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Editorial

Last year as editor and as author, I became aware of papers in the AWAAP been found on the internet by scientists and colleagues working in the veterinary field. In particular articles on emerging poultry pathology, such as the duodenal ulcers of layers and woody breast (on the European continent called wooden breast) gave e-mail reactions of several colleagues from all over the world. Whereas, of course, we cannot compete with journals being included in the citation index system, this tells us that minimally by seeking software such as Google our primary papers find their way. And this may encourage you as a reader to proceed submitting manuscripts. To help making the primary papers more accessible for readers, in this volume the titles from those published in former issues will become included.

24-11-2014, Erik Gruys

A. Letters

Dear Prof. Gruys,

Thanks a lot for your information about AWAAP journal. Sorry for not contributing any article since. Hoping in the near future

Best regards, Dai Tan Vo.

Dear Professor,

Thank you very much for sending me the new issue of AWAAP Journal. It is always a great pleasure to remember you and all the great time spent in Holland! I use this opportunity to wish you a Marry Christmas and Happy New Year!

All the best from Cluj!

Yours, Bogdan Sevastre

Dear Erik,

Many thanks for your email. I was able to load the journal with many thanks. Since 2009, I am back to Mozambique, after finishing my PhD Studies in Austria at Innsbruck Medical University. My full time job is still at Eduardo Mondlane University, Faculty of Veterinary Medicine as lecturer and pathologist. Since 2006 I am managing a family company called InterMed Mozambique, which imports and distribute veterinary medicines and biologicals, animal feed and does technical veterinary consultancy. In fact, our first international partner was a Dutch company called Kepro BV based in Deventer. Actually, we are growing with them since 2006. It has been an enjoyable challenge. By April, I might be visiting Holland and it will be a great pleasure meeting you there.

Greetings to you and your family. Custodio

Dear Erik,

As always it is great to hear from you and thank you for this year's AWAAP issue. I appreciate you for spending time on this. What you doing is important. Do you also do
editorial to other journals or serve as reviewer? I think you could help a lot some African journals. Perhaps you doing that.
I agree that when a man has excess free time, it often leads to stress, may be depression and unleashes harmful destructive behaviors. I am doing well. Work is ok and busy especially management. I (we) now thinking of settling, buy a house and establish, so far we been in moving mode. Sarin is now 2.5 yrs and will soon need a good school.
Regards to Barbara. Have a great holiday season.

Bereket

On Dec 4, 2013, at 9:04 AM, "E.Gruys" wrote:

Dear Bereket,
Hope you and your family are fine keeping it not too cold and not too hot. Hope you enjoy your present job. It is recognisable that optimum jobs don't exist. You have to do that work to get a salary and to keep you off from the road. Probably the latter is most important. I see now being retired that it is most important to keep busy with some science trying to avoid brain damage. When a person has too much time for free, that will become filled up with alcohol and other odd stuff.
We have our terrible December time with dark foggy days, but good times for children. For 2013 I have prepared again an AWAAP issue. It should be possible to load from the www.awAAP.nl website, from where you get on the place I have put it on the webspace of my www.gruys.com site.
Please, when it fails to get it, mail me. It contains a paper on oxalosis in Indian elephant of Eli and some discussions of me on wooden breast syndrome in broilers and on grey gut in layers.
Cheers,
Erik

Dear Prof. Erik Gruys,
Merry Christmas and Happy New Year- 2014.
May this new year be a year of love, peace, harmony, and success for you and your family.
Most of all, I wish you very long and healthy life.
With my all best wishes,
Michael K.
Dr.Michael Kahsay Ghebreremariam,DVM,MSc(AnimalPathology),Dip.AST
Head, Department
Department of Veterinary Sciences, Hamelmalo Agricultural College
Hamelmoalo, Eritrea E-mail:Michaelvet@yahoo.com

12-22-2013
Dear Prof Erik,
Marry Christmas and Happy New Year 2014 and also thank you very much to put the article "Oxalosis in Indian Elephant" in AWAAP 2013. Hope all of us, alumni of Master student on Animal Pathology, Utrecht University are happy and in good health and also success on their profession. For me AWAAP is very good media to share any information regarding to animal pathology issues, to know alumni activities in their country, etc. Who knows, one day Utrecht University invite their international alumni attending international seminar hosted by UU. Hope my dream realized. Regards,
Eli BBVet Denpasar, Bali
On Saturday, August 3, 2013 5:16 PM, I Ketut Eli Supartika <iketutelisupartika@yahoo.com> wrote:

Dear Prof. Erik,
Thank you for the email and the draft. I am sorry for the late in replying it, because I have been out of Bali for several days and its hard to find internet access there. Unfortunately there is no breeding program at Bali Elephant Camp. I will complete the draft you've sent and send back to you when it is ready. Have a nice weekend.
With best regards,
Eli

Dear Prof Erik
Thank you very much. Happy new year for you and all your family member. Cheers,
Soleman
Kind Regards, Dr. Soleman O. Al-Garib, Tripoli University
Faculty of Veterinary Medicine, Tripoli-Libya
Cellular phone 00218.91.370.6300, P.O.Box 7494 AinZara

B. Found
Interesting abstracts

In vertebrates and arthropods, blood clotting involves the establishment of a plug of aggregated thrombocytes (the cellular clot) and an extracellular fibrillar clot formed by the polymerization of the structural protein of the clot, which is fibrin in mammals, plasma lipoprotein in crustaceans, and coagulin in the horseshoe crab, Limulus polyphemus. Both elements of the clot function to staunch bleeding. Additionally, the extracellular clot functions as an agent of the innate immune system by providing a passive anti-microbial barrier and microbial entrapment device, which functions directly at the site of wounds to the integument. Here we show that, in addition to these passive functions in immunity, the plasma lipoprotein clot of lobster, the coagulin clot of Limulus, and both the platelet thrombus and the fibrin clot of mammals (human, mouse) operate to capture lipopolysaccharide (LPS, endotoxin). The lipid A core of LPS is the principal agent of gram-negative septicemia, which is responsible for more than 100,000 human deaths annually in the United States and is similarly toxic to arthropods. Quantification using the Limulus Amebocyte Lysate (LAL) test shows that clots capture significant quantities of LPS and fluorescent-labeled LPS can be seen by microscopy to decorate the clot fibrils. Thrombi generated in the living mouse accumulate LPS in vivo. It is suggested that capture of LPS released from gram-negative bacteria entrapped by the blood clot operates to protect against the disease that might be caused by its systemic dispersal.

Grouper fish mortality at the East Cost Libyan area of the Mediterranean Sea is one of the major problems encountered at autumn season starting from October. Sudden environmental changes associated
with water pollution were recorded. Adult fish especially the grouper types were affected. This phenomenon was previously recorded in Libya at 1985. The present study was carried out to demonstrate the most prevalent isolates that may lead to this phenomenon. It was found that the gram negative oxidase positive bacterial group (Pasteurella, Vibrio and Aeromonas spp.) were the most isolated bacteria with high incidence refers especially to Pasteurella piscicida with an incidence of 64%. Black or metallic colour cysts (Microspordiosis, Glugea spp. and Plistophora spp.) representing spores or larvae of this parasite were observed on the visceral organs and abdominal cavity. Other parasites included larval stages of Contracaecum spp. and Gonapodasmius epinepheli (Didymozoid digenes). The clinical and postmortem lesions were mostly characterized by unilateral or bilateral corneal opacity and haemorrhagic spots on the skin with ulcer formation in some cases. Abdominal distension and anal prolaps were also recorded. All the internal organs were congested. The swim bladder was greatly swollen and filled with gas.


Infectious laryngotracheitis (ILT) is a highly contagious acute respiratory disease of chickens that may result in high mortality and decrease in egg production. The disease is caused by herpesvirus 1 (an alpha-herpes virus). This report deals with an outbreak of infectious laryngotracheitis in layer chickens in Tripoli, Libya.

A total number of 432 day-old broiler chicks from Ross 308 parent stock were used in this study. These chicks were divided randomly into equal groups at 21 days of age under normal conditions and under heat stress. These birds were fed different concentrations of amino acids (Lysine, Threionine and Methionine) according to the NRC and higher than that. These birds were vaccinated according to the program provided by the Animal Health Center, and the antibodies level against Newcastle disease virus was measured. The results show that an increase of lysine has no significant effect on antibody production whereas the increases of threionine and methionine have good effect on immunity as the titer of antibodies against Newcastle disease was increased (higher for methionine under normal conditions). Moreover, heat stressed birds fed higher concentrations of threionine has no significant effect on antibody production compared to birds under normal conditions, whereas there was drop of antibodies level for heat stressed birds fed higher concentrations of methionine compared to birds under normal conditions.

16 world veterinary poultry association congress; 11/2009

Abdulatif Asheg et al. Conference Paper: Effect of pH on Total Intestinal Bacterial Count. 26th Maghrib Veterinary Congress; 05/2009

S.O. Al-Garib et al. An outbreak of a respiratory infection of multi-agents occurred in poultry flocks in Tripoli, Libya. 01/2006;
During March-May / 2005, the poultry industry in Tripoli faced heavy losses that was characterized by high mortality and respiratory distress. A field analysis of 800.000 broilers of various ages with
clinical respiratory infections were conducted. Serum was collected from a 92-broiler at ages 6, 10, 19, 23, 31, 43, 48 and 53 days of age. The seroconversion of antibody titers were detected by Enzyme-Linked immunosorbent assay (ELISA) against NDV, IBV, CAV and ORT. Although vaccination against NDV was performed at day 5 using Hitchner B1 and the birds were boosted at day 21 and 28 by La Sota, a very low antibody titer (2^2) was recorded between 6 to 43 days of age. However, the titer was significantly raised (2^5.5) at 48 and 53 days of age. Vaccination against IBV was conducted only at one-day-old using H120. The antibodies titer were dropped to 2^4 at day 23 whereas, it were significantly increased to 2^12 between day 31 to 53 of age. No vaccination were used against both CAV and ORT. The majority of broiler have detectable antibodies titer against CAV and ORT at day 6 and10, dropped to minimum between day 19 to 43 of age and again sharply raised at the end of the fattening period (day 48 and 53). In conclusion, a field infection of IBV as early as day 25-30 of age were recorded. In addition NDV field infection could also considered at older birds (day 40-50). Vertical transmission of both CAV and ORT were documented as well as a field infection. A mishandling of vaccine in either a storage or application were speculated to be the actual cause of this outbreaks. Moreover, the vaccination schedule need to be revised (e.g. changes in vaccine strains used and in the time of application) and an excellent monitoring system for various locations should be validated.

Laisse CJ et al. Characterization of tuberculous lesions in naturally infected African buffalo (Syncerus caffer). Vet Diagn Invest. 2011 Sep;23(5):1022-7. doi: 10.1177/1040638711416967. Tuberculosis pathology was studied on 19 African buffalo (Syncerus caffer) from a herd in the Hluhluwe-iMfolozi Park in South Africa. The animals tested positive with the comparative intradermal tuberculin test and were euthanized during a test-and-cull operation to decrease prevalence of bovine tuberculosis (bTB) in the park. The lymph nodes and lungs were examined grossly for presence of tuberculous lesions, which were scored on a 0-5 scale for macroscopic changes. The gross lesions were examined histologically and classified into grade I, II, III, or IV according to a grading system used for bTB lesions in domestic cattle. Macroscopic lesions were limited to the retropharyngeal, bronchial, and mediastinal lymph nodes and the lungs. The most frequently affected lymph nodes were the bronchial (in 16 animals) and mediastinal (in 11 animals). All four grades of microscopic lesions were observed, grade II lesions were the most frequent. Mycobacterium bovis was detected by PCR in 8 out of 19 animals, and acid-fast bacilli were seen in 7 out of 19 animals, together both techniques identified mycobacteria in 5 out of 19 animals. Lesions were paucibacillary, as acid-fast bacilli were only rarely observed. The absence of lesions in the mesenteric lymph nodes and the high frequency of lesions in respiratory tract associated lymph nodes suggest that the main route of M. bovis infection in African buffalo is by inhalation.

Bila C et al. Complement opsonization enhances Friend virus infection of B cells and thereby amplifies the virus-specific CD8+ T cell response. J Virol. 2011 Jan;85(2):1151-5. doi: 10.1128/JVI.01821-10. Epub 2010 Nov 3. B cells are one of the targets of Friend virus (FV) infection, a well-established mouse model often used to study retroviral infections in vivo. Although B cells may be effective in stimulating cytotoxic T lymphocyte responses, studies involving their role in FV infection have mainly focused on neutralizing antibody production. Here we show that polyclonal...
activation of B cells promotes their infection with FV both in vitro and in vivo. Furthermore, we demonstrate that complement opsonization of Friend murine leukemia virus (F-MuLV) enhances infection of B cells, which correlates with increased potency of B cells to activate FV-specific CD8(+) T cells.

The incidence and range of spontaneous pathology findings were determined in the eyes of male and female control Crl:CD-1(ICR)BR mice. Data were collected from 250, 430, 510, and 2,266 mice from control dose groups of 4-, 13-, 80- and 104-week studies, respectively, carried out between 2005 and 2013. Lesions of the eye were very rare in 4- and 13-week studies, uncommon in 80-week studies, and were of relatively higher incidence in 104-week studies. No sex predilection in the incidence of eye lesions was apparent. No neoplastic lesions were observed, and congenital lesions were very rare. The most common findings were cataracts, retinal degeneration, mineral deposits in the iris, keratitis, anterior uveitis, and mineral deposits in the corneal stroma. These lesions were observed only in animals from 80- and 104-week studies, except retinal degeneration which was observed in animals from all age-groups. There are no previous reports of mineral deposits in the iris in this strain of mice. It is hoped that reference to the incidences reported here will facilitate the differentiation of spontaneous lesions from compound-induced lesions in toxicology studies in this strain of mouse.

Saarnak CF et al. ADVANZ: Establishing a Pan-African platform for neglected zoonotic disease control through a One Health approach.

Advocacy for neglected zoonotic diseases (ADVANZ) is a One Health Neglected Zoonotic Diseases (NZDs) project, funded by the European Commission through its 7th framework programme. The initiative aims at persuading decision makers and empowering stakeholders at local, regional, and international levels towards a coordinated fight against NZDs. ADVANZ is establishing an African platform to share experiences in the prevention and control of NZDs. The platform will compile and package existing knowledge or data on NZDs and generate evidence-based algorithms for improving surveillance and control with the ultimate aim of eliminating and eradicating these diseases. The platform will serve as a forum for African and international stakeholders, as well as existing One Health and NZD networks and harness and consolidate their efforts in the control and prevention of NZDs. The platform had its first meeting in Johannesburg, South Africa in March 2013.

The environment, the on-going global climate change and the ecology of animal species determine the localisation of habitats and the geographical distribution of the various species in nature. The aim of this study was to explore the effects of such changes on snail species not only of interest to naturalists but also of importance to human and animal health. The spatial distribution of freshwater snail intermediate hosts involved in the transmission of schistosomiasis, fascioliasis and paramphistomiasis (i.e. Bulinus globosus, Biomphalaria pfeifferi

No controlled studies have been conducted to determine the predilection muscles of Trichinella zimbabwensis larvae in Nile crocodiles (Crocodylus niloticus) or the influence of infection intensity on the distribution of the larvae in crocodiles. The distribution of larvae in muscles of naturally infected Nile crocodiles and experimentally infected caimans (Caiman crocodilus) and varans (Varanus exanthematicus) have been reported in literature. To determine the distribution patterns of T. zimbabwensis larvae and predilection muscles, 15 crocodiles were randomly divided into three cohorts of five animals each, representing high infection (642 larvae/kg of bodyweight average), medium infection (414 larvae/kg of bodyweight average) and low infection (134 larvae/kg of bodyweight average) cohorts. In the high infection cohort, high percentages of larvae were observed in the triceps muscles (26%) and hind limb muscles (13%). In the medium infection cohort, high percentages of larvae were found in the triceps muscles (50%), sternomastoid (18%) and hind limb muscles (13%). In the low infection cohort, larvae were mainly found in the intercostal muscles (36%), longissimus complex (27%), forelimb muscles (20%) and hind limb muscles (10%). Predilection muscles in the high and medium infection cohorts were similar to those reported in naturally infected crocodiles despite changes in infection intensity. The high infection cohort had significantly higher numbers of larvae in the sternomastoid, triceps, intercostal, longissimus complex, external tibial flexor, longissimus caudalis and caudal femoral muscles (p < 0.05) compared with the medium infection cohort. In comparison with the low infection cohort, the high infection cohort harboured significantly higher numbers of larvae in all muscles (p < 0.05) except for the tongue. The high infection cohort harboured significantly higher numbers of larvae (p < 0.05) in the sternomastoid, triceps, intercostal, longissimus complex, external tibial flexor, longissimus caudalis and caudal femoral muscles compared with naturally infected crocodiles. Results from this study show that, in Nile crocodiles, larvae of T. zimbabwensis appear first to invade predilection muscles closest to their release site in the small intestine before occupying those muscles situated further away. The recommendation for the use of masseter, pterygoid and intercostal muscles as sampling sites for the detection of T. zimbabwensis in crocodiles is in contrast to the results from this study, where the fore- and hind limb muscles had the highest number of larvae. This study also supports the use of biopsy sampling from the dorso-lateral regions of the tail for
surveillance purposes in both wild and commercial crocodile populations.

Bertrand L et al. Incidence of Spontaneous Central Nervous System Tumors in CD-1 Mice and Sprague-Dawley, Han-Wistar, and Wistar Rats Used in Carcinogenicity Studies. Toxicol Pathol. 2014 Feb 4. [Epub ahead of print]
The incidence and range of spontaneous central nervous system tumors were determined in control Charles River rodents (Sprague-Dawley, Han-Wistar, Wistar rats, and CD-1 mice) from regulatory carcinogenicity studies carried out over the period 2002 to 2013 and were compared with the previously published data. In both species, the brain was notably more affected than the spinal cord. Incidences were comparable overall between rat strains (2.33%, 2.54%, and 2.89% in Wistar, Sprague-Dawley, and Han-Wistar strains, respectively) and were low in CD-1 mice (0.42% in 104-week studies and 0.2% in 80-week studies). Predominant tumor types were granular cell tumors in Wistar and Han-Wistar rats and malignant astrocytoma in Sprague-Dawley rats. Male rats were more frequently affected than females, but no sex predilection was apparent in CD-1 mice. Occasional early-onset tumors were diagnosed in rats from study week 23 onward. It is hoped that these results will provide the pathologist and the toxicologist with an up-to-date database of background neoplastic findings in widely used rodent strains.

The non-antibiotic anti-inflammatory theory of antimicrobial growth promoters (AGP) predicts that alternatives can be selected by simple in vitro tests. In vitro, the known AGP oxytetracycline (OTC) and a Macleaya cordata extract (MCE) had an anti-inflammatory effect with a half-maximal inhibitory concentration of 88 and 132 mg/l, respectively. In vivo, chickens received three different concentrations of MCE in drinking-water, OTC in feed and a control. Body weight (BW), feed intake (FI) and gain:feed (G:F) ratio were determined on days 14, 21 and 35. On day 35, body composition was determined. Plasma α1-acid glycoprotein (α1-AG) concentration was measured on days 21 and 35, and the expression of several jejunal inflammatory genes was determined on day 35. OTC-fed chickens showed a significantly higher BW, FI and G:F ratio compared with the control group at all time points. MCE had a significant linear effect on BW on days 21 and 35, and the G:F ratio was improved only over the whole period, whereas FI was not different. Only MCE but not OTC decreased the percentage of abdominal fat. Plasma α1-AG concentration increased from day 21 to 35, with the values being lower in the treatment groups. Both OTC and MCE significantly reduced the jejunal mucosal expression of inducible NO synthase. For most parameters measured, there was a clear linear dose-response to treatment with MCE. In conclusion, the results are consistent with the anti-inflammatory theory of growth promotion in production animals.

Most commonly, salivary cortisol is used in pig stress assessment, alternative salivary biomarkers are scarcely studied. Here, salivary cortisol and two alternative salivary biomarkers, haptoglobin and chromogranin A were measured in a pig
stress study. Treatment pigs (n = 24) were exposed to mixing and feed deprivation, in two trials, and compared to untreated controls (n = 24). Haptoglobin differed for feed deprivation vs control. Other differences were only found within treatment. Treatment pigs had higher salivary cortisol concentrations on the mixing day (P < 0.05). Chromogranin A concentrations were increased on the day of refeeding (P < 0.05). Haptoglobin showed a similar pattern to chromogranin A. Overall correlations between the salivary biomarkers were positive. Cortisol and chromogranin A were moderately correlated (r = 0.49, P < 0.0001), correlations between other markers were weaker. The present results indicate that different types of stressors elicited different physiological stress responses in the pigs, and therefore including various salivary biomarkers in stress evaluation seems useful.


One of the major challenges in pig production is managing digestive health to maximize feed conversion and growth rates, but also to minimize treatment costs and to warrant public health. There is a great interest in the development of useful tools for intestinal health monitoring and the investigation of possible prophylactic/therapeutic intervention pathways. A great variety of in vivo and in vitro intestinal models of study have been developed in the recent years. The understanding of such a complex system as the intestinal system (IS), and the study of its physiology and pathology is not an easy task. Analysis of such a complex system requires the use of systems biology techniques, like proteomics. However, for a correct interpretation of results and to maximize analysis performance, a careful selection of the IS model of study and proteomic platform is required. The study of the IS system is especially important in the pig, a species whose farming requires a very careful management of husbandry procedures regarding feeding and nutrition. The incorrect management of the pig digestive system leads directly to economic losses related suboptimal growth and feed utilization and/or the appearance of intestinal infections, in particular diarrhea. Furthermore, this species is the most suitable experimental model for human IS studies. Proteomics has risen as one of the most promising approaches to study the pig IS. In this review, we describe the most useful models of IS research in porcine and the different proteomic platforms available. An overview of the recent findings in pig IS proteomics is also provided.


The present study investigates the potential use of the scrapie-protective Q211 S146 and K222 caprine PRNP alleles as targets for selective breeding in Greek goats. Genotyping data from a high number of healthy goats with special emphasis on bucks, revealed high frequencies of these alleles, while the estimated probabilities of disease occurrence in animals carrying these alleles were low, suggesting that they can be used for selection. Greek goats represent one of the largest populations in Europe. Thus, the considerations presented here are an example of the expected effect of such a scheme on scrapie occurrence and on stakeholders.


Fifty cases of Shih Tzu ocular vitreoretinopathy were selected from the database of the Comparative Ocular
Pathology Laboratory of Wisconsin. Cases with severe coexisting conditions (e.g., corneal disease, uveitis or endophthalmitis) were excluded. Microscopical changes were evaluated and immunohistochemistry was used to define spindle cells, gliosis and the presence of basement membranes in the vitreous. Expression of glial fibrillary acidic protein, vimentin and smooth muscle actin was also performed. The mean age of the 50 cases was 10.1 years (range 2.5-15 years). The most characteristic microscopical abnormalities (50/50 cases) were retinal detachment and extensive retinal tear. Additionally, extracellular, eosinophilic matrix material admixed with few spindle cells, and pre-iridal fibrovascular membrane, goniodysgenesis, secondary glaucoma, hypermature and subcapsular cataract were detected. The spindle cells within the collagen matrix were strongly labelled for expression of vimentin, with weaker expression of smooth muscle actin.


Alzheimer's disease (AD) represents a highly common form of dementia, but can be diagnosed in the earlier stages before dementia onset. Early diagnosis is crucial for successful therapeutic intervention. The introduction of new diagnostic biomarkers for AD is aimed at detecting underlying brain pathology. These biomarkers reflect structural or biochemical changes related to AD. Examination of cerebrospinal fluid has many drawbacks; therefore, the search for sensitive and specific blood markers is ongoing. Investigation is mainly focused on upstream processes, among which oxidative stress in the brain is of particular interest. Products of oxidative stress may diffuse into the blood and evaluating them can contribute to diagnosis of AD. However, results of blood oxidative stress markers are not consistent among various studies, as documented in this review. To find a specific biochemical marker for AD, we should concentrate on specific metabolic products formed in the brain. Specific fluorescent intermediates of brain lipid peroxidation may represent such candidates as the composition of brain phospholipids is unique. They are small lipophilic molecules and can diffuse into the blood stream, where they can then be detected. We propose that these fluorescent products are potential candidates for blood biomarkers of AD.


The effect of three-day fasting on cardiac ischemic tolerance was investigated in adult male Wistar rats. Anesthetized open-chest animals (pentobarbitone 60 mg/kg, i.p.) were subjected to 20-min left anterior descending coronary artery occlusion and 3-h reperfusion for infarct size determination. Ventricular arrhythmias were monitored during ischemia and at the beginning (3 min) of reperfusion. Myocardial concentrations of beta-hydroxybutyrate and acetoacetate were measured to assess mitochondrial redox state. Short-term fasting limited the infarct size (48.5+/−3.3 % of the area at risk) compared to controls (74.3+/−2.2 %) and reduced the total number of premature ventricular complexes (12.5+/−5.8) compared to controls (194.9+/−21.9) as well as the duration of ventricular tachycardia (0.6+/−0.4 s vs. 18.8+/−2.5 s) occurring at early reperfusion. Additionally, fasting increased the concentration of beta-hydroxybutyrate and beta-hydroxybutyrate/acetoacetate ratio (87.8+/−27.0) compared to controls (7.9+/−1.7), reflecting altered mitochondrial redox state. It is concluded that three-day fasting effectively protected rat hearts against major endpoints of acute I/R injury. Further studies are needed to find out
whether these beneficial effects can be linked to altered mitochondrial redox state resulting from increased ketogenesis.


Using a panel of genus-specific probes this was the first study identifying the involvement of Treponema spp in porcine ulcerative stomatitis. Three sows encountered with necrotic stomatitis, necrotising Mortellaro-like inflammation of interdigital skin and with necrosis of the vaginal mucosa revealed polytreponemal infections with Treponema species/phytotypes commonly associated with bovine digital dermatitis and human periodontal disease.

Some photo from the Internet

Hello! I’m Sydney. I moved from Zimbabwe to work at Charles River in Scotland. Scotland has been a really good move for me. And I’d recommend it to others. It’s a wonderful place to explore, experience and develop an exciting career.

Animal pathologist Sydney studied veterinary medicine in his native Zimbabwe then lived in the Netherlands before making the exciting move to Scotland to develop his career. He now works for Charles River and is thoroughly enjoying life in Edinburgh.
C. Primary papers

Pathology related to the physiological process of calf birth

E Gruys

During birth of a mammal a tremendous transition occurs. From a placental oxygenation of the blood, there is a sudden change to a gas exchange by respiration. This is a physiological process, which is associated with the first inhalation of air in the lungs, but also with contractions of the muscular wall of some blood vessels during their closure. Moreover, in these vessels pathological changes occur, such as luminal thrombosis and media necrosis. Final closure of those vessels is by clearance of necrotic tissue and debris, and subsequent scar formation. In particular after closure of the navel with the remnants of the umbilical arteries, and closure of the ductus arteriosus Botalli, lateron after birth during necropsy one can find the marks of it. Besides those ‘normal’ changes one can find in each neonate, developmental disorders in some cases may be encountered. Finally during the birth process, or soon after it, infections may have developed, that become associated with secondary death of the calf. After parturition, the calf needs to receive maternal immunity by ingestion of colostrum. This is particularly important against the commensal bacteria at the farm which, when the calf has no immunity against them, easily may induce bacteriaemia. Often the consequences from such infection can be encountered in the ‘back streets’ of the body with inflammatory changes they have induced and which may be interpreted as due to the infection which became the cause of death.

At birth of a mammal the originally developed placental oxygenation of the blood stops and the body has to switch over, suddenly, to a pulmonary gas exchange. This physiological transition phase is associated with a period of acidosis and then a start of the respiration of air to the lungs. In the past the old Greek people called it “pneuma” (in Hebrew language “ruach”), a term that Bible translators often have wrongly interpreted. In addition, the muscular walls of the rupturing umbilical arteries and of the ductus arteriosus Botalli contract during which the blood circulation switches from a placental one (Fig 1) to the system we know from the adult animal. Often it is forgotten, however, that associated with this physiological birth process consecutive pathological processes develop, such as luminal thrombosis and media necrosis with subsequent scar formation in some vessels. One has to look only for its own navel to get proof of those processes to having developed in the past.

When birth has just passed and the flow of venous blood back to the body stops due to rupture, cutting or iatrogenic closure of the umbilical cord, a life-threatening problem starts to develop. Within the neonate the amount of CO$_2$ is increasing and the concentration of O$_2$ is decreasing. This in turn induces perception (chemo-receptors) of the higher concentration of carbon dioxide with activation (nervous system) of the pulmonary respiration. In the past within the Department of Pathology in Utrecht within the group of Prof P Wensvoort those processes have been subject of research. In this paper for veterinary practice most important phenomena concerning the calf will be discussed.
End of the embryonal period
In the unborn calf the low-oxygen blood in the caudal part of the caudal cava vein, the cranial cava vein and in the hepatic portal vein is mixed with oxygen-rich blood from the umbilical vein. Herewith a reasonable oxygen-rich mixblood reaches the right heart that by the open foramen ovale, the non-working lungs and the ductus arteriosus Botalli fills the left heart and aorta respectively. By this system oxygen is added to the poorly oxygenated blood of the large circulation of the fetus. Finally by the umbilical arteries which branch off from the aorta by the *aa hypogastricae*, the blood reaches the placenta where exchange to oxygenated blood can occur (Fig. 1).

The intra-abdominal parts of the umbilical arteries are located in the connective tissue suspensory bands of the urinary bladder, and together with the *urachus* and umbilical vein they enter the body by the umbilical cord (Fig. 2).

**Oxygenation of the blood before birth**

The oxygen-rich blood enters the body by the umbilical vein and flows to the R part of the heart where some of it enters by the foramen ovale, the *D. a. Botalli* and the pulmonary circulation the L side of the heart and the aorta.

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**Fig. 1. Oxygenation of the blood in a fetus before birth.** This drawing is after a necropsied calf laying on its left side.

Oxygen-rich blood is stained red-coloured. The low-oxygenated blood in the caudal part of the *vena cava caudalis* and within the *vena cava cranialis*, just as in the *vena portae* (blue coloured), is mixing with the oxygen-rich umbilical vein blood. As a consequence in the right heart the blood is well-oxygenated. By the open foramen ovale, the lungs and the ductus arteriosus Botalli the oxygen reaches the blood in left heart and the aorta (red arrows within and directly above the heart). Herewith some oxygen is added to the low-oxygenated blood (purple coloured) of the large circulation bringing blood to everywhere in the body. Finally by the umbilical arteries, which are located along the urinary bladder, the blood is entering the placenta, where gas exchange occurs.
Fig. 2. Calf lying on its left side and viewed from the back. The intra-abdominal parts of the umbilical arteries are located along the urinary bladder. They have ruptured and are retracted intra-abdominally at birth, and now are exposed on necropsy by the hands in blue gloves. In between the thumbs of the person wearing the gloves who was lifting the arteries, the urachus is located.

Intra-abdominally at the craniodorsal aspect of the navel the umbilical vein enters the liver (Fig. 3). Within the liver it has a shortcut to the caudal cava vein, by the ductus venosus Arantii. This ductus venosus closes shortly before birth, thus at time of parturition the venous blood from the umbilicus on its way to the cava vein passes the smaller vessels of the left liver half. The umbilical vein closes after birth. Later on, remnants of it still are found as ligamentum teres at the ventral aspect of the liver within the thin ligamentum falciforme which connects the liver in the median line with the diaphragm.

Transition at birth
Immediately after birth the situation has dramatically changed. At parturition the umbilical arteries rupture, they contract and retract. The ends of the ruptured arteries often with a haemorrhage around them during necropsy can be encountered intra-abdominally being situated left and right of the urinary bladder (Fig. 4). The urinary bladder itself can be filled with urine. Probably that is in relationship to the time period the total birth process has taken. Within the uterus the calf urinates in the amniotic sac, while after birth more or less mentally controlled is urinated. During the period if time within the birth canal then the calf will not have been busy urinating.
The blood in the umbilical vein no longer is oxygen-rich. A metabolic acidosis is developing and due to recognition of that by chemoreceptors the respiration centre of the brain is activated and pulmonary inspiration is started. A just-born human baby gets a bluish colour, starts breathing and typically for the human species, loudly vocalises because the young person had frightening death. Then, the gas exchange normalises the concentrations of oxygen and carbon dioxide, and as a consequence the colour of the neonatus.

The foramen ovale in the heart is closed by an increasing amount of blood with some rising of the local blood pressure in the left atrium. In most cases quickly a sticking of the atrial septum secundum to the primary septum develops. In a number of cases the foramen may remain a little bit patent, what is not abnormal. During the period of immediate postpartum acidosis contraction of the musculature in the wall of the ductus arteriosus Botalli had been activated. Within the lumen then thrombosis occurs and in the wall due to lack of the intraluminal circulation, the inner part of the tunica media becomes necrotic. Permanent closure is by removal of the necrotic muscular tissue and the fibrin followed by cicatrisation. The processes happening in the navel are just like those in the arteries. At its outer aspect epithelialisation of the wound closes the skin.
Fig. 4. The end of the ruptured and retracted-contracted umbilical arteries can be found near the urinary bladder. The black colour of the arterial end is due to blood that left the vessel during its rupture and retraction. It is not an uncommon finding and easily can be explained, because the contraction-retraction in time follows the rupture. The filling of the urinary bladder may probably depend on the duration of the parturition, when the calf was not urinating during birth.

Abnormalities related to birth

When the calf is not born alive, but is dead (Fig. 5), often in fact it concerns a suffocated animal that not has had the ability to breath. Probably because it still was in the amniotic sac when the respiration centre became activated. During this phase of asphyxia defecation, and oesophageal swallowing and tracheal aspiration of amniotic fluid may result in the following series of findings:

1. On the outer aspect of the skin of the dead body meconium may be encountered.
2. In the abomasum a large mass of fluid with meconium particles can be found. In the wall some asphyctic haemorrhages (petechiae) may be encountered.
3. In the lungs grossly in the bronchi and microscopically in bronchioli and alveoli meconium particles and cornified material from skin can be found.
4. Due to heavy agonal movements during terminal asphyxia larger haemorrhages may occur in internal organs such as kidneys and liver. In the calf sometimes one may encounter large haemorrhages in the perirenal area (Fig. 6). After removal of muscles a vertebral fracture can be shown (Fig. 7). Whereas some German colleague once suggested a developmental bone lesion, such lesion on histological investigation in our Dutch calves never has been found. Thus, it has been suggested to consider the heavy agonal contractions during asphyxia, or trauma during birth (by falling down from a standing cow, or due to iatrogenic pulling).
Fig. 5. Two photos of intrathoracic lungs of calves, which have been positioned as mirror pictures from each other. A feature of fetal atelectasis in the lung at the left panel (dark colour) contrasts with an air-containing pale-coloured lung in the right panel. A fetally atelectatic lung in relationship to atelectasis lateron in life is voluminous, and that can be explained by intrapulmonary amniotic fluid.

Fig. 6. Haemorrhage in the perirenal area, on which location on deeper seeking in the tissues a vertebral fracture was encountered (Fig. 7).

Fig. 7. Vertebral fracture (same as Fig. 6).
Umbilical infection

A well-known clinical item is an omphalitis due to local penetration of bacteria during the parturition / postpartum period. This infection can occur during rupture of the umbilical cord, and can happen as long as the umbilical wound has not been closed. Most often infections of the umbilical vein are encountered, but also the arteries may become infected. During their retraction the infection can enter the anatomical region (Fig. 8).

Infection of the umbilical vein may lead to a mass of pus and/or thrombosis of the veins within the liver (Figs. 9 and 10). Because the ductus ateriosus Arantii is closed shortly before birth such a neonatal thrombosis process on healing may be associated with closure of the left branch of the portal vein. When the calf survives this hepatic venous closure, in the liver this may be followed by left hepatic atrophy. In slaughtered animals one may encounter liver lesions with overt atrophy of the left lobe, and that may be explained by such pathogenesis (Fig. 11).
Fig. 10. Thrombosis of the umbilical vein (opened with scissors). Note, that the umbilical vein enters the portal vein (in the centre of the photo running from right to left). When the ductus venosus Arantii is closed, infectious material in the umbilical vein may reach the left hepatic lobe by the left branch of the portal vein.

Fig. 11. Atrophy of the left hepatic lobe: a small tissue mass is left (black arrow). The ligamentum teres, which is the remnant of the umbilical vein, runs to the anatomical hepatic median area.

Congenital abnormalities that may interfere with the calf's health after transition to postnatal pulmonary gas exchange

As described above, within the heart the closure of the embryonic right-left blood flow, and of the ductus arteriosus, are necessary for a well-oxygenated arterial blood after birth. That is why on necropsy we start to control the septum in the heart and patency of the ductus Botalli (Fig. 12).

Normally the foramen ovale is closed or a small gap can be found. When the opening is larger and is located more ventrally (low atrium septum-defect; (Fig. 13) this finding may be explained by non-closure of the septum primum.

Defects in the ventricular septum often are seen after opening of the aortic trunk (Fig. 14). Gaps can be smaller of larger and be associated with lesions of the cardiac valves or the position of the large arteries.
(Remember the in animals very rarely occurring tetralogy of Fallot (ventricular septum defect, narrowing of the de a. pulmonalis, transposition – riding of the aorta and a hypertrophic wall of the right ventricle). The outer aspect of such hearts, also without Fallot, is more round than expected.

Fig. 12. In the ductus arteriosus an anatomical wire has been put for controlling its patency (from below to the upper part of the photo). The ductus has been lifted by a wooden teeth cleaner (from left to right).

Fig. 13. Atrium septum-defect. Sometimes defects of the ventricular septum concern large holes in the septum primum, what develops earlier than the second septum which finally should have closed the foramen ovale.
**Errors of the vessels**

On controlling the heart one looks for the large vessels, the aorta and the pulmonary trunk. The offspring of the coronary arteries should be located directly after the aortic semilunar valves. On transposition of the great arterial vessels both coronary arteries start from the wrong vessel. It also is possible that one of the coronary arteries originates from the pulmonary artery. The control of the origin of the coronary arteries best can be done from the inner aspect of the aorta. (Fig.15).

When the left coronary artery branches off from the pulmonary artery, that lesion is easily missed during necropsy without its proper control. The calf had died soon after its birth due to lack of oxygen in the left cardiac muscle.

When the right coronary artery has its origin from the pulmonary artery, the animal can survive by adaptation of the wall of the right heart. It is a very rare finding whereby an enlarged heart with overt right-sided widened torturing arteries can be encountered (Fig. 16).

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**Fig. 14. Ventricular septum-defect.**

This specimen contains a small hole, a high ventricular septum defect immediately below the aortic valves. In addition a fibrous thickening of the ventricular intima can be encountered.

**Fig. 15. Opening of the entrance of the aorta from the left ventricular side offers best view on a septum defect.** And it gives best possibilities to control the origin of the coronary arteries (in the yellow scheme directly behind the green semilunar valves). (Note that the entrance of right coronary artery is located outside of the present photo (left arrow pointing to the white surrounding rim)).
Fig. 16. Drawing of the outer aspect of three hearts. In the middle panel is the normal situation: the coronary arteries are masked by adipose tissue in the coronary groove. In the heart at the right panel the left coronary artery branches from the pulmonary artery. The calf has died due to immediate cardiac failure because it developed asphyxia of the left ventricular wall. In the left panel there is a right coronary artery branching from the arteria pulmonalis. The right heart develops adaptation with widening and torturing of its coronary artery branches.

Kachexia
Normally on the outer aspect of the heart the coronary arteries are hardly visible due to supporting adipose tissue within the coronary grooves. It always is recommendable to look for that fat tissue. When the colour is dark and/or edematous, these things may be interpreted as an indication of usage of the lipid reserves in that tissue (Fig. 17).

Fig. 17. The coronary fat is edematous and hyperaemic-brownish coloured. This gives an indication for usage of the lipids stored and a diagnosis of cachexia.
**Back streets of the body**

In some anatomical structures of the body there are not many blood vessels, the $O_2$ concentration is marginal and the amount of carbon dioxide is becoming high. Some people call them the ‘back streets’ of the body. It concerns in particular the joints, meninges, and the eye. A problem of less blood circulation is that, when bacteria have colonised them, it becomes more difficult to get rid of them because leukocytes need oxygen for functions such as phagocytosis and killing (respiratory burst).

In the calf a neonatal infection with non-pathogenic *Escherichia coli* strains against which the first mother’s milk (colostrum) supplies maternal immunity often is the current problem. The germs apparently had passed the enteric barrier before ingestion of the colostrum. This may be caused by infection in the vaginal canal of the mother, by the fingers of the farmer, or on lack of colostrums, or a too late gift of it to the calf. Some colleagues mention it as consequence of an umbilical infection.

In view of this problem frequently found in a dead calf, we control the frontal chamber of the eyes for hypopyon, the joints for fibrin indicating arthritis, and when needed to open the skull for meningitis. Routinely the joints of hip, carpus, tarsus and stifle always are opened. The cartilage should be shiny and whitish coloured. The synovial fluid has a small volume and is viscous. The capsule of the joint is thin (Fig. 18). Sometimes there is more blood than expected or even haemarthros may be found. Then, one may consider cold weather and trauma respectively.

After infection with bacteriaemia there often is a lot of fibrin and/or pus in the joint cavity (Figs. 19 and 20). When there is positively polyarthritis, in most cases hypopyon of the eyes is positive as well, and one might expect a fibrino-purulent meningitis (Fig. 21). Grossly the features of the brain surface are not clearly changed in most occasions, because the brain tissue itself is pale coulored already and because in a dead calf the brain can be hyperaemic (hypoxia?). A picture of a meningitis as given in Fig. 21, is a rare necropsy finding, but it is a reason to like to control the meninges in animals with polyarthritis and signs of bacteriaemia.

![Fig.18 Normal joint (stifle) with shiny hyalin cartilage, a very little amount but viscous synovial fluid, and a thin capsule.](image-url)
Fig. 19. The joint cavity (tarsus) is overfilled with fibrin.

Fig. 20. In this tarsal joint there is an evident increase of fluid. Moreover, this synovial fluid is not clear but turbid: pus.
Fig. 21. Features of a fibrino-purulent meningitis. There is hyperaemia and in the sulci a pale material has accumulated.

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The author likes to thank drs Rytis Cepulis, Sanita Straume and Boguslaw Zakrzewski for supplying him with photos made during recent MSD-Intervet-workshops in the Baltic countries and Poland.

Osteoarthritis

Erik Gruys

Chronic degeneration of articular cartilage in osteoarthritis (OA), continental pathologists call arthrosis, is a frequent lesion in dogs and cats, horses and human patients. The joint lesion does not have a good prognosis regarding complete recovery, whereas a fibrous repair layer on the location of the lost hyaline cartilage and muscular strengthening exercise have positive effects. Various systems for anatomical repair are in a developmental phase, but have not led to a significantly good clinical method yet other than complete removal and replacement by a prosthesis in humans.

People perform trials with cartilage transplantation, implantation of stem cells, application of thrombocytes, etc. Many colleagues promote such methods, but convincing scientific evidence of a positive effect often is lacking. Also various oral supplements are promoted. Literature is quite positive, but again, evidence based medicine in most instances is not supporting their application. Proof of a positive effect often is difficult to deduct from the papers. Because patients suffer from a chronic change of the anatomical structure one is trying everything that comes into scope. When it has no success, no problem, as long as it gives no harm. Of the oral supplements, unsaturated fatty acids (fish oil), vitamin E and NSAIDs probably give most
positive results due to mitigation of secondary inflammatory changes. In a state of steady pain, it helps to interrupt the pain cycle. Inhibition of the inflammation as cause of the pain includes best chance for clinical result.

Chronic degeneration of the articular hyaline cartilage is a common finding in dogs, cats, horses and human patients. The term arthrosis indicates primary degenerative lesions, which as wear and tear lesions often are seen at older age. In young growing animals cartilage damage occurs in osteochondrosis due to a developmental error occurring during growth of the cartilage pre-stage of their bones (dyschondroplasia), what is seen in the growth plates and the articular cartilage of the skeleton. In the adult animals and at old age the osteoarthritis will manifest itself. Overload of the joint structures due to body weight overload in addition to less movement and muscular strength are major causative factors. The cartilage damage often is followed in time by a reactive inflammatory process and start of reparative processes that seldom result is a wished outcome. The pathology in the joint in osteochondrosis and osteoarthritis is comparable: the joint cartilage gets damaged. Some reparative cartilage growth may occur, free parts may float around in the joint cavity (joint mice), periarticular osteophytes may be formed and a secondary inflammation (associated with pain) can start.

In this paper the anatomy of the joint cartilage will be reviewed, possibilities for pathological changes described, the cartilage damage will be outlined and causes for its damage will be discussed. Experiments performed in animals to induce comparable lesions in humans will be mentioned and various therapeutical experiments reviewed, followed by a comment on oral options. Finally some conclusions are drawn.

**Anatomy of the articular cartilage**

The articular cartilage (Fig. 1) is a hyaline variant that contains diffusely spread chondrocytes, which are surrounded by cartilage matrix (Figs. 2 and 3). This intercellular material contains type-II collagen fibres, proteogycans and hyaluronic acid (Fig. 4). In between these molecules, which are connected to each other primarily by electrostatic bonds and in which the proteoglycans with hyaluronic acid (glycosaminoglycans; GAGs) form the majority, a mass of water is trapped. This structure all together gives the cartilage a function of an elastic cussion. The cartilage tissue is highly specialised, is metabolic slow, it does not contain a lot of cells per gram tissue and contains a high amount of matrix components. In adult animals the tissue is not vascularised resulting in nutrition and oxygen to reach the tissue by a long way of diffusion from the capsule. The tension of O2 is low and of CO2 high. Using the joint stimulates the exchange of those molecules by diffusion and thus the integrity of the tissue. This encloses a gliding scale, within limits of the anatomical borders, for articular physiology – pathophysiology that is related to the amount of its regular usage.
Because the skeleton cannot move without its musculature, the situation in the joint depends on muscular activity and parallels it. Moreover a stronger musculature ensures better mechanical dimming of load forces reducing the chance for traumatic lesions. And, just this is in favour of some movement activity such as an hour walking per day.

**Possibilities for pathological changes**

Starting from a normal joint one can discern various types of cartilage damage. They start with loss of proteoglycans giving the tissue a more fibrous histological appearance and less volume (grade 1). Often this feature is seen in normal ageing (11). Then, small defects with loss of tissue can be expected (grade 2), followed by larger gaps in the articular cartilage (grade 3). A fourth option (grade 4) is large gaps with reaction of the subchondral bone with an increase of its density. A schematic view on those lesions is given in Fig. 5.

Such a scale often is used clinically to describe lesions encountered and visualised by X-ray, whereas MRI has much potencies to visualise the loss of tissue (7).

Of course, the scales of lesions may increase to more severe as shown in Fig. 6. Veterinary examples of such joint pathologies are known in septic arthritis of young animals such as lambs and calves with an E.coli-infection, piglets with streptococcosis and foals with salmonella involvement (Fig. 6).

In young dogs of the large breeds with dyschondroplasia (osteochondrosis) examples of *osteochondrosis dissecans* in the shoulder joint may result in large defects as well (Fig. 7). Again clinically MRI is the technique of choice when available.

In older dogs with hip dysplasia (Fig. 8) or osteoarthritis (Fig. 9) good examples of chronic lesions of the hip joint or femur condyles can be encountered. In both examples not only the cartilage is damaged but also the subcondral bone, and the capsule is thickened.

One may ask which characteristics in those joint lesions can be encountered? Those are: 1. loss of cartilage; 2. formation of a layer of reparative fibrous cartilage upon the denuded subchondral bone; and 3. thickening of the joint capsule with formation of bone spicules around the original joint (acetabulum / stifle joints).
Figs. 3 (H&E) and 4 (Ruthenium red EM). On larger magnification (3) or electron microscopy (4) under a superficial fibrillary layer (F) a tissue is encountered with a large amount of intercellular substance (matrix) and dispersed chondrocytes. The matrix consists of type-II collagen fibres and GAGs which by the ruthenium red staining are visualized as stars and clumps inbetween and upon the collagen fibres. From chemical literature it is known that the intercollagenous substance consists of a backbone of hyaluronic acid on which by connecting protein proteoglycans join. The proteoglycans bind in turn chondroitin sulphate representing the major GAG in cartilage.

**Cartilage damage**

On damage is loss of GAGs easily develops, the morphology is becoming ruffled, and then larger defects of the tissue may occur. From damaged cells pro-inflammatory cytokines are released and there is an influx of inflammatory cells from the blood. Thrombocytes may release growth factors, such as TGF-β. Leukocytes (PMNs, monocytes) let increase the mass of cytokines released (such as IL-1, IL-6 and TNF-α). In addition lysosomal hydrolases are released and matrix metalloproteinases (MMPs) are activated (with MMP-9 and -13 for catabolism and MMP-2, -3 and 14 for remodelling). After some time the cartilage tissue may react by the formation of regenerative brood capsules and formation of new tissue along its borders.

Possibilities to explain the cartilage damage are:

- Age (too old); in rats within a couple of months clear changes are shown (11).
- Too less or too much usage of the joint; non-using a leg reduces oxygen supply and nutrition of the tissues due to reduced diffusion what is activated by movements. Too much usage, on the contrary, can result in damage due to trauma.
- Trauma. On blunt trauma often also the underlying tissues are involved. In sports horses subchondral bone fractures are known which may become fatal for the animal (10). It is evident that during sports the borders of a for-buoyancy build anatomy may become over rised.
- Overweight (too heavy); this often results in a combination of too less using the legs with an increased chance for traumatic damage.
Various authors have performed model studies and in particular on the stifle joint of the dog as model for the human knee (9). Studies that have to be mentioned here are: @a. binding up of a leg (Brandt et al.; 12); @b. iatrogenic joint damage by scarification of joint cartilage, osteotomy, meniscus damage and in particular cutting the frontal cross band (9). Moreover @c. as animal models for human disease, studies regarding spontaneous osteoarthritis in dogs have been performed (9).

Ad a. Binding-up a leg. When dogs were left for 6 days in a cage with one hind leg bound up a loss of 41% of proteoglycans was found. After a longer period of time adaptation occurred and cartilage changes could not be shown (12). During a lecture in the early eighties, however, Brandt told that after one day rest with a hind leg bound up followed by running after release of the stifle joint, it resulted in a significant loss of cartilage matrix. The combination of total rest followed by loading should have a bad outcome.

Ad b. Cross band cutting; this surgical lesion resulted in cartilage lesions of the medial femur condyl and the medial meniscus (3, 4, 5, 9, 14). In recent investigations (9) they found that MRI features and gross measurements of the cartilage of the medial femur condyl after 8 weeks significantly appeared to be reduced. In addition the peak vertical force (PVF) measured during kinetic movement analysis using a pressure plate showed a worse situation (pain-related) that later on went up again, but never reached the original level.

Ad c. Spontaneous OA in dogs; PVF studies in association with recording of movement analysis revealed that more walking was associated with a significant better PVF (P=0.001). Also the body condition ameliorated at walking more than an hour a day, which finding was in accordance with similar findings in human patients with OA.

**Treatment of osteoarthritis**
For therapy many different things have been tried: 1. surgical cleaning and smoothening of damaged cartilage; 2. repair of cartilage using implants, stem cells, growth factors (ao from own thrombocytes); 3. protheses (in particular human); 4. exercise and strengthening of musculature; and 5. addition of substances.
Fig. 6. Septic arthritis in a foal with salmonellosis. A total destruction of the femoral head cartilage has developed (right panel, with left as normal control).

Fig. 7. Osteochondrosis dissecans of the proximal humerus of both front legs of a large breed dog.

Fig 8. Hip dysplasia (dog). The cartilage of the femoral head is largely lost and replaced by a fibrous layer. The joint capsule is thickened. The acetabulum has widened and along its border new bone formation has developed.
Ad 1. Surgical cleansing. Herein the surgeon removes irregularities, joint mice and osteophytes with the aim of a final repair from the remaining cartilage. Often it results in growth of a layer of fibrous cartilage with which the animal may proceed in particular when the muscles have enough strength. In an old-fashioned hip operation formerly used for humans and still in use for dogs, the head of the femur with the acetabulum is simply removed trying to induce formation of a nearthros. The patients might reach a stage of acceptable pain but were not very mobile.

Ad 2. Repair of damaged cartilage. Implants of cartilage tissue have limited success. Better should be stem cells when given together with a matrix and growth factors. De Schauwer et al (6) reviewed instillation of mesenchymal stem cells in the horse. The results mentioned vary and when one critically looks for the described outcome in comparison to controls, it was less positive than the authors interpreted it. One upon three cases had no positive result at all.

Ahmed and Hincke (1) described an overview of all types of stem cells and techniques used so far. Best results were with bone marrow cells in a matrix such as fibrin, collagen or agarose. Remarkable was that many combinations are described by different authors, but no standard system had been reached. It is doubtful whether good results are repeatable. For clinical application they said it is too early. Remarkable is the fact that instilled stem cells search their own way and often later are found on other locations than they were instilled. Some authors try to activate the own regenerative capabilities of the cartilage tissue using growth factors from the alpha granules of thrombocytes (Table 1). Platelet-rich plasma (PRP) fractions are prepared and used (13). The outcome is doubtful, however, the thrombocytes contain more granules and components. The authors concluded (13), that there are good prospects for the future concerning application of matrix, cells and PRP combined or separate. At present methods used are too varying and scientifically weak. It is difficult to make a good choice for clinical use. The risk exists that without thorough knowledge some steps described are applied to patients before solid scientific results give fundaments to those treatments.
Table 1. Granular organelles with their major active components in thrombocytes.

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<th>Type of organelle</th>
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<tr>
<td>Alpha granule</td>
<td>cytokines, chemokines and proteins with functions in chemotaxis, cell proliferation and differentiation, and inflammation</td>
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<tr>
<td>Dense granule</td>
<td>ADP, ATP, Ca2+, histamin, serotonin and dopamine, substances that modulate homeostasis and tissue regeneration</td>
</tr>
<tr>
<td>Lysosomal granule</td>
<td>hydrolases, cathepsion D and E, elastases and lysozyme</td>
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From such statements it becomes clear to the reader that all clinical activities trying to repair damaged cartilage in fact represent experiments. Most are applied based on the statement that when it does not help, the damage would not become much larger. And then, one has to take care not to cheat yourself and the customers. Also clinicians have to remain critical on their own handling.

Ad 3. Prostheses. In particular in human patients prostheses are used with a reasonable success. In dogs as well total joint replacement techniques can be performed with methods based on human knowledge. Probably, however, outer joint consolidations (such as mentioned on www.animaloandp.com) commercially are more attractive.

Ad 4. Muscular strengthening and exercise. In a review paper Musumeci et al (11) mention that in man activities related to physical activity aimed to strengthen the muscles of the leg are in favour of prevention, treatment and rehabilitation of knee joint osteoarthritis. All data show that exercise is an effective and payable method for treatment and prevention, and are in line with the comparable above mentioned dog stifle joint results.

Ad 5. Addition of substances. Many different molecules, often based on components of normal cartilage tissue, have been tested in experimental settings. The molecules were injected intra-articularly or given orally. It concerns substances such as glucosamin, hyaluronic acid, undenatured type-II collagen, gelatin hydrolysate, and combinations thereof. Despite many positive results published and reviewed (fi by Gruys (8) and Beynen (2)), it remains difficult to get hard scientific proof of the effects wished. And a comment of the present author then yet is: kook soup of animal bones and/or fish bones and let the carnivores chew on a good bone with joint cartilage attached to it. That minimally is in favour of clean teeth.

Finally there is much attention to the oral application of fish oil, unsaturated fatty acids (omega-3) and high doses of vitamin-E. Due to their activity within the inflammatory cascade is will be expectable these materials to have a positive effect. Also NSAIDs by suppressing formation of prostaglandins and thus pain will result in a better clinical state. By interruption of pain sensation (suppression of “hypertrophy” of the receptor-effector neuron cycle) already much has been done in favour of a better clinical situation.
Conclusions

Chronic osteoarthritis does not have a good prognosis regarding repair to the original state. Formation of a fibrous layer in association with muscular strengthening, however, may enclose a more or less acceptable future for the clinical outcome. Various therapeutical methodologies, such as instillation of thrombocytes, stem cells, collagen type-II, etc aimed for cartilage repair, by many people are cheered, but strong scientific proof often still is lacking (evidence-based medicine). Most wise is to wait until the variety of experimental methodologies has crystallised to an acceptable system for practical application.

Prostheses for total joint replacement used with success in many human patients can be applied in animals, but appear too expensive for most companion animals. Possibly outer consolidation devices are more attractive. A series of oral supplements is on the market. In case they don’t help, they appear not to be harmful. Most effect is expected from unsaturated fatty acids and vitamin-E. On a state of sustained pain inhibition of the neurological pain loop by using NSAIDs is expected to give most chances for result, in particular when on relief of the pain muscular strengthening by exercise is favoured.

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### D. Primary paper titles from former AWAAP issues (can be obtained after request)

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<td>9-18. Subjects of theses for Master of Science in Animal Pathology defended at the Faculty of Veterinary Medicine, Utrecht University, The Netherlands (1998-2003).</td>
<td>E Gruys</td>
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<tr>
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<tr>
<td>Fibrillary protein pathology.</td>
<td>E Gruys</td>
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<td>TKA Nguyen, TD Vo, A Bekele Tilahun, T Negash Alkie</td>
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<td>Stroma and extracellular matrix proteins in canine tumours.</td>
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<td>Endproducts of lipid peroxidation in various pathological states.</td>
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<td>Pathology of swine.</td>
<td>E Gruys and G Strijkstra</td>
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<td>Effect of Newcastle disease virus on the spleen of experimental chickens.</td>
<td>SO Al-Garib et al</td>
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<td>Monitoring health by acute phase proteins.</td>
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<td>Coexistence of PCV2 and PRRSV infection in postweaning multisystemic wasting syndrome (PMWS).</td>
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<td>Effect of an anti-oxidant enriched diet on deposition of oxidative damage products and amyloid in brain tissue of old dogs</td>
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<td>Lameness scoring in a flock of broilers.</td>
<td>AA Adewuyi et al</td>
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<td>Detection of porcine reproductive and respiratory syndrome virus (PRRSV) genotypes in tissue sections.</td>
<td>H Kothalawaha et al</td>
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<td>Efficacy and characteristics of different methods of coccidiosis infection in broiler chickens</td>
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