).

## Results

OARSI histological scores were the highest in the axial part of the medial femoral, medial tibial and lateral tibial condyles. Scores varied significantly with anatomical region (H = 90.13; p < 0.001).

Thickness of CC and HC varied significantly (respectively, H = 52.18, p < 0,001, and H = 166.56, p < 0,001) between subregions FCMAx (median CC = 0.39 mm; median HC = 1.46 mm), FCMAb (0.34 mm; 0.98 mm), FCLAx (0.36 mm; 0.94 mm), FCLAb (0.27 mm; 0.51 mm), TCMAx (0.34 mm; 1.14 mm), TCMAb (0.35 mm; 0.68 mm), TCLAx (0.36 mm; 1.30

mm) and TCLAb (0.32 mm; 0.59 mm). Thickness of CC and HC varied significantly (respectively, H = 29.87, p < 0,001, and H = 98.70, p < 0,001) between regions FCM (median CC = 0.37 mm; median HC = 1.24 mm), FCL (0.30 mm; 0.72 mm), TCM (0.34 mm; 0.89 mm) and TCL (0.34 mm; 0.91 mm).

The median numbers of tidemarks, in the group of samples with OARSI score for structure of 0 and 1, were 0.0, 4.4 and 6.4 in categories of age 1 (0 to 3 years), 2 (4 to 6 years) and 3 (7 and 8 years) respectively; it varied significantly with categories of age (H = 100,761; p < 0,001). In the group of samples with a OARSI score for structure of 2 to 10, the mean number of tidemarks (2.2., 4.5 and 6.5) varied significantly between categories of age 1, 2 and 3 (F = 52.64; p < 0,001). There was no significant correlation between the number of tidemarks and the histological measure of osteoarthritis as determined by the modified OARSI score. When considering all slides (all OARSI scores), no tidemark was visible in the three sheep aged between 5 and 6 months (Letter B, figure 1), and median numbers of tidemarks were 1.7 and 3.0 for animals aged respectively 2 and 3 years.



Figure 1. Anatomical regions. Measure of thickness. Tidemark count.

## Discussion

In this population of sheep aged between 0 and 8 years old without clinical signs of OA, CC and HC thickness varied with anatomical localization. There was no evidence of association between CC thickness and loads. CC and HC thickness did not vary significantly with ageing. The number of tidemarks varied with age although there was no correlation between the number of tidemarks and other changes that are histologically characteristic of OA. While the current study provided useful documentation about the cartilage-bone interface in healthy animals, no information was obtained about changes associated with clinical naturally occurring or induced OA. It will be useful to enroll older animals (over 9 years old) in a future study to assess changes at a time when OA is more likely to occur. It will also be useful to study younger animals (1 year old) to determine the time of apparition of the first tidemark.

# How does the biochemical composition of the cartilage vary in the knee in a population of sheep without clinical signs of OA and of various ages?

The study "**Biochemical changes in the cartilage of the ovine knee**" was presented at the Worl congress on osteoarthrtitis and osteoporosis congress in Sevilla in 2014.

## Take home message

The current study provided useful reference values in normal cartilage or subclinical stages of OA. Water and proteoglycans seem to be in higher concentration in loaded regions (medial condyles, axial parts). Water increase might be an early event of cartilage deterioration.

## Introduction

As the ovine knee is commonly used to study OA, it is important to characterize and understand the biochemical composition of its articular cartilage The objectives of this study were (1) to assess whether cartilage composition varies with anatomical regions, age, and histological changes associated with OA in the ovine naturally ageing femoro-tibial (FT) joint, (2) to map the composition of the cartilage in the FT joint.

# Material and methods

70 knees from thirty five research ewes were assessed. They were 8, 15, 12 respectively in three categories of age (between 0 and 3 years old; 4 to 6; 7 and 8). Osteochondral slabs (for histology) and cartilage samples (for biochemistry) were collected in 8 anatomical regions (axial and abaxial areas of the median part of medial and lateral tibial and femoral condyles). The OARSI criteria were used for histological evaluation. Cartilage samples were weighted before and after freeze drying to determine water content. Glycosaminoglycans (GAGs) content was measured by using the dimethylene blue assay, and collagen through the hydroxyproline assay.

# Results

Water and GAGs contents were significantly higher in the medial condyle than in the lateral condyle both for the tibia and the femur. No significant difference was identified for collagen. Water and GAGs were significantly in higher concentration in axial regions than abaxial regions, while collagen tended to be higher abaxially. There was no significant influence of age on biochemical content, except for the axial part of the medial tibial condyle where proteoglycans decreased with increasing age.

Water content increased significantly with increasing OARSI subscore for structure, while contents in proteoglycans and collagen did not vary significantly.

A map of biochemical contents was designed in function of age and histological (OARSI) score for structure (Figure 1).





### Discussion

Conclusions of this study are that: (1) the content in the different biochemical components of articular cartilage (water, collagen and proteoglycans) vary with anatomical regions of the femoro-tibial joint. This variation could fit to differences of compression and shear stresses; (2) age influences the OARSI score, that increases in older sheep, but does not influence contents in the different biochemical components. This may be due to the fact that lesions are subclinical and correspond to a naturally occurring process of slow deterioration of cartilage; (3) water content increased with the OARSI score while collagen and proteoglycans did not. This could be explained also by the slow deterioration of the cartilage network allowing attraction of water while collagen and proteoglycans are still present in the cartilage.

# What are the existing compositional imaging techniques that can be used to assess biochemical composition of articular cartilage?

The current review "Magnetic resonance compositional imaging of articular cartilage: what can we expect in veterinary medicine?" is in press in The Veterinary Journal

## Take home message

Technical issues still limit the use of some techniques both for research and clinical use, especially in veterinary medicine. Gag-CEST (glycosaminoglycan chemical-exchange saturation transfer) and sodium imaging necessitate 7T magnets, which have limited availability. Long acquisition times are sometimes required, for instance in T1 rho and DWI (Diffusion-Weighted Imaging), and necessitate general anesthesia. With dGEMRIC (delayed Gadolinium-Enhanced MR Imaging of Cartilage), the optimal delay between two acquisitions is longer. Even in human medicine, some techniques are not fully validated such as UTE (Ultra-short echo T2), and nearly all techniques require validating for veterinary research or clinical practice. So far, T2 mapping seems to be most applicable for compositional imaging of animal cartilage.

# Introduction

Since cartilage has limited ability for repair, it is useful to determine its biochemical composition as soon as possible in clinical cases. It is also important to assess cartilage content in research animals in longitudinal studies in vivo. In recent years, compositional imaging techniques using magnetic resonance imaging (MRI) have been developed to assess the biochemical composition of cartilage. This article describes the MR compositional imaging techniques, and discusses their use and interpretation.

# Application in veterinary medicine

The current review showed that compositional imaging of cartilage in animals is primarily undertaken for research purposes, using small or large animal models. Technical issues still limit the use of some techniques both for research and clinical use. Gag-CEST and Sodium imaging necessitate 7T magnets and long acquisition times (respectively approximately 15 and 30 min).

T2 mapping can be performed at 1.5 T with an acceptable acquisition time (about 4 mins) and is easily applicable in veterinary medicine. The intra- and interobserver variability of T2 mapping seems low and its ability to differentiate repair cartilage from normal cartilage has been demonstrated in animal studies. However, human studies have shown that several factors can influence T2 values such as the age, the level of exercise and the orientation of the collagen fibrils to the Z-axis, hence the anatomy and the curvature of the articular surface. In addition, this orientation effect can be different in vivo and ex vivo. Therefore, it would be advised to validate the technique for each species and joint to account for all the above variables, and to keep the same protocol between studies to enable comparison. The use of T2\* mapping could shorten acquisition time (around 2 minutes).

T1p could be a good complement to T2 mapping as it seems to be more correlated to PG content, while T2 is more an indicator of collagen network. One difficulty with T1p, highlighted by human studies, is to reach a good resolution in a reasonable acquisition time despite the use of a 3.0 T magnet (14 mins). Its use in veterinary medicine has been

reported in only one study in cadaver specimens. In addition, the sequence is not available for all MRI systems (for example, it is not provided by Siemens).

dGEMRIC can be used with a 1.5 T system. However the time of the MRI investigation is very long (60 to 120 mins) and would increase the anesthaetic risk. In addition, penetration of Gd-DTPA<sup>2-</sup> varies with injecton technique (intravenous or intra-articular), time after administration, mobilisation of the joint, thickness of cartilage, or other inherent cartilage differences between species and joints. This should be taken into account to compare studies.

The poor spatial resolution of the thin cartilage encountered in some articular surfaces in animals (for example, the metatarsus-metacarpus in horses) is a frequent difficulty. It occurs not only with the compositional MRI techniques discussed in the current review but also with conventional MRI even when using high field magnet and sequences optimal for the study of cartilage. Higher field strength magnet could probably improve resolution and acquisition time. However, due to the the cost of such equipment, it is unlikely this adaptation will be in use soon.

The UTE-T2 mapping has not been investigated in veterinary research. DCW was initially tested in vitro on fragments of canine cartilage in 1995 but has not received much interest since.

*Figure 1. Example of a T2 mapping in the ovine knee.* The color scale assigns a color to each T2 relaxation time. Tissue with a long relaxation time (RT) will appear green, to yellow (like synovial fluid SF). Short-RT tissue (cartilage [C], for example) will appear blue.



## What is the magnetic resonance imaging of the lumbar spine in the sheep?

The study "Magnetic resonance imaging (MRI) anatomy of the ovine lumbar spine" was presented at the ECVS congress in Barcelona in 2012 and published in Anatomia, Histologia, Embryologia in 2013.

## Take home message

MRI was able to define most anatomical structures of the ovine spine in a similar way as what can be performed in humans. In both T1W and T2W, the signals of ovine IVDs were similar to those observed in man. Salient anatomical features were identified. These results provide data that may be helpful to plan future studies that contemplate the use of sheep as a model for the human spine.

## Introduction

Although there is an interest in using MRI for research studies evaluating the progress of a spinal disease or a therapeutic, there is little peer-reviewed information regarding the imaging anatomy of the ovine spine. The objective of this study was to describe the ovine lumbar spine by MRI and compare it with anatomy in man.

## Material and methods

10 ewes (n = 10) euthanized for reasons (e.g. mastitis) not related to lameness or back pain were used for the study. The animals weighted between 50 and 65 kg and were 4 to 7 years old. The lumbar segments of the spines were detached after evisceration and by transection with a band saw. 1.5 Tesla MR (Magnetom Symphony, Siemens, Germany) imaging of the spines was performed. Sagittal and transverse sequences were obtained in T1 and T2 weighting (T1W, T2W). Acquisition parameters are reported in Table 1. A four channel abdominal coil (Siemens, Germany) was positioned dorsally to the lumbar region. Images were then transferred on a medical digital imaging system (PACS, Telemis) for analysis. In order to validate MR reference images, these were compared to gross anatomic sections performed with a thin band saw through four of those lumbar spines after freezing. The sections obtained were about 5 mm thick. The gross sections were photographed and compared with MR images for identification of anatomic structures.

	Sag T1	Sag T2	Tra T1	Tra T2
Acquisition time	3:07	2:21	4:32	2:20
(minute:secs)				
Voxel	1.2×0.7×4	1.2×0.7×4	0.7×0.4×2	1×0.7×4
FOV	360	360	200	320
Bandwidth(Hz/Px)	181	150	180	130

Table 1. Acquisition parameters.

## Results

MRI was able to define most anatomical structures of the ovine spine in a similar way as can be imaged in humans. In both T1W and T2W, the signals of ovine IVDs were similar to those observed in man. Salient anatomical features were identified: (1) a 2 to 3 mm linear zone of hypersignal was noticed on both extremities of the vertebral body parallel to the vertebral plates in sagittal planes ; (2) the tendon of the crura of the diaphragm appeared as a

hypointense circular structure between hypaxial muscles and the aorta and caudal vena cava; (3) dorsal and ventral longitudinal ligaments and ligamentum flavum were poorly imaged; (4) no ilio-lumbar ligament was present; (5) the spinal cord ended between S1 to S2 level, and the peripheral white matter and central gray matter were easily distinguished on T1W and T2W images.

Examples of reference images are shown in figures 1 and 2.

*Figure 1 Transverse image obtained at the middle of L3.* 1. Vertebral body, 2. Epidural fat, 3. Longitudinal veins, 4. Cerebrospinal fluid, 5. Spinous process, 6. M. multifidus, 7. M. longissimus dorsi, 8. M. intertransversarii dorsales, 9. Transverse process.



*Figure 2. Sagittal view obtained at the median plane.* 1.Spinal cord, 2.Cerebrospinal fluid, 3.First sacral vertebra, 4.Spinous process, 5.Supraspinous ligament, 6.Interspinous ligament, 7.Basivertebral veins, 8.Subcutaneous fat, 9.Epidural fat, 10.Nucleus pulposus and inner part of annulus fibrosus, 11.Outer part of annulus fibrosus, 12.Vertebral body, 13.End of spinal cord. Note in T2W the nuclear cleft that appears as a hypointense spot in the center of the nucleus.



## Discussion

MRI was able to define most anatomical structures of the ovine spine in a similar way as what can be performed in humans. In both T1W and T2W, the signals of ovine IVDs were similar to those observed in man. Further studies should be performed in living animals.

# How can we inject lumbar disks by CT guidance?

The study "**CT – guided injection technique into intervertebral discs in the ovine lumbar spine**" was presented at the ECVS congress in Barcelona in 2012 and published in European Spine in 2013.

## Take home message

The technique described in this study is accurate. The model used herecould also be useful to train less experienced surgeons or radiologists to disc injection. This CT-guided injection seems to offer several advantages like ease of use, good success rate, the possibility to avoid important nervous and vascular structures, and low radiation.

## Introduction

CT is a modality of choice for guidance in many percutaneous minimal invasive interventional procedures and several CT-guided interventional procedures have been described for management of lumbosacral pain, e.g. periradicular infiltration, percutaneous laser disc decompression, intraarticular steroid injection, percutaneous facet joint denervation, and percutaneous lumbar vertebroplasty.

The objective of this study was to design and evaluate a CT-guided injection technique of the IVDs in the ovine lumbar spine, to report technical difficulties, and to measure the rate of successful injection. The current study might provide useful clinical information that will help researchers to induce IVDD in ovine models and to test minimal invasive therapies in those models.

## Material and methods

Insertion of needles into the nucleus pulposus was assessed by gross anatomic dissection in 2 lumbar segments (group A), and injection of liquid within the disc was assessed by discography in 6 segments (group B). Two spines (n = 2) were used for training and developing the technique. The injection technique is illustrated in figure 1. A  $21G \times 3^{1}/_{2}$  inch (0.7mm×90mm) needle (Becton Dickinson, Madrid, Spain) was used.

## Identification of the injection plane

The transverse processes of the lumbar vertebra were identified by palpation. A metallic marker (a stainless steel pin) was positioned on the skin longitudinally at the vertical projection of the abaxial extremity of the transverse processes of the lumbar vertebrae corresponding to the targeted IVD (for example, processes of L2 and L3 if the L2-3 IVD was targeted). The researcher left the room and a CT scan was performed to assess whether the marker was adequately placed (figure 1a). One rotation of CT scan was 18 mm long with 6 slices and covered the whole IVD. In this study, acquisition parameters for monitoring scans were 50 MAs, 130 KV, scan time 0,65 second, slice thickness 1 mm. The slice which best identified the IVD was determined.

## Identification of the injection axis

Then the virtual axis of the needle was drawn on the CT image using software, starting from the metallic landmark and targeting the center of the nucleus pulposus of the IVD. The angle of the axis to the median plane was measured. This angle was used to generate a laser beam from a laser generator at the top of the gantry. The laser beam had an orientation corresponding to the measured angle, and was in the plane of the slice which best identified the IVD (figure 1b).

## Identification of the entry site for the needle

The intersection of the beam and the metallic marker constituted the landmark on the skin to introduce the needle in the skin.

Identification of the injection depth

The direction of the needle was determined by the laser beam. The insertion of the needle consisted of pushing the needle forward while keeping it in the axis of the laser beam (figure 1c). The needle was inserted to a distance equal to the distance between the skin and the border of the IVD measured on the initial CT image. Additional CT scans were performed to re-orientate or reposition the needle as necessary. During scans, the clinicians left the room to avoid radiations. When adequately orientated, the needle was advanced into the IVD according to the distance initially measured between the skin and the nucleus pulposus on the CT image. Usually a "crushing" sensation confirmed penetration of the IVD. Final position was confirmed by CT.

## Figure 1. Injection technique



## Discussion

The current study provides useful clinical information that will help researchers to develop an ovine model of IVDD with this technique of needle insertion. However, the method introduced in this study remains to be validated at a larger scale and in living animals.

Some technical points were important in this study. Firstly, the length of the needle used in this study was 70 mm. It has been reported that skin is usually about 2 mm thick and the fat thickness of ovine back may reach 10mm. So longer needles may be necessary in other breeds or animals with more subcutaneous fat. Secondly, due to the compactness of the IVD, needles with an obturator could be used to avoid obstruction of the needle with cartilaginous tissue. Larger needles may also be necessary with more viscous liquids. In this study, injection of contrast necessitated exerting a very strong pressure in the syringe, and three injections could not deposit contrast agent in the IVD. Larger needles might also be stronger and easier to introduce due to limited bending, thus probably facilitating insertion. However, needle punctures may directly alter mechanical properties via nucleus pulposus depressurization and/or annulus fibrosus damage, depending on the needle size. Thirdly, the laser beam was employed to control the pathway of the needle after penetration of the skin. The major difficulty of this technique was to keep the needle in the axis of the laser beam during insertion. Fortunately, when the needle was inserted 2-3 cm into muscles, a CT scan was performed and the needle could be re-orientated if necessary.

## Is there naturally occurring disc disease in the sheep?

The study "Intervertebral disc degeneration occurs naturally at the lumbar-sacral disc in the sheep " has been submitted for the ECVS congress in Berlin in 2015.

## Take home message

This study indicated that the sheep could be used as a model of naturally occurring IVDD. The L6-S1 disc was more often affected. Since it is the level where dorsoventral movement is the highest, the hypothesis of a biomechanical etiology of the disease can be suggested.

## Introduction

Sheep are commonly used as animal models to study IVDD. Ovine models are induced mechanically or chemically. However, a naturally occurring disease would be extremely interesting since it could better mimic the progress of the disease in man. Computed tomography (CT) and Magnetic Resonance Imaging (MRI) are used to diagnose and assess the progress of IVDD. T2 weighted sequence are now commonly used in man to assess IVDs. A good correlation between T2 relaxation time values and IVD biochemical content has been demonstrated. In the current study, our hypothesis was that IVDD occurs naturally at the lumbar-sacral disc in the sheep. Our objective was therefore (1) to document and score lesions identified at the lumbar-sacral disc by CT and MRI; (2) to compare degenerative scores to those of other discs; (3) to correlate scores with age.

## Materials and methods

## Animals

19 sheep (Suffolk, Ile-de-France and Texel ewes), from six months to 10 years, euthanized for reasons not related to IVDD, were used.

## Imaging

Sheep were CT scanned after euthanasia in sternal recumbence with an Emotion 6 Philips Ingenuity. Structural abnormalities (osteophytes, intradiscal calcifications, endplate integrity and sclerosis) were scored. Sheep were scanned in sternal recumbence with a 1.5 T MRI (Magneton Symphony SIEMENS) with two body-coils. Field of view included five discs starting from the lumbar-sacral disc. Different MRI techniques were used to assess the discs. (1) In T2 mapping, a color scale was used to identify variations in T2 time. Areas of short T2 time appeared in blue color (high score, indicating disease) while areas of long T2 time appeared in red color (low score, indicating normality). This color code scoring is illustrated in **Figure 1**. (2) T2-weighted (T2W) images were used for scoring the discs. T2 signal intensity, disc extension beyond the interspace (DEBIT), nucleus shape and annular tears were assessed. (3) Besides scoring, T2 times were measured. The nucleus pulposus was the Region of Interest (ROI).

# Results

Higher scores appeared to be more frequent in the oldest sheep at T2 mapping, T2W and CT. Higher scores seemed to be more frequent at the lumbar-sacral disc in comparison to the other levels. Scores at T2 mapping and T2W was always higher than other levels, except in two sheep (3 years and 6 years) where partial fusion of the last vertebra and sacrum was observed and L5-L6 had higher scores than L6-S1. Scores at T2 mapping correlated

significantly with scores at T2W (r = 0.794, p = 0.000) and with T2 times (r = -0.881; p = 0.000), but not with scores at CT. Scores at CT correlated significantly with those at T2W (r = 0.310, p = 0.002). Scores at T2 mapping (p = 0.005), T2W (p = 0.001) and CT (p = 0.045) as well as T2 time (0.006) were significantly different in L6-S1 than other levels. Categories of age had a significant effect on scores at T2 mapping, T2W and on T2 times both at the L6-S1 level (p = 0.012, 0.004, 0.008) and other levels (p = 0.029, 0.014, 0.019). Scores at CT were not significantly influenced by categories of age.

**Figure 1. Scoring of color-coded T2 maps.** As degeneration advances, uniformity of T2 signal in the annulus fibrosus and the nucleus pulposus decreases and, at the last stage, the distinction between the annulus fibrosus and the nucleus pulposus is lost. In this scale, blue represents short T2 time and red represents long T2 time. Score 1 corresponds to a healthy IVD and score 4 to a strongly damaged IVD.



### Discussion

Our study demonstrated that degenerative changes occur naturally in the intervertebral discs in the sheep. To our knowledge this is the first prevalence study documenting IVDD in a population of sheep. In our study, the L6-S1 level was more often affected. Wilke et al. compared the quantitative biomechanical properties of the sheep spine to human and concluded there are biomechanical similarities between ovine and human spine. They showed that dorsoventral movement was the highest at the L6-S1 level. Interestingly, in the current study, partial ossification was seen in two younger animals. In that case, the highest scores were in L6-L5. This may strengthen the hypothesis of a biomechanical etiology of the disease and that IVDD occurs where mobility is the highest. This hypothesis could be tested also by comparing L6-S1 lesions to those identified at the sacro-iliac joint.

## How can we inject the L6-S1 disk in the sheep?

The study **"CT-guided injection into the lumbar-sacral intervertebral disc in the ovine lumbar spine"** has been submitted for the ECVS congress in Berlin in 2015.

## Take home message

The injection of the lumbar-sacral disc under CT guidance is feasible and the technique described in this study is accurate. However it will be necessary to avoid the injection of substances that could damage retroperitoneal tissues. The injection necessitated considerable pressure. This might be due to the compactness of the IVD and the viscosity of the contrast medium. The viscosity of injected substances should be considered to account for the difficulty of injection on one hand and the risk of extravasations on the other hand. Furthermore, large volumes will be difficult to inject.

**Introduction.** Recent data indicate that degeneration of intervertebral disc (IVD) occurs naturally in sheep at the level of the lumbar-sacral disc. Accessing the lumbar-sacral disc would be essential if researchers want to use that disc for study. This ex vivo study aimed to report a computer tomography (CT)-guided injection technique into the last IVD (L6-S1 or L7-S1) in the ovine lumbar spine.

**Material and methods.** Twelve adult ewes (mean age 6 years) and 3 young ewes of 6 months were euthanized. Group A (N = 8) was used to develop the technique and to assess whether relevant anatomical structures (large vessels, peritoneal cavity, organs) had been penetrated; the needle was directed to the lumbar-sacral disc but was not introduced into the disc. In group B (N = 7), the disc was injected with contrast medium to confirm the precision of the technique developed in group A. The technique is described in **Figure 1**.

**Results.** Two ewes could not be used because of tympanism. This problem was solved in two other ewes by trocarding their rumen. In group A, six ewes (N = 6) were injected. The needle did not penetrate any relevant anatomical structure and remained in a retroperitoneal position. The tract was only intramuscular. In group B, seven ewes (N = 7) were injected. The injection was successful with contrast medium present in the nucleus pulposus in 4 animals. In one sheep, the contrast agent did not stay in the nucleus pulposus and was noticed partly outside the disc after injection. In two animals, the contrast was noticed totally outside the disc though the tip of the needle was appropriately positioned before injection Injection necessitated considerable pressure on the syringe in all cases. We were able to inject 0,5ml of contrast agent in adults and 0,3ml in young animals.

**Figure 1. Insertion technique** A. The virtual axis of the needle was drawn on the CT image. A landmark was introduced perpendicularly to the laser beam generated at the level of the lumbar-sacral disc. B. The intersection of the laser beam and the landmark constituted the entry point of the guide (trocar). The insertion of the guide consisted in pushing it forward while keeping its base in the plane of the laser beam. C. The needle was inserted to a distance equal to the distance between the skin and the border of the intervertebral disc.



## Discussion/conclusion.

This study showed that the injection of the lumbar-sacral disc under CT guidance is feasible. The needles avoided relevant anatomical structure and their path was intramuscular and retroperitoneal. The injection was successful in 4 cases on 7 while it had an intermediate outcome in one case and failed in two cases. However, in these 3 sheep, the needle tip was perfectly in the center of the nucleus. The three sheep were the animals that were injected first, and a learning curve could be necessary to master the technique. They were all adults and it is also possible that the discs were degenerated with fissures allowing extravasations of the contrast medium after the injection. This has clinical implications. It will be necessary to avoid the injection of substances that could damage retroperitoneal tissues. The injection necessitated considerable pressure. This might be due to the compactness of the IVD and the viscosity of the contrast medium. The viscosity of injected substances should be considered to account for the difficulty of injection on one hand and the risk of extravasations on the other hand. Furthermore, large volumes are difficult to inject.

## What is the anatomy of iliac arteries by contrast arteriography in the sheep?

The study **"Angiographic anatomy of iliac arteries in the sheep"** has been submitted for the ECVS congress in Berlin in 2015.

## Take home message

The sheep model appears to be sufficiently similar to man to test stent properties. This study provides useful reference images and measures of lengths and diameters of relevant arteries.

**Introduction**: Iliac artery atherosclerotic disease occurs in man. Large animal surgeons are commonly involved in research on stents using animal models, such as sheep. However, little information is published regarding the angiographic anatomy of the iliac arteries in the ovine species. The objective of this study was to describe the angiographic anatomy of the iliac arteries in the sheep.

## Materials and methods

Computed tomography (CT) angiography of 10 adult ewes were performed and analysed. Diameters and lengths of the arteries were measured.

## Results

The angiographic anatomy is shown in **Figure 1**. Diameters of arteries are reported in **Table 1**. Lengths of arteries are reported in **Table 2**. The abdominal aorta divides into a primitive iliac trunk and a left external iliac artery. The left external iliac artery divides into a left femoral artery and a left deep femoral artery. The primitive trunk bifurcates into the right external artery and the common trunk of the internal iliac arteries. The right external iliac artery divides into a left internal iliac artery and a right deep femoral artery. The common internal iliac trunk divides into a left internal iliac artery and a right deep femoral artery. The common internal iliac artery divides into a left internal iliac artery and a right internal iliac artery. The salient differences between the anatomy in man and in sheep are (1) the absence of common iliac arteries in the sheep, (2) the common trunk at the origin of internal iliac arteries in the sheep, and (3) the position in the pelvis (versus in the limb) of the bifurcation of the external iliac arteries into femoral arteries in the sheep. Lengths of arteries are slightly different between man and sheep, while diameters are rather similar.

## Conclusion

Those findings have clinical implications. Diameters of arteries in the sheep are similar to those in man; in consequence, the size of stents used in medicine should be adequate in research using the ovine model. Conversely, some segments in man are not comparable to those in the sheep: absence of common iliac arteries in the sheep, origin of internal iliac arteries. Most of the time, a stent placement on iliac arteries is performed by a femoral access. This study did not include femoral arteries. To see if the femoral access is possible in the ovine model, an additional study including the angiography of the hind limb of the sheep should be conducted.

**Figure 1. 3D representation of the aorta and iliac arteries.** The abdominal aorta begins where the thoracic aorta crosses the diaphragm through the aortic hiatus. It divides into a primitive iliac trunk and a left external iliac artery. The left external iliac artery divides into a left femoral artery and a left deep femoral artery. The primitive trunk bifurcates into the right external artery and the common trunk of the internal iliac arteries. The right external iliac artery divides into a right femoral artery and a right deep femoral artery divides into a right femoral artery and a right deep femoral artery. The common internal iliac trunk divides into a left internal iliac artery and a right internal iliac artery. A human model is shown for comparison.

1.abdominal aorta; 2.primitive trunk; 3.left external iliac artery; 4. left femoral artery; 5.left deep femoral artery; 6.right external iliac artery; 7.common internal iliac trunk; 8.right femoral artery; 9.right deep femoral artery; 10.left internal iliac artery; 11.right internal iliac artery; 12.left kidney; 13.vertebra L5; 14.left common iliac artery; 15.right common iliac artery



Sheep model

## Human model

Table 1. Mean diameters (mm; with standard deviations, SD) of arteries in the sheep in this study

Artery	Diameter	SD
Abdominal aorta	14	3
Iliac primitive trunk	13	2
Right external iliac artery	8	1
Left external iliac artery	7	1
Proximal right femoral artery	5	1
Proximal left femoral artery	5	1
Proximal right deep femoral artery	5	1
Proximal left deep femoral artery	5	1
Common internal iliac trunk	9	2
Right internal iliac artery	4	1
Left internal iliac artery	5	1

### Table 2. Mean lengths (mm; with standard deviations, SD) of arteries in the sheep in this study

Artery	Diameter	SD
Abdominal aorta	240	17
Iliac primitive trunk	12	3
Right external iliac artery	87	11
Left external iliac artery	84	10
Common internal iliac trunk	5	4
Right internal iliac artery	16	3
Left internal iliac artery	15	4

## What is the anatomy of coronary arteries by contrast arteriography in the sheep?

The study "Angiographic anatomy of coronary arteries in the sheep" has been submitted for the ECVS congress in Berlin in 2015.

## Take home message

The anatomy of coronary arteries is similar in man and sheep. The left and right coronary arteries, and circumflex arteries are larger in the sheep. The diameter of the left interventricular artery was similar in both species. This study provides useful information about the anatomy of coronary arteries and shows that the ovine model can be used in research studies on coronary diseases.

## INTRODUCTION

Atherosclerosis is the main cause of death in the United States, the developed countries of European Union, and in Canada and occurs most commonly in coronary arteries. Stents can be used to dilate vessels at the occlusion site. Introduction of the stent and its progress along the vessel must be minimal invasive. Usually, the course of the vessel is identified by angiography. These techniques are often tested in animal models, such as sheep. However, there is limited peer reviewed information about the angiographic anatomy of the coronary arteries in the sheep. The objective of this study was to describe the angiographic anatomy of the coronary of the coronary arteries in the sheep.

**Material and methods.** Computed tomography (CT) angiography of 5 adult ewes was performed and analyzed. Diameters of coronary arteries were measured.

## RESULTS

Coronary arteries arise from the base of the ascending aorta. The left coronary artery presents a common trunk. The trunk divides into two main branches: the circumflex artery located in the atrio-ventricular groove and the left inter-ventricular artery, which descends in the left inter-ventricular groove (**Figure 1**). The right coronary artery courses first from the ascending aorta to the atrio-ventricular groove. Then it presents a circumflex course in the groove. Finally, it detaches a left retro-ventricular artery, and a right inter-ventricular artery coursing in the right inter-ventricular groove (**Figure 1**). Mean diameters (mm, with their SD) are reported in **Table 1**.

## DISCUSSION

The supply of heart is led through the coronary arteries that spring from sinuses above the semilunar cusps at the beginning of the aorta. The anatomy of coronary arteries is rather similar in man and sheep.

In man and sheep, the left coronary artery courses between the pulmonary trunk and the left auricle. The left coronary artery presents a common trunk in its initial portion. The artery then divides into left (anterior, paraconal) inter-ventricular artery (also called left descending artery) and circumflex artery. The left inter-ventricular artery descends in the inter-ventricular groove. The circumflex artery courses to the left (caudal) side in the atrio-ventricular groove. In man, most of the time, the circumflex artery originates from the left coronary artery (left dominance) but can also originate from the right coronary artery (right dominance). In this series of sheep, all circumflex arteries came from the left coronary artery, in accordance to what has been reported in the literature.

In man, the right coronary artery presents different portions. Firstly, it courses between the right auricle and the pulmonary trunk and reaches the atrio-ventricular groove. Then it pursues a circumflex course in the groove. Finally the artery detaches branches that reach the margin and the wall of the posterior (right) side of the heart, including a right (posterior, subsinusoal) interventricular artery (also called right descending artery). The anatomy in this series of ewes was similar.

In man, the diameter of the left coronary at its origin is 3 to 5 mm. The diameter of the left common trunk was 8 mm in this series of sheep. The right coronary has a diameter of 2 to 3 mm at its origin in man while it was 4 mm in this series of sheep. The diameter of the circumflex artery in sheep was larger too (4 mm versus 3 mm in man). The diameter of the left inter-ventricular artery was similar in both species (3 mm). The left retro-ventricular artery has not been measured in the ewes. Those arteries are very small and there was not enough contrast medium in it.

**Figure 1. 3D** volume of coronary arteries showing the sites where diameters of arteries were measured. A: ascending aorta, B1 and B2: common trunk of the left coronary artery, C: circumflex artery, D: left inter-ventricular artery, E : base of the right coronary artery, F1, F2,: right coronary artery, F2 : right coronary artery. The right inter-ventricular artery is not shown on this 3D reconstruction.



#### Table 1. Diameters of arteries.

	Mean (mm)	SD
Ascending aorta (A)	29	3
Left coronary artery (B1)	7	1
Left coronary artery (B2)	9	1
Left coronary artery (mean)	8	
Circumflex artery (C)	4	1
Left inter-ventricular artery (D)	3	1
Base of right coronary artery	12	2
Right coronary artery (F1)	5	2
Right coronary artery (F2)	3	<1
Right coronary artery (mean)	4	
Right inter-ventricular artery	1	<1

# Does fosfomycine trometamol prevent urinary tract infection after urodynamic studies?

The study "Effectiveness of prophylactic antibiotic intervention (fosfomycin trometamol) to prevent urinary tract infections (uti) due to urodynamic studies (uds) in women: a randomized controlled trial" was presented at the congress of the SIFUD in Nice in 2013 and at the congress of UK Continence Society in London in 2014.

## Take home message

Despite the small sample size limits generalization of the conclusions, this study suggests that urinary tract infection (UTI) after urodynamic studies (UDS) is not frequent. It also shows that antibiotic prophylaxis with fosfomycin trometamol might prevent significant bacteriuria in menopausal women.

## Introduction

Antibiotics are sometimes used to reduce the incidence of de novo urinary tract infection after urodynamic studies (UDS). This study assessed whether 2 doses of 3 g oral fosfomycin trometamol (Monuril\*) (administered to women 3 hours before and 24 hours after UDS) reduced the incidence of significant bacteriuria and clinical signs of urinary tract infection (UTI).

Fosfomycin trometamol (Monuril\*) is a phosphonic acid derivative. It acts by inhibiting transferase, a cytoplasmic enzyme that catalyses the first step in the biosynthesis of peptidoglycans. It has in vitro activity against a broad range of gram-positive and gram-negative aerobic microorganisms.

## Material and methods

A double-blind randomized controlled trial (RCT) with placebo (EudraCT 2008-007669-21) was conducted. Primary outcome measure was significant bacteriuria (colony forming unit >10<sup>5</sup>/ml of clean catch urine). Secondary outcome was the presence of clinical signs of UTI. The voiding diary and questionnaires (at UDS consultation, and by telephone after UDS) were used to assess whether signs of urinary tract inflammation were present such as urgency episodes, dysuria, pollakiuria, suprapubic tenderness , report of pyrexia (above 37°), and reports of hematuria. Presence of pyuria (finding of more than 100 leucocytes / mm3) was also considered as a clinical sign of UTI. The patients' urinary tract status was classified as: "no clinical signs of UTI", "no diagnosis", "clinical signs of UTI". A per protocol analysis was performed.

**Figure 1** shows the flow of patients from enrolment to analysis. Between October 2010 and October 2012, 116 patients were eligible and assessed for inclusion at the telephone enquiry. 81 were selected and randomized. 14 patients were excluded at the UDS consultation, and 14 dropped out. 27 patients in the placebo group and 25 in the treatment group were analyzed. Randomization succeeded in balancing the two study groups on all baseline characteristics (age, weight, size, body mass index, quality of life, and menopausal status). There were 20 women in a menopausal state in the placebo group, and 19 in the treatment group.





### Results

Between 2010 and 2012, among 116 eligible patients, 81 were included and randomized. Then 9 were excluded before UDS (positive urinary stick) and 20 were lost to follow up. Women in menopausal status had an increased risk of positive urinary stick (p = 0.064). Age, weight, size, body mass index, quality of life, and menopausal status, were not different between the placebo (n = 27) and the treatment (n = 25) groups. Log-linear analysis retained three parameters (menopause, bacteriuria, Monuril\* versus placebo) to explain observed interactions ( $\chi^2$  (7) = 49.34, p = 0.000). When they received Monuril\*, none of the patients in menopausal status were 1.6 times (odds ratio) more likely to develop significant bacteriuria than non menopausal women. No patient developed signs of UTI after UDS. No side effect was reported in the placebo group, while two patients developed mild diarrhea in the Monuril\* group.

## Conclusion

Though the limited sample size should be considered, this study suggests that UTI after UDS is not frequent. It also shows that antibiotic prophylaxy with Monuril might prevent significant bacteriuria in women in menopause.

## Focus 2. Diseases in in animals



# Does desmotomy of the interspinous ligament improve back pain in kissing spines in the horse?

The research work "A Controlled Study Evaluating a Novel Surgical Treatment for Kissing Spines in Standing Sedated Horses" was published in Veterinary Surgery in 2012.

## Take home message

This surgical technique allowed horses with back pain and radiographic ORDSP to return to work without further clinical signs of back pain and to show radiographic evidence of improvement.

## Introduction

Overriding dorsal spinous processes (ORDSP; kissing spines) occurs commonly in horses. In contrast to traditional surgical techniques, desmotomy of the interspinous ligament (ISLD) aims to relieve tension on afferent nociceptive receptors located in the ligament insertion, thereby abolishing the sensation of pain. Combining this with a controlled exercise program to release epaxial muscular spasm, permanent resolution of kissing spines is achieved. Encouraging early results in severe chronic cases led to this technique becoming our preferred treatment for all but mildly affected horses. The objective of the current study was to compare the results of a novel minimally invasive surgical technique with intralesional corticosteroid medication, as treatment for overriding dorsal spinous processes (ORDSP) in horses.

## Material and methods

ORDSP was diagnosed in 68 horses, based on history, clinical, and radiographic examination. All narrowed spaces were treated. Horses undergoing medical treatment had methylprednisolone acetate injected directly in the affected space under radiographic control. Surgical cases had interspinous ligament desmotomy (ISLD) using Mayo scissors; supraspinous ligaments were left intact. All horses had the same controlled exercise plan and returned to work 3–6 weeks after treatment. The surgical technique is described in figure 1.

## Results

Methylprednisolone was administered in 1–7 spaces in 38 cases, compared with ISLD in 3–8 spaces in 37 cases. Thirty-four horses (89%) treated medically initially resolved signs of back compared with 35 horses (95%) treated surgically. From these, back pain recurred in 19 medical cases and in none of the surgical cases. Horses having ISLD were 24 times more likely to experience long-term resolution of signs of back pain (OR 24; 95%CI: 5–115; P = < .0001). Repeat radiographs in 19 surgical cases indicated that interspinous spaces widened significantly postoperatively (P = < .0001)

## Figure 1. Surgical technique

The horse was moved to surgical stocks and incremental further doses of detomidine administered to maintain a profound level of sedation. Hair was clipped from the surgical site leaving the guide marks visible on the right side. The central position of each proposed interspinous space was located with a 13.3 MHZ linear ultrasound probe orientated sagittaly on midline. Mepivacaine hydrochloride (5 mL) was infiltrated subcutaneously on midline and to the left of midline at each site using a 5/8" 25 g needle. Next, 2" 21 g needles were inserted in the sagittal plane from midline directly into the interspinous space. Mepivacaine (15 mL) was injected for each interspinous space, 5 mL at a depth of 6 cm, 5 mL distributed in the muscle on each side of the space, and the remainder as the needle was withdrawn.

After routine draping, the stylet from a 3.5" 18 g needle was inserted ventrally from midline directly in to the interspinous space to serve as a guide to triangulate the orientation of the space. A 1-cm paramedian skin incision was made 3 cm left of midline using a #10 blade. Curved 7" Mayo scissors were then used to bluntly penetrate the thoracolumbar fascia and then rotated 90° to be parallel with the orientation of the interspinous space. In this orientation, they were passed axially from left to right across the top of the interspinous space ventral to the SSL; the spinal needle was removed as contact was made with the scissor tip. Having created a path through the top of the ISL just below the SSL they were then used to cut the ISL ventrally for up to 6 cm. A curved round-tipped 6-mm AO elevator was sometimes used to probe the operated space and its adjacent DSPs to verify that the ISLD had been successful. The incision was suture closed using 2 simple interrupted sutures of 4-monofilament nylon. A sterile adhesive dressing was applied for 24 hours. Horses were discharged on the day of surgery, or later if necessary or preferred by the owner. Prophylactic enrofloxacin (7.5 mg/kg, orally once daily) was administered for 5 days with a reducing dose of phenylbutazone (starting at 4.4 mg/kg orally once daily) for 10 days. Sutures were removed at 12-14 days.



## Discussion

We concluded that ISLD technique provides comparable success rates and patient safety, along with reduced cost, complication rate and postoperative rehabilitation time. This treatment proved superior to medical treatment despite being applied to a group of more severely affected horses. Further validation of the technique with larger case numbers is warranted, in tandem with research in to its mechanism of action.

# Can cryosurgery of lumbar nerves be used for treatment of back pain in horses: a preliminary histological study?

The study "Can cryosurgery of lumbar nerves be used for treatment of back pain in horses: a preliminary histological study?" was presented at the ECVS congress of Copenhagen and the AVEF congress in Pau in 2014.

## Introduction

The nerves innervating the articular facets of lumbar vertebrae and their ultrasound guided injection have been described in the horse. In humans, cryodernervation of lumbar facets has been used. Limited peer reviewed information about the effect of freezing on nerves is available. The objective of the current study was to demonstrate that cryosurgery induces histological damages to the medial branches of the dorsal ramus of lumbar nerves in the horse.

## Material and methods

Animals and cryosurgery. Two male ponies (3 and 5 years old) were euthanized for teaching purposes. After death, the lumbar spine was dissected to access the lumbar nerves. For each of the six lumbar spinal nerves on both sides (right and left), one of the two nerve branches of the medial branch of the dorsal ramus was identified where they course along the articular processes towards the articular facets. In each pony, 6 nerve branches were randomly selected to be used as controls while the other 6 were exposed to cryosurgery (three cycles of 2 min freezing [at minus 60°C] and 2 min thawing, using a cryogenic probe of 1.5 mm diameter, and a nitrous oxide cryogenic unit). Optic and electronic microscopy. After freezing, the nerves were gently dissected from bone. A 1 cm long sample was obtained centered on the frozen area. This portion was resected to obtain a frozen segment of 2mm long and was fixed. For light microscopy, semi-thin sections were died with toluidine blue. For electron microscopy, ultra-thin (80 nm) sections were stained in uranyl acetate and lead citrate. Semi-thin section slices were viewed with light microscopy by 4 blinded different observers. Three criteria were observed: (1) general color of the nerve at 5X magnification, (2) color of axons at 10 and 40 X magnification, (3) morphology of myelin sheaths at 10 and 40 X magnification. Scores for the 3 criteria are described in **Figure 1**. The mean score was calculated for each criterion and the means were summed to obtain a total histological score.

# Results

**Light microscopy**. Histological scores were significantly different between control and frozen nerves for general color of the nerve (U 0,000; p 0,005), color of axons (U 0,500; p 0,007), morphology of myelin sheaths (U 0,000; p 0,005). Total histological scores were also significantly different (U 0,000; p 0,005). **Electron microscopy**. Several morphological changes were visible in frozen nerves in comparison to unfrozen nerves. In myelinated fibers the axoplasm of frozen nerves was darker and the concentric aspect of equidistant myelin lamellae was lost. The different layers of myelin appeared detached from each other with white lines in between. The axoplasm of frozen non myelinated fibers was also darker than in control nerves. Damaged mitochondria were numerous in frozen nerves. Sometimes they formed clusters. No changes were identified in the endonevrium (the conjunctive tissue between fibers composed of vessels, fibroblasts and collagen fibrils).

**Figure 1. Scores for histological assessment by light microscopy.** 1. Color of the nerves. A score from 1 (figure A1) to 4 (figure B1) was given while considering the color of the nerve from clear to dark. The observation was performed at 5x magnification. 2. Color of axons. When all nerve fibers had one white disk in the middle of the myelin sheaths, a score 1 was given (Figure A2). When only a few fibers did not present a central white disk and they were localized in a precise place (i.e. not randomly dispersed), a score 2 was given. In score 3, most nerve fibers did not have a white disk in the middle of the myelin sheaths. When all nerve fibers did not have a white disk in the middle of the myelin sheaths. When all nerve fibers did not have a white disk in the middle of the myelin sheaths. When all nerve fibers did not have a white disk in the middle of the myelin sheaths. The thickness of myelin sheaths may appear increased or decreased. When the thickness was « normal » and the sheath appeared regular in transversal section, a score 1 was given (Figure A3). When a few nerve fibers presented an abnormal myelin sheath in a precise region (i.e. not randomly dispersed), a score 2 was given. When most fiber nerves presented abnormal myelin sheaths, a score 3 was given. When all nerve fibers had an irregular myelin sheath, a score 4 was given (Figure B3). T = thicker. D = disrupted.



### Discussion

Experimental studies suggested that a selective destruction of large myelinated fibers, sparing small unmyelinated fibers, would follow mild freezing, while all fiber types were damaged by severe freezing. The protocol used in the current study induced structural changes both in large myelinated and small nociceptive fibers. In human patients, different techniques have been used for denervation such as injections of alcohol or phenol, surgical section and thermal coagulation. Subsequent neuroma or fibrosis was described with secondary neuralgia. Conversely, it seemed that the inflammatory response and subsequent fibrosis were reduced with cryosurgery. It was shown that when the endoneurium remained intact, as is the case after cryosurgery, neuroma formation did not occur. In this study, the endoneurium appeared intact. This preservation of neural connective tissue sheaths following controlled freezing might limit inflammatory response and neurogenic pain after surgery in clinical cases.

This study showed that histological changes can be identified. In the future, the ultrasound guided technique that will be developed for cryosurgery will be assessed ex vivo by using those histological changes as outcome measures. Characterization of nerve fibers would also be useful to understand potential impact on proprioception and movement.

# How can we compare arthro CT and 3T MRI optimal sequences to identify cartilage defects in the fetlock joint?

The study "Comparison of 3 Tesla Magnetic Resonance Imaging (MRI) and Computed Tomography Arthrography (CTA) to identify structural cartilage defects of the fetlock joint in the horse" was presented at the Voorjaarsdagen of Amsterdan and the AVEF congress of Reims in 2012. It was published in The Veterinary Journal in 2014.

## Take home message

CTA, due to its short acquisition time and its specificity and sensitivity, is a valuable tool to assess cartilage defects in the MCP/MTP joint, and is more accurate than MRI. Though MRI allows assessment of soft tissues and subchondral bone, and as such remains a useful technique for joint evaluation, clinicians need to be aware of the limitations of MRI in assessing articular cartilage, as even with a high-field magnet and specific sequences, its accuracy remains relatively unsatisfactory

## Introduction

In horses, computed tomography (CT) and CT arthrography (CTA) have been used to detect cartilaginous and non-cartilaginous changes of the MCP/MTP joint. CTA involves the intraarticular injection of a radiopaque contrast medium. However, CTA was not reported to improve the detection of AC defects unlike results from human studies.

In the current study, we aimed to assess the limits of MRI and CTA to identify structural articular cartilage defects of the equine MCP/MTP joint. Our ex-vivo study compared the sensitivity and specificity of high resolution CTA and 3-T MRI cartilage specific sequences.

## Material and methods

Forty cadaveric distal limbs were imaged by CTA (after injection of contrast medium) and by 3 Tesla-MRI using specific sequences: Dual-Echo in the Steady-State (DESS), Sampling Perfection with Application-optimized Contrast using different flip-angle Evolutions (SPACE). Gross anatomy was used as a gold standard for the evaluation of sensitivity and specificity of both imaging techniques.

# Results

In CTA, cartilage appears as a radiolucent layer covering the high attenuating SB and underlying the radiopaque contrast medium. Using DESS sequence, cartilage and synovial fluid have a high signal intensity (bright) while SB and trabecular bone have a low signal intensity (dark). With the SPACE sequence, cartilage and SB are dark whereas synovial fluid and trabecular bone are bright. Examples of cartilage defects identified by CTA and MRI are shown in Figure 1.

Mean acquisition time (not including preparation of limbs and injection of contrast), for CTA and MRI was respectively 2 and 26 min. Sen, Spe, PPV and NPV for identification of AC defects for the different observers and both imaging techniques are reported in Table 3. Mean CTA Sen and Spe were respectively  $0.82 \pm 0.03$  (mean ± standard error) and  $0.96 \pm 0.02$ . Those values taken together were significantly higher than those of MRI ( $0.41 \pm 0.01$  and  $0.93 \pm 0.01$ ) (Mc Nemar test; P<0,001). Correlation between CTA and macroscopic scores was 0.99 (P<0,0001), while correlation between MRI and macroscopic scores was 0.58 (P<0.05). Sensitivities and specificities to detect defects did not significantly differ between anatomical regions. At CTA and MRI, interobserver agreement was very good for scoring the different regions ( $\kappa$ = 0.82 and 0.88, respectively). Intraobserver agreement was

very good for CTA and for MRI ( $\kappa$ = 0.96 and 0.92, respectively). Out of the 123 defects, CTA adequately classified in average (mean of the three assessments, two by observer 1 and one by observer 2) 48 score-1 defects, 5 score-2 defects and 13 score-3 defects. MRI was able to adequately classify in average 34 score-1 defects, 2 score-2 defects and 5 score-3 defects. Combining both techniques helped to detect 3 additional score-1 defects.

*Figure 1. Examples of cartilage defects.* This figure illustrates the difficulty to detect subtle articular cartilage defects with magnetic resonance imaging (MRI). The appearance of three different types of defect (score 1, 2 and 3) is illustrated for computed tomography arthrography (CTA), dual-echo in steady state sequence (DESS), and sampling perfection with application-optimized contrast using different flip-angle evolutions sequence (SPACE). Figures of line 1 refer to a partial-thickness defect < 5 mm in diameter (score 1 cartilage defect) on the middle area of the medial part of the condyle of the third metacarpus (red arrow). Figures of line 2 refer to a partial-thickness defect > 5 mm in diameter (score 2 cartilage defect) in the dorsal portion of proximal phalanx detected. Line 3 refers to a full cartilage defect (score 3) on the middle area of the medial part of the condyle of the third metacarpus (yellow arrow).



### Discussion

Our study suggests that CTA is a potentially valuable tool to assess superficial articular cartilage defects in the MCP/MTP joint. It is superior to MRI with regard to its short acquisition time, its good correlation to macroscopic evaluation, its specificity and its sensitivity, in the detection of subtle articular cartilage defects. As CTA provides a good contrast resolution and a high spatial resolution ( $0.2 \times 0.2 \times 0.3$  mm), it allows the assessment of the entire cartilage surface without any loss of data. Though MRI is a useful technique for joint evaluation, as it detects important abnormalities such as synovitis and bone marrow lesions that have been associated with pain in OA, clinicians need to be aware of its limitations. In this study, even with a high-field magnet and using specific sequences designed for cartilage assessment, in comparison to CTA, MRI was less effective in subtle cartilage defects detection.

# Can we improve learning of anatomy by using CT and MRI imaging?

The study "**Impact of cross-sectional magnetic resonance images on learning anatomy: a single-blind randomized controlled study**" was presented at the Congress of the association Internationale de Pedagogie Universitaire (AIPU) in Mons in 2014. <sup>P158</sup>

## Take home message

The current study showed that combination of cross-sectional MRI images and dissection can improve ability to position anatomical structures in the immediate period (short term) after the teaching session.

## Introduction

All medical professions depend on a detailed understanding of anatomy to perform clinical examinations, diagnoses and surgeries. This involves mastering spatial concepts such as the shape of anatomical structures, where they are located relative to each other, and how they are connected. Spatial cognition is also important for understanding and interpreting medical images produced by radiography, ultrasonography, computed tomography and magnetic resonance imaging (MRI). Furthermore, surgery relies strongly on mental models of internal three-dimensional anatomy based on surface views or cross sectional images.

In order to understand the spatial correlation or interaction of objects, humans appear to depend on their ability to rotate the object mentally (mental rotation) and to look at it from different perspectives. Shepard and Cooper proposed the concept of a "Mental Imagery" facility, which is responsible for the ability to mentally rotate visual forms. Mental rotation allows recognition of "key viewpoints," which in turn will result in understanding of the position, shape and relation of anatomical structures. Vandenberg and Kuse developed a test (the Mental Rotation Test (MRT) to assess mental imagery. The MRT has been reported to be correlated to anatomy exam scores and has been considered as a reliable predictor of success in learning anatomy.

The objective of the current study was to conduct a single-blind randomized controlled trial on "fresh" 1<sup>st</sup> year veterinary students to compare traditional "lecture-laboratory dissection" (LLD) with a new intervention (NI), that combined cross-sectional MRI scans with cadaveric dissection, in their ability to teach anatomy. We hypothesized that NI would result in immediate (short term) improved ability to position anatomical structures in comparison with LLD.

## Material and methods

A single-blind randomized controlled trial was conducted, with 2 comparison groups. A multiple choice questionnaire (MCQ), including questions about the position of anatomical structures and their relation to each other, was conducted immediately after the interventions (Figure 1)

# Results

There were no significant differences between the LLD and the NI groups for all variables (age, sex ratio, nationality, passed education, previous training, dyslexia, scores at pre-test and MRT). MRT was not correlated with the score at the anatomy test at the end of the interventions. Results of the MRT were significantly better in boys than in girls (U = 623.00; p 0.003). Students who had experienced the new intervention differed in performance at the anatomy test from those who had experienced the conventional intervention (U = 1125.50, p

0.038). Scores ranged from 1 to 8/10, with means of 4.34/10 (0.21) for the LLD group and 4.94/10 (0.64) for the NI group. Sex did not influence performance.

**Figure 1.** Study design. MRT = mental rotation test. LLD = lecture-laboratory dissection. NI = new intervention



## Discussion

This study suggests that it might be useful to integrate cross-sectional MRI images into anatomy courses. Combining MRI images in different planes with cadaveric dissection is an easy way to achieve that integration. The importance of mental rotation has been proved in medical education and it is possible that cross-sectional images force learners to see anatomical structures from different view-points. Since there are indications that spatial understanding is enhanced when learners are allowed to "rotate" the models by themselves, we feel there is a need for more scientific data on how to utilize computer-based images with their corresponding cross-sections to prepare novice veterinary students during their medical education.

What is the correlation between responses to the "plank test" and lesions of anatomical structures in the foot identified using low-field MRI?

The study "Associations between responses to hoof extension test and lesions identified by magnetic resonance imaging (MRI)" has been submitted for the ECVS congress of Berlin in 2015.

## Take home message

Scores for MRI changes of the facies flexoria and of the trabecullar bone of the distal sesamoid bone were significantly correlated with the scores to the "plank test". In show jumpers and dressage horses with a front limb lameness abolished by palmar digital nerve block and no significant finding at radiography and ultrasonography of the foot, the "plank test" was indicative of distal sesamoid disease as assessed by MRI. A positive "plank test" could therefore strengthen the decision to refer the horse for MRI investigation of the foot.

## Introduction

Several clinical tests can be used in lameness investigation. The hoof extension wedge test, for example, is performed by elevating the toe with a block, holding the opposite limb elevated for 60 seconds, and then trotting the horse away. Another method to perform hoof extension is to place the foot at the extremity of a wooden plank ("plank test") and progressively extend the distal interphalangeal joint. It induces tension in the deep digital flexor tendon, and consequently the distal sesamoid bone is then compressed against the articular surfaces of the distal and medial phalanges. Strains on the deep digital flexor tendon and on the sesamoidean ligaments (collateral and distal) might also affect the response to the test. It is commonly reported that clinical tests are not specific. However, since no study so far has correlated the responses to the "plank test" with lesions, it is also possible that this test has more clinical significance than expected.

The objective of this study was to determine the correlation between responses to the "plank test" and lesions of anatomical structures in the foot identified using low-field MRI, in show jumpers and dressage horses with a positive response to a palmar digital nerve block and no significant lesion identified by radiography or ultrasonography of the foot.

## Material and methods

Study population - In our veterinary practice, lame horses with a positive response to a palmar digital nerve block undergo a thorough radiographic and ultrasonographic investigation of the foot. When no significant lesion is identified, MRI is performed. This means, for example, that cases with an ultrasonographic lesion in the deep digital flexor tendon are not investigated by MRI. Selection of scans- The medical records of all horses undergoing standing MRI for foot pain in the Equine Clinic Desbrosse (between January 2010 and July 2013) were reviewed. 143 scans of adequate quality were eligible. Only scans of cases where a "plank test" had been performed at the time of initial clinical assessment and MRI were selected. We excluded scans of the non-lame foot (in horses where a bilateral MRI had been performed). We included the scans of lame feet where the lameness, identified on a straight line or a circle, was totally improved after a palmar digital nerve block. A total of 84 scans were retained. Variables- Data collected were (1) demographic data (sex, age, breed, use) (2) scores at "plank test", (3) scores of lesions identified by MRI. "Plank test" - The foot being tested was placed on the extremity of a 1.2 m wooden plank. The opposite limb was held up by an assistant and the plank was gradually lifted to reach a 30 ° angle with the floor. The horse's reaction was assessed. Observations were considered abnormal when the horse demonstrated backwards deviation of the cannon from the vertical (horse putting

weight on hind quarters), backward deviation of the cannon from the vertical with extension and lowering of the neck or jumping off of the plank. Observation was recorded as normal, suspect or abnormal (scores 0, 1 and 2). The test was performed first on the non-lame limb. *MRI scoring* - MR images were acquired using a standing 0.27T MR unit (Hallmarq<sup>a</sup>), using a dedicated coil. The imaging protocol included sagittal (T1 weighted [W] 3D, T2W 3D, Short TI Inversion Recovery [STIR]), transverse (perpendicular to deep digital flexor tendon) (T1 gradient echo [GRE], T2 fast spin echo [FSE], STIR FSE), and frontal (T2W, STIR FSE) sequences. Abnormalities were only recorded if they were visible in more than one plane. Abnormalities were scored as normal (score 0), mild (score 1), moderate (score 2), or severe (score 3) for every assessed structure. Scoring parameters were adapted from the scale described by Murray et al. for high-field MRI (2006).

Figure 1. Plank test



## Results

84 scans were used for analysis (n = 85). They were from 33 mares, 37 geldings and 14 stallions. The horses were used either for show jumping (66), dressage (15), endurance (1), polo (1) and standardbred racing (1). Mean age was 9.1 years (SD 2.6). The lameness was identified and blocked on the straight line in 73 horses and on the circle in 11 horses.

Mean score at the "plank test" was 0.8 (SD 0.8). A low but significant correlation was identified between MRI changes of the facies flexoria (r = 0.24, p 0.03) and of the trabecullar bone (r = 0.22, p 0.04) of the distal sesamoid bone. No other correlation was statistically significant between scores at the test and at MRI.

## Discussion

The current study showed that the plank test was correlated with disease of the distal sesamoid bone. However, the characteristics of the current population of horses and the MRI scoring criteria can influence largely this conclusion.

The absence of association with synovial effusion might be due to a higher prevalence of synovial changes in our research population of intensively used high level show jumpers and dressage horses. Since horses in our clinic initially undergo foot ultrasonography and MRI is not performed if tendon or bursa changes are identified, the prevalence of lesions of the deep digital flexor tendon at MRI was too low to conclude on the accuracy of the test to detect tendon lesions.

# What can we expect from CT to assist orthopaedic surgery?

The study "The use of computed tomography (CT) to assist orthopaedic surgery in 86 horses (2002-2010)" was published in Equine Veterinary Education in 2011.

## Take home message

We report our positive experience with the system, owing to the variety of the cases we were unable to compare CT assisted surgery to conventional techniques such as radiography and fluoroscopy. The system is safe (low radiation dose) but is slow and subject to metallic artefacts.

## Introduction

Imaging-assisted orthopaedic surgery is becoming part of routine orthopaedic practice in horses. CT allows the production of cross-sectional images with spatial separation of structures which assist in identification of the number and direction of fracture lines within the bone in comminuted fractures. The aim of this report was to retrospectively report CT assisted orthopaedic surgical cases in our practice.

## Material and methods

The medical records of all horses undergoing orthopaedic surgery assisted by CT in our clinic between April 2002 and March 2010 were reviewed. All procedures were performed with the assistance of a pQCT (peripheral quantitative computerized tomography) scanner (Equine XCT 3000, Norland-Stratec Medical Sys.). pQCT is a technique used predominantly in research to assess bone mineral density. The Norland-Stratec pQCT has been adapted to equine practice and to specifically image the limbs of the horse both in standing or recumbent position (Figures 1 to 3).

*Figure 1 and 2. Forelimb positioned through the gantry* (1) of the Equine XTC 3000 scanner. The limb is wrapped in stockinet (2) and laid on a splint within an arthroscopic sheath (3). Tension is applied to the distal limb via a stainless steel wire connected to the frame of the scanner (4 and 5). Use of markers (A) Draping of the limb and scanner. (B) Foot within the gantry with radiopaque markers and laser beam. (C) Splint (1), radiopaque markers (2) and C Clamp (3) used in osteosynthesis of the proximal phalanx. *Example of surgery* Picture A is a pre-operative scan of a comminutive fracture of P1. Picture B is the per-operative scan after placement of the first screw.



# Results

This descriptive study has shown that pQCT may be successfully used to facilitate different equine orthopaedic surgical procedures under general anaesthesia. CT scanning of the front foot in the standing horse was also used successfully to identify surgical landmarks before a procedure, thereby facilitating surgery and reducing anaesthetic time. However, in this case series, standing CT was used in only four cases prior to surgery. We only saw a few relevant cases for which intra-operative foot CT was indicated. Furthermore, conditions that affect hind feet, or that are associated with severe lameness, are not amenable to standing CT.

Figure 3. Examples of lesions that ere identified by CT



In this case series, CT was also used for conditions of more proximal regions such as the stifle (2 cases) and the proximal tibia (1 case). However, the size of the gantry limited investigation of very proximal joints to small sized horses or foals.

This study also illustrated potential uses of CT before surgery to identify the localisation and extent of keratoma and distal phalanx osteitis, to localize osteochondral or bone fragments in or near joints, to assess the position and extent of exostoses or bone remodelling, to evaluate the direction and length of implant in internal fixation, to localize subchondral bone cysts before injection with steroids, and to evaluate the localisation and extent of foreign body in wounds or sequestra.

Interestingly in our case series pQCT was used to assess surgical outcome at the end of the procedure in four cases. Adequate placement of screws, removal of splint bone exostosis and curettage of a spur on the sustentaculum tali were monitored post-operatively.

# Discussion

To our knowledge, this is the first report of the intra-operative use of CT. Conventional CT is little used intra-operatively for logistical and safety reasons. The mean level of exposure to natural background radiation each year in Germany is 2.4 mSv/year and the dose delivered at conventional chest radiography is 0.2 mSv (Hunold et al, 2003). For comparison, the radiation dose with the pQCT described here is less than 0.5  $\mu$ Sv per slice in a human radius and less than 1.5  $\mu$ Sv for a human femur. This is an advantage when multiple slices must be obtained during a surgical procedure such as internal fixation.

Metallic implants and instruments cause artefacts that may obscure the area of interest. Another limitation is the time of acquisition of the pQCT in comparison to more powerful scanners. Draping was another issue as it has to allow for movements of the gantry.

The study remains purely descriptive and was not intended to demonstrate the superiority of the system. However it may be a useful demonstration of what can be achieved by CT assisted surgery and a guide for practitioners who wish to develop the technique.

# Can we perform neurectomy of peroneal and tibial nerves by cryosurgery to treat bone spavin?

The study "Proposal for a surgical technique for cryodenervation of the tibial and peroneal nerves in the horse: an ex-vivo study" has been submitted for the ECVS congress of Berlin in 2015.

## Take home message

This study demonstrated that tibial, deep peroneal and superficial peroneal nerves can be frozen reliably. Histological changes occurred immediately after freezing. However it is difficult to evaluate the functional impact of those changes. Obviously, changes should be assessed at long term.. The histological changes seemed also to vary with the size of the nerve. It is likely that the tibial nerve would necessitate longer application of the probe in cryosurgery.

**Introduction.** Different treatments have been reported for bone spavin, including neurectomy of tibial and peroneal nerves. In humans, percutaneous cryodernervation has been described, for example of the lumbar facet joints. The objectives of this ex vivo study were : (1) to identify the path of the tibial and peroneal nerves and their anatomical landmarks; (2) to propose a technique to perform cryosurgery of the tibial and peroneal nerves; (3) to assess the accuracy of the proposed technique to freeze the nerves.

## Material and methods.

The limbs of four non-lame horses of various sizes were used. Observations by gross anatomy were used to document the anatomy of the nerves and develop a surgical technique. Nerves were exposed to three cycles of 2 min freezing [at minus 60°C] and 2 min thawing, using a cryogenic probe of 1.5 mm diameter, and a nitrous oxide cryogenic unit. To assess the accuracy of the surgical technique, the nerves were observed immediately after the intervention. Immuno-histology (anti-SMI31) was used to identify the nerves, quantify axonal density, and assess their morphological characteristics before and after freezing. The surgical technique is shown in **Figure 1**.

## **Results.**

For surgery of a medium size horse (1.65 m), the superficial peroneal nerve was accessed in the groove between long and lateral extensors, 9 cm proximally to the point of the hock and 5 cm caudally to the cranial limit of the long extensor muscle. The landmark was similar for the deep peroneal nerve, located 3 cm deeper between the long and lateral extensor muscles. The tibial nerve was palpated subcutaneously on the medial side of the calcanean tendon, 8 cm proximally to the point of the hock. The typical macroscopic aspect after freezing was observed in all nerves. Histology demonstrated morphological changes in frozen nerves in comparison to non frozen nerves.
**Figure 1. Description of surgical access to the nerves in this ex vivo study** (A) The cadaver was kept standing via a hoist passed through the thigh. (B) The tibial nerve was palpated subcutaneously on the medial side of the calcanean tendon, 6 to 8 cm proximally to the top of the calcaneus and about 4cm cranially. It was pushed caudally with the thumb towards the calcanean tendon. A 2 cm stab incision was performed just caudally to the thumb. The tibial nerve was then easily identified due to its size (about 6 mm diameter) at a depth of approximately 7mm. (C) The cryosurgical probe was applied on the nerve. Nerve was exposed to three cycles of 2 min freezing [at minus 60°C] and 2 min thawing, using a cryogenic probe of 1.5 mm diameter, and a nitrous oxide cryogenic unit. (D) The path of the superficial peroneal nerve was identified in the groove between long and lateral extensors, about 9 cm proximally to the point of the hock and 5 cm caudally to the cranial limit of the long extensor muscle. A 3 cm incision was performed through the skin and the subcutaneous tissues were gently dissected until the superficial peroneal nerve (diameter of 2mm) was visible subcutaneously. (E) The nerve was frozen with the cryogenic probe.(F) To reach the deep peroneal nerve, the cryogenic probe was introduced horizontally (via the incision made for the superficial peroneal nerve) on about 3 cm between the long and lateral extensor muscles until the fibrous peroneous tertius was felt at the tip of the probe. Then the cryogenic probe was directed caudally toward the tibialis cranialis, at the expected position of the deep peroneal nerve.



**Discussion.** This ex vivo study demonstrated that tibial, deep peroneal and superficial peroneal nerves can be frozen surgically. Histological changes occurred immediately after freezing. However it is difficult to evaluate the functional impact of those changes. The histological changes seemed also to vary with the size of the nerve. It is likely that the tibial nerve would necessitate longer application of the probe in cryosurgery. The clinical effects on lameness associated to bone spavin should be assessed in a clinical trial, as well as the short term and long term consequences of the technique on nociception, proprioception and locomotion.

# How can we inject the navicular bursa under ultrasonographic guidance?

The study **"Ex vivo assessment of an ultrasound guided injection technique of a distended navicular bursa**" has been submitted for the ECVS congress of Berlin in 2015.

#### Take home message

This ultrasound-guided technique was reliably performed with a success rate of 68 %, by a clinician not trained to the technique. It has the advantage not to rely upon external landmarks. This technique is feasible only if the bursa is distended, and if ultrasound images are of adequate quality

#### Introduction

Five injection techniques have been described to inject the navicular bursa. Ultrasonography is commonly used to evaluate the deep digital flexor tendon and the navicular bursa. Distention of the navicular bursa is frequent in performance horses. Our experience shows it is possible to guide the injection of the navicular bursa by ultrasonography when the bursa is distended. The objective of this study was to describe and assess a new ultrasound-guided technique to inject the distended navicular bursa.

#### Materials and methods

Specimens. Twenty distal limbs of horses of various breeds and sizes were collected in a slaughter house. Preliminary distension of navicular bursa. To obtain synovial distension, the navicular bursa was injected with 2 ml of contrast medium (Hexabrix 320mg/ml) using a lateral approach. After injection, radiography was performed to confirm that the contrast medium was distending the bursa. Figure 1 illustrates the anatomy of the navicular bursa and its distension when it is injected with 2 ml of contrast medium. Ultrasound-guided technique. The digit was placed on a table with the distal interphalangeal joint in hyperextension, as it is the case in living animal. A microconvex probe (6 MHz; ESAOTE MyLab ClassC)) was placed in the hollow of the pastern palmar to the deep digital flexor tendon and the region was assessed in a transverse plane. The probe was slightly oriented distally (Figure 1). The tendon of the deep digital flexor and the distended navicular bursa were identified. The probe was rotated (only very slightly) laterally or medially (the more distended recess, either lateral or medial, was selected for injection) (Figure 2). A 21G 0.8 x 50mm needle was inserted abaxially to the probe in the plane of the ultrasound beam in the direction of the recess of the navicular bursa. 1 ml methylene blue was injected, and the distension of the bursa was visualized. Outcome The technique had been developed and used in clinical cases by the first author. The injections were performed in this study by one clinician who had never used the technique but had experience of ultrasonography of the navicular bursa. A dissection was performed afterwards to assess whether the navicular bursa had been successfully injected and methylene blue could be observed in its synovial cavity.

**Figure 1. Position of the limb and the probe.** A. Computed tomography 3D volume. The navicular bursa has been injected with 2 ml of contrast medium. The dorsal recess (white arrow) of the bursa can be injected when it is distended. B. Position of the limb for ultrasonography of the navicular bursa in a living animal. C. The probe was placed in the hollow of the pastern transversally and was slightly oriented distally. A very slight abaxial rotation was then necessary to identify the recess and allow for introducing the needle. **Figure 2.** Ultrasonographic image before (A), during (B) and after (C) the injection. NB: Navicular Bursa; DDFT: Deep Digital Flexor Tendon.



### Results

One foot was not injected because a scar in the pastern impaired ultrasonographic visualization. The success rate of this ultrasound-guided technique was 13 /19 (68%). In 3 of the 13 feet, a small amount of methylene blue was found in the distal interphalangeal joint too. 6 injections were not successful (2 injections in the distal interphalangeal joint, 3 in the peripheral tissues, 1 in the digital sheath). With this technique, the average number of attempts (reorientations of the needle) in successful injections was 3 and the mean time of the procedure was 8 minutes 30.

#### Discussion

This ultrasound-guided technique was reliably performed with a success rate of 68 %, even by a clinician not trained to the technique. Our experience showed that extension of the foot is important; however, it is even easier in living animals than in this series of cadaveric limbs. Techniques Nb1, Nb2 and Nb3 rely on external landmarks that can vary with foot conformation, which is not the case in the current technique. Another advantage is that we can follow (on the screen) the insertion of the needle and the distension of the bursa during injection. Furthermore the deep digital flexor tendon is not penetrated. This technique has limitations. It is feasible only if the bursa is distended, and if ultrasound images are of adequate quality. Scars and introduction of air (reorientation of the needle) can create artifacts. Three feet were successfully injected but the distal interphalangeal joint was slightly colored by methylene blue. It might be due to communications created by repeated introduction of the needle through the tissues either during the initial distension of the bursa with contrast medium or during the injection with methylene blue solution.

# What are the histological characteristics of the synovial plica in the dorsal compartment of the fetlock joint in the horse?

The study "Histological structure of the synovial pad of the dorsal synovial recess of the metacarpo-phalangeal joint in the horse" has been submitted for the ECVS congress of Berlin in 2015.

#### Take home message

#### The pad did not present the

classical histological structure of synovium with type A (cuboid) cells and type B cells. The pavimentous cells could be type B synoviocytes. We could hypothesize the pad is lined by type B synoviocytes and would have a function in the production of the synovial fluid. , that synthesize synovial fluid. The pad is innervated; this may have clinical implications.

#### Introduction

A structure, named synovial pad, can be found in the dorsal recess of the metacarpophalangeal joint of horses<sup>2</sup>. The histological structure and function of this synovial pad is still unknown. The objective of the current ex vivo study was (1) to study the histological structure of the synovial pad, (2) to determine whether it is innervated.

#### Material And Methods

Samples. Five distal forelimbs from horses of different ages were collected in a slaughter house. Since the history of the animals was unknown, several parameters were assessed to determine whether disease, such as osteoarthtitis (OA), was present or not. Synovial fluid analysis (white blood cell count and total protein) was performed to detect signs of inflammation. Radiographies (latero-medial and dorso-palmar projections) were obtained to identify osteophytes and subchondral bone changes. Then the skin and the joint capsule were transected in a transversal plane, immediately distally to the articular interface, and reflected proximally. Two samples including a portion of the synovial membrane distally and the synovial pad proximally were obtained, one medially and one laterally. The capsule and the collateral ligaments were transected to expose the articular surfaces of MC3, proximal phalanx and proximal sesamoid bones. The articular surfaces were assessed to identify and score cartilage defects. Histology. Specimens were fixed in 4% formaldehyde for 24 to 36 hours. They were transferred in a PBS (Phosphate Buffered Saline) solution. They were embedded in paraffin. Slices of 6µm were obtained and placed in microslides (Super-Frost+). To maximize the possibility to identify nerves on the microslide, slices were obtained at two levels of the embedded specimen: one superficially and one at a depth of 1000µm. Slices stained with HES (Hematein Eosin Saffron) for conventional histology. were Immunohistology was used to assess whether the synovial pad was innervated, by marking the samples with SMI-31 (neurofilament antibody), colored with DAB (3,3'DiAminoBenzidine) chromogen. Nneuro-filaments appear brown with this coloration. A total of 20 samples were processed (5 [limbs] x 2 [medial, lateral] x 2 [superficial, deep]).

#### Results

The cartilage was normal at gross anatomy. White blood cells (0.5 to 0.8  $10^9$  cells/L) and total proteins (9,1 to 9,8 g/L) were within normal limits, indicating that no acute synovitis was present. The dorso-proximal edge of the proximal phalanx was moderately remodeled in 2 limbs and small osteophytes were identified on the dorso-proximal edge of the proximal phalanx in 2 limbs on latero-medial projections, and on its lateral edges on the dorso-palmar projection in one limb.

HES slides showed that flat cells were lining the pad giving a pavimentous (squamous) aspect. No cuboidal cells were visible. At the level of the synovial membrane, 1 to 4 layers of synoviocytes were observed. The dense irregular connective tissue was rich in fibroblasts; blood vessels and adipocytes were visible (**Figure 1**).

Six samples could not be assessed by immunohistology due to artifacts. Immunohistology showed that nerve fibers were present in the synovial pad in 8 out of 14 samples, both deeply and superficially. Between 1 and 10 tags (positive immunohistochemical tagging) were visible per field of view.

#### Figure 1. Samples obtained superficially and laterally, and colored with HES.

**A**. Hematein is a basic dye and the nucleus appears purple. Eosin is an acidic dye and cytoplasm appears pink. Saffron stains collagen in yellow. Flat cells gave a pavimentous (squamous) aspect to the pad (left). **B**. 1 to -3 layers of synoviocytes A and B were visible on the synovium (right).



#### Discussion

The synovium of the dorsal recess of the synovial cavity of the metacarpo-phalangeal joint presents the classical histological features described in the literature: a continuous surface layer of cells, the intima, with the underlying tissue, the subintima. The intima has a 1-4 cells thickness, with type A and type B synoviocytes, whereas the subintima includes a type 1 collagenous extracellular matrix, with fibroblasts, blood vessels and adipocytes. Type A cells have a polygonal or cuboidal aspect and can be considered as macrophages. Type B cells have a pavimentous (squamous) aspect and can be considered as pure synoviocytes involved in the production of the synovial fluid<sup>6.</sup> For the synovial pad, despite the limited number of samples, we observed the same structure for the intima, at any level (medial or lateral, superficial or deep): some flat cells giving a pavimentous (squamous) aspect, but no cuboid cells. As for the synovium, we observed a subintima with a collagenous extracellular matrix, with fibroblasts, blood vessels and adipocytes. We could hypothesize the pad is lined by type B synoviocytes and would have a function in the production of the synovial fluid. To ensure that these cells are real type B synoviocytes, we could use immunohistochemical techniques. Various markers exist but none of them are really specific to type B cells.

Thanks to immunohistochemical tagging, we demonstrated, at least qualitatively, the presence of neurofilaments. It would be very interesting to characterize neurofilaments, and determine the proportion of nociceptive fibers of the synovial pad, to know whether structural abnormalities of the pad could be associated with pain.

# Can we use VAC (vacuum assisted) therapy to treat wounds?

The study « La gestion des plaies par la thérapie VAC est-elle applicable chez le cheval? » was presented at the French session of the ECVS congress of Nantes in 2009 and published in Le Point Vétérinaire in 2011. It was awarded the "Grand Prix de la Presse Médicale".

### Take home message

La VAC thérapie est applicable chez le cheval moyennant l'amélioration de divers points techniques (réalisation du vide sur une surface irrégulière, perception d'un stimulus cutané, temps consacré à la mise en place, bouchage du filtre). Les preuves scientifiques de son efficacité chez l'homme sont faibles.

# Introduction

Chez l'homme, le traitement des plaies est, avec l'étude des procédés de cicatrisation, aussi vieux que la médecine. En France, il fut considéré pendant longtemps comme une discipline mineure, jusqu'à ce que se crée, sous l'impulsion de Luc Téot et de Sylvie Meaume, un comité scientifique regroupant des spécialités d'origine diverse appelé Société Française et Francophone des Plaies et Cicatrisations. Cette spécialité est devenue incontournable car il faut guérir vite tout en dépensant moins ou mieux. Certaines thérapeutiques nouvelles sont apparues comme la cicatrisation par larvothérapie (asticots-thérapie) et la greffe tissulaire. Par ailleurs, des pansements très variés ont été développés et des biotechnologies diverses on été initiées (facteurs de croissance, substituts cutanés, culture d'épiderme).

Parmi ces différents moyens thérapeutiques, une autre technique, la thérapie par pression négative (TPN) ou la thérapie Vac (si on privilégie l'acronyme anglais) est préconisée dans la phase de débridement des plaies. Elle consiste à appliquer localement une pression négative (sous-atmosphérique) au travers de la surface de la plaie grâce à une structure spongieuse. Les objectifs revendiqués par ses utilisateurs et promoteurs sont d'éliminer les exsudats, de réduire la charge bactérienne, de rapprocher les marges de la plaie, d'améliorer la circulation sanguine et notamment la perfusion dermique, de mobiliser le liquide interstitiel (décompression tissulaire), et de favoriser un environnement humide propice à la cicatrisation. Parallèlement à ses effets directs sur le processus de cicatrisation, la TPN permettrait une gestion des exsudats dans un système clos parfaitement étanche avec des répercussions importantes sur la réduction du renouvellement des pansements, la prévention du risque infectieux ainsi que le maintien de l'intégrité et de l'hygiène corporelle du patient. La technique a été principalement décrite et évaluée pour le traitement des ulcères du pied diabétique, les ulcères de jambe complexes, les escarres, les déhiscences sternales, les plaies abdominales ouvertes et les plaies traumatiques

Chez le cheval, les plaies peuvent aussi être fortement exsudatives et comme dans les autres espèces il est utile de favoriser la granulation et le contrôle de l'environnement de la plaie (exsudats, microbes). En ce sens, la thérapie Vac pourrait être utile. Il était donc intéressant d'envisager l'applicabilité de la technique, notamment en raison de la taille de l'animal et de sa propension à bouger, se coucher, ou se rouler, qui pourraient rendre impossible le maintien en place du système. En outre, vu l'absence de données scientifiques quant à son utilisation chez les équidés, une analyse critique de la littérature humaine semblait indispensable pour évaluer l'évidence scientifique actuelle de son efficacité.

#### Matériel et méthode

Le système de thérapie Vac fut testé sur cinq chevaux présentant une plaie cutanée. Le nombre de cas de notre étude étant insuffisant pour envisager une analyse de l'efficacité de

la technique chez le cheval, nous nous sommes limités à décrire son utilisation avec les difficultés rencontrées et les solutions apportées. A défaut de pouvoir évaluer statistiquement ces résultats chez le cheval, une revue systématique de la littérature en médecine humaine fut effectuée afin d'évaluer le niveau d'évidence de son efficacité chez l'homme.

Figure 1. Le système d'aspiration avec son récipient-collecteur et le dispositif de recharge de la batterie. Figure 2. Films autocollants, bandelettes à double-face autocollante, tuyau d'aspiration et mousse noire. Figure 3. Des bandelettes autocollantes sur chaque face sont placées au long des marges de la plaie. La mousse est découpée aux dimensions exactes de la plaie. Figure 4. Le film collant est posé et la tubulure d'aspiration est fixée. Figure 5. Série d'images illustrant le cas 5 :(1) la plaie, (2) l'installation de la mousse et du tube d'aspiration, (3) le renfort de l'étanchéité avec un bandage de Robert Jones, (4) les sécrétions obtenues au bout de x heures. Figure 6. Lors du retrait du film plastique, on observe parfois un agglomérat d'apparence gélatineuse.



#### Discussion

Notre étude a montré que la Vac thérapie peut être appliquée au cheval. Elle nous a permis d'identifier certaines difficultés techniques (réalisation du vide sur une surface irrégulière, perception d'un stimulus cutané, temps consacré à la mise en place, bouchage du filtre). Des solutions techniques ont été apportées ou devraient l'être facilement sur de futurs cas. Clairement, l'entraînement devrait réduire le temps nécessaire au placement du système sur l'animal et sa mise en route. Le dispositif a subjectivement semblé efficace pour collecter une quantité importante d'exsudat .Vu le petit nombre de cas, il fut impossible d'objectiver l'efficacité de la technique sur la vitesse de granulation. Ces deux paramètres devraient être évalués au cours d'une étude prospective. Enfin, la littérature actuelle existant en médecine humaine et qui évalue l'efficacité de la technique est de qualité moyenne. Il faut donc en tenir compte lorsqu'il s'agit de l'utiliser pour discuter les cas clinique qu'il nous serait amené de traiter par TPN, ou lorsqu'il s'agit de justifier la décision d'instaurer une thérapie VAC. Tous ces éléments d'information, que fournit notre étude, constituent un point de départ utile pour l'utilisation et l'évaluation scientifique de la thérapie VAC chez le cheval.

# What's the use of biopsies of the pelvic flexure at surgery?

The study "Intérêt de la biopsie intestinale réalisée lors de laparotomie exploratrice » was presented at the AVEF congress 2014 in Pau.

# Take home message

La biopsie intestinale au site d'entérotomie de l'anse pelvienne pourrait informer sur l'état de parasitisme et constituer à postériori un outil pour la gestion de la prévention de coliques futures. Cette étude devrait être poursuivie de façon analytique (statistique) sur un nombre plus important d'animaux et un groupe témoin.

# Introduction

Lors de chirurgie de colique, on pratique fréquemment une entérotomie au niveau de la courbure pelvienne du colon ascendant pour en réaliser la vidange. A cette occasion, il est facile de réaliser une biopsie intestinale en vue d'une analyse histopathologique. L'objectif de cette étude est d'analyser rétrospectivement les informations obtenues par la biopsie et d'en envisager l'intérêt potentiel.

# Matériel et méthode

Etude descriptive portant sur 16 chevaux (1 mâle, 9 hongres, et 6 femelles) âgés de 7 mois à 15 ans. Lors de laparotomie exploratrice sous anesthésie générale, une biopsie de la muqueuse intestinale est réalisée sur la courbure pelvienne, au site de l'entérotomie réalisée pour la vidange du colon ascendant. Un échantillon de muqueuse intestinale de 1cm de côté est prélevé et envoyé pour analyse histopathologique à l'Université de Liverpool.

Figure 1. Entérotomie de l'anse pelvienne



#### Résultats

*Etat de parasitisme des chevaux* : 15 chevaux sur 16 sont déclarés en ordre de vermifugation par leur propriétaire. L'éosinophilie sanguine, mesurée avant l'intervention chirurgicale, est anormalement élevée pour 2 chevaux sur 16.

Anomalies intestinales rencontrées: 10 chevaux présentent une atteinte du colon ascendant (2 entrappements néphro-spléniques, 3 déplacements à droite, 2 torsions, 1 sablose et 2 impactions), 4 une atteinte de l'intestin grêle (3 incarcérations et 1 occlusion), et 2 une atteinte du colon ascendant et de l'intestin grêle (impaction marquée de l'iléon et du colon ascendant).

Lésions histologiques: l'épithélium de la muqueuse est intègre dans 13 cas sur 16. Dans les 3 autres cas, une nécrose avec dépôts fibrineux et débris cellulaires est observée. Une

hyperhémie et un œdème paracellulaire sont toujours notés. Une infiltration de cellules inflammatoires est également toujours observée, mais d'intensité et de type variables. L'intensité de l'infiltration est discrète (1 cas sur 16), modérée (12 cas sur 16), marquée (2 cas sur 16) ou sévère (1 cas sur 16). On observe des infiltrations lymphoplasmocytaire (5 cas sur 16), neutrophilique (3 cas sur 16), et éosinophilique (8 cas sur 16).

Figure 2 à 4. Coupes histologiques montrant deux différents types d'infiltrat (lymphocytaire et neutrophilique).



#### Discussion

Grosche et al (2011) ont étudié les modifications structurelles de la muqueuse intestinale après ischémie et après reperfusion. Ils montrent que le processus s'accompagne d'une infiltration sous-épithéliale et paracellulaire de neutrophiles, de lymphocytes, de macrophages, et de quelques éosinophiles. Cet infiltrat cellulaire inflammatoire non spécifique est identifié dans une moitié des biopsies dans notre étude et pourrait témoigner de la souffrance tissulaire. Par ailleurs, notre étude révèle une prévalence de 50 % d'infiltrat à dominance éosinophilique. L'origine de cet infiltrat éosinophilique est probablement parasitaire. Collobert-Laugier et al (2002) ont montré qu'il existe une bonne corrélation entre le nombre d'éosinophiles et de mastocytes présents dans la muqueuse colique et la charge de cyathostomes. Il est intéressant de constater que parmi les 8 chevaux avec un infiltrat éosinophilique, seulement 2 présentent une éosinophilie sanguine.

Les propriétaires sont souvent convaincus de la bonne gestion parasitaire de leurs animaux. Les vétérinaires pourraient aussi êtres amenés à penser que la situation sanitaire est bonne de nos jours. Bien que le parasitisme ait été associé aux coliques, le propriétaire peut banaliser l'explication donnée par le vétérinaire sur l'origine possible de la colique. La biopsie intestinale au site d'entérotomie de l'anse pelvienne pourrait informer sur l'état de parasitisme et constituer à postériori un outil pour la gestion de la prévention de coliques futures. Toutefois, cette étude devrait être poursuivie de façon analytique (statistique) sur un nombre plus important d'animaux ainsi qu'en envisageant un groupe contrôle visant à identifier la prévalence de l'éosinophilie de la muqueuse chez des individus sains. Le nombre trop faible d'individus dans notre étude ne permet pas encore d'évaluer la valeur pronostique de la biopsie intestinale sur le devenir de l'animal.

#### How to interpret venous lactate in the horse with colics?

The study **« Can blood lactate help in identifying medical and surgical colic: a retrospective study**» was presented at the AVEF congress 2014 in Pau. It was also published in Pratique Vétérinaire Equine in 2014. It has been proposed for the ECVS congress in Berlin in 2015.

#### Take home message

Venous lactate dosage had a fair performance to specify the therapeutic indication, medical or surgical, in horses with colic by considering a threshold value of 2 mmol/L. It was more performant to predict survival.

#### Introduction

Blood or peritoneal lactate dosage is used to determine prognosis for horses that are hospitalized for emergency and intensive care, including patients with colic. In colic, lactate concentration can be measured in peritoneal fluid and arterial or venous blood. L-lactate is easier to measure than D-lactate. The objective of our study was to assess the accuracy of venous blood lactate dosage to identify the therapeutic indication, medical versus surgical, in horses with colic. Our research question was: can blood lactate be used on the field or at the initial clinical examination at the referral clinic to predict the therapeutic indication, either medical or surgical?

#### Material and methods.

Medical records of horses hospitalized for colics (surgical or not) in our clinic between January 2013 and February 2014 were reviewed. Venous lactate was always measured with an Accutrend®Lactate analyzer upon arrival at the clinic and every morning and night until normalization (0 or near 0 mmol/L). The following variables were also recorded for the current study: level of pain (light, moderate or severe), vital parameters (heart and respiratory rates, temperature) and blood parameters (packed cell volume [PCV], total proteins and acid-base excess [ABE]). The performance of the test to predict (1) indication of surgery or medical treatment, (2) survival in all cases, and (3) survival in surgical cases only, was analyzed with a ROC curve (Receiver Operating Characteristic). Surgical and medical cases were defined as in **Table 1.** An Area Under Curve (AUC) of 0.9 to 1, 0.8 to 0.9, 0.7 to 0.8, 0.6 to 0.7 and 0.5 to 0.6 indicated respectively excellent, good, fair, poor and worthless test. A cut off point maximizing sensitivity and specificity was identified.

Table 1. Definitions of surgica	I and medical indications.
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Surgical cases	Medical cases			
Horses with a lactate concentration above cut-off value and	Horses with a lactate concentration above cut-off value and			
<ol> <li>who were operated and for whom the surgery was justified on the basis of the surgical findings</li> </ol>	who were not operated despite the indication (due to financial constraints) and who survived			
(2) who were not operated despite the indication, (due to financial constraints) and who were euthanized or died due to failure of conservative treatment as expected				
Horses with a lactate concentration below cut-off value and	Horses with a lactate concentration below cut-off value and			
who were treated medically unsuccessfully because they should have had surgery according to findings at postmortem	<ol> <li>who were successfully treated medically according to initial indication</li> <li>who were treated medically according to initial indication but died due to a non surgical complication (e.g.enterotoxemia)</li> </ol>			

# **Results.**

84 horses were referred for colic that did not answer to treatment by the first opinion veterinarian. There were 32 mares, 42 geldings, 10 stallions, aged between 3 months and 29 years. 43 horses were treated medically and 41 surgically (19 displacements or torsion of the ascending colon, 24 incarcerations or strangulations of the small intestine). Blood lactate concentration at arrival was significantly different between medical cases and surgical cases (U = 416.5; p 0.000), with medians of 1.3 and 3.0 mmol/L respectively. Blood lactate was significantly higher in horses who died (4.1 mmol/L) than in those who survived (1.3 mmol/L) (U = 180.0, p 0.000). The performance of the test for differentiating horses between medical and surgical indications was fair (AUC 0.77). A threshold value of 2 mmol/L maximized the sensitivity and specificity of the test. In horses treated surgically, the test was good at predicting (AUC = 0.81) their survival when the concentration was lower than 3.1mmol/L. The test presented a good performance to identify horses who would survive (AUC [Area Under Curve] = 0.87) regardless of the treatment modality. The lactate concentration was significantly correlated to PCV (r = 0.42), ABE (r = -0.38), pain (r = 0.42), heart rate (r = 0.53) and respiratory rate (r = 0.55).

# Discussion

This study shows that dosage of lactate had an acceptable performance to specify the therapeutic indication, medical or surgical, in horses with colic by considering a threshold value of 2 mmol/L (AUC 0.77). It was more accurate to predict survival (AUC = 0.87 and 0.81). We observed lower values (4.1 mmol/L in horses who died and 1.3 mmol/L in horses who survived) than those reported in the literature. This is probably explained by differences in target populations. For example, Johnston et al (2007) studied cases of torsion of the ascending colon, and Radcliff et al (2012) did not included medical cases in their study. However, the threshold for predicting survival of horses who had surgery in our study (3.1mmol/L) is similar to the median concentration of surviving horses after surgery in the study of Radcliff et al (2012).

The threshold values identified in our study should of course be considered in light of other clinical parameters and potential sources of bias. The time between sampling and analysis can influence blood levels because erythrocytes can produce lactate ex vivo (Fall et al, 2005; Tennent-Brown et al, 2011). In our study, blood was analyzed immediately after collection, which is not the case in other studies where blood was analyzed after freezing (Nappert et al, 2001), sometimes one hour after collection (Radcliff et al, 2012). Collection tubes can also influence the dosage. Lactate can be produced ex vivo when blood is collected in a lithium heparin tube (not EDTA) and stored at room temperature (Tennent-Brown and al, 2010). It is also necessary to take into account the variability due to age, breed and size of horses. It has been demonstrated that ponies and newborn foals (up to 48-72 hours) have blood lactate concentrations higher than adult horses (Tennent-Brown, 2012; Dunkel et al, 2013).

We conclude that lactate concentration in venous blood can provide useful information to veterinarians in making the therapeutic decision in horses with colic.

# What is the Computed Tomography (CT) and Magnetic Resonance Imaging (MRI) anatomy of the eye and orbit?

The study "Computed Tomography and Magnetic Resonance Anatomy of the normal orbit and eye of the horse" was presented at the ECVS congress of Copenhagen and the AVEF congress in Pau in 2014. It has been published in Anatomia Histologia and Embryologia.

### Take home message

CT and MRI are useful techniques to image the equine orbit and eye that can have clinical applications. The bony limits of the orbital cavity, the relationship of the orbit with sinuses, and foramina of the skull were well identified by CT. MRI was useful to observe soft tissues and was able to identify adnexae of the ocular globe (eyelids, periorbital fat, extraocular muscles, lacrymal and tarsal glands). Though MRI was able to identify all components of the eye (including the posterior chamber), it could not differentiate sclera from choroid and retina. The only nerve identified was the optic nerve.

# Introduction

The horse has a prominent eye and orbit which is prone to both traumatic and infectious diseases. For the diagnosis and monitoring of such diseases, medical imaging is sometimes necessary. CT and MRI are now common in referral centers, as well as in numerous equine and small animal practices in the UK, USA and Europe. However, there is few peer reviewed information about CT and MRI ocular anatomy. The objective of the current study was to describe CT and MRI anatomy of the equine orbit and ocular globe.

# Material and methods

The heads from 4 adult horses were scanned with a 6-slice Emotion 6 CT (Siemens, Erlangen), and a 3.0 Tesla Siemens Verio 6 MRI using T1 and T2-weighted sequences. For acquisitions, the heads were positioned at 45 degrees to the plane of the gantry. In order to validate CT and MR reference images, these were compared to anatomic models and gross anatomic sections performed with a thin band saw through the heads after freezing.

# Results

The bony limits of the orbital cavity, the relationship of the orbit with sinuses, and foramina of the skull were well identified by CT. MRI was useful to observe soft tissues and was able to identify adnexae of the ocular globe (eyelids, periorbital fat, extraocular muscles, lacrymal and tarsal glands). Though MRI was able to identify all components of the eye (including the posterior chamber), it could not differentiate sclera from choroid and retina. The only nerve identified was the optic nerve. Vessels were not seen in this series of cadaver heads. The current study showed that CT and MRI are useful techniques to image the equine orbit and eye that can have clinical applications. Examples of images are shown in figures 1 and 2.

**Figure 1 and 2.** *CT volume rendered image of the orbit.* 1 Frontal bone (1a zygomatic process of frontal bone). 2 Nasal bone. 3 Lacrimal bone. 4 Zygomatic bone (4a temporal process of zygomatic bone). 5 Sphenoid bone. 6 Palatine bone. 7 Mandible (7a condylar process of mandible. 7b coronoid process of mandible). 8 Temporal bone (8a zygomatic process of temporal bone). *Frontal T2W.* 1. Cornea. 2 Iris. 3 Ciliary body. 4 Vitreous body. 5 Anterior chamber. 6.-Posterior chamber. 7.-Lens. 8.-Retina, choroid, sclera.





#### Discussion

Bones do not totally limit the orbital cavity and do not totally encircle the globe and its retrobulbar soft tissues. The rostral, dorsal, lateral and ventral limits of the orbit are classically described in textbooks while the anatomical caudal landmarks are not commonly highlighted. The current study demonstrated that the caudal limit of the orbit is delineated by a part of the temporal bone, the coronoid process of the mandible, the temporal muscle and the lateral and medial pterygoideus muscles. This absence of bony "coat" covering the entire orbital cone caudally has clinical implications since this soft tissue compartment could permit access of foreign bodies to the orbit.

T1W images have been reported to provide the best spatial resolution and anatomic details of the eye and orbital structures. In this study, T1W images showed a contrast difference inside the lens, but did not allow the accurate examination of the iris, the ciliary body and of the posterior chamber, in contrast to T2W images.

Ultrasonography has been reported to be able to identify lesions of the anterior segment of the ocular globe associated with trauma. Intraocular masses (inflammatory, neoplastic, cystic) can be assessed by US; since many lesions arise from the anterior uvea, they can be difficult to diagnose without an offset device, extra-coupling gel, or scanning through closed eyelids. In addition, the posterior chamber is not always seen ultrasonographically in normal equine eyes because of its small dimensions. MRI consistently identified the anterior and posterior chambers in the four heads in the current study.

US can also be used to evaluate the posterior segment for abnormalities of the vitreous and the retina. The retina cannot be clearly delineated from the underlying choroid and sclera. This was also the case with MRI in this study. One advantage of US is that echoes may demonstrate motion such as in vitreous hemorrhage and retinal detachment. This is not possible with MRI.

One disadvantage of US is that structures beyond bony surfaces are not imaged. It is difficult to assess the extent and severity of bony involvement using this technique. Therefore, in bony invasion/lysis, CT or MRI is recommended.

Nevertheless, US is a rapid, safe and practical method. On the other hand, CT and MRI suffer from technical difficulties, including the need for general anesthesia and limited availability.

# What do we know about gaz anesthesia in pigeons?

This research question was answered by a systematic review of the existing literature. The review « **Anesthésie gazeuse des oiseaux : une synthèse méthodique** » was published in French in Le Point Vétérinaire in 2014.

#### Take home message

Les deux gaz, isoflurane et sévoflurane, sont comparables pour la plupart des paramètres. Néanmoins, le sévoflurane présenterait moins d'effets secondaires que l'isoflurane (moindre diminution de la fréquence respiratoire et moins d'arythmies cardiaques) et offrirait un temps de réveil plus bref.

### Introduction

En médecine aviaire, il faut parfois recourir à l'anesthésie générale pour réaliser une intervention diagnostique ou thérapeutique. L'anesthésie par voie gazeuse, comparativement à l'anesthésie fixe, offre une plus grande sécurité et flexibilité. Parmi les anesthésiques volatils disponibles, l'isoflurane est la molécule la plus fréquemment utilisée. Elle permet une induction et un réveil rapide et elle présente peu d'effets cardio-dépresseurs. Néanmoins, apnées, tachycardie, hypertension et arythmies ont été décrites. Depuis un peu plus d'une dizaine d'années, d'autres molécules ont fait leur apparition en anesthésie, dont le sévoflurane. Celui-ci causerait moins d'effets cardiovasculaires indésirables chez l'oiseau. L'objectif de cet article est de réaliser une synthèse méthodique des effets de l'isoflurane et du sévoflurane chez les oiseaux.

#### Matériel et méthode

Une recherche documentaire a été effectuée dans la base de donnée PubMed, en introduisant l'équation de recherche «[ isoflurane OR sevoflurane] AND [birds] ». Seuls des articles publiés après 2000 ont été répertoriés. Pour être sélectionnés et analysés, il devait s'agir d'essais cliniques sur les effets physiologiques de l'anesthésie à l'isoflurane ou au sévoflurane chez les oiseaux (Figure 1). 11 études sont retenues. Afin d'apprécier la qualité méthodologique et le niveau de preuve scientifique des articles sélectionnés, un score est attribué à chacun d'entre eux, selon une grille de lecture inspirée du *Consort Statement (CONsolidated Standards of Reporting Trials)* 

Figure 1. Pigeon anesthésié à l'isoflurane



1. Pigeon anesthésié, intubé et sous examen électrocardiographique.

2a, 2b et 2c. Tracés d'électrocardiogramme chez un pigeon anesthésié : tracé normal (2a) ; blocs du deuxième degré (2b) ; blocs du troisième degré (2c). PHOTOS : I. BOTMAN

# Résultats

**Tableau 1.** Effets de l'isoflurane et du sévoflurane sur la fréquence et le ryhtme cardiaques, la pression artérielle, la fréquence respiratoire, l'end-parabronchial CO2 et la température corporelle. La flèche indique une diminution ou une augmentation significative. O signifie qu'aucune différence significative n'est notée. / indique que le paramètre n'a pas été mesuré dans l'étude. > indique que la valeur du paramètre mesuré pour un gaz est supérieure à celle pour l'autre gaz. \* indique une différence significative

GAZ	QUESTION POSÉE	ESPÈCE [RÉFÉRENCE]	FRÉQUENCE CARDIAQUE	PRESSION ARTÉRIELLE	ARYTHMIE	FRÉQUENCE RESPIRA- TOIRE	PE'CO <sub>2</sub>	TEMPÉRATURE CORPORELLE
Isoflurane	Comparaison à l'état éveillé	Caracara huppé [7]	0	↓*	0	↓*	^∗	↓*
		Buse variable [21]	↓*	/	/	/	/	/
		Canard fuligule [13]	↓*	/	oui	↓* (apnées)	/	↓*
		Conure à gros bec [14]	↓*	/	/	↓*	/	↓*
	Si la durée est augmentée	Conure à gros bec [14]	↓*	/	/	↓*	0	↓*
		Poule [16]	0	0	0	/	/	/
		Vautour moine [12]	0	0	0	/	0	0
		Pygargue à tête blanche [11]	↓*	^*	Oui	↓*	/	↓*
	Si la dose est augmentée (1 à 2x MAC)	Poule [16]	0	↓*	0	/	/	/
		Vautour moine [12]	0	↓*	0	/	0	0
Sévoflu- rane	Comparaison à l'état éveillé	Caracara huppé [6]	0	↓*	0	↓*	^∗	↓*
		Conure à gros bec [17]	↓*	/	0	↓*	/	/
	Si la durée est augmentée	Conure à gros bec [17]	↓*	/	0	0	0	0
		Pygargue à tête blanche [11]	↓*	↑*	oui	↓*	/	↓*
	Si la dose est augmentée (1 à 2x MAC)	Poule [15]	0	↓*	0	/	/	/
		Conure à gros bec [17]	0	/	0	Non précisé	0	0
lsoflu- rane/ sévoflu- rane	Comparaison	Buse à queue rousse [8]	0	0	0	Sévoflurane > isoflurane	0	0
		Pygargue à tête blanche [11]	lsoflurane > sévoflurane	lsoflurane > sévoflurane	Isoflurane > sévoflurane	0	/	lsoflurane > sévoflurane

# Discussion

Les deux gaz sont comparables pour la plupart des paramètres. Néanmoins, le sévoflurane permettrait un réveil plus rapide, une moindre diminution de la fréquence respiratoire, et entraînerait moins d'arythmies cardiaques. Les deux gaz induisent en général une baisse de la pression artérielle, de la fréquence respiratoire et de la température corporelle. Ils sont également à l'origine d'une diminution de la fréquence cardiaque au cours de l'anesthésie, dont l'ampleur semble dépendre davantage de la durée de l'intervention que de la concentration en gaz. L'évolution des paramètres respiratoires et des valeurs des gaz sanguins semble liée à la technique de ventilation utilisée (spontanée ou contrôlée).

Aucune des études dont le but est une comparaison des effets cardiorespiratoires induits par l'isoflurane et le sévoflurane n'ont anesthésié les oiseaux à la MAC respective de ces gaz. Dès lors, il est délicat d'interpréter les résultats puisqu'aucune preuve ne peut garantir que la profondeur de l'anesthésie était la même pour le sévoflurane et pour l'isoflurane.

Les rares articles fiables existants sur l'anesthésie des oiseaux concernent des espèces aussi variées que le pygargue à tête blanche et le poulet, en passant par le conure à gros bec. Or, dans le domaine de l'anésthésie, d'importantes différences peuvent exister parmi les espèces aviaires.

De futures et rigoureuses études contrôlées et randomisées sont donc requises afin d'évaluer les effets spécifiques de l'isoflurane et du sévoflurane chez un maximum d'espèces aviaires, à une profondeur d'anesthésie équivalente. Il est donc important que des études déterminent aussi avec précision la MAC, préalable à la comparaison de gaz de nature différente).

# What are the cardio-respiratory parameters of the awake pigeon and the effect of isoflurane at MAC in the anesthetized pigeon?

The study "Cardio-respiratory parameters in the awake pigeon and during anaesthesia with isoflurane" is in press in Veterinary Anesthesia and Analgesia.

#### Take home message

Isoflurane MAC in pigeons appeared to be higher than in other avian species. Isoflurane anaesthesia in pigeons resulted in hypercapnia, hypotension, mild hypothermia and second-degree and third-degree atrioventricular blocks.

# Introduction

In birds, anaesthesia can be provided either by injectable agents or by inhalation agents. Inhalation anaesthesia is the preferred technique and isoflurane has been traditionally used for avian anaesthesia. Isoflurane anaesthesia is characterized by minimal cardiovascular adverse effects, rapid induction and short recovery times, though short periods of excitement during induction and recovery, and apnoea or cardiac arrhythmias during maintenance, have been reported in bald eagles. In the pigeon, anaesthetic protocols with isoflurane have been described where the delivered concentrations (according to vaporizer dial setting) were 3 to 5% for induction and 1.5 to 3% for maintenance.

An isoflurane MAC (the end-parabronchial concentration of anaesthetic agent at which 50% of anaesthetized individuals will not move in response to a supramaximal noxious stimulus of  $1.51 \pm 0.15\%$  has been reported in pigeons.

However, information is lacking about cardiovascular and respiratory parameters when pigeons are anaesthetized with isoflurane without surgical or diagnostic procedures being performed and while breathing spontaneously. In addition, respiratory parameters and blood pressure have not been documented in the awake pigeon.

This study aimed to assess cardiovascular and respiratory variables in the awake pigeon and during anaesthesia at the individual's MAC of isoflurane while breathing spontaneously.

# Material and methods

#### Birds

Seven adult pigeons were used in this study. All seven pigeons were acclimatized to handling during a 2 month period before this study. All animals entered and completed all phases of the study.

#### Baseline monitoring parameters in awake animals

Several physiological variables were measured in each pigeon in the awake state without tranquillization. An ECG was used to assess HR and rhythm. A Doppler blood flow probe and occlusive cuff with sphygmomanometer was used to monitor indirect SAP (Figure 1). For gas analysis by capnography (inspired CO2, PE'CO2), the sectioned blind end of a latex glove finger (size small) was fixed around the beak of the pigeon proximal to the nares (Figure 1). To measure the body temperature an electronic thermometer was inserted into the cloaca.

#### Induction and maintenance

Prior to anaesthesia, birds were allowed to breathe 100% oxygen (1L minute<sup>-1</sup>) via a facemask for 5 minutes. Anaesthesia was induced with isoflurane delivered in 100 % oxygen (1L minute<sup>-1</sup>) using a Bain non-rebreathing system and an initial vaporizer setting of 4 %. Once the bird was sufficiently relaxed and voluntary movement of the eyelids had ceased, the face mask was removed and the trachea was intubated with a 3 mm noncuffed endotracheal tube. Oxygen delivery was adjusted to 0.3L minute<sup>-1</sup> for the remainder of the procedure. Birds were allowed to breathe spontaneously.

#### **MAC** determination

The MAC was determined by using the "bracketing" method. After stabilization at a predetermined anaesthetic concentration, jaws of a Rochester-Carmalt forceps were used to clamp a digit until either a gross purposeful movement occurred, such as kicking of the limbs or moving of the wings, or for up to 60 seconds. The end-parabronchial anaesthetic concentration was decreased by 10 % if no movement occurred. The procedure was repeated until the bird reacted. At this moment, end-parabronchial anaesthetic concentration was defined and calculated as "the median value between the maximal end-parabronchial concentration that allowed movement and the minimal end-parabronchial concentration that prevented movement".

Figure 1. Measure of blood pressure and gas



#### Results

In the awake pigeon, mean  $\pm$  SD HR, SAP, *f*R, PE'CO2 and T were respectively 155  $\pm$  28 beats minute<sup>-1</sup>, 155  $\pm$  21 mmHg, 34  $\pm$  6 breaths minute<sup>-1</sup>, 38  $\pm$  8 mmHg and 41.8  $\pm$  0.5 °C. Mean isoflurane MAC was 1.8  $\pm$  0.4%. During maintenance of anaesthesia at MAC, while no significant decreases in HR or respiratory rates were noticed between the awake and anesthetized states, significant decreases of SAP and cloacal temperature and an increase of PE'CO2 were observed. No arrhythmia was identified in awake pigeons while second-degree and third-degree atrioventricular blocks occurred under isoflurane.

#### Discussion

The MAC of isoflurane determined in pigeons in this study was higher than that previously reported for pigeons and also that reported for other birds. Isoflurane resulted in a smooth and rapid induction of anaesthesia in pigeons, similar to other species, although in this study the recovery time was longer than that reported in other studies. Isoflurane resulted in mild hypothermia and hypotension. A salient finding was the significant development of second-degree and third-degree cardiac blocks. Hypercapnia was also observed suggesting that ventilatory assistance could be recommended.

Focus 3. Education to Evidence Based Practice and to Research



# What is the efficacy of nutraceuticals to alleviate clinical signs of osteoarthritis?

Our group has already been previously involved in research on such compounds. This larger question about the efficacy of nutraceuticals in OA was answered by a "Systematic review of efficacy of nutraceuticals to alleviate clinical signs of osteoarthritis (oa)" that was published in Journal of Internal Veterinary Medicine in 2012. It was awarded the top five downloaded publication of the journal.

#### Take home message

The evidence of efficacy of nutraceuticals is poor, with the exception of diets supplemented with omega-3 fatty acids in dogs. Greater access to systematic reviews must be part of the objectives of the veterinary science in the future. Their reporting would be improved by internationally agreed-upon criteria for standards and guidelines.

#### Introduction

Clinically, animals with OA present with stiffness or lameness. Lameness is due to a combination of joint pain and restricted movement of the joint. There are many medical therapeutic options. Products called "nutraceuticals" have recently appeared on the market. The veterinary profession has ethical obligations to ensure effective and safe service and to base therapeutic decisions on scientific evidence. The objective of the current study was to conduct a systematic review to know the efficacy of nutraceuticals that are used in veterinary medicine for the treatment of OA.

#### Material and methods

The main investigator of this study conducted a document search using Medline, CAB Abstracts and Google Scholar databases. In order to identify other studies and to confirm the effectiveness of our study, published reviews focusing on therapy of OA in dogs and horses were consulted. Only controlled experimental studies or clinical trials published in English or French before December 2010 were included. To be eligible, the articles had to cover the effects of oral supplements of one or more natural substances in the form of granulated, drinkable solution, capsule or feed. Only *in vivo* studies evaluating clinical signs of pain or abnormal locomotion were considered. Due to the limited number of clinical studies available in horses, the results obtained from experimental studies on induced OA in this species were included in the review, although their conclusions are less able to be generalized than those obtained from controlled trials in naturally occurring OA. Our system of evaluation included an evaluation of internal validity (step 1) and of external validity (steps 2 and 3). The statistical significance of the effect (step 4) and global level of evidence (step 5) were determined. 22 publications were selected.

#### Results

In horses, soybean and avocado unsaponifiables extracts had no effect and global strength of evidence of efficacy was low for non-denatured type II collagen; chondroitin sulfate; combinations of oligo-elements, amino acids and vitamins (AOV); and combinations of myristoleic acid, glutamine, methylsulfonylmethane, hydrolyzed collagen, and AOV.

In dogs, hydroxycitric acid and extract of Indian and Javanese turmeric were not effective, though for the latter veterinarians reported an improvement in clinical signs in contradiction with objective data that was obtained with a force plate. Global strength of evidence of efficacy was low in studies demonstrating a significant effect for the use of  $\beta$ -1,3/1,6 glucans ;gelatine hydrolysate; non-denatured type II collagen alone or combined with hydroxycitric acid or with chromium nacinate; special milk protein concentrate. Two different compounds

containing glutamine and chondroitin sulfate showed contradictory results: one compound had beneficial effects, although the other one (combined with manganese) had no effect. The highest global strength of evidence of efficacy was demonstrated by omega three fatty acids supplemented diets. Green lipped mussel powder had a significant effect in 3 out of 4 studies, and due to this inconsistency between studies, we could not conclude to a strong indication for its clinical use.

In cats, it was not possible to recommend the use of diets supplemented with omega 3 fatty acids, green lipped mussel powder, glutamine, and chondroitin sulfate, as only one study, though of high quality, had been performed for this product.

This review identified several major methodological issues in clinical trials: the limited numbers of rigorous randomised controlled trials and of patients, the lack of objective outcome measures, the uncommon use of the concept of "effect size", the risk of conflict of interest, the lack of standardization of dosages and duration of treatments.

# Discussion

In this review, it is the potential of nutraceuticals to alleviate the clinical signs of osteoarthritis that was evaluated, rather than any potential disease (structure)-modifying effects. In addition, only clinical and *in vivo* experimental studies were selected. This does not mean that we considered *in vitro* studies as being of low quality. Their conclusions are simply less easy to generalize to the population of animals and are less useful to answer clinical questions referring to improvement of signs of pain and abnormal locomotion.

This review also showed the limited numbers of rigorous randomised controlled trials and of participants in clinical studies. Several methodological weaknesses in the methodology of trials were identified. Firstly, objective outcome measures were rarely used. Lameness is traditionally evaluated semi-objectively by clinicians. Promulgators of evidence based research recommend the use of objective instruments to validate outcomes and provide a standardised means for clinical assessment of the efficacy of veterinary treatments, like kinematics and force plates. Secondly, the difficulty in recruiting patients and the importance of considering the power of studies and sample size in veterinary research have been emphasised. This review shows that a low number of individuals were studied. Thirdly, a statistically significant result does not indicate whether the observed effect has any clinical importance. The concept of "effect size", a unitless measure of the degree to which the apparent treatment effect exceeds the placebo effect, has not been widely reported in veterinary trials.

There were also other methodological elements that influenced the evaluation of the efficacy of nutraceuticals such as uncontrolled composition of the marketed product and combination of the nutraceuticals with others.

It must also be noted that the duration of treatment was variable. Initial standardisation is difficult as there will always be regimens (mean dosage, treatment frequency and treatment duration) that are not or less effective than others. Nevertheless, once a nutraceutical has been suggested to be effective in one group, conditions of administration should be defined if the purpose is to assess whether it is also effective in another group or to compare it with another product.

# What is the scientific evidence of efficacy of nutraceuticals in canine liver disease?

This question was answered in a paper published in Veterinary Clinics of North America ("Nutraceuticals for canine liver disease: assessing the evidence.") and in anoter one published in Companion Animals ("An Evidence-Based Approach to Nutraceuticals"), both in 2013.

#### Take home message

Minimal information is available in the scientific literature about commonly used nutritional supplements, i.e. S-adenosylmethionine, Silymarin, and Vitamine E. Only nine publications are useful to evaluate clinical evidence of efficacy of these compounds as hepatoprotectants in canine liver disease, and none of these provides strong clinical conclusions. At this stage, individual veterinarians must decide whether or not to use nutraceuticals in their patients. Studies in animal models and humans can provide preliminary information regarding the use of nutritional supplements as adjunctive treatments for a variety of acute and chronic diseases affecting liver function. However, controlled studies for specific indications are mandatory to assess evidence-based clinical application of these products. Until such evidence exists, individual veterinarians must assume responsibility for their decision to use nutritional supplements in their canine patients with liver disease.

#### Introduction

The veterinary profession has ethical obligations to ensure effective and safe treatment and to base therapeutic decisions on scientific evidence. It is important to know the true efficacy and safety of products, such as dietary supplements, that are used in veterinary medicine. Because these products are often promoted as "natural", pet owners may have the misconception that they are safer than drugs. These products may be used widely by pet owners since they can purchase them directly at health food stores or by internet. It is therefore extremely important for veterinarians to have a thorough understanding of the safety and efficacy of dietary supplements in veterinary patients. We will focus this review on several dietary supplements that have been suggested to be useful for liver diseases in dogs.

#### Hepatoactive dietary supplements: what can we find in the scientific literature?

With Medline, no "MESH term" (Medical Subject Headings term) can be identified for "Nutraceuticals". Instead "Dietary Supplements" is proposed. When the equation ("Dietary Supplements"[Mesh]) AND "Liver Diseases"[Mesh] AND "Dogs"[Mesh]) is used in Medline, Cab Abstracts and Google Scholar, very few research papers are identified. A total of only nine research papers are useful for analysis; they are referenced in Table 1. No relevant reference is identified in the databases about efficacy of vitamin E. However, very detailed narrative reviews exist about hepatoprotectants, published by Center in 2004 ([2], a review of 105 pages), and by Webster and Cooper in 2009 [6], in Veterinary Clinics of North America; those papers provide a thorough description of the physiopathology of liver diseases (mechanisms of hepatocyte cell death, oxidative stress) and possible actions of food and non-food products that are called "hepatoprotectants". Dietary supplements that are commonly listed as "hepatoprotectants" include S-adenosylmethionine (SAMe), yutan (which contains urodeoxycholic acid), and silymarin (derived from the milk thistle plant).

#### Hepatoactive nutraceuticals: what can we glean from available scientific literature?

Currently there is limited available evidence regarding efficacy of nutritional supplements. Two detailed reviews have been conducted regarding the use of dietary supplements as hepatoprotectants for dogs. However, only eight clinically relevant publications that focused on the use of so-called hepatoprotectants in dogs were referenced in these two reviews. Only under very limited circumstances were some nutritional supplements shown to have a favorable biochemical or clinical outcome in treating or preventing hepatic disease.

Minimal regulatory oversight of veterinary nutritional supplements may explain this lack of scientific evidence regarding the clinical efficacy of these products. Because nutritional supplements are not considered by the FDA to be drugs, manufacturers are not currently required to provide efficacy data as long as the label does not make medicinal claims.

When evaluating the scientific literature available for nutritional supplements it is important to consider the route of administration and composition of the compound used in the research population as compared to the composition of the marketed product. In some research publications, a highly purified compound with verified potency was used rather than the commercially available nutritional supplement. Additionally, the use of multiple nutritional supplement makes it difficult to ascertain which particular compound may have provided a beneficial effect.

#### Conclusion

Until greater regulatory oversight of nutritional supplements is required, individual veterinarians will have to weigh the costs, risks and potential benefits of nutritional supplements for their patients on an individual basis. Veterinarians should strive to maintain a critical view of nonscientific promotional material and rely primarily on scientific evidence. Prior to recommending or administering a nutritional supplement to a patient with the intent of providing "hepatoprotection", veterinarians should obtain informed consent from the dog's owner to ensure that the owner understands that there is little to no evidence to support the use of these products for treatment or prevention of liver disease. It is equally as important for veterinarians to be aware that lack of adequate regulation of so-called "nutraceuticals" increases the risk of lack of quality control, labeling inaccuracies, and omission of cautionary statements. While some dietary supplements have shown beneficial effects under limited in vitro conditions or for a very specific hepatotoxin, their general use as global "hepatoprotectants" remains questionable.

# What is the scientific evidence about the effects of corticosteroids on articular cartilage?

The review « Les corticostéroïdes, amis ou ennemis du cartilage ? » was published in French in Pratique vétérinaire équine in 2014.

# Take home message

Bien que les corticostéroïdes soient largement utilisés par voie intra-articulaire dans différentes espèces, la littérature relative à leurs effets sur le cartilage reste peu abondante, contrairement à ce qu'on pourrait penser. Des études complémentaires sont nécessaires. En conclusion, l'utilisation des corticostéroides, à défaut de preuves d'efficacité et d'innocuité manifestes, doit être réfléchie. L'utilisation intra-articulaire ne doit pas être routinière et serait plutôt utile lorsque l'inflammation (synovite) est importante.

# Introduction

Chez l'homme, une synthèse méthodique conclut que les corticostéroïdes administrés en intra-articulaire améliorent la douleur pour une durée d'une semaine mais pas beaucoup plus. Une autre synthèse montre que cette amélioration ne dépasse pas trois semaines. Il semble que la triamcinolone soit plus efficace que les autres corticostéroïdes. L'hexacétonide de triamcinolone aurait un effet plus puissant et plus prolongé que l'acétonide de triamcinolone.

Au-delà des effets cliniques sur la douleur et la locomotion, les effets sur la structure histologique et la composition biochimique du cartilage continuent à faire débat. La multiplicité des molécules et des doses ne facilitent pas les choses. On a l'impression que tant a déjà été écrit sur le sujet, et pourtant il reste utile de se poser la question suivante : quelles preuves avons-nous des effets positifs ou négatifs des corticostéroïdes sur le cartilage articulaire ?

L'objectif de cet article est de réaliser une synthèse méthodique de la littérature afin d'évaluer les éléments de preuves portant sur l'effet des corticostéroïdes sur le cartilage lorsqu'ils sont administrés par voie intra-articulaire chez le cheval et dans d'autres espèces.

# Matériel et méthode

Pour répondre à cette question, une synthèse méthodique est réalisée. Elle identifie 38 études contrôlées réalisées in vivo évaluant l'effet des corticostéroïdes sur la structure ou la composition biochimique du cartilage, ou sur la membrane et le liquide synovial. Ces effets sont documentés par plus de quatre études chez le cheval, le lapin et le chien.

# Résultats

Chez le cheval, l'effet de la méthylprednisolone sur le cartilage est plutôt défavorable.Les travaux qui évaluent son impact sur le cartilage sain mettent en évidence une augmentation de la mort cellulaire des chondrocytes, une diminution de la synthèse des glycosaminoglycans et du collagène, une augmentation du clivage du collagène, une augmentation du turnover de l'aggrécan, une inhibition de la synthèse du procollagène, une augmentation des produits de dégradation de l'aggrécan et une augmentation de la concentration en eau du cartilage. Les études utilisant un modèle d'ostéoarthrite induite démontrent également les effets négatifs de la méthylprednisolone sur le cartilage ; l'étude de Frisbie et coll. montre en outre que les effets bénéfiques sur l'inflammation de la membrane synoviale ne compensent pas les effets négates sur le cartilage. Une seule étude

porte sur la betamethasone ne rapporte ni effet néfaste, ni effet bénéfique. Le cas de la triamcinolone est différent puisqu'à son action anti-inflammatoire semble aussi associée une action favorable sur le cartilage; cette unique étude in vivo a donné à cette molécule son image chondroprotectrice. Si différentes études concluent à une action favorable des corticostéroïdes sur la membrane et le liquide synovial, une seule autre rapporte toutefois une accentuation de l'inflammation après injection de méthylprednisolone.



L'Injection Intraarticulaire de corticostéroïdes fait partie des thérapeutiques de l'ostéo-arthrite. Cliché : J.-M. Vandeweerd

# Discussion

Notre étude se limite pour l'instant à établir un état des lieux des publications réalisées jusqu'à ce jour. Les équations de recherche de notre étude portant sur le cartilage, il faut rester prudent sur les conclusions que nous pourrions tirer concernant l'effet des corticostéroïdes sur la membrane synoviale et la synovie. En outre, il se pourrait que l'effet des corticostéroïdes soient différent en fonction de la présence ou non d'inflammation de la membrane synoviale. Enfin, il sera intéressant d'envisager, dans le futur article, les doses et la fréquence d'administration des corticostéroïdes ainsi que leur association avec d'autres molécules (anesthésiques locaux, acide hyaluronique).

Professor JM Vandeweerd University of Namur (UNamur) Department of Veterinary Medicine Unit of Integrated Veterinary Research Namur Research Institute for Life Sciences Rue de Bruxelles, 61 5000 Namur Belgium Tel. +32 (0)81724374 Mobile. +32 (0)495703728 jean-michel.vandeweerd@unamur.be

Professor JF Nisolle Université Catholique de Louvain (UCL) Centre Hospitalier Universitaire (CHU) de Mont Godinne-Dinant Avenue Docteur Gaston-Therasse 1 5530 Yvoir Belgium Tel. +32 (0)81423513 Mobile. +32 (0)474353164 jean-francois.nisolle@uclouvain.be

