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Research outputs:

Ascaris Suum Infection Downregulates Inflammatory Pathways in the Pig Intestine In Vivo and in Human Dendritic Cells In Vitro.

Ascaris suum is a helminth parasite of pigs closely related to its human counterpart, A. lumbricoides, which infects almost 1 billion people. Ascaris is thought to modulate host immune and inflammatory responses, which may drive immune hyporesponsiveness during chronic infections. Using transcriptomic analysis, we show here that pigs with a chronic A. suum infection have a substantial suppression of inflammatory pathways in the intestinal mucosa, with a broad downregulation of genes encoding cytokines and antigen-processing and costimulatory molecules. A. suum body fluid (ABF) suppressed similar transcriptional pathways in human dendritic cells (DCs) in vitro. DCs exposed to ABF secreted minimal amounts of cytokines and had impaired production of cyclooxygenase-2, altered glucose metabolism, and reduced capacity to induce interferon-gamma production in T cells. Our in vivo and in vitro data provide an insight into mucosal immune modulation during Ascaris infection, and show that A. suum profoundly suppresses immune and inflammatory pathways.

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Characterization of the enhancer and promoter landscape of inflammatory bowel disease from human colon biopsies

Inflammatory bowel disease (IBD) is a chronic intestinal disorder, with two main types: Crohn's disease (CD) and ulcerative colitis (UC), whose molecular pathology is not well understood. The majority of IBD-associated SNPs are
located in non-coding regions and are hard to characterize since regulatory regions in IBD are not known. Here we profile transcription start sites (TSSs) and enhancers in the descending colon of 94 IBD patients and controls. IBD-upregulated promoters and enhancers are highly enriched for IBD-associated SNPs and are bound by the same transcription factors. IBD-specific TSSs are associated to genes with roles in both inflammatory cascades and gut epithelia while TSSs distinguishing UC and CD are associated to gut epithelia functions. We find that as few as 35 TSSs can distinguish active CD, UC, and controls with 85% accuracy in an independent cohort. Our data constitute a foundation for understanding the molecular pathology, gene regulation, and genetics of IBD.

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Expression of selected genes isolated from whole blood, liver and obex in lambs with experimental classical scrapie and healthy controls, showing a systemic innate immune response at the clinical end-stage

Background: Incubation period, disease progression, pathology and clinical presentation of classical scrapie in sheep are highly dependent on PRNP genotype, time and route of inoculation and prion strain. Our experimental model with precolostrum inoculation of homozygous VRQ lambs has shown to be an effective model with extensive PrPSc dissemination in lymphatic tissue and a short incubation period with severe clinical disease. Serum protein analysis has shown an elevation of acute phase proteins in the clinical stages of this experimental model, and here, we investigate changes in gene expression in whole blood, liver and brain. Results: The animals in the scrapie group showed severe signs of illness 22 weeks post inoculation necessitating euthanasia at 23 weeks post inoculation. This severe clinical presentation was accompanied by changes in expression of several genes. The following genes were differentially expressed in whole blood: TLR2, TLR4, C3, IL1B, LF and SAA, in liver tissue, the following genes differentially expressed: TNF-α, SAA, HP, CP, AAT, TTR and TF, and in the brain tissue, the following genes were differentially expressed: HP, CP, ALB and TTR. Conclusions: We report a strong and evident transcriptional innate immune response in the terminal stage of classical scrapie in these animals. The PRNP genotype and time of inoculation are believed to contribute to the clinical presentation, including the extensive dissemination of PrPSc throughout the lymphatic tissue.

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Forensic age determination of human inflicted porcine bruises inflicted within 10h prior to slaughter by application of gene expression signatures

Prediction-models based on gene expression profiles from experimental bruises are capable of determining the age of bruises with a precision of ±2h. However, these models have not yet been applied on tissue from pigs in forensic cases requested by the police. We applied two prediction-models, based on mRNA expression of 13 (prediction-model no. 1) and 4 genes (prediction-model no. 2) involved in inflammation, on forensic cases of porcine bruises in order to determine if gene expression profiles can be used for age determination in forensic cases.

Subcutaneous fat tissue from bruises notified to the police was sampled: 1) within 6h after slaughter (group no. I, n=142), and 2) after freezing the skin for up to 1year (group no. II, n=40). qPCR of genes involved in inflammation was performed to predict the bruise age after partial least squares analysis.

mRNA expression data were obtained for 52.8% and 7.5% bruises in group nos. I and II, respectively. Prediction-model no. 2, based on the mRNA expression of Selectin E, Selectin P, Interleukin 6 and Nuclear Factor Kappa Beta Subunit1, was most suitable for predicting the age of bruises within 8h prior to slaughter.

In conclusion, mRNA expression profiles can assist in estimating the age of bruises. However, when applying gene expression signatures in forensic cases the age estimate should be interpreted together with histological manifestations. Subcutaneous tissue must be stabilized hours after the bruises are detected in order to obtain mRNA of a sufficient quality.
Histological evaluation of experimental porcine bruises

Age estimation is a crucial part of the forensic investigation of bruises in livestock pigs [1-3]. Currently, age estimations are based on histological evaluation of the lesions in the skin and underlying muscle tissue [2]. However, the intensity of inflammation and tissue damage depends not only on the age of bruises but also on sampling site, anatomical location and the speed, mass and force used to inflict the lesions [1, 4, 5]. Twelve experimental slaughter pigs were anesthetized and on each animal, four blunt traumas were inflicted on the back (area of impact Nos. 1–4). The pigs were euthanized at 2, 5 or 8 h after infliction. Skin and underlying muscle tissue were sampled from the center (B) and both ends of bruises (A, C) and evaluated histologically. Descriptive statistics were performed on the data obtained and presented in figures and tables. Differences (odds ratios) between sampling sites (A, B and C), object used to inflict bruises (plastic tube or iron bar), anatomical location (area of impact Nos. 1–4) and bruise age (2, 5 and 8 h) were evaluated using the GENMOD procedure in SAS Enterprise Guide 7.1 and presented in tables. In addition, the agreements (estimated as Cohen's kappa) between two observers evaluating the histological parameters were calculated and presented. Data have been further analyzed and discussed in a recent paper [1]

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IFN-λ and microRNAs are important modulators of the pulmonary innate immune response against influenza A (H1N2) infection in pigs

The innate immune system is paramount in the response to and clearance of influenza A virus (IAV) infection in non-immune individuals. Known factors include type I and III interferons and antiviral pathogen recognition receptors, and the cascades of antiviral and pro- and anti-inflammatory gene expression they induce. MicroRNAs (miRNAs) are increasingly recognized to participate in post-transcriptional modulation of these responses, but the temporal dynamics of how these players of the antiviral innate immune response collaborate to combat infection remain poorly characterized. We quantified the expression of miRNAs and protein coding genes in the lungs of pigs 1, 3, and 14 days after challenge with swine IAV (H1N2). Through RT-qPCR we observed a 400-fold relative increase in IFN-lambda 3 gene expression on day 1 after challenge, and a strong interferon-mediated antiviral response was observed on days 1 and 3 accompanied by up-regulation of genes related to the pro-inflammatory response and apoptosis. Using small RNA sequencing and qPCR validation we found 27 miRNAs that were differentially expressed after challenge, with the highest number of regulated miRNAs observed on day 3. In contrast, the number of protein coding genes found to be regulated due to IAV infection peaked on day 1. Pulmonary miRNAs may thus be aimed at fine-tuning the initial rapid inflammatory response after IAV infection. Specifically, we found five miRNAs (ssc-miR-15a, ssc-miR-18a, ssc-miR-21, ssc-miR-29b, and hsa-miR-590-3p)-four known porcine miRNAs and one novel porcine miRNA candidate-to be potential modulators of viral pathogen recognition and apoptosis. A total of 11 miRNAs remained differentially expressed 14 days after challenge, at which point the infection had cleared. In conclusion, the results suggested a role for miRNAs both during acute infection as well as later, with the potential to influence lung homeostasis and susceptibility to secondary infections in the lungs of pigs after IAV infection.
Necrotizing enterocolitis is associated with acute brain responses in preterm pigs

BACKGROUND: Necrotizing enterocolitis (NEC) is an acute gut inflammatory disorder that occurs in preterm infants in the first weeks after birth. Infants surviving NEC often show impaired neurodevelopment. The mechanisms linking NEC lesions with later neurodevelopment are poorly understood but may include proinflammatory signaling in the immature brain. Using preterm pigs as a model for preterm infants, we hypothesized that severe intestinal NEC lesions are associated with acute effects on the developing hippocampus. METHODS: Cesarean-delivered preterm pigs (n = 117) were reared for 8 days and spontaneously developed variable severity of NEC lesions. Neonatal arousal, physical activity, and in vitro neuritogenic effects of cerebrospinal fluid (CSF) were investigated in pigs showing NEC lesions in the colon (Co-NEC) or in the small intestine (Si-NEC). Hippocampal transcriptome analysis and qPCR were used to assess gene expressions and their relation to biological processes, including neuroinflammation, and neural plasticity. Microglia activation was quantified by stereology. The neuritogenic response to selected proteins was investigated in primary cultures of hippocampal neurons. RESULTS: NEC development rapidly reduced the physical activity of pigs, especially when lesions occurred in the small intestine. Si-NEC and Co-NEC were associated with 27 and 12 hippocampal differentially expressed genes (DEGs), respectively. These included genes related to neuroinflammation (i.e., S100A8, S100A9, IL8, IL6, MMP8, SAA, TAGLN2) and hypoxia (i.e., PDK4, IER3, TXNIP, AGER), and they were all upregulated in Si-NEC pigs. Genes related to protection against oxidative stress (HBB, ALAS2) and oligodendrocytes (OPALIN) were downregulated in Si-NEC pigs. CSF collected from NEC pigs promoted neurite outgrowth in vitro, and the S100A9 and S100A9/S100A8 proteins may mediate the neuritogenic effects of NEC-related CSF on hippocampal neurons. NEC lesions did not affect total microglial cell number but markedly increased the proportion of Iba1-positive amoeboid microglial cells. CONCLUSIONS: NEC lesions, especially when present in the small intestine, are associated with changes to hippocampal gene expression that potentially mediate neuroinflammation and disturbed neural circuit formation via enhanced neuronal differentiation. Early brain-protective interventions may be critical for preterm infants affected by intestinal NEC lesions to reduce their later neurological dysfunctions.
Oral Supplementation with Bovine Colostrum Prevents Septic Shock and Brain Barrier Disruption During Bloodstream Infection in Preterm Newborn Pigs

Preterm infants have increased risk of neonatal sepsis, potentially inducing brain injury, and they may benefit from early initiation of enteral milk feeding. Using preterm pigs as models, we hypothesized that early provision of bovine colostrum to parentally nourished newborns protects against sepsis and neuroinflammation during bloodstream infection. Preterm newborn pigs were administered $10^9$ CFU/kg of intra-arterial Staphylococcus epidermidis (SE, an opportunistic pathogen often causing sepsis in preterm infants), followed by administration of total parenteral nutrition (TPN, SE + TPN, n = 15) or oral provision of bovine colostrum with supplementary parenteral nutrition (SE + COL, n = 14), and compared with uninfected, TPN-nourished controls (CON + TPN, n = 11). SE-infected animals showed multiple signs of sepsis, including
lethargy, hypotension, respiratory acidosis, internal organ hemorrhages, cellular responses (leukopenia, thrombocytopenia), brain barrier disruption and neuroinflammation. At 24 h, colostrum supplementation reduced the SE abundance in blood and cerebrospinal fluid (CSF, both p < 0.05). Further, colostrum feeding normalized arterial blood pressure (38.5 ± 1.20 vs 30.6 ± 3.79 mmHg), pH (7.37 ± 0.02 vs 7.10 ± 0.07) and lactate (1.01 ± 0.11 vs 4.20 ± 1.20 mM, all p < 0.05), and increased motor activity, to levels in controls (p < 0.001). Finally, colostrum-fed animals showed reduced blood-CSF barrier permeability and CSF leukocyte levels, and this was accompanied by normalized gene expression of tight junction proteins (Occludin, Claudin-5, both p < 0.05) and reduced expression of leukocyte chemoattractants (CXCL9-11, all p < 0.01). Early oral supplementation with bovine colostrum prevents septic shock and ameliorates brain barrier disruption and neuroinflammation during bloodstream infection in preterm pigs. Bovine colostrum supplementation may improve resistance against systemic infection in immature, immune-compromised preterm infants.

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**Pigs are useful for the molecular study of bone inflammation and regeneration in humans**  
Pigs are used with increased frequency to model different kinds of orthopedic surgical conditions. In order to show the full potential of porcine models in orthopedic research, it is therefore required to examine the expression of bone regulatory genes in pigs affected by orthopedic surgery and compare it to the expression in humans and mice as mice, are one of the most applied animal species in orthopedics today. In the present study, the local molecular response to drilling of a tibial implant cavity, and the subsequent insertion of a steel implant was examined in a porcine model. Pigs were euthanized five days after drilling of the bone. The molecular response of 73 different genes was analyzed using a high-throughput quantitative polymerase chain reaction platform and compared to histopathology. Histologically, it was found that bone remodeling was initiated on day 5 after surgery and was associated with upregulation of several genes involved in bone degradation and formation (CTSK, ACP5, IBSP, RANK, RANKL and COL1A1). Interleukin-6 and several acute-phase proteins (C3, SAA and ITIH4) were significantly upregulated, indicating their importance in the initial process of healing and osseointegration. All tested bone morphogenic proteins (BMP2, -4 and -7) including their inhibitor noggin were also significantly upregulated. Surprisingly, vascular endothelial growth factor A was not found to be regulated five days after surgery while several other vascular growth factors (ANGPT1, ANGPT2 and PTN) were upregulated. The pig was found to be a useful model for elucidation of bone regulatory genes in humans.
Prenatal Intra-Amniotic Endotoxin Induces Fetal Gut and Lung Immune Responses and Postnatal Systemic Inflammation in Preterm Pigs

Prenatal inflammation is a major risk for preterm birth and neonatal morbidity, but its effects on postnatal immunity and organ functions remain unclear. Using preterm pigs as a model for preterm infants, we investigated whether prenatal intra-amniotic (IA) inflammation modulates postnatal systemic immune status and organ functions. Preterm pigs exposed to IA lipopolysaccharide (LPS) for 3 days were compared with controls at birth and postnatal day 5 after formula feeding. IA LPS induced mild chorioamnionitis but extensive intra-amniotic inflammation. There were minor systemic effects at birth (increased blood neutrophil counts), but a few days later, prenatal LPS induced delayed neonatal arousal, systemic inflammation (increased blood leukocytes, plasma cytokines, and splenic bacterial counts), altered serum biochemistry (lower albumin and cholesterol and higher iron and glucose values), and increased urinary protein and sodium excretion. In the gut and lungs, IA LPS-induced inflammatory responses were observed mainly at birth (increased LPS, CXCL8, and IL-1β levels and myeloperoxidase-positive cell density, multiple increases in innate immune gene expressions, and reduced villus heights), but not on postnatal day 5 (except elevated lung CXCL8 and diarrhea symptoms). Finally, IA LPS did not affect postnatal gut brush-border enzymes, hexose absorption, permeability, or sensitivity to necrotizing enterocolitis on day 5. Short-term IA LPS exposure predisposes preterm pigs to postnatal systemic inflammation after acute fetal gut and lung inflammatory responses.

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Subtyping of Swine Influenza Viruses Using a High-Throughput Real-Time PCR Platform

Influenza A viruses (IAVs) are important human and animal pathogens with high impact on human and animal health. In Denmark, a passive surveillance program for IAV in pigs has been performed since 2011, where screening tests and subsequent subtyping are performed by reverse transcription quantitative real-time PCR (RT-qPCR). A disadvantage of the current subtyping system is that several assays are needed to cover the wide range of circulating subtypes, which makes the system expensive and time-consuming. Therefore, the aim of the present study was to develop a high-throughput method, which could improve surveillance of swine influenza viruses (swIAVs) and lower the costs of virus subtyping. Twelve qPCR assays specific for various hemagglutinin and neuraminidase gene lineages relevant for swIAV and six assays specific for the internal genes of IAV were developed and optimized for the high-throughput qPCR platform BioMark (Fluidigm). The qPCR assays were validated and optimized to run under the same reaction conditions using a 48.48 dynamic array (48.48DA). The sensitivity and specificity was assessed by testing virus isolates and field samples with known subtypes. The results revealed a performance of the swIAV 48.48DA similar to conventional real-time analysis, and furthermore, the specificity of swIAV 48.48DA was very high and without cross reactions between the assays. This high-throughput system provides a cost-effective alternative for subtyping of swIAVs.

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The intensity of the inflammatory response in experimental porcine bruises depends on time, anatomical location and sampling site

The assessment of the age of bruises inflicted on livestock is an important component of veterinary forensic pathology investigations. However, the sampling site within a bruise, the anatomical location and the mass and speed of the object inflicting the blunt trauma might influence the intensity of the inflammatory reaction. In the present study, the variation of the inflammatory reaction within and along experimental porcine bruises was evaluated in order to determine the optimal sampling site. Moreover, we evaluated if a combination of histological characteristics and gene expression signatures was able to differentiate bruises according to anatomical location, age of bruises and the speed and mass of the object used to cause the impact.

Twelve experimental slaughter pigs were anesthetized, and on each animal four blunt traumas were inflicted on the back using either a plastic tube or an iron bar, respectively. The pigs were euthanized at 2, 5 or 8h after infliction. Following gross examination, skin and underlying muscle tissue were sampled from the center and both ends of bruises and evaluated histologically. Subcutaneous fat tissue from the center of the bruises was sampled for quantitative real-time polymerase chain reaction to evaluate mRNA expression of 13 selected genes. Uninjured tissue was sampled from the right thigh of all pigs and served as control tissue.

The amount of tissue damage and the intensity of the inflammatory reaction in bruises depended on the sampling site within and along a bruise, the anatomical location and the age of the bruise. The optimal site for sampling, i.e. the most pronounced inflammatory reaction, was at the center of the bruises where the plastic tube or iron bar first struck the skin. Moreover, bruises inflicted in areas with a thin layer of subcutaneous fat tissue showed more damage and inflammation in the underlying muscle tissue compared to bruises inflicted in areas with a thicker layer of subcutaneous fat tissue. In addition, hemorrhage in the muscle tissue was more likely present when bruises were inflicted with an iron bar compared to a plastic tube. Combining histology and mRNA expression of the 13 genes showed that the age of bruises could be determined with a precision of ±2.04h. Moreover, the age of bruises could be determined with a precision of ±1.84h based solely on mRNA expression of a selection of four genes.
A polyphenol-enriched diet and Ascaris suum infection modulate mucosal immune responses and gut microbiota composition in pigs

Polyphenols are a class of bioactive plant secondary metabolites that are thought to have beneficial effects on gut health, such as modulation of mucosal immune and inflammatory responses and regulation of parasite burdens. Here, we examined the interactions between a polyphenol-rich diet supplement and infection with the enteric nematode Ascaris suum in pigs. Pigs were fed either a basal diet or the same diet supplemented with grape pomace (GP), an industrial by-product rich in polyphenols such as oligomeric proanthocyanidins. Half of the animals in each group were then inoculated with A. suum for 14 days to assess parasite establishment, acquisition of local and systemic immune responses and effects on the gut microbiome. Despite in vitro anthelmintic activity of GP-extracts, numbers of parasite larvae in the intestine were not altered by GP-supplementation. However, the bioactive diet significantly increased numbers of eosinophils induced by A. suum infection in the duodenum, jejunum and ileum, and modulated gene expression in the jejunal mucosa of infected pigs. Both GP-supplementation and A. suum infection induced significant and apparently similar changes in the composition of the prokaryotic gut microbiota, and both also decreased concentrations of isobutyric and isovaleric acid (branched-chain short chain fatty acids) in the colon. Our results demonstrate that while a polyphenol-enriched diet in pigs may not directly influence A. suum establishment, it significantly modulates the subsequent host response to helminth infection. Our results suggest an influence of diet on immune function which may potentially be exploited to enhance immunity to helminths.

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Dietary cinnamaldehyde enhances acquisition of specific antibodies following helminth infection in pigs

Dietary phytoneutrients such as cinnamaldehyde (CA) may contribute to immune function during pathogen infections, and CA has been reported to have positive effects on gut health when used as feed additive for livestock. Here, we investigated whether CA could enhance antibody production and specific immune responses during infection with an enteric pathogen. We examined the effect of dietary CA on plasma antibody levels in parasite-naïve pigs, and subsequently acquisition of humoral immune responses during infection with the parasitic nematode Ascaris suum. Parasite-naïve pigs fed diets supplemented with CA had higher levels of total IgA and IgG in plasma, and A. suum-infected pigs had higher levels of parasite-specific IgM and IgA in plasma 14 days post-infection. Moreover, dietary CA increased expression of genes encoding the B-cell marker CD19, sodium/glucose co-transporter1 (SCL15A1) and glucose transporter 2 (SLC2A2) in the jejunal mucosa of A. suum-infected pigs. Dietary CA induced only limited changes in the composition of the prokaryotic gut microbiota of A. suum-infected pigs, and in vitro experiments showed that CA did not directly induce proliferation or increase secretion of IgG and IgA from lymphocytes. Our results demonstrate that dietary CA can significantly enhance acquisition of specific immune responses in pigs. The underlying mechanism remains obscure, but apparently does not derive simply from direct contact between CA and host lymphocytes and appears to be independent of the gut microbiota.

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Forensic aspects of gene expression signatures for age determination in bruises as evaluated in an experimental porcine model

Determining the age of bruises and the force used to inflict the trauma is of crucial importance in both human and veterinary forensic pathology. In the present study, the expression of more than 50 different genes in subcutaneous fat and muscle tissue from experimental bruises in pigs was investigated. The aim was to evaluate if expression signatures of selected genes were capable of determining bruises according to age and the force of impact. Eighteen experimental pigs were anesthetized, and on each animal four blunt traumas were inflicted on the back with a low, moderate or high force. The pigs were euthanized from 1 to 10 h after infliction of the trauma and subcutaneous fat and muscle tissues were sampled. As control, subcutaneous fat and muscle tissues were sampled from two un-injured pigs. Quantitative real-time polymerase chain reaction was performed to evaluate mRNA expression of genes involved in inflammation, tissue damage and repair. Expression signatures of thirteen selected genes in subcutaneous fat but not in muscle tissue reflected the age of bruises with a precision of approximately ±2 h. Moreover, the gene expression signature in the subcutaneous fat was to some extend able to separate bruises inflicted with different forces. Expression signatures of selected genes in the subcutaneous fat will increase the precision of the age determination of bruises in pigs. Further, due to the similarity of porcine and human skin physiology and immunity, these results might also provide valuable information in human forensic science.

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Contributors: Barington, K., Jensen, H. E., Skovgaard, K.
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Web of Science (2017): Impact factor 2.027
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 1
Scopus rating (2016): CiteScore 1.05 SJR 0.605 SNIP 0.798
Web of Science (2016): Impact factor 1.842
BFI (2015): BFI-level 1
Scopus rating (2015): CiteScore 1.01 SJR 0.736 SNIP 0.837
Web of Science (2015): Impact factor 1.896
Immunological effects of reduced mucosal integrity in the early life of BALB/c mice

Certain stimuli at the gut barrier may be necessary in early life to establish a proper balance of immune tolerance. We evaluated a compromised barrier in juvenile mice in relation to microbiota and local and systemic immunity. BALB/c mice were treated with a low dose of dextran sulfate sodium (DSS) with or without ampicillin and lipopolysaccharide (LPS) to clarify the importance of microbial antigens and interaction between microbial-associated patterns and toll-like receptors. The barrier breach resulted in increased plasma LPS, which was highest in mice treated simultaneously with ampicillin. Adding LPS in the food reduced its levels in plasma. Regulatory T cells were acutely increased in mesenteric lymph nodes (MLN) and spleen during DSS treatment regardless of simultaneous ampicillin treatment. In contrast, NK T and NK cells decreased in MLN and in spleen. This acute DSS effect was reflected in fold changes of haptoglobin and Il1a in colon, and this was also more pronounced in mice simultaneously treated with ampicillin. On day 1 post-treatment, major upregulations of Ifng, Foxp3, Il1b, Il2, and Il6 genes in colon were only observed in the mice simultaneously treated with ampicillin. A two-fold upregulation of colonic Foxp3 and Il1a was evident 25 days post-treatment. DSS skewed the microbiota in favor of Gram negative phyla. Therefore, increased permeability induced tolerogenic immunity independent of microbiota, and this was enhanced by LPS stimulation.

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Increased Intestinal Inflammation and Digestive Dysfunction in Preterm Pigs with Severe Necrotizing Enterocolitis

The risk factors for necrotizing enterocolitis (NEC) are well known, but the factors involved in the different NEC presentations remain unclear. We hypothesized that digestive dysfunction and intestinal inflammation are mainly affected by severe NEC lesions. In 48 preterm pigs, the association between the macroscopic NEC score (range 1-6) and the expression of 48 genes related to inflammation, morphological, and digestive parameters in the distal small intestine was investigated. Only severe NEC cases (score of 5-6) were associated with the upregulation of genes involved in inflammation (CCL2, CCL3, CD14, CD163, CXCL8, HP, IL1B, IL1RN, IL6, IL10, NFKBIA, PTGS2 and TNFAIP3) compared to pigs that appeared healthy (score of 1-2) or showed mild NEC (score of 3-4). Pigs with a score of 5-6 had higher
intestinal tissue IL-1β levels and a lower mucosal mass, villus height, and aminopeptidase N activity compared to pigs with a score of 1-4, and lower crypts and activities of lactase, dipeptidylpeptidase IV, and aminopeptidase A than pigs with a score of 1-2. The expression of a range of inflammation-related genes was increased only in pigs with severe NEC, concomitant with morphological changes and decreased hydrolase activity. A severe inflammatory response and digestive dysfunction are associated mainly with severe NEC. Still, it remains difficult to separate the initial causes of NEC and the later intestinal consequences of NEC in both infants and experimental models.

General information
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Organisations: National Veterinary Institute, Section for Immunology and Vaccinology, University of Copenhagen, Aarhus University
Contributors: Støy, A. C. F., Heegaard, P. M. H., Skovgaard, K., Bering, S. B., Bjerre, M., Sangild, P. T.
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Web of Science (2017): Impact factor 2.688
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 1
Scopus rating (2016): CiteScore 2.47 SJR 1.355 SNIP 1.262
Web of Science (2016): Impact factor 2.598
BFI (2015): BFI-level 1
Scopus rating (2015): CiteScore 2.41 SJR 1.501 SNIP 1.288
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Web of Science (2015): Indexed yes
BFI (2014): BFI-level 1
Scopus rating (2014): CiteScore 2.26 SJR 1.366 SNIP 1.223
Web of Science (2014): Impact factor 2.649
BFI (2013): BFI-level 1
Scopus rating (2013): CiteScore 2.38 SJR 1.423 SNIP 1.357
Web of Science (2013): Impact factor 2.369
BFI (2012): BFI-level 1
Scopus rating (2012): CiteScore 2.46 SJR 1.21 SNIP 1.325
Web of Science (2012): Impact factor 2.573
BFI (2011): BFI-level 1
Scopus rating (2011): CiteScore 2.4 SJR 1.231 SNIP 1.107
Web of Science (2011): Impact factor 2.656
BFI (2010): BFI-level 1
Scopus rating (2010): SJR 1.064 SNIP 1.089
Web of Science (2010): Impact factor 2.289
BFI (2009): BFI-level 1
Scopus rating (2009): SJR 0.901 SNIP 0.885
BFI (2008): BFI-level 1
Scopus rating (2008): SJR 0.834 SNIP 0.843
Scopus rating (2007): SJR 0.712 SNIP 0.754
Scopus rating (2006): SJR 0.639 SNIP 0.769
Scopus rating (2005): SJR 0.52 SNIP 0.64
Loss of prion protein induces a primed state of type I interferon-responsive genes

The cellular prion protein (PrP\(^{C}\)) has been extensively studied because of its pivotal role in prion diseases; however, its functions remain incompletely understood. A unique line of goats has been identified that carries a nonsense mutation that abolishes synthesis of PrP\(^{C}\). In these animals, the PrP-encoding mRNA is rapidly degraded. Goats without PrP\(^{C}\) are valuable in re-addressing loss-of-function phenotypes observed in Prnp knockout mice. As PrP\(^{C}\) has been ascribed various roles in immune cells, we analyzed transcriptomic responses to loss of PrP\(^{C}\) in peripheral blood mononuclear cells (PBMCs) from normal goat kids (n = 8, PRNP\(^{+/+}\)) and goat kids without PrP\(^{C}\) (n = 8, PRNP\(^{Ter/Ter}\)) by mRNA sequencing. PBMCs normally express moderate levels of PrP\(^{C}\). The vast majority of genes were similarly expressed in the two groups. However, a curated list of 86 differentially expressed genes delineated the two genotypes. About 70% of these were classified as interferon-responsive genes. In goats without PrP\(^{C}\), the majority of type I interferon-responsive genes were in a primed, modestly upregulated state, with fold changes ranging from 1.4 to 3.7. Among these were ISG15, DDX58 (RIG-1), MX1, MX2, OAS1, OAS2 and DRAM1, all of which have important roles in pathogen defense, cell proliferation, apoptosis, immunomodulation and DNA damage response. Our data suggest that PrP\(^{C}\) contributes to the fine-tuning of resting state PBMCs expression level of type I interferon-responsive genes. The molecular mechanism by which this is achieved will be an important topic for further research into PrP\(^{C}\) physiology.

General information

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Web of Science (2017): Indexed yes
BFI (2016): BFI-level 1
Scopus rating (2016): CiteScore 3.11 SJR 1.236 SNIP 1.101
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 1
Scopus rating (2015): CiteScore 3.32 SJR 1.427 SNIP 1.136
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 1
Scopus rating (2014): CiteScore 3.54 SJR 1.559 SNIP 1.148
Influenza A virus infections are a major public health concern. Many million cases of disease associated with influenza A virus occur every year during seasonal epidemics, and especially vulnerable populations such as the elderly, pregnant women, young children, and individuals with underlying conditions such as diabetes and patients of autoimmune diseases are at higher risk of severe complications from influenza A virus infection. However, in otherwise healthy individuals, influenza A virus infection is relatively short-lived, commonly being cleared within one to two weeks. Influenza A virus causes respiratory infection, primarily infecting the respiratory epithelial cells. In the time span from influenza A virus infection until specific antibodies and cytotoxic T lymphocytes arrive at the site of infection, innate immunity is highly important for restricting viral spread and facilitating development of a tailored adaptive immune response. Upon infection, the influenza A virus is recognized by innate viral pathogen sensors which initiate the induction of a balanced pro- and anti-inflammatory cytokine response as well as the hallmark interferon response, inducing an ‘antiviral state’ in the infected cell as well as neighboring cells. As with numerous other cellular processes, the innate host response is modulated by microRNAs, a class of short non-coding RNAs important for the regulation of translation of protein-coding gene transcripts. Comprehensive assessment of the transcriptional host response to influenza A virus infection requires the joint expression profiling of protein-coding gene and microRNA expression. Paper 1 is a review which emphasizes the importance of the pig in the study of influenza A virus infections. Pigs are themselves natural hosts for influenza A virus, and our close relationship with this species poses an ever present risk of emergence of zoonotic influenza A virus strains. The porcine response to influenza A virus infection greatly mirrors human conditions, and the pig thus represents an important animal model with great translational value for the study of human influenza A virus infection. Paper 2 presents results demonstrating the temporal dynamics of microRNA expression in circulating leukocytes from pigs after influenza A virus infection.
challenge, and emphasizes the need for control of the time parameter in suitable animal models for the evaluation of the biomarker potential of circulating microRNAs. Differential microRNA expression in circulating leukocytes peaks two weeks after challenge, suggesting that microRNAs may influence susceptibility to secondary infections. The study likewise shows that the expression profile of protein-coding genes in porcine circulating leukocytes mirrors what is seen in humans after natural or experimental influenza A virus infection. Paper 3 examines the local innate immune and microRNA response in the lungs of pigs after influenza A virus challenge. In contrast to observations in circulating leukocytes, differential microRNA expression peaks three days after challenge, suggesting that pulmonary microRNA expression may be aimed at modulating the rapid transcriptional pro-inflammatory response which peaks already one day after challenge. Paper 4 compares the local lung microRNA expression in vaccinated and unvaccinated pigs after influenza A virus challenge. Vaccinated and unvaccinated pigs displayed significantly different clinical signs, with a more severe course of disease observed in unvaccinated pigs presenting. This difference in disease severity was reflected in the pulmonary transcriptional innate host response of protein-coding genes and microRNA during infection. Target analysis of the differentially expressed microRNA between the two groups of pigs indicated the involvement of microRNAs in host innate and adaptive immune responses, apoptosis, and lung regeneration.

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Organisations: National Veterinary Institute, Innate Immunology, Virology
Contributors: Brogaard, L., Skovgaard, K., Larsen, L. E.
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Subtyping of swine influenza viruses using a high-throughput real time PCR platform
Introduction. Swine influenza is a respiratory disease caused by multiple subtypes of influenza A virus (IAV). The genome of IAV consists of 8 segments and subtype classification is based on the surface glycoproteins hemagglutinin (HA) and neuraminidase (NA). In Denmark, the influenza screening test and subsequent subtyping is performed by real-time RT-PCR (RT-qPCR) but several assays are needed to cover the wide range of circulating subtypes which is expensive, resource- and time-demanding. To mitigate these restrictions the high-throughput qPCR platform BioMark (Fluidigm) has been explored. The BioMark platform uses less sample and reagent volume compared to standard qPCR platforms and allows for up to 9,216 parallel reactions on one chip. Materials and methods. A total of 14 PCR assays specific for the different subtypes of HA and NA genes relevant for swine influenza and 6 assays specific for the internal genes of IAV were validated and optimised to run under identical reaction conditions and assembled on a dynamic array chip (Fluidigm). Results. The sensitivity and specificity of the chip was assessed by testing cell culture isolates and field samples with known subtypes (based on sequencing). The results revealed that the performance of the dynamic chip was similar to conventional real-time analysis. Discussion and conclusion. Application of the chip for subtyping of swine influenza has resulted in a significant reduction in time, cost and working hours. Thereby, it is possible to offer diagnostic services with reduced price and turnover time which will facilitate choice of vaccines and by that lead to reduction of antibiotic used.

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The impact of a diet with fructan-rich chicory roots on Oesophagostomum dentatum worm population dynamics and host immune responses in pigs
Oesophagostomum infections in pigs persist for months. We hypothesized that feeding fructans (dried chicory roots) may improve immunity and facilitate worm expulsion. We therefore examined the effects of long-term chicory on O. dentatum population dynamics and host immune responses. Methods: Seventy-two pigs were allocated to four groups in a 2-factorial design. Group O was fed regular feed and trickle inoculated with 15 O. dentatum L3/kg/day 0-12 weeks post-
infection (pi.) start. Group OC was also trickle inoculated but switched to a chicory-rich diet (12% inulin in DM) weeks 3-12 pi. Group C was uninfected but switched to chicory diet while Group Ctr remained uninfected on regular feed. Six pigs per group were necropsied 5, 9 and 12 weeks pi. for worm counts and qRT-PCR for gene expression in the gut. Faecal egg counts (FEC) and specific antibody levels were assessed regularly. Results: When group OC switched to chicory diet, FECs dropped within 3-4 days and remained very low. Worm counts were reduced 50-65% by chicory feeding (Group OC versus O; p<0.001) and was accompanied by a 2-fold higher O. dentatum-specific IgG1 response. In group O, a build-up of a typical Th2-type immune response was seen but leveled out later and worm counts remained stable. Group C had a down-regulated Th1-type response and thus an anti-inflammatory effect in colon. Conclusions: We found little evidence that chicory feeding improved host protective immunity against Oesophagostomum. It seems more likely, as previously suggested, that physico-chemical changes in caeco-colon are responsible for the observed anthelmintic effects.

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Transcriptional immune response in mesenteric lymph nodes in pigs with different levels of resistance to Ascaris suum
A single nucleotide polymorphism on chromosome 4 (SNP TXNIP) has been reported to be associated with roundworm (Ascaris suum) burden in pigs. The objective of the present study was to analyse the immune response to A. suum mounted by pigs with genotype AA (n = 24) and AB (n = 23) at the TXNIP locus. The pigs were repeatedly infected with A. suum from eight weeks of age until necropsy eight weeks later. An uninfected control group (AA; n = 5 and AB; n = 5) was also included. At post mortem, we collected mesenteric lymph nodes and measured the expression of 28 selected immune-related genes. Recordings of worm burdens confirmed our previous results that pigs of the AA genotype were more resistant to infection than AB pigs. We estimated the genotype difference in relative expression levels in infected and uninfected animals. No significant change in expression levels between the two genotypes due to infection was observed for any of the genes, although IL-13 approached significance (P = 0.08; Punadjusted = 0.003). Furthermore, statistical analysis testing for the effect of infection separately in each genotype showed significant up-regulation of IL-13 (P

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BFI (2017): BFI-level 1
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Web of Science (2017): Impact factor 1.039
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 1
Activation of innate immune genes in caprine blood leukocytes after systemic endotoxin challenge

Sepsis is a serious health problem associated with a range of infectious diseases in animals and humans. Early events of this syndrome can be mimicked by experimental administration of lipopolysaccharides (LPS). Compared with mice, small ruminants and humans are highly sensitive to LPS, making goats valuable in inflammatory models. We performed a longitudinal study in eight Norwegian dairy goats that received LPS (0.1 μg/kg, Escherichia coli O26:B6) intravenously. A control group of five goats received corresponding volumes of sterile saline. Clinical examinations were performed continuously, and blood samples were collected throughout the trial. Characteristic signs of acute sepsis, such as sickness...
behavior, fever, and leukopenia were observed within 1 h of LPS administration. A high-throughput longitudinal gene expression analysis of circulating leukocytes was performed, and genes associated with the acute phase response, type I interferon signaling, LPS cascade and apoptosis, in addition to cytokines and chemokines were targeted. Pro-inflammatory genes, such as IL1B, CCL3 and IL8, were significantly up-regulated. Interestingly, increased mRNA levels of seven interferon stimulated genes (ISGs) were observed peaking at 2 h, corroborating the increasing evidence that ISGs respond immediately to bacterial endotoxins. A slower response was manifested by four extrahepatic acute phase proteins (APP) (SAA3, HP, LF and LCN2) reaching maximum levels at 5 h. We report an immediate induction of ISGs in leukocytes in response to LPS supporting a link between the interferon system and defense against bacterial infections. The extrahepatic expression of APPs suggests that leukocytes contribute to synthesis of these proteins at the beginning of a systemic inflammation. Taken together, these findings provide insights into the dynamic regulation of innate immune genes, as well as raising new questions regarding the importance of ISGs and extrahepatic APPs in leukocytes after systemic endotoxin challenge.

**General information**

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Organisations: National Veterinary Institute, Section for Immunology and Vaccinology, Norwegian University of Life Sciences
Contributors: Salvesen, Ø., Reiten, M. R., Heegaard, P. M. H., Tranulis, M. A., Espenes, A., Skovgaard, K., Ersdal, C.
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Web of Science (2017): Impact factor 1.958
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 1
Scopus rating (2016): CiteScore 1.83 SJR 0.87 SNIP 1.011
Web of Science (2016): Impact factor 1.75
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 1
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BFI (2014): BFI-level 1
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BFI (2013): BFI-level 1
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ISI indexed (2013): ISI indexed yes
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Scopus rating (2012): CiteScore 1.94 SJR 0.779 SNIP 1.023
Web of Science (2012): Impact factor 1.861
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Web of Science (2012): Indexed yes
BFI (2011): BFI-level 1
Scopus rating (2011): CiteScore 2.66 SJR 1.165 SNIP 1.447
Characterization and differentiation of equine experimental local and early systemic inflammation by expression responses of inflammation-related genes in peripheral blood leukocytes

Local inflammation may progress into systemic inflammation. To increase our understanding of the basic immunological processes during transition of equine local inflammation into a systemic state, investigation into the equine systemic immune response to local inflammation is warranted. Therefore, the aim of this study was to investigate the innate peripheral blood leukocyte (PBL) immune response to local inflammation in horses, and to compare this response with the PBL immune response during the early phase of acute systemic inflammation. Expression of 22 selected inflammation-related genes was measured in whole blood leukocytes from 6 horses in an experimental cross-over model of lipopolysaccharide- (LPS-) induced acute synovitis (3 μg LPS intraarticularly; locally inflamed [LI] horses) and endotoxemia (1 μg LPS/kg intravenously; systemically inflamed [SI] horses). Multiple clinical and hematological/biochemical examinations were performed, and serial blood samples were analyzed by reverse transcription quantitative real-time PCR. Post-induction expression profiles of all genes were compared between study groups using principal component analysis (PCA) and hierarchical clustering. Moderate synovitis and mild systemic inflammation of approximately 24 h duration was confirmed by clinical and paraclinical observations in LI and SI horses, respectively. In the LI group, samples obtained 3-16 h post-injection showed distinct clustering in the PCA compared with baseline levels, indicating a transcriptional response to local inflammation in PBLs in this time interval. There was no clinical or hematological indication of actual systemic inflammation. There was a clear separation of all LI samples from all SI samples in two distinct clusters, indicating that expression profiles in the two study groups were different, independent of time since LPS injection. Co-regulated genes formed four clusters across study groups which were distinctly differently regulated. Only few of individual genes displayed different expression between the study groups at all times after LPS injection. Local inflammation in horses initiated an innate transcriptional response in PBLs, which differed from the transcriptional response during the early phase of systemic inflammation. This study may provide new insights into the immunobiology of PBLs during the transition of local inflammation into a systemic state.

General information

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Organisations: National Veterinary Institute, Section for Immunology and Vaccinology, University of Copenhagen, Swedish University of Agricultural Sciences
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Scopus rating (2014): CiteScore 1.81 SJR 0.943 SNIP 1.018
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BFI (2009): BFI-level 1
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Comparison of innate and Th1-type host immune responses in Oesophagostomum dentatum and Trichuris suis infections in pigs

The present study investigated details of the innate and Th1/Treg type associated host immune responses in Trichuris suis and Oesophagostomum dentatum mono- and co-infected pigs and in vitro in stimulated porcine dendritic cell cultures. Forty-eight pigs were allocated into a 2-factorial design with two groups trickle inoculated with 10 T. suis eggs/kg/day (Group T) or 20 O. dentatum L3/kg/day (O). Another group (OT) was infected with both parasites. Group C remained uninfected. Expression of innate and Th1/Treg cell associated genes in gut mucosa and associated lymph nodes was determined by qPCR at necropsy day 35 and 71. Gene expression showed suppressed/inhibited Th1 and Treg type immune reactions, in accordance with previous findings of a predominant Th2 type immune response to both nematodes. The in vitro part examined production of TNF-α in porcine dendritic cells (DC) exposed to T. suis and/or O. dentatum excretory/secretory (E/S) products. Further, binding capacity and structure of E/S products were characterized. Glycan and lectin binding capacity were generally lower in O. dentatum E/S products compared to T. suis which may explain the earlier found weaker Th2 response to the former. Surprisingly, O. dentatum E/S products induced a significant (p < 0.0001) increase in TNF-α DC production, potentially indicating a new mode of helminth-host immune response interaction.

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BFI (2016): BFI-level 1
Scopus rating (2016): CiteScore 2.37 SJR 1.11 SNIP 0.82
Web of Science (2016): Impact factor 2.493
Web of Science (2016): Indexed yes
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Web of Science (2013): Impact factor 1.849
ISI indexed (2013): ISI indexed yes
BFI (2012): BFI-level 1
Scopus rating (2012): CiteScore 2.49 SJR 0.957 SNIP 0.881
Web of Science (2012): Impact factor 2.208
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Early enteral feeding reduces sepsis response and neuroinflammation in a pig model of neonatal bloodstream infection

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Contributors: Brunse, A., Worsoe, P., Pors, S. E., Skovgaard, K., Sangild, P. T.
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Gut, immunity and brain development is immature in preterm pigs and responsive to different milk diets

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Contributors: Ryom, K., Andersen, A. D., Nguyen, D. N., Bergström, A., Skovgaard, K., Thymann, T., Sangild, P. T., Bering, S. B.
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Hepatic expression of inflammatory genes and microRNAs in pigs with high *cholesteryl ester transfer protein* (CETP) activity

Human obesity and obesity-related diseases (ORD) are growing health problems worldwide and represent a major public health challenge. Most of these diseases are complex conditions, influenced by many genes (including microRNAs) and environmental factors. Many metabolic perturbations are associated with obesity; e.g., low levels of high-density lipoproteins (HDL) are high risk factors of cardiovascular events. A number of genetic, lifestyle, and environmental factors have been shown to contribute to the lowering of HDL-cholesterol. One of these factors is cholesteryl ester transfer protein (CETP) promoting the redistribution of cholesteryl esters, triglycerides, and phospholipids between plasma proteins.

Moreover, obesity and ORD are often linked with chronic low-grade inflammation leading to insulin resistance and endothelial and microvascular dysfunctions. The aim of this study was to detect differences in the hepatic expression of genes involved in low-grade inflammation and of obesity- and cholesterol-related microRNAs in two mixed breed populations of pigs (Yorkshire-Göttingen minipig, YM and Duroc-Göttingen minipig, DM) including males and females, with extreme phenotypes for CETP activity levels (designated as CETP-high and CETP-low, respectively). Furthermore, breed and gender differences were also investigated. We found significant difference (P <0.05) in hepatic expression levels of several mRNAs and microRNAs between the CETP-high and -low groups (C5, IL1RN, IL18, and miR-223-5p); between the two mixed breeds (IL1RAP and miR-140-5p); and between gender (APOA1, IL1RN, and FBLN1). Furthermore, when taking breed into account we show that the transcriptional levels of TNF, miR20a, miR33b, and miR130a differed between the two CETP groups. We conclude that increased CETP activity is accompanied by a modest differential hepatic expression of several microRNAs and inflammatory-related genes. Furthermore, our study demonstrates that when modeling the analysis of expression data, it is important to take gender- and breed-specific effects into account.
Investigating the Role of Surface Materials and Three Dimensional Architecture on In Vitro Differentiation of Porcine Monocyte-Derived Dendritic Cells

In vitro generation of dendritic-like cells through differentiation of peripheral blood monocytes is typically done using two-dimensional polystyrene culture plates. In the process of optimising cell culture techniques, engineers have developed fluidic micro-devices usually manufactured in materials other than polystyrene and applying three-dimensional structures more similar to the in vivo environment. Polydimethylsiloxane (PDMS) is an often used polymer for lab-on-a-chip devices but not much is known about the effect of changing the culture surface material from polystyrene to PDMS. In the present study the differentiation of porcine monocytes to monocyte-derived dendritic cells (moDCs) was investigated using CD172apos pig blood monocytes stimulated with GM-CSF and IL-4. Monocytes were cultured on surfaces made of two- and three-dimensional polystyrene as well as two- and three-dimensional PDMS and carbonised three-dimensional PDMS. Cells cultured conventionally (on two-dimensional polystyrene) differentiated into moDCs as expected. Interestingly, gene expression of a wide range of cytokines, chemokines, and pattern recognition receptors was influenced by culture surface material and architecture. Distinct clustering of cells, based on similar expression patterns of 46 genes of interest, was seen for cells isolated from two- and three-dimensional polystyrene as well as two- and three-dimensional PDMS. Changing the material from polystyrene to PDMS resulted in cells with expression patterns usually associated with macrophage expression (upregulation of CD163 and downregulation of CD1a, FLT3, LAMP3 and BATF3). However, this was purely based on gene expression level, and no functional assays were included in this study which would be necessary in order to classify the cells as being macrophages. When changing to three-dimensional culture the cells became increasingly activated in terms of IL6, IL8, IL10 and CCR5 gene expression. Further stimulation with LPS resulted in a slight increase in the expression of maturation markers (SLA-DRB1, CD86 and CD40) as well as cytokines (IL6, IL8, IL10 and IL23A) but the influence of the surfaces was unchanged. These findings highlights future challenges of combining and comparing data generated from microfluidic cell culture-devices made using alternative materials to data generated using conventional polystyrene plates used by most laboratories today.

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MicroRNAs (miRNAs) are a class of short regulatory RNA molecules which are implicated in modulating gene expression. Levels of circulating, cell-associated miRNAs in response to influenza A virus (IAV) infection has received limited attention so far. To further understand the temporal dynamics and biological implications of miRNA regulation in circulating leukocytes, we collected blood samples before and after (1, 3, and 14 days) IAV challenge of pigs. Differential expression of miRNAs and innate immune factor mRNA transcripts was analysed using RT-qPCR. A total of 20 miRNAs were regulated after IAV challenge, with the highest number of regulated miRNAs seen on day 14 after infection at which time the infection was cleared. Targets of the regulated miRNAs included genes involved in apoptosis and cell cycle regulation. Significant regulation of both miRNAs and mRNA transcripts at 14 days after challenge points to a protracted effect of IAV infection, potentially affecting the host’s ability to respond to secondary infections. In conclusion, experimental IAV infection of pigs demonstrated the dynamic nature of miRNA and mRNA regulation in circulating leukocytes during and after infection, and revealed the need for further investigation of the potential immunosuppressing effect of miRNA and innate immune signaling after IAV infection.
Limited effects of preterm birth and the first enteral nutrition on cerebellum morphology and gene expression in piglets

Preterm pigs show many signs of immaturity that are characteristic of preterm infants. In preterm infants, the cerebellum grows particularly rapid and hypoplasia and cellular lesions are associated with motor dysfunction and cognitive deficits. We hypothesized that functional brain delays observed in preterm pigs would be paralleled by both structural and molecular differences in the cerebellum relative to term born piglets. Cerebella were collected from term (n = 56) and preterm (90% gestation, n = 112) pigs at 0, 5, and 26 days after birth for stereological volume estimations, large-scale qPCR gene expression analyses (selected neurodevelopmental genes) and western blot protein expression analysis (Sonic Hedgehog pathway). Memory and learning was tested using a T-maze, documenting that preterm pigs showed delayed learning. Preterm pigs also showed reduced volume of both white and gray matter at all three ages but the proportion of white matter increased postnatally, relative to term pigs. Early initiation of enteral nutrition had limited structural or molecular effects. The Sonic Hedgehog pathway was unaffected by preterm birth. Few differences in expression of the selected genes were found, except consistently higher mRNA levels of Midkine, p75, and Neurotrophic factor 3 in the preterm cerebellum postnatally, probably reflecting an adaptive response to preterm birth. Pig cerebellar development appears more affected by postconceptional age than by environmental factors at birth or postnatally. Compensatory mechanisms following preterm birth may include faster white matter growth and increased expression of selected genes for neurotrophic factors and regulation of angiogenesis. While the pig cerebellum is immature in 90% gestation preterm pigs, it appears relatively mature and resilient toward environmental factors.

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Provision of Amniotic Fluid During Parenteral Nutrition Increases Weight Gain With Limited Effects on Gut Structure, Function, Immunity, and Microbiology in Newborn Preterm Pigs

Background: Small enteral boluses with human milk may reduce the risk of subsequent feeding intolerance and necrotizing enterocolitis in preterm infants receiving parenteral nutrition (PN). We hypothesized that feeding amniotic fluid, the natural enteral diet of the mammalian fetus, will have similar effects and improve growth and gastrointestinal (GI) maturation in preterm neonates receiving PN, prior to the transition to milk feeding. Materials and Methods: Twenty-seven pigs, delivered by cesarean section at ~90% of gestation, were provided with PN and also fed boluses with amniotic fluid (AF; n = 13, 24-72 mL/kg/d) or no oral supplements (nil per os [NPO]; n = 14) until day 5 when blood, tissue, and fecal samples were collected for analyses. Results: Body weight gain was 2.7-fold higher in AF vs NPO pigs. AF pigs showed slower gastric emptying, reduced meal-induced release of gastric inhibitory peptide and glucagon-like peptide 2, changed gut microbiota, and reduced intestinal permeability. There were no effects on GI weight, percentage mucosa, villus height, plasma citrulline, hexose absorptive capacity, and digestive enzymes. Intestinal interleukin (IL)-1β levels and expression of IL1B and IL8 were increased in AF pigs, while blood biochemistry and amino acid levels were minimally affected. Conclusion: Enteral boluses of AF were well tolerated in the first 5 days of life in preterm pigs receiving PN. Enteral provision of AF before the initiation of milk feeding may stimulate body growth and improve hydration in preterm infants receiving PN. Furthermore, it may improve GI motility and integrity, although most markers of GI maturation remain unchanged.

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Spray Dried, Pasteurised Bovine Colostrum Protects Against Gut Dysfunction and Inflammation in Preterm Pigs

OBJECTIVE: Feeding bovine colostrum (BC) improves gut maturation and function, and protects against necrotizing enterocolitis (NEC), relative to formula in newborn preterm pigs. Before BC can be used for preterm infants, it is important to test if the milk processing, required to reduce bacterial load and increase shelf life, may affect bioactivity and efficacy of a BC product. METHODS: We investigated if spray dried, and pasteurised, spray dried BC had protective effects on gut function in preterm pigs, relative to formula. After a 2-day total parenteral nutrition period, preterm pigs were fed formula for a few hours (to induce a pro-inflammatory state) followed by 2 days of formula (FORM, n = 14), BC (COLOS, n = 14), spray dried BC (POW, n = 8), or pasteurised, spray dried BC (POWPAS, n = 9). RESULTS: Spray drying and pasteurisation of BC decreased the concentration of TGF-β1, TGF-β2 and increased protein aggregation. All three BC groups had reduced NEC severity, small intestinal levels of IL-1β, IL-8 and colonic lactic acid levels, and increased intestinal villus height, hexose absorption, and digestive enzyme activities, relative to the FORM group (all P < 0.05). All three BC diets stimulated epithelial cell migration in a wound-healing model with IEC-6 cells. CONCLUSION: Spray drying and pasteurisation affect BC proteins, but do not reduce the trophic and anti-inflammatory effects on the immature intestine. It remains to be studied if BC products will benefit preterm infants just after birth when human milk is often not available.
The preterm pig as a model of premature infant gait ataxia

Aims/background Compromised gait, balance and motor coordination (ataxia) as observed in cases of cerebral palsy is a serious complication to premature birth. The cerebellum is a central region with regards to these brain functions and its development shows high sensitivity to premature birth. Our group has over many years refined a pig model of premature birth focusing on gut and immune system development. Phenotypically, we have observed distinct motoric problems e.g. falls, tiptoe walking and swaying in preterm pigs relative to term born counterparts, indicating compromised brain function. The aim of this study was to compare gait patterns and cerebellar neurodevelopmental gene expression of preterm and term piglets. Methods We compared gait patterns and T-maze performance of caesarean born preterm (3 litters, 90% gestation) and term born pigs (1 litter, 100% gestation) recorded at five distinct postnatal days. MatLab was used to determine a list of spatiotemporal gait characteristics e.g. stride length/ frequency, “duty factor” and asymmetry indices. These data were paralleled by qPCR of >60 selected neurodevelopmental genes of isolated cerebellar tissue. Results While most genes did not differ significantly, we found higher (fold change [1,5-2]) mRNA levels of Midkine, Doublecortin, Neurotrophin3, p75 and Ephrin-B1 in preterms. Preliminary results from gait and T-maze showed significant functional differences between terms and preterms. Conclusions The preterm pig shows functional delays relative to terms, yet the limited cerebellar gene expression differences (mainly related to angiogenesis) suggest other brain regions e.g. motor cortex and basal ganglia to also be involved in compromised gait.

Characterization of the bacterial gut microbiota of piglets suffering from new neonatal porcine diarrhoea

Background: In recent years, new neonatal porcine diarrhoea (NNPD) of unknown aetiology has emerged in Denmark. NNPD affects piglets during the first week of life and results in impaired welfare, decreased weight gain, and in the worst-case scenario death. Commonly used preventative interventions such as vaccination or treatment with antibiotics, have a limited effect on NNPD. Previous studies have investigated the clinical manifestations, histopathology, and to some extent, microbiological findings; however, these studies were either inconclusive or suggested that Enterococci, possibly in interaction with Escherichia coli, contribute to the aetiology of NNPD. This study examined ileal and colonic luminal contents of 50 control piglets and 52 NNPD piglets by means of the qPCR-based Gut Microbiotassay and 16 samples by 454 sequencing to study the composition of the bacterial gut microbiota in relation to NNPD. Results: NNPD was associated with a diminished quantity of bacteria from the phyla Actinobacteria and Firmicutes while genus Enterococcus was more than 24 times more abundant in diarrhoeic piglets. The number of bacteria from the phylum Fusobacteria was also doubled in piglets suffering from diarrhoea. With increasing age, the gut microbiota of NNPD affected piglet and control piglets became more diverse. Independent of diarrhoeic status, piglets from first parity sows (gilts) possessed significantly more bacteria from family Enterobacteriaceae and species E. coli, and fewer bacteria from phylum Firmicutes. Piglets born to gilts had 25 times higher odds of having NNPD compared with piglets born to multiparous sows. Finally, the co-occurrence of genus Enterococcus and species E. coli contributed to the risk of having NNPD. Conclusion: The results of this study support previous findings that points towards genus Enterococcus and species E. coli to be involved in the pathogenesis of NNPD. Moreover, the results indicate that NNPD is associated with a disturbed bacterial composition and larger variation between the diarrhoeic piglets.
Circulating Extracellular microRNA in Systemic Autoimmunity

MicroRNAs (miRNAs) are differentially regulated in healthy, activated, inflamed, neoplastic, or otherwise pathological cells and tissues. While their main functions are executed intracellularly, many miRNAs can reproducibly be detected extracellularly in plasma and serum. This circulating, extracellular miRNA is protected against degradation by complexation with carrier proteins and/or by being enclosed in subcellular membrane vesicles. This, together with their tissue- and disease-specific expression, has fuelled the interest in using circulating microRNA profiles as harbingers of disease, i.e., as diagnostic analytes and as clues to dysregulated pathways in disease. Many studies show that inflammation and immune dysregulation, e.g., in autoimmune diseases, are associated with distinct miRNA expression changes in targeted tissues and in innate and adaptive immunity cells such as lymphocytes, natural killer cells, neutrophil granulocytes, and monocyte-macrophages. Exploratory studies (only validated in a few cases) also show that specific profiles of circulating miRNAs are associated with different systemic autoimmune diseases including systemic lupus erythematosus (SLE), systemic sclerosis, and rheumatoid arthritis. Even though the link between cellular alterations and extracellular profiles is still unpredictable, the data suggest that circulating miRNAs in autoimmunity may become diagnostically useful. Here, we review important circulating miRNAs in animal models of inflammation and in systemic autoimmunity and summarize some proposed functions of miRNAs in immune regulation and dysregulation. We conclude that the studies suggest new hypotheses and additional experiments, and that further diagnostic development is highly dependent on analytical method development and on obtaining sufficient numbers of uniformly processed samples from clinically well-characterized patients and controls.

Concurrent host-pathogen gene expression in the lungs of pigs challenged with Actinobacillus pleuropneumoniae

Background: Actinobacillus pleuropneumoniae causes pleuropneumonia in pigs, a disease which is associated with high morbidity and mortality, as well as impaired animal welfare. To obtain in-depth understanding of this infection, the interplay between virulence factors of the pathogen and defense mechanisms of the porcine host needs to be elucidated. However, research has traditionally focused on either bacteriology or immunology; an unbiased picture of the transcriptional responses can be obtained by investigating both organisms in the same biological sample. Results: Host and pathogen responses in pigs experimentally infected with A. pleuropneumoniae were analyzed by high-throughput RT-qPCR. This approach allowed concurrent analysis of selected genes encoding proteins known or hypothesized to be important in the acute phase of this infection. The expression of 17 bacterial and 31 porcine genes was quantified in lung samples obtained within the first 48 hours of infection. This provided novel insight into the early time course of bacterial genes involved in synthesis of pathogen-associated molecular patterns (lipopolysaccharide, peptidoglycan, lipoprotein) and genes involved in pattern recognition (TLR4, CD14, MD2, LBP, MYD88) in response to A. pleuropneumoniae. Significant up-regulation of proinflammatory cytokines such as IL1B, IL6, and IL8 was observed, correlating with protein levels, infection status and histopathological findings. Host genes encoding proteins involved in iron metabolism, as well as
bacterial genes encoding exotoxins, proteins involved in adhesion, and iron acquisition were found to be differentially expressed according to disease progression. By applying laser capture microdissection, porcine expression of selected genes could be confirmed in the immediate surroundings of the invading pathogen. Conclusions: Microbial pathogenesis is the product of interactions between host and pathogen. Our results demonstrate the applicability of high-throughput RT-qPCR for the elucidation of dual-organism gene expression analysis during infection. We showed differential expression of 12 bacterial and 24 porcine genes during infection and significant correlation of porcine and bacterial gene expression. This is the first study investigating the concurrent transcriptional response of both bacteria and host at the site of infection during porcine respiratory infection.

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Scopus rating (2010): SJR 2.142 SNIP 1.037
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Web of Science (2008): Indexed yes
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Dynamic expression of leukocyte innate immune genes in whole blood from horses with lipopolysaccharide-induced acute systemic inflammation

Background: In horses, insights into the innate immune processes in acute systemic inflammation are limited even though these processes may be highly important for future diagnostic and therapeutic advances in high-mortality disease conditions as the systemic inflammatory response syndrome (SIRS) and sepsis. Therefore, the aim of this study was to investigate the expression of 31 selected blood leukocyte immune genes in an equine model of acute systemic inflammation to identify significantly regulated genes and to describe their expression dynamics during a 24-h experimental period. Systemic inflammation was induced in 6 adult horses by the intravenous injection of 1 µg lipopolysaccharide (LPS) per kg bw. Sixteen blood samples were collected for each horse at predetermined intervals and analyzed by reverse transcription quantitative real-time PCR. Post-induction expression levels for each gene were compared with baseline levels. Results: Systemic inflammation was confirmed by the presence of clinical and hematological changes which were consistent with SIRS. The clinical response to LPS was transient and brief as all horses except one showed unaltered general demeanor after 24 h. Twenty-two leukocyte genes were significantly regulated at at least one time point during the experimental period. By close inspection of the temporal responses the dynamic changes in mRNA abundance revealed a very rapid onset of both pro-and anti-inflammatory mediators and a substantial variation in both expression magnitudes and duration of changes between genes. A majority of the 22 significantly regulated genes peaked within the first 8 h after induction, and an on-going, albeit tightly controlled, regulation was seen after 24 h despite approximate clinical recovery. Conclusions: This first broad study of gene expressions in blood leukocytes during equine acute LPS-induced systemic inflammation thoroughly characterized a highly regulated and dynamic innate immune response. These results provide new insights into the molecular mechanisms of equine systemic inflammation.

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Organisations: National Veterinary Institute, Section for Immunology and Vaccinology, University of Copenhagen, Swedish University of Agricultural Sciences
Contributors: Vinther, A. M. L., Skovgaard, K., Heegaard, P. M. H., Andersen, P. H.
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BFI (2014): BFI-level 1
Scopus rating (2014): CiteScore 1.81 SJR 0.943 SNIP 1.018
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BFI (2013): BFI-level 1
Scopus rating (2013): CiteScore 1.85 SJR 0.861 SNIP 0.853
Web of Science (2013): Impact factor 1.743
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High-throughput Gene Expression Analysis In Pigs As Model For Respiratory Infections

Influenza A virus infections have great impact on human health and welfare and significant resources are linked to influenza epidemics due to excess hospitalizations and lost productivity. Up to 15% of the human population is affected when Influenza spreads around the world in seasonal epidemics (WHO).

Animal models are essential in understanding the mechanisms involved in human infectious disease and for the development of effective prevention and treatment strategies. It is increasingly realized that large animal models like the pig are exceptionally human like and serve as an excellent model for disease and inflammation. Pigs are fully susceptible to human influenza, and have been demonstrated to be involved in influenza evolution and ecology. Pigs share many similarities with humans regarding lung physiology and innate immune cell infiltration of the respiratory system and thus seem to be an obvious large animal model for respiratory infections. This study aimed at providing a better understanding of the involvement of circulating non-coding RNA and innate immune factors in porcine blood leukocytes during influenza virus infection. By employing the pig as a model we were able to perform highly controlled experimental infections and to study changes of symptoms, viral titer, and expression of microRNAs/mRNAs as the influenza infection progresses in time, generating information that would be difficult to obtain from human patients.

The Gram-negative bacterium Actinobacillus pleuropneumoniae causes pneumonia in pigs, a disease which is associated with high morbidity and mortality, as well as impaired animal welfare. The rapidly evolving pneumonia is characterized by large areas of lung necrosis resulting from the combined effect of tissue damage caused by the bacteria, and a strong proinflammatory immune response. To obtain in-depth understanding of this infection, concurrent gene expression of host and pathogen in lung samples collected from pigs experimentally infected with A. pleuropneumoniae was studied. We
applied high-throughput RT-qPCR for the simultaneous analysis of host and pathogen gene expression. This parallel analysis was done in lung tissue samples as well as in the immediate surroundings of infection loci after laser capture microdissection. Regulation of gene expression of several immune factors was observed in agreement with protein levels of these factors in lung tissue, infection status and histopathological findings.

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Organisations: National Veterinary Institute, Section for Immunology and Vaccinology, Section for Bacteriology, Pathology and Parasitology, Section for Virology, IDT Biologika GmbH, Technical University of Denmark
Contributors: Skovgaard, K., Brogaard, L., Schou, K. K., Larsen, L. E., Mortensen, S., Dürrwald, R., Schengel, M., Heegaard, P. M. H.
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Source-ID: 122152957
Research output: Research - peer-review › Conference abstract for conference – Annual report year: 2016

Immune and inflammatory responses in pigs infected with Trichuris suis and Oesophagostomum dentatum
The aim of the present study was to investigate parasite induced immune responses in pigs co-infected with Trichuris suis and Oesophagostomum dentatum as compared to mono-species infected pigs. T. suis is known to elicit a strong immune response leading to rapid expulsion, and a strong antagonistic effect on O. dentatum populations has been observed in co-infected pigs. Forty-eight helminth naive pigs were allocated into 4 groups in a 2-factorial design. Two groups were trickle inoculated with either 10 T. suis eggs/kg/day (Group T) or 20 O. dentatum L3/kg/day (Group O). Group OT was infected with same levels of both T. suis and O. dentatum (Group OT) and Group C remained uninfected. In each group, six pigs were necropsied after 35 days and the remaining pigs after 71 days. Parasite E/S-antigen specific serum antibodies were quantified by an in-direct ELISA. qPCR was used to measure the expression of immune function related genes in the mucosa of proximal colon and the draining lymph node. Highly significant interactions were identified for O. dentatum specific IgG1 (p < 0.0001) and IgG2 (p < 0.0006) antibodies with a remarkable 2-fold higher antibody response in group OT pigs as compared to group O. These findings indicated that T. suis enhanced the antibody response against O. dentatum in Group OT. The gene expression data confirmed a strong Type 2 response to T. suis (e. g. marked increase in IL-13, ARG1 and CCL11) and clearly weaker in amplitude and/or delayed onset response to O. dentatum in the single infected group. Interactions were found between the two nematodes with regard to several cytokines, e.g. the increase in IL-13 observed in Group T was absent in Group OT (p = 0.06, proximal colon mucosa, 35 and 71 p.i.). Some of these immune response-related interactions may support, or even partially explain, the observed interactions between the two worm populations in co-infected pigs.

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Web of Science (2017): Impact factor 2.422
Web of Science (2017): Indexed yes
Immune gene expression in the spleen of chickens experimentally infected with Ascaridia galli

Ascaridia galli is a gastrointestinal nematode infecting chickens. Chickens kept in alternative rearing systems or at free-range experience increased risk for infection with resulting high prevalences. A. galli infection causes reduced weight gain, decreased egg production and in severe cases increased mortality. More importantly, the parasitised chickens are more susceptible to secondary infections and their ability to develop vaccine-induced protective immunity against other diseases may be compromised. Detailed information about the immune response to the natural infection may be exploited to enable future vaccine development. In the present study, expression of immune genes in the chicken spleen during an experimental infection with A. galli was investigated using the Fluidigm (R) BioMark (TM) microfluidic qPCR platform which combines automatic high-throughput with attractive low sample and reagent consumption. Spleenic transcription of immunological genes was compared between infected chickens and non-infected controls at week 2, 6, and 9 p.i. corresponding to different stages of parasite development/maturation. At week 2 p.i. increased expression of IL-13 was observed in infected chickens. Increased expression of MBL, CRP, IFN-alpha, IL-1 beta, IL-8, IL-12 beta and IL-18 followed at week 6 p.i. and at both week 6 and 9 p.i. expression of DEF beta 1 was highly increased in infected chickens. In summary, apart from also earlier reported increased expression of the Th2 signature cytokine IL-13 we observed only few differentially expressed genes at week 2 p.i. which corresponds to the larvae histotrophic phase. In contrast, we observed increased expression of pro-inflammatory cytokines and acute phase proteins in infected chickens, by week 6 p.i. where the larvae re-enter the intestinal lumen. Increased expression of DEF beta 1 was observed in infected chickens at week 6 p.i. but also at week 9 p.i. which corresponds to a matured stage where adult worms are present in the intestinal lumen. (C) 2015 Elsevier B.V. All rights reserved.

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Scopus rating (2016): CiteScore 1.63 SJR 0.742 SNIP 0.708
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Web of Science (2016): Indexed yes
BFI (2015): BFI-level 2
Scopus rating (2015): CiteScore 1.67 SJR 0.862 SNIP 0.749
Web of Science (2015): Impact factor 1.664
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BFI (2014): BFI-level 2
Scopus rating (2014): CiteScore 1.6 SJR 0.777 SNIP 0.718
Web of Science (2014): Impact factor 1.535
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 2
Scopus rating (2013): CiteScore 1.89 SJR 0.834 SNIP 0.797
Web of Science (2013): Impact factor 1.748
ISI indexed (2013): ISI indexed yes
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Scopus rating (2012): CiteScore 2.15 SJR 0.841 SNIP 0.913
Web of Science (2012): Impact factor 1.877
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
BFI (2011): BFI-level 2
Scopus rating (2011): CiteScore 2.16 SJR 0.859 SNIP 0.995
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ISI indexed (2011): ISI indexed yes
Web of Science (2011): Indexed yes
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Scopus rating (2010): SJR 0.792 SNIP 0.948
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Scopus rating (2009): SJR 0.784 SNIP 0.851
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Scopus rating (2008): SJR 0.705 SNIP 0.87
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Increased microbe-receptor contact in early life – approaching immune regulation

The crucial colonization in early life educates immune receptors and cells of the body to form the immune system that we depend on during maintenance, disease, and repair. When regulatory mechanisms fail and the system itself becomes the cause of disease, we should look to the proposed early window of opportunity, where it may be possible to affect the developing immune system towards tolerance.

We hypothesize that increased contact in early life between immune receptors and microbial-associated molecular patterns (MAMP), like TLR-4 and LPS, favors a regulatory immune environment later in life. Dextran Sulphate Sodium interrupts the barrier function of the gut wall by shaving the mucus layer. In low doses it may have the desired contact-increasing effect without inducing colitis-related disease.

Following low-dose DSS treatment in early life of BALB/c mice, we did a gene expression screening in ileum and colon together with cell counts in the spleen and mesenteric lymph nodes combined with sequencing the gut microbiota. We investigated the effect of DSS alone, and in combination with Ampicillin and LPS to elucidate the importance of bacterial ligands.

Our study shows that DSS changes the gut microbiota, and Ampicillin itself can act protective as well as activating on inflammatory markers in a time-dependent manner. It is apparent that DSS works differently in the ileum and colon for some genes. In some cases LPS as only ligand reduces inflammatory markers, but overall it is confirmed that the abundance in bacterial ligands is the most important factor for immune regulation.

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Intrabronchial Microdialysis: Effects of Probe Localization on Tissue Trauma and Drug Penetration into the Pulmonary Epithelial Lining Fluid

Recent intrabronchial microdialysis data indicate that the respiratory epithelium is highly permeable to drugs. Of concern is whether intrabronchial microdialysis disrupts the integrity of the respiratory epithelium and thereby alters drug penetration into the pulmonary epithelial lining fluid (PELF). The objective of this study was to investigate the effect of intrabronchial microdialysis on the integrity of the bronchial epithelium. Microdialysis sampling in PELF in proximal (n=4) and distal bronchi (n=4) was performed after intravenous inulin and florfenicol administration in anaesthetized pigs. Inulin was used as a marker molecule of permeability of the epithelium, and florfenicol was used as test drug. Bronchial tissue was examined by histopathology (distal and proximal bronchi) and gene expression analysis (RT-qPCR, proximal bronchi) at the termination of the experiment (6.5hr). The microdialysis probe caused overt tissue trauma in distal bronchi, whereas no histopathological lesions were observed in proximal bronchi. A moderate up-regulation of the pro-inflammatory cytokines IL1B, IL6 and acute-phase reactant serum amyloid A was seen in proximal bronchi surrounding the microdialysis probes suggesting initiation of an inflammatory response. The observed up-regulation is considered to have limited impact on drug penetration during short-term studies. Inulin penetrated the respiratory epithelium in both proximal and distal bronchi without any correlation to histopathological lesions. Likewise, florfenicol penetration into PELF was unaffected by bronchial histopathology. However, this independence of pathology on drug penetration may not be valid for other antibiotics. We conclude that short-term microdialysis drug quantification can be performed in proximal bronchi without disruption of tissue integrity.

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MicroRNA regulation of TLRs in a post-influenza animal model

Introduction
Substantial morbidity and mortality is caused by secondary bacterial infections occurring in individuals after influenza A virus (IAV) infection. Results from studies in mice suggest that this may in part be due to a lack of responsiveness to Toll-like receptor (TLR) ligands in the post-IAV infected individual. Using the pig as an animal model, we have identified microRNAs (miRNAs) that are differentially expressed in lung tissue two weeks after challenge compared to uninfected controls, i.e. well after the infection has cleared. The role for differential expression of miRNA at this late time point...
remains unclear. We therefore seek to examine the potential involvement of miRNAs in the translational regulation of TLRs and associated proteins, thus contributing to the lowered responsiveness to bacterial TLR ligands at this late time point, making the individual vulnerable to secondary infections.

Methods and outcome

Pigs were experimentally challenged with a Danish reassortant IAV strain (A/sw/Denmark/12687/03(H1N2)). Lung tissue was harvested 14 days after challenge, as well as from uninfected control animals. Using RNAseq and high-throughput RT-qPCR, we quantified the expression of relevant miRNAs (e.g. miR-335 and miR-146a-5p) and mRNA levels of relevant miRNA targets.

Transcriptional analysis at the site of infection reveals a set of miRNAs to be regulated one week after the pigs had cleared the IAV infection (i.e. two weeks after challenge). This set included miRNAs experimentally validated or in silico predicted to bind to and regulate transcripts of TLRs and relevant co-factors and transcription factors (online tools). The antiviral immune response elicited by IAV infection thus includes late miRNA regulation, which in turn may be at the expense of host responsiveness to bacterial TLR ligands.

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**Necrotizing Enterocolitis in Preterm Pigs Is Associated with Increased Density of Intestinal Mucosa-Associated Bacteria Including Clostridium perfringens**

Background: Necrotizing enterocolitis (NEC) is associated with changes in the luminal gut microbiota. It is not known whether the mucosa-associated microbiota is affected by NEC and stimulates inflammatory lesions. Objective: We hypothesized that the density of the mucosa-associated microbiota correlates with NEC severity in preterm pigs and that in vitro infection with increasing densities of Clostridium perfringens, which has been associated with NEC in preterm infants, would lead to a transcriptional response related to the inflammatory conditions of NEC. Methods: First, we determined the density of total bacteria and C. perfringens in the distal small intestinal mucosa of 58 NEC and healthy preterm pigs using quantitative PCR. Next, we analyzed in IPEC-J2 cells the effect of different infection densities of C. perfringens type A on the expression of genes related to intestinal function and immune response. Results: Total bacterial and C. perfringens densities were higher in NEC versus healthy pigs and correlated positively with NEC severity. In IPEC-J2 cells expression levels of inflammation-related genes (CCL5, NFKBIA, IL8, IL1RN, and TNFAIP3) increased, while the expression of the sodium/glucose co-transporter (SLC5A1) decreased, with increasing density of C. perfringens. Conclusions: Total bacterial and C. perfringens densities were higher in NEC versus healthy pigs and correlated positively with NEC severity. In IPEC-J2 cells expression levels of inflammation-related genes (CCL5, NFKBIA, IL8, IL1RN, and TNFAIP3) increased, while the expression of the sodium/glucose co-transporter (SLC5A1) decreased, with increasing density of C. perfringens.
Preterm Birth Reduces Nutrient Absorption With Limited Effect on Immune Gene Expression and Gut Colonization in Pigs

The primary risk factors for necrotizing enterocolitis (NEC) are preterm birth, enteral feeding, and gut colonization. It is unclear whether feeding and colonization induce excessive expression of immune genes that lead to NEC. Using a pig model, we hypothesized that reduced gestational age would upregulate immune-related genes and cause bacterial imbalance after birth. Preterm (85%-92% gestation, n=53) and near-term (95%-99% gestation, n=69) pigs were delivered by cesarean section and euthanized at birth or after 2 days of infant formula or bovine colostrum feeding. At birth, preterm
delivery reduced 5 of 30 intestinal genes related to nutrient absorption and innate immunity, relative to near-term pigs, whereas 2 genes were upregulated. Preterm birth also reduced ex vivo intestinal glucose and leucine uptake (40%-50%), but failed to increase cytokine secretions from intestinal explants relative to near-term birth. After 2 days of formula feeding, NEC incidence was increased in preterm versus near-term pigs (47% vs 0%-13%). A total of 6 of the 30 genes related to immunity (TLR2, IL1B, and IL8), permeability (CLDN3, and OCLN), and absorption (SGLT) decreased in preterm pigs without affecting Gram-negative bacteria-related responses (TLR4, IkBa, NFkB1, TNFAIP3, and PAFA). Bacterial abundance tended to be higher in preterm versus near-term pigs (P=0.09), whereas the composition was unaffected. Preterm birth predisposes to NEC and reduces nutrient absorption but does not induce upregulation of immune-related genes or cause bacterial dyscolonization in the neonatal period. Excessive inflammation and bacterial overgrowth may occur relatively late in NEC progression in preterm neonates.

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Contributors: Østergaard, M. V., Cilieborg, M. S., Skovgaard, K., Schmidt, M., Sangild, P. T., Bering, S. B.
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Web of Science (2012): Impact factor 2.196
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BFI (2011): BFI-level 1
Scopus rating (2011): CiteScore 2.37 SJR 0.943 SNIP 1.2
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ISI indexed (2011): ISI indexed yes
Web of Science (2011): Indexed yes
BFI (2010): BFI-level 1
The pig as a large animal model for influenza a virus infection

It is increasingly realized that large animal models like the pig are exceptionally human like and serve as an excellent model for disease and inflammation. Pigs are fully susceptible to human influenza, share many similarities with humans regarding lung physiology and innate immune cell infiltration of the respiratory system.

This study aimed at providing a better understanding of the involvement of innate immune factors and non-coding RNA in blood leukocytes during influenza A virus infection. By using the pig as a model we were able to perform highly controlled experimental infections and study early clinical signs of disease, viral titer, and transcriptional response of coding and non-coding RNA. This was completed during the first two weeks after experimental viral infection, generating information that would be difficult to obtain from human patients.

Expression of a wide range of immune factors including several genes known to be centrally involved in the viral defence was quantified by high throughput qPCR (BioMark, Fluidigm). Likewise, miRNAs were quantified using the BioMark (Fluidigm) as well as by MiRCURY LNATM (Exiqon).

During the first 24 hours of infection we found the expression of several antiviral genes, including interferon and interferon-related genes, to mimic key findings from human studies. Finally, several circulating miRNAs isolated from blood leukocytes was found to hold great potential as biomarkers for progression of viral lung infection. These results further consolidate the pig as a valuable model for influenza A virus infection.
Vascular gene expression in diet-induced atherosclerotic göttingen minipigs

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Contributors: Blom, L., Skovgaard, K., Ludvigsen, T., Kirk, R., Christoffersen, B. Ø., Pedersen, H. D., Heegaard, P. M. H., Olsen, L. H.
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Bovine colostrum improves intestinal function following formula-induced gut inflammation in preterm pigs

Background & aims
Only few hours of formula feeding may induce proinflammatory responses and predispose to necrotizing enterocolitis (NEC) in preterm pigs. We hypothesized that bovine colostrum, rich in bioactive factors, would improve intestinal function in preterm pigs following an initial exposure to formula feeding after some days of total parenteral nutrition (TPN).

Methods
After receiving TPN for 2 days, preterm pigs were fed formula (FORM, n = 14), bovine colostrum (COLOS, n = 6), or formula (6 h) followed by bovine colostrum (FCOLOS, n = 14). Intestinal lesions, function, and structure, abundance and location of bacteria, and inflammation markers were investigated.

Results
NEC severity and interleukins (IL)-1β and -8 protein concentrations were lower, while villus height, galactose absorption, and brush-border enzyme activities were increased in the distal small intestine in COLOS and FCOLOS pigs, relative to FORM pigs. Intestinal gene expression of serum amyloid A, IL-1β, -6 and -8, and bacterial abundance, correlated positively with NEC severity of the distal small intestine.

Conclusions
Bovine colostrum restores intestinal function after initial formula-induced inflammation in preterm pigs. Further studies are required to test if bovine colostrum may also benefit preterm infants during the challenging transition from total parenteral nutrition to enteral nutrition, when human milk is unavailable.

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Web of Science (2017): Indexed yes
BFI (2016): BFI-level 1
Scopus rating (2016): CiteScore 3.83 SJR 1.724 SNIP 1.667
Characterization of the bacterial gut microbiota in new neonatal porcine diarrhoea

During the last decade farmers and veterinarians have reported the emergence of a new neonatal porcine diarrhoea (NNPD) affecting piglets up to 7 days old. Routine laboratory testing for common pathogens are inconclusive and vaccination and treatment with antibiotics or alternative zootechnical interventions have limited effect. NNPD is not associated with an increased mortality, but have been reported to cause significant morbidity within herds and litters. Piglets born to gilts are in particularly affected by NNPD. NNPD impairs the welfare of the piglets, and results in decreased weight gain which is of economic importance to the farmer. Despite the limited effect of antibiotics, farmers often treat affected piglets with antibiotics to prevent secondary infections to NNPD resulting in increased consumption of antibiotics.
Thus, there are several encouraging reasons for identifying the aetiology behind NNPD. Consequently an interdisciplinary project called: "New neonatal porcine diarrhoea in Denmark. Elucidation of aetiology, diagnostics, and effect of treatments" (freely translated) was initiated. The project enrolled three PhD students with different approaches and hypotheses. The aim of this project was to investigate whether the aetiology to NNPD could be identified in the bacterial gut microbial changes.

In order to be able to characterize the bacterial gut microbiota of numerous samples simultaneously the Gut Microbiotassay was developed. This is an assembly of 24 different primer sets targeting 16S or 23S rRNA genes of the major bacterial groups constituting the gut microbiota. This approach was applied due to the limited number of intestinal bacteria that currently can be cultivated. Primers were found in published literature, tested in silico and modified or designed if necessary. The Gut Microbiotassay was optimized for the high-throughput quantitative real-time PCR-based 48.48 Access Array™ Integrated Fluidic Circuit (Fluidigm). The efficiency and sensitivity of the primer sets were tested against 15 different pure-cultured bacterial strains. Finally the Gut Microbiotassay was tested on DNA extracted from ileal or colonic contents from piglets with or without NNPD and verified via 454 next generation sequencing of the PCR amplicons. Bioinformatics was conducted using BION-meta customized for this specific setup.

With the Gut Microbiotassay in place gut microbial profiles of ileal and colonic contents of 50 control piglets and 52 case piglets from four Danish pig farms affected by NNPD were obtained and deeper taxonomic insight was acquired by sequencing the PCR amplicons. Statistic results from qPCR data revealed that the gut microbiota of NNPD-affected piglets differed from that of control piglets by a depletion of the phyla Firmicutes, Bacteroidetes, and Actinobacteria, while the numbers of genus Enterococcus and the class Beta- and Gammaproteobacteria (including family Enterobacteriaceae and species Escherichia coli), but also phylum Fusobacteria were elevated. Moreover, piglet born to gilts possessed more members from family Enterobacteriaceae including species E. coli and a reduced number of bacteria from phylum Firmicutes. Piglets born to gilts were estimated to have 25 higher odds of being affected by NNPD. Sequence results revealed genus Enterococcus to be comprised of high numbers of species Enterococcus hirae but also Enterococcus durans. Conversely, particularly Lactobacillus acidophilus was scarcely represented in piglets suffering from NNPD.

As part of one of the other enrolled PhD projects a NNPD-infection model was established by inoculating healthy neonate piglets with intestinal NNPD-material (case piglets) or healthy intestinal material (control piglets), while some piglets not were inoculated. Diarrhoea was successfully reproduced in case piglets while control piglets remained healthy. In order to assess whether the diarrhoea was characterized by similar gut microbial changes as detected for field cases of NNPD, ileal and colonic intestinal contents from 49 control piglets (13 un-inoculated) and 32 control piglets (18 un-inoculated) were analyzed using the Gut Microbiotassay. The corresponding regulation of selected intestinal genes involved in diarrhoea was examined for a subset of piglets by qPCR using the 96.96 Dynamic Array™ Integrated Fluidic Circuits (Fluidigm). Similar to NNPD-field cases the gut microbiota of case piglets were characterized by reduced numbers of the phyla Firmicutes, Bacteroidetes, and Actinobacteria. Furthermore, they were inhabited by increased numbers of genus Enterococcus as well as class Beta- and Gammaproteobacteria including species E. coli. The expression of several genes involved in recognition of pathogen-associated molecular patterns, inflammation, and intestinal barrier function were significantly up- or down-regulated reflecting the complex immunological response to being inoculated and/or infected with NNPD-material. Finally, a high abundance of genus Enterococcus (characteristic of case piglets) was associated with high expressions of several transcripts involved in epithelial integrity.

Altogether, the results of the studies included in this thesis reveal that NNPD is associated with a disturbed gut microbial composition, and all points towards members from the genus Enterococcus are involved in the pathogenesis of NNPD.
(NDV). A. galli infection influenced both humoral and cell-mediated immune responses after ND vaccination. Thus, significantly lower NDV serum titres were found in the A. galli-infected group as compared to the non-parasitized group early after vaccination. In addition, the A. galli-infected chickens showed significantly lower frequencies of NDV-specific T cells in peripheral blood three weeks after the first ND vaccination as compared to non-parasitized chickens. Finally, A. galli significantly increased local mRNA expression of IL-4 and IL-13 and significantly decreased TGF-ß4 expression in the jejunum two weeks after infection with A. galli. At the time of vaccination (six and nine weeks after A. galli infection) the local expression in the jejunum of both IFN-? and IL-10 was significantly decreased in A. galli-infected chickens. Upon challenge with the NDV LaSota strain, viral genomes persisted in the oral cavity for a slightly longer period of time in A. galli-infected vaccinees as compared to non-parasitized vaccinees. However, more work is needed in order to determine if vaccine-induced protective immunity is impaired in A. galli-infected chickens.
Expression studies of six human obesity-related genes in seven tissues from divergent pig breeds

Obesity has reached epidemic proportions globally and has become the cause of several major health risks worldwide. Presently, more than 100 loci have been related to obesity and metabolic traits in humans by genome-wide association studies. The complex genetic architecture behind obesity has triggered a need for the development of better animal models than rodents. The pig has emerged as a very promising biomedical model to study human obesity traits. In this study, we have characterized the expression patterns of six obesity-related genes, leptin (LEP), leptin receptor (LEPR), melanocortin 4 receptor (MC4R), fat mass and obesity associated (FTO), neuronal growth regulator 1 (NEGR1) and adiponectin (ADIPOQ), in seven obesity-relevant tissues (liver; muscle; pancreas; hypothalamus; and retroperitoneal, subcutaneous and mesenteric adipose tissues) in two pig breeds (production pigs and Göttingen minipigs) that deviate phenotypically and genetically from each other with respect to obesity traits. We observe significant differential expression for LEP, LEPR and ADIPOQ in muscle and in all three adipose tissues. Interestingly, in pancreas, LEP expression is only detected in the fat minipigs. FTO shows significant differential expression in all tissues analyzed, and NEGR1 shows significant differential expression in muscle, pancreas, hypothalamus and subcutaneous adipose tissue. The MC4R transcript can be detected only in hypothalamus. In general, the expression profiles of the investigated genes are in accordance with those observed in human studies. Our study shows that both the differences between the investigated breeds and the phenotypic state with respect to obesity/leanness play a large role for differential expression of the obesity-related genes.

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Extensive changes in innate immune gene expression in obese Göttingen minipigs do not lead to changes in concentrations of circulating cytokines and acute phase proteins

The usefulness of Göttingen minipigs as models for obesity and obesity-related pathologies is well established. The low-grade inflammation associated with obesity involves a range of innate immune factors; however, to our knowledge, the impact of obesity on innate immune factor expression has not been studied in Göttingen minipigs. Therefore, we studied the expression of innate immune genes in liver and adipose tissues as well as serum concentrations of cytokines and acute phase proteins in obese vs. lean Göttingen minipigs. In the liver, of 35 investigated genes, the expression of nine was significantly different in obese pigs (three up-regulated, six down-regulated). Of 33 genes in adipose tissues, obesity was associated with changed expression of 12 genes in the visceral adipose tissue (VAT) (three up-regulated), 11 in the abdominal retroperitoneal adipose tissue (RPAT) (seven of these up-regulated) and eight in the subcutaneous adipose tissue (SAT) from the neck (five of which were up-regulated). Obesity-associated expression changes were observed for three genes in all adipose tissues, namely chemokine (C-C motif) ligand 3-like 1 (up-regulated), CD200 molecule (down-regulated) and interleukin 1 receptor antagonist (up-regulated) with interleukin 1 receptor antagonist being the most highly regulated gene in both VAT and RPAT. Looking at patterns of expression across the three types of adipose tissues, obesity was associated with an increased number of acute phase proteins differentially expressed between adipose tissues and a decreased tissue-specific expression of cytokines and chemokines. In contrast to obese humans, no changes in serum concentrations of haptoglobin, C-reactive protein, serum amyloid A, tumor necrosis factor-α and interleukin 6 were found in obese Göttingen minipigs.

General information
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Organisations: National Veterinary Institute, Section for Immunology and Vaccinology, Novo Nordisk A/S, University of Copenhagen
Contributors: Højbege, T. R., Skovgaard, K., Moesgaard, S. G., Cirera, S., Christoffersen, B. Ø., Heegaard, P. M. H.
Pages: 67–73
Publication date: 2014
Peer-reviewed: Yes
High-throughput gene expression analysis in pigs as model for respiratory infections

Animal models are essential in understanding the mechanisms involved in human infectious disease and for the development of effective prevention and treatment strategies. It is increasingly realized that large animal models like the pig are exceptionally human like and serve as an excellent model for disease and inflammation. Pigs are fully susceptible to human influenza, and have been demonstrated to be involved in influenza evolution and ecology. Pigs share many similarities with humans regarding lung physiology and innate immune cell infiltration of the respiratory system and thus seem to be an obvious large animal model for respiratory infections. This study aimed at providing a better understanding of the involvement of circulating non-coding RNA and innate immune factors in porcine blood leukocytes during influenza virus infection. By employing the pig as a model we were able to perform highly controlled experimental infections and to study changes of symptoms, viral titer, and expression of microRNAs/mRNAs as the influenza infection progresses in time, generating information that would be difficult to obtain from human patients.

General information

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Organisations: National Veterinary Institute, Section for Immunology and Vaccinology, Section for Bacteriology, Pathology and Parasitology, Section for Virology
Contributors: Skovgaard, K., Brogaard, L., Schou, K. K., Larsen, L. E., Mortensen, S., Dürrwald, R., Schengel, M., Heegaard, P. M. H.
Publication date: 2014
Peer-reviewed: Yes
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Electronic versions:
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Source: PublicationPreSubmission
Source-ID: 103606591
Research output: Research - peer-review › Conference abstract for conference – Annual report year: 2014
Profiling gene expression in mesenteric lymph nodes in pigs with different levels of resistance to *Ascaris suum*

A single nucleotide polymorphism on chromosome 4 (SNP TXNIP) has been reported to be associated with roundworm (*Ascaris suum*) burden in pigs. The objective of the present study was to profile the immune response mounted by pigs with two SNP TXNIP genotypes following an *A. suum* infection. We selected pigs with genotypes AA (*n*=24) and AB (*n*=23) and trickle-infected them with *A. suum* from eight weeks of age until necropsy eight weeks later. An uninfected control group (AA; *n*=5 and AB; *n*=5) was also included. At postmortem, we collected mesenteric lymph nodes and measured the expression of 28 selected genes. Recordings of worm burdens confirmed our previous results that pigs of the AA genotype were more resistant to infection than AB pigs. By estimating the genotype difference in relative expression levels in infected and uninfected animals, we found that IL-13 levels tended to change with genotype (*P*=0.077); specifically, pigs of the AA genotype had increased IL-13 expression following *A. suum* infection but IL-13 expression was unchanged in AB pigs. Furthermore, IL-13 expression tended to be associated with total worm burden in AB pigs (*P*=0.07). The expression of chemokine ligand 17 (CCL17) was up-regulated in AA pigs (*P*<0.05) but not in AB pigs following *A. suum* infection. Pigs of genotype AB had higher expression of the high-affinity IgG receptor (FCGR1A) than AA pigs in both infected and non-infected animals (*P*=1.85*10⁻¹¹). In conclusion, our results suggest the two genotypes differ in the magnitude of their Th2-type response following *A. suum* infection.

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**General information**

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Pages: 27
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URLs:
Research output: Research - peer-review | Conference abstract in proceedings – Annual report year: 2014

The pig as a large animal model for characterization of host-pathogen interactions

Large animal models are essential in understanding the mechanisms involved in human infectious disease. To study the expression of host and bacterial genes involved in defense and survival mechanisms, we analyzed lung tissue from pigs experimentally infected with the Gram-negative bacterium *A. pleuropneumoniae*. All steps including RNA extraction and high-throughput real-time qPCR were carried out simultaneously for the two organisms. By applying this dual-organism approach, we obtained unique insights into the host-pathogen interaction at the site of infection. Differential expression of host genes involved in innate immune responses towards Gram-negative infections, including pattern recognition receptors and cytokines concurrent with expression of bacterial genes involved in lipopolysaccharide biosynthesis and adhesion was demonstrated.

We also studied the gene expression in blood leukocytes after experimental H1N2 virus infection of pigs, and found the regulation of several swine encoded miRNAs and cytokines to mimic key findings from influenza studies in human patients. By employing the pig as a model we were able to perform highly controlled experimental infections and to study changes of symptoms, viral titer, and expression of microRNAs/mRNAs as the influenza infection progressed in time, generating information that would be difficult to obtain from human patients.

**General information**

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Organisations: National Veterinary Institute, Section for Immunology and Vaccinology, Section for Bacteriology, Pathology and Parasitology
Contributors: Skovgaard, K., Brogaard, L., Heegaard, P. M. H., Schou, K. K.
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Event: Abstract from Immunology and Infectious Diseases, Sandbjerg, Denmark.
Electronic versions: Immunology_and_Infectious_Diseases.pdf
Source: PublicationPreSubmission
Source-ID: 100371558
Research output: Research - peer-review | Conference abstract for conference – Annual report year: 2014
Wet-lab tested microRNA assays for qPCR studies with SYBR®Green and DNA primers in pig tissues

MicroRNAs are key post-transcriptional regulators of gene expression that are involved in several biological processes including those that mediate disease pathophysiology. Hence, quantifying microRNA expression levels can provide important and novel insights into disease biology. In recent years, the pig has emerged as an excellent large animal model for studying human diseases and conditions (e.g. obesity) due to similarities in organ size, gastro-intestinal tract, metabolism, immune response, genetics and the availability of relevant tissues that are not normally easily available in humans. We have previously developed two useful tools in the field of microRNA quantitative real time PCR (qPCR): 1) a very specific, sensitive and simple qPCR method based on DNA primers, MiR-specific qPCR; and 2) the free primer-design software miRprimer. The present study integrates in a publicly accessible database all available information on validated porcine microRNA qPCR assays that have utilized these tools. Due to the high phylogenetic conservation in microRNA sequence between pig, humans and other domestic species this database is a very valuable resource for the broader scientist community who are working on microRNAs and want to use readily tested qPCR assays in a simple and cost-effective manner.

Ampicillin-Improved Glucose Tolerance in Diet-Induced Obese C57BL/6NTac Mice Is Age Dependent

Ampicillin has been shown to improve glucose tolerance in mice. We hypothesized that this effect is present only if treatment is initiated prior to weaning and that it disappears when treatment is terminated. High-fat fed C57BL/6NTac mice were divided into groups that received Ampicillin at different ages or not at all. We found that both diet and Ampicillin significantly changed the gut microbiota composition in the animals. Furthermore, there was a significant improvement in glucose tolerance in Ampicillin-treated, five-week-old mice compared to nontreated mice in the control group. At study termination, expressions of mRNA coding for tumor necrosis factor, serum amyloid A, and lactase were upregulated, while the expression of tumor necrosis factor (ligand) superfamily member 15 was downregulated in the ileum of Ampicillin-treated mice. Higher dendritic cell percentages were found systemically in high-fat diet mice, and a lower tolerogenic dendritic cell percentage was found both in relation to high-fat diet and late Ampicillin treatment. The results support our hypothesis that a "window" exists early in life in which an alteration of the gut microbiota affects glucose tolerance as well as development of gut immunity and that this window may disappear after weaning.
A novel multi-stage subunit vaccine against paratuberculosis induces significant immunity and reduces bacterial burden in tissues (P4304)

Effective control of paratuberculosis is hindered by lack of a vaccine preventing infection, transmission and without diagnostic interference with tuberculosis. We have developed a novel multi-stage recombinant subunit vaccine in which a fusion of four early expressed MAP antigens is combined with a MAP protein expressed in latent infection (FET11 vaccine). FET11 vaccine proteins were formulated with CAF01 adjuvant and injected to MAP challenged calves at two different ages. 28 calves divided into two FET11 vaccine groups, a commercial vaccine and a control group were used in the study and followed for a year. The FET11 vaccine induced a significant T cell response against constituent vaccine proteins characterized by a high percentage of CD4+ T cells and participation of polyfunctional CD4+ T cells. Of the two different age groups, late FET11 vaccination conferred protective immunity characterized by a significant containment of bacterial burden in gut tissues compared to non-vaccinated animals. There was no cross-reaction with bovine tuberculosis in vaccinated animals. This novel multi-stage vaccine has the potential to become a marker vaccine for paratuberculosis.

General information
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Organisations: National Veterinary Institute, Section for Immunology and Vaccinology, Statens Serum Institut
Contributors: Thakur, A., Aagaard, C., Riber, U., Skovgaard, K., Andersen, P., Jungersen, G.
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Web of Science (2017): Impact factor 4.539
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 2
Scopus rating (2016): CiteScore 4.79 SJR 3.474 SNIP 1.176
Web of Science (2016): Impact factor 4.856
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 2
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Web of Science (2015): Impact factor 4.985
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 2
Scopus rating (2014): CiteScore 5.03 SJR 3.744 SNIP 1.271
Web of Science (2014): Impact factor 4.922
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 2
Scopus rating (2013): CiteScore 5.61 SJR 3.909 SNIP 1.35
Web of Science (2013): Impact factor 5.362
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 2
Scopus rating (2012): CiteScore 5.82 SJR 4.011 SNIP 1.362
Web of Science (2012): Impact factor 5.52
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
BFI (2011): BFI-level 2
Scopus rating (2011): CiteScore 5.67 SJR 4.06 SNIP 1.347
In preterm infants, the serious gastrointestinal disease, necrotizing enterocolitis (NEC), is caused by the combined effect of abnormal bacterial colonization, enteral feeding, and prematurity, including immaturity of the immune system. In a well-established preterm pig model of NEC, the effect of diet on disease development has been studied thoroughly; however, the inflammatory response during NEC needs to be characterized to improve and promote the use of this model as a model for human disease. We investigated how expression of genes related to immune function and gut maturation in distal small intestinal tissue was affected by NEC development in a number of experimental diet groups. Preterm pigs delivered by Cesarean section received total parenteral nutrition for 2 d followed by enteral nutrition for an additional 2 d: bovine colostrum (n = 6), infant formula (FORM, n = 13), FORM for 6 h followed by bovine colostrum (n = 14), spray dried bovine colostrum (n = 8) or pasteurized, spray dried bovine colostrum (n = 9). At euthanasia, the gastrointestinal tract (stomach to colon) was evaluated for NEC lesions using a severity score ranging from 1–6 (6 being severe NEC). Pigs with a severity score of minimum three in any gastrointestinal region was regarded as a case of NEC. High throughput qPCR was used to investigate the gene expression of 48 genes in intestinal tissue. Across all enteral diet groups, a relatively higher expression of IL-6, IL-8, IL1-RA and CCL3 was seen in pigs suffering from NEC compared to healthy pigs irrespective of enteral diet group, which points to inflammation as being an important component of NEC. Further studies will address the relationship of inflammation related gene expression and the development of NEC in order to elucidate cause-effect relationships leading to NEC.

Characterization of the gene expression response in a preterm pig model of necrotizing enterocolitis

In preterm infants, the serious gastrointestinal disease, necrotizing enterocolitis (NEC), is caused by the combined effect of abnormal bacterial colonization, enteral feeding, and prematurity, including immaturity of the immune system. In a well-established preterm pig model of NEC, the effect of diet on disease development has been studied thoroughly; however, the inflammatory response during NEC needs to be characterized to improve and promote the use of this model as a model for human disease. We investigated how expression of genes related to immune function and gut maturation in distal small intestinal tissue was affected by NEC development in a number of experimental diet groups. Preterm pigs delivered by Cesarean section received total parenteral nutrition for 2 d followed by enteral nutrition for an additional 2 d: bovine colostrum (n = 6), infant formula (FORM, n = 13), FORM for 6 h followed by bovine colostrum (n = 14), spray dried bovine colostrum (n = 8) or pasteurized, spray dried bovine colostrum (n = 9). At euthanasia, the gastrointestinal tract (stomach to colon) was evaluated for NEC lesions using a severity score ranging from 1–6 (6 being severe NEC). Pigs with a severity score of minimum three in any gastrointestinal region was regarded as a case of NEC. High throughput qPCR was used to investigate the gene expression of 48 genes in intestinal tissue. Across all enteral diet groups, a relatively higher expression of IL-6, IL-8, IL1-RA and CCL3 was seen in pigs suffering from NEC compared to healthy pigs irrespective of enteral diet group, which points to inflammation as being an important component of NEC. Further studies will address the relationship of inflammation related gene expression and the development of NEC in order to elucidate cause-effect relationships leading to NEC.

General information
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Organisations: National Veterinary Institute, Section for Immunology and Vaccinology
Contributors: Støy, A. C. F., Sangild, P. T., Skovgaard, K., Heegaard, P. M. H.
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Peer-reviewed: Yes
Event: Abstract from 10th International Veterinary Immunology Symposium, Milano, Italy.
Cloning Changes the Response to Obesity of Innate Immune Factors in Blood, Liver, and Adipose Tissues in Domestic Pigs

The objective of this study was to evaluate the usefulness of cloned pigs as porcine obesity models reflecting obesity-associated changes in innate immune factor gene expression profiles. Liver and adipose tissue expression of 43 innate immune genes as well as serum concentrations of six immune factors were analyzed in lean and diet-induced obese cloned domestic pigs and compared to normal domestic pigs (obese and lean). The number of genes affected by obesity was lower in cloned animals than in control animals. All genes affected by obesity in adipose tissues of clones were downregulated; both upregulation and downregulation were observed in the controls. Cloning resulted in a less differentiated adipose tissue expression pattern. Finally, the serum concentrations of two acute-phase proteins (APPs), haptoglobin (HP) and orosomucoid (ORM), were increased in obese clones as compared to obese controls as well as lean clones and controls. Generally, the variation in phenotype between individual pigs was not reduced in cloned siblings as compared to normal siblings. Therefore, we conclude that cloning limits both the number of genes responding to obesity as well as the degree of tissue-differentiated gene expression, concomitantly with an increase in APP serum concentrations only seen in cloned, obese pigs. This may suggest that the APP response seen in obese, cloned pigs is a consequence of the characteristic skewed gene response to obesity in cloned pigs, as described in this work. This should be taken into consideration when using cloned animals as models for innate responses to obesity.

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Organisations: National Veterinary Institute, Section for Immunology and Vaccinology, Aarhus University
Contributors: Højboege, T. R., Skovgaard, K., Stagsted, J., Heegaard, P. M. H.
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Ratings:
Disseminated Intravascular Coagulation in a Novel Porcine Model of Severe \textit{Staphylococcus aureus} Sepsis Fulfills Human Clinical Criteria

Summary Sepsis is a common and often fatal complication in human patients in intensive care units. Relevant and well-characterized animal models of sepsis may provide valuable information on pathophysiological mechanisms and be a mean of testing new therapeutic strategies. Large animal models of \textit{Staphylococcus aureus} sepsis are rare, even though \textit{S. aureus} increasingly affects human patients. Sepsis changes the haemostatic balance and leads to endothelial cell (EC) activation, coagulopathy and, in severe cases, disseminated intravascular coagulation (DIC). The aim of this study was to characterize the haemostatic and vascular alterations in a novel porcine model of severe \textit{S. aureus} sepsis, investigating whether the changes fulfill the human clinical criteria for DIC. Five pigs were inoculated intravenously with \textit{S. aureus} and two control animals were sham-inoculated. Blood samples were collected for thromboelastography (TEG) and assessment of plasma-based haemostatic parameters. Tissue was collected for histopathology and reverse transcriptase quantitative real-time polymerase chain reaction for measurement of mRNA encoding EC markers. All infected animals developed DIC; including procoagulant activation represented by hypercoagulable TEG profiles and prolonged clotting time. Histologically, numerous pulmonary thrombi were present in one pig. Inhibitor consumption was represented by decreasing antithrombin levels in infected pigs. Hyaline globules were found in three infected pigs, confirming fibrinolytic activation. EC activation was identified by expression of von Willebrand factor in small vessels together with elevated mRNA encoding activated EC markers. Severe haemostatic and vascular changes fulfilling the human criteria for DIC were therefore seen in all infected pigs. A tendency towards uncompensated DIC was seen in two animals.

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Ratings:
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Web of Science (2018): Indexed yes
BFI (2017): BFI-level 1
Scopus rating (2017): CiteScore 1.36 SJR 0.594 SNIP 0.828
Web of Science (2017): Impact factor 1.364
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 1
Scopus rating (2016): CiteScore 1.17 SJR 0.671 SNIP 0.697
Web of Science (2016): Impact factor 1.214
BFI (2015): BFI-level 1
Scopus rating (2015): CiteScore 1.23 SJR 0.698 SNIP 0.868
Web of Science (2015): Impact factor 1.173
BFI (2014): BFI-level 1
Scopus rating (2014): CiteScore 1.17 SJR 0.562 SNIP 0.773
Web of Science (2014): Impact factor 1.142
BFI (2013): BFI-level 1
Scopus rating (2013): CiteScore 1.32 SJR 0.635 SNIP 0.896
Web of Science (2013): Impact factor 1.1
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 1
Scopus rating (2012): CiteScore 1.57 SJR 0.728 SNIP 1.047
Web of Science (2012): Impact factor 1.376
ISI indexed (2012): ISI indexed yes
Expression of innate immune genes, proteins and microRNAs in lung tissue and leukocytes of pigs infected with influenza virus

This study aimed at providing a better understanding of the involvement of innate immune factors including microRNA (miRNA) in the local and systemic host response to influenza virus infection. Twenty pigs were challenged by influenza A virus subtype H1N2. Expression of miRNA, mRNA and proteins were quantified at different time points after challenge (24h, 72h, and 14days post infection (pi)). Gene expression was quantified using 48.48 Dynamic Arrays (Fluidigm Corporation, CA, USA) combining 48 samples with 48 primer sets for 2304 individual and simultaneous qPCR reactions. Several groups of genes were significantly regulated according to time point and infection status: Pattern recognition receptors (TLR2, TLR3, TLR7, RIG1, MDA5), IFN and IFN induced genes (IFNB, IFNG, IRF7, STAT1, ISG15 and OASL), cytokines (IL1B, IL1RN, IL6, IL7, IL10, IL12A, TNF, CCL2, CCL3 and CXCL10), and several acute phase proteins. Likewise, the following miRNAs were differentially expressed in one or more time groups compared to the control pigs: miR-15a, miR-21, miR-146, miR-206, miR-223 and miR-451. At day one pi lung tissue protein levels of IL-6, IL-12 and IFN-α were significantly increased compared to the control group, and haptoglobin and C-reactive protein were at significantly increased at day three pi. MiRNA are small non coding RNA molecules, that regulate gene expression in a wide range of organisms. Cellular miRNAs might be involved in influenza infection, both by targeting immune related host transcripts but also by targeting viral gene products. Our results suggest that in addition to a wide range of immune factors, miRNAs are involved in fine tuning of an efficient innate immune response to influenza infection.

General information
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Organisations: National Veterinary Institute, Section for Immunology and Vaccinology, Section for Virology, Technical University of Denmark, IDT Biologika GmbH, University of Copenhagen
Expression of innate immune genes, proteins and microRNAs in lung tissue of pigs infected experimentally with influenza virus (H1N2)

This study aimed at providing a better understanding of the involvement of innate immune factors, including miRNA, in the local host response to influenza virus infection. Twenty pigs were challenged by influenza A virus subtype H1N2. Expression of microRNA (miRNA), mRNA and proteins were quantified in lung tissue at different time points after challenge (24 h, 72 h and 14 d post-infection (p.i.). Several groups of genes were significantly regulated according to time point and infection status including pattern recognition receptors (TLR2, TLR3, TLR7, retinoic acid-inducible gene I, melanoma differentiation associated protein-5), IFN and IFN-induced genes (IFN-β, IFN-γ, IRF7, STAT1, ISG15 and OASL), cytokines (IL-1 β, IL-1RN, IL-6, IL-7, IL-10, IL-12A, TNF-α, CCL2, CCL3 and CXCL10) and several acute phase proteins. Likewise, the following miRNAs were differentially expressed in one or more time groups compared with the control pigs: miR-15a, miR-21, miR-146, miR-206, miR-223 and miR-451. At d 1 p.i. lung tissue protein levels of IL-6, IL-12 and IFN-α were significantly increased compared with the control group, and haptoglobin and C-reactive protein were significantly increased at d 3 p.i. Our results suggest that, in addition to a wide range of innate immune factors, miRNAs may also be involved in controlling acute influenza infection in pigs.

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State: Published
Organisations: National Veterinary Institute, Section for Immunology and Vaccinology, Section for Virology, Technical University of Denmark, IDT Biologika GmbH, University of Copenhagen
Contributors: Skovgaard, K., Cirera, S., Vasby, D., Podolska, A., Breum, S. Ø., Dürwald, R., Schlegel, M., Heegaard, P. M. H.
Pages: 531-544
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BFI (2018): BFI-level 1
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 1
Scopus rating (2017): CiteScore 2.34 SJR 1.031 SNIP 0.686
Web of Science (2017): Impact factor 2.312
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 1
Scopus rating (2016): CiteScore 2.7 SJR 1.169 SNIP 0.816
Web of Science (2016): Impact factor 2.342
BFI (2015): BFI-level 1
Scopus rating (2015): CiteScore 2.55 SJR 1.264 SNIP 0.836
Web of Science (2015): Impact factor 2.83
BFI (2014): BFI-level 1
Scopus rating (2014): CiteScore 2.65 SJR 1.275 SNIP 0.965
Web of Science (2014): Impact factor 3.271
BFI (2013): BFI-level 1
Scopus rating (2013): CiteScore 2.47 SJR 1.146 SNIP 0.825
Gene Expression Analysis of the IPEC-J2 Cell Line: A Simple Model for the Inflammation-Sensitive Preterm Intestine

The IPEC-J2 cell line was studied as a simple model for investigating responses of the newborn intestinal epithelium to diets. Especially, the small intestine of immature newborns is sensitive to diet-induced inflammation. We investigated gene expression of epithelial- and immune response-related genes in IPEC-J2 cells stimulated for 2h with milk formula (CELL-FORM), colostrum (CELL-COLOS), or growth medium (CELL-CONTR) and in distal small intestinal tissue samples from preterm pigs fed milk formula (PIG-FORM) or colostrum (PIG-COLOS). High throughput quantitative PCR analysis of 48 genes revealed the expression of 22 genes in IPEC-J2 cells and 31 genes in intestinal samples. Principal component analysis (PCA) discriminated the gene expression profile of IPEC-J2 cells from that of intestinal samples. The expression profile of intestinal tissue was separated by PCA into 2 groups according to diet, whereas no diet-dependent grouping was seen for IPEC-J2 cells. Expression differences between PIG-FORM and PIG-COLOS were found for DEFB1, CXCL10, IL1RN, and ALPI, while IL8 was upregulated in CELL-FORM compared with CELL-CONTR. These differences, between IPEC-J2 cells and intestinal tissue from preterm pigs, both used as models for the newborn intestine, underline that caution must be exercised prior to analysis and interpretation of diet-induced effects on gene expression.

General information
State: Published
Organisations: National Veterinary Institute, Section for Immunology and Vaccinology, University of Copenhagen
Contributors: Støy, A. C. F., Heegaard, P. M. H., Sangild, P. T., Østergaard, M. V., Skovgaard, K.
Number of pages: 7
Publication date: 2013
Peer-reviewed: Yes
Gene expression in lymph nodes in pigs with two different genotypes associated with resistance or susceptibility to *Ascaris suum*

**General information**
State: Published
Organisations: National Veterinary Institute, Section for Immunology and Vaccinology, University of Copenhagen
Publication date: 2013
Peer-reviewed: Yes
Event: Abstract from 8th European Congress on Tropical Medicine and International Health (ECTMIH 2013), Copenhagen, Denmark.
Source-ID: u::9323
Research output: Research - peer-review › Conference abstract for conference – Annual report year: 2013

Gene expression in mesenteric lymph nodes of pigs with two different genotypes associated with resistance or susceptibility to *Ascaris suum*

**General information**
State: Published
Organisations: National Veterinary Institute, Section for Immunology and Vaccinology, University of Copenhagen
Pages: 102-103
Publication date: 2013
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**Publication information**
Journal: Tropical Medicine & International Health
Volume: 18
Issue number: S1
Article number: 0.6.6.002
ISSN (Print): 1360-2276
Ratings:
BFI (2018): BFI-level 2
Web of Science (2018): Indexed yes
Gene expression patterns in multiple organs in experimentally induced *Staphylococcus aureus* sepsis in pigs

**General information**

State: Published
Organisations: National Veterinary Institute, Section for Immunology and Vaccinology, University of Copenhagen

**DOI:** 10.1111/tmi.12162
High-throughput Leukocyte expression analysis of innate immune mediators in equine systemic inflammation

General information
State: Published
Organisations: National Veterinary Institute, Section for Immunology and Vaccinology
Contributors: Lindberg, A. M. H., Skovgaard, K., Tarp, K., Heegaard, P. M. H., Andersen, P. H.
Publication date: 2013
Peer-reviewed: Yes
Event: Abstract from 12th European Veterinary Emergency and Critical Care Society Annual Congress (EVECCS 2013), Copenhagen, Denmark.
Source: dtu
Source-ID: u::9325
Research output: Research - peer-review › Conference abstract for conference – Annual report year: 2013

Induction of tolerence in the gut by low-dose DSS treatment

General information
State: Published
Organisations: National Veterinary Institute, Section for Immunology and Vaccinology, Novo Nordisk A/S, University of Copenhagen
Publication date: 2013
Peer-reviewed: Yes
Event: Abstract from The Graduate Programme for In Vivo Pharmacology and Experimental Animals, Hillerød, Denmark.
Source: dtu
Source-ID: u::9324
Research output: Research - peer-review › Conference abstract for conference – Annual report year: 2013

Mild heat treatment does not reduce the colitis-protective effects of bovine colostrum in preterm pigs
Objective and study: Fresh bovine colostrum (BC) prevents development of necrotizing enterocolitis (NEC) in preterm pigs. Spray drying and pasteurization are required to use BC in clinical settings but this may also reduce its bioactivity. In studies on preterm pigs, we compared raw BC with spray dried and pasteurized BC.
Methods: Preterm pigs were fed total parenteral nutrition for 2 d, followed by two boluses of milk formula (15 mL/kg/3h) and continued enteral feeding with milk formula (FORM, n = 14), fresh BC (COLOS, n = 14), spray dried, powdered BC (POW, n = 8), or spray dried, pasteurized BC (POWPAS, n = 9). Pigs were euthanized after two days of enteral feeding and NEC lesions, intestinal structure, digestive and absorptive functions, microbiota, and tissue protein and mRNA levels of immune factors were analyzed. Finally, we determined the concentrations of some bioactive proteins in the colostrum products and studied treatment-related aggregation of proteins.
Results: POW and POWPAS pigs showed lowered gut NEC severity, IL-1β and IL-8 levels and lactic acid levels, and higher intestinal villus heights, hexose absorption, hydrolase activities (lactase, maltase, peptidases) than FORM pigs (all P < 0.05). These values in POW and POWPAS groups were similar to those in the COLOS group. Intestinal expression of IL1B, IL6 and IL8 and bacterial abundance score were positively correlated with NEC severity (P < 0.05). Spray drying, and especially pasteurization, increased the breakdown of growth factors (TGF-β1 and -β2) and aggregation of milk proteins.
Conclusion: Spray drying and pasteurization affect BC proteins but such treatments do not necessarily decrease its trophic and anti-inflammatory effects on the immature intestine. It remains to be studied if such colostrum products also improve gut maturation in preterm infants.

General information
State: Published
Organisations: National Veterinary Institute, Section for Immunology and Vaccinology, Section for Bacteriology, Pathology and Parasitology, Aarhus University, University of Copenhagen
Organization and Biology of the Porcine Serum Amyloid A (SAA) Gene Cluster: Isoform Specific Responses to Bacterial Infection.

Serum amyloid A (SAA) is a prominent acute phase protein. Although its biological functions are debated, the wide species distribution of highly homologous SAA proteins and their uniform behavior in response to injury or inflammation in itself suggests a significant role for this protein. The pig is increasingly being used as a model for the study of inflammatory reactions, yet only little is known about how specific SAA genes are regulated in the pig during acute phase responses and other responses induced by pro-inflammatory host mediators. We designed SAA gene specific primers and quantified the gene expression of porcine SAA1, SAA2, SAA3, and SAA4 by reverse transcriptase quantitative polymerase chain reaction (RT-qPCR) in liver, spleen, and lung tissue from pigs experimentally infected with the Gram-negative swine specific bacterium Actinobacillus pleuropneumoniae, as well as from pigs experimentally infected with the Gram-positive bacterium Staphylococcus aureus. Our results show that: 1) SAA1 may be a pseudogene in pigs; 2) we were able to detect two previously uncharacterized SAA transcripts, namely SAA2 and SAA4, of which the SAA2 transcript is primarily induced in the liver during acute infection and presumably contributes to circulating SAA in pigs; 3) Porcine SAA3 transcription is induced both hepatically and extrahepatically during acute infection, and may be correlated to local organ affection; 4) Hepatic transcription of SAA4 is markedly induced in pigs infected with A. pleuropneumoniae, but only weakly in pigs infected with S. aureus. These results for the first time establish the infection response patterns of the four porcine SAA genes which will be of importance for the use of the pig as a model for human inflammatory responses, e.g. within sepsis, cancer, and obesity research.
Pig α1-Acid Glycoprotein: Characterization and First Description in Any Species as a Negative Acute Phase Protein.

The serum protein α1-acid glycoprotein (AGP), also known as orosomucoid, is generally described as an archetypical positive acute phase protein. Here, porcine AGP was identified, purified and characterized from pooled pig serum. It was found to circulate as a single chain glycoprotein having an apparent molecular weight of 43 kDa by SDS-PAGE under reducing conditions, of which approximately 17 kDa were accounted for by N-bound oligosaccharides. Those data correspond well with the properties of the protein predicted from the single porcine AGP gene (ORM1, Q29014 (UniProt)), containing 5 putative glycosylation sites. A monoclonal antibody (MAb) was produced and shown to quantitatively and specifically react with all microheterogenous forms of pig AGP as analyzed by 2-D electrophoresis. This MAb was used to develop an immunoassay (ELISA) for quantification of AGP in pig serum samples. The adult serum concentrations of pig AGP were in the range of 1-3 mg/ml in a number of conventional pig breeds while it was lower in Göttingen and Ossabaw minipigs (in the 0.3 to 0.6 mg/ml range) and higher in young (2-5 days old) conventional pigs (mean: 6.6 mg/ml). Surprisingly, pig AGP was found to behave as a negative acute phase protein during a range of experimental infections and aseptic inflammation with significant decreases in serum concentration and in hepatic ORM1 expression during the acute phase response. To our knowledge this is the first description in any species of AGP being a negative acute phase protein.

General information
State: Published
Organisations: National Veterinary Institute, Section for Immunology and Vaccinology, Section for Public sector service and commercial diagnostics, University of Veterinary Medicine Vienna, University of Copenhagen
Contributors: Heegaard, P. M. H., Miller, I., Sørensen, N. S., Seerensen, K. E., Skovgaard, K.
Number of pages: 13
Publication date: 2013
Postnatal amniotic fluid intake reduces gut inflammatory responses and necrotizing enterocolitis in preterm neonates

Preterm neonates are susceptible to gastrointestinal disorders such as necrotizing enterocolitis (NEC). Maternal milk and colostrum protects against NEC via growth promoting, immunomodulatory, and antimicrobial factors. The fetal enteral diet amniotic fluid (AF), contains similar components, and we hypothesized that postnatal AF administration reduces inflammatory responses and NEC in preterm neonates. Preterm pigs (92% gestation) were delivered by caesarean section and fed parental nutrition (2 days) followed by enteral (2 days) porcine colostrum (COLOS, n = 7), infant formula (FORM, n = 13), or AF supplied before and after introduction of formula (AF, n = 10) in experiment 1, and supplied only during the enteral feeding period in experiment 2 (FORM, n = 16; AF, n = 14). The NEC score was reduced in both AF and COLOS pigs, relative to FORM, when AF was provided prior to full enteral feeding (9.9 and 7.7 compared with 17.3, P <0.05). There was no effect of AF when provided only during enteral feeding. AF pigs showed decreased bacterial abundance in colon and intestinal inflammation-related genes (e.g., TNF-α, IL-1α, IL-6, NOS) were downregulated, relative to FORM pigs with NEC. Anti-inflammatory properties of AF were supported by delayed maturation and decreased TNF-α production in murine dendritic cells, as well as increased proliferation and migration, and downregulation of IL-6 expression in intestinal cells (IEC-6, IPEC-J2). Like colostrum, AF may reduce NEC development in preterm neonates by suppressing the proinflammatory responses to enteral formula feeding and gut colonization when provided before the onset of NEC.

General information
State: Published
Organisations: National Veterinary Institute, Section for Immunology and Vaccinology, Center for Biological Sequence Analysis, Department of Systems Biology, Section for Bacteriology, Pathology and Parasitology, Aarhus University, University of Copenhagen
Contributors: Siggers, J., Østergaard, M. V., Siggers, R. H., Skovgaard, K., Mølbak, L., Thymann, T., Schmidt, M., Møller, H. K., Purup, S., Fink, L. N., Frokiaer, H., Boye, M., Sangild, P. T., Bering, S. B.
Pages: G864-G875
Publication date: 2013
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Journal: American Journal of Physiology: Gastrointestinal and Liver Physiology
Volume: 304
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BFI (2018): BFI-level 1
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 1
Scopus rating (2017): CiteScore 3.24 SJR 1.822 SNIP 0.918
Web of Science (2017): Impact factor 3.293
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 1
Scopus rating (2016): CiteScore 3.62 SJR 1.877 SNIP 1.037
Web of Science (2016): Impact factor 3.468
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 1
Scopus rating (2015): CiteScore 3.59 SJR 1.981 SNIP 1.005
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 1
Scopus rating (2014): CiteScore 4.06 SJR 2.189 SNIP 1.181
Web of Science (2014): Impact factor 3.798
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 1
Simultaneous analysis of host and pathogen gene expression changes during bacterial infection

General information
State: Published
Organisations: National Veterinary Institute, Section for Immunology and Vaccinology, Section for Bacteriology, Pathology and Parasitology
Contributors: Brogaard, L., Hansen, M. S., Jensen, T. K., Heegaard, P. M. H., Skovgaard, K., Schou, K. K.
Publication date: 2013
Peer-reviewed: Yes
Event: Abstract from 9th Cold Spring Harbor meeting on Microbial Pathogenesis and Host Response, Cold Spring Harbor, NY, United States.
Source: dtu
Source-ID: n:oai:DTIC-ART:highwire/386709110::28538
Research output: Research - peer-review > Journal article – Annual report year: 2013

The Gut Microbiotassay: a high-throughput qPCR approach combinable with next generation sequencing to study gut microbial diversity
Background
The intestinal microbiota is a complex and diverse ecosystem that plays a significant role in maintaining the health and well-being of the mammalian host. During the last decade focus has increased on the importance of intestinal bacteria. Several molecular methods can be applied to describe the composition of the microbiota. This study used a new approach, the Gut Microbiotassay: an assembly of 24 primer sets targeting the main phyla and taxonomically related subgroups of the intestinal microbiota, to be used with the high-throughput qPCR chip 'Access Array 48.48', AA48.48, (Fluidigm®) followed by next generation sequencing. Primers were designed if necessary and all primer sets were screened against DNA extracted from pure cultures of 15 representative bacterial species. Subsequently the setup was tested on DNA extracted from small and large intestinal content from piglets with and without diarrhoea. The PCR amplicons from the 2304 reaction chambers were harvested from the AA48.48, purified, and sequenced using 454-technology.

Results
The Gut Microbiotassay was able to detect significant differences in the quantity and composition of the microbiota according to gut sections and diarrhoeic status. 454-sequencing confirmed the specificity of the primer sets. Diarrhoea was associated with a reduced number of members from the genus Streptococcus, and in particular S. alactolyticus.

Conclusion
The Gut Microbiotassay provides fast and affordable high-throughput quantification of the bacterial composition in many samples and enables further descriptive taxonomic information if combined with 454-sequencing.

General information
State: Published
Organisations: National Veterinary Institute, Section for Bacteriology, Pathology and Parasitology, Section for Immunology and Vaccinology, Department of Applied Mathematics and Computer Science, Statistics and Data Analysis, Danish Genome Institute
Number of pages: 14
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Peer-reviewed: Yes

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Volume: 14
Article number: 788
ISSN (Print): 1471-2164
Ratings:
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Web of Science (2018): Indexed yes
BFI (2017): BFI-level 1
Scopus rating (2017): CiteScore 4.08 SJR 2.11 SNIP 1.151
Web of Science (2017): Impact factor 3.73
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 1
Scopus rating (2016): CiteScore 4.05 SJR 2.163 SNIP 1.096
Web of Science (2016): Impact factor 3.729
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 1
Scopus rating (2015): CiteScore 4.3 SJR 2.348 SNIP 1.159
Web of Science (2015): Impact factor 3.867
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 1
Scopus rating (2014): CiteScore 4.18 SJR 2.327 SNIP 1.199
Web of Science (2014): Impact factor 3.986
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 1
Scopus rating (2013): CiteScore 4.39 SJR 2.195 SNIP 1.188
Web of Science (2013): Impact factor 4.041
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
The need for transparency and good practices in the qPCR literature.

Two surveys of over 1,700 publications whose authors use quantitative real-time PCR (qPCR) reveal a lack of transparent and comprehensive reporting of essential technical information. Reporting standards are significantly improved in publications that cite the Minimum Information for Publication of Quantitative Real-Time PCR Experiments (MIQE) guidelines, although such publications are still vastly outnumbered by those that do not.

General information
State: Published
Organisations: National Veterinary Institute, Section for Immunology and Vaccinology

Bibliographical note
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Source: dtu
Source-ID: u::10285
Research output: Research - peer-review › Journal article – Annual report year: 2013

DOI:
10.1186/1471-2164-14-788
Expression of Innate Immune Response Genes in Liver and Three Types of Adipose Tissue in Cloned Pigs

The pig has been proposed as a relevant model for human obesity-induced inflammation, and cloning may improve the applicability of this model. We tested the assumptions that cloning would reduce interindividual variation in gene expression of innate immune factors and that their expression would remain unaffected by the cloning process. We investigated the expression of 40 innate immune factors by high-throughput quantitative real-time PCR in samples from liver, abdominal subcutaneous adipose tissue (SAT), visceral adipose tissue (VAT), and neck SAT in cloned pigs compared to normal outbred pigs. The variation in gene expression was found to be similar for the two groups, and the expression of a small number of genes was significantly affected by cloning. In the VAT and abdominal SAT, six out of seven significantly differentially expressed genes were downregulated in the clones. In contrast, most differently expressed genes in both liver and neck SAT were upregulated (seven out of eight). Remarkably, acute phase proteins (APPs) dominated the upregulated genes in the liver, whereas APP expression was either unchanged or downregulated in abdominal SAT and VAT. The general conclusion from this work is that cloning leads to subtle changes in specific subsets of innate immune genes. Such changes, even if minor, may have phenotypic effects over time, e.g., in models of long-term inflammation related to obesity.

General information
State: Published
Organisations: National Veterinary Institute, Division of Veterinary Diagnostics and Research, Innate Immunology, Aarhus University
Contributors: Højbøge, T. R., Skovgaard, K., Stagsted, J., Heegaard, P. M. H.
Pages: 407-417
Publication date: 2012
Peer-reviewed: Yes

Publication information
Journal: Cellular Reprogramming
Volume: 14
Issue number: 5
ISSN (Print): 2152-4971
Ratings:
BFI (2018): BFI-level 1
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 1
Scopus rating (2017): CiteScore 1.66 SJR 0.549 SNIP 0.476
Web of Science (2017): Impact factor 1.43
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 1
Scopus rating (2016): CiteScore 1.55 SJR 0.676 SNIP 0.431
Web of Science (2016): Impact factor 1.255
BFI (2015): BFI-level 1
Scopus rating (2015): CiteScore 1.65 SJR 0.72 SNIP 0.629
Web of Science (2015): Impact factor 1.462
BFI (2014): BFI-level 1
Scopus rating (2014): CiteScore 1.94 SJR 0.877 SNIP 0.701
Web of Science (2014): Impact factor 1.788
BFI (2013): BFI-level 1
Scopus rating (2013): CiteScore 2.57 SJR 1.058 SNIP 0.856
Expression of microRNAs and innate immune factor genes in lung tissue of pigs infected with influenza virus (H1N2)

Swine influenza is a highly infectious respiratory disease in pigs caused by influenza A virus. Activation of a frontline of pattern-recognition receptors (PRRs) expressed by epithelial cells as well as immune cells of the upper respiratory tract, leads to a potent type 1 interferon (IFN) release and simultaneous proinflammatory cytokine expression. A transient induction of cytokines is required for an efficient antiviral defence; however, an over-reactive and prolonged inflammatory response may lead to excessive infiltration of immune cells, contributing to immunopathology of the infected lung. Thus, this response must be tightly regulated. Recently, microRNA (miRNA) has been proposed to play an important role in modulating and fine tuning the innate immune response in order to avoid such harmful overreactions. Little is known about the significance of miRNA regulation in the lung during acute influenza A infection. The present work aimed of providing a better understanding of the involvement of innate immune factors including miRNA in the host response to establishment and progression of influenza virus infection. Twenty pigs were challenged by aerosol containing H1N2 (A/swine/Denmark/12687/03) influenza virus. Expression of mRNA coding for cytokines, chemokines, pattern recognition receptors and other antiviral effector molecules were quantified in lung tissue at different time points after challenge (24h PI, 72h PI, and 14days PI). Likewise, microRNA in the lung tissue was quantified at the same time points. Our results demonstrate a significant regulation of several microRNAs and their targeted mRNA in the lungs of pigs during acute influenza.

General information
Incubation of human blood fractions leads to changes in apparent miRNA abundance

A basic investigation on the presence and composition of miRNA species and their reaction to in vitro incubation and stimulation (borosilicate glass beads), in plasma, platelet-rich plasma (PRP), red blood cells (RBC), peripheral blood mononuclear cells (PBMC) and polymorphonuclear (PMN) cells was performed. 19 specific miRNAs were compared in control samples (0 hours), incubated 24 hour samples, and incubated 24 hour samples with glass bead stimulation for each blood fraction.

All 19 miRNAs were expressed in all blood fractions albeit at different levels for different miRNAs. Incubation resulted in significant changes in the abundance of miR-21, miR-155, Let-7c and Let 7f in plasma, miR-21, miR-23a and miR-150 in RBC and miR-15b, miR126, miR155 and Let-7g in PBMC, while no change was seen in PRP and PMN. Interestingly, in the samples incubated with glass beads, no miRNAs were significantly affected in plasma, RBC, PBMC and PMN, while expression of miR-25, miR15a, miR-126 and miR223 was significantly changed in PRP. Thus, PRP, as the only blood fraction depended on stimulation to change its miRNA profile upon incubation. For the other fractions, stimulation either leveled out the changes induced by incubation alone (plasma, RBC and PBMC) or no effect was seen in either case. This study confirms the presence of miRNA in anucleate cells like RBC and platelets. The up-regulation of four specific miRNAs in isolated plasma upon incubation was surprising; one possible explanation is that miRNA complexes in plasma may become more accessible for cDNA synthesis and qPCR upon incubation. Accessibility could be a new point of regulation for soluble miRNAs.

General information
State: Published
Organisations: National Veterinary Institute, Section for Immunology and Vaccinology, Technical University of Denmark
Contributors: Skovgaard, K., Jørgensen, S. T., Heegaard, P. M. H.
Publication date: 2012
Peer-reviewed: Yes
Event: Abstract from International Workshop on Small RNA in Cancer, Inflammation and Aging, Copenhagen, Denmark.
Electronic versions:
Source: dtu
Source-ID: u::8351
Research output: Research - peer-review › Conference abstract for conference – Annual report year: 2013

Innate immune responses to obesity in cloned and normal outbred domestic pig

Pigs are widely used as biomedical models for obesity and obesity-induced inflammation underlying the metabolic syndrome in humans because of similar physiology and metabolic features. It was the objective of this study to evaluate if pigs cloned by somatic cell nuclear transfer (n=17) could serve as a refined pig model for obesity-induced innate host responses by reducing pig-to-pig biological variation compared to wild-type (WT) pigs (n=19). Pigs were fed ad libitum with a high fat/high sucrose diet to induce obesity or kept lean on a restricted diet (60% of ad libitum intake) beginning at three months of age. mRNA expression levels were determined for 39 innate immune factors on a high-throughput qPCR system in samples from liver, abdominal fat, mesenteric fat and subcutaneous fat. Previous findings have suggested that
cloning may affect certain phenotypic traits of pigs including basic concentrations and responsiveness of components of the innate immune system. Terminal body weights at 7½ - 9½ months of age were significantly higher for both (WT and cloned) obese groups compared to the lean groups. However, obese WT pigs weighed significantly more than obese cloned pigs (P<0.01). In particular, mRNA expression profiles of certain acute phase proteins were significantly affected by cloning, being expressed at higher levels in the liver of both cloned groups compared to both WT groups but at lower levels in adipose tissues of cloned lean pigs as opposed to WT lean pigs. Also there were significant differences between WT and cloned pigs in the gene response to obesity. Thus, significant phenotypic differences were established for central innate immune factors between cloned and WT pigs, including differences in the response of these factors to an obesity-promoting diet. This should be taken into consideration when using cloned animals as models for innate responses to obesity.

General information
State: Published
Organisations: National Veterinary Institute, Section for Immunology and Vaccinology, Aarhus University
Contributors: Højbøge, T. R., Skovgaard, K., Stagsted, J., Heegaard, P. M. H.
Publication date: 2012
Peer-reviewed: Yes
Event: Abstract from 7th World Immune Regulation Meeting, Davos, Switzerland.
Source: dtu
Source-ID: u::9438
Research output: Research - peer-review › Conference abstract for conference – Annual report year: 2013

Intestinal colonization, gut function and inflammatory responses are moderately influenced by gestational age at birth

General information
State: Published
Organisations: National Veterinary Institute, Section for Bacteriology, Pathology and Parasitology, Section for Immunology and Vaccinology, University of Copenhagen
Contributors: Cilieborg, M. S., Skovgaard, K., Nørgaard, L. M., Sangild, P. T., Boye, M.
Number of pages: 2
Publication date: 2012
Peer-reviewed: Yes
Event: Abstract from 14th International Symposium on Microbial Ecology, Copenhagen, Denmark.
Electronic versions:
Cilieborg_ISME_2012.pdf
Source: dtu
Source-ID: u::5722
Research output: Research - peer-review › Conference abstract for conference – Annual report year: 2012

Profiling microRNAs in lung tissue from pigs infected with Actinobacillus pleuropneumoniae

Background: MicroRNAs (miRNAs) are a class of non-protein-coding genes that play a crucial regulatory role in mammalian development and disease. Whereas a large number of miRNAs have been annotated at the structural level during the latest years, functional annotation is sparse. Actinobacillus pleuropneumoniae (APP) causes serious lung infections in pigs. Severe damage to the lungs, in many cases deadly, is caused by toxins released by the bacterium and to some degree by host mediated tissue damage. However, understanding of the role of microRNAs in the course of this infectious disease in porcine is still very limited.

Results: In this study, the RNA extracted from visually unaffected and necrotic tissue from pigs infected with Actinobacillus pleuropneumoniae was subjected to small RNA deep sequencing. We identified 169 conserved and 11 candidate novel microRNAs in the pig. Of these, 17 were significantly up-regulated in the necrotic sample and 12 were down-regulated. The expression analysis of a number of candidates revealed microRNAs of potential importance in the innate immune response. MiR-155, a known key player in inflammation, was found expressed in both samples. Moreover, miR-664-5p, miR-451 and miR-15a appear as very promising candidates for microRNAs involved in response to pathogen infection.

Conclusions: This is the first study revealing significant differences in composition and expression profiles of miRNAs in lungs infected with a bacterial pathogen. Our results extend annotation of microRNA in pig and provide insight into the role of a number of microRNAs in regulation of bacteria induced immune and inflammatory response in porcine lung.

General information
State: Published
Organisations: National Veterinary Institute, Section for Immunology and Vaccinology, University of Copenhagen
Contributors: Podolska, A., Anthon, C., Bak, M., Tommerup, N., Skovgaard, K., Heegaard, P. M. H., Gorodkin, J., Cirera, S., Fredholm, M.
Pages: 459
Publication date: 2012
The gut microbiotassay – a high-throughput real-time PCR chip combined with next generation sequencing.

During the last decade it has become evident that there is a relation between certain medical conditions and the composition of the gut microbiota. To get a better understanding of this complex interaction it is important with high-throughput methods which are sensitive and specific but also informative. Many methods can be used to try to define and characterize the gut microbiota. Here we designed an assay consisting of twenty-four different primer systems targeting the most common bacterial groups of the intestine on different hierarchical levels. The aim of this study was to implement and test this assay with the high-throughput real-time PCR chip "Access Array 48.48" from Fluidigm. The chip executes 2304 individual reactions in parallel and afterwards it is possible to harvest the amplicons for next-generation sequencing. This approach gives a taxonomical overview of the gut microbiota, hence the name: 'the gut microbiotassay'.

The assay was tested on fifteen different bacterial type strains each functioning as target for one or more of the primer systems. In this way the sensitivity and the specificity of the primers were assessed. Next the assay was tested on complex ecosystems by extracting DNA from luminal content from small and large intestine, respectively. A 454-barcode library was added to the samples, and incorporated in the amplicons. Subsequently the amplicons were harvested, and any PCR bi-products were removed in a purification step. Finally detailed information on the bacterial composition for each sample was obtained with the Roche 454 GS FLX sequencing.

The gut microbiotassay had a high specificity and sensitivity, detecting from 50 down to at least 0.05ng/μl when tested on dilution series of pure cultures of bacterial type strains. When applied to complex ecosystems it demonstrated distinct quantities of bacteria in the different gut sections, with the highest number found in colon as expected. From the sequence data it was evident that primer systems targeting lower taxonomical levels, contributed with a higher resolution, revealing species that primer system targeting higher taxonomical levels could not detect. At the same time the results for the different primer systems confirmed one another, as some bacteria were detected on various phylogenetic levels, but all in line with their respective taxonomical classification.

The gut microbiotassay in combination with next generation sequencing both provides a quantitative measure in terms of Cq-values achieved from the real-time PCR, as well as the deeper information obtained from next-generation sequencing of the amplicons. It is quick to perform and offers a high-throughput at a relatively low cost. These features make the gut microbiotassay worth considering, when choosing between current methods used to characterize the gut microbiota.
The Impact of Staphylococcus Aureus Concentration on the Development of Pulmonary Lesions and Cytokine Expression After Intravenous Inoculation of Pigs

Acute respiratory distress syndrome is a common complication in severe sepsis. In pigs, the lungs play an important role in clearing systemic bacterial infections due to pulmonary intravascular macrophages found specifically in pigs. However, this increases the exposure of the porcine lungs to pathogens and potential injury. The authors propose that increasing the concentration of the inoculum without changing the bacterial dose will lead to severe sepsis with pronounced pulmonary lesions. This could potentially create a risk of cytokine spillover to the circulation, leading to an increased systemic response. Eight Danish Landrace pigs, approximately 10 weeks old, were inoculated twice with a low or once with a high concentration of Staphylococcus aureus. Three pigs were sham-inoculated. The animals were grouped based on macro- and microscopic lung lesions. The mRNA expression of local pulmonary inflammatory markers was compared to protein levels of systemic inflammatory markers. The most severe pulmonary lesions were observed in animals receiving the high S. aureus concentration, indicating that severity of lesions is dependent on inoculum concentration rather than total numbers of bacteria. Furthermore, local mRNA expression of inflammatory cytokines appeared to be dependent on the magnitude and severity of tissue destruction, including the ability to confine the lesions. Increasing mRNA levels of serum amyloid A could be a confident marker of severity of pulmonary lesions. Since no correlation was observed between local and systemic levels of inflammatory cytokines, this finding could indicate an ability of the porcine lung to compartmentalize the local inflammatory response and thus restrict systemic contribution.
Fetal lipopolysaccharide exposure modulates diet-dependent gut maturation and sensitivity to necrotising enterocolitis in pre-term pigs

Uterine infections during pregnancy predispose to pre-term birth and postnatal morbidity, but it is unknown how prenatal bacterial exposure affects maturation of the immature gut. We hypothesised that a prenatal exposure to gram-negative lipopolysaccharide (LPS) has immunomodulatory effects that improve resistance towards necrotising enterocolitis (NEC) in pre-term neonates. At approximately 85% gestation, pig fetuses were injected intramuscularly with saline or LPS (0.014 mg/kg), or intra-amniotically with LPS (0.4 mg/kg). Pigs were delivered by caesarean section 3–5 d later and fed colostrum (C) or formula (F) for 48 h. Gut indices did not differ between pigs injected intramuscularly with saline or LPS, and these groups were therefore pooled into two control groups according to diet (control-F, n 32 and control-C, n 11). Control-F pigs showed reduced villus heights, mucosal structure, gut integrity, digestive enzymes, elevated NEC incidence (38 v. 0%, P <0.05) and several differentially expressed immune-related genes, relative to control-C pigs. Compared with the control-F and control-C groups, values in formula-fed pigs given intra-amniotic LPS formula (n 17) were intermediate for villus height, enzyme activities, intestinal permeability and NEC incidence (18%, P = 0.2 relative to control-F), and numbers of differentially expressed immune genes. In conclusion, prenatal exposure of the fetal gut to Gram-negative bacteria may modulate the immediate postnatal response to an enteral diet and colonising bacteria. Copyright © The Authors 2011.

Original language: English
Keywords: Bacterial concentration, Cytokine, Histopathology, Lung, RT-qPCR, Porcine model, Staphylococcus aureus, Swine

DOIs: 10.1177/0300985812439726

Research output: Research - peer-review › Journal article – Annual report year: 2012

General information
State: Published
Organisations: Microbial Ecology, Division of Veterinary Diagnostics and Research, National Veterinary Institute, Innate Immunology, Baylor College of Medicine, University of Copenhagen
MicroRNA expression in lung tissue and blood isolated from pigs suffering from bacterial pneumonia

MicroRNAs (miRNAs) are a highly evolutionarily conserved group of small non-coding RNA molecules, which regulate the activity of other genes at the post-transcriptional level. Recently it has become evident that miRNA plays an important role in modulating and fine tuning of the innate and adaptive immune responses. Still, little is known about the impact of miRNAs in the development and pathogenesis of lung infections. Expression of miRNA, known to be induced by bacterial (i.e., LPS) ligands and thus supposed to play a role in the regulation of antimicrobial defence, were studied in lung tissue from pigs experimentally infected with Actinobacillus pleuropneumoniae serotype 2 and 6. Circulating miRNAs were studied in blood from pigs infected with A. pleuropneumoniae serotype 2 using real time-qPCR (RT-qPCR). Expression profiles of miRNA in blood of seven animals before and after infection, where also studied using miRCURY™ LNA arrays (Exiqon, Denmark). Piglets were inoculated by dripping 1ml bacterial suspension, into each nostril during inhalation. Each time group is a different set of 4-6 pigs. Most of the inoculated pigs revealed characteristic, well demarcated, lung lesions. No pathological changes were seen in lungs from control animals. All AP infected animals had a significantly higher level of mRNA coding for the acute-phase protein SAA-2 in the liver compared to the control group. Whole Blood samples were collected in PAXgene Blood RNA Tubes (PrenalytiX) before (control) and after infection of piglets (6 h., 12 h., 24 h. and 48 h.). Total RNA was extracted from blood samples using PAXgene™ Blood RNA kit (Qiagen/ PrenalytiX). The quantity of extracted total RNA was determined using a Nanodrop ND-1000 and the quality of extracted RNA was estimated by on-chip electrophoresis (Nanochip 6000) on an Agilent 2100 Bioanalyzer, a RNA integrity number (RIN) was assigned to each sample. Expression levels of selected miRNA were further studied in lung tissue collected at two time points (6 h. and 24 h.) after A. pleuropneumoniae serotype 2 and 6 infection. 600 ng total RNA from blood samples before and after infection were labelled with Hy5™ and Hy3™ fluorescent label, respectively, using the miRCURY™ LNA Array power labelling kit (Exiqon, Denmark). Sample were mixed pair-wise and hybridized to miRCURY™ LNA array version 11.0 (Exiqon, Denmark), which contains capture probes targeting all miRNAs for human, mouse and rat. The miRCURY™ LNA array microarray slides were scanned, and image analysis was carried out using the LmaGene 8.0 software (BioDiscovery, Inc., USA). A two-tailed T-test calculated between infected and control identified 10 of 1263 miRNA to be differentially expressed (p-values lower than 0.05). MicroRNA expression in lung tissue over time in response to the two different serotypes were very similar. miR-223 was found to be highly up regulated, followed by miR-146a and to a lesser degree miR-233 in lung tissue of the AP serotype 2 infected animals. MiR-233 was also found to be up regulated in blood based on both microarray and RT-qPCR, mir-233 is a negative regulator of neutrophil proliferation and activation and might act to limit the potentially harmful consequences of the accumulation of infiltrating neutrophils in AP infected lungs. More data of microRNA expression in blood of pigs infected with A. pleuropneumonia serotype 2 will be presented.

General information
State: Published
Organisations: Innate Immunology, Division of Veterinary Diagnostics and Research, National Veterinary Institute
Contributors: Skovgaard, K., Wendt, K. T., Heegaard, P. M. H.
Publication date: 2011
Porcine blood mononuclear cell cytokine responses to PAMP molecules: comparison of mRNA and protein production

Pathogen-associated molecular patterns (PAMPs) are conserved molecules of microorganisms inducing innate immune cells to secrete distinct patterns of cytokines. In veterinary species, due to a lack of specific antibodies, cytokines are often monitored as expressed mRNA only. This study investigated the induction of IFN-α, IL-12 p40, IL-1β, TNF-α, IL-6 and IL-10 by PAMP-molecules [CpG oligonucleotide D19 (CpG), peptidoglycan (PGN), lipopolysaccharide (LPS), Pam3Cys and poly-U] in porcine blood mononuclear cells (BMC) within a 24h period. As expected, cytokine responses were PAMP-specific, CpG inducing IFN-α and IL-12 p40, and PGN, LPS and Pam3Cys inducing varying amounts of IL-12 p40, IL-1β, TNF-α, IL-6 and IL-10. Surprisingly, the ssRNA-mimic poly-U induced IL-6 and IL-1β only. Using CpG, PGN and LPS, the kinetics of cytokine production measured as mRNA (reverse transcription (RT)-qPCR) and protein (ELISA), respectively, correlated well, mRNA responses preceding protein responses. With the exception of IL-1β and IL-6, mRNA-responses were transient, whereas protein responses, except for TNF-α, followed saturation kinetics. Remarkably, LPS-induced TNF-α mRNA was not followed by a protein response. These results provide guidelines concerning the timing and use of protein and mRNA determinations for the characterization of porcine cytokine responses to PAMPs, although given the low number of animals used here results are preliminary and need confirmation in a larger study.

General information
State: Published
Organisations: National Veterinary Institute, Innate Immunology, Division of Veterinary Diagnostics and Research
Contributors: Sørensen, N. S., Skovgaard, K., Heegaard, P. M. H.
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Web of Science (2017): Impact factor 1.632
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 2
Scopus rating (2016): CiteScore 1.63 SJR 0.742 SNIP 0.708
Web of Science (2016): Impact factor 1.718
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 2
Scopus rating (2015): CiteScore 1.67 SJR 0.862 SNIP 0.749
Web of Science (2015): Impact factor 1.664
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 2
Scopus rating (2014): CiteScore 1.6 SJR 0.777 SNIP 0.718
Web of Science (2014): Impact factor 1.535
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 2
Scopus rating (2013): CiteScore 1.89 SJR 0.834 SNIP 0.797
Web of Science (2013): Impact factor 1.748
ISI indexed (2013): ISI indexed yes
Transcriptional profiling at different sites in lungs of pigs during acute bacterial respiratory infection

The local transcriptional response was studied in different locations of lungs from pigs experimentally infected with the respiratory pathogen Actinobacillus pleuropneumoniae serotype 5B, using porcine cDNA microarrays. This infection gives rise to well-demarcated infection loci in the lung, characterized by necrotic and haemorrhagic lesions. Lung tissue was sampled from necrotic areas, from visually unaffected areas and from areas bordering on necrotic areas. Expression pattern of these areas from infected pigs was compared to healthy lung tissue from un-infected pigs. Transcription of selected genes important in the innate defence response were further analysed by quantitative realtime reverse-transcriptase PCR. A clear correlation was observed between the number of differentially expressed genes as well as the magnitude of their induction and the sampling location in the infected lung, with the highest number of differentially expressed genes, and the most highly induced genes found in necrotic areas. Interestingly, a group of differentially regulated genes was represented in all three areas, comprising genes encoding cytokines, acute phase proteins, and...
factors related to regulation of apoptosis and the complement system. Interferon-g was downregulated in both necrotic and bordering areas. Evidence of neutrophil recruitment was seen by the up-regulation of chemotactic factors for neutrophils. In conclusion, we found subsets of genes expressed at different levels in the three selected areas of the infected lung as compared to the control group. Thus it is demonstrated that an infection with clearly defined infected loci leads to a rapid disseminated intra-organ response in neighbouring seemingly unaffected tissue areas of the infected organ. Within the lung, we found a clear division of induced genes as in unaffected areas a large part of differently expressed genes were involved in systemic reactions to infections, while differently expressed genes in necrotic areas were mainly concerned with homeostasis regulation.
Scopus rating (2009): SJR 1.229 SNIP 0.834
BFI (2008): BFI-level 1
Scopus rating (2008): SJR 1.207 SNIP 0.771
Scopus rating (2007): SJR 2.081 SNIP 0.859
Scopus rating (2006): SJR 1.703 SNIP 0.755
Scopus rating (2005): SJR 1.457 SNIP 0.792
Scopus rating (2004): SJR 1.083 SNIP 0.626
Scopus rating (2003): SJR 0.961 SNIP 0.672
Scopus rating (2002): SJR 0.672 SNIP 0.249
Scopus rating (2001): SJR 0.262 SNIP 0.161
Scopus rating (2000): SJR 0.33 SNIP 0.161
Scopus rating (1999): SJR 0.289 SNIP 0.432

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Research output: Research - peer-review › Journal article – Annual report year: 2011

Transition from parenteral to enteral nutrition induces immediate diet-dependent gut histological and immunological responses in preterm neonates

Necrotizing enterocolitis (NEC) in preterm infants develops very rapidly from a mild intolerance to enteral feeding into intestinal mucosal hemorrhage, inflammation, and necrosis. We hypothesized that immediate feeding-induced gut responses precede later clinical NEC symptoms in preterm pigs. Fifty-six preterm pigs were fed total parenteral nutrition (TPN) for 48 h followed by enteral feeding for 0, 8, 17, or 34 h with either colostrum (Colos, n = 20) or formula (Form, n = 31). Macroscopic NEC lesions were detected in Form pigs throughout the enteral feeding period (20/31, 65%), whereas most Colos pigs remained protected (1/20, 5%). Just 8 h of formula feeding induced histopathological lesions, as evidenced by capillary stasis and necrosis, epithelial degeneration, edema, and mucosal hemorrhage. These immediate formula-induced changes were paralleled by decreased digestive enzyme activities (lactase and dipeptidylpeptidase IV), increased nutrient fermentation, and altered expression of innate immune defense genes such as interleukins (IL-1α, IL-6, IL-18), nitric oxide synthetase, tight junction proteins (claudins), Toll-like receptors (TLR-4), and TNF-α. In contrast, the first hours of colostrum feeding induced no histopathological lesions, increased maltase activity, and induced changes in gene expressions related to tissue development. Total bacterial density was high after 2 days of parenteral feeding and was not significantly affected by diet (colostrum, formula) or length of enteral feeding (8–34 h), except that a few bacterial groups (Clostridium, Enterococcus, Streptococcus species) increased with time. We conclude that a switch from parenteral to enteral nutrition rapidly induces diet-dependent histopathological, functional, and proinflammatory insults to the immature intestine. Great care is required when introducing enteral feeds to TPN-fed preterm infants, particularly when using formula, because early feeding-induced insults may predispose to NEC lesions that are difficult to revert by later dietary or medical interventions.

General information
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Organisations: Bacteriology & Pathology, Division of Veterinary Diagnostics and Research, National Veterinary Institute, Innate Immunology, Microbial Ecology, Aarhus University, University of Copenhagen
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Ratings:
BFI (2018): BFI-level 1
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 1
Scopus rating (2017): CiteScore 3.24 SJR 1.822 SNIP 0.918
Web of Science (2017): Impact factor 3.293
Cytokine and acute phase protein mRNA expression in liver tissue from pigs with severe sepsis caused by intravenous inoculation of Staphylococcus aureus

The aim was to substantiate previous findings of hepatic dysfunction in a porcine model of Staphylococcus aureus induced severe sepsis. Nine pigs were inoculated intravenously once or twice with 10^8 S. aureus per kilogram body weight and killed 12, 24 and 48 h later. Three pigs served as controls. Blood was sampled for bacteriology, haematology and clinical pathology. Tissues were collected at necropsy for bacteriology, gene expression analysis and histology. Bacterial counts in blood remained low, decreased in the lungs, liver and spleen, but increased in bone. All infected pigs developed sepsis characterized by fever, neutrophilia, increased serum levels of C-reactive protein (CRP) and interleukin (IL)-6, and decreased levels of serum iron. CRP and IL-6 serum levels peaked at 36 h. Serum IL-1β and tumour necrosis factor-α (TNFα) did not change. Serum aspartate aminotransferase (AST) and bilirubin were elevated at 36 and 48 h. Microabscesses were found in the livers from pigs killed at 12 h only. The livers from pigs killed at 48 h also showed light, diffuse fibrin exudation (vascular leakage). Real-time PCR showed a decreased hepatic expression of mRNA coding for albumin and increased hepatic expression of IL-6, IL-8, IL-1β, and CRP. No increase could be detected in the IL-1α or TNFα liver-mRNA levels. IL-6, IL-8 and IL-1β expression peaked at 24 hours (2-5 fold compared to the control group). In conclusion, the increased liver cytokine mRNA levels indicate a local hepatic, non-infectious inflammatory response, and supports evidence of liver dysfunction indicated by increase in AST and bilirubin, and liver histopathology. Although hepatocyte IL-8 production has been proposed to indicate hepatocyte stress, the increased mRNA IL-8 levels, as the increase of the other cytokines, could have originated from cellular sources not constitutive to the liver. This warrants future examination of the liver by e.g. laser capture microdissection. Supported by grant no. 271-07-0417 from the Danish Medical Research Council.

MicroRNAs are small non-coding RNA molecules (18-23 nt), that regulate the activity of other genes at the post-transcriptional level. Recently it has become evident that microRNA plays an important role in modulating and fine tuning innate and adaptive immune responses. Still, little is known about the impact of microRNAs in the development and pathogenesis of lung infections. Expression of microRNA known to be induced by bacterial (i.e., LPS) ligands and thus supposed to play a role in the regulation of antimicrobial defence, were studied in lung tissue and in blood from pigs experimentally infected with Actinobacillus pleuropneumoniae (AP). Expression differences of mRNA and microRNA were quantified at different time points (6h, 12h, 24h, 48h PI) using reverse transcription quantitative real-time PCR (Rotor-Gene and Fluidigm). Expression profiles of miRNA in blood of seven animals were further studied using miRCURY™ LNA arrays (Exiqon, Denmark). All AP infected animals had significantly higher levels of mRNA coding for inflammatory mediators as IL-6 and IL-8 as well as the acute phase protein SAA, in the lung compared to the control group. MiR-223 was found to be highly up regulated, followed by miR-146a and to a lesser degree miR- 155 in lung tissue of the AP infected animals. MiR-233 was also found to be up regulated in blood based on both microarray and real-time PCR. MiR-233 has been found to be a negative regulator of neutrophil proliferation and activation, and might act to limit the potentially harmful consequences of the accumulation of infiltrating neutrophils in AP infected lungs.
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Source-ID: 274597

Research output: Research - peer-review › Conference abstract in proceedings – Annual report year: 2011

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**Extraction of mRNA from coagulated horse blood and analysis of inflammation-related cytokine responses to coagulation**

Coagulated blood is a rich source of mRNA that allows the study of the regulation of expression of cytokine and other genes. However, while several methods are available for isolation of RNA from whole blood and tissues, protocols for purification of mRNA from clotted blood are not generally available. Here, a protocol for RNA extraction from highly clotted blood was optimized and the regulation of a number of cytokine genes compared to stabilized blood was studied. Whole blood samples from 10 clinically healthy horses were incubated for 24 hours at 37°C and RNA was extracted from the peripheral blood mononuclear cells present in the blood clot, homogenizing the clot by rotating knife homogenization (GentleMACS, Miltenyi Biotec) in the presence of QiAzoL extraction buffer (Qiagen). The RNA extracted yielded high concentrations of total RNA (50-265 ng/μl) and quality measures (RIN=8.5-9.2), comparable with that purified by standard methods from stabilized blood. Cytokine mRNA expression was assessed by reverse transcribed quantitative real time PCR and it was found that 24-hour clotting led to a significant increase in the concentrations of mRNA of the pro-inflammatory cytokines interleukin-1β (IL-1β), interleukin-1-receptor antagonist (IL-1ra), interleukin-15 (IL-15), and interleukin-8 (IL-8). These findings that a coagulation-induced inflammation-related cytokine response takes place in whole blood upon clotting. The extraction method provides reproducible and reliable results allowing the recovery of quantifiable high-quality RNA for molecular expression analysis.

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**General information**

State: Published

Organisations: Innate Immunology, Division of Veterinary Diagnostics and Research, National Veterinary Institute

Contributors: Bovbjerg, K. K. L., Heegaard, P. M. H., EFSA Publication

Number of pages: 224

Publication date: 2010

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Title of host publication: 2010 Annual Meeting SLB & IEIIS : Abstracts

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**Hepatic gene expression changes in pigs experimentally infected with the lung pathogen Actinobacillus pleuropneumoniae as analysed with an innate immunity focused microarray**

Knowledge on gene expression in the liver during respiratory infections is limited although it is well-established that this organ is an important site of synthesis of several systemic innate immune components as response to infections. In the present study, the early transcriptional hepatic response of genes associated with innate immune responses was studied in pigs 14–18 h after intranasal inoculation with Actinobacillus pleuropneumoniae, using innate immune focused microarrays and quantitative real-time PCR (qPCR). The microarray analysis of liver tissue established that 51 genes were differentially expressed. A large group of these genes encoded proteins involved in the acute phase response, including serum amyloid A, C-reactive protein, fibrinogen, haptoglobin and tumor necrosis factor-a the expression of which were all found to be up-regulated and glutathione S-transferase, transthyretin, transferrin and albumin which were down-regulated. Additional genes associated with innate immune responses were investigated using qPCR; genes encoding interleukin (IL)1, IL6, IL8, lipopolysaccharide binding protein, lactotransferrin, and PigMAP were up-regulated and interferon 1a, a1-acid glycoprotein, mannann-binding lectin A, surfactant protein D, and surfactant protein A1 were down-regulated in the liver of infected animals. Down-regulation of a1-acid glycoprotein during infection has not been described previously in any species. These results confirm that the liver plays an important role in initiating and orchestrating the innate immune response to A. pleuropneumoniae infection. Keywords: acute phase protein, hepatic transcriptional response, innate defence, gene expression, pig

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**General information**

State: Published
Local and disseminated acute phase response during bacterial respiratory infection in pigs

The acute phase response is playing an important role, aiming to restore the healthy state after tissue injury, inflammation and infection. The biological function of this response and its interplay with other parts of innate defense reactions remain somewhat elusive. Expression of acute phase proteins (APP) outside the liver is increasingly recognized, still little is known of extra-hepatic production of APP in pigs. 14-18 h after experimental infection with Actinobacillus pleuropneumoniae, causing acute pleuropneumonia in pigs, we studied local APP gene expression changes in different locations of the infected lung (necrotic areas, areas bordering on necrotic areas, and from visually unaffected areas). Expression differences was also studied in the liver and in peripheral lymphoid tissue (tracheobronchial lymph nodes, spleen, tonsils) of infected (n=10) and non-infected (n=5) pigs using reverse transcription quantitative real-time PCR (RT-qPCR). SAA, CRP and IL-6 were further measured in serum samples before and after the infection using ELISA.

Expression of ALB, A1AG, APOA1, CRP, Hp, PigMAP, SAA, TF, and TTR were found in all tissues investigated. SAA, Hp, LTF, LBP, TRF, SpA, SpD, IL-1α and IL-6 were found to be differentially expressed in all three areas of the infected lung compared to lung tissue from control pigs. Expression differences were general highest in necrotic areas. We found good correlation between ELISA and RT-qPCR data the in serum and whole blood respectively. mRNA coding for CRP, Hp, IL-6, and SAA were found to be significantly up regulated in infected pigs in whole blood and tracheobronchial lymph nodes. In the spleen we found mRNA coding for APOA1, FIB, Hp, PigMAP, SAA, TF, and IL-6 to be differentially expressed. In the tonsils IL-6 and SAA were also found differentially expressed between infected and control animals. We demonstrated that acute pleuropneumonia caused by A. pleuropneumoniae leads to a rapid disseminated local intra-lung APP response, also in apparently unaffected areas of the infected lung. Further extrahepatic expression of several acute-phase proteins was found 14-18h after experimental infection with A. pleuropneumoniae. This firmly establishes that expression of APPs is widely disseminated, involving changes in the expression of APPs at a dynamic scale comparable to the hepatic response. These results suggest that many different cell-types in the organism are involved in production of APP and further supports that extrahepatic APP might be important players of the innate defence system.
that the qPCR based quantifications reduced the cluster density variability as compared to fluorometer based quantifications. Furthermore, it was found that the fluorometer based measurements tended to deviate for dilute as well as for more concentrated libraries. Following the sequencing of the lung lymph node samples analyses are to be conducted to study the time and serotype dependent transcriptional response to Ap infection.

A two-locus DNA sequence database for typing plant and human pathogens within the Fusarium oxysporum species complex.

We constructed a two-locus database, comprising partial translation elongation factor (EF-1alpha) gene sequences and nearly full-length sequences of the nuclear ribosomal intergenic spacer region (IGS rDNA) for 850 isolates spanning the phylogenetic breadth of the Fusarium oxysporum species complex (FOSC). Of the 850 isolates typed, 101 EF-1alpha, 203 IGS rDNA, and 256 two-locus sequence types (STs) were differentiated. Analysis of the combined dataset suggests that two-thirds of the STs might be associated with a single host plant. This analysis also revealed that the 26 STs associated with human mycoses were genetically diverse, including several which appear to be nosocomial in origin. A congruence analysis, comparing partial EF-1alpha and IGS rDNA bootstrap consensus, identified a significant number of conflicting relationships dispersed throughout the bipartitions, suggesting that some of the IGS rDNA sequences may be non-orthologous. We also evaluated enniatin, fumonisin and moniliformin mycotoxin production in vitro within a phylogenetic framework.
Pathogen-associated molecular patterns (PAMPs) are conserved microbial structures recognized by pattern-recognition receptors (PRRs) of the innate immune system. Binding of PAMPs by certain PRRs on dendritic cells induces these to express costimulatory molecules and cytokines, enabling an inductive antigen-presentation. Different PAMPs will activate different signalling pathways, resulting in specific cytokine signatures, which will influence the orientation of a developing immune response. In the pig, the range of antibodies available for cytokine-detection is limited, and so cytokines are often
monitored at mRNA-level only. However, mRNA levels do not always correlate with corresponding protein levels, and translational regulation is abundant, e.g. exerted by microRNAs through inhibition of mRNA-translation. Here, the kinetics and magnitude of induction of cytokines (IFN-α, IL-12 p40, IL-1β, TNF-α, IL-6 and IL-10) by PAMP-structures (oligonucleotides, single-stranded RNA, peptidoglycan, lipopeptides and lipopolysaccharide) were investigated in porcine PBMCs comparing expression at mRNA (quantitative real-time PCR, qPCR) and protein level (ELISA). Overall, the two levels correlated well, with the protein response in most cases being slower than the mRNA response, as expected. Different PAMPs induced different cytokines with varying kinetics of induction. In some cases qPCR appeared more sensitive than ELISA, but to what degree this could be explained by translational inhibition or by different detection-sensitivities of the two techniques is not known at this point.

**General information**

State: Published
Organisations: Innate Immunology, Division of Veterinary Diagnostics and Research, National Veterinary Institute
Contributors: Sørensen, N. S., Skovgaard, K., Vorsholt, H., Heegaard, P. M. H.
Publication date: 2009
Peer-reviewed: Yes
Event: Abstract from 3rd European Veterinary Immunology Workshop, Berlin, Germany.
Keywords: Pathogen-associated molecular patterns, Peripheral blood mononuclear cells, Pig, ELISA, Cytokines, Quantitative real-time PCR
Source: orbit
Source-ID: 244212
Research output: Research - peer-review › Conference abstract for conference – Annual report year: 2009

**Rapid and widely disseminated acute phase protein response after experimental bacterial infection of pigs**

The acute phase protein response is a well-described generalized early host response to tissue injury, inflammation and infection, observed as pronounced changes in the concentrations of a number of circulating serum proteins. The biological function of this response and its interplay with other parts of innate host defence reactions remain somewhat elusive. In order to gain new insight into this early host defence response in the context of bacterial infection we studied gene expression changes in peripheral lymphoid tissues as compared to hepatic expression changes, 14-18 h after lung infection in pigs. The lung infection was established with the pig specific respiratory pathogen Actinobacillus pleuropneumoniae. Quantitative real-time PCR based expression analysis were performed on samples from liver, tracheobronchial lymph node, tonsils, spleen and on blood leukocytes, supplemented with measurements of interleukin-6 and selected acute phase proteins in serum. C-reactive protein and serum amyloid A were clearly induced 14-18 h after infection. Extrahepatic expression of acute phase proteins was found to be dramatically altered as a result of the lung infection with an extrahepatic acute phase protein response occurring concomitantly with the hepatic response. This suggests that the acute phase protein response is a more disseminated systemic response than previously thought. The current study provides to our knowledge the first example of porcine extrahepatic expression and regulation of C-reactive protein, haptoglobin, fibrinogen, pig major acute phase protein, and transferrin in peripheral lymphoid tissues.

**General information**

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Organisations: Innate Immunology, Division of Veterinary Diagnostics and Research, National Veterinary Institute, Microbial Ecology
Contributors: Skovgaard, K., Mortensen, S., Boye, M., Wendt, K. T., Campbell, F. M., Eckersall, P. D., Heegaard, P. M. H.
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Scopus rating (2017): SJR 1.266 SNIP 1.139
Web of Science (2017): Impact factor 2.903
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 2
Scopus rating (2016): SJR 1.44 SNIP 1.303
Web of Science (2016): Impact factor 2.798
Rapid and widely disseminated acute phase protein response after experimental bacterial infection of pigs

General information
State: Published
Organisations: National Veterinary Institute, Innate Immunology, Division of Veterinary Diagnostics and Research, Microbial Ecology
Contributors: Skovgaard, K., Mortensen, S., Boye, M., Wendt, K. T., Campbell, F. M., Eckersall, P. D., Heegaard, P. M. H.
Publication date: 2009
Peer-reviewed: No
Event: Poster session presented at 3rd Annual Meeting of EPIZONE, Antalya, Turkey.
Source: orbit
Source-ID: 240743
Research output: Research › Poster – Annual report year: 2009

Stability of microRNA in partly degraded RNA extracted from porcine lung tissue

General information
State: Published
Organisations: Innate Immunology, Division of Veterinary Diagnostics and Research, National Veterinary Institute
Contributors: Skovgaard, K., Mortensen, S., Wendt, K. T., Lauritsen, K. T., Heegaard, P. M. H.
Publication date: 2009
Peer-reviewed: No
Event: Poster session presented at 4th International qPCR Symposium & Industrial Exhibition & Application Workshop, Freising, Germany.
Source: orbit
Source-ID: 240739
Research output: Research › Poster – Annual report year: 2009

Stability of microRNA in partly degraded RNA extracted from porcine lung tissue

General information
State: Published
Organisations: Innate Immunology, Division of Veterinary Diagnostics and Research, National Veterinary Institute
Contributors: Skovgaard, K., Mortensen, S., Wendt, K. T., Lauritsen, K. T., Heegaard, P. M. H.
Publication date: 2009
Peer-reviewed: No
Event: Poster session presented at 3rd Annual Meeting of EPIZONE, Antalya, Turkey.
Source: orbit
Source-ID: 240742
Research output: Research › Poster – Annual report year: 2009

Enteral formula feeding causes acute intestinal dysfunction and inflammation in preterm pigs during development of necrotizing enterocolitis

General information
State: Published
Organisations: Innate Immunology, Division of Veterinary Diagnostics and Research, National Veterinary Institute, Microbial Ecology
Contributors: Siggers, J., Siggers, R., Skovgaard, K., Thymann, T., Boye, M., Sangild, P.
Publication date: 2008
Peer-reviewed: No
Event: Abstract from Asian Society for Pediatric Research Joint Meeting, Honolulu, HI, United States.
Source: orbit
Source-ID: 241226
Research output: Research › Conference abstract for conference – Annual report year: 2008

Enteral formula feeding causes acute intestinal dysfunction and inflammation in preterm pigs during development of necrotizing enterocolitis.

General information
State: Published
Enteral formula feeding causes acute intestinal dysfunction and inflammation prior to development of necrotizing enterocolitis in preterm pigs

General information
State: Published
Organisations: Innate Immunology, Division of Veterinary Diagnostics and Research, National Veterinary Institute, Microbial Ecology
Contributors: Siggers, J., Siggers, R., Skovgaard, K., Thymann, T., Boye, M., Sangild, P. T.
Publication date: 2008
Peer-reviewed: No
Event: Poster session presented at Asian Society for Pediatric Research Joint Meeting, Honolulu, HI, United States.
Source: orbit
Source-ID: 231670
Research output: Research › Poster – Annual report year: 2008

Extrahepatic Acute Phase Protein Response in Pigs with Pleuropneumonia Caused by Experimental Actinobacillus pleuropneumoniae Infection

General information
State: Published
Organisations: Adaptive Immunology & Parasitology, Division of Veterinary Diagnostics and Research, National Veterinary Institute, Virology
Contributors: Skovgaard, K., Mortensen, S., Poulsen, K. T., Boye, M., Heegaard, P. M. H.
Publication date: 2008
Peer-reviewed: No
Source: orbit
Source-ID: 222707
Research output: Research › Poster – Annual report year: 2008

Extrahepatic Expression of Acute Phase Proteins in Pigs undergoing Actinobacillus pleuropneumoniae Infection

General information
State: Published
Organisations: Innate Immunology, Division of Veterinary Diagnostics and Research, National Veterinary Institute, Adaptive Immunology & Parasitology, Microbial Ecology
Contributors: Skovgaard, K., Mortensen, S., Boye, M., Heegaard, P. M. H.
Publication date: 2008
Peer-reviewed: No
Source: orbit
Source-ID: 241227
Research output: Research › Conference abstract for conference – Annual report year: 2008

Gene expression profiling of the early response in lungs of pigs experimentally infected with Actinobacillus pleuropneumoniae

General information
State: Published
Organisations: Adaptive Immunology & Parasitology, Division of Veterinary Diagnostics and Research, National Veterinary Institute, Aarhus University
Contributors: Mortensen, S., Skovgaard, K., Hedegaard, J., Heegaard, P. M. H.
Oral administration of amniotic fluid reduces necrotizing enterocolitis in preterm pigs

General information
State: Published
Organisations: Innate Immunology, Division of Veterinary Diagnostics and Research, National Veterinary Institute, Microbial Ecology
Contributors: Siggers, J., Siggers, R., Skovgaard, K., Schmidt, M., Møller, H., Boye, M., Sangild, P.
Publication date: 2008
Peer-reviewed: No
Event: Abstract from Asian Society for Pediatric Research Joint Meeting, Honolulu, HI, United States.
Source: orbit
Source-ID: 241225
Research output: Research › Conference abstract for conference – Annual report year: 2008

The disseminated acute phase protein response to experimental bacterial lung infection in pigs

General information
State: Published
Organisations: National Veterinary Institute, Division of Veterinary Diagnostics and Research, Adaptive Immunology & Parasitology
Contributors: Skovgaard, K., Mortensen, S., Heegaard, P. M. H.
Publication date: 2008
Peer-reviewed: No
Source: orbit
Source-ID: 222669
Research output: Research › Poster – Annual report year: 2008

Time–dependent functional and immunological changes in the intestine of the preterm pig susceptible to necrotizing enterocolitis

General information
State: Published
Organisations: Innate Immunology, Division of Veterinary Diagnostics and Research, National Veterinary Institute, Microbial Ecology
Contributors: Siggers, J., Siggers, R., Skovgaard, K., Thymann, T., Boye, M., Sangild, P.
Publication date: 2008
Peer-reviewed: No
Event: Abstract from Digestive Disease Week 2008, San Diego, CA, United States.
Source: orbit
Source-ID: 241223
Research output: Research › Conference abstract for conference – Annual report year: 2008

Time–dependent functional and immunological changes in the intestine of the preterm pig susceptible to necrotizing enterocolitis.

General information
State: Published
Organisations: National Veterinary Institute
Contributors: Siggers, J., Siggers, R., Skovgaard, K., Thymann, T., Boye, M., Sangild, P.
Publication date: 2008
Peer-reviewed: No
Event: Poster session presented at Digestive Disease Week 2008, San Diego, CA, United States.
Source: orbit
Source-ID: 231643
Microarray analysis of pig innate immune responses after experimental infection with Actinobacillus pleuropneumoniae

General information
State: Published
Organisations: Innate Immunology, Division of Veterinary Diagnostics and Research, National Veterinary Institute, Adaptive Immunology & Parasitology
Contributors: Skovgaard, K., Mortensen, S., Poulsen, K., Boye, M., Jungersen, G., Heegaard, P. M. H.
Publication date: 2007
Peer-reviewed: No
Event: Abstract from FoodDTU, Lyngby, Denmark.
Source: orbit
Source-ID: 241217
Research output: Research › Conference abstract for conference – Annual report year: 2007

Molecular characterisation of the early response in pigs to experimental infection with Actinobacillus pleuropneumoniae using cDNA microarrays

Background: The bacterium Actinobacillus pleuropneumoniae is responsible for porcine pleuropneumonia, a widespread, highly contagious and often fatal respiratory disease of pigs. The general porcine innate immune response after A. pleuropneumoniae infection is still not clarified. The objective of this study was hence to characterise the transcriptional response, measured by using cDNA microarrays, in pigs 24 hours after experimental inoculation with A. pleuropneumoniae. Methods: Microarray analyses were conducted to reveal genes being differentially expressed in inflamed versus non-inflamed lung tissue sampled from inoculated animals as well as in liver and tracheobronchial lymph node tissue sampled from three inoculated animals versus two non-inoculated animals. The lung samples were studied using a porcine cDNA microarray with 5375 unique PCR products while liver tissue and tracheobronchial lymph node tissue were hybridised to an expanded version of the porcine microarray with 26879 unique PCR products. Results: A total of 357 genes differed significantly in expression between infected and non-infected lung tissue, 713 genes differed in expression in liver tissue from infected versus non-infected animals and 130 genes differed in expression in tracheobronchial lymph node tissue from infected versus non-infected animals. Among these genes, several have previously been described to be part of a general host response to infections encoding immune response related proteins. In inflamed lung tissue, genes encoding immune activating proteins and other pro-inflammatory mediators of the innate immune response were found to be up-regulated. Genes encoding different acute phase reactants were found to be differentially expressed in the liver. Conclusion: The obtained results are largely in accordance with previous studies of the mammalian immune response. Furthermore, a number of differentially expressed genes have not previously been associated with infection or are presently unidentified. Determination of their specific roles during infection may lead to a better understanding of innate immunity in pigs. Although additional work including more animals is clearly needed to elucidate host response to porcine pleuropneumonia, the results presented in this study demonstrate three subsets of genes consistently expressed at different levels depending upon infection status.

General information
State: Published
Oral administration of amniotic fluid reduces necrotizing enterocolitis in preterm pigs

General information
State: Published
Organisations: Innate Immunology, Division of Veterinary Diagnostics and Research, National Veterinary Institute, Microbial Ecology
Contributors: Siggers, J., Siggers, R., Skovgaard, K., Schmidt, M., Møller, H., Boye, M., Sangild, P.
Publication date: 2007
Peer-reviewed: No
Event: Abstract from Digestive Disease Week 2007, San Diego, CA, United States.
Source: orbit
Source-ID: 214350
Research output: Research - peer-review › Journal article – Annual report year: 2007

Validation of putative reference genes for qRT-PCR normalization in tissues and blood from pigs infected with Actinobacillus pleuropneumoniae

The quantitative real-time reverse transcriptase polymerase chain reaction (qRT-PCR) is a sensitive and very efficient technique for quantification of gene expression. However, qRT-PCR relies on accurate normalization of gene expression data, as RNA recovery and cDNA synthesis efficiency might vary from sample to sample. In the present study, six putative reference genes were validated for normalization of gene expression in three different tissues and in white blood cells from pigs experimentally infected with the common respiratory pathogen Actinobacillus pleuropneumoniae. Two dedicated validation programs (geNorm and Normfinder) were used to rank the six reference genes from best to worst. qRT-PCR data for the proinflammatory cytokine IL-6 was normalized using the proposed genes from geNorm and Normfinder as well as the commonly used reference gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH). IL-6 expression was quantified in white blood cells, liver, lymph nodes and tonsils from 10 infected pigs and 5 control pigs. After normalization using either geNorm or Normfinder IL-6 was shown to be significantly up-regulated (P <0.05) in all of the tissues from infected animals compared to non-infected control animals with a good agreement of expression differences between the two programs. On the contrary, normalization of IL-6 expression data from blood using GAPDH rendered the difference between infected and non-infected groups non-significant, and resulted in significantly different values compared to geNorm (P = 0.01). Based on these results, we recommend to validate putative reference genes before normalization.

General information
State: Published
Organisations: Innate Immunology, Division of Veterinary Diagnostics and Research, National Veterinary Institute, Bacteriology & Pathology
Contributors: Skovgaard, K., Mortensen, S., Poulsen, K., Angen, Ø., Heegaard, P. M. H.
Pages: 140-146
Publication date: 2007
Peer-reviewed: Yes
Analysis of three different dyes for cDNA labelling in oligonucleotide microarrays

General information
State: Published
Organisations: Innate Immunology, Division of Veterinary Diagnostics and Research, National Veterinary Institute, Microbial Ecology
Contributors: Mortensen, S., Skovgaard, K., Boye, M., Heegaard, P. M. H.
Publication date: 2006
Peer-reviewed: No
Event: Abstract from International Symposium on Animal Functional Genetics, Michigan, USA.
Source: orbit
Source-ID: 241214
Research output: Research › Conference abstract for conference – Annual report year: 2006

Analysis of three different fluorescent dyes for cDNA labelling in oligonucleotide microarrays

General information
State: Published
Organisations: Innate Immunology, Division of Veterinary Diagnostics and Research, National Veterinary Institute, Microbial Ecology
Contributors: Mortensen, S., Skovgaard, K., Boye, M., Heegaard, P. M. H.
Publication date: 2006
Peer-reviewed: Yes
Event: Poster session presented at Dyrlægefaglig uge, Nyborg, Denmark.
Source: orbit
Source-ID: 241744
Research output: Research › peer-review › Poster – Annual report year: 2006

Characterization of porcine host response after infection with Actinobacillus pleuropneumoniae using cDNA microarrays

General information
State: Published
Organisations: Innate Immunology, Division of Veterinary Diagnostics and Research, National Veterinary Institute, Section for Veterinary Diagnostics
Contributors: Skovgaard, K., Hedegaard, J., Mortensen, S., Sørensen, P., Jensen, T. K., Bendixen, C., Heegaard, P. M. H.
Publication date: 2006
Peer-reviewed: Yes
Event: Poster session presented at 19th International Pig Veterinary Society Congress, Copenhagen, Denmark.
Source: orbit
Source-ID: 241758
Research output: Research › peer-review › Poster – Annual report year: 2006
Differential expression of genes encoding CD30L and P-selectin in cattle with Johne's disease: Progress toward a diagnostic gene expression signature
Mycobacterium avium subspecies paratuberculosis (Mycobacterium paratuberculosis), the causative agent of paratuberculosis (paraTB) or Johne's disease in ruminants, is a health problem for the global cattle industry with significant economic losses related to decreased milk production and reduced fertility. Commonly paraTB in cattle is diagnosed by antibody detection by serum enzyme-linked immunosorbent assay (ELISA), by detection of the pathogen by cultivation of individual faecal samples, or by in vitro measurement of cell mediated immune responses using the IFN-gamma test. There is an ongoing need for developing new diagnostic approaches as all currently available diagnostic tests for paraTB may fail to detect sub-clinical infection. We used cDNA microarrays to simultaneously measure expression of over 1300 host genes to help identify a subset of gene expression changes that might provide a unique gene expression signature for paraTB infection. In the present study, non-stimulated leukocytes isolated from 10 sub-clinical paraTB infected cows were examined for genes being expressed at significantly different levels than in similar cells from control cows with the same herd background. We included cattle (Holstein) from two locations (Denmark and USA) for the microarray experiment. Our results indicate that expression profiles of at least 52 genes are different in leukocytes from M. paratuberculosis infected cattle compared to control cattle. Gene expression differences were verified by quantitative real-time reverse transcriptase polymerase chain reactions (qRT-PCR) on the same group of cattle (Holstein) used for the microarray experiment. In order to assess the generality of the observed gene expression, a second and different group of cattle (Jersey) was also examined using qRT-PCR. Out of the seven genes selected for qRT-PCR, CD30 ligand (CD30L) and P-selectin were consistently differentially expressed in freshly isolated leukocytes from paraTB infected and control animals of both breeds of cattle. Although further work is clearly needed to develop a more complete gene expression signature specific for paraTB, our results demonstrate that a subset of genes in leukocytes are consistently expressed at different levels, depending upon M. paratuberculosis infection status.
Web of Science (2016): Impact factor 1.718
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 2
Scopus rating (2015): CiteScore 1.67 SJR 0.862 SNIP 0.749
Web of Science (2015): Impact factor 1.664
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 2
Scopus rating (2014): CiteScore 1.6 SJR 0.777 SNIP 0.718
Web of Science (2014): Impact factor 1.535
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 2
Scopus rating (2013): CiteScore 1.89 SJR 0.834 SNIP 0.797
Web of Science (2013): Impact factor 1.748
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 2
Scopus rating (2012): CiteScore 2.15 SJR 0.841 SNIP 0.913
Web of Science (2012): Impact factor 1.877
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
BFI (2011): BFI-level 2
Scopus rating (2011): CiteScore 2.16 SJR 0.859 SNIP 0.995
Web of Science (2011): Impact factor 2.076
ISI indexed (2011): ISI indexed yes
Web of Science (2011): Indexed yes
BFI (2010): BFI-level 2
Scopus rating (2010): SJR 0.792 SNIP 0.948
Web of Science (2010): Impact factor 2.176
Web of Science (2010): Indexed yes
BFI (2009): BFI-level 2
Scopus rating (2009): SJR 0.784 SNIP 0.851
Web of Science (2009): Indexed yes
BFI (2008): BFI-level 2
Scopus rating (2008): SJR 0.705 SNIP 0.87
Scopus rating (2007): SJR 0.773 SNIP 0.92
Web of Science (2007): Indexed yes
Scopus rating (2006): SJR 0.791 SNIP 0.999
Web of Science (2006): Indexed yes
Scopus rating (2005): SJR 0.681 SNIP 0.925
Web of Science (2005): Indexed yes
Scopus rating (2004): SJR 0.751 SNIP 0.976
Web of Science (2004): Indexed yes
Scopus rating (2003): SJR 0.665 SNIP 0.757
Web of Science (2003): Indexed yes
Scopus rating (2002): SJR 0.578 SNIP 0.92
Web of Science (2002): Indexed yes
Scopus rating (2001): SJR 0.628 SNIP 0.862
Web of Science (2001): Indexed yes
Scopus rating (2000): SJR 0.499 SNIP 0.792
Web of Science (2000): Indexed yes
Scopus rating (1999): SJR 0.443 SNIP 0.655

Original language: English
Keywords: gene expression signature, Mycobacteria, cDNA microarray, Johne's disease, paratuberculosis
Source: orbit
Establishment of an in vitro infection model for Actinobacillus pleuropneumoniae: Characterisation of cell lines and evaluation of gene expression response

General information
State: Published
Organisations: Innate Immunology, Division of Veterinary Diagnostics and Research, National Veterinary Institute
Contributors: Mortensen, S., Petersen, C. B., Skovgaard, K., Aasted, B., Heegaard, P. M. H.
Publication date: 2006
Peer-reviewed: Yes
Event: Poster session presented at Dyrlægefaglig uge, Nyborg, Denmark.
Source: orbit
Source-ID: 241747
Research output: Research - peer-review › Poster – Annual report year: 2006

Establishment of an in vitro infection model for Actinobacillus pleuropneumoniae: Characterisation of cell lines and evaluation of gene expression response

General information
State: Published
Organisations: Innate Immunology, Division of Veterinary Diagnostics and Research, National Veterinary Institute
Contributors: Mortensen, S., Petersen, C. B., Skovgaard, K., Aasted, B., Heegaard, P. M. H.
Publication date: 2006
Peer-reviewed: Yes
Source: orbit
Source-ID: 241755
Research output: Research - peer-review › Poster – Annual report year: 2006

Molecular and functional characterization of the lung surfactant protein C (SP-C) gene in pig

General information
State: Published
Organisations: Innate Immunology, Division of Veterinary Diagnostics and Research, National Veterinary Institute, Microbial Ecology
Contributors: Cicera, S., Nygård, A., Jensen, H., Skovgaard, K., Boye, M., Fredholm, M.
Publication date: 2006
Peer-reviewed: No
Event: Abstract from 1st European Conference on Pig Genomics, Lodi, Italy.
Source: orbit
Source-ID: 241053
Research output: Research › Conference abstract for conference – Annual report year: 2006

Molecular characterization of the porcine surfactant, pulmonary-associated protein C gene
The surfactant, pulmonary-associated protein C (SFTPC) is a peptide secreted by the alveolar type II pneumocytes of the lung. We have characterized the porcine SFTPC gene at genomic, transcriptional, and protein levels. The porcine SFTPC is a single-copy gene on pig chromosome 14. Two transcripts were found in a newborn pig lung cDNA library: a full-length clone and a clone missing exon 5. cDNA sequence comparison revealed four synonymous and two nonsynonymous substitutions and in-frame insertions at the beginning of exon 5. Comparison of the SFTPC coding region between several mammals showed high levels of conservation. Northern blot studies showed lung-specific expression of the full-length SFTPC transcript, appearing in 50-day-old fetus and increasing during lung development. Both SFTPC transcripts were detected mainly in lung by real-time RT-PCR and they were significantly down-regulated in necrotic lungs of pigs infected with Actinobacillus pleuropneumoniae. Additionally, the protein levels were also decreased or absent in the necrotic tissue.

General information
State: Published
Organisations: Innate Immunology, Division of Veterinary Diagnostics and Research, National Veterinary Institute, Microbial Ecology
Contributors: Cirera, S., Nygård, A., Jensen, H., Skovgaard, K., Boye, M., Fredholm, M.
Pages: 659-668
Using pig cDNA microarrays to study the immune response and inherited differences in expression

General information
State: Published
Organisations: Innate Immunology, Division of Veterinary Diagnostics and Research, National Veterinary Institute, Section for Veterinary Diagnostics
Contributors: Hedegaard, J., Skovgaard, K., Mortensen, S., Sørensen, P., Jensen, T. K., Heegaard, P. M. H., Bendixen, C.
Publication date: 2006
Peer-reviewed: Yes
Event: Poster session presented at Norwegian School of Veterinary Science, Oslo.
Source: orbit
Research output: Research - peer-review › Poster – Annual report year: 2006

Validation of housekeeping genes for normalisation of quantitative real-time RT-PCR data for IL-6 expression in four different tissues in pigs infected with Actinobacillus pleuropneumoniae

General information
State: Published
Organisations: Innate Immunology, Division of Veterinary Diagnostics and Research, National Veterinary Institute, Bacteriology & Pathology
Contributors: Skovgaard, K., Mortensen, S., Angen, Ø., Heegaard, P. M. H.
Publication date: 2006
Peer-reviewed: Yes
Event: Poster session presented at Dyrlægefaglig uge, Nyborg, Denmark.
Source: orbit
Research output: Research - peer-review › Poster – Annual report year: 2006

Validation of housekeeping genes for normalisation of quantitative real-time RT-PCR data for IL-6 expression in four different tissues in pigs infected with Actinobacillus pleuropneumoniae

General information
State: Published
Organisations: Innate Immunology, Division of Veterinary Diagnostics and Research, National Veterinary Institute, Bacteriology & Pathology
Contributors: Skovgaard, K., Mortensen, S., Angen, Ø., Heegaard, P. M. H.
Publication date: 2006
Peer-reviewed: Yes
Source: orbit
Research output: Research - peer-review › Poster – Annual report year: 2006

Differential expression of genes encoding CD30L and P-selectin in cattle with Johne's disease: Progress toward a diagnostic gene expression signature

General information
State: Published
Organisations: Innate Immunology, Division of Veterinary Diagnostics and Research, National Veterinary Institute, Adaptive Immunology & Parasitology
Contributors: Skovgaard, K., Pudrith, C. B., Grell, S. N., Heegaard, P. M. H., Jungersen, G., Coussens, P. M.
Publication date: 2005
In vitro screening of probiotic properties of Saccharomyces cerevisiae var. boulardii and food-borne Saccharomyces cerevisiae strains

The probiotic potential of IS Saccharomyces cerevisiae strains used for production of foods or bevel-ages or isolated from such, and eight strains of Saccharomyces cerevisiae var. boulardii, was investigated. All strains included were able to withstand pH 2.5 and 0.3% Ox-all. Adhesion to the nontumorigenic porcine jejunal epithelial cell line (IPEC-J2) was investigated by incorporation of H-3-methionine into the yeast cells and use of liquid scintillation counting. Only few of the food-borne S. cerevisiae strains exhibited noteworthy adhesiveness with the strongest levels of adhesion (13.6-16.8%) recorded for two isolates from blue veined cheeses. Merely 25% of the S. cerevisiae var. boulardii strains displayed good adhesive properties (16.2-28.0%). The expression of the proinflammatory cytokine IL-1α decreased strikingly in IPEC-J2 cells exposed to a Shiga-like toxin 2e producing Escherichia coli strain when the cells were pre- and coincubated with S. cerevisiae var. boulardii even though this yeast strain was low adhesive (5.4%), suggesting that adhesion is not a mandatory prerequisite for such a probiotic effect. A strain of S. cerevisiae isolated from West African sorghum beer exerted similar effects hence indicating that food-borne strains of S. cerevisiae may possess probiotic properties in spite of low adhesiveness. 

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Johne's disease in cattle is associated with enhanced expression of genes encoding IL-5, GATA-3, tissue inhibitors of matrix metalloproteinases 1 and 2, and factors promoting apoptosis in peripheral blood mononuclear cells

Infection of ruminants with Mycobacterium avium subspecies paratuberculosis (M. para tuberculosis) leads to a chronic and often fatal granulomatous enteritis known as Johne's disease. Most infections with M. paratuberculosis occur during the first 6 months of life, and there is some evidence for transmission in utero. Once established, infections typically exist in a subclinical state for several years. Recent gene-expression profiling studies suggested the hypothesis that inherent gene-expression profiles in peripheral blood mononuclear cells (PBMCs) from M. paratuberculosis-infected cattle may be different than expression profiles in PBMCs from uninfected controls. If true, this would suggest that it is possible to identify an M. paratuberculosis infection "signature" through transcriptional profiling of peripheral immune cells. In addition, identification of groups or classes of genes showing inherently different expression in PBMCs from M. paratuberculosis-infected cattle relative to PBMCs from uninfected controls might highlight important interactions between this pathogen
and the host immune system. In this report, we describe studies aimed at testing this hypothesis. Our novel results indicate that, indeed expression profiles of at least 42 genes are inherently different in freshly isolated PBMCs from M. paratuberculosis-infected cattle when compared to similar cells from uninfected controls. Gene-expression differences observed following microarray analysis were verified and expanded upon by quantitative real-time PCR (Q-RT-PCR). Our results indicate that T cells within PBMCs from M. paratuberculosis-infected cows have adopted a predominant Th 2-like phenotype (enhanced expression of IL-5, GATA 3, and possibly IL-4 mRNA), that cells within infected cow PBMCs may exhibit tissue remodeling deficiencies through higher expression of tissue inhibitor of matrix metalloproteinase (TIMP) 1 and TIMP2 RNA and lower expression of matrix metalloproteinase (MMP) 14 RNA than similar cells from healthy controls, and that cells within the PBMC population of M. paratuberculosis-infected cows are likely poised for rapid apoptosis (upregulation of CIDE-A, Bad, TNFRI, and Fas).
Molecular characterization of the early pig response to experimental infection with Actinobacillus pleuropneumoniae using cDNA microarrays

**General information**
State: Published
Organisations: Innate Immunology, Division of Veterinary Diagnostics and Research, National Veterinary Institute, Section for Veterinary Diagnostics
Contributors: Sørensen, J., Skovgaard, K., Mortensen, S., Sørensen, P., Jensen, T. K., Heegaard, P. M. H., Bendixen, C.
Publication date: 2005
Peer-reviewed: Yes
Event: Poster session presented at 1st European Farm Animal Functional Genomics Workshop, Edinburgh, UK.
Source: orbit
Source-ID: 241760
Research output: Research - peer-review › Poster – Annual report year: 2005

What has functional genomics taught us about Johne's disease in cattle?

**General information**
State: Published
Organisations: Innate Immunology, Division of Veterinary Diagnostics and Research, National Veterinary Institute
Contributors: Coussens, P., Skovgaard, K., Heegaard, P. M. H.
Pages: 5-5
Publication date: 2005
Peer-reviewed: Yes
What has functional genomics taught us about Johne's disease in cattle?

General information
State: Published
Organisations: Innate Immunology, Division of Veterinary Diagnostics and Research, National Veterinary Institute
Contributors: Coussens, P., Skovgaard, K., Heegaard, P. M. H.
Pages: 5-5
Publication date: 2005
Peer-reviewed: Yes

Publication information
Journal: Journal of Animal Science
Volume: 83
Issue number: Suppl. 1
ISSN (Print): 0021-8812
Ratings:
BFI (2018): BFI-level 1
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 2
Scopus rating (2017): CiteScore 1.61 SJR 0.848 SNIP 0.982
Web of Science (2017): Impact factor 1.711
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 2
Scopus rating (2016): CiteScore 1.51 SJR 1.035 SNIP 1.152
Web of Science (2016): Impact factor 1.863
BFI (2015): BFI-level 2
Scopus rating (2015): CiteScore 1.56 SJR 1.307 SNIP 1.28
Web of Science (2015): Impact factor 2.014
BFI (2014): BFI-level 2
Scopus rating (2014): CiteScore 2.26 SJR 1.412 SNIP 1.397
Web of Science (2014): Impact factor 2.108
BFI (2013): BFI-level 2
Scopus rating (2013): CiteScore 2.11 SJR 1.211 SNIP 1.307
Web of Science (2013): Impact factor 1.92
ISI indexed (2013): ISI indexed yes
BFI (2012): BFI-level 2
Scopus rating (2012): CiteScore 1.71 SJR 1.421 SNIP 1.569
Web of Science (2012): Impact factor 2.093
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
Fusarium commune is a new species within the Gibberella clade identified by morphological and molecular phylogenetic data

Fusarium commune sp. nov. was isolated from soil and Pisum sativum in Denmark and several widespread locations within the northern hemisphere from diverse substrates including white pine, Douglas fir, carnation, corn, carrot, barley and soil. Fusarium commune is characterized by and distinguished from its putative sister taxon, the E oxysporum complex, in having long, slender monophialides and polyphialides when cultured in the dark. Based on the combined DNA sequence data from translation elongation factor 1alpha (EF-1alpha) and the mitochondrial small subunit ribosomal DNA (mtSSU rDNA), the 15 isolates of F commute analyzed formed a strongly supported clade closely related to but independent of the F oxysporum and Gibberella fujikuroi species complexes.

General information
State: Published
Organisations: Innate Immunology, Division of Veterinary Diagnostics and Research, National Veterinary Institute
Contributors: Skovgaard, K., O'Donnell, K., Nirenberg, H. I., Rosendal, S.
Pages: 630-636
Publication date: 2003
Peer-reviewed: Yes

Publication information
Journal: Mycologia
Volume: 95
Issue number: 4
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Ratings:
BFI (2018): BFI-level 1
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 1
Scopus rating (2017): CiteScore 2.7 SJR 1.317 SNIP 1.307
Web of Science (2017): Impact factor 2.762
Web of Science (2017): Indexed yes
Fusarium commune is a new species within the Gibberella clade identified by morphological and molecular phylogenetic data

General information
Population structure and pathogenicity of Fusarium oxysporum isolated from soil and root necrosis of pea

General information
State: Published
Organisations: Innate Immunology, Division of Veterinary Diagnostics and Research, National Veterinary Institute
Contributors: Skovgaard, K., Bødker, L., Rosendahl, S.
Publication date: 2002
Peer-reviewed: Yes
Event: Poster session presented at Seventh International Mycological Congress (IMC7), Oslo, Norway.
Source: orbit
Source-ID: 241765
Research output: Research - peer-review › Poster – Annual report year: 2002

Forty-nine strains of the Fusarium oxysporum complex were isolated from five different sample locations within two neighboring pea fields. Of these, 39 strains were isolated from soil and 10 from pea plants showing symptoms of root rot. Twenty-eight of the isolates were tested for pathogenicity towards pea. Based on percentage discoloration of the roots and stem base, the isolates were divided into three groups: seven strains were pathogenic, 14 strains were weakly pathogenic, and seven strains were non-pathogenic towards pea. To assess the genetic relatedness of all 49 strains, gene genealogies were constructed from aligned DNA sequences from part of the translation elongation factor, nitrate reductase, beta tubulin, and mitochondrial small subunit rDNA. Maximum parsimony analysis of the combined data set yielded a single most-parsimonious tree containing three strongly supported clades which may represent cryptic species. No correlation was observed between the multigene phylogeny and pathogenicity toward pea, strain geographic origin and substrate (soil or plant) from which the strains were isolated. Strains that were non-pathogenic, weakly pathogenic or pathogenic sometimes shared the same multilocus genotype. These results suggest that strains pathogenic and putatively non-pathogenic to pea are very closely related genetically. (C) 2002 Federation of European Microbiological Societies. Published by Elsevier Science B.V. All rights reserved.
Evolution of Fusarium oxysporum f. sp. vasinfectum races inferred from multigene genealogies

General information
State: Published
Organisations: Julius-Kuhn-Institut, National Center for Agricultural Utilization Research, University of Copenhagen
Contributors: Skovgaard, K., Nirenberg, H., O’Donnell, K., Rosendahl, S.
Pages: 1231-1237
Publication date: 2001
Peer-reviewed: Yes

Publication information
Comparison of variation in intra- and extracellular isozymes of Fusarium oxysporum.

General information
State: Published
Organisations: University of Copenhagen
Contributors: Skovgaard, K., Rosendahl, S.
Pages: 1077-1084
Publication date: 1998
Peer-reviewed: Yes

Publication information
Journal: Mycological Research
Volume: 102
ISSN (Print): 0953-7562
Ratings:
BFI (2018): BFI-level 1
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 1
Scopus rating (2017): CiteScore 2.63 SJR 1.134 SNIP 1.262
Web of Science (2017): Impact factor 2.571
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 1
Scopus rating (2016): CiteScore 2.46 SJR 0.946 SNIP 1.046
Web of Science (2016): Impact factor 2.184
BFI (2015): BFI-level 1
Scopus rating (2015): CiteScore 2.36 SJR 0.962 SNIP 0.969
Web of Science (2015): Impact factor 2.244
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 1
Scopus rating (2014): CiteScore 2.56 SJR 1.075 SNIP 1.103
Web of Science (2014): Impact factor 2.342
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 1
Scopus rating (2013): CiteScore 2.32 SJR 0.93 SNIP 1.105
Web of Science (2013): Impact factor 2.139
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 1
Scopus rating (2012): CiteScore 2.18 SJR 0.971 SNIP 1.304
Web of Science (2012): Impact factor 2.082
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
BFI (2011): BFI-level 1
Scopus rating (2011): CiteScore 1.42 SJR 0.961 SNIP 1.303
Web of Science (2011): Impact factor 2.809
ISI indexed (2011): ISI indexed yes
Web of Science (2011): Indexed yes
BFI (2010): BFI-level 1
Scopus rating (2010): SJR 1.089 SNIP 1.584
Web of Science (2010): Impact factor 2.259
BFI (2009): BFI-level 1
Scopus rating (2009): SJR 1.146 SNIP 1.55
Web of Science (2009): Indexed yes
BFI (2008): BFI-level 1
Optimeret sygdomsforebyggelse i slagtesvinebesætninger


Jorsal, S. E. L., Project Manager, National Veterinary Institute, Diagnostic & Development
Goecke, N. B., PhD Student, National Veterinary Institute, Virology
Larsen, L. E., Project Participant, National Veterinary Institute, Virology
Hjulsager, C. K., Project Participant, National Veterinary Institute, Virology
Skovgaard, K., Project Participant, National Veterinary Institute, Innate Immunology
Svineafgiftsfonden: DKK1,776,000.00
01/01/2015 → 31/12/2017
Collaborators: SEGES Pig Research Center
Documents:
Optimeret sygdomsforebyggelse i slagtesvinebesætninger
Project: Research
Impact of low-grade inflammation on influenza
Starbæk, S. M. R., PhD Student
Skovgaard, K., Main Supervisor
Heegaard, P. M. H., Supervisor
Jungersen, G., Supervisor
Larsen, L. E., Supervisor
Institut stipendie (DTU)
01/12/2016 → 30/11/2019
Award relations: Impact of low-grade inflammation on influenza
Project: PhD

Diagnostic methods for veterinary pathogens
Goecke, N. B., PhD Student, National Veterinary Institute
Larsen, L. E., Main Supervisor, National Veterinary Institute
Hjulsager, C. K., Supervisor, National Veterinary Institute
Skovgaard, K., Supervisor, National Veterinary Institute
Bøtner, A., Examiner, National Veterinary Institute
Salicio, S. C., Examiner
Simon, G., Examiner
Samfinansieret - Andet
15/12/2014 → 16/05/2018
Award relations: Diagnostic methods for veterinary pathogens
Project: PhD

The establishment of the microbiota in piglets
Hermann-Bank, M. L., PhD Student, National Veterinary Institute
Skovgaard, K., Main Supervisor, National Veterinary Institute
Boye, M., Supervisor, National Veterinary Institute
Mølbak, L., Supervisor, National Veterinary Institute
Schou, K. K., Examiner, National Veterinary Institute
Jacobson, M., Examiner
Nielsen, D. S., Examiner
Institut stipendie (DTU) Samf.
01/07/2010 → 01/04/2015
Award relations: The establishment of the microbiota in piglets
Project: PhD

Expression of rhabdovirus-induced fish-specific microribonucleic acids in rainbow trout (Oncorhynchus mykiss)
Bela-Ong, D., PhD Student, National Veterinary Institute
Lorenzen, N., Main Supervisor, National Veterinary Institute
Schyth, B. D., Supervisor, National Veterinary Institute
Skovgaard, K., Examiner, National Veterinary Institute
Giehm Mikkelsen, J., Examiner
Wiegertjes, G., Examiner
Institut stipendie (DTU) Samf.
01/02/2011 → 26/09/2014
Award relations: Expression of rhabdovirus-induced fish-specific microribonucleic acids in rainbow trout (Oncorhynchus mykiss)
Project: PhD

Non-coding RNA mediated gene regulation during in fluenza infection
Brogaard, L., PhD Student, National Veterinary Institute
Skovgaard, K., Main Supervisor, National Veterinary Institute
Larsen, L. E., Supervisor, National Veterinary Institute
Lorenzen, N., Examiner, National Veterinary Institute
Salicio, S. C., Examiner
Tchilian, E. Z., Examiner
Institut stipendie (DTU)
15/07/2013 → 31/01/2018
Award relations: Non-coding RNA mediated gene regulation during in fluenza infection
Project: PhD
Enkelt blodprøve eller indsendt lungevæv kan identificere hvilke mikroorganismer der ligger til grund for grisens gen-aktivitet som den udspiller sig under forskellige lunge infektioner. Det vil blive undersøgt om man ved hjælp af Luftvejsinfektioner forårsager kolossale økonomiske tab for danske og udenlandske svineproducenter. Vi vil undersøge Genetic response to lung infection: on the quest for distinctive expression signatures

**Project: Research**
**Award relations:** Nutriomics - functional foods for cloned, lean/obese pigs
**Collaborators:** Aarhus University, Chr. Hansen A/S
**01/01/2007 → 31/03/2011**
**Forskningsprojekter - Andre ministerier og styrelser:** DKK960,000.00
**Project ID:** 22023 og 22024
**Flambard, B., Project Participant, Chr. Hansen A/S**
**Heegaard, P. M. H., Project Manager, National Veterinary Institute, Division of Veterinary Diagnostics and Research**
**Boye, M., Project Manager, National Veterinary Institute, Division of Veterinary Diagnostics and Research**
**Callesen, H., Project Participant, Aarhus University**
**Valadi, H., Project Participant, Dept. of Rheumatology and Inflammation Research, University of Gothenburg**
**01/11/2013 → 31/10/2016**
**Collaborators:** Aarhus University, University of Gothenburg, Dept. of Rheumatology and Inflammation Research, University of Gothenburg
**Project: Research**

Nutriomics - functional foods for cloned, lean/obese pigs


Stagsted, J., Project Manager, Aarhus University
Hedegaard, J., Project Participant, Aarhus University
Bendixen, E., Project Participant, Aarhus University
Knudsen, K. E. B., Project Participant, Aarhus University
Bendixen, C., Project Participant, Aarhus University
Berg, P., Project Participant, Aarhus University
Vajta, G., Project Participant, Aarhus University
Purup, S., Project Participant, Aarhus University
Bertram, H., Project Participant, Aarhus University
Skovgaard, K., Project Participant, National Veterinary Institute, Division of Veterinary Diagnostics and Research
Boye, M., Project Manager, National Veterinary Institute, Division of Veterinary Diagnostics and Research
Heegaard, P. M. H., Project Manager, National Veterinary Institute, Division of Veterinary Diagnostics and Research
Flambard, B., Project Participant, Chr. Hansen A/S

**Project ID:** 22023 og 22024
**Forskningsprojekter - Andre ministerier og styrelser:** DKK960,000.00
**01/01/2007 → 31/03/2011**
**Collaborators:** Aarhus University, Chr. Hansen A/S
**Award relations:** Nutriomics - functional foods for cloned, lean/obese pigs
**Project: Research**

Genetic response to lung infection: on the quest for distinctive expression signatures

Luftevejsinfektioner forårsager kolossale økonomiske tab for danske og udenlandske svineproducenter. Vi vil undersøge grisens gen-aktivitet som den udspiller sig under forskellige lunge infektioner. Det vil blive undersøgt om man ved hjælp af en enkelt blodprøve eller i indsendt lungevæv kan identificere hvilke(n) mikroorganismer der ligger til grund for

Skovgaard, K., Project Manager, National Veterinary Institute
Heegaard, P. M. H., Project Participant, National Veterinary Institute
Tarp, K., Project Participant, National Veterinary Institute

Forskningsrådene - STVF: DKK2,340,000.00
01/01/2008 → 31/12/2010
Award relations: Genetic response to lung infection: on the quest for distinctive expression signatures
Project: Research

Activities:

CBioVikings Virus Symposium
Period: 25 Jun 2015
Kerstin Skovgaard (Invited speaker)
National Veterinary Institute
Section for Immunology and Vaccinology

Description
The pig as a large animal model for influenza

Oral presentation

Related event

CBioVikings Virus Symposium
25/06/2015 → …
Copenhagen, Denmark
Activity: Talks and presentations › Conference presentations

Sharing Advances on Large Animal Models
Period: 15 Dec 2014 → 17 Dec 2014
Kerstin Skovgaard (Invited speaker)
National Veterinary Institute
Section for Immunology and Vaccinology

Description
High-throughput gene expression analysis in pigs as model for respiratory infections
Degree of recognition: International

Related event

Sharing Advances on Large Animal Models
15/12/2014 → 17/12/2014
Munich, Germany
Activity: Talks and presentations › Conference presentations
Immunology and Infectious Diseases  
Period: 4 Sep 2014  
Kerstin Skovgaard (Invited speaker)  
National Veterinary Institute  
Section for Immunology and Vaccinology

Description
The pig as a large animal model for characterization of host-pathogen interactions

Related event
Immunology and Infectious Diseases  
03/09/2014 → 05/09/2014  
Sandbjerg, Denmark  
Activity: Talks and presentations › Conference presentations