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COMPARATIVE EVALUATION OF IMMUNE RESPONSES OF SWINE IN PRRS-STABLE AND UNSTABLE HERDS

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Porcine Reproductive and Respiratory Syndrome (PRRS) is an elusive model of host/virus relationship in which disease is determined by virus pathogenicity, pig breed susceptibility and phenotype, microbial infectious pressure and environmental conditions. Successful disease control corresponds to “stability”, i.e. a condition with no clinical signs of PRRS in the breeding-herd population and no viremia in weaning-age pigs. The aim of this work was to compare the profile and time-course of humoral and cell-mediated immunity of replacement gilts in one stable and one unstable herd, respectively. In particular, we investigated PRRS virus (PRRSV) in serum and group oral fluid samples by Real-time RT-PCR, PRRSV-specific IgA and IgG in oral fluids, serum IgG antibody and the cell-mediated response (PRRSV-specific release of interferon-gamma) in whole blood samples. These parameters were measured in order to identify possible discrepancies in the development and kinetics of the immune response against PRRSV. Gilts got regularly infected around 7-9 weeks after entering the stable farm, and at the very beginning in the unstable one. Four main results must be highlighted: A) the precocity of the Ab response in group oral fluids was similar to that seen in sera; B) circulation of PRRSV was consistently detected in the unstable herd, as opposed to the stable one; C) a balanced IgA and IgG response in oral fluids was only observed in the stable herd; D) an IFN-gamma response was regularly observed in the stable herd, whereas gilts of the unstable one were partly positive at arrival day, only (transfer of maternal immunity). The above findings indicate that a peculiar profile of immune response to PRRSV underlies herd stability. Therefore, the outlined immune parameters can represent a useful readout system to evaluate successful adaptation to PRRSV based on acclimatization of breeding animals and management of pig flow. In this respect, failure of disease control measures could be traced back to farm management and/or peculiar virus “immunotypes”, affecting the immune response of PRRSV-infected animals.

RESPONSE OF PORCINE MONOCYTE DERIVED MACROPHAGE SUBSETS AND DENDRITIC CELLS TO ASFV STRAINS OF DIFFERENT VIRULENCE

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African swine fever (ASF) is a severe disease of domestic pigs and wild boar, which is currently present in Africa and some European countries [1, 2]. There is no vaccine available and the only control measures are stamping out and movement control [3]. The aetiological agent is the ASF virus (ASFV), a large enveloped DNA virus which primarily infects cells of the myeloid lineage [4, 5]. An improved characterization of the interactions between these immune cells with ASFV strains of different virulence may help underpin vaccine development efforts. Blood derived monocytes were differentiated *in vitro* into macrophages (moM Φ) or dendritic cells (moDC). moM Φ were left untreated or were classically (moM1) or alternatively (moM2) activated, whereas, moDC were left untreated or matured with TNF- α and IFN- α . Cells were infected with an attenuated strain (BA71V) and a virulent Sardinian isolate (22653/14) of ASFV, alongside mock-infected controls. Virus-cells interaction was investigated using flow cytometry, ELISA and confocal microscopy. 22653/14 presented a greater ability to infect moM1 compared to the avirulent strain and higher expression of early (p30) compared to late (p72) proteins was observed in BA71V-infected moM1. Infected macrophages displayed a lower expression of CD16 compared to un-infected bystander cells and no differences were observed in the expression of CD163 between infected and bystander cells. The levels of MHC I were similar between mock and 22653/14-infected macrophages, instead BA71V-infected moM Φ and moM2 presented lower percentages of MHC I compared to the mock-infected control. Differences in cytokine responses were observed, with higher levels of IL-18, IL1- α and IL-1 β release by moM1 in response to BA71V compared to 22653/14. Both isolates are able to infect moDC and 22653/14 presented greater ability to infect matured moDC compared to BA71V. Higher resistance of matured moDC to BA71V infection is due to inhibition of late protein synthesis. As macrophages, ASFV-infected moDC displayed lower expression of CD16. Our results revealed significant

differences in the response of macrophage subsets and moDC with these ASFV isolates: the attenuated BA71V strain presented higher susceptibility to type I and type II IFN antiviral activity and promoted a higher release of cytokines which might influence the development of protective immunity.

1. Sanchez-Vizcaino JM (2006) African Swine Fever. In: Straw BE, Zimmerman JJ, D'Allaire S, Taylor DJ (eds) Diseases of Swine 9th edition, pp 291-298. Blackwell Publishing, Ames, Iowa.
2. Sanchez-Vizcaino JM et al. (2015) New insights into the role of ticks in African Swine fever epidemiology. *Rev Sci Tech* 34:503-11.
3. Costard S et al. (2013) Epidemiology of African swine fever virus. *Virus Res* 173:191-197.
4. Dixon LK et al.: Asfarviridae. In *Virus Taxonomy*, VIIIth Report of the ICTV. Edited by Fauquet CM, Mayo MA, Maniloff J, Desselberger U, Ball LA. London: Elsevier/Academic Press; 2005:135-143.
5. Sánchez-Cordón PJ et al. (2008). Role of hepatic macrophages during the viral haemorrhagic fever induced by African swine fever virus. *Histol Histopathol* 23:683–691.

PHENOTYPIC AND FUNCTIONAL CHARACTERIZATION OF PORCINE MONOCYTE-DERIVED MACROPHAGES PRODUCED WITH DIFFERENT METHODS

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Macrophages maintain tissue homeostasis and are key players in the immune response to pathogens [1]. Porcine macrophages can be generated in vitro from monocytes differentiated through incubation in media supplemented with porcine serum-plasma [2, 3, 4] or with recombinant human macrophage-colony stimulator factor (hM-CSF) [5], but currently there is no standardized protocol. In this study blood derived monocytes were differentiated into macrophages using six different culture conditions (10-20-30% (v/v) of autologous plasma or 50-100-200 ng/ml of hM-CSF), then differentiation was assessed using light and confocal microscopy, flow cytometry and ELISA. Monocytes cultured with either plasma or hM-CSF increased in dimension (forward scatter; FSC) and granularity (side scatter; SSC), but SSC was higher in macrophages differentiated with porcine plasma and these cells displayed an increased number of elongated projections protruding from cell surfaces. CD163, MHC II DR and CD203a expression were up-regulated following monocytes differentiation into macrophages in all conditions, but CD163 was slightly lower in macrophages differentiated using 30% autologous plasma. Macrophages differentiated with hMCSF and high percentages of porcine plasma showed an ability to proliferate in vitro. Macrophages differentiated with all the methods displayed higher susceptibility to ASFV infection than monocytes and increased release of TNF-alpha in response to lipopolysaccharide (LPS) stimulation compared to monocytes. Macrophages cultured in autologous plasma showed a higher basal release of IL-1RA compared to those cultured with hM-CSF and displayed a lower ability to release IL-1 and TNF-alpha in response to classical activation. In addition, using porcine plasma great variability among animals was observed. Data generated in this study suggest that

all the protocols are suitable to differentiate porcine monocytes into macrophages, although the use of high percentages of porcine plasma lead to formation of more elongated cells, with lower expression of CD163 and less able to release pro-inflammatory cytokines in response to classical activation stimuli. Moreover with hM-CSF a higher reproducibility between experiments can be obtained. We hope that information generated from this study will facilitate in vitro studies with porcine macrophages.

1. Martinez FO et al. 2009. Alternative activation of macrophages: an immunologic functional perspective. *Annu Rev Immunol* 27:451-83.
2. Garcia-Nicolas O et al. 2014. Virulence and genotype-associated infectivity of interferon-treated macrophages by porcine reproductive and respiratory syndrome viruses. *Virus Res* 179:204-211.
3. Chamorro S et al. 2000. Phenotypic characterization of monocyte subpopulations in the pigs. *Immunobiology* 202:82-93.
4. McCullough KC et al. 1997. The phenotype of porcine monocytic cells: modulation of surface molecule expression upon monocyte differentiation into macrophages. *Vet Imm Immunopath* 58: 265-275.
5. Kapetanovic R et al. 2013. The impact of breed and tissue compartment on the response of pig macrophages to lipopolysaccharide. *BMC Genomics* 14:581.

DEVELOPMENT OF AN ALTERNATIVE ANIMAL-FRIENDLY HOUSING SYSTEM FOR RABBIT DOES AND KITS

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In Europe an increased interest in the “ethical quality” of animal husbandry is leading to study breeding systems that allow a higher welfare level, without impairment of environmental sustainability, economy and food security. Italy is still the second world rabbit meat producer, while the other traditional producers (France and Spain) significantly reduced their production. The aim of this study was to evaluate the impact of an alternative animal- friendly housing system for rabbit does and kits on animal welfare.

The experiment was managed from February to April 2015, using prototypes colony cages. These cages were characterized by interior spaces divided by removable partitions. Four nests were positioned at the two ends of the cage and equipped with a sliding door to allow controlled lactation. Sixteen nulliparous White New Zealand does were artificially inseminated and divided in two groups:

Control group (C) reared in standard colony cages; eperimental group reared in colony cages with septa (S). Five days before the kindling septa were closed and removed a week later.

Rabbits, for three consecutive reproductive cycles, had been checked for the following reproductive parameters: sexual receptivity, fertility, number of born and weaned rabbits, milk production in the first sixteen days of lactation, kits' weight at birth and at weaning. Moreover, blood samples were collected to evaluate oxidative status and innate immunity at the following intervals:

T0 - a week before the release in colony cages;

T1 - a week after going into colony cages;

T2 - a week after delivery, corresponding to the removing of the partitions in the S group;

T3 - at the weaning of kits.

Free Radicals and Anti-Oxidant activity were performed using enzymatic kits (Diacron, Italy); Serum lysozyme was assessed by the lyso-plate assay (Osserman and Lawlor, 1966); total hemolytic complement was evaluated following the procedure described by Seyfarth, (1976); serum bactericidal activity (SBA) was performed with a microtitre format assay (Amadori et al., 1997) and haptoglobin was measured by using a commercial kit (Tridelta Development Ltd, Kildare, Ireland). Data were analyzed using a linear STATA model (2015). The level of statistical significance was set at $P < 0.05$.

Regarding to production performance, the colony prototype (S) showed better results than the classic colony (C) in terms of overall productivity.

Regarding the innate immunity, it was observed that lysozyme is strongly affected by the rearing system. In this test, higher values were observed in the postpartum period in rabbits of C group, indicating a state of suffering of the animals as a result of continuous nest invasions by other rabbits. The complement activity was significantly lower in does of group C7 days after delivery, suggesting a predisposition of these animals to infections. Bactericidal activity and haptoglobin were influenced only by the physiological condition, while the oxidative activity was affected by both the farming system and the physiological status. This study demonstrates that the innate immunity parameters are useful tools to evaluate an animal friendly housing system.

SALMONELLA SEROVAR INTERACTION WITH JEJUNAL EPITHELIAL CELLS

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Salmonella spp infections are an important source of foodborne illnesses and therefore a major public health concern (1). Important studies highlighted the molecular basis of pathogenesis of *S. Typhimurium* infection, while scanty data are available about other environmental serotypes, often isolated in cases of foodborne disease but not included in pathogenicity studies. Owing to the above, the aim of our work was to verify invasiveness in the IPEC-J2 swine jejunal cell line and the modulation of intestinal innate immunity by six different environmental *Salmonella* strains. In our study, overnight cultures of 7 different *Salmonella enterica* strains: *S. Coeln*, *S. Ablogame*, *S. enterica* sub-specie diarizone (Strain 1), *S. Veneziana*, *S. enterica* sub-specie diarizone (strain 2), *S. Typhimurium* and *S. Thompson* isolated from wild boar livers were sub-cultured for 2 h at 37 °C in BHI medium. Each bacterial strain was re-suspended at 1×10^8 CFU/ml in DMEM/F12 medium (2) and used to infect IPEC-J2 cells; untreated cells were employed as negative control. Bacterial penetration and innate immune responses were evaluated as previously described (2, 3). Differences between data sets were checked for significant differences by Kruskal-Wallis test, followed by a Dunn's post-test. The significance threshold was set at $P < 0.05$. All the strains were able to penetrate inside IPEC-J2 cells. In particular, our results demonstrated greater penetration of *S. Coeln* ($P < 0.0001$) and *S. Thompson* ($P = 0.0059$) compared with *S. Typhimurium* (control strain). *S. Diarizonae* 1 ($P = 0.0408$) showed lesser penetration with respect to the control strain. Concerning innate immunity, our results showed different abilities to modulate gene expression by the strains under study. In particular, in accordance with another study (2), *S. Typhimurium* infection determined a pro-inflammatory effect characterized by up-regulation of IL-8 ($P = 0.022$), TNF- α ($P = 0.0003$), IL-1 β ($P < 0.0001$), p38 MAPK ($P = 0.0027$) and IL-18 ($P = 0.041$) and an increase of antimicrobial peptide gene expression: bD1 ($P = 0.001$), bD2 ($P = 0.002$), bD4 ($P = 0.0006$). At the same time we observed down-

regulation of IL-4 (P=0.03) and MD2 (P=0.0018). On the contrary, *S. Coeln* caused a significant decrease of p38 MAPK and CD14 (P=0.0157, P=0.0431) gene expression and no modulation of antimicrobial peptides. *S. Thompson* caused a significant increase of JNK1 (P=0.0196), NFK- κ p65 (P=0.0046) gene expression. *S. Ablogame* down-regulated p38 MAPK (P=0.03), TLR4 (P< 0.05) and TLR5(P< 0.05). Treatment with *S. Diarizonae* strain 1 caused a significant decrease of p38 MAPK (P=0.0412), MD2 (P=0.0044) and bD4 (P=0.0344) gene expression. The adopted cell line had been shown to give valuable information about pathogenicity of *Salmonella* spp. (4). Our data suggest a potential pathogenic role of all the strains under study and different interactions with the host. In particular, our findings about *S. Coeln* and *S. Thompsonson* are in agreement with an EFSA report (1).

- 1) EFSA, 2012.
- 2) Schmidt et al 2008.
- 3) Razzuoli et al., 2013. J Interferon Cytokine Res.
- 4) Brosnahan and Brown. 2012. Vet Microbiol.

GUT INNATE IMMUNE RESPONSE TO CADMIUM EXPOSURE

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Cadmium (Cd) is a toxic and carcinogenic heavy metal widely distributed in the environment. The ingestion of contaminated food and drinking water is the major source of exposure to Cd for humans and animals and the gastrointestinal tract is the first target of interaction. The toxicity of Cd is related to its ability to modulate the activity of cellular enzymes, to initiate oxidative stress, to suppress mitochondrial functions and disrupt calcium homeostasis. However, little is known about Cd interaction with the intestinal tract (1). Owing to the above, the aim of our study was to investigate the effects of Cd on innate immunity using swine jejunal IPEC-J2 cells (2). Cells were seeded into 12-well tissue culture plates (2 mL per well, 2×10^5 cells/mL) and incubated at 37°C in 5% CO₂ until confluence. Cells were then treated for 3 hours with 1 μM and 10 μM Cd solutions at 37°C in 5% CO₂. We tested five wells for each concentration and untreated wells were used as negative control. Total RNA was extracted and following the reverse transcription step (2) the change in mRNA expression profiles of porcine cytokines IL-1β, IL-6, IL-8, Nf-κb1, Nf-κb-p65, MYD88, IL-18, IFN-β, P38, β2-M, TLR4, TLR5, MD2, CD14, TNF-α, bD1, bD2, bD3, bD4, JNK, STAT3 and SOCS1 was investigated using primer sets described in previous studies (2). HPRT1 and GAPDH were used as housekeeping control genes (3). In each sample of IPEC-J2 cells, the relative expression of the selected genes was calculated using the formula $\Delta Ct = Ct(\text{target gene}) - Ct(\text{housekeeping})$. The average intensity of expression ($\Delta Ct \text{ sample} - \Delta Ct \text{ negative control}$) of the genes under study was compared among treatment groups by one-way analysis of variance (ANOVA). The threshold for significance was set at $P < 0.05$. In cells treated with 1 μM Cd we showed a significant increase ($P < 0.05$) of IL-6, IL-8, Nf-κb1 and Nf-κb-p65 gene expression and down-regulation of TNF-α expression ($P = 0.002$). These data are in agreement with previous studies (1) and highlight a pro-inflammatory effect of low concentrations of Cd. Concerning the treatment with 10 μM Cd we observed up-regulation ($P < 0.05$) of bD1, bD2, bD3, IFN-β, IL-18, TNF-α and β2-M and down-regulation of IL-8, Nf-κb1 and STAT3 gene expression. These results suggest activation of the Type I IFNs system; in particular

we observed an IFN- β response after treatment with 10 μ M of Cd. Moreover, we also observed up-regulation of β 2-M, indicated as an in vivo marker of Cd exposure in previous studies (4). In conclusion, our results support the hypothesis that Cd exposure may modify the basal level of cytokine expression; specifically, different concentrations of this heavy metal seem to influence different compartments of the innate immune response. These data confirm the ability of non-infectious stressors to modulate innate immunity; hence, they might cause an alteration of gut interaction with bacteria.

- 1)Ninkov M., et al. *Toxicol Lett.* 2015; 237:89-99.
- 2)Razuoli E., et al. *J Interferon Cytokine Res.* 2013; 33 597–605.
- 3)Uddin MJ., et al. *BMC Research Notes.* 2011; 4:441.
- 4)Xiaosheng W., et al. *Toxicological Science.* 2012; 128:524-531.

RUMEN FLUID, A NEW DIAGNOSTIC MATRIX IN DAIRY CATTLE FARMS?

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Production diseases of dairy cows include several pathologies and are considered man-made problems caused by the inability of cows to achieve a feed energy intake matching their high production requirements (1). A correct management of production diseases demands early diagnostic and prognostic parameters, in order to implement the necessary adjustments in the management system and reduce the prevalence of clinical cases (2). A previous study of our group showed that forestomachs walls express immune receptors and cytokines, and the rumen liquor contains leukocytes able to produce IFN- γ (3), suggesting an integrated system including receptors, signaling molecules, cytokines and infiltrating leukocytes. Forestomach immune response could react to “dangers” arising within the forestomach environment, but also act as reporter system of disease conditions arising elsewhere in the body. Our working hypothesis implied that ruminal fluids could be an important source of diagnostic information for the identification of herds at risk for production diseases, in addition to the traditional blood and faecal analysis. We first demonstrated that the diet can influence the immune response in forestomachs. Diverse leukocyte populations at very low concentrations and IFN- γ were revealed in some samples of rumen fluids, with a clear inhibition of the response observed in all the animals fed the maize-supplemented diet, compare to a normal and a soy-supplemented diet. We better characterized the leukocytes subpopulations in the rumen liquor, isolating B cells, monocytes, and $\gamma\delta$ T cells. We also compared the leukocyte composition in ruminocentesis versus nasal probe sampling, and some differences seem to occur, probably due the fact the samples come from different areas of the rumen, however no significant statistical difference between samples collection

techniques was found. Finally we performed a field survey (146 cows from 13 farms) in order to find correlation among the immune profile of the rumen liquor (FACS and molecular analysis), blood, and faecal parameters. Clinically healthy animals showed a farm specific immunologic pattern of the rumen liquor: low CD45 mRNA expression, low or absent IFN- γ , few or absent B-cells. Whereas farms at risk for general wellness presented high levels of CD45 and IFN- γ , increased numbers of B-cells and other leukocyte populations, such as myeloid cells. This immunological pattern of the rumen liquor seems to be associated to inflammatory markers of acute phase response in blood. We can conclude that the epithelial cells of ruminant forestomachs can react to disturbances of the fermentation processes due to improper diets, and the inflammatory response can be sustained by infiltrating leukocytes, able to release cytokines in the rumen liquor. Our data points into the idea that dairy farms could be ranked according to a risk score using the inflammatory markers in rumen fluids (leukocyte populations, CD45 expression). These markers could integrate the usual, consolidated information (e.g. rumen pH and VFA, milk cell counts, blood/faecal analysis).

1)Mulligan et al., 2007.

2) Ingvarlsen et al., 2003.

3) Trevisi et al., 2014.

PIGLETS FED SEED-BASED ORAL VACCINE AGAINST VEROCYTOTOTOXIC *ESCHERICHIA COLI* – IN VIVO STUDY

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Verocytotoxic *Escherichia coli* (VTEC) is responsible of severe enterotoxaemia in swine. VTEC pathogenicity is strictly related to VT2e toxin and F18 adhesive fimbriae. Nowadays no vaccines are available and an outbreak of the disease requires antibiotic medication, therefore novel strategies, alternative to antibiotics, are required. Plant-based oral vaccines offer an innovative approach to vaccination with the main advantages of avoidance of injections and ability to induce specific antibodies in the mucosa, where the major pathogens gain access to the body.

The aim of this study was to evaluate the efficacy of tobacco seeds-based oral vaccines directed against VTEC infection (ethical authorization: 102/2015-PR).

A competitive indirect ELISA was developed to measure respectively the F18 adhesive fimbriae and the B subunit of verocytotoxin, antigens expressed in *Nicotiana tabacum* seeds previously produced (Rossi et al. 2013). 36 weaned piglets were divided randomly into 4 experimental groups (CC, challenged control; CT, challenged treated; UC, unchallenged control; UT, unchallenged treated). Treated piglets were fed five times, on day 0, 1, 2, 7 and 14, with 20 g of engineered milled tobacco seeds (expressing 6.6-7.4 µg of F18 plus 34-37 µg of VT2eB) mixed with 20 g of milk powder. Controls received 20 g of wild type of tobacco seeds. The animals were challenged at day 20 with 10E10 CFU of O138 VTEC *E. coli*. During the entire experimental period body weight (BW), average daily gain (ADG), feed intake (FI) and feed conversion (FC) were registered individually. From day 20 to 30, animals were evaluated for the general health status and scored daily for specific clinical signs (respiratory, palpebral oedema, epiphora, vitality, faecal consistency, and rectal temperature) with a point-score scale described by Rossi et al. (2014). The oral delivery strategy guaranteed the total consumption of the treated feed. The uninfected piglets did not show any VTEC-related clinical sign. In the first post-

challenge period (days 21-25) CT showed a reduction of ADG and FI lower than CC. CT showed an average total score (from day 1 to day 9 post-challenge) significantly lower than CC for oedema, epiphora, vitality and depression. Death, respiratory and neurologic signs were not observed. These results show that piglets fed tobacco seeds expressing VTEC antigens have overall a better clinical status. This oral delivery strategy appeared effective in reducing the development of clinical signs after challenge with O138 VTEC *E. coli* strain.

1. L. Rossi, A. Di Giancamillo, S. Reggi, C. Domeneghini, A. Baldi, V. Sala, V. Dell'Orto, A. Coddens, E. Cox, C. Fogher. "Expression of porcine verocytotoxic *Escherichia coli* antigens in tobacco seeds and evaluation of gut immunity after oral administration in mouse model" *JVS*; 2013,14(3):263-270.
2. L. Rossi, V. Dell'Orto, S. Vagni, V. Sala, S. Reggi, A. Baldi. Protective effect of oral administration of transgenic tobacco seeds against verocytotoxic *Escherichia coli* strain in piglets. *Vet Res Comm*; 2014, 38(1):39-49.

EVALUATION OF THE IMMUNE RESPONSE IN PIGLETS FED SEED-BASED ORAL VACCINE AGAINST VEROCYTOTOXIC *ESCHERICHIA COLI*

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The gut mucosal surface is considered the largest immunologic organ bearing specialized components of the immune system that protect the host against pathogens such as verocytotoxic *Escherichia coli* (VTEC), control responses to food components, and maintain tolerance to harmless external antigens. Oral vaccines can selectively elicit mucosal secretory immunoglobulins A, that play a pivotal role in the mucosal immunity, and a variety of cell-mediated immune responses, including the expression of antigen presenting molecules (major histocompatibility complex, MHC), Toll-like receptors (TLRs) and soluble factors (cytokines and chemokines). Transgenic plants are a valuable platform to produce genetically modified oral vaccines for a large number of human and animal diseases. This study focuses on the immune response elicited in piglets after administration of tobacco seeds-based oral vaccine expressing F18 adhesive fimbriae and the B subunit of verocytotoxin genes from VTEC (Rossi et al. 2014a,b).

36 weaned piglets were divided randomly into 4 experimental groups (CC, challenged control; CT, challenged treated; UC, unchallenged control; UT, unchallenged treated). Treated piglets were fed five times, on day 0, 1, 2, 7 and 14, with 20 g of engineered milled tobacco seeds (expressing 6.6-7.4 µg of F18 plus 34-37 µg of VT2eB) mixed with 20 g of milk powder. Controls received 20 g of wild type of tobacco seeds. The animals were challenged at day 20 with 10E10 CFU of O138 VTEC *E. coli*. Blood samples and small intestinal scrapes were collected from all sacrificed piglets and processed for ELISA determination of serum IgA and intestinal mucosa IgA, TNF-α, IL-8 and CXCL9 (MIG). Samples of jejunum and mesenteric lymph nodes were collected and processed for gene expression study. RNA was extracted, reverse transcribed and assayed with specific primer pairs by Real-Time PCR to quantify the gene expression of the innate immunity receptors TLRs 2 and 4 and the proinflammatory cytokines IFN-γ and IL-1β in jejunum, and the expression of the antigen presenting molecules MHC type I and II in mesenteric

lymph nodes. The challenge significantly increased the expression of MHC-I, which is the molecule responsible for antigen presentation to CD8⁺ T lymphocytes (CC had the highest expression, followed by CT). UC group showed the lowest expression of MHC-I, demonstrating that the upregulation of this gene expression occurs only in the presence of antigen, either vaccine or pathogen. No statistically significant differences were found for MHC-II and IFN- γ . TLR2, TLR4, IL-1 β , TNF- α and CXCL9 were maximally expressed in UT group and minimally expressed in UC group. The proinflammatory cytokine IL-1 β resulted highly expressed in CT group in jejunum by PCR. Overall the immune parameters measured showed the highest values in the vaccinated animals versus controls; these results indicate that vaccination with both antigens stimulates the IgA and cytokine production more than the infectious agents.

1. L. Rossi, L. Pinotti, A. Agazzi, V. Dell'Orto, A. Baldi. *Ital J Anim Sci* 2014a, 13:23-29
2. L. Rossi, V. Dell'Orto, S. Vagni, V. Sala, S. Reggi, A. Baldi. *Vet Res Comm* 2014b, 38:39-49

EFFECT OF THE HEALTH STATUS IN LATE PREGNANCY ON THE CONCENTRATION OF THE IMMUNOGLOBULIN IN THE COLOSTRUM OF DAIRY COWS

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The success of growth of calves is influenced by the quality of colostrum administered after calving and in particular by its content of immunoglobulins (IgG). The quality of the colostrum depends on many factors, some of these are known, but others have been poorly studied, such as the health conditions in the late gestation. The presence of an infectious-inflammatory condition is able to alter the level of IgG in the blood and, likely, also their transfer in the colostrum. The aim of the work was to study the consequence of an inflammation before the calving, recognized on the basis of the increase of plasma acute phase proteins (APP), on the content of the IgG in the colostrum. Thirty-two Italian Friesian cows were monitored with weekly blood samples between -28 and 28 days after calving on which was performed a large metabolic and inflammatory profile. A homogeneous sample of colostrum was collected at the first milking, for the determination of the chemical-physical characteristics and of the content of IgG. No clinical problem has been detected during the dry period, while some cows showed increased concentrations of positive APP (ceruloplasmin = cucp, and haptoglobin = Hp), which indicated the presence of inflammatory phenomena. Thus, cows were divided retrospectively into two groups, according to the concentrations in cucp, determined 3 days before calving: cucp higher than 2.25 $\mu\text{mol/L}$ (10 cows; HCP), and cucp lower than 2.25 $\mu\text{mol/L}$ (22 cows; LCP). HCP vs LCP group showed: higher levels of Hp at -14 days post calving (0.46 vs 0.15 g/L $P < 0.1$) and the day of calving (0.74 vs 0.30 g/L, $P < 0.05$); higher levels of reactive oxygen metabolites (15.6 vs 12.5 mg/100 mL H_2O_2 , $P < 0.01$) and higher levels of bilirubin at 3 days before (6.19 vs 2.93 $\mu\text{mol/L}$, $P < 0.05$) and 3 days post calving (10.14 vs 7.95 $\mu\text{mol/L}$, $P < 0.01$). These data indicate that HCP cows suffered of an oxidative stress condition and a liver difficulty to dispose of bile acids, likely because the liver is involved in the acute phase response, and then engaged in the production of other proteins (i.e. positive APP). The colostrum of HCP cows had a

lower content of IgG compared with LCP (61.7 vs 72.3 g/L, $P < 0.05$) and a higher fat content (8.55 vs 5.79 g/L, $P < 0.01$). The average body weight of calves resulted lower in HCP than of LCP cows (44.5 vs 47.3 kg, $P < 0.1$), while the average body weight increase in the first month of life was similar (0.40 kg/d). These data support that the presence of subclinical inflammatory phenomena in the pre calving period could modify the biology of the synthesis of colostrum, reducing the production or the transfer of IgG. Thus, inflammatory events in the pre-partum can contribute to reduce IgG in the colostrum and can impair the immunity of the calf. The higher milk fat content in HCP cows seems likely due to the increase in the blood precursors coming from a more intense body fat mobilization, associated to infective and inflammatory conditions, despite plasma NEFA were not different. Results suggest that a check-up of the metabolic and inflammatory status of cows in late pregnancy provides useful information to improve the care of calves.

COMPARISON OF SKIN TEST AND IFN- γ ASSAY FOR DIAGNOSIS OF BOVINE TUBERCULOSIS IN BLACK NEBRODI PIGS

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Pigs, sheep, goats, buffalo and a variety of wildlife and farmed species are susceptible to *Mycobacterium bovis*. Intra-vitam tests which are officially used to detect bovine tuberculosis in cattle are skin test and IFN- γ assay. The need of a repeated immobilization, the difficulties in the reading and interpretation and the black color of the bristles complicate the routine use of the skin test in black Nebrodi pigs (especially if they are feral pigs, wild boars or crossbreed) (Pesciaroli et al., 2012). The authors compare the use of both tests for the detection of *Mycobacterium bovis* infection in pigs evaluating the possibility to use the IFN- γ assay as alternative for the in vivo diagnosis of the infection in this species. 124 pigs were submitted to in vivo e post- mortem investigations for bovine tuberculosis. The skin test was carried out on the external surface of the ear canal, before the inoculation of bovine PPD a blood sample for the INF- γ assay was collected. On the same animals an anatomo-pathological examination was conducted. Tissue samples were collected and processed for bacteriological investigations. Statistical analysis about concordance of skin test and IFN- γ assay was performed using classical concordance tests (Cohen's Kappa index and McNemar's test). Forty- five resulted positive to skin test while 48 to IFN- γ assay. Moreover comparison between skin test and IFN- γ assay of the 124 subjects showed a concordance of 89.5% and a Cohen's Kappa index of 0.786. During the abattoir inspection twenty nine carcasses showed tuberculous-like lesions while *Mycobacterium* spp. was isolated in 44 animals. The McNemar's test between the two in vivo tests of the latter showed a value of $\chi^2=1.8$ with a p-value of 0.1797. This low χ^2 value indicates a non-significant difference. The correlation was of 88.6% and the Cohen's kappa index was 0.77. This means that in our case the two tests tend to have overlapping performances. We, also, considered the 20 subjects positive for *Mycobacterium bovis*. In this case 18 subjects resulted positive

while 2 were negative to both tests. Therefore the correlation was 100% and K=1.00. Overall, we can hypothesize that the results obtained by the two tests are equivalent, making possible the use of IFN- γ assay as alternative to skin test in situations where the control of bovine tuberculosis in pigs is required.

1. Pesciaroli M., Russo M., Mazzone P., Aronica V., Fiasconaro M., Boniotti M.B., Corneli S., Cagiola M., Pacciarini M., Di Marco V., Pasquali P. (2012). Evaluation of the interferon-gamma (IFN- γ) assay to diagnose *Mycobacterium bovis* infection in pigs. *Veterinary Immunology and Immunopathology* 148: 369-372.

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