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

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INTRODUCCIÓN

Las citas bibliográficas son la piedra angular de la propagación del conocimiento y el medio principal para evaluar la calidad de la investigación, así como para dirigir las inversiones en ciencia. Por tanto, y considerando que la ciencia se está haciendo cada vez más intensiva en datos, la práctica de citación de datos es fundamental para considerarlos como objeto de primera clase con la misma relevancia que los productos científicos que reflejan.

El anexo del año 2017 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 2017.

Esta nueva actualización se compone de 46 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, 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 el anexo de 2016, respectivamente.
<http://ria.asturias.es/RIA/handle/123456789/5541>
<http://ria.asturias.es/RIA/handle/123456789/7765>

BIBLIOGRAFÍA CIENTÍFICA. AÑO 2017

ALVAREZ, I., PEREZ-PARDAL, L., TRAORE, A., KOUDANDE, D. O., FERNANDEZ, I., SOUDRE, A., DIARRA, S., SANOU, M., BOUSSINI, H. & GOYACHE, F. 2017. Differences in genetic structure assessed using Y-chromosome and mitochondrial DNA markers do not shape the contributions to diversity in African sires. *Journal of Animal Breeding and Genetics*, 134, 393-404.

Abstract: Up to 173 African sires belonging to 11 different subpopulations representative of four cattle groups were analysed for six Y-specific microsatellite loci and a mitochondrial DNA fragment. Differences in Y-chromosome and mtDNA haplotype structuring were assessed. In addition, the effect of such structuring on contributions to total genetic diversity was assessed. Thirty-five Y-chromosome and 71 mtDNA haplotypes were identified. Most Y-chromosomes analysed (73.4%) were of zebu origin (11 haplotypes). Twenty-two Y-haplotypes (44 samples) belonged to the African taurine subfamily Y2a. All mtDNA haplotypes belonged to the African taurine T1 haplogroup with 16 samples and nine haplotypes belonging to a recently identified subhaplogroup (T1e). Median-joining networks showed that Y-chromosome phylogenies were highly reticulated with clear separation between zebu and taurine clusters. Mitochondrial haplotypes showed a clear star-like shape with small number of mutations separating haplotypes. Mitochondrial-based F-ST-statistics computed between cattle groups tended to be statistically non-significant ($p > .05$). Most F-ST values computed among groups and subpopulations using Y-chromosome markers were statistically significant. AMOVA confirmed that divergence between cattle groups was only significant for Y-chromosome markers ($\phi(CT) = 0.209$). At the mitochondrial level, African sires resembled an undifferentiated population with individuals explaining 94.3% of the total variance. Whatever the markers considered, the highest contributions to total Nei's gene diversity and allelic richness were found in West African cattle. Genetic structuring had no effect on patterns of contributions to diversity. (DOI: 10.1111/jbg.12278)

BALSEIRO, A., ALTUZARRA, R., VIDAL, E., MOLL, X., ESPADA, Y., SEVILLA, I. A., DOMINGO, M., GARRIDO, J. M., JUSTE, R. A., PRIETO, M. & PEREZ DE VAL, B. 2017. Assessment of BCG and inactivated *Mycobacterium bovis* vaccines in an experimental tuberculosis infection model in sheep. *Plos One*, 12

Abstract: Background/Aims Animal tuberculosis (TB) is a complex animal health problem that causes disruption to trade and significant economic losses. TB involves a multi-host system where sheep, traditionally considered a rare host of this infection, have been recently included. The aims of this study were to develop an experimental TB infection model in sheep with a *Mycobacterium caprae* field strain isolated from a tuberculous diseased ewe, and to use this to evaluate the safety and efficacy of two vaccines against TB in sheep, the live-attenuated *M. bovis* BCG vaccine (Danish strain) and a heat-inactivated *M. bovis* (HIMB) vaccine. Methods Eighteen 2 month-old lambs were experimentally challenged with *M. caprae* by the endotracheal route (1.5×10^3 CFU). They were separated per treatment group into parenterally vaccinated with a live BCG Danish strain vaccine ($n = 6$), orally vaccinated with a suspension of HIMB ($n = 6$) and unvaccinated controls ($n = 6$). Clinical, immunological, pathological and bacteriological parameters of infection were measured. Results All lambs were successfully infected and developed gross TB lesions in the respiratory system. The BCG vaccine conferred considerable protection against experimental TB in lambs, as measured by a reduction of the gross lesion volumes and bacterial load. However, HIMB vaccinated animals did not show protection. Conclusions This study proposes a reliable new experimental model for a better understanding of tuberculosis in sheep. BCG vaccination offers an effective prospect for controlling the disease. Moreover alternative doses and/or routes of administration should be considered to evaluate the efficacy of the HIMB vaccine candidate. (DOI: 10.1371/journal.pone.0180546)

BEZOS, J., CASAL, C., ALVAREZ, J., ROY, A., ROMERO, B., RODRIGUEZ-BERTOS, A., BARCENA, C., DIEZ, A., JUSTE, R., GORTAZAR, C., PUENTES, E., AGUILO, N., MARTIN, C., DE JUAN, L. & DOMINGUEZ, L. 2017. Evaluation of the Mycobacterium tuberculosis SO2 vaccine using a natural tuberculosis infection model in goats. *Veterinary Journal*, 223, 64-71

Abstract: The development of new vaccines against animal tuberculosis (TB) is a priority for improving the control and eradication of this disease, particularly in those species not subjected to compulsory eradication programmes. In this study, the protection conferred by the Mycobacterium tuberculosis SO2 experimental vaccine was evaluated using a natural infection model in goats. Twenty-six goats were distributed in three groups: (1) 10 goats served as a control group; (2) six goats were subcutaneously vaccinated with BCG; and (3) 10 goats were subcutaneously vaccinated with SO2. Four months after vaccination, all groups were merged with goats infected with Mycobacterium bovis or Mycobacterium caprae, and tested over a 40 week period using a tuberculin intradermal test and an interferon-gamma assay for mycobacterial reactivity. The severity of lesions was determined at post-mortem examination and the bacterial load in tissues were evaluated by culture. The two vaccinated groups had significantly lower lesion and bacterial culture scores than the control group ($P < 0.05$); at the end of the study, the SO2 vaccinated goats had the lowest lesion and culture scores. These results suggest that the SO2 vaccine provides some protection against TB infection acquired from natural exposure. (DOI: 10.1016/j.tvjl.2017.04.006)

CAAMAÑO, J. N., SALMAN, A., MARTINEZ-PASTOR, F., FUEYO, C., TAMARGO, C., CARBAJO, M. & HIDALGO, C. O. 2017. Assessment of chromatin status in the semen bank of the Spanish native cattle breed "Asturiana de la Montaña". *Reproduction in Domestic Animals*, 52, 86-87. (Poster-P10)

Abstract: Germplasm banks are a tool for the preservation of native, rare and/or genetically relevant breeds. We present a partial study of the SERIDA semen bank for the cattle breed "Asturiana de la Montaña", assessing chromatin status (a critical parameter for sperm fertility) in 8 bulls that were sampled in 4 seasons. Two straws were thawed (37°C, 30 s) for each bull and season and analysed by Sperm chromatin Structure Assay (SCSA, flow cytometry) just after thawing and after 5 hr at 37°C (for detecting susceptibility to post-thawing damage), obtaining DNA fragmentation (%DFI) and chromatin immaturity (%HDS). Data were analysed by linear mixed-effects models (results as mean $\pm$ SD of %). All values indicate very low chromatin alterations (ranging 1.0 ± 0.9 to 3.6 ± 1.0 for %DFI, 2.5 ± 0.4 to 6.3 ± 0.7 for %HDS). Bull effect was significant in both cases ($p < .001$). We detected a significant interaction bull $\times$ season ($p < .001$), with season being significant in 4 bulls for %DFI ($p < .05$) and in 2 bulls for %HDS ($p < .001$). The seasonal dynamics differed between bulls, but changes were small. Incubation induced a small, non-significant increase of %DFI and a decrease of %HDS ($p < .1$). In conclusion, this survey shows excellent chromatin status after thawing, with no susceptibility to undergo further damage. Chromatin status seems to be an individual characteristic of each bull.

CACHI, A. M., WUNSCH, A., VILANOVA, A., GUARDIA, M., CIORDIA, M. & ALETA, N. 2017. S-locus diversity and cross-compatibility of wild Prunus avium for timber breeding. *Plant Breeding*, 136, 126-131

Abstract: Prunus avium is primarily cultivated for its fruit, sweet cherries. However, it is also used to produce high-quality timber. In a P. avium seed orchard, gametophytic self-incompatibility is a restriction for free pollen flow and should be considered when establishing basic forest materials. In this study, S-locus diversity and cross-incompatibility of wild cherry individuals in clonal banks established for breeding for timber production were investigated. Wild cherry trees (140) with outstanding forest growth habit, collected in northern Spain, grafted and planted in two clonal banks, were genotyped at the S-locus. The self-incompatibility S-locus genes, S-RNase and SFB, were analysed by PCR. Twenty-two S-haplotypes, resulting in 72 different S-genotypes, were identified. The genotypes were grouped into 33

incompatibility groups and 39 unique genotypes. This initial S-locus analysis revealed large genetic diversity of wild cherry trees from the Spanish northern deciduous forest, and provides useful information for seed orchard design. Wild *P. avium* displays significantly more genetic diversity than what is detected in local cultivars, revealing a narrowing of genetic diversity during local domestication. (DOI: 10.1111/pbr.12450)

CAMPA, A. & FERREIRA, J.J. 2017. Gene coding for an elongation factor is involved in resistance against powdery mildew in common bean. *Theoretical and Applied Genetics*, 130, 849-860.

Abstract: Key message Genetic control of the resistance response against powdery mildew in common bean was studied combining genetic, genomic and transcriptomic analyses. A candidate resistance gene in cultivar Porrillo Sintetico was proposed. The species causing the fungal disease powdery mildew (PM) in the local common bean crop was identified as *Erysiphe polygoni* through the molecular analysis of the internal transcribed spacer region. A genetic analysis of the resistance in cultivar Porrillo Sintetico was conducted using different F-2:3 populations, and a dominant gene conferring total resistance against a local PM isolate was physically located between 84,188 and 218,664 bp of chromosome Pv04. An in silico analysis of this region, based on the common bean reference sequence, revealed four genes candidate to be involved in the resistance reaction. Relative expression levels of these genes after PM infection showed a significant over-expression of the candidate gene Phvul.004G001500 in the resistant genotype Porrillo Sintetico. This gene was re-sequenced in the parental genotypes X2776 and Porrillo Sintetico to explain their different phenotypic responses against PM. Several substitutions were identified in exon regions, all of them synonymous, so differences in the produced amino acid sequence were not expected. However, a total of 37 mutations were identified in non-coding regions of the gene sequence, suggesting that intron variation could be responsible for the different gene expression levels after PM infection. No evidence of other regulatory mechanisms, such as alternative splicing or methylation, was identified. Candidate resistance gene Phvul.004G001500 codes for an elongation factor that is not a typical gene related to recognition of specific pathogens in plants, suggesting its involvement in the resistance through plant immune system. (DOI: 10.1007/s00122-017-2864-x)

CAMPA, A., TRABANCO, N. & FERREIRA, J.J. 2017. Identification of Clusters that Condition Resistance to Anthracnose in the Common Bean Differential Cultivars AB136 and MDRK. *Phytopathology*, 107, 1515-1521

Abstract: The correct identification of the anthracnose resistance systems present in the common bean cultivars AB136 and MDRK is important because both are included in the set of 12 differential cultivars proposed for use in classifying the races of the anthracnose causal agent, *Colletotrichum lindemuthianum*. In this work, the responses against seven *C. lindemuthianum* races were analyzed in a recombinant inbred line population derived from the cross AB136 x MDRK. A genetic linkage map of 100 molecular markers distributed across the 11 bean chromosomes was developed in this population to locate the gene or genes conferring resistance against each race, based on linkage analyses and chi-square tests of independence. The identified anthracnose resistance genes were organized in clusters. Two clusters were found in AB136: one located on linkage group Pv07, which corresponds to the anthracnose resistance cluster Co-5, and the other located at the end of linkage group Pv11, which corresponds to the Co-2 cluster. The presence of resistance genes at the Co-5 cluster in AB136 was validated through an allelism test conducted in the F2 population TU x AB136. The presence of resistance genes at the Co-2 cluster in AB136 was validated through genetic dissection using the F2:3 population ABM3 x MDRK, in which it was directly mapped to a genomic position between 46.01 and 47.77 Mb of chromosome Pv11. In MDRK, two independent clusters were identified: one located on linkage group Pv01 corresponding to the Co-1 cluster, and the second located on LG Pv04 corresponding to the Co-3 cluster. This report enhances the understanding of the race-specific *P. vulgaris*-*C. lindemuthianum* interactions and will be useful in breeding programs. (DOI: 10.1094/phyto-01-17-0012-r)

CASAL, C., INFANTES, J. A., RISALDE, M. A., DIEZ-GUERRIER, A., DOMINGUEZ, M., MORENO, I., ROMERO, B., DE JUAN, L., SAEZ, J. L., JUSTE, R., GORTAZAR, C., DOMINGUEZ, L. & BEZOS, J. 2017. Antibody detection tests improve the sensitivity of tuberculosis diagnosis in cattle. *Research in Veterinary Science*, 112, 214-221.

Abstract: We evaluated the sensitivity (Se) of the single cervical intradermal tuberculin (SIT) test, two interferon-gamma (IFN-gamma) assays and three different antibody detection techniques for bovine tuberculosis (bTB) diagnosis in 131 mixed beef breed cattle. The results of the diagnostic techniques performed over the whole herd, and over the animals confirmed as infected based on the presence of lesions compatible with the disease and/or *M. bovis* isolation were compared to determine apparent prevalence (AP) and Se. The Se of the SIT test (severe interpretation) was 63.7% (95% CI, 54.54-72.00), while the Se of the IFN-gamma assays ranged between 60.2% and 92%. The proportion of infected cattle detected by the different antibody detection techniques ranged from 65.5% to 87.6%. Three of the antibody detection techniques yielded a significant higher ($p < 0.05$) Se than that achieved with the official diagnostic techniques. In addition, the interpretation in parallel of cellular and antibody detection techniques reached the highest Se: 98.2% (95% CI, 93.78-99.51) suggesting that the use of diagnostic techniques detecting both cellular and humoral responses could be considered as an alternative in the control of bTB outbreaks in high prevalence settings. (DOI: 10.1016/j.rvsc.2017.05.012)

DE LA ROZA-DELGADO, B., GARRIDO-VARO, A., SOLDADO, A., GONZALEZ ARROJO, A., CUEVAS VALDES, M., MAROTO, F. & PEREZ-MARIN, D. 2017. Matching portable NIRS instruments for in situ monitoring indicators of milk composition. *Food Control*, 76, 74-81.

Abstract: The real time knowledge of dairy milk composition can be used as a tool to guarantee milk quality and safety, offering additional information for dairy producers and consumers. To carry out these in situ analyses, methodologies based on Near Infrared (NIR) portable sensors have a great potential as an advisory tool. The main goals of the present work have been to develop a methodology using a hand-held portable NIR spectrophotometer to collect raw milk spectra, including the development of calibration models for the analysis of protein, fat and solids-non-fat (SNF) of raw milk and further to transfer the developed models to another portable unit. A total of 542 fresh milk samples were scanned over the NIR spectral range (1600-2400 nm) using a hand-held MicroPhazir (TM) (MP) NIR spectrometer and different instrumental configurations. The best results for repeatability and reproducibility calculated as root mean squared (RMS) were obtained using a 17 mm cuvette thickness. The displayed predictive ability of calibration models measured as Standard error of prediction/Standard error of cross validation were 0.96; 0.72 and 0.83 for fat, protein and SNF contents, respectively. For cloning purposes an additional MP unit (satellite) has been used. A standardization set of 10 samples enabled standardization of both instruments. After applying standardization matrix, Standard error of differences between master and satellite reached great reduction, 68% for fat, 66% for protein and 54% for SNF. Moreover, the demonstrated ability of sharing calibration models among several units is essential for implementation of portable instruments for in-situ analysis to provide indicators of milk composition at farm level. (DOI: 10.1016/j.foodcont.2017.01.004)

ESPESCHIT, I. F., SCHWARZ, D. G. G., FARIA, A. C. S., SOUZA, M. C. C., PAOLICCHI, F. A., JUSTE, R. A., CARVALHO, I. A. & MOREIRA, M. A. S. 2017. Paratuberculosis in Latin America: a systematic review. *Tropical Animal Health and Production*, 49, 1557-1576.

Abstract: Latin America is the definition of the American group, where languages of Latin origin are spoken, including countries in South, Central, and North America. Paratuberculosis is a gastrointestinal contagious chronic disease that affects ruminants, whose etiological agent is the bacilli *Mycobacterium avium* subsp. *paratuberculosis* (MAP). Paratuberculosis is characterized by intermittent diarrhea, decreased milk production, dehydration, and progressive weight loss and is possibly involved in Crohn's disease, a human intestinal disease. MAP is resistant to environmental factors, pasteurization, and water disinfection, which coupled with the subclinical-clinical nature of the disease, and makes paratuberculosis a relevant socioeconomic and public health issue, justifying the descriptive review of research on the disease carried out in Latin American countries. A survey of articles, published until September 2016, on the Scopus database, PubMed, Agris, and Science Direct, about detection of the agent and the disease in Latin America, without restrictions to the date of the research was performed. The keywords were as follows: "paratuberculosis," "*Mycobacterium avium* subsp. *paratuberculosis*," "cattle," "milk," "wildlife," "goat," "ovine," "dairy," and the name of each country in English. Studies found from nine of the 20 Latin America countries, 31 related to Brazil, 17 to Argentina, 14 to Chile, eight to Colombia, six to Mexico, two to Peru, two to Venezuela, and one to Panama and to Bolivia, each. The agent was detected in cattle, goats, sheep, domesticated water buffalo, and wild animals. Microbiological culture, PCR, and ELISA were the frequent techniques. The small number of studies may result in overestimation or underestimation of the real scenario (.DOI: 10.1007/s11250-017-1385-6)

ESPI, A., DEL CERRO, A., SOMOANO, A., GARCIA, V., PRIETO, J. M., BARANDIKA, J. F. & GARCIA-PEREZ, A. L. 2017. *Borrelia burgdorferi* sensu lato prevalence and diversity in ticks and small mammals in a Lyme borreliosis endemic Nature Reserve in North-Western Spain. Incidence in surrounding human populations. *Enfermedades Infecciosas Y Microbiología Clínica*, 35, 563-568.

Abstract: To determine the prevalence and diversity of *Borrelia burgdorferi* sensu lato (s.l.) in an endemic Nature Reserve (Sierra del Sueve) in North-Western Spain, and the risk of human exposure to infected ticks in Asturias, 1013 questing ticks and 70 small mammals were collected between 2012 and 2014. A retro-spective descriptive analysis was also carried out on human Lyme borreliosis (LB) cases reported to the local hospital (Cabezas). Samples were screened for *B. burgdorferi* s.l. presence by a nested PCR assay, and genospecies were confirmed by sequencing. *B. burgdorferi* s.l. was detected in 1.4% (12/845) of *I. ricinus* questing nymphs, 9.1% (2/33) of questing adults, and 12.9% (9/70) of small mammals, as well as in the other tick species. PCR positive samples of 17 questing tick and 6 small mammals were sequenced. Four genospecies were identified: *B. afzelii*, *B. garinii*, *B. lusitaniae*, and *B. valaisiana*. Phylogenetic analyses based on the *flaB* genes showed the heterogeneity of *B. afzelii* in this area. The detection of *B. burgdorferi* s.l. among questing ticks and small mammals in the study area, as well as the abundance of ticks and of large wild and domestic mammals, indicate a high risk of infection by *B. burgdorferi* s.l. in the area. Reporting of LB cases to the local hospital support this, and shows the need of thorough monitoring of *B. burgdorferi* infection in ticks and hosts in the area. More investigations are needed to assess the role of different wildlife species and the risk of transmission to humans. (DOI: 10.1016/j.eimc.2016.06.011)

FUEYO, C., TAMARGO, C., CAAMAÑO, J. N., SALMAN, A., MARTINEZ-PASTOR, F., FERNANDEZ-ALEGRE, E., CARBAJO, M. & HIDALGO, C. O. 2017. Characterization of overall motility and subpopulations in cryopreserved semen of the Spanish native cattle breed "Asturiana de la Montaña". *Reproduction in Domestic Animals*, 52, 100-100.

Abstract: Germplasm banks must be surveyed in order to assure the integrity and usefulness of the material. We carried out a survey of the SERIDA semen bank for the cattle breed "Asturiana de la Montaña", assessing motility by CASA (Computer-aided Sperm Analysis) in 34 bulls, with semen collected in different seasons. Three straws were thawed (37°C, 30 s) for each bull and season. Standard motility parameters and subpopulations (clustering of CASA data), were analysed by general linear models (results as mean $\pm$ SEM). Clustering yielded 3 subpopulations: (S1) slow and non-linear, (S2) fast and linear and (S3) slow and linear (overall % were: 18.1 ± 0.7 , 41.5 ± 1.8 , 40.4 ± 1.8), with S2 and S3

moderately correlating with total motility ($r = .33$ and $-.29$ respectively with $p < .01$). The season did not influence motility parameters or subpopulation proportions. The bull effect was always significant ($p < .001$). Total and progressive motility (MOT) were $39.1\% \pm 1.2\%$ and $27.2\% \pm 1.1\%$ overall, with a great dispersion among bulls. Bulls clustered roughly in three groups: Higher MOT/S2 and low S1, low S3 and variable MOT/S1/S2, and high S1 and low MOT/S2 with variable S3. In conclusion, the survey shows that the doses in the cryobank widely vary in motility, possibly reflecting bull freezability. (Poster 53).

GOMEZ, E., CARROCERA, S., MARTIN, D., SANCHEZ-CALABUIG, M. J., GUTIERREZ-ADAN, A., MURILLO, A. & MUÑOZ, M. 2017a. Hepatoma-derived growth factor: Protein quantification in uterine fluid, gene expression in endometrial-cell culture and effects on in vitro embryo development, pregnancy and birth. *Theriogenology*, 96, 118-125.

Abstract: Artemin a member of the glial cell line-derived neurotrophic factor (GDNF) family is present in mice and human preimplantation embryos, and reproductive tract, during early pregnancy promoting embryo development in vitro. The presence of artemin in cattle embryos and reproductive tract, however, is unknown. In the present work we identified for first time artemin in bovine uterine fluid (UF) (Western blot), endometrium (RT-PCR, Western blot and immunohistochemistry) and embryos (RT-PCR and immunohistochemistry) during early preimplantation development. In addition, GFRalpha3, a component of the artemin receptor was localized in blastocysts produced in vitro. Individually developing embryos released ARTEMIN in culture medium and triggered ARTEMIN mRNA down-regulation in epithelial cells from endometrial cell cultures. Our results suggest that ARTEMIN derived from early embryos and maternal reproductive tract may exert important roles during early development in cattle. (DOI: 10.1016/j.theriogenology.2016.12.007)

GOMEZ, E., CARROCERA, S., UZBEKOVA, S., MARTIN, D., MURILLO, A., ALONSO-GUERVOS, M., GOYACHE, F. & MUÑOZ, M. 2017b. Protein in culture and endogenous lipid interact with embryonic stages in vitro to alter calf birthweight after embryo vitrification and warming. *Reproduction Fertility and Development*, 29, 1932-1943.

Abstract: Short-term protein removal in vitro improves long-term blastocyst competence to survive vitrification. We investigated the mechanisms and effects underlying protein removal. Day-6 morulae and early blastocysts were cultured individually with and without protein for 24 h. Development and lipid content were analysed in expanded blastocysts derived from morulae (M-XB) and from early blastocysts (EB-XB). Expression of genes involved in lipid metabolism, stress responses and apoptosis was analysed in fresh and vitrified-warmed M-XB produced with and without protein. Pregnancy rates, birth rates and birthweight (BW) were recorded after transfer of embryos. Day-7 EB-XB production rates (with, 66.9 ± 6.2 and without, 68.8 ± 6.0 protein) were higher than M-XB rates (with, 21.4 ± 4.6 and without, 9.4 ± 4.6 protein; $P < 0.005$). EB-XB showed fewer lipids than M-XB ($P = 0.03$). In fresh M-XB, expression of sterol regulatory element binding protein (SREBP1) was lower with (4.1 ± 2.2) than without (13.6 ± 2.2) protein, contrary to results obtained for Patatin-like phospholipase domain containing 2, Hormone-sensitive lipase and Bcl-2-associated X protein ($P < 0.05$). Protein did not affect pregnancy rates and birth phenotypes ($P = 0.05$). However, BW was higher ($P < 0.01$) in calves born from vitrified M-XB (48.6 ± 3.4 kg) than from EB-XB (39.8 ± 2.9 kg). Such effects were more pronounced in females ($P < 0.001$). Calves from fresh embryos did not show BW differences. These results indicate that embryonic kinetics and vitrification impact birth phenotypes, at least in females. Alterations might involve exogenous protein and mobilisation of lipid stocks. (DOI: 10.1071/rd16213)

GOMEZ, E., HUMBLLOT, P., NTALLARIS, T., DALBIES, R., RICHARD, C., FERNANDEZ-GONZALEZ, A., MARTIN, D. & MUNOZ, M. **2017c**. Lack of impact of low energy diets on metabolomic analysis of cow follicular and uterine fluids. *Reproduction in Domestic Animals*, 52, 78-78.

GOMEZ, E., MARTIN, D., CARROCERA, S., SANCHEZ-CALABUIG, M. J., GUTIERREZ-ADAN, A., ALONSO-GUERVOS, M., PEYNOT, N., GIRAUD-DELVILLE, C., SANDRA, O., DURANTHON, V. & MUÑOZ, M. **2017d**. Expression and localization of ARTEMIN in the bovine uterus and embryos. *Theriogenology*, 90, 153-162.

Abstract: Artemin a member of the glial cell line-derived neurotrophic factor (GDNF) family is present in mice and human preimplantation embryos, and reproductive tract, during early pregnancy promoting embryo development in vitro. The presence of artemin in cattle embryos and reproductive tract, however, is unknown. In the present work we identified for first time artemin in bovine uterine fluid (UF) (Western blot), endometrium (RT-PCR, Western blot and immunohistochemistry) and embryos (RT-PCR and immunohistochemistry) during early preimplantation development. In addition, GFRalpha3, a component of the artemin receptor was localized in blastocysts produced in vitro. Individually developing embryos released ARTEMIN in culture medium and triggered ARTEMIN mRNA down-regulation in epithelial cells from endometrial cell cultures. Our results suggest that ARTEMIN derived from early embryos and maternal reproductive tract may exert important roles during early development in cattle.(DOI: 10.1016/j.theriogenology.2016.12.007)

GOMEZ, E., MUÑOZ, M., SIMO, C., IBANEZ, C., CARROCERA, S., MARTIN-GONZALEZ, D. & CIFUENTES, A. **2017e**. Non-invasive metabolomics for improved determination of embryonic sex markers in chemically defined culture medium (vol 1474, pg 138, 2017). *Journal of Chromatography A*, 1526, 167-167.

GOMEZ, E., SANCHEZ-CALABUIG, M. J., MARTIN, D., CARROCERA, S., MURILLO, A., CORREIA-ALVAREZ, E., HERRERO, P., CANELA, N., GUTIERREZ-ADAN, A., ULBRICH, S. & MUÑOZ, M. **2017f**. Invitro 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 invitro-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. (DOI: 10.1016/j.theriogenology.2017.11.038)

GREMA, M., TRAORE, A., ISSA, M., HAMANI, M., ABDOU, M., FERNANDEZ, I., SOUDRE, A., ALVAREZ, I., SANOU, M., TAMBOURA, H. H., ALHASSANE, Y. & GOYACHE, F. 2017. Morphological assessment of Niger Kuri cattle using multivariate methods. *South African Journal of Animal Science*, 47, 505-515

Abstract: A total of 406 adult cows and 34 bulls belonging to the Niger Kuri cattle population were assessed for 16 body measurements and 11 qualitative traits to contribute to the characterization of this unique cattle breed. Body measurements included facial, horn, ear and rump lengths; facial, cranial, shoulder, pelvic and ischium widths; height at withers and at hips; muzzle circumference; heart girth; body and tail lengths; and thorax depth. Qualitative traits included cephalic profile, ear shape, muzzle pigmentation, eyelid pigmentation, hoof pigmentation, horn colour, dewlap size, backline profile, horn shape, spotting pattern, and coat colour pattern. Data were analysed jointly with 377 individuals from four other West African taurine cattle breeds (N'Dama, Lagunaire, Lobi and Somba) using multivariate statistical methods, including canonical and correspondence analyses. Among the breeds analysed, Kuri cattle had the highest mean values for all body measurements: height at withers and body length had mean values of 124.0 $\pm$ 0.4 and 146.8 $\pm$ 1.0 cm in Kuri cows, respectively, and 126.0 $\pm$ 2.2 and 155.7 $\pm$ 3.6 cm, respectively, in Kuri bulls. Canonical analysis allowed the construction of contour plots to illustrate the high differentiation between Kuri cattle and the other breeds, regardless of the sex of the individuals. Further, the Mahalanobis distance matrices showed that pairs involving Kuri cattle had the higher differentiation of these populations. Correspondence analysis carried out on these 11 qualitative traits allowed the researchers to ascertain a clear differentiation between the Kuri and the other taurine cattle breeds. The N'Dama, Lagunaire, Lobi, and Somba breeds did not show clear differentiation at qualitative-type trait level with 75% confidence regions computed for these four breeds being highly intermingled. In the current data, Kuri cattle had the highest frequency in qualitative features, such as concave cephalic profile, dropped ears, non-pigmented muzzle and grey-coloured horns, which are absent in West African taurine and zebu cattle breeds, according to the literature. The current evidence would suggest that unique Kuri cattle type features may result from breeding decisions rather than from zebu admixture. This work confirms that at type trait level Kuri cattle is a unique population within the West African taurine cattle group. The implementation of genetic analyses aiming at ascertaining the degree of uniqueness of the breed is advised. (DOI: 10.4314/sajas.v47i4.9)

JORDANA, J., GOYACHE, F., FERRANDO, A., FERNANDEZ, I., MIRO, J., LOARCA, A., MARTINEZ LOPEZ, O. R., CANELON, J. L., STEMMER, A., AGUIRRE, L., LARA, M. A. C., ALVAREZ, L. A., LLAMBI, S., GOMEZ, N., GAMA, L. T., MARTINEZ, R. D., PEREZ, E., SIERRA, A., CONTRERAS, M. A., LANDI, V., MARTINEZ, A. & DELGADO, J. V. 2017. Contributions to diversity rather than basic measures of genetic diversity characterise the spreading of donkey throughout the American continent. *Livestock Science*, 197, 1-7.

Abstract : Donkey was introduced into the Americas soon after its discovery in the 15th century. However, there is no historical consensus on how they spread across the continent. In a previous study, two distinct genetic pools (Clusters A -Southern part - and B -Northern part of South America and Central America) were identified, with likely confluence in Colombia. The aim of this study was to evaluate whether the main genetic diversity parameters, such as gene diversity (GD) and allelic richness (k), or the relative contributions of various breeds to these parameters are useful indicators to give genetic support to historical information on putative routes of the spreading of donkeys across the American continent. In full agreement with historical sources suggesting that Greater Antilles were the first breeding nucleus, both total contributions to gene diversity (gGDT) and to allelic richness (C-T((k))) showed a higher ability to identify the "abundant centre" of the species on the Continent. Even though there are historical reports suggesting various entry points of the donkey into the continent (e.g. in Brazil), these parameters

suggested that, in our dataset, the Cuban donkey population was the more likely representative of the first breeding nucleus of the species. Central and South American donkey populations in the surroundings of the Caribbean Gulf would more likely be early derivatives of Antillean donkey. The strong North-South genetic structure was confirmed for the American donkey metapopulation. Current analyses suggest that populations classified into Cluster A (South) are essentially a sample of the genetic background of Cluster B (North). The Andean route had the highest importance in the formation of the South American populations. The extinction of either population belonging to Cluster B could lead to a decrease in overall genetic diversity both at the gene diversity level (negative gGDT values) and the allelic richness level (positive $Or(k)$ contributions). The opposite pattern is found for populations belonging to Cluster A. The extinction of the populations belonging to Cluster B would decrease the overall American donkey gene diversity in roughly 8% and would dramatically affect the number of alleles in the metapopulation (19.1%). However, the extinction of the donkey populations classified into Cluster A would increase overall gene diversity by 2.2%. Although, the genetic scenario of each individual population varies substantially, the joint conservation of the donkey populations classified into both Clusters A and B is highly advised. (DOI: 10.1016/j.livsci.2016.12.014)

LOPEZ LOPEZ, C., FERREIRA, L. M. M., GARCIA, U., MORENO-GONZALO, J., RODRIGUES, M. A. M., OSORO, K., FERRE, I. & CELAYA, R. 2017. Diet selection and performance of horses grazing on different heathland types. *Animal*, 11, 1708-1717

Abstract: The number of horses in northern Spanish mountains has increased in recent decades, but little is known about their grazing behaviour, performance and potential for foal meat production. This research aimed to study the diet selection, liveweight (LW) changes and parasitic status of dry and lactating mares, and foals' LW gains, grazing on heathlands with different botanical composition. The experimental design consisted of three vegetation types: dominated by heather (*Ericaceae*) species (H), dominated by gorse (*Ulex gallii*; G) and co-dominated by gorse and heath-grasses (G-G), with four replicates per treatment (12 paddocks of 1.2 ha). The study lasted three grazing seasons (2010-12). Each year, 24 crossbred mature mares (310 $\pm$ 52 kg LW) were used, managing one lactating mare with her foal plus one non-lactating mare per paddock from May to late summer or early autumn. In the case of H paddocks, animals had to be removed before (late August to early September) because of apparent loss of body condition. Animals were periodically weighed. Mares' diet composition was estimated using alkane markers, analysing the discrepancies in alkane concentrations between dietary plant components and faeces. Faecal samples were also analysed for gastrointestinal nematodes ova. Chemical composition of the main plant components (i.e. heather, gorse and grasses) revealed a low nutritive value, averaging 79, 115 and 113 g CP/kg dry matter (DM), respectively, that could restrict livestock performance. Mares initially selected gorse and grasses (0.47 and 0.40, respectively, in 2010), increasing heather consumption over time (from 0.13 in 2010 to 0.29 in 2012) as gorse availability decreased. The performance of both mares and foals was lower in H compared with G and G-G paddocks (-216 v. 347 g/day for mares, $P < 0.01$; 278 v. 576 g/day for foals, $P < 0.05$), whereas LW changes were more favourable in dry mares than in lactating ones (241 v. 78 g/day; $P < 0.05$). Small strongyle (*Cyathostominae*) egg counts in mares' faeces increased across the grazing season with no differences between treatments. These results indicate that grazing by horses on gorse- and grass-gorse-dominated shrublands could be sustainable at least during part of the year (4 to 6 months). However, heather-dominated heathlands are not able to meet the nutritional needs of horses even for a short time (2 to 4 months). Nevertheless, the low nutritive quality of these vegetation communities, especially in autumn, requires animal access to other pastures with a higher nutritive value, or supplementary feeding, to enhance foals' growth and maintain sustainable grazing systems with productive herds. (DOI: 10.1017/s1751731117000465)

MADRERA, R. R., BEDRIÑANA, R. P. & VALLES, B. S. 2017. Enhancement of the nutritional properties of apple pomace by fermentation with autochthonous yeasts. *Lwt-Food Science and Technology*, 79, 27-33.

Abstract: This paper focuses on the enhancement of the nutritional composition and phenolic compounds of apple pomace by solid state fermentation with autochthonous cider yeasts. Under the tested fermentation conditions (7 days, 25 degrees C), 3 yeast strains (*S. cerevisiae*, ref: 32; *S. bayanus*, ref: C6; and *H. uvarum*, ref: 62) were able to deplete fermentable sugars. Significant increases in protein (23-49%), fat (17-39%) and dietary fibre (30-41%) were detected in all cases with respect to unfermented apple pomace. The biotransformation increased the content of phenolic compounds, mainly quercetin and phloretin derivatives, as well as that of oleic and linoleic acids. The information derived from this study is relevant to revalorize apple pomace as a nutritive and functional foodstuff allowing the production of enriched foods. (DOI: 10.1016/j.lwt.2017.01.021)

MARTINEZ-SASTRE, R., RAVERA, F., GONZALEZ, A. J., LOPEZ SANTIAGO, C., BIDEGAIN, I. & MUNDA, G. 2017. Mediterranean landscapes under change: Combining social multicriteria evaluation and the ecosystem services framework for land use planning. *Land Use Policy*, 67, 472-486.

Abstract: Mediterranean cultural landscapes are currently undergoing intense transformations, resulting in a polarization of land uses across an intensification-abandonment continuum. This transformation is characterized by uncertain and non-linear interactions and impacts on ecosystem services and human wellbeing. Our study focuses on a particular multifunctional Mediterranean cultural landscape composed of different land uses (i.e., dehesas, olive groves, pine forests, Mediterranean forests and scrublands) in the Sierra Morena mountain range (Jaén, Spain), which is undergoing a rapid process of land use change. In this context, the involvement of local stakeholders through participatory processes is critical for proper landscape management and decision-making. The aim of this paper is to combine Social Multicriteria Evaluation (SMCE) and ecosystem services frameworks for an assessment of likely future scenarios under different drivers of land use change, analysing how such changes would affect people living or making use of ecosystem services. Among four plausible future scenarios, the so-called "Mosaic landscape" scenario was widely recognized as the most desirable future landscape configuration for Sierra Morena, as it allows the supply of a balanced flow of ecosystem services and reduces ecosystem services trade-offs and conflicts among stakeholders. The combination of social multicriteria evaluation with the ecosystem services framework and future scenario analysis allows a robust co-creation of knowledge and provides insights pertinent to participatory management of cultural landscapes and stakeholders' relationships through a socially relevant but also rigorous methodology (DOI:10.1016/j.landusepol.2017.06.001)

MATEO, M., HERNANDEZ DE MINGO, M., DE LUCIO, A., MORALES, L., BALSEIRO, A., ESPI, A., BARRAL, M., LIMA BARBERO, J. F., ANGEL HABELA, M., FERNANDEZ-GARCIA, J. L., CALERO BERNAL, R., KOSTER, P. C., CARDONA, G. A. & CARMENA, D. 2017. Occurrence and molecular genotyping of *Giardia duodenalis* and *Cryptosporidium* spp. in wild mesocarnivores in Spain. *Veterinary Parasitology*, 235, 86-93.

Abstract: There is a surprisingly scarce amount of epidemiological and molecular data on the prevalence, frequency, and diversity of the intestinal protozoan parasites *Giardia duodenalis* and *Cryptosporidium* spp. in wildlife in general and mesocarnivore species in particular. Consequently, the extent of the cyst/oocyst environmental contamination attributable to these wild host species and their potential implications for public veterinary health remain largely unknown. In this molecular epidemiological survey a total of 193 individual faecal samples from badgers (*Meles meles*, *n* = 70), ferrets (*Mustela putorius furo*, *n* = 2), genets (*Genetta genetta*, *n* = 6), Iberian lynxes (*Lynx pardinus*, *n* = 6), beech martens (*Martes foina*, *n* = 8), mongooses (*Herpestes ichneumon*, *n* = 2), otters (*Lutra lutra*, *n* = 2), polecats (*Mustela putorius*, *n* = 2), red foxes (*Vulpes vulpes*, *n* = 87), wildcats (*Felis silvestris*, *n* = 2), and wolves (*Canis lupus*, *n* = 6) were obtained from road-killed, hunted, and accidentally found carcasses,

and from camera-trap surveys or animals entering rescue shelters, during the period December 2003 to April 2016. Investigated specimens were collected in five Spanish autonomous regions including Andalusia ($n = 1$), Asturias ($n = 69$), Basque Country ($n = 49$), Castile-La Mancha ($n = 38$), and Extremadura ($n = 36$). The presence of cysts/oocysts was confirmed by PCR-based methods targeting the small subunit (*ssu*) ribosomal RNA gene of these parasite species. Genotyping of the obtained isolates were attempted at appropriate markers including the glutamate dehydrogenase (*Guodenalis*) and the 60-kDa glycoprotein (*C. parvum* and *C. ubiquitum*) loci. Overall, *G. duodenalis* was detected in 8% (7/87) of red foxes, a single beech marten, and a single wolf, respectively. *Cryptosporidium* was identified in 3% (2/70) of badgers, 8% (7/87) of red foxes, a single genet, and a single mongoose, respectively. None of the nine *G. duodenalis* isolates generated could be genotyped at the assemblage/sub assemblage level. Out of the nine *Cryptosporidium* isolates successfully characterized, three were identified as *C. canis* (one in a mongoose and two in red foxes), and three as *C. parvum* (one in a badger and three in red foxes). The remaining three isolates were assigned to *C. felis* (in a red fox), *C. hominis* (in a badger), and *C. ubiquitum* (in a red fox), respectively. Two additional *Cryptosporidium* isolates infecting a badger and a genet, respectively, were untypable. The red fox was confirmed as a suitable host of potentially zoonotic *Cryptosporidium* species, mainly *C. parvum* and *C. ubiquitum*. The high mobility and wide home range of red foxes, together with their increasing presence in urban and peri-urban settings, may lead to the overlapping of sylvatic and domestic cycles of the parasite, and consequently, to an increased risk of cryptosporidiosis in production animals and humans. The detection of *C. hominis* oocysts in a badger raises the question of whether this finding represents a true infection or a sporadic event of mechanical passage of *C. hominis* oocyst of anthroponotic origin. (DOI: 10.1016/j.vetpar.2017.01.016)

MIÑARRO, M., SOMOANO, A. & VENTURA, J. 2017. Intra-annual continuous reproduction of the apple pest *Microtus lusitanicus*: Implications for management. *Crop Protection*, 96, 164-172.

Abstract: The Lusitanian pine vole (*Microtus lusitanicus*) is a key pest of apple in Asturias (NW Spain). The sustainable management of populations of this vole requires a deep knowledge of relevant aspects of its biology and ecology. To improve our knowledge on the breeding characteristics of *M. lusitanicus*, we analysed a sample of 700 individuals collected monthly in apple orchards during two years. The continuous occurrence of immature specimens and pregnant females during the whole study period indicates that *M. lusitanicus* can breed continuously throughout the year in apple orchards in Asturias. The relatively mild weather conditions in winter and the presence of a permanent grass cover throughout the year seem to be favourable conditions for continuous breeding of the vole. Nevertheless, some data (decrease in the size of testes and seminal vesicle of mature males in winter, lower percentage of pregnant females in December and of non-mature specimens in March) suggest that reproduction activity decreases slightly in winter. Mean litter size was relatively low in comparison with that reported for other *Microtus* species. The balanced adult sex-ratio and the relatively small testis size are congruent with a monogamous mating system. Nevertheless, the frequent coexistence of matures with two or more other adult voles of the opposite sex, and even the cohabitation of one male with two pregnant females, suggest that monogamy was facultative in our population. This study adds significantly to the current knowledge on the biology of this vole pest and can help in the design and implementation of sustainable and efficient management plans. As a first step, control practices should be deseasonalized and applied throughout the year. (DOI:10.1016/j.cropro.2017.02.020)

MODROÑO, S., SOLDADO, A., MARTINEZ-FERNANDEZ, A. & DE LA ROZA-DELGADO, B. 2017. Handheld NIRS sensors for routine compound feed quality control: Real time analysis and field monitoring. *Talanta*, 162, 597-603.

Abstract: Significant advances achieved in different sensor technologies and computer processing data have made possible to respond the needs of livestock sector, providing precise and rapid information on feed composition, being an alternative to real time quality control on compound feed the use of handheld NIRS sensors. This work aimed to evaluate two hand-held portable NIR spectrophotometers for on-site and real time analysis of nutritive parameters in raw compound feed: Phazir 1624 Polychromix Inc (PhIR) and MicroNIR™ 1700 by JDSU (MICRO). For computing data, different

combinations of pre-treatments and multivariate statistical methods have been assayed to extract the valuable information of spectra data and to develop appropriate calibrations. The calibration models displayed greatest predictive capacity for Crude Protein, Crude Fiber and Starch and the determination coefficients of cross validation were 0.90 – 0.88 for CP, 0.85-0.91 for CF, 0.89- 0.88 and 0.89-0.91 for STCH using PhIR and MICRO instruments respectively. Dry Matter showed the lowest determination coefficients of cross validation 0.67-0.73. Accuracy achieved 99-101 % for both NIRS instruments and no differences were found when applying tstudent-test comparing reference and predicted data. Results obtained with both instruments were compared by using standard deviation and not significant differences were observed at the 5% level. Results so far have demonstrated the potential of these handheld NIRS instruments proposed here to estimate the individual compound feeds composition changes at farms level instantly, time avoiding the disadvantage of moving the samples to the lab. (DOI: 10.1016/j.talanta.2016.10.075)

MOUSSA, M. M. A., ISSA, M., TRAORE, A., GREMA, M., HAMANI, M., FERNANDEZ, I., SOUDRE, A., ALVAREZ, I., SANOU, M., TAMBOURA, H. H., ALHASSANE, Y. & GOYACHE, F. **2017**. Morphological assessment of the Zebu Bororo (Wodaabe) cattle of Niger in the West African zebu framework. *Archives Animal Breeding*, 60, 363-371.

Abstract: A total of 357 adult cows and 29 sires belonging to the long-horned Niger Zebu Bororo cattle population were assessed for 13 body measurements and 11 qualitative traits. Data were jointly analysed with 311 cows and 64 sires belonging to other four West African zebu cattle populations, sampled in Burkina Faso and Benin, representative of both the short-horned and the long-horned West African zebu groups using multivariate statistical methods. Besides the other long-horned zebu breed analysed (Zebu Mbororo of Burkina Faso), Zebu Bororo cattle tended to have the highest mean values for all body measurements. Mahalanobis distance matrices further informed that pairs involving Zebu Bororo cattle had the higher differentiation of the dataset. However, contour plots constructed using eigenvalues computed via principal component analysis (PCA) illustrated a lack of differentiation among West African zebu cattle populations at the body measurements level. Correspondence analysis carried out on the 11 qualitative traits recorded allowed for ascertaining a clear differentiation between the Zebu Bororo and the other zebu cattle populations analysed which, in turn, did not show a clear differentiation at the qualitative type traits level. In our data, Zebu Bororo cattle had in high frequency qualitative features such as dropped ears, lyre-shaped horns and red-pied coat colour that are not frequently present in the other West African zebu populations analysed. A directional selection due to a rough consensus of the stock-keepers may be hypothesised. Performance of further analyses to assess the degree in which such breeding differences may be related to genetic or production differences are advised. (DOI: 10.5194/aab-60-363-2017)

MUÑOZ, M., MARTIN, D., CARROCERA, S., ALONSO-GUERVOS, M., MORA, M. I., CORRALES, F. J., PEYNOT, N., GIRAUD-DELVILLE, C., DURANTHON, V., SANDRA, O. & GOMEZ, E. **2017**. Localisation of stem cell factor, stanniocalcin-1, connective tissue growth factor and heparin-binding epidermal growth factor in the bovine uterus at the time of blastocyst formation. *Reproduction Fertility and Development*, 29, 2127-2139.

Abstract: Early embryonic losses before implantation account for the highest rates of reproductive failure in mammals, in particular when in vitro-produced embryos are transferred. In the present study, we used molecular biology techniques (real-time quantitative polymerase chain reaction), classical immunohistochemical staining coupled with confocal microscopy and proteomic analysis (multiple reaction monitoring and western blot analysis) to investigate the role of four growth factors in embryo-uterine interactions during blastocyst development. Supported by a validated embryo transfer model, the study investigated: (1) the expression of stem cell factor (SCF), stanniocalcin-1 (STC1), connective tissue growth factor (CTGF) and heparin-binding epidermal growth factor-like growth factor (HB-EGF) in bovine uterine fluid; (2) the presence of SCF, STC1, CTGF and HB-EGF mRNA and protein in the bovine endometrium and embryos; and (3) the existence of reciprocal regulation between endometrial

and embryonic expression of SCF, STC1, CTGF and HB-EGF. The results suggest that these growth factors most likely play an important role during preimplantation embryo development in cattle. The information obtained from the present study can contribute to improving the performance of in vitro culture technology in cattle and other species.(DOI: 10.1071/rd16383)

MURILLO, A., MUÑOZ, M., MARTIN-GONZALEZ, D., CARROCERA, S., MARTINEZ-NISTAL, A. & GOMEZ, E. **2017**. Low serum concentration in bovine embryo culture enhances early blastocyst rates on Day-6 with quality traits in the expanded blastocyst stage similar to BSA-cultured embryos. *Reproductive Biology*, 17, 162-171.

Abstract: In bovine, single in vitro embryo culture in protein-free medium from Day-6 to Day-7 leads to expanded blastocyst (XB) with improved pregnancy and birth rates after cryopreservation. Under these conditions, early blastocysts (EB) progress to the XB stage at higher rates than morulae (M). However, embryo production with BSA in culture prior to Day-6 leads to low EB rates. We investigated whether a very low FCS concentration (0.1%) in culture from Day-1 to Day-6 would improve EB rates and, subsequently, increase XB rates on Day-7 after single culture in protein-free medium. The quality of embryos produced was evaluated in terms of survival to cryopreservation, apoptosis percentage, lipid accumulation and transfer to recipients. On Day-6, EB rates from embryos cultured with FCS were higher than with BSA ($P = 0.022$). On Day-7, XB rates were higher in embryos from Day-6 EB than from Day-6 M, both with and without FCS ($P < 0.005$). After vitrification/warming of Day-7 XB, 100% embryos survived at 24h in all treatments, and total cell number and apoptosis percentage were not affected by the presence of FCS or embryonic stage on Day-6. Cryopreserved and fresh embryos produced with FCS until Day-6, and then deprived of protein and cultured individually, led to pregnancies after ET. In conclusion, minute FCS concentration improves EB rates on Day-6 leading, after one-day single culture without protein, to more XBs. The quality of XB produced with FCS compares well with XB produced with BSA in terms of apoptosis, lipid accumulation and pregnancy. (C) 2017 Society for Biology of Reproduction & the Institute of Animal Reproduction and Food Research of Polish Academy of Sciences in Olsztyn.(DOI: 10.1016/j.repbio.2017.04.002)

MURILLO-RIOS, A., MAILLO, V., MUÑOZ, M., GUTIERREZ-ADAN, A., CARROCERA, S., MARTIN-GONZALEZ, D., FERNANDEZ-BUZNEGO, A. & GOMEZ, E. **2017**. Short- and long-term outcomes of the absence of protein during bovine blastocyst formation in vitro. *Reproduction Fertility and Development*, 29, 1064-1073.

Abstract: In cattle, individual in vitro embryo culture after Day 6 benefits development, allowing non-invasive analysis of culture medium. However, undefined supplements in culture reduce analytical reliability. In this study we assayed the short- and long-term performance of embryos after bovine serum albumin removal over a 24-h period in individual culture. The absence of protein decreased embryo development and cell counts in the inner cell mass without affecting blastocyst sex ratio. However, the absence of protein produced embryos with an improved tendency to survive vitrification after 24h in culture ($P=0.07$). After transfer to recipients, birth rates of embryos that had been cultured with protein tended to decrease ($P < 0.06$) mostly as a result of a higher number of miscarriages ($P < 0.013$), reflecting lower viability. Birthweight, gestation length, height and thorax circumference did not differ between embryos cultured with or without protein. In fresh blastocysts cultured without protein, gene expression analysis showed higher abundance ($P < 0.05$) of insulin-like growth factor 2 receptor (IGF2R; imprinting) and activating transcription factor 4 (ATF4) and DNA-damage-inducible transcript 3 (DDIT3; endoplasmic reticulum stress) transcripts, with DNA methyltransferase 3A (DNMT3A; imprinting) tending to increase ($P=0.062$). However, in hatched blastocysts that survived cryopreservation, glucose-6-phosphate dehydrogenase (G6PD) was overexpressed in embryos cultured without protein ($P < 0.01$). The absence of protein results in fewer blastocysts but improved long-term viability after cryopreservation. (DOI: 10.1071/rd15485)

MURUBE, E., CAMPA, A. & FERREIRA, J.J. 2017. Identification of new resistance sources to powdery mildew, and the genetic characterisation of resistance in three common bean genotypes. *Crop & Pasture Science*, 68, 1006-1012

Abstract: Powdery mildew (PM) is a devastating disease of many legume species, including common bean. In this work, we assessed the responses of 108 dry and snap bean accessions to PM, and characterised the genetic control of the resistance in three bean genotypes. Resistance tests under controlled conditions led to the identification of 11 dry bean accessions with total resistance. However, no snap bean accessions showed total resistance, although two cultivars showed mixed phenotypes including seedlings with total resistance. The inheritance of resistance was analysed in three F-2:3 populations involving the resistant bean genotypes BelNeb, G19833 and BGE003161. In the three populations, the segregation for PM resistance fit the expected ratio for one dominant gene. The resistance loci were mapped to the beginning of the linkage group Pv04. The physical positions of the flanking markers indicated that the three resistance genes were located between the physical positions 0 and 1.09Mb. This work provides new PM-resistance sources and markers linked to resistance genes, which will be very useful in common bean breeding programs focussed on protecting bean crops against this disease. (DOI: 10.1071/cp16460)

OCEJO, M., OPORTO, B., JUSTE, R. A. & HURTADO, A. 2017. Effects of dry whey powder and calcium butyrate supplementation of corn/soybean based diets on productive performance, duodenal histological integrity, and *Campylobacter* colonization in broilers. *Bmc Veterinary Research*, 13.

Abstract: *Campylobacter* is the main cause of gastroenteritis in humans in industrialized countries, and poultry is its principal reservoir and source of human infections. Dietary supplementation of broiler feed with additives could improve productive performance and elicit health benefits that might reduce *Campylobacter* contamination during primary production. The aim of this study was to assess the effect of dietary supplementation with whey (a prebiotic) and calcium butyrate (a salt of a short-chain fatty acid) on productive traits, duodenal histological integrity, and *Campylobacter* colonization and dissemination in broiler chickens during the 42-day rearing period. Results: Six hundred one-day-old Ross-308 chickens were placed into 20 ground pens and assigned to one of 4 corn/soybean-based dietary treatments (5 replicates of 30 chicks per treatment) following a randomized complete block design: 1) basal diet with no supplementation as the control, 2) diet supplemented with 6% dry whey powder, 3) diet containing 0.1% coated calcium butyrate, and 4) diet containing 6% whey and 0.1% calcium butyrate. At age 15 days, 6 chickens per pen were experimentally inoculated with *Campylobacter jejuni*. The results showed that supplementation of the corn/soybean-based diet with 6% whey alone or, preferably, in combination with 0.1% coated calcium butyrate improved growth and feed efficiency, had a beneficial effect on duodenal villus integrity, and decreased mortality. These favourable effects were particularly significant during the starter period. Six days after oral challenge, *Campylobacter* was widespread in the flock, and the birds remained positive until the end of the rearing period. Although *Campylobacter* was not isolated from environmental samples, it was detected by real-time polymerase chain reaction (PCR) in dust, air filters, and drinkers while birds shed culturable *C. jejuni* cells. No differences ($p > 0.050$) in colonization or shedding levels that could be attributed to the diet were observed during the assay. Conclusions: Beneficial effects on performance and intestinal health were observed, particularly during the starter period, when chickens were fed a diet supplemented with both whey and coated calcium butyrate. However, none of the tested diets provided the chicks any differential degree of protection against *Campylobacter* infection. (DOI: 10.1186/s12917-017-1121-5)

OSORO, K., FERREIRA, L. M. M., GARCIA, U., MARTINEZ, A. & CELAYA, R. 2017. Forage intake, digestibility and performance of cattle, horses, sheep and goats grazing together on an improved heathland. *Animal Production Science*, 57, 102-109

Abstract. A mixed herd of five cows, five mares, 32 ewes and 32 goats was managed with their offspring during two consecutive years in a paddock (22.3 ha) with 76% of heathlands and 24% of improved pasture area, with the aim of studying their differences in ingestive behaviour and performance. Diet composition, dry matter intake (DMI) and digestibility (DMD) were estimated on three occasions using the alkane technique, and all animals were periodically weighed to calculate bodyweight (BW) changes per livestock unit (LU). Goats selected higher proportions of shrubs than the other herbivore species ($P < 0.001$). Overall, daily DMI was higher ($P < 0.001$) in equines (218 g/kg BW^{0.75}) than in ruminants (174, 121 and 80 g/kg BW^{0.75} for cattle, goats and sheep, respectively), whereas DMD was lower ($P < 0.001$) in equines (569 g/kg DM) compared with ruminants (733–791 g/kg DM). During the first half of the grazing season (from late April to mid-July), dams' BW changes per LU did not differ between species. However, during late summer–autumn lower ($P < 0.001$) BW losses per LU were observed in sheep and goats (–89 g/day) than in cattle (–534 g/day), being intermediate in horses (–254 g/day). For the overall grazing season, ewes and goats showed greater ($P = 0.056$) BW gains per LU (338 g/day) than cows and mares (178 g/day). Regarding the offspring, BW gains per LU were greater ($P < 0.001$) in lambs (3612 g/day) than in other species. Calves presented greater gains than kids (2647 vs 1909 g/day.LU), whereas foals showed intermediate gains (2385 g/day.LU). Therefore, under these conditions of partially improved heathlands, sheep was the most productive species. However, looking at the diet selection and digestibility, goats could complement sheep or cattle herds by achieving a more efficient utilisation of heathland vegetation, and increasing overall productivity per hectare. By contrast, horses, having high levels of grass intake, compete with cattle and sheep for pasture utilization. (DOI: 10.1071/an15153)

PANDO BEDRIÑANA, R., MANGAS ALONSO, J. J. & SUAREZ VALLES, B. 2017. Evaluation of autochthonous *Saccharomyces bayanus* strains under stress conditions for making ice ciders. *Lwt-Food Science and Technology*, 81, 217-225.

Abstract: The product diversification policy being carried out by the Asturian cider industry includes the development of beverages similar to so-called ice ciders. During apple juice fermentations, yeast cells are affected by several stress conditions. In this study, 74 *Saccharomyces bayanus* strains isolated from Asturian cider, have been analyzed in synthetic media for their growth ability, sugar fermentation capacity and acetic acid production under the stress conditions that occur during the production of ice cider. According to the data obtained 23 strains high proliferation capabilities, sugar fermentation capacity and low acetic acid production have been differentiated. Ten strains were chosen to produce ice ciders from apple juice (31.8 °Brix) at 12 °C. The products obtained were characterized by not having developed the malolactic conversion and their low contents of acetic acid, methanol and ethyl acetate. Significant differences among ciders were detected for alcoholic degree, total acidity, and contents of glycerol, pyruvic, fumaric and shikimic acids due to the yeast strain used. A Multiple Correspondence Analysis showed four strains associated with sensory quality variables. Our results open up the possibility of using autochthonous *S. bayanus* strains as starters in the making of Asturian ice ciders. (DOI: 10.1016/j.lwt.2017.03.055)

PELAYO, R., PENEDO, M. C. T., VALERA, M., MOLINA, A., MILLON, L., GINJA, C. & ROYO, L. J. 2017. Identification of a new Y chromosome haplogroup in Spanish native cattle. *Animal Genetics*, 48, 450-454.

Abstract: The aim of this work was to perform a thorough analysis of the diversity of Y-haplotypes in Spanish cattle. A total of 207 *Bos taurus* males were sampled across 25 European breeds, with a special focus on rare, local Spanish populations. Animals were genotyped with five Y-specific microsatellites (INRA189, UMN0103, UMN0307, BM861 and BYM1), two indels (ZFY10 and USP9Y) and one SNP (UTY19). A new haplogroup, distinct from those described by Gotherstrom et al. (2005), was identified and named Y1.2. Samples representing the three *B. taurus* Y-haplogroups were genotyped for four additional Y chromosome SNPs (rs121919254, rs121919281, rs121919323 and rs137049553). Among these SNPs, only rs121919281 was informative in *B. taurus* and helped to confirm the new Y1.2 haplogroup. Analysis of a larger dataset of standardized haplotypes for 1507 individuals from 57 populations from Spain, other European countries and Africa showed the new Y1.2 haplogroup to be found exclusively in Spanish breeds. This finding reinforces the importance of local Spanish cattle as

reservoirs of genetic diversity as well as the importance of the Iberian Peninsula in the history of cattle. (DOI: 10.1111/age.12549)

PRIETO-MARTINEZ, N., MORATO, R., MUINO, R., HIDALGO, C. O., RODRIGUEZ-GIL, J. E., BONET, S. & YESTE, M. **2017**. Aquaglyceroporins 3 and 7 in bull spermatozoa: identification, localisation and their relationship with sperm cryotolerance. *Reproduction Fertility and Development*, 29, 1249-1259.

Abstract: The present study aimed to determine the localisation of aquaglyceroporins 3 (AQP3) and 7 (AQP7) in bull spermatozoa and their relationship with the sperm cell's resilience to withstand cryopreservation (i.e. cryotolerance). A total of 18 bull ejaculates were cryopreserved and their sperm quality analysed before and after freeze-thawing. The presence and localisation of AQP3 and AQP7 was determined through immunoblotting and immunocytochemistry. AQP3 was found in the mid-piece and AQP7 in the mid-piece and post-acrosomal region of bull spermatozoa. Immunoblotting showed specific signal bands at 30 and 60kDa for AQP3 and at 25kDa for AQP7. Neither the relative abundance of AQP3 and AQP7 nor their localisation patterns was altered by cryopreservation but individual differences between bull ejaculates were found in immunoblots. In order to determine whether these individual differences were related to sperm cryotolerance, bull ejaculates were classified as having good (GFE) or poor freezability (PFE) on the basis of their sperm quality after thawing. While the relative abundance of AQP3 before cryopreservation did not differ between ejaculates with GFE and PFE, the abundance of AQP7 was higher in GFE than in PFE ejaculates. This finding was further confirmed through principal component and linear regression analyses. In conclusion, the relative abundance of AQP7 in fresh semen may be used as a marker to predict bull sperm cryotolerance. (DOI: 10.1071/rd16077)

SALINAS, L. M., CASAIS, R., GARCIA MARIN, J. F., DALTON, K. P., ROYO, L. J., DEL CERRO, A., GAYO, E., DAGLEISH, M. P., ALBERDI, P., JUSTE, R. A., DE LA FUENTE, J. & BALSEIRO, A. **2017a**. Vaccination against Louping Ill Virus Protects Goats from Experimental Challenge with Spanish Goat Encephalitis Virus. *Journal of Comparative Pathology*, 156, 409-418.

Abstract: Spanish goat encephalitis virus (SGEV) is a recently described member of the genus *Flavivirus* belonging to the tick-borne encephalitis group of viruses, and is closely related to louping ill virus (LIV). Naturally acquired disease in goats results in severe, acute encephalitis and 100% mortality. Eighteen goats were challenged subcutaneously with SGEV; nine were vaccinated previously against LIV and nine were not. None of the vaccinated goats showed any clinical signs of disease or histological lesions, but all of the non-vaccinated goats developed pyrexia and 5/9 developed neurological clinical signs, primarily tremors in the neck and ataxia. All non-vaccinated animals developed histological lesions restricted to the central nervous system and consistent with a lymphocytic meningoencephalitis. Vaccinated goats had significantly ($P < 0.003$) greater concentrations of serum IgG and lower levels of IgM ($P < 0.0001$) compared with unvaccinated animals. SGEV RNA levels were below detectable limits in the vaccinated goats throughout the experiment, but increased rapidly and were significantly ($P < 0.0001$) greater 2-10 days post challenge in the non vaccinated group. In conclusion, vaccination of goats against LIV confers highly effective protection against SGEV; this is probably mediated by IgG and prevents an increase in viral RNA load in serum such that vaccinated animals would not be an effective reservoir of the virus. (DOI: 10.1016/j.jcpa.2017.03.004)

SALINAS, L. M., CASAIS, R., GARCIA MARIN, J. F., DALTON, K. P., ROYO, L. J., DEL CERRO, A., GAYO, E., DAGLEISH, M. P., JUSTE, R. A. & BALSEIRO, A. **2017b**. Lambs are Susceptible to Experimental Challenge with Spanish Goat Encephalitis Virus. *Journal of Comparative Pathology*, 156, 400-408

Abstract: Spanish goat encephalitis virus (SGEV) is a member of the genus *Flavivirus*, family *Flaviviridae*, and causes encephalomyelitis in goats. The aim of this study was to determine whether sheep are susceptible to experimental challenge with SGEV by two different routes. The results show that

SGEV can infect sheep by both the subcutaneous and intravenous routes, resulting in neurological clinical disease with extensive and severe histological lesions in the central nervous system. Lambs challenged subcutaneously developed more severe lesions on the ipsilateral side of the brain, but the lesion morphology was similar irrespective of the route of challenge. The clinical presentation, pathogenesis, lesion morphology and distribution shows that SGEV is very similar to louping ill virus (LIV) and therefore any disease control plan must take into account any host species and SGEV vectors as potential reservoirs. Furthermore, discriminatory diagnostics need to be applied to any sheep or goat suspected of disease due to any flavivirus in areas where SGEV and LIV co-exist. (DOI: 10.1016/j.jcpa.2017.03.003)

SERRANO, M., ELGUEZABAL, N., SEVILLA, I. A., GEJO, M. V., MOLINA, E., ARRAZURIA, R., URKITZA, A., JONES, G. J., VORDERMEIER, M., GARRIDO, J. M. & JUSTE, R. A. 2017. Tuberculosis Detection in Paratuberculosis Vaccinated Calves: New Alternatives against Interference. *Plos One*, 12.

Abstract: Paratuberculosis vaccination in cattle has been restricted due to its possible interference with the official diagnostic methods used in tuberculosis eradication programs. To overcome this drawback, new possibilities to detect *Mycobacterium bovis* infected cattle in paratuberculosis vaccinated animals were studied under experimental conditions. Three groups of 5 calves each were included in the experiment: one paratuberculosis vaccinated group, one paratuberculosis vaccinated and *M. bovis* infected group and one *M. bovis* infected group. The performance of the IFN-gamma release assay (IGRA) and the skin test using conventional avian and bovine tuberculins (A- and B-PPD) but also other more specific antigens (ESAT-6/CFP10 and Rv3615c) was studied under official and new diagnostic criteria. Regarding the IGRA of vaccinated groups, when A- and B-PPD were used the sensitivity reached 100% at the first post-challenge sampling, dropping down to 40-80% in subsequent samplings. The sensitivity for the specific antigens was 80-100% and the specificity was also improved. After adapting the diagnostic criteria for the conventional antigens in the skin test, the ability to differentiate between *M. bovis* infected and non-infected animals included in paratuberculosis vaccinated groups was enhanced. Taking for positive a relative skin thickness increase of at least 100%, the single intradermal test specificity and sensitivity yielded 100%. The comparative intradermal test was equally accurate considering a BPPD relative skin increase of at least 100% and greater than or equal to that produced by APPD. Using the specific antigens as a proteic cocktail, the specificity and sensitivity reached 100% considering the new relative and absolute cut-offs in all experimental groups ($0 > 30\%$ and $\Delta \text{mm} \geq 2$, respectively). Results suggest that the interference caused by paratuberculosis vaccination in cattle could be completely overcome by applying new approaches to the official tuberculosis diagnostic tests. (DOI: 10.1371/journal.pone.0169735)

SEVILLA, I. A., MOLINA, E., TELLO, M., ELGUEZABAL, N., JUSTE, R. A. & GARRIDO, J. M. 2017. Detection of *Mycobacteria* by Culture and DNA-Based Methods in Animal-Derived Food Products Purchased at Spanish Supermarkets. *Frontiers in Microbiology*, 8.

Abstract: *Mycobacteria* include obligate and opportunistic pathogens that cause significant human and animal disease. The burden of tuberculosis has been largely reduced in developed territories but remains a huge problem worldwide. The significance of nontuberculous *mycobacteria* is growing considerably, especially in developed regions with higher life expectancy and more therapy-related immunosuppressed individuals. Due to their robustness *mycobacteria* can contaminate animal products by direct transmission from infected individuals or by environmental contamination during processing. The situation at market level is poorly known. Most studies analyzing commercially available foods are limited to a small or local scale and mainly focused on a particular *mycobacterial* species. There is a need to investigate if animal products that have passed the established controls to be for sale at main supermarkets could represent a route of contact with any *mycobacteria*. Thus, our goal was to study the prevalence of *mycobacteria* in these foods to assess if this could represent a source of human exposure. Five stores from the main supermarket chains in Spain were selected. 138 dairy and 119 meat products were purchased. All were processed using culture and multiplex real-time PCR methods. Additional molecular methods were used to specifically identify any positive result. *Mycobacterium avium* subsp. *hominissuis* (2), *M. avium* subsp. *avium* (1), and *M. fortuitum* (1) were isolated from powdered infant formula and ground beef, chicken

sausage, and mortadella cold cut, respectively. Mycobacterial DNA (*M. avium*, *M. tuberculosis* complex and other nontuberculous mycobacteria) was detected in 15% of dairy products and 2% of meat products. These results show that the prevalence of viable mycobacteria in foods of animal origin obtained at the supermarket was not substantial although a considerable proportion of them contained mycobacterial DNA. Contact with mycobacteria through this route could be ensured over time. Further investigation is necessary to determine the real impact of foodborne mycobacterial exposure on human health and identify critical points in the food production system to enable setting up more stringent control measures.(DOI: 10.3389/fmicb.2017.01030)

SOMOANO, A., VENTURA, J. & MIÑARRO, M. 2017. Continuous breeding of fossorial water voles in northwestern Spain: potential impact on apple orchards. *Folia Zoologica*, 66, 37-49.

Abstract: The montane water vole, *Arvicola scherman*, can be found at low altitude in northwestern Spain, where it has become a key pest in apple orchards. Its sustainable control entails the knowledge of its reproduction pattern, which is unknown for this region. Thus, we conducted a study on more than 800 voles caught monthly over two years in apple orchards located in Asturias. Sexual characteristics, body measurements and relative age class were recorded. Intra-annual changes in body mass, testicular volume and seminal vesicle length of males did not affect significantly reproduction at a population scale, since pregnant females were detected over the whole year and their occurrence did not show significant intra-annual differences. Consequently, the recruitment of young specimens was also continuous during the study period. Mild temperatures, even in winter, and ample food all the year around presumably meets the demands of physiological cost of continuous reproduction of *A. scherman* in this area. The implications of our results for facing this vole control in this agroecosystem are discussed. Furthermore, the information here reported might be useful to accurately assess the effect of sustainable control strategies on the reproductive biology of fossorial water voles in this and in similar environments.

THOMAS, J., ANGELES RISALDE, M., SERRANO, M., SEVILLA, I., GEJO, M., ANTONIO ORTIZ, J., FUERTES, M., FRANCISCO RUIZ-FONS, J., DE LA FUENTE, J., DOMINGUEZ, L., JUSTE, R., GARRIDO, J. & GORTAZAR, C. 2017. The response of red deer to oral administration of heat-inactivated *Mycobacterium bovis* and challenge with a field strain. *Veterinary Microbiology*, 208, 195-202

Abstract: Deer species (family Cervidae) are often part of the *Mycobacterium tuberculosis* complex maintenance host community, and tuberculosis (TB) control in deer, including vaccination, is consequently an area of ongoing research. However, most research into deer vaccination against TB is focused on using the live bacillus Calmette Guérin (BCG). Oral inactivated vaccines represent an interesting alternative to either oral or parenteral BCG, since neither diagnostic cross-reactions nor vaccine strain survival are likely to occur. In order to describe the red deer response to heat-inactivated *M. bovis* (IV) as compared to BCG and to unvaccinated controls (n = 5/group), we ran an experiment with five month-old vaccinated red deer, which were challenged with a virulent *M. bovis* strain 70 days later and necropsied at 60 days post-challenge. A reduction in the IV group infection burden was discovered. There were significant differences between the IV group and the control group (53% lesion reduction) as regards to the TB lesion scores, but not between other pairs. Complement component 3 plasma levels increased after challenge, and there were no differences between groups. The plasma cytokines (IL-1 beta, TNF alpha, IFN gamma, IL-10 and IL-12) levels did not change after vaccination, but IL-1 beta, TNF alpha and IL-10 did so following the challenge. The IL-10 level increased in all the groups while TNF alpha levels had a distinct response pattern in the IV group and IL-10 had a distinct response pattern in control group. The results showed that oral vaccination with IV reduces the TB lesion score in red deer challenged with a *M. bovis* field strain without interfering with the in vivo diagnosis of infection in this species. (DOI: 10.1016/j.vetmic.2017.08.007)

TRAPIELLO, E., RIGLING, D. & GONZALEZ, A. J. 2017. Occurrence of hypovirus-

infected *Cryphonectria parasitica* isolates in northern Spain: an encouraging situation for biological control of chestnut blight in Asturian forests. *European Journal of Plant Pathology*, 149, 503-514.

Abstract: The presence of hypovirulent (hypovirus-infected) isolates of the chestnut blight fungus *Cryphonectria parasitica* in Asturias, northern Spain, is first reported by using both morphological and molecular assessments. Hypovirulence was detected in 16 of the 69 councils where the fungus was previously observed. In total 30 of the 863 *C. parasitica* isolates analyzed were found to be hypovirus-infected. The isolates belonged to the two main vegetative compatibility (vc) types (EU-1 and EU-13) of the region. Asturian hypoviruses were genetically characterized by sequencing of ORFA and ORFB specific regions of the hypoviral RNA. The results show that 70% of the investigated hypovirulent isolates contained the Spanish subtype (subtype E) while 30% isolates the German subtype (D) of *Cryphonectria hypovirus 1* (CHV-1). The presence of hypovirulent isolates compatible with the dominant vc types and the low vc diversity in this region favours the application of biological control. An initial disease management effort could be applied by using the hypovirus of subtype D, whereas further research related to the biocontrol properties of the subtype E hypovirus should be conducted.

VAN DER HEIJDEN, E. M. D. L., CHILESHE, J., VERNOOIJ, J. C. M., GORTAZAR, C., JUSTE, R. A., SEVILLA, I., CRAFFORD, J. E., RUTTEN, V. P. M. G. & MICHEL, A. L. 2017. Immune response profiles of calves following vaccination with live BCG and inactivated *Mycobacterium bovis* vaccine candidates. *Plos One*, 12.

Abstract: Conventional control and eradication strategies for bovine tuberculosis (BTB) face tremendous difficulties in developing countries; countries with wildlife reservoirs, a complex wildlife-livestock-human interface or a lack of veterinary and veterinary public health surveillance. Vaccination of cattle and other species might in some cases provide the only suitable control strategy for BTB, while in others it may supplement existing test-and-slaughter schemes. However, the use of live BCG has several limitations and the global rise of HIV/AIDS infections has furthermore warranted the exploration of inactivated vaccine preparations. The aim of this study was to compare the immune response profiles in response to parenteral vaccination with live BCG and two inactivated vaccine candidates in cattle. Twenty-four mixed breed calves (*Bos taurus*) aged 4-6 months, were allocated to one of four groups and vaccinated sub-cutaneously with live *M. bovis* BCG (Danish 1331), formalin-inactivated *M. bovis* BCG, heat-killed *M. bovis* or PBS/Montanide T (control). Interferon. responsiveness and antibody production were measured prior to vaccination and at weekly intervals thereafter for twelve weeks. At nine weeks post-priming, animals were skin tested using tuberculin and MTBC specific protein cocktails and subsequently challenged through intranodular injection of live *M. bovis* BCG. The animals in the heat-killed *M. bovis* group demonstrated strong and sustained cell-mediated and humoral immune responses, significantly higher than the control group in response to vaccination, which may indicate a protective immune profile. Animals in this group showed reactivity to the skin test reagents, confirming good vaccine take. Lastly, although not statistically significant, recovery of BCG after challenge was lowest in the heat-killed *M. bovis* group. In conclusion, the parenteral heat-killed *M. bovis* vaccine proved to be clearly immunogenic in cattle in the present study, urging further evaluation of the vaccine in challenge studies using virulent *M. bovis* and assessment of vaccine efficacy in field conditions. (DOI: 10.1371/journal.pone.0188448).

VICENTE, F., SANTIAGO, C., JIMENEZ-CALDERON, J. D. & MARTINEZ-FERNANDEZ, A. 2017. Capacity of milk composition to identify the feeding system used to feed dairy cows. *Journal of Dairy Research*, 84, 254-263

Abstract: This Research Paper addresses the hypothesis that is possible to identify the type of feed used for dairy cows by means of the analysis of milk composition and the fatty acid profile of milk fat. Sixteen dairy farms were monitored during 1 year with quarterly visits between summer 2014 and spring 2015. Rations varied throughout the year due to annual dynamic change of forage production, forage rotation, variation of nutrient requirements according to physiological state of the animal, etc. The ingredients of the rations were analysed by cluster identifying five feeding systems based on the main ingredient of the

diet: grazing, maize silage, grass silage, dry forage and concentrate. Milk composition could explain up to 91·3% of the total variability among feeding systems, while fatty acid profile could explain only up to 61·2% of total variability. However, when the sum of types of fatty acids and their ratios are taken, up to 93·5% of total variability could be explained. The maize silage system had the greatest milk yield, protein, solid non-fat and urea proportions, as well as the highest proportion of saturated fatty acid and lowest concentration of trans11 18 : 1, cis9 18 : 1 and 18 : 3 n3. Principal component analysis distinguishes the maize silage system from other feeding systems, both from milk composition and milk fatty acid profile. Concentrate system overlapped partially with the grazing, grass silage and dry forage systems. The latter systems had the highest concentrations of cis9 18 : 1, trans11 18 : 1 and 18 : 3, but there was no clear differentiation among them. (DOI: 10.1017/s0022029917000383)

VIDAL, O., DROGEMUELLER, C., OBEXER-RUFF, G., REBER, I., JORDANA, J., MARTINEZ, A., BALTEANU, V. A., VICENTE DELGADO, J., EGHBALSAIED, S., LANDI, V., GOYACHE, F., TRAORE, A., PAZZOLA, M., VACCA, G. M., BADAOU, B., PILLA, F., D'ANDREA, M., ALVAREZ, I., CAPOTE, J., SHARAF, A., PONS, A. & AMILLS, M. **2017**. Differential distribution of Y-chromosome haplotypes in Swiss and Southern European goat breeds. *Scientific Reports*, 7.

Abstract: The analysis of Y-chromosome variation has provided valuable clues about the paternal history of domestic animal populations. The main goal of the current work was to characterize Y-chromosome diversity in 31 goat populations from Central Eastern (Switzerland and Romania) and Southern Europe (Spain and Italy) as well as in reference populations from Africa and the Near East. Towards this end, we have genotyped seven single nucleotide polymorphisms (SNPs), mapping to the *SRY*, *ZFY*, *AMELY* and *DDX3Y* Y-linked loci, in 275 bucks from 31 populations. We have observed a low level of variability in the goat Y-chromosome, with just five haplotypes segregating in the whole set of populations. We have also found that Swiss bucks carry exclusively Y1 haplotypes (Y1A: 24%, Y1B1: 15%, Y1B2: 43% and Y1C: 18%), while in Italian and Spanish bucks Y2A is the most abundant haplotype (77%). Interestingly, in Carpathian goats from Romania the Y2A haplotype is also frequent (42%). The high Y-chromosome differentiation between Swiss and Italian/Spanish breeds might be due to the post-domestication spread of two different Near Eastern genetic stocks through the Danubian and Mediterranean corridors. Historical gene flow between Southern European and Northern African goats might have also contributed to generate such pattern of genetic differentiation. (DOI: 10.1038/s41598-017-15593-1)