IV-01

Macrophages regulate neutrophils' IL-1β release in the presence of *Mycobacterium avium* subsp. *paratuberculosis*

Iraia Ladero[1], Angela Holder[2], Jeannine Kolakowski[2], Heather Harris[2], Joseba Garrido[1], Natalia Elguezabal[1], Dirk Werling[2].

[1]NEIKER – Basque Institute for Agricultural Research and Development, Derio, Spain; [2]Royal Veterinary College (RVC), Hatfield, United Kingdom.

Objective: The main objective was to test the impact of IL-1β secretion and extracellular trap (ET) formation by neutrophils on inflammasome activation of macrophages in response to *Mycobacterium bovis* BCG (Mbv) and *Mycobacterium avium* subsp. *paratuberculosis* (Map).

Material & Methods: Blood was extracted from healthy cows (300 ml, n=5). Monocytes were isolated using anti-CD14-coupled microbeads (Miltenyi®) after peripheral blood mononuclear cell (PBMC) isolation with Lymphoprep®. $1x10^5$ monocytes were grown in 96 well plates supplemented with recombinant bovine M-CSF at 37°C and 5% CO2 for 7 days to generate monocyte derived macrophages (MDMs). Neutrophils were isolated after the Lymphoprep® isolation step, by hypotonic lysis to eliminate red blood cells and were seeded $2x10^5$cells/well in 96 well plates. Cultures of MDMs and neutrophils from the same cow were incubated separately and together. Cultures were stimulated with PMA, zymosan, Map K10-GFP and Mbv-GFP and incubated for 4 hours at 37°C and 5% CO2 in 96 well plates for fluorimetric estimation of ET and in 24 well plates containing round-coverslides for ET visualization by confocal microscopy after immunostaining against histones and neutrophil elastase. IL-1β levels were measured in culture supernatants with a specific bovine IL-1β ELISA kit (Invitrogen®).

Results: Fluorimetric assay results revealed that ET release was higher in MDM-neutrophil co-cultures (Map: 16%, Mbv: 18%) compared to neutrophils (Map: 12%, Mbv: 11%) and MDM cultures (Map: 5%, Mbv: 9%) after stimulation with mycobacteria.

MDM cultures showed more IL-1β release against Mbv (55-60pg/ml) than MDM-neutrophil co-cultures (20-24pg/ml) and neutrophil (21-22pg/ml) cultures. However, neutrophils released more IL-1β when challenged with Map (57-68pg/ml) than MDM- neutrophil co-cultures (13-19pg/ml) and MDM cultures (27-38pg/ml).

ET formation was seen in neutrophil cultures stimulated with Mbv or Map. MDMs with internalized Mbv and Map were seen in MDM cultures. In MDM-neutrophil co-cultures, Mbv and Map stimulation caused a loss of cell integrity leaving only a few MDMs with internalized mycobacteria.

Conclusions: Neutrophils have been implicated in the pathogenesis of bovine paratuberculosis (PTB) and tuberculosis (TB), but their role is not yet well defined and increasing knowledge in this field can aid in better vaccine designing. IL-1β has shown to be important for host resistance to human tuberculosis infection. Here we present data generated by using an *ex vivo* assay designed to study the interaction of bovine neutrophils with MDMs during mycobacterial infection.

ET release results suggest that this mechanism may not depend on the mycobacterial species. In contrast, the fact that IL-1β release was higher in neutrophil cultures in response to Map and higher in MDM cultures in response to Mbv suggests a different mode of action that can be species dependent. As far as we know, no one has described before the IL-1β production in response to Map by neutrophils. These findings support an important role of bovine neutrophils against Map triggering the Th17 response through IL-1β release and starting an effective response phagocytosing and immobilising bacteria with their NETs. As a consequence macrophages are attracted by IL-1β production and phagocytose neutrophils, being provided with the antimicrobial compounds that result from neutrophils' first contact with MAP.

Keywords: Mycobacterium avium subsp. paratuberculosis, Mycobacterium bovis, Neutrophils, Macrophages, IL-1β.

IV-02

Efficacy of a live *Trichophyton verrucosum* vaccine for control of bovine dermatophytosis in veal calves

Flaminia Valentini[1], Isabella Nicola[2], Edoardo Ramacciotti[3], Alberto Garofalo[1], Giulia Cagnotti[1], Antonio D'Angelo[1], Paola Gianella[1], Claudio Bellino[1].

[1]Department of Veterinary Sciences, Torino, Italy; [2]Université de Montréal, Saint-Hyacinthe, Canada; [3]Veterinary practitioner, Torino, Italy.

Objective: The aim of this study was to assess the safety and the efficacy of a single dose injection of a commercially available live *Trichophyton verrucosum* vaccine for control of ringworm in veal calves.

Materials e methods: A blinded case-control clinical trial was carried out on 709 veal calves of both sexes reared in multiple stalls in two farms of Piedmont region, in which the prevalence of dermatophytosis was about 30%. Animals of both herds were randomly divided into two groups: ***Exp)*** experimental group (n = 340) and *Ctr*) control group (n =369). In each group, calves with and without dermatological lesions were matched. The *Exp* group received a single dose of the commercially available live vaccine against *Trichophyton verrucosum* (TRICHOBEN, *Bioveta a.s*) inoculated intramuscularly in the neck 20 days after their arrival in farm (T0), while the control group did not receive anything. Calf were examined at T0 and at 5 subsequent experimental times: T1=35 d; T2= 55 d; T3= 75 d; T4= 80d; T5 = 95d. A thorough physical examination was performed in order to identify possible local or systemic side effects and a dermatological examination was performed in order to assessed the number of lesions of ringworm (typical annular, rick, grayish-white crust lesion) present on each calf. Data was expressed as mean ± standard deviation (SD). The Student *t* test and the *z* statistic were then used in order to assess differences between groups over experimental times. Statistical differences were set at $p < .05$. The Bonferroni *p* correction method was used for multiple comparisons. Statistical analysis was performed using R 3.4.3.

Results: No local or systemic side effects were observed in animals that received a single dose of the vaccine.

Overall, 217 out 709 calves (30.6%) showed lesions of ringworm at T0. The percentage of animals of the *Exp* group that showed dermatological lesions was 32,4% (n=110) at T0, 17,4% (n=59) at T1, 10,3% (n= 35) at T2, 1,2% (n=4) at T3, 0,6% (n= 2) at T4 and 0,3% (n=1) at T5. The percentage of animals of the *Ctr* group that showed dermatological lesions was 29,0% (n=107) at T0, 19.5% (n=72) at T1, 8,4% (n= 31) at T2, 3,2% (n=12) at T3, 1,3% (n= 5) at T4 and 0,5% (n=2) at T5. Overall, the number of calves that showed dermatological lesions decreased significantly in both herds and groups over all experiment intervals ($p < .0001$). No statistical differences were found between *Ctr* and *Exp* group over experimental times ($p < .05$). The average number of lesions recorded per animal in *Exp* group was 9,01 (± 9,9) at T0, 7,1 (± 10,1) at T1, 3,2 (± 2,9) at T2 and 3 (± 1,8) at T3. Two calves showed 2 lesions at T4 while one showed 3 lesions at T5. The average number of lesions per animal recorded in *Ctr* group was 9,2 (± 13,3) at T0, 9,1 (± 12,1) T1, 8,7 (± 14,0) at T2 and 5,5 (± 6,4) a T3 and 5,8 (± 4,3) at T4. Two calves showed 1 and 9 lesions respectively at T5. The average number of lesions did not significantly differ between *Ctr* and *Exp* group over the five experimental times ($p > .05$).

Conclusions: The results of the present study suggest that ringworm had a high prevalence among veal calves. Since *Trichophyton verrucosum* infection is influenced largely by immunological status, young animals are probably the most susceptible. As previously reported for different vaccine formulations, any local or systemic effect following vaccine administration was observed in the present study. When comparing the experimental and control groups, a single administration of a *T. verrucosum* live vaccine to veal calves with ringworm did not seem to hasten the resolution of lesions. However, the significantly decrease of infected animals in the non vaccinate group suggests that spontaneous recovery, with a low spread of the disease, is likely in younger veal calves. Further studies are needed to evaluated if a protocol with 2 vaccine injection could increase the efficacy of the commercial live vaccine in preventing/treating *Trichophyton verrucosum* ringworm in veal calves.

Keywords: Bovine dermatophytosis, Trichophyton verrucosum, live vaccine, veal calves.

IV-03

A step toward an effective vaccine against *Staphylococcus aureus* mastitis in a mouse model

Kamila Reis Santos[1], Fernando Nogueira Souza[2], Marcos Bryan Heinemann[1], Eduardo Milton Ramos Sanchez[3], Luiza Campos Reis[3], Hiro Goto[3], Adriano França Da Cunha[4], Mônica Maria Oliveira Pinho Cerqueira[4], Camila Freitas Batista[5], Alice Maria Melville Paiva Della Libera[1].

[1]Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo, São Paulo, Brazil; [2]Universidade Federal da Paraíba, Paraíba, Brazil; [3]Instituto Medicina Tropical da Universidade de São Paulo, São Paulo, Brazil; [4]Escola de Veterinária da Universidade Federal de Minas Gerais, Minas Gerais, Brazil; [5]Universidade Cruzeiro do Sul, São Paulo, Brazil.

Vaccines for bovine *S. aureus* intramammary infections (IMIs) have been pursued for decades and approaches have focused mainly on opsonic antibody response aiming antibody-mediated bacterial clearance. However, immunoglobulins do not provide enough protection, and consequently the available mastitis vaccines only result in the reduction of the severity of clinical cases and increase the odds of cure of pre-existing cases of *S. aureus* IMIs. To be effective, the ideal *S. aureus* mastitis vaccine should either prevent infection or clear the bacteria from the mammary gland shortly after infection. It would seem that *S. aureus* mastitis vaccine that meets these criteria has not been developed yet. Thus, here we aimed to evaluate the expansion of a protective population of γδ T cells subpopulation ($V\gamma4^+$) in mice vaccinated with three *Staphylococcus aureus* recombinant proteins with a potential to prevent new intramammary infections in association or not with granulocyte-macrophage colony-stimulating factor (GM-CSF) DNA vaccine, which could boost antigen presentation. For the present study were used 18 mice (six mice per group) C57bl/6J lineage with six weeks of age. The animals were divided in three groups, each group with a different vaccine protocol. Firstly, we produced three *S. aureus* recombinant proteins ATP synthase subunit alpha (SAS); succinyl-diaminopimelate desuccinylase (SDD); and cysteinyl-tRNA synthetase (CTS), that was previously identified as potential candidates for preventing new IMIs in a previous study carried out by our research group using serum immunoproteomics. Beyond that, we also produce a DNA vaccine for granulocyte-macrophage stimulating factor (GM-CSF). The first group (G1) received the recombinant proteins and saponin as adjuvant at days 14, 28 and 42, and the second group (G1) received the same recombinant proteins and the GM-CSF at day 0. The third group (G3) received just the adjuvant saponin and serve as a unvaccinated control group. The spleen's γδ T cells subpopulation ($V\gamma4^+$) was determined by flow cytometry using a specific monoclonal antibody. We choose this population because the expansion of this lymphocyte population has been associated with protective against subsequent infections. The data were analyzed using the FlowJo software (Becton, Dickinson and Company, Oregon, United States). Statistical analysis was performed using GraphPad Prism 7 (GraphPad Software, Inc., San Diego, USA). Firstly, the data were tested for normality of the distribution using the Shapiro-Wilk test. As the data showed Gaussian distribution, the data were subjected to a variance analysis followed by Tukey's test. The animals that received the three *Staphylococcus aureus* recombinant proteins with a potential to prevent new intramammary infections together with the GM-CSF DNA vaccine showed the highest percentage of this cell population .(4.40 ± 8.53; $P = 0.01$), followed by those that received just the recombinant proteins (2.94 ± 0.28; $P = 0.03$), which both are statistically different from the unvaccinated control group (2.65 ± 0.38). Thus, our immunogenicity findings indicated that these recombinant proteins identified in a previous serum immunoprotemic study have an important property associated with protective immunity against *S. aureus* infection.

Keywords: Mastitis, immune response, protective immunity, vaccine, Staphylococcus aureus.

IV-04

Assessment of in vivo immune and health status parameters in Holstein-Friesian calves fed milk replacer supplemented with microalgae displaying in vitro macrophage-stimulatory activity

Ana Rita Violante Pedro[1], Margarida Rosa Garcez Maia[2], Elisabete Gomes Martins[3], Carla Mendonça[4], Isabel Costa Ramos[5], Catarina Gonçalves[6], Tânia Lima[7], Cátia Mota[8], Joana Laranjeira[9], Ana Rita Cabrita[2], António Mira Da Fonseca[2], Manuel Vilanova[10], Alexandra Correia[10].

[1]ICBAS/i3S/LAQV/REQUIMTE-UP, Porto, Portugal; [2]LAQV/REQUIMTE/ICBAS-UP, Porto, Portugal; [3]ADM/EPIUnit-UP/EUVG, Coimbra, Portugal; [4]Centro de Estudos de Ciência Animal-Instituto de Ciências, Tecnologias e Agroambiente-UP, Porto, Portugal; [5]Cooperativa Agrícola de Vila do Conde CRL, Vila do Conde, Portugal; [6]International Iberian Nanotechnology Laboratory, Braga, Portugal; [7]i3S/IBMC-UP, Porto, Portugal; [8]ICBAS/LAQV/REQUIMTE-UP, Porto, Portugal; [9]Allmicroalgae, Pataias, Portugal; [10]ICBAS/i3S-UP, Porto, Portugal.

Objectives: Morbidity in newborn calves derives most commonly from gastrointestinal infections and respiratory syndromes. Although difficult to precise and extremely variable among regions, it has devastating effects on animal welfare and on long-term productivity and profitability. Most neonatal diseases derive from an imbalance in the pressure posed by pathogens in the surrounding environment and the host immune response to these pathogens. Maintaining good management practices is crucial to control morbidity rates; nevertheless, strategies that could enhance calves' immunity would be helpful in preventing disease, animal loss, suboptimal performance, and the overuse of antibiotics. Dietary supplements have been used to enhance calves' innate and acquired immunity. Yeast-derived beta-glucans trigger Dectin-1-mediated signalling, inducing epigenetic changes in innate immune cells, which result in a higher response to a subsequent stimulus (innate memory). Although scarcely studied, microalgae are promising alternatives as immune-modulators, due to their content of polyunsaturated fatty acids, organic minerals, beta-glucans, and antioxidants. This work addressed the effects of digestion products of microalgae on bovine innate immune cells and the use of microalgae as dietary supplements to newborn calves. We aimed at characterizing the *in vitro* response of macrophages to these dietary supplements and the zootechnical performance and immune parameters of newborn calves fed milk replacer supplemented with 1% *Chlorella vulgaris*.

Material and methods: *In vitro* assay: Peripheral blood monocyte-derived macrophages (MDM), from eight Holstein-Friesian calves, were stimulated with digestion products of *C. vulgaris*, *Nannochloropsis oceanica* and *Tetraselmis* sp. Monogastric (pre-ruminant) *in vitro* digestion of microalgae was performed according to previously described methodology. Two different dilutions of digestion products (1:10; 1:100) were used as representative of 2 and 0.2% supplementation, respectively, considering a daily intake of 8 L of milk replacer. Cytokine production (TNF-α, IL-6, IL-8, IL1β, IL-10), cytokine mRNA expression (TNFA, IL6, IL8, IL10, IL1 β, IL12A, IL12B and IL23A), and reactive-oxygen species (ROS) production were evaluated.

I*n vivo* assay: fourteen male Holstein-Friesian calves, 10 days old, were randomly allocated to control (milk replacer) and experimental (milk replacer with 1% *C. vulgaris*) groups. Prior to the beginning of the trial, animals were weighted, clinical evaluation was performed, and blood was collected for serum IgG and complete hemogram evaluation. Calves were fed 7 L of milk replacer (140 g/L) in two equal meals for 42 days. Starter feed, meadow hay and freshwater were provided *ad libitum*. Milk intake and refusals, faecal scores and health parameters were recorded. Animals were periodically weighed to evaluate average daily gain. Faeces were collected for pH, short-chain fatty acids and microbiome profiling. Blood was collected for hemogram and serum cytokine evaluation and to isolate peripheral blood mononuclear cells (PBMC) for assessment of proliferation and cytokine production in response to mitogens; peripheral blood monocytes were challenged with several toll-like receptor (TLR) and C-type Lectin-like receptor (CLR) agonists to evaluate cytokine expression, and MDM were used for phagocytosis assays.

Results: Digestion products of *C. vulgaris*, *N. oceanica*, and *Tetraselmis* sp., induced mRNA expression and production of all assessed cytokines, as well as of ROS by MDM. These results indicate that microalgae supplementation has a stimulatory effect on bovine macrophages. The *in vivo* supplementation of replacer milk with *C. vulgaris* had no negative impact on average feed intake, average daily gain, and health parameters. Serum cytokines and innate immune cells' response (cytokine mRNA and protein expression, phagocytosis) were similar between control and experimental groups. Also, no differences were found between groups regarding MDM phagocytosis capacity or PBMC proliferation. Assessment of faecal pH, short-chain fatty acids and microbiome profiling is currently ongoing.

Conclusions: Microalgae had an immunostimulatory effect on bovine macrophages. However, supplementation of calves' milk replacer with 1% *C. vulgaris* did not result in significant alterations in the evaluated parameters. Higher inclusion levels of *C. vulgaris* may be necessary to evidence putative immunomodulatory effects of *C. vulgaris* supplementation on immune and growth parameters in newborn calves.

Funding: This work received financial support from Fundação para a Ciência e Tecnologia/Ministério da Ciência, Tecnologia e Ensino Superior (FCT/MCTES) through the project UIDB/50006/2020 and through the Project AlgaValor from Portugal 2020 (POCI-01-0247-FEDER-035234; LISBOA-01-0247-FEDER-035234; ALG-01-0247-FEDER-035234). AP was supported by phD grant PD/BDE/135540/2018 from FCT/MCTES, ADM Portugal S.A., and Cooperativa Agrícola de Vila do Conde. MM was supported by FCT through program DL57/2016–Norma transitória (SFRH/BPD/70716/2010).

Keywords: Bovine, microalgae, dietary supplementation, cytokines.

IV-06

Comparison between immune response observed in cattle vaccinated with a traditional toxoid vaccine or with the recombinant Hc-domain of botulinum neurotoxin type C and D

Luca Zandonà[1], Elena Tonon[1], Matteo Cornaggia[1], Adriana Di Castri[1], Ilenia Dirigo[1], Christelle Mazuet[2], Alessia Rizzardi[1], Joachim Frey[3], Luca Bano[1].

[1]Istituto Zooprofilattico Sperimentale delle Venezie, Diagnostic and Microbiology Laboratory, Treviso, Italy; [2]Institut Pasteur, Centre National de Référence des Bactéries anaérobies et du Botulisme, Paris, France; [3]Vetsuisse Faculty, University of Berne, Bern, Switzerland.

Objective: In the present study, we compared the immune response occurring in cows immunized with the recombinant heavy chain (Hc) domain of *Clostridium botulinum* neurotoxin (BoNT) type C and D (HcBoNT/C and HcBoNT/D), with that elicited by a commercial toxoid-based vaccine.

Materials and methods: Ten male calves, four-month-old, were vaccinated twice with a bivalent (C and D) traditional toxoid-based vaccine and ten male calves, hosted in the same stable, at the same age, were vaccinated with three doses of the Hc-BoNT recombinant vaccine. HcBoNT/C and HcBoNT/D were expressed in *Escherichia coli* and purified as previously described (1). Besides, five calves were injected three times with the only adjuvant, while other five were untreated and were included in the study as negative control. All in vivo experiments were approved by the ethical committee and authorized by the Italian Ministry of Health (n° 417/2017-PR).

Serum samples were collected before the first vaccination and three weeks after the second and the third vaccination. To evidence the antibody response, two ELISA tests were developed in-house using the immunogens as capture antigens. The neutralizing antibodies were investigated by means of a mouse protection assay (MPA) performed with BoNT type C, D and D/C (2). The neutralizing titer was quantified using two reference antitoxins.

Results: The group of cows vaccinated with the recombinant vaccine showed an average antibody titre of 1.031 ELISA units (EU) for type C and 1.14 EU for type D. In contrast, the average titre in animals vaccinated with the bivalent toxoid vaccine was lower, i.e. 0.461 EU for type C and 0.182 for type D.

The average antibody titers in untreated animals and animals inoculated with the adjuvant were 0.171 EU and 0.097 EU, respectively.

After two vaccinations, the mouse protection assay performed with BoNT type C revealed average neutralizing titers of 5.1 International Units (UI)/ml in bovines vaccinated with the recombinant vaccine or 0.625 UI/ml in those vaccinated with the toxoid vaccine. Despite the high humoral response evidenced by ELISA, the mouse protection assay performed with BoNT type D/C did not show any protection titre after two vaccinations and the antibody response recorded after the third vaccination was equal (1.25-2.5 UI/ml) to that obtained with the toxoid vaccine after two vaccinations. This result is probably due to the limited homology of the HcBoNT/DC with the HcBoNT/D (37%) and HcBoNT/C (77%) (3). The means of the neutralizing serum titers against BoNT/D were 2.06 UI for bovines vaccinated with the toxoid-based vaccine and 4.87 UI for bovines immunized with the recombinant vaccine.

Conclusions: A two-dose subcutaneous immunization with the recombinant vaccine stimulated a robust humoral response compared with control groups.

Our results demonstrate that the neutralizing antibodies titers against BoNT/C and BoNT/D stimulated by the recombinant vaccine were respectively 8 and 2 times higher than those of the classic toxoid vaccine included in the study. The recombinant vaccines encompass the immunogenic part of the protein that is biologically inactive, and does not contain formalin, hence it represents a safer tool for operators. The lack of protective antibodies against BoNT D/C in sera of bovines vaccinated with the recombinant peptides can be explained with the low structural homologies between the C-terminal-Hc of BoNT/DC and the C-terminal-Hc of BoNT/D (37%) and BoNT/C (77%). To prevent bovine botulism sustained by BoNT/DC, a new subunit vaccine set up with the C-terminal part of the heavy chain of the mosaic BoNT/DC should be tested.

References:

1. Stahl C., Unger L., Mazuet C., Popoff M., Straub R., Frey J. (2009) “Immune response of horses to vaccination with the recombinant Hc domain of botulinum neurotoxin types C and D”. *Vaccine* 27: 5661–5666.
2. Hatheway C.L., Dang C. (1994) “Immunogenicity of the neurotoxins of *Clostridium botulinum*”. In: Jankovic J., Hallett M. (eds) Therapy with botulinum toxin. Marcel Dekker, New York, pp 93–107
3. Nakamura K., Kohda T., Shibata Y., Tsukamoto K., Arimitsu H., Hayashi M., Mukamoto M., Sasakawa N., Kozaki S. (2012) “Unique biological activity of botulinum D/C mosaic neurotoxin in murine species”. *Infect Immun* 80:2886-2893.

Keywords: Botulism, recombinant vaccine, cattle.

IV-07

Reduction in BRD cases and antibiotics consumption using dry cow vaccination combined with early intranasal calf vaccination in a commercial Danish dairy farm

Jørgen Kragsig Olesen[1], Jonna Hjorth[2], Henrik Schmidt[2], Marina Solé[3], Martijn Seelie[3].

[1]Kvægdyrlægerne Midt, Bording, Denmark; [2]HIPRA Nordic ApS, VEJEN, Denmark; [3]HIPRA laboratorios, Amer, Spain.

Objectives: Bovine Respiratory Disease (BRD) has a considerable negative impact on production economics and on calf welfare, as well as a major impact on antibiotic consumption. The objective of this case study was to measure the changes in number of BRD treatments and the impact on antibiotic usage in the period 1-120 days of life when vaccinating dry cows with HIPRABOVIS® SOMNI/Lkt (*M. haemolytica* leucotoxoid & *H. somni* vaccine - HIPRA) in combination with

early intranasal calf vaccination with NASYM (live attenuated BRSV vaccine - HIPRA).

Materials and methods: A closed 285-cow dairy herd located in Denmark, with an average of 14 heifer calves born per month, experienced massive BRD problems among calves from the first days of life until 2 months of age.

Newborn calves are fed 3.8 L of good quality colostrum (Brix>22%) and are housed individually the first two days of life. After this they are housed in pairs. From approximately three weeks of age, they are housed 6 calves per pen.

Vaccination protocol: Dry cows are vaccinated subcutaneously with HIPRABOVIS® SOMNI/Lkt around dry-off and a 2nd dose 3-4 weeks later. NASYM is applied intranasally in calves during the first week of life.

Treatment data were gathered from April 2019 until December 2021.

A period of 12 months of vaccination is compared with a 12-month period prior to vaccination. An analysis of the first 120 days of life of all heifers born during the two periods has been performed. Bull calves are not included in the dataset as they are sold at the age of 2-5 weeks.

The number of BRD treatments and days under treatment pre- and post-vaccination was compared in order to estimate the impact of vaccination on the disease. Treatments are considered as a new case if there are 14 days or more between cases.

Results: Heifers born during the vaccination period had a significantly lower risk of experiencing one or more BRD treatments during their first 120 days of life compared to the heifers born during the 12-month period prior to the start of vaccination. The average number of BRD treatments per heifer was significantly reduced, from 1.04 before the start of vaccination to 0.36 in the vaccination period ($p<0.001$). A significant reduction was seen in the percentage of animals treated (from 73.5% to 30.8% $p<0.001$), and in the risk of being treated more than once (from 23.9% to 4.1% $p<0.001$).

If we consider the number of therapeutic days, there is a significant reduction from 8.62 before vaccination to 1.92 days of treatment per animal in the vaccination period, which means a reduction of 78%, with a corresponding decrease in antibiotic consumption.

The reduction in therapeutic days was significant throughout the entire study ($p<0.001$) but was highly significant from 1-60 days of life.

Conclusions: On a 285-cow dairy farm with known respiratory problems in calves, dry cow vaccination with HIPRABOVIS® SOMNI/Lkt and intranasal vaccination of the young calves with NASYM resulted in a reduction of average number of BRD treatments per animal from 1.04 to 0.36 ($p<0.001$). The number of therapeutic days was reduced significantly by 78% ($p<0.001$), with a corresponding decrease in antibiotic consumption. The reduction was noted in both the number of animals treated, and in the risk of being treated more than once. The reduction in therapeutic days was significant throughout the entire study ($p<0.001$) but was highly significant from 1-60 days of life.

Keywords: BRD, VACCINATION, Intranasal, passive immunity, cow vaccination.

IV-09

Single-shot vaccines against bovine respiratory syncytial virus (BRSV): comparative evaluation of long-term protection after immunisation in presence of BRSV-specific maternal antibodies

Jean François Valarcher[1], Sara Hägglund[1], Katarina Näslund[1], Luc Jouneau[2], Ester Malmström[3], Olivier Boulesteix[4], Anne Pinard[4], Dany Leguéré[4], Alain Deslis[4], David Gauthier[4], Catherine Dubuquoy[2], Vincent Pietralunga[2], Aude Rémot[5], Alexander Falk[6], Ganna Shevchenko[7], Sara Bergström Lind[7], Claudia Von Brömssen[8], Karin Vargmar[9], María Jose Rodriguez[10], Marga Garcia Duran[10], Isabelle Schwartz-Cornil[2], Geraldine Taylor[11], Sabine Riffault[2].

[1]Swedish University of Agricultural Sciences, Dept. of Clinical Sciences, Ruminant medicine, Host Pathogen Interaction Group, Uppsala, Sweden; [2]Université Paris-Saclay, UVSQ, INRAE, VIM, Jouy-en-Josas, France; [3]Swedish University of Agricultural Sciences, Dept. of Clinical Sciences, Ruminant medicine, Host Pathogen Interaction Group, Uppsala, France; [4]INRAE, PFIE, Nouzilly, France; [5]Université de Tours, INRAE, ISP, Jouy-en-Josas, France; [6]Uppsala University, Analytical Chemistry, Department of Chemistry-BMC, Uppsala, Sweden; [7]Uppsala University, Analytical Chemistry, Department of Chemistry-BMC, Uppsala, Sweden; [8]Swedish University of Agricultural Sciences, Dept. of Energy and Technology, Unit of Applied Statistics and Mathematics, Uppsala, Sweden; [9]Swedish University of Agricultural Sciences, Dept. of Biomedicine and Veterinary Public Health, Uppsala, Sweden; [10]Inmunología y Genética Aplicada, S.A. (INGENASA), Madrid, Spain; [11]The Pirbright Institute, Woking, United Kingdom.

Objectives: The objectives of this work were to compare the safety and the long-term clinical and virological protection induced by three single-shot vaccines against bovine respiratory syncytial virus (BRSV) in calves with BRSV-specific maternally derived antibodies (MDA).

Materials and methods: Four groups of six 3.5 to 8 week-old calves with BRSV-specific MDA were vaccinated either with i) 100 µg of the stabilised pre-fusion form of the BRSV F protein (PreF) in 2 ml ISA 61 VG adjuvant (MontanideTM, SEPPIC, France) intramuscularly (i.m) ii) 5 x 10^6 PFU of ΔSHr-BRSV intranasally (i.n) or iii) one dose of a commercial vaccine (CV) containing BRSV strain 375 ($10^{5.0}$-$10^{7.2}$ Cell Culture Infective Dose 50 %, Zoetis, France) i.n., or iv) were injected with a placebo consisting of 2 ml of ISA 61 VG i.m. Three months later, calves were challenged with BRSV Snook strain by aerosol and were monitored during 13 days post challenge. Clinical, immunological, virological and pathological investigations as well as mass spectrometry-based proteomic analyses were performed to assess the vaccine efficacy and safety.

Results: In line with previous results from this experimental model, all controls developed mild to severe clinical signs of respiratory disease. All vaccinated calves were clinically protected to some extent. The PreF vaccine tended to afford better clinical protection than either ΔSHrBRSV or the CV.

Virus RNA was detected by RT-qPCR, in all controls and in all ΔSHrBRSV- and CV-immunised calves, but not in 3/6 and 4/6 PreF-immunised calves, in nasal secretions and bronchoalveolar lavage (BAL), respectively. Based on the area under the curves of the nasal virus shedding, PreF induced a 10-fold better protection than ΔSHrBRSV and a 100-fold better pro-

tection than the CV.

At necropsy, although performed 13 days post challenge, lesions of interstitial pneumonia and emphysema were present in 6/6 controls and 3/6 CV-vaccinated calves, whereas none or only very limited lesions were observed in calves vaccinated with PreF or ΔSHrBRSV. Histopathological analysis revealed an acute to subacute bronchointerstitial pneumonia in controls, with an increased infiltration of neutrophils in the airways, compared to that in vaccinated calves. Based on BAL cytology and proteomic data with a focus on neutrophil proteins, calves immunised with PreF or ΔSHrBRSV were significantly better protected than those immunised with the CV. Three PreF-immunised calves had the highest number of eosinophils in a lung section, however no adverse clinical reactions were observed.

In contrast to ΔSHrBRSV and the CV, PreF induced BRSV-specific humoral responses pre challenge in most animals, such as BRSV-neutralising antibodies in 4/6 calves by D56 post vaccination (PV) (36 days pre challenge). On D84 PV (8 days pre-challenge), PreF-vaccinated calves had significantly higher titres of BRSV-specific serum IgG, and IgG1, than those vaccinated with ΔSHrBRSV or the CV. Additionally, BRSV-specific serum IgG2 levels tended to be higher in PreF-vaccinated calves than in calves immunized with ΔSHrBRSV or the CV on D84 PV. All three vaccines primed for rapid IgG responses following BRSV challenge and PreF primed for neutralising antibodies, but not IgA, in contrast to ΔSHrBRSV and the CV. BRSV-specific and IFNγ expressing T cells were detected by ELISpot in two CV-immunised calves 84 days PV and in several calves in all groups 13 days PI, but without any significant differences between calves from the different groups.

Conclusions: The i.m. PreF-based BRSV-vaccine induced slightly better clinical protection and a stronger virological protection than the two i.n. live-attenuated vaccines (ΔSHrBRSV and CV), in calves having BRSV-specific MDA at vaccination. Overall, ΔSHrBRSV performed better than the CV for pathological and virological protection. No safety problems were observed after vaccination and challenge. Finally, as a subunit vaccine, PreF allows the development of a DIVA (differentiation between infected and vaccinated animals) test that can facilitate monitoring the efficacy and the safety of the vaccine in the field. The vaccine can additionally be used in control programs.

Keywords: BRSV, vaccine, PreF, ΔSHrBRSV, long-term protection.

IV-10

Immunoglobulin G levels in beef cow colostrum in Finland

Tuomo Kähkönen[1], Heli Simojoki[1], Heidi Härtel[2], Tuire Tuukkanen[2], Maiju Pesonen[3].

[1]University of Helsinki, Helsinki, Finland; [2]HKScan Finland Oy, Turku, Finland; [3]Natural Resources Institute Finland, Ruukki, Finland.

Objectives: The aim of the study was to evaluate and determine the immunoglobulin G content of colostrum in Finnish beef cows by digital Brix refractometer and ELISA (enzyme-linked immunosorbent assay). Colostrum quality of beef cows has not previously been studied in Finland at the cow or farm level.

Material & Methods: The study was conducted on Finnish beef cow farms in the spring of 2021. Colostrum samples were collected from 19 farms. The farm collected calving and colostrum related data and took colostrum samples; in total 137 colostrum samples; 6 to 25 samples per farm. The colostrum samples were frozen in the farm and sent later to laboratory. The breeds of the cows were Hereford (n=37), Aberdeen angus (n=42) and other beef breed (n=43). Immunoglobulin G (IgG) levels were evaluated via total solids with a digital Brix meter (Atago PAL-S, Atago CO, Japan) and determined by ELISA (Bovine IgG ELISA kit, Bethyl Laboratories, Inc., Texas, USA). The measurement results were collected in an Excel (Microsoft Office 365) and the statistical analysis was performed with Stata software version 17.0 MP-Parallel Edition, (Texas, USA). The association of IgG content and variables was evaluated with a regression model. Variables included to the colostrum model were farm, parity and breed of the dam. The correlation between Brix measurement and IgG was tested with Pearson pairwise correlation test.

Results: Total solids Brix percentages of the beef cow's colostrum were median 24.5%, mean 24.7% and standard deviation (sd) 3.84%. Immunoglobulin G levels in the beef cow's colostrum were median 122.0 g / l, mean 128.7 g / l and sd 40.5 g / l. Dam's parity did not affect the IgG results of colostrum(p> 0.05). Breed and farm were highly associated to each other and both were associated with IgG when set separately into the final model. Aberdeen angus cows had higher IgG content in colostrum compared to Herefords or the group other breeds (coeff 23.8; p=0.006). Also the farm was associated to the IgG content of colostrum (Wald's test p=0.02). The lowest herd level mean IgG content was 89.3 g / l, and highest 173,0 g /l.

Conclusion: IgG levels in beef cow's colostrum were sufficient in all farms. IgG levels in beef cow colostrum differed between breeds and farms. Affordable Brix meters can be used as a tool to predict colostrum quality to ensure sufficient calf immunity in beef farms.

Keywords: Beef cow, Colostrum, IgG, Brix, ELISA.

IV-11

Evaluation of the influence of vaccination with a Type 1 and Type 2 BVDV vaccine on the milk yield of lactating cows

Lucy Metcalfe[1], Kathrin Sommer[2], Christian Guidarini[1], Matthew Yarnall[1].

[1]Boehringer Ingelheim, Ingelheim, Germany; [2]Boehringer Ingelheim, Hannover, Germany.

Objective: To determine whether there was an influence of vaccination with Bovela on milk yield in lactating dairy cows

under field conditions.

Materials and Methods: This field study was a randomized, blinded and negative controlled study according to the principles of Good Clinical Practice (GCP) in lactating cows. A total of 139 lactating cows in their high lactation phase, between 1 month after calving and 6 months of lactation, with different serological BVDV status were included in the study. Seven of the study animals were excluded before administration of IVP. Sixty-six (66) animals (group 2) were vaccinated once with a commercial titre of 10^5 $TCID_{50}$ / 2 ml vaccine. The same number of animals in the control group (group 1) received 2 ml of solvent (WPBS). The test and control items were administered intramuscularly (i.m.) in the same manner.

Daily milk yield, compared between both groups, was the primary parameter measured using an automatic milking system from one week before (day post vaccination (DPV) -7) vaccine administration until day 21, termination of the study. The experimental unit was the lactating cow. For each animal a baseline was calculated as the mean of the daily milk yields of DPV-7 – 0. The absolute changes from baseline and percent changes from the preceding seven days were calculated per animal for each day post vaccination (study days 1 – 21) and the mean daily milk yields were calculated per animal for each study week post vaccination. The period after vaccination was divided into 3 segments (DPV 1-7, DPV 8-14 and DPV 15-21).

Other parameters investigated were BVD-related clinical signs, rectal temperature, mastitis, injection site and BVDV-1 and BVDV-2 specific antibodies.

Results: The mean daily milk yield ranged between minimum of 26.43L on DPV 9 and a maximum of 29.07L on DPV 17 in group 1 and a minimum of 26.40L on DPV 7 and a maximum of 29.13L on DPV 17 in group 2.

No statistically significant difference were observed between group 1 and group 2 with regard to mean daily milk yield when comparing the three different periods (DPV 1-7, DPV 8-14 and DPV 15-21) after vaccination as well as between group 1 and group 2 with regard to changes from baseline when comparing the three periods after vaccination. No changes in mean milk yield of 10% or more was detected when comparing the daily mean milk yield of both groups to the milk yield of the seven preceding days.

The mean rectal temperature post vaccination stayed within the physiological range. There were no BVD-related clinical signs or reactions at the injection site reported. Antibody titres of the vaccinated group showed a statistically significant increase from study day (SD) 0 to 21 for both BVDV types. The antibody titre of the vaccinated group was also significantly higher than of the control group on SD21.

Conclusion: The primary parameter milk yield was evaluated between both study groups and revealed no statistically significant difference between vaccinated and control groups after vaccination. This demonstrates that there is no influence of vaccination with Bovela on milk yield. The vaccine is safe for use in lactating cows under field conditions.

Keywords: Vaccination, BVDV, bovine viral diarrhoea, milk yield.

IV-12

Impacts of meloxicam on IBR, BRSV, PI3, and CV titers and morbidity when administered concurrently with a modified-live respiratory vaccine in abruptly-weaned beef calves

Elizabeth R. Homerosky, Craig Dorin.

Veterinary Agri-Health Services, Ltd., Airdrie, Canada.

Objectives: The use of non-steroidal anti-inflammatories (NSAIDs) to mitigate pain and inflammation following stressful events or painful procedures has become common practice in the beef industry. However, a recent in-house study revealed that calves administered meloxicam on arrival at the feedlot were more likely to be treated for bovine respiratory disease (*P*=0.035). It remains unknown if the increased risk was due to impaired inflammatory response, decreased vaccination response, or a combination thereof. The objective of this study was to determine the effect of meloxicam on vaccination response and health by measuring titers for infectious bovine rhinotracheitis (IBR), bovine respiratory syncytial virus, parainfluenza 3, and coronavirus at three time points during the feeding period.

Materials and Methods: For this randomized controlled blinded clinical trial, 271 abruptly-weaned 650lb. steer calves were inducted into a single pen at an Alberta feedlot during October 2018. Upon induction, a venous blood sample was collected and calves received a 5-way modified-live respiratory vaccine, 8-way clostridial vaccine, growth implant, anthelmintic, and macrolide antibiotic. Calves randomly allocated to the control group (N=135) were administered saline, whereas calves allocated to the experimental group (N=136) received injectable meloxicam. Subsequent blood samples were collected at day 7 and 21 to measure early and peak immune response. Antibody titers were quantified using an ELISA test and results were interpreted by an immunologist. Calves were monitored daily for clinical signs of disease and treatments were recorded until an out-weight was measured at day 45.

Results: There were no differences in the direction and magnitude of antibody titers between treatment groups. Additionally, there were no differences in the proportion of calves that seroconverted to any of the viruses at day 7 and 21 between treatment groups except for IBR. At day 21 a greater proportion of calves in the meloxicam group seroconverted to IBR compared to the saline group (*P*=0.01). Morbidity was associated with treatment group as all four treated calves received meloxicam on arrival (*P*<0.05) and a single calf subsequently died. There were no differences in performance parameters at any handling events between treatment groups.

Conclusion: The association between meloxicam and increased morbidity is unclear, however decreased vaccination response is likely not a contributing factor. Stress associated with abrupt weaning methods, coupled with concurrent administration of vaccines and long-acting NSAIDs likely creates a complex immunological response. Further research is warranted to explore the relationship between NSAID administration and health outcomes.

Keywords: NSAIDs, immunity, bovine respiratory disease, feedlot, ELISA.

IV-13

Disease Protection and Immunogenicity of Two Commercial Intranasal Vaccines Evaluated with a BHV-1 Challenge of Weaned Beef Calves

Michael Bolton[1], Philip Griebel[2], Kevin Hill[3], Scott Nordstrom[3], Geert Vertenten[4].

[1]Veterinary Consultant, Michigan, MI, United States; [2]Intervac University of Saskatchewan, Saskatchewan, Canada; [3]Merck Animal Health, Madison, NJ, United States; [4]MSD Animal Health, Boxmeer, Netherlands.

Objective: This study compared both immunogenicity and disease protection between 2 commercial intranasal vaccines (Nasalgen IP® and Inforce 3®), when used in beef calves and challenged with BHV-1.

Materials and Methods: One hundred, 4 to 10 week-old calves at processing were randomly assigned to five intranasal treatment groups (n = 20/group): Group A - vaccine diluent at processing and weaning, Group B – NasIP (Nasalgen IP®) at processing and booster at weaning; Group C – vaccine diluent at processing and primary NasIP at weaning; Group D- Inf3 (Inforce 3®), at processing and booster at weaning; and Group E –vaccine diluent at processing and primary Inf3 at weaning. The analysis of BHV-1 serum neutralizing antibody titres confirmed IN vaccination was performed with maternal antibodies present. All calves were removed from dams at 5- 6 months of age and 14 healthy, BVDV negative calves from each group were shipped the same day from the ranch to the research station. The day after arrival, calves received the designated weaning vaccination. Three days later all calves were aerosol challenged with BHV-1 and monitored daily for clinical respiratory signs, body weight, and rectal temperature. Nasal secretions and serum samples were collected to quantify innate and acquired immune responses.

Results: Analyses of clinical responses following BHV-1 challenge revealed mean rectal temperatures among all vaccinated animals were significantly ($p \leq 0.0001$) lower than animals receiving diluent. Weight loss following BHV-1 infection was reduced compared to controls in all vaccination groups ($p < 0.0001$). Differences in temperature and weight were significant among vaccination groups but were numerically small.

Over the duration of the study all vaccinate groups shed less virus than the diluent control calves. Inf3 booster vaccination significantly ($p = 0.0008$) reduced virus shedding when comparing to primary Inf3 vaccination. NasIP booster group shed significantly ($p < 0.0007$) less virus beginning on day 4 post-infection. However, a significant reduction in virus shedding was not observed until day 5 post-infection with both Inf3 booster vaccination ($p = 0.0001$) and NasIP primary vaccination ($p = 0.03$). The primary Inf3 vaccination significantly reduced virus shedding only on day 6 ($p = 0.04$) and day 9 ($p = 0.001$) post-infection and this group did not significantly ($p = 0.197$) reduce the number of days virus was shed compared to the control group. In contrast, NasIP primary ($p = 0.017$), NasIP boost ($p = 0.0007$), and Inf3 boost ($p < 0.0001$) significantly reduced the number of days on which individual animals shed virus.

IFN alpha and gamma secretion was significantly ($p < 0.0001$) lower in all vaccinate groups compared to the control group. Both booster groups had significantly lower IFN alpha and IFN gamma secretion ($p < 0.0001$) compared to primary vaccination groups.

The NasIP booster group was the only group displaying a significant increase in BHV-1 serum IgG antibody titres three days after booster vaccination compared to controls ($p < 0.0001$) and all other vaccine groups ($p < 0.0003$). There were no significant differences in BHV-1-specific IgA antibody responses among treatment groups during the post challenge period ($p = 0.60$).

Conclusions: Differences in primary and boostered groups demonstrated that NasIP and Inf3 intranasal vaccination of young calves, when neutralizing maternal antibody was present in blood, induced BHV-1 specific immune memory that persisted for at least 4 months in the upper respiratory tract, evident primarily as significantly greater reductions in body temperature and IFN secretion post challenge. Primary vaccination with NasIP resulted in a more rapid onset of reduction in virus shedding compared to primary vaccination with Inf3 and primary NasIP vaccination resulted in a significant reduction in the number of days virus was shed but primary Inf3 vaccination did not. When comparing booster vaccination with NasIP and Inf3 there were small but significant differences in clinical disease but not virus shedding. The NasIP booster group was the only group to show a significant ($p = 0.0003$) increase in specific serum IgG three days after booster vaccination.

Keywords: Intranasal vaccines, BHV-1, protection, immunogenicity.

IV-14

Iron supplementation modulates the early immune response after intranasal vaccination of calves

Hans-Joachim Schuberth[1], Alina Kauke[1], Lennart Golbeck[2], Walter Grünberg[2], Imke Cohrs[3], Theresa Scheu[3], Esther Humann-Ziehank[4].

[1]University of Veterinary Medicine / Institute for Immunology, Hannover, Germany; [2]University of Veterinary Medicine / Clinic for Cattle, Hannover, Germany; [3]Educational and Research Centre for Animal Husbandry, Hofgut Neumuehle, Münchweiler an der Alsenz, Germany; [4]LABVETCON - Laboratory Veterinary Consulting, Burgdorf, Germany.

Objectives: Iron is involved in immune cell differentiation, metabolism and function. We addressed the question whether iron supplementation and the route of supplementation affects the response of calves towards an early intranasal vaccination with a live vaccine.

Materials and methods: A total of 29 healthy, purebred Holstein-Friesian calves were randomly assigned into one of three groups. Calves were born to pluriparous cows over the course of 11 months. Within 1h of birth calves received 4L of fresh colostrum from their respective dam (voluntary + rest drenched) and were treated with either a subcutaneous injection of 1000mg Fe3+ (INJ, n = 10), 1050 mg Fe3+ mixed into the colostrum (ORAL, n = 9) or with 0.9% NaCl as a sham treatment (CON, n = 10). Calves were then transferred to iglus

for 21 days and grouped thereafter. 10L of fresh milk from the own dam were fed for 5 days, feeding was then switched to 10L of milk replacer (1500 g/d, 15% DM, 65 mg FE/kg).

Calves were intranasally (i.n.) vaccinated at day 11 (between day 8 and 14 post natum) with a modified life vaccine against parainfluenza 3 and bovine respiratory syncytial virus (Bovalto Respi® intranasal). Heparinized blood was taken immediately before i.n. vaccination and 24 hours later. Major blood leukocyte subpopulations (granulocytes, lymphocytes, monocytes) were quantified flow cytometrically after hypotonic blood lysis. Mononuclear cells obtained after density gradient centrifugation were incubated with bovine-specific directly labelled monoclonal antibodies and cellular subpopulations (CD4+, CD8+, and gd T-cells, CD21+ B-cells, CD21-/MHC class II+ lymphoid cells, NK cells, classical, intermediate and non-classical monocytes) were determined flow cytometrically. Changes in leukocyte subpopulation numbers and ratios between cell types (24 h after vaccination versus before vaccination) were recorded as fold changes (FC). At day 30 (between day 28 and 32 post natum) all calves were vaccinated subcutaneously (s.c.) with an inactivated vaccine (Bovalto Respi® 3). Calves were categorized into those showing a swelling within 2 days at the injection site (SWELL+) and those with no side effect (SWELL-).

Results: Within the ORAL group, intranasal vaccination resulted in significant FCs > 1 of total leukocytes (FC: 1.10, p = 0.008), lymphocytes (FC: 1.35, p = 0.006), CD2+ T cells (FC = 1.6, p = 0.04), gd T cells (FC = 1.32, p = 0.012). The monocyte/lymphocyte ratio was significantly lower (FC = 0.79, p = 0.009) and the neutrophil/lymphocyte ratio dropped in tendency (FC = 0.87, p = 0.098). Within the INJ group, the numbers of CD4+ T cells dropped in tendency (FC = 0.85, p = 0.078) whereas the ratio between intermediate and non-classical monocytes displayed a significant rise (FC = 1.34, p = 0.0425). CON calves responded with a significant higher CD4/CD8 ratio (FC = 1.29, p = 0.033). The numbers of CD21-/MHC II+ lymphoid cells dropped in tendency (FC 0.86, p = 0.094), whereas numbers of intermediate (FC = 1.28, p = 0.078) and non-classical monocytes (FC = 1.38, p = 0.080) tended to rise. After s.c. vaccination on day 30, 1/9 ORAL calves, 4/10 INJ calves and 3/10 CON calves were SWELL+ (chi2, p > 0.1). SWELL- calves differed significantly (p < 0.02) from SWELL+ regarding their previous response after i.n. vaccination: SWELL- calves displayed positive FCs of lymphocytes (FC= 1.24 ± 0.08), CD4+ T-cells (FC = 1.61 ± 0.22) and CD8+ T cells (FC = 1.04 ± 0.12). FCs of SWELL+ calves were < 1 (lymphocytes: FC = 0.85 ± 0.07; CD4+ T cells: FC = 0.66 ± 0.09; CD8+ T cells: FC = 0.69 ± 0.06).

Conclusions: Intranasal vaccination generates signals resulting in an altered circulation behavior of immune cell subpopulations and/or their release from primary and secondary immune organs. Iron supplementation and the route of supplementation alters vaccination-induced changes of leukocyte subpopulation numbers in blood. A lack of side effects after s.c. vaccination in calves responding with raised numbers of circulating lymphocytes after i.n. vaccination, more frequently seen in orally iron supplemented calves, supports route-specific immune modulatory effects of iron supplementation.

Keywords: Iron supplementation, calves, vaccination, immune response.

IV-15

Comparing the effects on calf health after use of a commercially available *Mycoplasma bovis* vaccine in dairy herds in Scotland

Graeme Fowlie.

Meadows Veterinary Centre, Oldmeldrum, Inverurie, United Kingdom.

Objectives: *Mycoplasma bovis* is a bacterial pathogen of cattle with rising significance in the UK. With variable clinical signs such as pneumonia, mastitis and lameness causing substantial morbidity and mortality in all age groups and cattle systems coupled with difficulties in diagnosis and treatment *Mycoplasma bovis* control presents a real challenge to the cattle industry. Control methods of *M. bovis* infection include extended antibiotic courses metaphylactically, segregating and culling animals and pasteurising colostrum. Improvements in herd biosecurity has been recommended to prevent disease incursion into negative herds. Vaccination using autogenous vaccines has been used for some time in the UK with positive results in published data. A commercial 3-strain *M. bovis* vaccine available in the US, but not Europe, was acquired for this study. There is very limited published data on the efficacy of any commercially available *M. bovis* vaccine. The objectives of this study were to evaluate this commercial vaccine under UK field conditions in terms of safety, ease of incorporation into existing vaccine protocols (i.e. proof-of-concept), reducing mortality and reducing antimicrobial use in dairy calves up to 200 days old.

Materials and Methods: 4 dairies in North-East Scotland with evidence of mycoplasma herd infection and calf pneumonia were selected for vaccination, 4 other dairies were observed as controls. 902 calves born prior to vaccination (first time-period) and 680 calves born after vaccination was commenced (second time-period) were observed up to 200 days old for antibiotic use and mortality. Cows were vaccinated once at drying off (8 weeks pre-calving). Calves were vaccinated at 4 weeks of age. No changes to pre-existing farm vaccine protocols or routine herd management were made on any of the 8 farms during the study. A Kruskal-Wallis Rank Sum Analysis was performed on mortality data. Antibiotic usage was analysed using a Student's Independent T-Test two-sample assuming unequal variance. Vaccine safety and ease of incorporation into existing vaccine protocols was assessed with herd manager discussions.

Results: No adverse reactions were reported during the study in either calves or cows. All farms enrolled in the study successfully completed the vaccine protocols in cows and calves. All farms continued to use the vaccine after the conclusion of the study. There was a significant reduction in post-weaning mortality after vaccination in the second time period (P<0.02). There were 31 deaths in pre-vaccination born calves during the post weaning period (n=534), there were 2 deaths in the post-vaccination born calves during the post-weaning period (n=398). Calves born on non-vaccinating farms showed a slight increased mortality risk in the second time-period. There was a significant reduction in antibiotic usage after vaccination compared to control farms (p<0.05). Vaccinating farms purchased 70.2% less antibiotics after vaccination. Control farms (with qualifying data) purchased 33.9%

more during the second time-period.

Conclusions: This preliminary study demonstrated the safety and proof-of-concept of a commercially available *Mycoplasma bovis* vaccine in UK dairy herds. Due to study design and data gathering insufficiencies the significant reductions in post-weaning mortality and antimicrobial usage can only be broadly suggestive of an effect of the vaccine. Further work on assessing the effect of this vaccine in European cattle herds infected with *M. bovis* is warranted.

Keywords: Mycoplasma bovis, vaccine, dairy calves.

IV-16

Evaluation of health and lactation performance of dairy heifers supplemented with colostrum during the pre-weaning period

Jenna Stockler[1], Manuel Chamorro[1], Thomas Passler[1], Herris Maxwell[1], Manuel Campos[2], Tom Earleywine[3], Debbie Haines[4].

[1]Auburn University College of Veterinary Medicine, Auburn, Alabama, United States; [2]Saskatoon Colostrum Company Ltd., Saskatoon, Canada; [3]Land O' Lakes Animal Milk Products, Dane County, United States; [4]University of Saskatchewan College of Veterinary Medicine, Saskatoon, Canada.

Objectives: To determine if supplementing dairy heifers with 60 g of colostrum-Immunoglobulin G (IgG) in the daily milk-replacer ration during the first 75 days of life resulted in lower incidence of health events, greater first-lactation milk production, and greater survival to second lactation compared with age-matched dairy heifers that did not receive IgG supplementation.

Materials and Methods: A randomized controlled clinical trial was performed. Thirty Holstein heifers from a single dairy herd were separated from the dam immediately after birth and received 300 g of IgG from a colostrum-derived commercial colostrum replacer within 6 hours of birth. At 24 hours of age (Day 1), heifers were assigned to two different treatment groups. Group CR (n=15) received 150 g of a commercial colostrum replacer containing 30 g of IgG mixed with 304 g of a commercial milk replacer twice daily until weaning (day 75). Group MR (n=15) received 453 g of milk replacer with no colostrum replacer supplementation twice daily until weaning and acted as the control group. Blood samples were collected from all heifers at birth, at 24 hours of life to evaluate serum total protein (STP) and serum IgG levels. On-farm personnel blinded to treatment allocation monitored the calves daily and recorded morbidity and mortality events as well as treatments. Individual body weights were collected from all heifers monthly until calving. Milk production was recorded during first lactation and the corrected 305 Mature-Equivalent (ME) milk production was calculated for each heifer.

Results: During the pre-weaning period (Days 1 to 75), a greater proportion of diarrhea events and treatments were recorded in the MR group compared with the CR group ($P < 0.05$); however, respiratory disease, umbilical infection, joint infection and other morbidity events were similar between treatment groups ($P > 0.05$). Mortality was not observed among treatment groups during the pre-weaning period. The mean serum IgG and STP at 24 hours were similar between CR (27.6 g/L and 6.32 g/dL, respectively) and MR (26.42 g/L and 6.17 g/dL, respectively) calves ($P > 0.05$). Individual body weights were similar among treatment groups from birth to weaning and from weaning to calving ($P > 0.05$). The mean corrected 305-ME lactation was similar among heifers from the CR and MR groups ($P > 0.05$); however, survival to second lactation was greater in the CR group ($P < 0.05$). A greater number of heifers in the MR group were culled due to health reasons compared with heifers from the CR group (4 vs. 0, respectively).

Conclusions: The administration of 300 g of IgG through a colostrum-based commercial colostrum replacer to newborn calves results in serum IgG levels superior to what is considered excellent transfer of passive immunity (25 g/L) in dairy calves. Supplementation of dairy heifers with colostrum IgG throughout the pre-weaning period results in disease-sparing effects that extend to the start of the second lactation.

Keywords: Colostrum, IgG, Diarrhea, Survival, Lactation.

IV-17

Cellular and humoral immune response elicited in cattle through combined mucosal and systemic immunization with *Neospora caninum* membrane antigens

Alexandra Correia[1], Carla Mendonça[2], Margarida Duarte Araújo[3], Mariana Resende[1], Elena Pérez Antón[4], Luzia Teixeira[5], António Rocha[2], Manuel Vilanova[1].

[1]Instituto de Ciências Biomédicas Abel Salazar, University of Porto; i3S/IBMC, University of Porto, Porto, Portugal; [2]Instituto de Ciências Biomédicas Abel Salazar, University of Porto; Centro de Estudos de Ciência Animal-Instituto de Ciências, Tecnologias e Agroambiente, University of Porto, Porto, Portugal; [3]Instituto de Ciências Biomédicas Abel Salazar, University of Porto; LAQV/REQUIMTE, University of Porto, Porto, Portugal; [4]i3S/IBMC, University of Porto, Porto, Portugal; [5]Instituto de Ciências Biomédicas Abel Salazar, University of Porto; Unidade Multidisciplinar de Investigação Biomédica, University of Porto, Porto, Portugal.

Neospora caninum is an obligatory intracellular protozoan causative of abortion and stillbirths in cattle posing an estimated global economic burden exceeding one billion dollars *per year* to dairy and beef industries and farms. Vaccination is considered the most cost-effective approach to manage neosporosis, however no commercial vaccine is currently available to prevent this disease. We have developed an experimental intranasal vaccine against neosporosis, using *N. caninum* membrane antigens (NcMP) plus CpG adjuvant, that conferred IFN-gamma-dependent long-term protection to mice challenged with this protozoan.

Objectives: We aimed at improving the immunogenicity and mucoadhesiveness of this experimental vaccine in cattle by adding an additional adjuvant and administration route.

Material&Methods: Holstein calves were immunized by in-

tranasal administration of bovine-specifc CpG and NcMP, plus a carbomer-based adjuvant. To enhance the systemic immune response elicited by the reformulated mucosal immunization, we combined the intranasal administration with a subcutaneous boost of NcMP plus CpG. Cellular and humoral responses were evaluated in immunized and sham-immunized calves. Expression of genes encoding putative antimicrobial proteins was evaluated in bovine monocyte-derived macrophages infected with N. caninum and stimulated with IFN-gamma.

Results: The immunized calves presented elevated levels of parasite-specific IgG and IgA antibodies. Peripheral blood mononuclear cells collected from the immunized animals restimulated *ex vivo* with *N. caninum* antigens showed a marked proliferative response and elevated production of IFN-gamma. This host protective cytokine induced the *in vitro* upregulation of genes related with NO and ROS production, and autophagy in bovine monocyte-derived macrophages. These results show that the used immunization strategy induces parasite-specific humoral and cellular immunity in the bovine host. Additionally, it also stimulates the IL-12/IFN-gamma axis, a key protective mechanism against neosporosis.

Conclusion: Taken together, these results suggest that this optimized immunization approach may induce a protective response in cattle, a hypothesis that is currently being tested in calves.

Funded by FEDER through COMPETE 2020 and FCT - PTDC/CVT-CVT/31020/2017.

Keywords: Neospora caninum, mucosa, vaccination, IFN-gamma.

IV-18

Effectiveness of two intranasal vaccines for the control of Bovine Respiratory Disease (BRD) in newborn beef calves

Nicolas Masset[1], François Meurens[2], Maxime Marie[1], Pauline Lesage[1], Anne Lehébel[2], Nadine Brisseau[2], Sébastien Assié[2].

[1]INRAE, Oniris, BIOEPAR / SELAS EVA, Réseau Cristal, Nantes / Argentonnay, France; [2]INRAE, Oniris, BIOEPAR, Nantes, France.

Objectives: Bovine Respiratory Syncytial Virus (BRSV) is a major cause of Bovine Respiratory Disease (BRD) in newborn calves worldwide. Vaccination is widely used to prevent BRD and BRSV intranasal vaccines were developed to overcome interference with BRSV-specific maternally derived antibodies (MDA). Many experimental challenge trials have proven BRSV intranasal vaccine efficacy, but evidence of effectiveness under field conditions is still lacking, especially for newborn beef calves. The objective of this study was to compare the effectiveness in preventing BRD of a newly available commercial BRSV and BPI-3 intranasal vaccine (Bovalto Respi® Intranasal, Boehringer-Ingelheim) with that of the benchmarked one (Rispoval® RS + PI3 Intranasal, Zoetis) in newborn beef calves reared in a cow-calf farming system in France.

Materials and methods: A randomized non-inferiority multicenter trial was carried out to assess whether Bovalto Respi® Intranasal was at least as effective as Rispoval® RS + PI3 Intranasal, with a pre-stated margin of non-inferiority δ for the prevention of BRD in newborn beef cattle. Sample size was determined to demonstrate non-inferiority assuming α = 0.05, β = 0.20, δ = 0.05 and a prevalence of BRD in the active control vaccine group of 10%. Primary outcome was BRD cases during the in-housed risk period up to 3 months after vaccination. The statistical analysis of the primary outcome was carried out using a mixed logistic regression model. The variable in-housed risk period was kept in the model to adjust BRD occurrence to the variation of the duration of exposition to pathogens between calves. Least squares means were calculated from the model and used to calculate the difference in BRD prevalence between vaccine groups P_{BRD}(Bovalto Respi® Intranasal) - P_{BRD}(Rispoval® RS PI3 Intranasal) and its 95% confidence interval (CI). Secondary outcomes were compared between the two vaccine groups using the chi-squared test or Fisher test (mortality and lethality of calves, as well as antibiotic, non-steroidal and steroidal anti-inflammatory treatments administered during the in-housed risk period) and Student's t-test (time between the vaccine administration and the occurrence of BRD).

Results: A total of 935 Charolais calves from 39 farms were enrolled and randomized into 2 vaccine groups (Bovalto Respi® Intranasal n = 468; Rispoval® RS PI3 Intranasal n = 467). Age at vaccination, duration of in-housed risk period, parity of dams, sex ratio, and occurrence of diseases before vaccination do not significantly differ between the two experimental groups. There was no significant difference between the two vaccines regarding the occurrence of BRD during in-housed risk period. Using least squares means of model outcome, the difference of prevalence P_{BRD}(Bovalto Respi® Intranasal) - P_{BRD}(Rispoval® RS PI3 Intranasal) was estimated at -0.4%, 95% CI = [-1.6% ; 0.8%]. Moreover, no significant differences were observed between vaccines regarding mortality, lethality, duration between vaccination and the occurrence of BRD or treatments in the 2 groups.

Conclusions: Bovalto® Respi Intranasal is at least as effective as Rispoval® RS PI3 Intranasal for the prevention of BRD in newborn beef calves in a cow-calf system in field conditions.

Keywords: BRD, BRSV, Newborn Calf, Intranasal Vaccine.

IV-19

Systemic and local immune responses of beef calves vaccinated post transportation and at the time of a mild respiratory tract infection

Victor Cortese[1], Amelia Woolums[2], Brandi Karisch[2], Thomas Short[3], Merrilee Thoresen[2], Peres Badial[2].

[1]Zoetis, Simpsonville, United States; [2]Mississippi State University, Mississippi State, United States; [3]Zoetis, Parsippany, United States.

Objective: To assess the effect of transportation on immune responses to vaccination in calves.

Materials and Methods: Seventy-five weaned beef calves were randomly assigned to one of three groups (n=25). Group 1 was not transported (NTV) while groups 2 (TV) and 3 (TUV) were transported for 12 hours (day 0). Twelve hours later, NTV and TV were vaccinated intranasally with modified live bovine respiratory syncytial virus (BRSV), bovine herpesvirus −1 (BHV-1) and parainfluenza virus type 3(PI3V), and subcutaneously with modified live bovine viral diarrhea (BVDV) 1 and 2 with Mannheimia haemolytica (Mh) leukotoxoid vaccine. Nasal secretions and serum were collected pre and post vaccination for measurement of nasal interferon alpha, beta, and gamma and IgA to BHV-1 and BRSV, and serum neutralizing (SN) titers to BHV-1, BRSV, and BVDV 1 and 2.

Results: At vaccination some cattle had nasal discharge, fever, and coughing. Nasal swabs tested for common respiratory viruses pre-vaccination were negative. During the study, 5 cattle were treated for naturally occurring BRD. The BHV-1 and BVDV 1 and 2 SN titers were significantly higher in vaccinated than nonvaccinated calves on days 14 and 21. BVDV2 titers were significantly higher in TV than NTV. Response to vaccination was demonstrated in the systemic, but not nasal antibody responses.

Conclusions: This study demonstrates that cattle can mount a humoral response to vaccination in spite of transport and respiratory disease. Differences in serum and nasal responses further demonstrates the division between the local and systemic immune systems.

Keywords: Mucosal, immunity, vaccination, stress, shipping.

IV-21

Vaccination against Salmonellosis in veal farms in the Netherlands is an effective prevention method to reduce the use of antimicrobials

Niels Geurts[1], Henk Kuijk[2], Geert Vertenten[2].

[1]DAP Thewi, Tilburg, Netherlands; [2]MSD Animal Health, Boxmeer, Netherlands.

Objectives: Salmonellosis has been recognized as a disease in cattle all over the world for several decades. It has primarily been associated with S. enterica subsp. enterica serovar Dublin (S. Dublin) and S. enterica subsp. enterica serovar Typhimurium (S.Typhimurium). In view of the economic importance to the cattle industry and the potential to infect the human population, different vaccines have been developed. The objective of this study was to evaluate effect of vaccination with an inactivated Salmonellosis vaccine on mortality and reduction of antimicrobial use in veal farms in the Netherlands.

Materials & Methods: Forty-one animal groups in 6 Dutch veal farms with a history of both S. Dublin and S. Typhimurium were involved in the studies. Sixteen groups were vaccinated with an inactivated Salmonellosis vaccine (Bovivac® S, MSD Animal Health) according to the product data sheet and the other groups remained unvaccinated against Salmonellosis. The vaccinated groups were compared to the non-vaccinated groups over a rearing period of 25 to 26 weeks for mortality, daily antibiotic dose, daily antibiotic dose as defined by the SDa (Netherlands Veterinary Medicines Institute), the rearing cost per calf and the rearing cost per calf excluding vaccination.

Results: In total 42620 animals were included in the study: 17839 vaccinated and 24781 not vaccinated. The average animal group was 1115 and 991 animals for respectively the vaccinated and non-vaccinated groups. The average mortality was 2.54% in the vaccinated and 3.54% in the non-vaccinated group ($p<0.001$). The daily antibiotic dose (real and the one as defined by SDa) was significantly lower in vaccinated animals compared to non-vaccinated animals: 30.56 vs 40.20 daily dosages ($p= 0.001$) for the real daily antibiotic use and 13.06 vs 18.20 daily dosages ($p=0.009$) for the daily antibiotic dose as defined by the SDa. Finally, the average rearing costs were slightly higher in vaccinated (17.64 euro) compared to non-vaccinated animals (17.21 euro), but if the rearing cost excluding vaccination is considered, it is significantly ($p=0.012$) lower in the vaccinated group.

Conclusions: Vaccination against Salmonellosis with an inactivated vaccine is beneficial in Dutch veal conditions as there is less mortality and a strong reduction in antimicrobial use.

Keywords: Salmonellosis, vaccination, veal, antimicrobial reduction.

IV-22

The effect of local and systemic passive immunity acquired from maternal colostrum on clinical protection of beef calves against experimental challenge with BRSV.

Manuel Chamorro[1], David Martinez[1], Thomas Passler[1], Ricardo Stockler[1], Gage Raithel[1], Scott Silvis[1], Merrilee Thoresen[2], Paul Walz[1], Amelia Woolums[2].

[1]Auburn University College of Veterinary Medicine, Auburn, Alabama, United States; [2]Mississippi State University College of Veterinary Medicine, Starkville, Mississippi, United States.

Objectives: 1. To determine if vaccination of beef cows during the last trimester of gestation with two doses of an inactivated bovine respiratory syncytial virus (BRSV) vaccine resulted in greater transfer of local and systemic BRSV-specific passive immunity through maternal colostrum. 2. To determine the role of nasal BRSV-IgG-1 and IgA and serum neutralizing antibodies transferred from maternal colostrum on clinical protection of beef calves against experimental challenge with BRSV.

Materials & Methods: A randomized, controlled, clinical trial was performed. Forty, 3-month-old Black Angus-cross beef steers were assigned to 1 of 2 treatment groups. Group Vacc (n=20) nursed colostrum from cows vaccinated with 2 doses of an inactivated-BRSV vaccine before calving. Group NoVacc (n=20) nursed colostrum from unvaccinated cows. At 3 months of age, calves were challenged with BRSV by intranasal nebulization. Following challenge, respiratory signs were

scored. Nasal secretion and serum samples were collected before and after challenge for BRSV-specific nasal IgG1, IgA, and serum neutralizing antibody testing. Nasal secretion samples were collected after challenge for identification of BRSV by RT-PCR.

Results: Following BRSV challenge, mild respiratory scores were recorded in both groups (P > 0.05). The proportion of calves with fever (rectal temperature > 39.7 C) was greater in NoVacc calves. Nasal BRSV IgG-1 titers and serum neutralizing antibodies were greater in Vacc calves at 48 hours of life (P < 0.05); however, decayed similarly in both groups by 3 months of age (P > 0.05). Nasal BRSV IgA titers were non-existent following colostrum intake and before BRSV challenge and increased similarly (P > 0.05) in both groups after challenge. Calves in the NoVacc group had a higher probability of shedding BRSV in nasal secretions after challenge (P < 0.05). A greater proportion of NoVacc calves tested positive by BRSV RT-PCR after challenge (P < 0.05).

Conclusion: Vaccination of beef cows during the last trimester of gestation with two doses of an inactivated BRSV-vaccine was safe and resulted in greater transfer of local and systemic passive immunity to their calves. Moderate to low levels of BRSV serum neutralizing antibodies provide clinical protection against experimental challenge with BRSV. Nasal BRSV IgG-1 and IgA transferred from maternal colostrum do not play an important role on clinical protection of 3-month-old beef calves against experimental challenge with BRSV.

Keywords: Colostrum, IgG-1, IgA, neutralizing, BRSV.

IV-23

Evaluation of the cell-mediated immune response of dairy cattle vaccinated with an autogenous vaccine against mastitis sustained by *Staphylococcus aureus* and *Streptococcus agalactiae*

Matteo Cornaggia[1], Genovese Serena[2], Valentina Bertazzo[2], Katia Capello[2], Giulia Zarpellon[2], Tiziana Ferro[2], Lucillo Cestaro[3], Giulio Severi[4], Annalisa Stefani[2], Luca Bano[2].

[1]Istituto Zooprofilattico Sperimentale delle Venezie, Padova - Istituto Zooprofilattico Sperimentale della Lombardia ed Emilia-Romagna, Brescia, Italy; [2]Istituto Zooprofilattico Sperimentale delle Venezie, Padova, Italy; [3]Veterinary Practitioner, San Donà di Piave, Italy; [4]Istituto Zooprofilattico Sperimentale dell'Umbria e Marche, Perugia, Italy.

Objective: Mastitis causes heavy economic losses in the dairy industry due to milk discharge, therapeutic costs, and culling rate of infected animals[4].

Staphylococcus aureus (SAU) and *Streptococcus agalactiae* (SAG) are important contagious bacteria agents of mastitis of cattle. In positive herds, their widespread is usually contained through the application of high hygienic standard measures, segregation, and blanket dry cow treatments that lead to a high consumption of antibiotics.

For this reason, authorities and consumers encourage the development of effective alternatives to the antimicrobial administration such as vaccines. Many vaccines against mastitis due to SAU have been tested with debated results, especially in field conditions[1,3].

The aim of our work was to evaluate the cell-mediated immune response elicited by a bivalent tailor-made vaccine produced with SAU and SAG as inactivated antigens, in field conditions.

Materials and methods: The herd was composed by 89 lactating Friesian cows hosted in the same experimental condition and served by an automatic milking system.

SAU and SAG were previously detected in the bulk tank milk. Bacteria were cloned and a tailor-made bivalent inactivated vaccine was prepared with a final concentration of $7.5x10^8$ UFC/ml.

Eighty-two subjects were vaccinated twice (V group) while 7 subjects served as negative controls (C) and received only the placebo composed of the adjuvants and vaccine medium without bacteria.

The peripheral whole blood and the individual milk samples were collected from 7 animals randomly selected within the V group and 7 from the C group at 0, 1, 2 and 3 months after the first immunization.

The cell mediated response was investigated by flow cytometry on the blood samples measuring the lymphocytes T CD4+ and CD8+ previously stimulated *in vitro* for 24h with heat-shocked cultures[2] (HSC) of SAU and SAG, and the lymphocyte activation pattern CD25+ was recorded. Concanavalin A (ConA) and PBS stimulation were used as a positive and negative control respectively.

Linear mixed models were adopted to evaluate the effects of groups (V and C), type of stimulation (SAG, SAU, ConA or PBS) and sampling time on parameters.

Results: Differences in cell response were significant (p=0.001) when blood was incubated in the wells with HSC, ConA and PBS. Statistical analysis revealed a significant difference in the increase expression of CD4+CD25+ against SAG (p= 0.0037) in the V group after the immunization. No differences were recorded for SAU vaccination.

Bacteriological evaluation of the individual milk resulted negative to SAG while 1 subject resulted positive to SAU in the V and C groups, respectively.

Conclusions: The study of the antigen-specific cellular subsets activated in response to SAG and SAU was aimed to achieve an *in vitro* test for cell-mediated response evaluation to study the effectiveness of vaccine against bovine mastitis.

The specific increase expression of $CD4^+CD25^+$ lymphocytes in the V group against SAG suggest that vaccination is able to stimulate properly the cells of the immune system and this observation is supported also by the nonproliferation in the C group which received only the adjuvant.

The poor results obtained with SAU are probably due to the expression of *S. aureus* exotoxins that act as superantigens and result in the overproduction of cytokines and activation of T cells that make the measurement inconclusive.

This preliminary study needs further confirmation in experimental conditions to better understand the role of the vaccines in the stimulation of the cell-mediated immune response for the prevention of mastitis in cattle.

References:

1. Bröker, B. M., Mrochen, D., Péton, V. (2016). The T cell response to *Staphylococcus aureus*. *Pathogens*.
2. Brown, A. F., Murphy, A. G., Lalor, S. J., Leech, J. M., O'Keeffe, K. M., Mac Aogáin, M., McLoughlin, R. M. (2015). Memory Th1 cells are protective in invasive *Staphylococcus aureus* infection. *PLoS Pathogens.*
3. Pereira, U. P., Oliveira, D. G. S., Mesquita, L. R., Costa, G. M., Pereira, L. J. (2011). Efficacy of *Staphylococcus aureus* vaccines for bovine mastitis: a systematic review. *Veterinary microbiology*.
4. Ruegg, P. L. (2017). A 100-Year Review: Mastitis detection, management, and prevention. *Journal of dairy science*.

Keywords: Dairy cows, flow cytometry, vaccine, mastitis.

IV-24

UK study: comparing antibody quantities in commercial calf scour pastes vs. the same volume of colostrum from cows vaccinated with a commercial calf scour vaccine

Kat Baxter-Smith[1], Robert Simpson[1], Geert Vertenten[2].

[1]MSD Animal Health, Milton Keynes, United Kingdom; [2]MSD Animal Health, Boxmeer, Netherlands.

Objectives: Neonatal calf diarrhoea (scour) is a common and costly disease on farm. There are many preventative approaches which can be undertaken to reduce the incidence and severity of this disease. It has been demonstrated previously that feeding colostrum with high levels of antibodies is protective against calf scour[1,2]. No published peer-reviewed evidence could be found on a literature search for the efficacy of scour pastes in preventing calf scour, however a recent survey completed by 479 UK cattle farmers revealed that they rated dam vaccination and scour pastes as equally efficacious scour prevention tools[3].

Little information is available regarding quantity of antibodies in commercial scour pastes, therefore the aim of this study was to measure antibody levels of rotavirus and coronavirus in commercially available calf scour pastes, and compare the results with the same volume of colostrum from cows vaccinated with a commercial calf scour vaccine (Rotavec® Corona, MSD Animal Health). This vaccine is licensed to reduce the incidence and severity of calf scour by raising the level of antibodies to rotavirus, coronavirus and E. coli K99 in the dam's colostrum.

Materials and Methods: Six different popular commercial scour paste brands were sourced from various agricultural trade stores. 2 tubes of each brand were purchased.

Samples of colostrum from 13 randomly selected heifers and cows which had been vaccinated during pregnancy with a commercial calf scour vaccine (Rotavec® Corona; as per SPC) were collected directly after calving and transported to the lab.

Rotavirus and coronavirus antibody titres in all samples were determined using a standard fluorescent antibody virus neutralisation test in all samples of colostrum and scour paste. The antibody titres reported were the reciprocal of the final dilution at which significant neutralisation of the virus by the sample was still seen.

Results: From the 12 scour pastes that were tested; there was an average virus neutralisation antibody titre of 10800 (min 80, max 81920) for coronavirus and 4341 (min 40, max 14482) for rotavirus.

The average virus neutralisation antibody titres of the same volume of colostrum from the vaccinated cows and heifers was 57383 (min 7241, max 81920) for coronavirus and 33846 (min 10240, max 81920) for rotavirus.

Conclusions: The 6 brands of calf scour pastes tested in this study contained on average markedly numerically lower levels of rotavirus and coronavirus antibodies compared to the same volume of colostrum from vaccinated cows. Although often listed as components of the tested scour pastes in this study, values for antibody quantities were not stated on the 'contents' or 'ingredients' labels. This study determined that on average commercial scour pastes do contain substantially lower levels of antibodies. The numerical value of antibody titres in the scour pastes varied considerably between brands.

Feeding a new-born calf 10% body weight (4L in a 40kg calf) of colostrum in the first hours of life is recommended to ensure a high level of passive immunity[4]. Using the results of this study based on average scour paste antibody levels, giving 4 Litres of scour vaccine-boosted colostrum would provide the calf with the antibody equivalent of 700-1000 scour pastes (paste size 30g).

Scour pastes are not licensed for the prevention of calf scour and evidence is lacking to support their use for this Therefore, this study illustrates that calf scour pastes are not a suitable substitute for good colostrum management and dam vaccination in preventing calf scour.

Farmers could be misinformed if they consider scour pastes and vaccination as equally efficacious at preventing calf scour; and more education from vets is needed.

Keywords: Calf scour, antibodies, scour paste, vaccine.

IV-25

Can an injectable trace element supplement increase the immune response of dairy calves?

Andrew Bates[1], Matt Wells[2], Richard Laven[3], Line Ferriman[4], Axel Heiser[5], Clare Fitzpatrick[6].

[1]Vetlife Scientific Ltd, Temuka, New Zealand; [2]Virbac Ltd, Hamilton, New Zealand; [3]School of Veterinary Sciences, Massey University, New Zealand; [4]Vetlife Ashburton, Ashburton, New Zealand; [5]AgResearch, Hopkirk Research institute, New Zealand; [6]Department of Microbiology and Immunology, Otago University, New Zealand.

Objectives: This study was carried out on a pastoral spring calving dairy farm in New Zealand with approval from Massey University Animal Ethics Committee. It was designed

to investigate the effect of TMS supplementation on the immune system in young calves before weaning.

Recent New Zealand (NZ) work suggests that neo-natal injection of a trace mineral supplement (TMS) containing copper, selenium, manganese, zinc and chromium can reduce morbidity and mortality on NZ dairy farms in the first 140 days of life. Overseas, evidence from older calves indicates this form of TMS leads to a heightened immune response. In light of the association between TMS and reductions in morbidity and mortality, this study was designed to investigate the effect of TMS supplementation under NZ pastoral conditions on the immune system in young calves before weaning.

Materials and methods*:* The first 40, Jersey-Friesian cross-bred heifer calves born after the mid-point of calving on a mid-Canterbury dairy farm (43.91° S, 171.75° E), were blood sampled within 24 hours of birth for serum total protein, Cu, Se and Zn. Thirty of these were selected, using stratified randomisation to form two equal groups (treatment and control) with the same distribution of serum total protein, Cu, Se, Zn and for the breed and age. From the remaining 10 calves, five were selected using stratified randomisation to form a sentinel group to verify no field exposure to *Salmonella sp*. occurred during the trial period.

All calves were housed in covered, open fronted bay-sheds with solid walls up to 1.5m in groups of 10-12 and 1.5m^2 allocated space per calf. Treatment and sentinel groups were randomly distributed amongst the housing groups. Calves were bedded on wood chips which were topped up weekly and all calves remained on the farm of origin for the period of study.

All calves received two injections of a killed vaccine containing *Salmonella typhimurium, S. bovis-morbificans*, *S. hindmarsh* and *S.Brandenburg* at two and six weeks of age. At the same time as vaccination, the treatment group received an injection of a TMS containing 40mg Zn, 10mg Mn, 5mg Se, 15mg Cu per ml. Sentinel animals received no injections. All animals were bled weekly from 2-9 weeks. Samples were analysed for neutrophil and monocyte phagocytic function, gamma interferon response, Salmonella sp. antibody titres and serum selenium, copper and zinc and differences examined using Bayesian statistics.

Results: At weeks 3 and 4 there was a wide difference between TMS and control calves in the percentage of white blood cells phagocytosing with the 95% predictive interval (PI) for the difference in the distributions excluding zero (+15% (95%PI=10.0-20.0) and +8% (95%PI=2.0-13.0) respectively. At weeks 3,4 and 5 phagocytosis per cell was also greater in the TMS calves (+12% (95%PI=2.1-21.6), +19% (95%PI=8.2-28.8) and +10% (95%PI=1.6-20.6) respectively). There was no statistical evidence of a difference in gamma interferon or antibody production between TMS and control claves although gamma interferon response was numerically greater in the TMS group.

Conclusions: This study adds to the evidence that TMS supplementation can increase some components of the innate immune systems in young calves. Neutrophil and monocyte function were increased, with a numerical increase in gamma interferon production. Although we found no conclusive evidence for an increase in antibody response in supplemented calves, both treatment groups showed an antibody response greater than the sentinel calves despite the presence of maternal immunity.

Keywords: Trace mineral supplement, mmunity, phagocytosis, calf.

IV-26

Field efficacy trials with a new intranasal BRD vaccine

Birgit Makoschey[1], Piet Nuijten[1], Björn Sander[2], Eva Zschiesche[2], Geert Vertenten[1].

[1]MSD Animal Health, Boxmeer, Netherlands; [2]MSD Animal Health, Schwabenheim an der Selz, Germany.

Objective: The objective of these studies was to determine the efficacy of a new intranasal BRD vaccine (Bovilis® INtranasal RSP™ Live) under field conditions. The vaccine contains live, attenuated BRSV and PI3 strains. During lab studies it was shown that it significantly reduced nasal shedding and clinical symptoms caused by BRSV and PI3 challenge infections as well as reduction of lung pathology and viral load in the lungs in a BRSV infection model.

Materials and methods: Two blinded, randomised, placebo-controlled field trials were conducted to investigate the efficacy. The first one was conducted in Portugal (3 farms) and the second one in Germany (2 farms) and France (6 farms). Calves (approx. 250 in total) aged 5 to 12 days received the vaccine or a placebo intranasally directly from the tip of a syringe. Groups were separated as much as possible during the first 2 weeks after vaccination as spreading of the live vaccine viruses is known to occur. Animals were monitored for signs of BRD like increased rectal temperature, coughing, increased breathing rate, nasal discharge, ocular discharge and depression. In case of BRD a bronchoalveolar lavage (BAL) was performed. Nasal swabs (NS) were taken weekly starting 2 weeks after vaccination. BAL and NS samples were tested by PCR for 8 different BRD pathogens, including BRSV, PI3, BCoV, BoHV-1, *Mycoplasma bovis, Histophilus somni, Pasteurella multocida and Mannheimia haemolytica*. Sera were collected on day 0, 14, 28 and 84 and tested in virus neutralisation (VN) assays for antibodies against BRSV and PI3. The efficacy of the vaccine was assessed based on differences between vaccinated and control calves in 1) clinical signs of BRD, 2) PCR results for BRSV and PI3 in BAL and NS samples, and 3) VN titers against BRSV and PI3.

Results: In the first study in Portugal, 124 calves were included, 62 in each group. In the vaccinated group 4 animals were excluded (N=58), whereas in the placebo group 17 animals were excluded (N=45), of which 15 animals were tested positive for the PI3 vaccine strain using a specific PCR. This proved the transmission of the PI3 vaccine strain from vaccinated to some of the placebo calves.

There was a notable difference in clinical signs between the two groups: more animals in the vaccinated group were without clinical scores than in the placebo group: 19% of the vaccinated calves had a total clinical score of 0 versus 11.1% in the placebo group, 55.2% of the vaccinated calves had BRD scores of 0 versus 37.8% in the placebo group, and

29.3% of calves in the vaccinated group had no BRD symptoms and a negative BRSV and PI3 PCR result versus only 15.6% in the placebo group. PCR analyses of many BAL and nasal swabs were positive for several other BRD pathogens like *Pasteurella, Mannheimia* and BCoV. As expected, serological responses were low on average and VN titers declined over time.

In the second study, a very low incidence of infection in France occurred but in Germany a BRSV field infection took place. Further detailed analysis of the different efficacy parameters for Germany showed: i) a significant difference (p=0.0406) for nasal swab samples positive for BRSV between the vaccinated group (61.5%) and in the placebo control group (85.7%), ii) that BRD clinical scores had the tendency to be higher in the placebo control group, iii) no clear differences in serological responses against BRSV or PI3, iv) a notable difference of BAL samples positive for BRSV: 30.8% in the vaccinated group was positive for BRSV in comparison to 42.9% in the placebo control group.

Conclusion: Field studies to determine the efficacy of vaccines against BRD pathogens are complex and influenced by animal health status, weather conditions, farm management and exposure to various pathogens including those that are not part of the vaccine. Indeed, in both studies most samples contained several other BRD pathogens, besides BRSV and PI3. Nevertheless, this new intranasal BRD vaccine (Bovilis® INtranasal RSP™ Live) induced significant protection against nasal shedding of BRSV during an outbreak. This is the first EU-licensed intranasal BRD vaccine for which efficacy has been proven in a field trial.

Keywords: BRD, intranasal vaccination, field efficacy, PI3, BRSV.

IV-27

Effect of periparturient intranasal vaccination on post parturient health parameters in Holstein cows

Victor Cortese[1], Pablo Pinedo[2], Juan Rodrigo Pedraza[3], Thomas Short[3], D. Manriquez[2], A. Velasquez-Munoz[2], G. Solano[2].

[1]Zoetis, Simpsonville, KY, United States; [2]Colorado State University, Boulder, United States; [3]Zoetis, Parsippany, United States.

Objective: The objective of this study was to evaluate the effect on health and reproductive performance of vaccinating Holstein cows were vaccinated with an intranasal modified live viral bovine respiratory syncytial virus (BRSV), modified live, temperature sensitive bovine herpesvirus −1 (BHV-1) and modified live, temperature sensitive parainfluenza virus type 3 vaccine (INV) during the peri-parturient period.

Materials and Methods: In a large commercial dairy, 4834 multiparous cows were vaccinated 18-24 days prior to expected calving date (n=1198), within twelve hours after parturition (n=1250) or at both time points (n=1141). A group was left as unvaccinated controls (n0 intranasal vaccination, n=1245). Cattle were blocked based on parity group and expected calving date and randomized to the experimental treatments (vaccination and control) within blocks. Health and reproductive outcomes were monitored and compared to matched, randomly assigned control cows.

Results: The greatest effects of vaccination were significant decreases in total cows removed from the herd throughout the lactation, decreased presentation of retained fetal membranes, and significantly lower incidences of pneumonia and mastitis. Overall, a greater impact was determined with two doses and, if one dose was administered, the results tended to favor administration at calving.

Conclusion: This study indicates that using this INV during the periparturient period can improved several postpartum health outcomes due to the up regulation of the immune system and supports earlier work indicating better immune responses when administered on the day of calving. While this vaccine can be used for immune modulation, more importantly this study suggests that future immune modulators may have a better outcome if administered to the local immune system.

Keywords: Postpartum health, postpartum immune suppression, calving stress, intranasal vaccination, immunomodulation.

IV-28

Benchmarking of the immune response induced by commercial intranasal vaccines against BRSV in dairy calves

Gilles Foucras[1], Lola Romanos[1], Nathan Cebron[2], Blandine Gausseres[2], Julie Cournet[2], Christian Tasca[2], Stéfanie Bernheim[3].

[1]ENVT, Toulouse, France; [2]IHAP, Toulouse, France; [3]Zoetis France, Malakoff, France.

Objectives: BRSV is a main respiratory agent in young calves. The intranasal vaccine Rispoval® Intranasal RS+PI3 (Zoetis) is available for more than ten years and has a long history of protective effects in bovine herds. Recently three new vaccines based on the same principle became available on the European market. However little is known about the local immune response provided by this way of immunization in cattle, and if there is any difference in the priming capacity of the viral strains used in these vaccines.

Materials and methods: Cross-bred dairy calves (n=40) were allocated randomly to one of the four commercial vaccines (10/group), with equal numbers of males and females. Calves had received 4L of fresh pooled colostrum (Brix index >22) at birth, and were housed in individual hutches. At the age of 8-17 days according to the label of the summary of product characteristics, calves were vaccinated intranasally with one of the four vaccines following the manufacturer's recommendations with the indicated material and modalities for application. Blood was collected before immunization and serum BRSV-specific antibodies were assessed (Monoscreen AbELISA BRSV, BioX Diagnostics), so that titres were not known at the time of vaccine administration. Mucosal lining was collected using nasal swabs (Copan) at 0, 7, and 14 days after vaccination. Virus load was quantified by RT-qPCR (Biosellal).

Local immune response was assessed by quantifying cytokine production with a Multiplex bovine cytokines assay (Milliplex, MERCK-Millipore) and by ELISA (Kingfisher Biotech).

Results: At the time of vaccination, all calves had BRSV-specific IgG1 antibodies with a mean value of 74% [43;105%] compared to the positive control of the ELISA kit. No group difference of the serum BRSV-specific antibodies of the calves before vaccination can be detected. BRSV was detected in 8/10 calves vaccinated with Rispoval® IN RS+PI3 at one of the two dates post-vaccination, but was more inconstantly detected in other vaccine groups. Cytokine concentrations were normalized to the total protein amount recovered by nasal swabing. Most of the 15 cytokines were detected in the mucosal lining and varied according to the date of sampling upon vaccination. Only trends were seen despite differences of BRSV strains, modalities of application, volume of vaccine and amount of virus administered in one or the two nostrils. Rispoval® IN RS+PI3 had a good capacity to induce Interferon gamma-induced protein 10 (IP-10 or CxCL10) production early at d7 in all calves tested.

Conclusions: With this design, no significant difference of the local immune response was noticed during a period of two weeks after vaccine application, despite some favorable trends and an homogeneous response induced by Rispoval® IN. Further data are needed to define further the difference of priming capacity among intranasal vaccines, and the consequences on the protection herewith afforded.

Keywords: BRSV, vaccine, intranasal, calves, immunity.

IV-29

Influence of vaccination on the seroconversion of 2 major respiratory pathogens in German beef rearing farms

Egon Thesing[1], Geert Vertenten[2].

[1]Intervet Deutschland GmbH, MSD Tiergesundheit, Unterschleißheim, Germany; [2]MSD Animal Health, Boxmeer, Netherlands.

Objectives: An important part of the German beef sector is the fattening of Simmental/crossbreed bulls. BRD (Bovine Respiratory Disease) is the main health problem on those farms. The causes are multifactorial, but the contribution of different pathogens is widely accepted. Bovine Respiratory Syncytial Virus (BRSV) and *Mannheimia haemolytica* (Mh) are 2 major pathogens playing a pivotal role in the BRD complex. Nowadays, the diagnostic of pathogens is mainly done by antigen detection on respiratory samples, rather than serological identification of antibodies. As serological studies during the rearing time in beef calves are not commonly available, a study was performed to obtain insights in the serodynamics of BRSV and Mh using paired blood samples including different BRD vaccination schemes.

Materials & Methods: The study was done on 2 beef rearing farms in different regions of Germany over 2 separate years (2017-2018) during the winter season. All calves were vaccinated with a live intranasal BRSV-Parainfluenza-3 vaccine (Rispoval® RS-Pi3, Zoetis) and received metaphylactic antimicrobial treatment at arrival on the rearing unit. The vaccination protocol was continued with either 2 administrations at 2 and 6 weeks after arrival with a monovalent attenuated vaccine (Rispoval® RS, Zoetis) (control group (cg)) or a multivalent, inactivated BRSV-Parainfluenza-3-Mh vaccine (Bovilis® Bovipast RSP (MSD Animal Health) trial group (tg)). Paired serum samples were taken from relevant subsets of animals in the cg and tg. The first sampling was performed just before the first vaccination and the second sampling 12 weeks later.

BRSV and Mh antibodies were measured in the Centre for Diagnostic Services (MSD Animal Health, Boxmeer, The Netherlands) by *in house* developed ELISAs and titers were expressed as log2.

Seroconversion for BRSV and Mh was defined as a titer change of 2 log2 steps or more.

A statistical evaluation was done for several parameters with significance level 0.05.

Results: In total 1.127 calves (farm A n=931, farm B n=196) were included in the study. Eventually, paired samples were taken from 196 calves corresponding to approx. 12 % (n=137) of the calves in cg and 5 % (n=59) of the calves in tg.

The baseline serum titers for BRSV and Mh ELISA at the time of the first sampling were not significantly different between groups (p=0.28) and there was no influence of age to the humoral response (p=0.44).

Most calves in the control group showed no humoral response in BRSV titer. Moreover, the titers dropped in a quarter of the calves. Only in 5 calves (4 %) the BRSV -titers increased 4 fold (>2 log2 steps) and consequently identified as a seroconversion. In contrast to this, 58 % of the animals in the trial group seroconverted. If one assumes that the infection pressure is equally high in both groups, one can establish a significant (p<0.0001) better induction of antibody formation after vaccination with the trivalent inactivated vaccine. Apart from the vaccination with the inactivated vaccine, additional field infections might play a role in this seroconversion. A good BRSV response to vaccination with the multivalent inactivated vaccine (Bovilis® Bovipast RSP) has already been described by Berge et al. (2021).

Concerning the Mh antibody induction, 42% of the cg calves had a seroconversion despite the absence of a Mh strain in the administered vaccine. This is indicative for a field infection in those animals. A clear humoral response for Mh under field conditions in young dairy calves is reported also by Jozan (2021). Almost twice the rate (81%) of animals showed serum conversion in the tg (17% > 2 log2 steps; 64% > 4 log2 steps) which is due to the combination of the vaccine effect and the field infection as demonstrated in the cg.

Conclusion: Serological screening of antibodies in beef calf rearing farms provides useful and practical insights into the occurrence of infections and the impact of vaccinations. Interpretation of this data supports the establishment of targeted prophylactic measures. *Mannheimia haemolytica* is a frequently circulating pathogen in beef calf rearing farms in which the use of a trivalent inactivated BRSV-Parainfluenza-3-*Mannheimia haemolytica* vaccine (Bovilis® Bovipast RSP) leads to a clear humoral response.

Keywords: Seroconversion, BRSV, Mannheimia haemolytica, vaccination, German beef farms.

IV-30

Impact of prepartum administration of an infectious calf diarrhea vaccine on nonspecific colostral immunoglobulin concentrations of dairy cows

Greg Chambers[1], William Kelton[2], Grant Smolenski[3], Emma Cuttance[1].

[1]VetEnt Research, Te Awamutu, New Zealand; [2]Te Huataki Waiora School of Health, The University of Waikato, Hamilton, New Zealand; [3]MS3 Solutions Ltd, Hamilton, New Zealand.

Objectives: Passive transfer of colostral immunoglobulins from the cow to the calf is essential for calf health. The objective of this study was to determine if prepartum administration of a vaccine stimulates increased concentrations of colostral immunoglobulins of dairy cows beyond what is explained by vaccine-specific immunoglobulins.

Materials & Methods: A prospective cohort study was conducted on a spring-calving commercial dairy farm that had applied a policy of only vaccinating cows with even ear tag numbers with a calf diarrhea vaccine, while cows with odd ear tag numbers were left unvaccinated. Cows in the vaccinated group (even ear tag numbers, n=204) received a sensitizer and booster vaccination with a vaccine against bovine rotavirus (serotypes G6 and G10), bovine coronavirus and *E. coli* having the K99 pili adherence factor. A sensitizer was given because the study vaccine was different to the vaccine previously used. Cows in the control group (odd ear tag numbers, n=194) received a 2 mL subcutaneous sterile saline solution. Both groups received two treatments at a three-week interval, completing the treatments approximately two weeks prior to the planned start of calving. During the calving period, technicians separated calves from cows immediately after parturition and prior to suckling, and cows were completely milked out within six hours of parturition. Using novel techniques, vaccine-specific, total, and nonvaccine-specific (total minus vaccine-specific) concentrations of immunoglobulin classes A, G1, G2a and M (IgA, IgG1, IgG2a and IgM respectively) were quantified by mass spectrometry for 20 colostrum samples from each treatment group.

Results: Technicians harvested colostrum from a total of 47 cows (n=24 control cows and n=23 vaccinated cows). Two cows were excluded due to low BCS (n=1) and a spurious colostrum volume (n=1), leaving 45 eligible samples. After matching, 20 samples from each treatment group were randomly selected for analysis. The mean colostrum Brix % was 24.65 and 26.20% for unvaccinated vaccinated cows respectively, but this difference was not significant at the 5% level. We found 2.2- to 3.0-fold higher concentrations of vaccine specific IgG1, IgG2a and total immunoglobulins in the colostrum of vaccinated cows compared to control cows, and no difference in the concentration of IgA. We also found a 1.6-fold increase in the concentration of IgM. IgG2a concentrations were lower but still within the detection range of our assay. While no significant differences were observed for the other immunoglobulin isotypes, we found a 1.5-fold increase in total IgM concentrations associated with vaccination.

After subtracting vaccine-specific from total immunoglobulin concentrations, we again found a 1.5-fold increase in total IgM concentrations associated with vaccination but no significant differences for the other immunoglobulin isotypes (Table 1). Furthermore, the difference in mean non-vaccine-specific colostral IgM concentration between vaccinated and control cows (2.72 mg/ml) was larger in magnitude than the difference in vaccine-specific IgM concentration (0.15 mg/ml). Though not statistically significant at the 5% level, the predicted difference in mean non-vaccine-specific IgG1 concentration between vaccinated and control cows was 10.78 (95% CI = -9.03 - 30.58) mg/ml, which was larger than what was explained by the difference in vaccine-specific IgG1 concentrations of 0.98 mg/ml. This increase in IgG1 concentrations drove the bulk of the 12.9 (95% CI = -10.26 - 36.07) mg/mL increase in overall non-vaccine-specific immunoglobulin concentrations.

Table 1. Predicted mean concentrations of non-vaccine-specific colostral immunoglobulins for control (n=20) and vaccinated (n=20) cows in a cohort study evaluating the effect of prepartum vaccination of pregnant dairy cows with a calf diarrhea vaccine on colostrum immunoglobulin concentrations.

	Mean (95% CI) concentration (mg/ml)		
Immunoglobulin class	**Control group**	**Vaccinated group**	**P-value**
IgG1	95.30 (81.30, 109.31)	106.08 (92.07, 120.08)	0.278
IgG2a	4.73 (3.96, 5.50)	4.71 (3.95, 5.48)	0.972
IgA[1]	9.87 (7.79, 12.50)	8.73 (6.88, 11.10)	0.462
IgM[1]	5.78 (4.74, 7.05)	8.76 (7.18, 10.67)	0.005
IgG	100.03 (85.57, 114.49)	110.79 (96.33, 125.24)	0.294
Total Ig	115.46 (99.08, 131.84)	128.37 (111.99, 144.75)	0.267

1. Values were log transformed and then transformed back to the original scale due to non-normality.

Conclusion: It is possible that the vaccine, in addition to managing infectious calf diarrhea, may also improve colostrum quality through increased non-vaccine-specific colostrum immunoglobulin concentrations. Further research is necessary to determine the mechanism for these preliminary findings and what impacts it may have on calf health outcomes.

Keywords: Vaccination, colostrum, immunoglobulin, dairy cow.

IV-31

Bovine Myeloid Antimicrobial Peptide-28 (BMAP-28) mRNA Expression by Bovine Cells and Effects of Synthetic BMAP-28 on Bovine Respiratory Disease Pathogens.

Santiago Cornejo Tonnelier[1], Cassandra Barber[1], Merrilee Thoresen[1], Daryll Vanover[2], Hannah Peck[2], Philip Santangelo[2], Amelia Woolums[1].

[1]Department of Pathobiology and Population Medicine, College of Veterinary Medicine, Mississippi State University., Mississippi State, MS, United States; [2]Wallace H. Coulter Department of Biomedical Engineering, Georgia Institute of Technology and Emory University, Atlanta, Georgia, United States.

Objectives: *Mannheimia haemolytica* (MH) is the principal bacterial pathogen associated with bovine respiratory disease (BRD) in cattle. Bovine Herpes virus type 1 (BHV-1) can cause BRD by itself or it can be associated with MH or other opportunistic bacteria. Existing antimicrobials do not consistently prevent BRD due to MH, and do not have an effect against viruses; bovine antimicrobial peptides (AMP) have immune-stimulating and nonspecific antimicrobial effects that could improve BRD control. Messenger RNA (mRNA) treatment could be used to induce AMP expression in cattle, but efficacy must first be confirmed *in vitro*. Synthetic AMP can be generated to use as standards when characterizing mRNA-expressed AMP. We hypothesized that bovine cells can express synthetic mRNA coding for the AMP BMAP-28 and that synthetic BMAP-28 can inhibit the growth of MH and elicit antiviral effects against BHV-1 virus.

Materials & Methods: Madin-Darby bovine kidney cells were cultured and transfected with mRNA coding for BMAP-28 linked to the reporter nanoluciferase. After 4, 12, 24, and 72 hrs, relative light units (RLU) and protein concentration were measured. Results were expressed as RLU/µg of protein.

MH at 500 CFU/ml was incubated with synthetic BMAP-28 at 10 or 100 µg/ml for 0, 12, or 24 hrs, and quantitative culture was performed.

BHV-1 at 10^3 and 10^4 IU/ml were treated with synthetic BMAP-28 at 10 or 100 µg/ml and incubated at 37ºC for 2 hrs. A TCID50 assay on Madin-Darby bovine kidney cells was performed for each treatment. TCID50 units were calculated after 5 days post-infection (dpi).

Results: Bovine kidney cells expressed mRNA coding for BMAP-28 with peak expression occurring at 24hs in cell lysates and supernatants. Synthetic BMAP-28 at 10µg/ml inhibited MH growth at 12 and 24hs post-treatment. Synthetic BMAP-28 at 100 µg/ml elicited antiviral effects against BHV-1.

Conclusions: Treatment of bovine cells with synthetic mRNA induces BMAP-28 expression *in vitro*. BMAP-28 can inhibit MH growth and BHV-1 replication. These results provide support for further research to test the mRNA-expressed product against BRD pathogens *in vitro* and *in vivo*. mRNA treatment to induce AMP expression could lead to new BRD control strategies.

Keywords: BMAP-28, mRNA, BRD, Mannheimia haemolytica, BHV-1.

IV-32

Q fever: Vaccinate as much as needed and as little as possible

Jens Böttcher[1], Gregor Siegl[2], Stephanie Geischeder[1], Frank Dautzenberg[1], Elisabeth Deckinger[1], Michaela Alex[1], Vanessa Turowski[1], Britta Janowetz[1].

[1]Bavarian Animal Health Service, Poing, Germany; [2]Meltl GmbH & Co.KG, Grabenstätt, Germany.

Objective: *Coxiella (C.) burnetii* is a zoonotic pathogen. Endemic infection in dairy cow farms is frequently observed. A vaccine (Coxevac™, Ceva Santé Animal) is available to control infection in cattle. In this case report a controlled vaccination scheme was implemented in an infected dairy cow farm over a time period of 4½ years. Shedding of *C. burnetii* with milk and during calving was monitored. A long-term follow-up and characterization of chronically infected cows was possible as these were not immediately removed. In order to avoid side effects of vaccination no regular revaccination of all cows every 9 months was performed, however, long-term vaccination consisted of a primary vaccination of heifers and a single booster vaccination before 1st and 2nd breeding (after 1st calving), respectively. The immune response of seronegative heifers was assessed by phase-specific antibody ELISA and IFN-γ-Recall assay (IFN-γ-RA) before/after primary vaccination and revaccination.

Materials and Methods: In 2015 acute infection (abortion and stillbirths) was observed in a dairy cow farm (225 lactating cows, Simmental cattle). *C. burnetii* was identified as a possible cause. The farmer asked for coordinated intervention measures. Since October 2015 until January 2020 pathogen-shedding during calving was monitored by vaginal swabs. Additionally, individual milk samples were collected yearly. In spring 2016 primary vaccination of cattle (>12 months) was performed.

Vaginal swabs and milk samples were tested by quantitative PCR (Böttcher et al., 2013); milk and serum samples were tested for phase-specific antibodies and Li-heparin blood samples were tested by IFN-γ-RA (Böttcher et al., 2017).

A group of 15 heifers (2017) were tested before (t0), 4 months (t1), 16 months after primary vaccination (t2 i.e. at revaccination) and 1 month after revaccination (t3) for phase-specific-antibodies and IFN-γ-reactivity.

Results: Detection rates of *C. burnetii* in vaginal swabs were 9/59 in 2015 (Oct-Dec), 20/142 in 2016 (Jan-Jun), 19/145 in 2016 (Jul-Dec), 1/236 (2017), 6/253 (2018), 5/292 (2019) and 0/56 (Jan/Feb 2020). Two chronically infected cows (#129, #934) shed *C. burnetii* at calving in 2018. Four cows in the same calving pen with #129 tested positive ($<10^3$ C.b./swab), too. In 2019, #129 tested negative at calving, however, 5 cows in the same calving group tested positive ($<10^{3,5}$ C.b./swab). Detection rates of *C. burnetii* in milk were 7/191 (2015), 5/225 (2016/Mar), 13/211 (2016/Aug), 8/190 (2017), 0/161 (2018), 2/229 (2019) and 3/225 (2020).

Five and three chronically infected cows were present in the herd in 2018 (## 126, 129, 133, 214, 934) and 2019 (##129, 214, 934), respectively. Cow #129 shed *C. burnetii* at 2nd, 3rd and 4th, cows #126 and #934 only once at 2nd and 6th parturition, respectively. Vaginal swabs of #133 and #214 tested negative at parturition. Cows #126, #129 and #934 constantly shed large amounts in milk (mean $10^{3,4}$, $10^{4,1}$, $10^{3,4}$/ml, respectively) whereas #133 and #214 showed intermittent low-level shedding in milk (mean $10^{1,4}$, $10^{1,2}$/ml, respectively). Mean PhI-/PhII-titres (milk) of cows #126, #129, #133, #214, #934 were 2705/5561, 1324/6053, 566/95, 294/114, 109/2639, respectively. Cow #214 was the last one that established a

chronic infection (since Aug2016).

Primary vaccination and revaccination of heifers was analysed: PhI-titre (serum) was negative until t2, after revaccination (t3) a mean titre of 10000 was reached. PhII-titre increased to 10000 at t1, decreased to 2000 at t2 and increased to 100.000 at t3. IFN-γ-RA increased from <15% to 40% of the poke-weed-mitogen-reactivity at t1, decreased negative (<15%) at t2 and increased to 80% at t3.

Conclusions: To our knowledge this is the first report describing the long-term shedding of *C. burnetii* in a vaccinated herd. Herd vaccination decreased shedding of *C. burnetii* at calving about 9 months later. In other words, vaccination had no effect on the infected uterus. Vaccination had no effect on chronic shedders. Primary vaccination and only one revaccination protected cows from becoming chronic shedders. The strong PhII-titres and IFN-γ-reactivity after revaccination exceeding the level after primary vaccination suggests the induction of an immunological memory by primary vaccination.

Acknowledgements: This study was financially supported by the Free State of Bavaria and the Bavarian Joint Founding Scheme for the Control and Eradication of contagious Livestock Diseases.

References:

Böttcher, J. et al. (2013) Berl Münch Tierärztl Wochenschr 126, 427-435.

Böttcher, J. et al. (2017) J Vet Med Res 4(9):1106.

Keywords: Q fever, Coxiella, vaccination, chronic shedding.

IV-33

Duration of immunity after a natural infection with bovine respiratory syncytial virus

Sara Hägglund[1], Katarina Näslund[1], Hakan Enul[1], Cecilia Lefverman[1], Leonore Pascal[1], Anna Svensson[1], Menno Holzhauer[2], Karin Alvåsen[3], Catherine Dubuquoy[4], Isabelle Schwartz-Cornil[4], Sabine Riffault[4], Geraldine Taylor[5], María Jose Rodriguez[6], Marga Garcia Duran[6], Jean François Valarcher[1].

[1]Swedish University of Agricultural Sciences, Dept. of Clinical Sciences, Unit of Ruminant Medicine, Host Pathogen Interaction Group, Uppsala, Sweden; [2]GD Animal Health, Deventer, Netherlands; [3]Swedish University of Agricultural Sciences, Dept. of Clinical Sciences, Unit of Epidemiology, Uppsala, Sweden; [4]Université Paris-Saclay, UVSQ, INRAE, VIM, Jouy-en-Josas, France; [5]The Pirbright Institute,, Woking, United Kingdom; [6]Inmunología y Genética Aplicada, S.A. (INGENASA), Madrid, Spain.

Objectives: Bovine respiratory syncytial virus (BRSV) infections commonly occur as epizootic outbreaks in nearby cattle herds. In parallel with improved biosecurity, the maintenance of herd immunity could contribute to minimize virus circulation. With the overall goal to identify vaccine targets, the objective of this work was to monitor the duration of BRSV-specific humoral and cellular immunity in cattle.

Materials and methods: Local and systemic BRSV-specific immunity was monitored in cattle in one Swedish dairy herd following a BRSV-outbreak. The herd consisted of 534 cattle with an average age of 33 months and an average yearly milk production of 9971 kg ECM/cow. Cows were housed in a free stall with cubicles, weaned calves in group pens and unweaned calves in hutches outdoor. During the outbreak, two cows died in respiratory distress and there was a loss of delivered milk that had an estimated value of 2300 Euro. BRSV was detected by ELISA (seroconversion) and/or virus isolation and/ or RTqPCR in cows, heifers and calves.

Blood and milk (when applicable) was collected from 33 female cattle of the Swedish red and white breed every two months during 26 months after the outbreak (post outbreak, PO). The sampled animals were either born during or just after the outbreak (n=5), or were aged 2-3 months (n=6), 4-5 months (n=6), 7-11 months (n=8), or 23-30 months (n=8) at the time of the outbreak. The oldest animals were either at < 6 (n=4) or > 6.5 (n=4) months of gestation.

Nasal secretions and saliva were repeatedly collected from five animals. To verify the absence of BRSV re-circulation in the herd, blood was collected at the end of the study from 24 additional animals born >4 months PO. BRSV-specific antibodies of different isotypes were analysed by ELISAs, along with BRSV-neutralising antibodies (NAb) by a virus neutralisation assay. Furthermore, attempts were made to detect BRSV-specific T cell responses in peripheral blood, by lymphoproliferation and ELISpot assays.

Results: All cattle that were older than 2 months at the time of the outbreak had or developed BRSV-specific serum IgG and BRSV-F competing antibodies, which remained stable during at least 26 months. The four cows in > 6.5 months of gestation had the lowest levels of such antibodies and two cows did not secrete detectable BRSV-specific IgG in milk. The youngest cattle, which were possibly infected in the presence of maternally derived antibodies, became seronegative within 4-6 months PO (within 3-5 months of age) and remained so for at least 26 months, inferring that no re-infections occurred. All animals that were born >4 months PO were seronegative at the end of the study.

The BRSV-NAb responses and the BRSV-specific serum IgG2 followed a similar pattern as IgG, but with a greater individual variation. The highest NAb-titres were detected in some of the heifers that were 5-11 months old at the outbreak, but titres dropped within 4-6 months. Another such heifer, from which virus had been isolated, developed very poor NAb responses but high levels of BRSV-specific IgG2.

BRSV-specific IgA was detected in nasal secretions of all sampled cattle two months PO, but these responses were not stable over time. In contrast, BRSV-specific local IgG2 responses were more stable but varied between individuals, in agreement with the data on BRSV-specific IgG2 in serum. Overall, BRSV-specific IgG2 and IgA were poorly detectable in saliva. It was not possible to detect BRSV-specific T cell responses in peripheral blood two months PO.

Conclusions: In conclusion, it was demonstrated that BRSV-specific IgG lasts for at least two years in animals infected above 2 months of age but are not always detectable in milk. The BRSV-specific IgG2 antibody responses and the BRSV-NAb titers varied between individuals. In young animals, a drop of NAb titers was observed within 4 months, whereas

in adults, the NAb titers were low but stable. Cows that were near calving during an outbreak and all calves born later than two months before an outbreak, appear as the most critical candidates for vaccination in order to limit virus circulation in the field. More investigations are needed to confirm these data and to identify if previously infected animals are virologically protected by their antibodies upon reinfection.

Keywords: BRSV, immunity, duration, neutralising antibodies, respiratory disease.

IV-34

Virus detection by PCR following vaccination of naive calves with intranasal or injectable multivalent modified-live viral vaccines

Victor Cortese[1], Paul H. Walz[2], Benjamin W. Newcomer[2], Kay P. Riddell[2], Daniel W. Scruggs[3].

[1]Zoetis, Simpsonville, United States; [2]Auburn University, Auburn, United States; [3]Zoetis, Parsippany, United States.

Objective: To evaluate the duration and cycle times of PCR-positive results following administration of modified-live viral (MLV) vaccines to sero-negative beef calves.

Materials and Methods: Twenty beef calves were randomly assigned to either group 1 and vaccinated intranasally with a MLV vaccine containing bovine alpha herpesvirus 1 (BoHV-1), bovine respiratory syncytial virus (BRSV), and bovine parainfluenza virus 3 (BPIV-3), or to group 2 and vaccinated subcutaneously with a MLV vaccine containing bovine viral diarrhea virus 1 and 2 (BVDV-1, -2), BoHV-1, BRSV, and BPIV-3. Deep nasopharyngeal swabs (NPS) and transtracheal washes (TTW) were collected from all calves, and whole blood was collected from group 2 calves and tested by PCR.

Results: In group 1, the proportions of calves that tested PCR-positive to BVDV, BoHV-1, BRSV, and BPIV-3 on any sample at any time were 0%, 100%, 100%, and 10%, respectively. In group 1 calves, 100% of calves became PCR-positive for BoHV-1 by day 3 post-vaccination and 100% of calves became PCR-positive for BRSV by day 7 post-vaccination. In group 2, the proportions of calves that tested positive to BVDV, BoHV-1, BRSV, and BPIV-3 on any sample at any time were 50%, 40%, 10%, and 0%, respectively. All threshold cycle (Ct) values were >30 in group 2 calves, irrespective of virus; however, Ct values <25 were observed in group 1 calves from PCR-positive results for BoHV-1 and BRSV. All calves were PCR-negative for all viruses after day 28.

Conclusion: Following intranasal MLV viral vaccination, PCR results and Ct values for BRSV and BoHV-1 suggest that attempts to differentiate vaccine virus from natural infection is unreliable.

Keywords: Bovine virus vaccination, deep nasopharyngeal swabs, PCR.

IV-35

Efficacy of a vaccine to control Coxiellosis in goats 1 year after primo-vaccination: assessment of the duration of immunity

Philippe Gisbert[1], Tamas Szalai[2], Jean De Foucauld[1], Georges Orszagh[2].

[1]Ceva Santé Animale, Libourne, France; [2]Ceva Phylaxia, Budapest, Hungary.

Objectives: In small ruminants, Coxiellosis also named Q fever is responsible for abortion, stillbirth and weak born. In addition, Q fever is a zoonosis which can cause flu-like syndromes in humans but also abortions or cardio-vascular disorders. It is therefore a major public health concern. Control of Q fever is therefore of main interest.

In this challenge study, the efficacy of an inactivated vaccine against *Coxiella burnetii* - Nine Mile strain phase I (Coxevac®, Ceva Santé Animale) one year after primo vaccination on goats was evaluated on two main criteria:

- Reduction of abortion rate.
- Reduction of shedding in milk, faeces and vaginal mucus.

Material and methods: The study was conducted in compliance with the provisions of Directive 2010/63/EU relative of the protection of animals used for scientific purposes.

Forty 3 months old goats were vaccinated twice 3 weeks apart according to the product's label (Coxevac® 2 mL subcutaneously); forty goats of the same age were not vaccinated and were included as control. Eleven months later, goats were mated after oestrus synchronization. Finally, 14 pregnant goats in the vaccinated group and 7 from the non-vaccinated were selected.

At 75 +/- 7d of pregnancy (1 year and 27 days after the second injection of the vaccine), goats were challenged subcutaneously with a heterologous field strain of *Coxiella burnetii* (CbC1).

Abortion rate and number of live kids were assessed. Faeces and vaginal shedding were measured by qPCR from 14 days post challenge to 35 days post abortion/kidding. Milk shedding was measured by qPCR from the day of abortion/kidding to 35 days. For these three types of samples, both ratio of shedders animals and quantity of excreted *Coxiella burnetii* were measured.

Results: Five out of seven goats of the control group aborted (71.4%) while there were only three out of 14 in the vaccinated group (21.4%). The difference between the two groups was significant ($p=0.0408$).

The rate of non-viable or aborted kids was 71.4% and 11.1% in the control group and in the vaccinated group, respectively ($p=0.0017$).

From d56 post challenge to d35 post kidding/abortion, the proportion of shedders in faeces and vaginal mucus was significantly higher ($p<0.003$ and $p<0.002$, respectively) in the control group than in the vaccinated group. The mean level of excretion in faeces (measured in bacteria per g) was reduced by 4 log10 between the control and the vaccinated group. This reduction was 5 log10 for vaginal mucus (measured in bacteria per mL).

Regarding the shedding in milk, the excretion of *Coxiella burnetii* was significantly higher in the control group than in the vaccinated group (p<0.0002). The mean quantity of bacteria excreted per milliliter was reduced by 4 log10 in the vaccinated group.

Conclusion: This study showed that goat vaccination (Coxevac®, Ceva Santé Animale) was effective for one year in reducing the abortion rate due to *Coxiella burnetii* and excretion of the bacterium. This is of main interest to control the disease in the flocks and to reduce the zoonotic risk.

Keywords: Q fever, goats, vaccine, immunity.

IV-36

A field study evaluating humoral immune response in calves vaccinated with two multivalent respiratory vaccines

Anna Catharina Berge[1], Thibault Jozan[2], Camille Levesque[3], Geert Vertenten[4].

[1]Berge Veterinary Consulting BV, Vollezele, Belgium; [2]MSD Santé Animale, Beaucouzé, France; [3]LABOCEA, Javené, France; [4]MSD Animal Health, Boxmeer, Netherlands.

Objectives: Calf vaccination may be adversely affected by maternal antibodies. Two trivalent inactivated Bovine Respiratory Disease (BRD) vaccines that are commercialized in Europe (Bovilis® Bovipast RSP or Bovilis® Bovigrip, MSD Animal Health (BPAST) and Bovalto® Respi 3, Boehringer Ingelheim (BTO)), can be administered to calves from 2 weeks of age. BPAST has proven efficacy in calves with maternal antibodies, while BTO is only indicated for use in seronegative animals. In field conditions, calves rarely remain seronegative for BRSV due its high prevalence in cattle herds. The objective of this field study was to evaluate the seroneutralising (SN) antibody responses against bovine respiratory syncytial virus (BRSV) and specific humoral (IgG) ELISA response to BRSV, bovine parainfluenza 3 virus (BPI3V) and *Mannheimia haemolytica* (Mh) in young calves vaccinated with either BPAST or BTO and after booster vaccination 9 to 11 months later.

Materials & Methods: This field study was performed on one dairy farm in France, with 30 dairy calves that received at least 3L colostrum during the first 6h of life. Three study groups were created of 12 calves vaccinated with BPAST, 13 with BTO and 5 non vaccinated negative controls. Vaccines were administered at 15-30 days of age (T0), 1 month (m) later (T0+1m) and at 9-11 months of age (T10). Blood samples were taken at T0, T0+0.5m, T0+1m, T0+1.5m, T0+2m, T0+2.5m, T0+3m, T0+4m, T0+5m, T0+6m, T10, T10+0.5m and T10+1m. Serum antibodies in the individual calves after primo (first 2 injections) and booster (3rd injection) vaccinations were evaluated by calculating the areas under the curve (AUC) of the Log2 transformed BRSV SN titres and the optic density measures of the ELISA tests for BRSV, BPI3V and Mh. A seroconversion was defined as a four-fold or more increase in titres. Multivariate general linear models were used to evaluate the influence of the vaccination on the AUC of the serum measures within 6 months after the primo vaccination and 1 month after the booster vaccination.

Results: There was no significant difference in T0 titres between the groups. No negative control calves seroconverted, whereas respectively 15 and 75% of the BTO and BPAST vaccinated calves seroconverted for BRSV after primovaccination and respectively 0% and 75% of the BTO and BPAST vaccinated calves seroconverted to BRSV after booster vaccination. The BPAST vaccinated calves had significantly higher BRSV SN titres AUC following the primo vaccination and booster vaccination compared to the negative control calves and the BTO vaccinated calves. The BTO vaccinated calves did not have a significantly different BRSV SN titres AUC response after the primo or booster vaccinations compared to the negative control calves.

Although some animals experienced a natural infection with BPI3V, Mh or both, some differences in the antibody responses between the different groups could still be identified. BPAST and BTO vaccinated calves mounted a significantly higher AUC ELISA OD for both BPI3V and Mh compared to negative control calves after primovaccination with the highest AUC measured in the BPAST vaccinated calves. This in contrast with booster vaccination where only the BPAST vaccinated calves mounted a significantly higher AUC ELISA OD for both BPI3V and Mh compared to negative control calves.

Conclusion: This study indicates that despite containing antigens targeting the same BRD pathogens, immunogenicity of vaccines can be very different. Early vaccination of calves with multivalent adjuvanted inactivated BRD vaccines (e.g. Bovilis® Bovipast® RSP) can elicit a humoral response with a memory effect as indicated by the serological response after booster vaccination.

Keywords: BRD vaccines, commercial calves, BRSV, PI3, Mannheimia haemolytica.

IV-37

Concurrent vaccination for pneumonia in pre-weaned calves; a longitudinal study on the safety, and serological response elicited by delivery of two live intranasal vaccines

Anna Flynn[1], Catherine Mcaloon[2], Katie Surgrue[1], Ricki Fitzgerald[1], Cara Sheridan[3], Bosco Cowley[3], Emer Kennedy[1].

[1]Grassland Science Research Department, Animal & Grassland Research and Innovation Centre, Teagasc Moorepark, Cork, Republic of Ireland; [2]School of Veterinary Medicine, University College Dublin, Dublin, Republic of Ireland; [3]MSD Animal Health, Dublin, Republic of Ireland.

Objectives: Bovine respiratory disease complex (BRD) is an economically important disease with a high mortality rate. In Ireland, it remains the leading cause of death in calves between 1 and 12 months of age. Moreover, as antimicrobial resistance and animal welfare are areas of growing public concern, control of BRD through prophylactic vaccination will be increasingly important in decreasing calf rearer reliance on antibiotics. It has been suggested that vaccination of pre-weaned

calves from two weeks of age in the face of maternally-derived antibodies (MDA) can provide efficacious protection from the viruses most commonly associated with pneumonia.

Although numerous respiratory vaccines suitable for calves are available, achieving early protection is often challenging due to a limited vaccination time-window and labour demand. Reportedly, it is now common practice on farms to administer multiple intranasal vaccines together to pre-weaned calves. However, this practice is currently unlicensed and there is limited information on the effects of concurrent intranasal administration of these vaccines. The objective of this longitudinal study was to determine the safety of concurrent administration, from 3 weeks of age, of two currently available intranasal vaccines for the viruses Bovine Respiratory Syncytial Virus (BRSV), Parainfluenza-3-virus (PI3V) and Bovine Herpes Virus 1 (BHV-1) and to examine the serological responses they elicited in the face of high levels of MDA.

Materials & Methods: Forty dairy (n=30) and dairy beef (n=10) calves, male (n=13) and female (n=27) were balanced in a randomised block design with 4 treatments; i) Live BHV-1 vaccine only (Bovilis® IBR Marker Live, MSD Animal Health) (BHV-1 only) ii) BRSV/PI3V vaccine only (Bovilis® INtranasal RSP Live, MSD Animal Health) (RSV/PI3V only) and iii) Concurrent vaccination with live BHV-1 and BRSV/PI3V vaccines (Bovilis® IBR Marker Live & Bovilis® INtranasal RSP® Live) (CV) and iv) non-vaccinated control group. Calves were vaccinated intranasally (IN) at an average age of 21.6 (±4.8) days with a 2 ml dose of each vaccine, 1 ml per nostril. Calves in the CV treatment received both vaccines separately, and consecutively. Control calves were given a placebo of the diluent used for both vaccines. All calves from vaccinated groups received booster vaccination against BHV-1 (Bovilis® IBR Marker Live, 2ml i.m) and RSV&PI3V (Bovilis® Bovipast® RSP, MSD Animal Health, 5ml, s.c.) at the age of 4 months. The serological response to vaccination was measured at 3 weeks post intranasal vaccination and following booster. The antibody response was measured with commercially available indirect ELISA test kits, and the results were compared between treatment groups.

In addition to serology, clinical health scores, including rectal temperature were taken before and after IN vaccination. Calves were weighed weekly and growth rates were compared between treatments. All data analysis was performed using SAS version 9.4 (SAS Institute Inc.) and significant associations in mixed models were confirmed at the $P<0.05$ level.

Results: There was no significant difference in rectal temperature before and after IN vaccine administration ($P= 0.219$). Concurrent vaccination had no adverse effect on weight gain or clinical score, with CV calves gaining on average 0.28 kg (±0.14) kg/week more than BHV-only and BRSV/PI3V only calves. Intranasal vaccination with BRSV/PI3V at three weeks of age resulted in an improved anamnestic response to the PI3V and BRSV antigens following subsequent administration of an inactivated vaccine against PI3V, BRSV and *M. haemolytica*. Therefore, intranasal vaccination at three weeks of age in the face of high levels of MDA acted to prime the immune response for a subsequent parenteral dose of inactivated BRSV/PI3V vaccine. BHV-1 antibodies increased in all treatment groups including the controls post-IN vaccination, which may indicate circulation of wild BHV-1 in the herd.

Conclusion: Although concurrent intranasal vaccination with a live vaccine against BHV-1 and a live vaccine against BRSV and PI3V is outside of the licensed use of the two products, in our study the concurrent use in 3-week old seropositive calves caused no adverse effect on weight gain or clinical parameters compared to calves vaccinated with the vaccines individually and non-vaccinated controls. Moreover, the enhanced immune response against BHV-1 and RSV following booster vaccination might suggest that the immune system had been primed. Any decision to use these vaccines concurrently needs to be made on a case-by-case basis by a veterinary professional.

Keywords: concurrent vaccination, intranasal, pneumonia, safety, serology.