

**Villaviciosa, 2018**

Bibliografía científica del **SERIDA** (Servicio Regional de Investigación y Desarrollo Agroalimentario) en **RIA**, Repositorio Institucional de Asturias

Anexo : año 2018

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## INTRODUCCIÓN

La investigación científica y la publicación del artículo científico son dos actividades íntimamente relacionadas. La publicación de los resultados alcanzados en las investigaciones es el último paso en el ciclo de la investigación científica.

Junto al deber moral de los investigadores de comunicar sus investigaciones, se asienta el reconocimiento por parte de los pares, y la promoción académica en las instituciones de investigación, ya que además de plasmar la investigación en un documento permanente, es imprescindible difundirla.

El anexo del año 2018 contiene la producción científica publicada en artículos de revistas incluidas en las bases de datos Thomson-ISI (SCI) por el personal investigador y colaborador del SERIDA durante el año 2018.

Esta nueva actualización se compone de 40 artículos y como en estudios anteriores, la recopilación se ha organizado y redactado en el estilo bibliográfico Harvard que asocia autor y año por orden alfabético.

Cada referencia, se ha completado con el resumen o abstract que resume el contenido del artículo a excepción de algún poster que constituía la cita bibliográfica, resultando un listado de referencias bibliográficas y descriptivas que completan la bibliografía científica del SERIDA publicada ya en RIA en años anteriores, la bibliografía hasta 2015 y los anexos correspondientes a los años 2016 y 2017, respectivamente.

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## BIBLIOGRAFÍA CIENTÍFICA. ANEXO 2018

ALVAREZ, I., et al. (2018) Usefulness of running animal models in absence of pedigrees: Estimation of genetic parameters for gastrointestinal parasite resistance traits in Djallonké sheep of Burkina Faso. *Small Ruminant Research*, **160**, 81-88.

**Abstract:** The main aim of the current study was to exemplify the usefulness of pedigree-free animal models in scenarios in which available information is limited. Up to 271 Djallonké sheep individuals of Burkina Faso were typed for 29 microsatellites. Performance records were obtained from a trial designed to assess environmental factors affecting gastrointestinal parasite resistance. Packed cell volume (PCV), log-transformed fecal egg count (lnFEC) and FAMACHA eye scores were analyzed fitting univariate and multivariate animal models. Molecular information was used to: a) construct an artificial pedigree maximizing the correlation between the molecular coancestry matrix and the coancestry of the artificial genealogies (P\* Matrix); and b) computing the Ritland's estimator of between-individuals molecular coancestry (R Matrix). All animal models fitted included as fixed effects the contemporary group, day of assessment after deworming and body weight as a linear covariate. As random effects, models included the additive genetic effect and the permanent environment associated to individual. Multivariate models further included the covariances between permanent environmental and additive genetic effects. Univariate models had difficulties to separate between additive genetic and permanent environmental effects. The P\* Matrix gave higher estimates of additive genetic variance than the R Matrix. Independently of the Matrix used, multivariate models gave more consistent estimates of heritability ( $h^2$ ) and permanent environmental effect: estimates of  $h^2$  for lnFEC varied from  $0.063 \pm 0.037$  to  $0.173 \pm 0.076$ ; estimates of  $h^2$  for FAMACHA scores ranged from  $0.206 \pm 0.070$  to  $0.343 \pm 0.111$ ; and c) estimates of  $h^2$  for PCV ranged from  $0.073 \pm 0.045$  to  $0.142 \pm 0.084$ . Genetic correlations estimated for the pair lnFEC-FAMACHA using multivariate models were significant in all cases varying from  $0.548 \pm 0.247$  to  $0.785 \pm 0.242$ . Pedigree-free animal models may be advantageous in developing countries at the beginning of selection programmes or to improve the outcome of trials designed to assess performance of local breeds for economically interesting traits.

ARRAZURIA, R., et al. (2018) Diet induced changes in the microbiota and cell composition of rabbit gut associated lymphoid tissue (GALT). *Scientific Reports*, **8**.

**Abstract:** The gut associated lymphoid tissue (GALT) is the largest immune organ of the body. Although the gut transient and mucosa-associated microbiota have been largely studied, the microbiota that colonizes the GALT has received less attention. The gut microbiome plays an important role in competitive exclusion of pathogens and in development and maturation of immunity. Diet is a key factor affecting the microbiota composition in the digestive tract. To investigate the relation between diet, microbiota and GALT, microbial and cell composition of vermiform appendix (VA) and sacculus rotundus (SR) were studied in two groups of New Zealand white rabbits on different diets. Diet shifted the lymphoid tissue microbiota affecting the presence and/or absence of certain taxa and their abundances. Immunohistochemistry revealed that a higher fibre content diet resulted in M cell hyperplasia and an increase of recently recruited macrophages, whereas T-cell levels remained unaltered in animals on both high fibre and standard diets. These findings indicate that diet has an impact on the microbiota and cell composition of the GALT, which could act as an important microbial recognition site where interactions with beneficial bacteria can take place favouring microbiota replacement after digestive dysregulations.

ARRIETA-VILLEGAS, C., et al. (2018) Efficacy of parenteral vaccination against tuberculosis with heat-inactivated *Mycobacterium bovis* in experimentally challenged goats. *Plos One*, **13** (5).

**Abstract:** Tuberculosis (TB) in animals is a re-emerging disease with a wide range of hosts that causes large economic losses in livestock. Goats are particularly susceptible to TB and, in endemic areas, vaccination may be a valuable measure to control the disease. The main aim of this study was to evaluate the efficacy of parenteral vaccination of goats with a heat-inactivated *Mycobacterium bovis* (HIMB) vaccine, and compare it to *M. bovis* Bacille Calmette-Guérin (BCG) vaccine. Twenty-four goat kids were divided in 3 groups as following: HIMB vaccinated group (n = 8), BCG vaccinated group (n = 8) and unvaccinated group (n = 8). Afterwards, goats were experimentally challenged with *Mycobacterium caprae* by the endobronchial route. Antigen specific interferon- $\gamma$  release assays and serology were performed after vaccination and challenge. Pathological and bacteriological parameters were evaluated after necropsy at 9 weeks post-challenge (p.c.) HIMB vaccine showed similar levels of

protection to BCG in terms of volume reduction of thoracic TB lesions, presence of extra-pulmonary lesions, as well as a slight reduction of bacterial load in pulmonary lymph nodes. Moreover, HIMB vaccine did not induce interferences on the interferon- $\gamma$  release assay based on reagents previously developed to differentiate infected from BCG vaccinated individuals. The results indicate that HIMB is a suitable vaccine candidate for further larger-scale trials under field conditions in goats

BERRUETA, L. A., et al. (2018) Relationship between hydroxycinnamic acids and the resistance of apple cultivars to rosy apple aphid. *Talanta*, **187**, 330-336.

**Abstract:** The phenolic profiles of apple cultivars from the SERIDA Asturian cider apple breeding program, including parents and progenies, were determined by ultrahigh-performance liquid chromatography-diode array detector-electrospray ionization-quadrupole time of flight/mass spectrometer in order to study the relationship between [phenols](#) and the resistance of apple tree cultivars to rosy apple aphid (RAA). A [pattern recognition](#) technique named partial least square discriminant analysis (PLS-DA) was used to classify apple cultivars based on resistance to RAA, resistant and susceptible, reaching scores with accuracy higher than 97% and 91% respectively. [Hydroxycinnamic acids](#), particularly 4-caffeoylquinic acid (4-CQA) and 4-*p*-coumaroylquinic acid (4-pCoQA), were identified as the major player in RAA resistance by the PLS-DA model. Indeed, the [isomerisation](#) 5-CQA  $\rightarrow$  4-CQA is favoured in resistant cultivars, whereas the isomerisation 5-pCoQA  $\rightarrow$  4-pCoQA is favoured in susceptible cultivars. As a result, resistant cultivars accumulate higher amounts of 4-CQA than susceptible ones, and the opposite occurs for 4-pCoQA. Also, minor isomerisations of 5-CQA to 1-CQA or 3-CQA show opposite behaviour for resistant and susceptible cultivars. Cultivar resistance to RAA is concluded to be related with the [phenylpropanoid](#) pathway, the isomerisation reactions being the key [metabolic reaction](#) for a cultivar to be resistant or susceptible to RAA.

CAMPA, A. and JOSE FERREIRA, J. (2018) Genetic diversity assessed by genotyping by sequencing (GBS) and for phenological traits in blueberry cultivars. *Plos One*, **13** (10).

**Abstract:** Blueberry is a small fruit crop which includes a complex group of different *Vaccinium* species of various ploidy levels. Commercial blueberries have been grown in Europe most recently, so there is not much information available about their adaptation into new regions. In this work we investigated adaptation to the environmental conditions of northern Spain, in terms of flowering and ripening seasons, of a set of 70 blueberry cultivars including several of the most important cultivated American species (*V. corymbosum*, *V. virgatum*, *V. macrocarpon* and *V. uliginosum*) in order to identify which types are best-suited in this geographical area of Europe. Most materials showed high chilling requirements for flowering under local conditions, while materials with low-chilling requirements showed problems in the maturation process of the flowers. Most cultivars were early or mid-season while a relative lack of late-season cultivars was observed. GBS was used for the analysis of genetic diversity in this sample of 70 cultivars. A total of 5255 SNP markers were obtained and a cluster analysis revealed three main groups associated with the ploidy level of the species. A Principal Component Analysis revealed a grouping of the *V. corymbosum* cultivars according to their chilling requirements. A total of 29 SNPs were identified as being highly informative for diversity analysis and potentially useful for cultivar identification and for breeding purposes. The results obtained from this research should contribute to the expansion of this crop, as well as providing data about genetic diversity useful for the preservation of genetic resources or for future breeding programs.

CAMPA, A., et al. (2018) Genetic Diversity, Population Structure, and Linkage Disequilibrium in a Spanish Common Bean Diversity Panel Revealed through Genotyping-by-Sequencing. *Genes*, **9** (11)

**Abstract:** A common bean (*Phaseolus vulgaris*) diversity panel of 308 lines was established from local Spanish germplasm, as well as old and elite cultivars mainly used for snap consumption. Most of the landraces included derived from the Spanish common bean core collection, so this panel can be considered to be representative of the Spanish diversity for this species. The panel was characterized by 3099 single-nucleotide polymorphism markers obtained through genotyping-by-sequencing, which revealed a wide genetic diversity and a low level of redundant material within the panel. Structure, cluster, and principal component analyses revealed the presence of two main subpopulations corresponding to the two main gene pools identified in common bean, the Andean and Mesoamerican pools, although most lines (70%) were associated with the Andean gene pool. Lines showing recombination between the two gene pools were also observed, most of them showing useful for snap bean consumption, which suggests that both gene pools were probably used in the breeding of snap bean cultivars. The usefulness of this panel for genome-wide association studies was tested by conducting association mapping for determinacy. Significant marker-trait associations were found on chromosome Pv01, involving the gene Phvul.001G189200, which was identified as a candidate gene for determinacy in the common bean.

DALTON, K. P., et al. (2018a) Clinical course and pathogenicity of variant rabbit haemorrhagic disease virus in experimentally infected adult and kit rabbits: Significance towards control and spread. *Veterinary Microbiology*, **220**, 24-32.

**Abstract:** RHDVb has become the dominant RHDV on the Iberian Peninsula. A better understanding of its pathogenicity is required to aid control measures. Thus, the clinical course, humoral immune response, viraemia and kinetics of RHDV-N11 (a Spanish RHDVb isolate) infection in different tissues at both viral RNA and protein levels were studied in experimentally infected young and adult rabbits. The case fatality rate differed between the two age groups, with 21% of kits succumbing while no deaths were observed in adults. Fever and viremia were strongly associated with death, which occurred 48 h post infection (PI) too fast for an effective humoral immune response to be mounted. A significant effect on the number of viral RNA copies with regard to the variables age, tissue and time PI ( $p < 0.0001$  in all cases) was detected. Histological lesions in infected rabbits were consistently more frequent and severe in liver and spleen and additionally intestine in kits, these tissues containing the highest levels of viral RNA and protein. Although no adults showed lesions or virus antigen in intestine, both kits and adults maintained steady viral RNA levels from days 1 to 7 PI in this organ. Analysis revealed the fecal route as the main dissemination route of RHDV-N11. Subclinically infected rabbits had detectable viral RNA in their faeces for up to seven days and thus may play an important role spreading the virus. This study allows a better understanding of the transmission of this virus and improvement of the control strategies for this disease.

DALTON, K. P., et al. (2018b) ELISA for detection of variant rabbit haemorrhagic disease virus RHDV2 antigen in liver extracts. *Journal of Virological Methods*, **251**, 38-42.

**Abstract:** The emergence and rapid spread of variant of the rabbit hemorrhagic disease virus (RHDV2) require new diagnostic tools ensure that efficient control measures are adopted. In the present study, a specific sandwich enzyme-linked immunosorbent assay (ELISA) for detection of RHDV2 antigens in rabbit liver homogenates, based on the use of an RHDV2-specific monoclonal antibody (Mab) 2D9 for antigen capture and an anti-RHDV2 goat polyclonal antibody (Pab), was developed. This ELISA was able to successfully detect RHDV2 and RHDV2 recombinant virions with high sensitivity (100%) and specificity (97.22%). No cross-reactions were detected with RHDV G1 viruses while low cross-reactivity was detected with one of the RHDVa samples analyzed. The ELISA afforded good repeatability and had high analytical sensitivity as it was able to detect a dilution 1:163,640 (6.10 ng/mL) of purified RHDV-N11 VLPs, which contained approximately  $3.4 \times 10^6$  molecules/mL particles. The reliable discrimination between closely related viruses is crucial to understand the epidemiology and the interaction of co-existing pathogens. In the work described here we design and validate an ELISA for laboratory based, specific, sensitive and reliable detection of RHDVb/RHDV2. This ELISA is a valuable, specific virological tool for monitoring virus circulation, which will permit a better control of this disease.

DEL CERRO, A., et al. (2018) A one-step TaqMan real-time qRT-PCR assay for the specific detection and quantitation of the Spanish goat encephalitis virus (SGEV). *Journal of Virological Methods*, **255**, 98-100.

**Abstract:** Louping ill-like virus (LI) has been recently detected in two different locations in the north of Spain and separated by only around 400 km. Using molecular approaches, the viruses causing both outbreaks have been shown to be different to LI virus, but also different to each other. They have been called SSEV (Spanish sheep encephalitis virus) and SGEV (Spanish goat encephalitis virus) taking into account the species from which they were isolated. The aim of this paper was to design a quantitative TaqMan real-time RT-PCR protocol, for the specific diagnostic and quantitation of SGEV. Linearity, efficiency and dynamic range as well as reproducibility and specificity of the method has been tested and established. The method has proved to be valid for the specific detection and viral load quantitation of SGEV genome in virus isolates and tissue samples from infected animals. This assay will be a useful analytical tool in early diagnosis and epidemiological surveys.

DIEZ-DELGADO, I., et al. (2018) Impact of piglet oral vaccination against tuberculosis in endemic free-ranging wild boar populations. *Preventive Veterinary Medicine*, **155**, 11-20.

**Abstract:** The Eurasian wild boar (*Sus scrofa*) is the main wild reservoir of the *Mycobacterium tuberculosis* complex in Mediterranean woodlands and a key risk factor for cattle tuberculosis (TB) breakdowns. Wild boar vaccination therefore has the potential to be a valuable tool for TB control. We tested two orally delivered vaccines, heat-inactivated *Mycobacterium bovis* (IV) and BCG, in four sites (two per vaccine type: one Managed and one Natural or unmanaged) during four years. TB was also monitored in 15 unvaccinated sites (spatial control), as well as in all sites from one year

prior to intervention (temporal control). The rationale is that by vaccinating 2–6 month old wild boar [piglets](#) we can reduce disease at the population level during the study period. This is achievable due to the fast turnover of wild boar populations. Vaccine [baits](#) were deployed using selective piglet feeders and this method proved highly successful with uptake rates of 50 to 74% in Natural sites and 89 to 92% in Managed sites. This is relevant for the potential delivery of vaccines to control other diseases, too. Local wild boar TB [prevalence](#) at the beginning of the study was already high ranging from 50 to 100%. TB prevalence increased in unvaccinated sites (6%), while a significant decline occurred in the Managed IV site (34%). Changes recorded in the remaining sites were not significant. The short-term impact of vaccination observed in the field was complemented by mathematical modelling, representative of the [field system](#), which examined the long-term impact and showed that vaccination of piglets reduced prevalence and increased abundance at the population level. We conclude that IV could become part of integrated TB control schemes, although its application must be tailored for each specific site.

DIEZMA-DIAZ, C., et al. (2018) Effect of parasite dose and host age on the infection with *Besnoitia besnoiti* tachyzoites in cattle. *Transboundary and Emerging Diseases*, **65** (6), 1979-1990.

**Abstract:** Bovine besnoitiosis is continuing to spread in Europe. Therefore, the development of ruminant animal models of infection is urgently needed to evaluate therapeutic and prophylactic tools. Herein, we studied the effect of parasite dose and host age on the infection dynamics with *Besnoitia besnoiti* tachyzoites in cattle in two independent experimental infections. In experiment A, twelve 3-month-old male calves were inoculated intravenously with either three different doses of tachyzoites (G1:  $10^8$ ; G2:  $10^7$ ; G3:  $10^6$ ) or with PBS (G4). In experiment B, six 14-month-old bulls were inoculated with  $10^6$  tachyzoites based on results obtained in experiment A. In both trials, clinical signs compatible with acute and chronic besnoitiosis were monitored daily; blood and skin samples were collected regularly for 70–115 days post-infection (pi). Finally, animals were killed, and tissues were collected for lesion and parasite detections. Infected animals developed mild–moderate signs compatible with acute besnoitiosis. Lymphadenopathy and fever were observed in both calves (from 12 hr until 7 days pi) and bulls (from 6 days until 9 days pi). Seroconversion was detected at 16–19 days pi, and antibody levels remained high. Infected animals did not develop characteristic clinical signs and macroscopic lesions of chronic besnoitiosis. However, successfully, parasite-DNA was detected in a reduced number of target tissues: conjunctiva, ocular sclera, epididymis, skin of the scrotum and carpus in calves ( $n = 10$ , 6 of which belonged to G3), and pampiniform plexus and testicular parenchyma in bulls. Remarkably, one tissue cyst and mild microscopic lesions were also detected. In summary, inoculated animals developed the acute besnoitiosis and chronic infection was evidenced by microscopic findings. However, our results suggest that tachyzoite dose and host age are not key variables for inducing clinical signs and macroscopic lesions characteristic of chronic besnoitiosis. Thus, a further refinement of this model should evaluate other parasite- and host-dependent variables.

FERREIRA, L. M. M., et al. (2018) Effects of animal species and diet composition on long-chain alcohol faecal recoveries and accuracy of subsequent diet composition estimates. *Grass and Forage Science*, **73** (2), 355-367.

**Abstract:** Long-chain alcohols (LCOH) were evaluated as markers to estimate diet composition of ruminant species fed with two distinct diets composed of herbage and “heather” (Ericaceae species). Diet composition was estimated using LCOH, combined or not with n-alkanes and/or long-chain fatty acids (LCFA), by least-squares procedures, using faecal concentrations corrected or not for their incomplete recovery. The effect of grouping plant species with similar marker profiles and the impact of feeding selectivity (FS) within the dietary group on the accuracy of diet composition estimates were also evaluated. Results showed the usefulness of LCOH as diet composition markers. The LCOH were incompletely recovered in the faeces of all animal species and tended to increase with carbon-chain length in a curvilinear manner in cattle and sheep, whereas in goats this association was better described by a linear function. Accuracy of estimates was higher when LCOH and n-alkane markers were combined. For all marker types, results indicated that a correction of marker faecal concentrations to incomplete faecal recovery is needed to obtain accurate diet composition estimates. Grouping plant species with similar marker profiles improved the accuracy of estimates, whereas combination of marker types minimized the FS effect within the dietary group.

FUENTE-GARCIA, C., et al. (2018) Search for proteomic biomarkers related to bovine pre-slaughter stress using liquid isoelectric focusing (OFFGEL) and mass spectrometry. *Journal of proteomics*.

**Abstract:** [Proteome](#) changes derived from animals that have suffered pre-slaughter stress are a fact. In this study, [Proteomic](#) analysis was carried out on 20 [bovine](#) loin samples from Asturiana de los Valles and [crossbreds](#) cattle previously classified as normal and DFD meat at 24 h post-mortem using pH measurements. Sarcoplasmic sub-proteome of *Longissimus thoracis* at 24 h post-mortem was fractionated by the use of liquid [isoelectric focusing](#) (OFFGEL) in the pH range 3–10, followed by [SDS-PAGE](#) analysis of each retrieved fraction. The protein [fractionation](#) profile showed high reproducibility along the different sample groups. Five protein bands showed significant differences ( $p < 0.05$ ) between the two groups, allowing discrimination between them. Proteins present in these bands, which were identified by LC-MS, were [actin](#), phosphoglucotase-1, alpha-crystallin B, [heat shock protein](#) beta-6 and heat shock protein beta-1.

GARCIA MARIN, J. F., et al. (2018) Canine adenovirus type 1 (CAV-1) in free-ranging European brown bear (*Ursus arctos arctos*): A threat for Cantabrian population? *Transboundary and Emerging Diseases*, **65** (6), 2049-2056.

**Abstract:** Canine adenovirus type 1 (CAV-1) is responsible for infectious canine hepatitis. The disease has been described in captive American black bear (*Ursus americanus*) and European brown bear (*Ursus arctos arctos*), with just one recently reported case in a cub of a free-ranging brown bear (*Ursus arctos horribilis*) from Alaska. The aim of this work is to summarize findings related to presence and associated mortality of CAV-1 in 21 free-ranging Cantabrian brown bears (*Ursus arctos arctos*) submitted to necropsy in Asturias and Castilla y León (northwestern Spain) from 1998 to 2018. On the basis of the anatomopathological findings and laboratory results three free-ranging brown bears died due to infectious canine hepatitis, which is to our knowledge the first description of death due to this disease in free-ranging bears in Europe. Gross lesions consisted of petechial haemorrhages and congestion in different internal organs, haemorrhagic fluid in internal cavities, friable and yellowish liver and thickening of gall bladder. Microscopic lesions were observed mainly in liver, kidney and brain and consisted of multifocal necrosis of cells with presence of basophilic intranuclear inclusions. Immunohistochemical (IHC) and real-time polymerase chain reaction (qPCR) techniques were used to assess the presence of CAV-1 in paraffin-embedded liver samples. Viral antigens were detected by IHC labelling within hepatocytes and Küppfer cells in the three animals. The presence of viral DNA was confirmed by qPCR in one of them. In order to evaluate the circulation of CAV-1 in brown bears, a retrospective study was performed using both IHC and qPCR techniques in 11 and 12 additional brown bears, respectively. An extra brown bear was found positive by IHC. This study shows that CAV-1 surveillance of brown bears and sympatric carnivores should be considered as major concern for the monitoring the population evolution throughout time in this endangered species.

GAYO, E., et al. (2018) Inflammatory Lesion Patterns in Target Organs of Visna/Maedi in Sheep and their Significance in the Pathogenesis and Diagnosis of the Infection. *Journal of Comparative Pathology*, **159**, 49-56.

**Abstract:** Ovine visna/maedi (VM) infection is characterized by the development of chronic inflammatory lesions in different organs, mainly in the [lung](#), [mammary gland](#) and [central nervous system](#) (CNS), with either histiocytic or lymphocytic pattern predominance being described in the CNS. To help to understand the role of host [immune response](#) in the development of these patterns, 50 naturally-infected [sheep](#) and eight non-infected sheep from intensive milk-producing [flocks](#) were studied. The histological lesion patterns in the three main target organs in each sheep were characterized. Lesion severity was determined, including minimal lesions. A histiocytic pattern was observed in 23 sheep (46%), a lymphocytic inflammatory pattern in 19 sheep (38%) and a mixed inflammatory pattern in eight sheep (16%). Forty animals showed moderate or severe lesions (80%), while 10 had minimal lesions (20%). Moderate or severe lesions affected only one target organ in 20 sheep (50%), two organs in 14 sheep (35%) and all three target organs in six sheep (15%). Infection was confirmed by [immunohistochemistry](#) (IHC) using an antibody specific for p28 of VM virus/caprine arthritis and encephalitis virus and by [polymerase chain reaction](#) (PCR) in all sheep. Minimal inflammatory lesions associated with positive IHC and PCR were observed. The results suggest that the development of a predominant inflammatory pattern in different organs within the same animal may be related to the host immune response. Minimal and focal lesions, not considered previously, should be taken into account when formulating a differential diagnosis in affected sheep.

GOMEZ, E., et al. (2018a) Differential release of cell-signaling metabolites by male and female bovine embryos cultured in vitro. *Theriogenology*, **114**, 180-184.

**Abstract:** Male and female early [bovine](#) embryos show dimorphic transcription that impacts [metabolism](#). Individual release of [metabolites](#) was examined in a 24h single culture medium from Day-6 male and female [morulae](#) that developed to Day-7 expanded [blastocysts](#). Embryos were produced in vitro, fertilized with a single [bull](#) and cultured in SOFaaci+6 g/L BSA. The embryonic sex was identified (amelogenin gene amplification). Embryos (N = 10 males and N = 10 females) and N = 6 blank samples (i.e. SOFaaci+6 g/L BSA incubated with no embryos) were collected from 3 replicates. [Metabolome](#) was analyzed by UHPLC-TOF-MS in spent culture medium. After tentative identification, N = 13 metabolites significantly (P < 0.05; ANOVA) differed in their concentrations between male and female embryos, although N = 10 of these metabolites showed heterogeneity (Levene's test; P > 0.05). LysoPC(15:0) was the only metabolite found at higher concentration in females (fold change [FC] male to female = 0.766). FC of metabolites more abundant in male culture medium (N = 12) varied from 1.069 to 1.604. Chemical [taxonomy](#) grouped metabolites as [amino-acids](#) and related compounds (DL-2 aminooctanoic acid, [arginine](#), 5-hydroxy-L-tryptophan, and palmitoylglycine); [lipids](#) (2-hexenoylcarnitine; Lauroyl diethanolamide; 5,6 dihydroxyprostaglandin F1a; LysoPC(15:0); DG(14:0/14:1(9Z)/0:0) and triterpenoid); endogenous amine ((S)-N-Methylsalsolinol/(R)-N-Methylsalsolinol); n-acyl-alpha-hexosamine (N-acetyl-alpha-D-galactosamine 1-phosphate); and dUMP, a product of [pyrimidine metabolism](#). Among the compounds originally contained in CM, female embryos significantly depleted more arginine than males and blank controls (P < 0.001). Male and female embryos induce different concentrations of metabolites with [potential](#) signaling effects. The increased abundance of metabolites released from males is consistent with the higher metabolic activity attributed to such blastocysts.



GOMEZ, E., et al. (2018b) In vitro cultured bovine endometrial cells recognize embryonic sex. *Theriogenology*, **108**, 176-184.

**Abstract:** Endometrial cell [co-culture](#) (ECC) with single embryo may reflect [endometrium](#) responses in vivo. [Bovine](#) Day-6 in vitro-produced [morulae](#) were cultured until Day-8 in modified synthetic [oviductal fluid](#) (mSOF), or on the epithelial side of ECC. Expression of [epithelial- and stromal-cell](#) transcripts was analyzed by RT-PCR in ECC with one male (ME) or female embryo (FE). Concentrations of ARTEMIN (ARTN) and total protein were determined in epithelial cell-conditioned medium. ECCs yielded embryos with more cells in the [inner cell mass](#) than embryos cultured in mSOF. Embryos altered transcript expression only in epithelial cells, not in stromal ones. Thus, ME induced larger [reductions](#) than FE and controls (i.e., no embryos cultured) in [hexose](#) transporter [solute carrier family](#) 2 member 1 (*SLC2A1*) and member 5 (*SLC2A5*), [connective tissue growth factor](#) (*CTGF*), [artemin](#) (ARTN), and [interferon](#) alpha and beta receptors subunit *IFNAR1* and *IFNAR2*. FE reduced *SLC2A1* and *SLC2A5*, and increased ARTN expression with respect to controls. ME tended to reduce total protein concentration ( $P < 0.082$ ) in ECC-conditioned medium, while ARTN protein and [gene expressions](#) strongly correlated ( $R > 0.90$ ;  $P < 0.05$ ) in the group of ME or FE, but not in controls (without embryo). Isolated male and female embryos may differentially release signaling factors that induce sexually dimorphic responses in endometrial cells.

JIMENEZ-CALDERON, J. D., et al. (2018) A winter intercrop of faba bean and rapeseed for silage as a substitute for Italian ryegrass in rotation with maize. *Archives of Agronomy and Soil Science*, **64** (7), 983-993.

**Abstract:** In order to combine the ability of legumes to fix atmospheric nitrogen and the cruciferous capacities to mobilize soil nutrients and herbicide action, the aim of this work was to evaluate an alternative winter intercrop (faba bean-rapeseed) as a replacement of Italian ryegrass culture in a rotational system with maize as summer crop. For this purpose, two adjacent plots were used during three agronomic years (2011–2012, 2012–2013 and 2013–2014) to evaluate the agronomic performance through the forage production, nutritional composition of forage and silage, and the effects on soil fertility. The Italian ryegrass was cultivated under conventional management: using chemical fertilization and recommended dosages of herbicides. The faba bean-rapeseed intercrop was cultivated under an alternative management: organic fertilization and less herbicide supply. The intercrop provides higher forage yield per hectare than Italian ryegrass, with greater protein ( $\text{kg ha}^{-1}$ ) and similar energy ( $\text{GJ ha}^{-1}$ ) yields. The intercrop allows reducing the inputs of chemical fertilization and herbicides, and it has a positive effect on the balance of soil nutrients, especially increasing the potassium, calcium and magnesium contents. The results show that faba bean-rapeseed intercrop could be an alternative to the Italian ryegrass as winter crop.

JUSTE, R. A., et al. (2018) Association between combinations of genetic polymorphisms and epidemiopathogenic forms of bovine paratuberculosis. *Heliyon*, **4** (2).

**Abstract:** Control of major mycobacterial diseases affecting livestock is a challenging issue that requires different approaches. The use of genetic markers for improving resistance to *Mycobacterium avium* subsp. *paratuberculosis* infection in cattle has been explored as a promising population strategy. We performed paratuberculosis epidemiopathogenic phenotypic and genotypic characterization involving 24 SNPs in six candidate genes (*NOD2*, *CD209*, *SLC11A1*, *SP110*, *TLR2* and *TLR4*) on 502 slaughtered Friesian cows. In the current study, we investigate whether recently proposed paratuberculosis (PTB) epidemiopathogenic (EP) forms (apparently free-AF, latent-LAT and patent-PAT) could be associated with some combination of these 24 SNPs. Best EP form grouping was obtained using a combination of 5 SNPs in four genes (*CD209*: rs210748127; *SLC11A1*: rs110090506; *SP110*: rs136859213 and rs110480812; and *TLR2*: rs41830058). These groups were defined according to the level of infection progression risk to patent epidemiopathogenic forms and showed the following distributions: LOWIN (low) with 39 (8%) cases (94.9% AF/5.1% LAT/0% PAT); LATIN (low) with 17 (3%) cases (5.9% AF/94.1% LAT/0% PAT); AVERIN (average) with 413 (82%) cases (52.1% AF/38.5% LAT/9.4% PAT) and PATIN (patent) with 33 (7%) cases (36.4% AF/24.2% LAT/39.4% PAT). Age of slaughter was significantly higher for LATIN (88.3 months) compared to AVERIN (65.3 months;  $p = 0.0007$ ) and PATIN (59.1 months;  $p = 0.0004$ ), and for LOWIN (73.9 months) compared to PATIN ( $p = 0.0233$ ), and nearly significant compared to AVERIN ( $p = 0.0572$ ). These results suggest that some selected genetic polymorphisms have a potential use as markers of PTB EP forms and thus add a new tool for the control of this widespread infection.

LLANO SUAREZ, P., et al. (2018) Rapid on-site monitoring of fatty acid profile in raw milk using a handheld near infrared sensor. *Journal of Food Composition and Analysis*, **70**, 1-8.

**Abstract:** This work is focused on development of a quick and simple to use analytical methodology for on-site monitoring the fatty acid (FA) profile in raw milk at farm level by using a near infrared handheld spectrometer. This novel methodology was developed using a total of 108 liquid milk samples, scanned at room temperature by NIRS



without pre-treatment, and analyzed by GC–MS for reference data. Calibration was carried out by multivariate regression combining math pre-treatments and Partial Least Square with internal and external validation. Calibration models displayed good predictive capacity for total saturated, monounsaturated (MUFA) and polyunsaturated FA (PUFA) with high coefficients of determination of cross validation ( $R^2_{cv} > 0.8$ ). Good results were also obtained for prediction of individual FA: caproic, capric, lauric, miristic, palmitic and arachidic as well as for unsaturated FA: oleic, conjugated linoleic acid and omega-6 acids with  $R^2_{cv}$  values ranged between 0.91–0.73. Validation statistics have confirmed that the highest  $R^2_v$  (coefficient of determination of external validation) values to quantify FA were for PUFA, linolenic acid ( $R^2_v = 0.92$ ), caproic acid and MUFA ( $R^2_v = 0.87$ ). These results establish that a profitable classification of milk can be carried out at farm level by including a fatty acid composition labeling.

LOBO, A., et al. (2018) Chemical, olfactometric and sensory description of single-variety cider apple juices obtained by cryo-extraction. *Lwt-Food Science and Technology*, **90**, 193-200.

**Abstract::** A set of ten single-apple juices obtained by cryo-extraction has been analysed for chemical and sensory profiles to evaluate their potential for the production of ice juices. Criteria for the selection of apple varieties were based on the equilibrium between sugars and acids and aromatic complexity. Verdialona, Durona de Tresali and Perico presented the highest ratios between total sugars and total acidity, while Raxao and Regona had the lowest. The results for olfactometry show that Xuanina, Limón Montés and Perico had the most complex olfactometric profiles and de la Riega, the simplest. Sensory analyses revealed that the juice from Durona de Tresali was the most appreciated, followed by those from de la Riega, Solarina and Blanquina cultivars. Verdialona was described by its sweet taste whereas the Regona cultivar was mainly defined by its tannin/bitter flavour.

MANGAS, J. J., et al. (2018) Validation of a gas chromatography-flame ionization method for quality control and spoilage detection in wine and cider. *Acta Alimentaria*, **47** (1), 17-25.

**Abstract:** A GC-FID method for major volatile analysis in cider and wine was validated under the requirements of the ISO/IEC 17025 regulation using a split mode injection. This method presented a good linearity ( $R^2 > 0.999$ ,  $C_m > 99\%$ ), a wide range of calibration, the maximum random errors for repeatability and intermediate precision were 5% and 8%, respectively, and the maximum limit of quantification and limit of detection were 14.1 mg l<sup>-1</sup> and 4.6 mg l<sup>-1</sup>, respectively. Trueness, expressed as recovery (%), was successfully validated by means of two different methods, standard addition (92–110%) and comparison against reference materials (93–115%). Expanded uncertainty estimated at 95% confidence level ranged between 11% for methanol and 7% for allyl alcohol. The validated method was successfully applied for the analysis of major volatiles in ciders and wines, and to detect yeast and lactic acid bacteria spoilage.

MIÑARRO, M. and GARCIA, D. (2018) Unravelling pest infestation and biological control in low-input orchards: the case of apple blossom weevil. *Journal of Pest Science*, **91** (3), 1047-1061

**Abstract:** Low-input farming is an alternative production system that provides a great opportunity to disentangle the natural mechanisms regulating crop pests, since neither pests nor their natural enemies are disrupted by pesticides. Here, we use a key apple pest in Europe, the apple blossom weevil (*Anthonomus pomorum*), as a model case to unravel the factors driving pest infestation and its biological control in a low-input context, namely the cider apple orchards of NW Spain. We applied a holistic approach based on the complete life cycle of the pest and combined large-scale observation (23 orchards) with small-scale experimental assessment. Weevil attack (0.4–37.4% of flowers) increased with the proportion on apple trees in the immediate orchard neighbourhood and with semi-natural woody habitat in the surrounding landscape and decreased with tree distance to orchard edge and apple bloom level. Thus, the prevalence of the pest depended on the availability of the various resources required for foraging, egg-laying and overwintering. Three types of natural enemies supplied complementary pest control by preying on weevils at different stages in their life cycle: seven parasitoid species attacked immature weevils (6.4–81.5%), while the additive effects of birds and crawling arthropods were evident in terms of the removal of adult weevils (31–44%). We conclude that the effective biological control of *A. pomorum* can be achieved in low-input systems to maintain the pest at non-harmful levels, through combined management of the pest, its habitat and its natural enemies

MORALES-ALMARAZ, E., et al. (2018) Parity and grazing-time effects on milk fatty acid profile in dairy cows. *Animal Production Science*, **58** (7), 1233-1238

**Abstract:** Milk fatty acid (FA) profiles of lactating Holstein dairy cows were determined to evaluate the effects of parity (primiparous or multiparous) and the grazing time (0, 6 and 12 h), complementary to a total mixed ration, in a 2 × 3 factorial design. The interaction of parity with grazing time did not affect milk production and protein content; however, dry matter intake of herbage, total dry matter intake and milk fat content were affected by the interaction ( $P < 0.05$ ). Both factors affected the milk concentration of monounsaturated FAs. The 18 : 3 cis9 cis12, 18 : 2 cis9 trans11, and 18 : 3 cis9 cis12 cis15 concentrations in milk were higher ( $P < 0.05$ ) in primiparous cows. Milk 18 : 2 cis9 trans11

concentration in primiparous cows with 12 h grazing (1.17 g/100 g FA) was 44% and 57% higher than with 6 h and 0 h grazing (0.66 and 0.50 g/100 g FA, respectively). Similar pattern was observed for 18 : 3 cis9 cis12 cis15. The milk 18 : 1 trans11 concentration was higher ( $P < 0.05$ ) with 6 h and 12 h grazing than without grazing. In conclusion, the parity would appear to mainly affect the proportion of short-chain FAs, and had an important influence on the proportions of 18 : 2 cis9 trans11, and 18 : 3 cis9 cis12 cis15. These FAs and also 18 : 1 trans11 were modified by grazing time.

MORATO, R., et al. (2018) Aquaporin 11 is related to cryotolerance and fertilising ability of frozen-thawed bull spermatozoa. *Reproduction Fertility and Development*, **30** (8), 1099-1108.

**Abstract:** Aquaporins (AQPs) are channel proteins involved in the transport of water and solutes across biological membranes. In the present study we identified and localised aquaporin 11 (AQP11) in bull spermatozoa and investigated the relationship between the relative AQP11 content, sperm cryotolerance and the fertilising ability of frozen-thawed semen. Bull ejaculates were classified into two groups of good and poor freezability and assessed through immunofluorescence and immunoblotting analyses before and after cryopreservation. AQP11 was localised throughout the entire tail and along the sperm head. These findings were confirmed through immunoblotting, which showed a specific band of approximately 50 kDa corresponding to AQP11. The relative amount of AQP11 was significantly ( $P < 0.05$ ) higher in both fresh and frozen-thawed spermatozoa from bull ejaculates with good freezability compared with those with poorer freezability. In addition, *in vitro* oocyte penetration rates and non-return rates 56 days after AI were correlated with the relative AQP11 content in fresh spermatozoa. In conclusion, AQP11 is present in the head and tail of bull spermatozoa and its relative amount in fresh and frozen-thawed spermatozoa is related to the resilience of the spermatozoa to withstand cryopreservation and the fertilising ability of frozen-thawed spermatozoa. Further research is needed to elucidate the actual role of sperm AQP11 in bovine fertility.

OLEAGA, A., et al. (2018) Leishmania in wolves in northern Spain: A spreading zoonosis evidenced by wildlife sanitary surveillance. *Veterinary Parasitology*, **255**, 26-31.

**Abstract:** Leishmaniosis is, to date, considered the second most important emerging vector-borne protozoal disease in the world after malaria. The form of zoonotic visceral leishmaniosis found in the Mediterranean basin is caused by *Leishmania infantum*, and its life cycle includes the domestic dog and a phlebotomine sandfly vector. This complex epidemiological cycle and its high prevalence of subclinical infection, hinder the surveillance and control of *L. infantum*, and allows it to go unnoticed at the geographical endemicity limits of the parasite or in recently colonized areas. We, therefore, tested 102 wolves (*Canis lupus*) and 47 other wild carnivores in order to detect *Leishmania* DNA by means of PCR. Samples were collected from 2008 to 2014 in Asturias (northern Spain), a region considered non-endemic for the parasite. The results obtained provided valuable information regarding the prevalence of *Leishmania* in wild carnivores in Asturias and its geographic distribution in the region: an average prevalence of 33% for wolves and an overall prevalence of 40% for all the wild carnivores studied were reported, with a widespread presence of the parasite in the region and an apparent increase in its prevalence in wolves during the last decade. This suggests the usefulness of the wolf as a sentinel species for the detection and study of *Leishmania* in the field and confirms the value of wildlife sanitary surveillance programs for the detection and monitoring of hitherto disregarded diseases that affect domestic animals and humans.

OLIVAN, M., et al. (2018) Effect of sex and RYR1 gene mutation on the muscle proteomic profile and main physiological biomarkers in pigs at slaughter. *Meat Science*, **141**, 81-90.

**Abstract:** Gender and RYR1 gene mutation might have an effect on the muscle metabolic characteristics and on the animal's stress at slaughter, which could influence the process of muscle-to-meat conversion. Forty-eight pigs were distributed in a design including two factors: sex (male/female) and RYR1 genotype (NN/Nn). At slaughter, physiological blood biomarkers and muscle proteome were analyzed and carcass and [meat quality](#) traits were registered. Females had higher serum levels of glucose, urea, C-reactive protein "CRP", Pig-MAP and glutation-peroxidase "GPx" and lower levels of lactate, showed faster muscle pH decline and higher meat exudation. RYR1 mutation increased serum [creatinine](#), creatine kinase and CRP and decreased GPx. The proteomic study highlighted significant effects of gender and RYR1 genotype on proteins related to fibre composition, [antioxidant](#) defense and *post mortem* glycolytic pathway, which correlate to differences of meat quality. This study provides interesting information on muscle biomarkers of the ultimate meat quality that are modulated by the animal's individual susceptibility to stress at slaughter.

PANEA, B., et al. (2018) Effects of breed-production system on collagen, textural, and sensory traits of 10 European beef cattle breeds. *Journal of Texture Studies*, **49** (5), 528-535.

**Abstract:** In the current study the collagen, texture, and sensory characteristics of meat from 712 yearling males of 10 local Spanish and French beef breeds raised in their typical production systems were described. The breed-production system affected collagen and texture variables but affected sensory variables only slightly. There was a large amount of

intra breed-production system variation for all the variables. French breeds had lower values for collagen solubility (~12%) than Spanish breeds (~40%). Stress (WB) varied from 36 N/cm<sup>2</sup> in Casina to 44 N/cm<sup>2</sup> in Salers, whereas compression stress at 80% ranged from 35 N/cm<sup>2</sup> in Asturiana de los Valles to 40 N/cm<sup>2</sup> in Salers. Oven cooking resulted in higher cooking losses (24%) than cooking on a grill (12%). Cooking losses increased as the grill temperature increased. Numerous significant correlations were found among variables. Carcass weight is associated with all the collagen and texture variables. Correlation coefficients among texture and collagen variables were statistically significant and these correlation coefficients were in general higher for solubility percentage than for total collagen content, highlighting the importance of the solubility of collagen rather than total collagen in determining meat textural properties.

PICINELLI LOBO, A., et al. (2018) Chemical, olfactometric and sensory description of single-variety cider apple juices obtained by cryo-extraction. *Lwt-Food Science and Technology*, **90**, 193-200.

**Abstract:** A set of ten single-apple juices obtained by cryo-extraction has been analysed for chemical and sensory profiles to evaluate their potential for the production of ice juices. Criteria for the selection of apple varieties were based on the equilibrium between sugars and acids and aromatic complexity. Verdialona, Durona de Tresali and Perico presented the highest ratios between total sugars and total acidity, while Raxao and Regona had the lowest. The results for olfactometry show that Xuanina, Limón Montés and Perico had the most complex olfactometric profiles and de la Riega, the simplest. Sensory analyses revealed that the juice from Durona de Tresali was the most appreciated, followed by those from de la Riega, Solarina and Blanquina cultivars. Verdialona was described by its sweet taste whereas the Regona cultivar was mainly defined by its tannin/bitter flavour.

RODRIGUEZ MADRERA, R., et al. (2018) Application of central composite design in the fermentation of apple pomace to optimize its nutritional and functional properties. *Acta Alimentaria*, **47** (3), 324-332.

**Abstract:** Simultaneous optimization of protein, soluble and insoluble dietary fibre, total phenolic content, and antioxidant activity was carried out in apple pomace by using response surface methodology (RSM) and solid state fermentation. A central composite design (CCD) was used to optimize the content of nutritional and functional properties, and two factors were studied: time and temperature of fermentation. The fermentation of apple pomace under the best conditions (29.5 °C and 4.9 days) allowed the depletion of fermentable sugars, an increase the contents of protein and insoluble dietary fibre (36% and 23%, respectively), while soluble dietary fibre, total phenolic content, and antioxidant activity remained constant.

RODRIGUEZ MADRERA, R. and SUAREZ VALLES, B. (2018) Characterization of apple seeds and their oils from the cider-making industry. *European Food Research and Technology*, **244** (10), 1821-1827.

**Abstract:** Apple seeds and their oils from an agro-food waste, derived from the cider industry protected by the Designation of Origin “Sidra de Asturias”, were chemically characterized. The average oil yield was 19.7%. The major fatty acids in apple seed oils are unsaturated fatty acids (90.3 ± 0.3%) belonging to the ω-3, ω-6 and ω-9 series (linolenic acid, 1.2 ± 0.1%; linoleic acid, 55.3 ± 1.2%; oleic acid, 33.4 ± 0.8%). Oils also showed an important antioxidant activity and high levels of tocopherols (total tocopherols 1280 ± 104.8 mg/kg oil) with β-tocopherol (794.5 ± 62.2 mg/kg oil) being most abundant in all cases followed by α-tocopherol (439.2 ± 34.5 mg/kg oil) and these compounds correlate with DPPH radical activity (r = 0.937). In the defatted apple seeds, protein (37.5 ± 1.8%) and fibre (20.3 ± 0.4%) were the major nutritional components detected and other interesting constituents, such as extractable polyphenols and hydrolysable tannins, were also relevant. The results suggest apple seeds from the cider-making industry could be a suitable raw material for food, pharmacological or cosmetic uses.

SALINAS, L. M., et al. (2018) Neurological syndrome in goats associated with *Ipomoea trifida* and *Ipomoea carnea* containing calystegines. *Toxicon : official journal of the International Society on Toxinology*, **157**, 8-11.

**Abstract:** A disease characterized by [ataxia](#), [tremors](#) and [nystagmus](#) had been observed in goats in Nicaragua. The main histologic lesions were loss and neuronal vacuolation of [Purkinje cells](#) and Wallerian-like [degeneration](#) mainly in the [cerebellum](#), suggesting a [glycoprotein storage disease](#). *Ipomoea carnea* and *Ipomoea trifida* found in the paddocks were negative for [swainsonine](#), but contained calystegines at 0.02% and 0.06% suggesting that the disease was caused by these substances, which are [competitive inhibitors](#) of [β-glucosidase](#) and [α-galactosidase](#) activities.

SALMAN, A., et al. (2018) Extended equilibration time affects motility and some kinematic parameters in thawed semen in Holstein and Asturiana de los Valles bull breeds. *Reproduction in Domestic Animals*, **53**, 192-192.

SANTIN, M., et al. (2018) Molecular Characterization of *Enterocytozoon bieneusi* in Wild Carnivores in Spain. *Journal of Eukaryotic Microbiology*, **65** (4), 468-474.

**Abstract:** Microsporidia comprises a diverse group of obligate intracellular parasites that infect a broad range of invertebrates and vertebrates. Among Microsporidia, *Enterocytozoon bieneusi* is the most frequently detected species in humans and animals worldwide bringing into question the possible role of animal reservoirs in the epidemiology of this pathogen. Although *E. bieneusi* is an emerging zoonotic pathogen able to infect many domestic and wild mammals that could act as reservoir of infection for humans and other animals, only few studies have documented its occurrence in wild carnivores. To determine the occurrence of *E. bieneusi* in wild carnivores, we examined 190 wild carnivores collected from different locations in Spain. Twenty-five fecal samples (13.2%) from three host species (European badger, beech marten, and red fox) were *E. bieneusi*-positive by PCR. Nucleotide sequence analysis of the ITS region revealed a high degree of genetic diversity with a total of eight distinct genotypes including four known (PtEbIX, S5, S9, and WildBoar3) and four novel (EbCar1-EbCar4) genotypes identified. Phylogenetic analysis showed that the four novel genotypes (EbCar1-EbCar4), S5, S9, and WildBoar3 clustered within the previously designated zoonotic Group 1. Our results demonstrate that human-pathogenic genotypes are present in wild carnivores, corroborating their potential role as a source of human infection and environmental contamination.

SERRANO, M., et al. (2018) Different lesion distribution in calves orally or intratracheally challenged with *Mycobacterium bovis*: implications for diagnosis. *Veterinary Research*, **49**.

**Abstract:** Animal tuberculosis (TB) remains a major problem in some countries despite the existence of control programmes focused mainly on cattle. In this species, aerogenous transmission is accepted as the most frequent infection route, affecting mainly the respiratory system. Under the hypothesis that the oral route could be playing a more relevant role in transmission, diagnosis and disease persistence than previously thought, this study was performed to assess the course of TB infection in cattle and its effects on diagnosis depending on the route of entry of *Mycobacterium bovis*. Two groups of five calves each were either endotracheally (EC) or orally (OC) challenged. Necropsies were carried out 12 weeks after challenge except for three OC calves slaughtered 8 weeks later. All animals reacted to the tuberculin skin test and the entire EC group was positive to the interferon-gamma release assay (IGRA) 2 weeks after challenge and thereafter. The first positive IGRA results for OC calves (3/5) were recorded 4 weeks after challenge. Group comparison revealed significant differences in lesion and positive culture location and scoring. TB-compatible gross lesions and positive cultures were more frequently found in the thorax ( $p < 0.001$ ) and lung ( $p < 0.05$ ) of EC animals, whereas OC animals presented lesions ( $p = 0.23$ ) and positive cultures ( $p < 0.05$ ) mainly located in the abdomen. These results indicate that the infection route seems to be a determining factor for both the distribution and the time needed for the development of visible lesions. Our study suggests that confirmation of TB infection in some skin reactor animals can be problematic if current post-mortem examination and diagnostics are not improved.

SUAREZ, M., et al. (2018a) Development of a novel 3D glass-ceramic scaffold for endometrial cell in vitro culture. *Ceramics International*, **44** (12), 14920-14924.

**Abstract:** [Glass-ceramic](#) and biphasic calcium phosphate materials with 3D architectures to be used as scaffolds for endometrial stromal cells (ESCs) were synthesized by freezing and lyophilising ceramic suspensions. The [microstructures](#) obtained by FESEM showed the presence of open and 3D interconnected porous structures, which are known to promote [cell growth and proliferation](#). Immunohistochemical staining showed that ESCs spread out and ingressed into the Glass-ceramic scaffold. On average, 2952 cells were found per mm<sup>3</sup> and the depth of cellular infiltration into the scaffold was 66.4 µm. Embryo development occurred on top of cell-cultured scaffold, which suggests that it is not embryotoxic. Taking all of this into account, we can state that the Glass-ceramic scaffold provides a promising tool to develop an *in vitro* endometrial 3D model.

SUAREZ, P., et al. (2018b) Rapid on-site monitoring of fatty acid profile in raw milk using a handheld near infrared sensor. *Journal of Food Composition and Analysis*, **70**, 1-8.

**Abstract:** This work is focused on development of a quick and simple to use analytical methodology for on-site

monitoring the fatty acid (FA) profile in raw milk at farm level by using a near infrared handheld spectrometer. This novel methodology was developed using a total of 108 liquid milk samples, scanned at room temperature by NIRS without pre-treatment, and analyzed by GC–MS for reference data. Calibration was carried out by multivariate regression combining math pre-treatments and Partial Least Square with internal and external validation. Calibration models displayed good predictive capacity for total saturated, monounsaturated (MUFA) and polyunsaturated FA (PUFA) with high coefficients of determination of cross validation ( $R^2_{cv} > 0.8$ ). Good results were also obtained for prediction of individual FA: caproic, capric, lauric, myristic, palmitic and arachidic as well as for unsaturated FA: oleic, conjugated linoleic acid and omega-6 acids with  $R^2_{cv}$  values ranged between 0.91- 0.73. Validation statistics have confirmed that the highest  $R^2_v$  (coefficient of determination of external validation) values to quantify FA were for PUFA, linolenic acid ( $R^2_v = 0.92$ ), caproic acid and MUFA ( $R^2_v = 0.87$ ). These results establish that a profitable classification of milk can be carried out at farm level by including a fatty acid composition labeling.

TRAORE, G. F., et al. (2018) Multivariate analyses of morphological traits characterise the Guinea fowl (*Numida meleagris*) of Burkina Faso as a homogeneous population. *European Poultry Science*, **82**.

TRAPIELLO, E., et al. (2018) First Report of *Gnomoniopsis castaneae* Causing Canker on Hybrid Plants of *Castanea saliva* x *C. crenata* in Spain. *Plant Disease*, **102** (5), 1040-1040.

**Abstract:** *Gnomoniopsis castaneae* Tamietti (syn. *G. smithogilvyi* L.A. Shuttlesworth, E.C.Y. Liew and D.I. Guest) is the causal agent of chestnut rot disease (Visentin et al. 2012). It has recently become known for being responsible for wood cankers on sweet chestnut, similar those caused by *Cryphonectria parasitica* on twigs and scions of sweet chestnut (Seddaiu et al. 2017). In November 2016, three samples of hybrid plants *C. sativa* x *C. crenata* showing chestnut blight symptoms such as bark coloration, were collected in a nursery in Asturias, northern Spain. Symptomatic branches were surface-sterilized in 98% ethanol for 30 s and dried on sterilized tissue paper, plated on potato dextrose agar (PDA) and incubated at room temperature with a natural photoperiod (about 11 h light, 13 h dark). Fungal colonies different from *C. parasitica* were consistently isolated from affected bark after 5 days of incubation in two of the samples. Colonies were transferred on PDA and next incubated at 25° C with a natural photoperiod (16 h light, 8 h dark). After 21 days, colonies showed abundant brownish-white aerial mycelium while in dark conditions the colour of the colony is whitish. Orange viscous conidia were observed under both, light and dark conditions, they occurred uniformly throughout the colony with concentric shape in light. Based on morphological characteristics, such as the morphology of the colony and the presence of subglobose pycnidia, with conidia ellipsoid, apically obtuse, with truncate base, non-septate and hyaline, *Gnomoniopsis castaneae* was suspected (Meyer et al. 2015). For molecular identification, DNA was extracted and the internal transcribed spacer (ITS) gene region of rDNA was amplified using the ITS1/ITS4 primers (White et al. 1990). The sequence of the isolate LPPAF935 was deposited in GenBank (accession number: LT837820) and showed the 100% identity with the *G. smithogilvyi* strain ICMP14079 (accession number: KC145860) according to the best BLAST match in the NCBI database. Pathogenicity tests were carried out on ten chestnut plants inoculated with the fungus by a cut in the bark. As a control, ten plants were used on which only a cut was made. All plants were placed in a greenhouse under natural conditions. Bark cankers symptoms similar to the ones observed in the samples analyzed were visible on chestnut plants two months after inoculation. No symptoms were observed on the controls. The assay was conducted twice. Fungal colonies morphologically identified as *G. castaneae* were reisolated from bark cankers on inoculated chestnut plants, fulfilling Koch's postulates.

VALLEJO, R., et al. (2018) Immunohistochemical characterization of tuberculous lesions in sheep naturally infected with *Mycobacterium bovis*. *Bmc Veterinary Research*, **14**.