Prevalence of bovine tuberculosis in Egyptian cattle and the standardization of the Interferon-gamma assay as an ancillary test

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Bovine tuberculosis (bTB) caused primarily by Mycobacterium bovis continues to cause significant losses in the cattle industry and is a major public health problem. Despite its worldwide application, the IFN-γ assay has not been applied in Egypt. The aim of this study was to determine the appropriate cut-off value of IFN-γ assay to complement the skin-test screening in Egypt. The relative sensitivity (Se) of PPD and antigen cocktail-based IFN-γ assays (IFN-γ-BA and IFN-γ-EC) were analyzed retrospectively, relative to bTB confirmatory tests (culture and PCR), using single cervical tuberculin (SCT) test reactors during 2011-2013. The absolute specificity (Sp) was studied using blood samples collected from cattle from one bTB-free herd. Analysis of the bTB database-generated sheets indicates the infection rate had decreased from 2009 to 2012, and then increased in 2013. The disease is concentrated in the Egyptian Nile Delta and Valley relative to elsewhere in the country. The cut-offs for IFN-γ-EC assay could be optimized to provide higher sensitivity, comparable to cut-offs for IFN-γ-BA assay. Data analysis suggests (PPDbOD > 0.1, PPDbOD - NILQD > 0.05 and PPDbOD > PPDaOD) and (ECOD - NILQD ≥ 0.1) cut-off strategies to get optimal IFN-γ-BA and IFN-γ-EC assays results respectively. To our knowledge, this is the first report describing the prevalence of bTB in cattle in Egypt and pointing out the appropriate cut-off criteria to optimize IFN-γ assay as a routine ancillary test for diagnosis of bTB in Egypt.

Evaluating cell-mediated immune response and the role of transfer factor in immunotherapy of tuberculosis

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Tuberculosis is a chronic zoonotic disease that causes major health problems on a global scale. To evaluate the potential protective efficacy of transfer factor as an immunotherapy option, cell-mediated immune response was evaluated in guinea pigs inoculated with prepared transfer factor and then infected with virulent Mycobacterium bovis.

When M. bovis was isolated from the lymph node of a tubercular cow and cultured on stonebrink media, colonies appeared within 58-72 days. Heat was used to kill bacterial antigens and new tuberculin was prepared. The heat killed M. bovis antigen was then used to immunize ten healthy animals. Ten days after immunization, transfer factor was prepared from the immunized animals according to protocol developed by Rozzo and Kirkpatrick (1992). This transfer factor was used to inoculate Group A of 12 test animals, while Group B of 12 test animals served as the control. Both groups were exposed to virulent M. bovis. Transfer factor activity was evaluated and delayed-type hypersensitivity skin reactions were determined in both groups.

The first group exhibited delayed-type hypersensitivity after 48 hours in the form of a skin reaction; erythrocytes rosette formations increased in Group A with significant differences [p<0.01] between Group A and B. The migration inhibitory factor was determined and showed significant differences [p<0.01] between Group A and Group B. The transfer factor recipient group remained healthy, while the non-recipient group appeared weak with weight loss,
loss of appetite, difficult respiration, as well as showed different sizes of tubercles in the liver, spleen and lungs.

The health of the transfer factor recipient group demonstrates that the cellular immunity of tuberculosis can be activated and transferred by transfer factor, a specific mediator to tuberculosis. This study concludes that transfer factor can be used as immunotherapy within a protective program against tuberculosis in animals.

POSTER NUMBER: 1003

Potential of the B. bovis 6-cys gene family members of as components of novel transmission blocking vaccines

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Babesia bovis, an intra-erythrocytic tick transmitted apicomplexan protozoan, responsible for bovine babesiosis has a complex life style including asexual reproduction in the mammalian host and sexual reproduction in its Rhipicephalus microplus tick vector. Currently used live attenuated vaccines target only the parasite blood stages and are unable to block transmission, thus developing B. bovis transmission blocking (TBD) vaccines remains a goal. Similarly to the related Plasmodium parasites, B. bovis differentially expresses a set of antigens during its distinct life stages, but expression of such antigens remains uncharacterized. The B. bovis genome encodes a 10 members B. bovis-6cys gene family (designated Bbo 6-cys A to J) encoding proteins containing a conserved sexual stage domain. The profile of transcription of Bbo 6-cys genes in blood stages, inside the tick midgut as well as kinetes was examined using RT-PCR and transcriptomic approaches, which demonstrated differential expression of the 6-cys genes A, B, E, H, I & J inside the tick midgut, upon termination of tick feeding. Transcriptome analysis suggests that genes A, B & E remain expressed during the kinete stage. Based on these, we began functional characterization of tick-specific antigens by disrupting the 6-cys gene E by transfection, targeting two stages inside tick, yielding the B. bovis clonal line termed 6-cys E-KO-4H. Growing of B. bovis 6-cys E-KO-4H in in vitro cultures suggests that expression of gene E is unrequired for survival in blood stages. The genotype and transmission phenotype of the 6-cys E-KO-4H clonal line is currently being investigated, and clonal lines with disruption in the 6-cys A & B genes are being generated. The data supports 6-cys proteins as suitable candidates for developing TBD vaccine candidates.

POSTER NUMBER: 1004

Immune responses to different doses of PCV2 vaccines

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Porcine Circovirus 2 (PCV2) is associated with a number of syndromes collectively referred to as porcine Circovirus-associated disease (PCVAD). PCVAD incidence has been strongly reduced by PCV2 vaccines worldwide. Because of the poor standardization of PCV2 vaccines, this study aimed (a) to correlate the inactivated virus mass content (measured by sucrose gradient analysis and UV spectroscopy) with in vivo vaccine potency, and (b) to define in vitro correlates of protection.

Using the same oil emulsion, 12 pigs were vaccinated with three different doses of inactivated, whole-virus antigen (211 to 844 ng/dose); 4 animals were injected with a commercial vaccine (positive control), and 4 other pigs were mock-vaccinated with PBS. 4 weeks later, pigs were challenged intranasally with 2x10^5 TCID_{50} of a PCV2a strain. Antibody was measured in blood and oral fluids (OF) by ELISA and a neutralization assay; PCV2 was quantified in serum by Real-time PCR for ORF2 gene. PCV2-specific cell-mediated responses were investigated by an interferon-γ release assay in whole blood, IFN-γ ELISPOT and lymphocyte proliferation (Ki-67 and BrDU incorporation assays).

All the vaccines under study but mock protected animals from PCV2 infection in terms of post challenge viremia, the lowest Ag payload performing even better than the others. A weak Ab response was observed in OF of vaccinated pigs. No correlation was observed between serum antibody titers (both ELISA and neutralizing) and protection. Instead, cell-mediated immune (CMI) parameters showed a good correlation with vaccine efficacy. In particular, the IFN-γ release assay was a useful marker for predicting protection in vaccinated pigs, in agreement with the Ki-67 and ELISPOT assays. All control pigs always tested negative in CMI assays.
Our results highlight an in vitro approach to potency testing of PCV2 vaccines towards a batch consistency policy. This may be conducive to effective control measures for veterinary vaccines, in the framework of the current 3R policies.

POSTER NUMBER: 1005

Identification of peptide motifs for equine Major Histocompatibility Complex class I molecules

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The peptide binding motif of the Equine Leukocyte Antigen ELA-A3.1 classical Major Histocompatibility Complex (MHC) class I molecule was determined by a standard immunochemical approach. Endogenously bound peptides were eluted from P-815 cells transfected with the ELA-A3.1 gene using an equine specific monoclonal antibody. The peptides were sequenced using tandem mass spectrometry and revealed a nonamer with anchoring amino acids at positions 2 (aspartic acid) and 9 (isoleucine or leucine). Using an independent method horse MHC class I expression was stabilized in RMA-S cells transfected with ELA-A3.1 by screening with a library of 15-mer peptides that overlapped by 10 amino acids and spanned the entire Immediate Early (IE) protein of Equine Herpesvirus type 1 (EHV-1). One of the IE-derived peptides (PPARDGARFGELAAS) stabilized the ELA-A3.1 MHC class I molecule. This was confirmed and the sequence further narrowed by testing with shorter peptides. The promiscuously binding nonamer SDYLELDTI also stabilized ELA-A3.1 expression. Taken together, these results point to the EHV-1 IE-derived peptide RDGARFGEL as a candidate for studies of cytotoxic T-cell immunity to EHV-1 in the horse. Other in vivo studies have shown that the IE protein contains immunogenic peptides that can induce protective immunity against this important viral pathogen.

POSTER NUMBER: 1006

Efficacy of a protective vaccine in young sheep

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Haemonchus contortus is one of the most important parasitic worms causing global production losses in the small ruminant industry. We have previously shown vaccine efficacy (70%) using with a surface larval antigen and the adjuvant, DEAE-dextran; protection was correlated with significant cellular responses in adult sheep (1). However, young animals are highly susceptible to nematode infections and successful vaccine-induced protection in these animals is often limited. This has been suggested to be due to inadequate immune responsiveness of these young animals. In the present study, we tested whether similar correlates of vaccine-induced protection would be detected in young, 3-4 months old Merino sheep as with our previous efficacious trials with adult sheep. Intradermal injections using type-1 and type-2 immune mediators were used to quantify innate responses (before vaccination) and specific anti-larval responses (after vaccination), by measuring wheal responses (hypersensitivity reactions) and by analysing cellular recruitment in skin biopsies at the sites of antigen injection. Vaccinated groups demonstrated significant differences in total IgG, cytokine and cellular (including eosinophil) responses post-vaccination but no protective efficacy was seen in young lambs. This study suggests that lack of protective efficacy in young sheep is not due to a lack of immune reactivity but that vaccination induces different qualitative or quantitative immune responses to adult animals to vaccine antigens.

Reference:
Variants in CXCL16 Gene are Associated with the Equine Arteritis Virus (EAV) Persistent Infection in the Stallion

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EAV establishes persistent infections in 30-70% of infected stallions as defined by continual shedding of virus in their semen. Flow cytometric analysis has demonstrated horses can be divided into two populations based on whether their CD3\(^+\) T lymphocyte cells are susceptible or resistant to infection with virulent Bucyrus strain of EAV in vitro. Furthermore, stallions possessing EAV susceptible CD3\(^+\) T lymphocytes are significantly more likely to become carriers than those with the resistant phenotype. A genome wide association study (GWAS) revealed the gene(s) responsible for this trait is located on chromosome 11 (ECA11) with a distribution that is consistent with a dominant mode of inheritance. Whole genome sequencing on one resistant and two susceptible horses revealed the region implicated by GWAS contained 12 non-synonymous variant nucleotides within 8 genes. Of these, only 4 were associated with CD3\(^+\) T cell EAV susceptibility and these were all located within exon 1 of the CXCL16 gene. Further testing of 240 horses demonstrated the existence of three alleles for CXCL16, two associated with susceptibility and one with resistance. Subsequent investigations conducted on 64 stallions from diverse breeds and characterized as being semen shedders or non-shedders following exposure to EAV, demonstrated a strong association between CXCL16 genotype and the ability of the virus to establish a persistent infection (P<0.000001). Recent studies have demonstrated that EAV preferentially infects both a subpopulation of T cells (CD3\(^+\)CD11a\(^+\)CD18\(^{low}\)CD49d\(^{low}\)) and a subpopulation of monocytes expressing CXCL16 (CD14\(^+\)CXCL16\(^+\)) that may play a critical role in persistent infection and semen shedding of EAV in stallions. Further characterization of these cell subpopulations is in progress.

Broad and Potent Neutralization of Simian Immunodeficiency Viruses from Chimpanzees and Gorillas by Host Receptor Antibodies and CD4 Immunoadhesins

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Broadly cross-reactive neutralizing antibodies represent powerful new tools to treat and prevent human immunodeficiency virus type 1 (HIV-1) infection. Here, we examined whether these reagents can inhibit more distantly related simian immunodeficiency viruses infecting chimpanzees (SIVcpz) and gorillas (SIVgor). Using a panel of genetically diverse infectious molecular clones of SIVcpz and SIVgor derived from fecal consensus sequences (n=12), we found that antibodies directed against the CD4 binding site (n=10), surface loop peptidoglycans (n=5) and quaternary epitopes (n=3) of the HIV-1 envelope glycoprotein (Env) generally failed to neutralize SIVcpz and SIVgor strains at concentrations of up to 10 g/ml. In contrast, antibodies directed against the membrane proximal external region (MPER) of the HIV-1 Env, immunoadhesins containing the first two immunoglobulin-like domains of human CD4 (D1D2) linked to a CCR5-mimetic sulfopeptide or a CD4-induced (CD4i) antibody, as well as mono- and bispecific antibodies directed against human CD4 and CCR5 neutralized SIVcpz and SIVgor strains with considerable breadth (>90%) and potency (geometric mean IC\(_{50}\) values: 0.8 - 8.4 nM). Importantly, the anti-receptor antibodies blocked SIVcpz and SIVgor entry in cells expressing the human and chimpanzee CD4 and CCR5 receptors, and neutralized SIVcpz infection in primary chimpanzee CD4\(^+\) T-cells with a geometric mean IC\(_{50}\) of 5.7 nM. These findings provide new insight into the mechanisms of action and protective efficacy of anti-HIV-1 neutralizing antibodies and identify candidate constructs for vector-mediated antibody gene transfer approaches aimed at combating SIVcpz infection in captive and select wild-living chimpanzee communities.

Mycobacterium tuberculosis lacking cyclopropane rings on mycolic acids is highly attenuated, but more immunogenic than BCG against human and bovine tuberculosis
Background: *Mycobacterium tuberculosis* and *Mycobacterium bovis* are major human and bovine pathogens, with a substantial health and economic burden. The best way to control such diseases is an effective vaccine. However, the only used vaccine, the attenuated *M. bovis* BCG, is only marginally effective and has little impact on disease prevalence. We previously showed that mycolic acids cyclopropanation in the mycobacterial cell wall affects pathogenicity and immunogenicity of the bacteria. We postulated a mutant totally lacking cyclopropane rings and/or oxygenated mycolates may serve as a base for an improved vaccine.

Methods: we constructed several deletion mutants of *M. tuberculosis*, including triple mutants completely defective in mycolic acids cyclopropanation and oxygenation. We then tested these mutants for acid fastness, detergent sensitivity, pathogenesis in mice, and as vaccines in a virulent *M. tuberculosis* challenge.

Results: Two mutants (MGM1990, MGM1991, lacking cyclopropane rings or cyclopropane rings and oxygenated mycolates) were found to be defective in acid fastness, detergent resistance and highly attenuated in mice. Mice infected with these strains had a more robust immune response in the lungs than those infected by wt. When one of these strains (MGM1991) was compared to BCG in a vaccination protection study, where mice were vaccinated and challenged with virulent Mtb, the bacterial burden in the lungs was 0.5 logs lower in MGM1991 vaccinated mice, compared to BCG vaccinated mice.

Conclusions: Oxygenation and cyclopropanation of the cell wall's mycolic acids are essential for pathogenesis of Mtb. Mutants lacking these genes are attenuated, but evoke more effective immune response, thus may serve as basis for an effective and safe vaccine against both human and bovine tuberculosis.
A complement-mediated cytotoxicity assay was used to assess functional serum alloantibody titres in BNP-dams, Pregsure-vaccinated dams with healthy calves, cows vaccinated with an alternative product and unvaccinated controls. Alloantibody specificity was investigated using MHC I-defined bovine cell lines and transfected mouse lines expressing the individual MHC I alleles identified from MDBK cells. All BNP-dams and 50% of Pregsure-vaccinated cows were shown to have MDBK-MHC I specific alloantibodies, which cross-reacted to varying degrees with other MHC I genotypes, with alloantibodies in BNP-dams predicted to cross-react with ~60% of calves. MHC I expression levels on different blood cell types, assessed by flow cytometry, were found correlate with levels of alloantibody-mediated damage in vitro and in vivo. Alloantibody-killed bone marrow cells were shown to express higher levels of MHC I than undamaged cells.

Overall, haematopoietic depletion in BNP was shown to depend on the titre of alloantibody produced by individual cows, its specificity for MHC I alleles expressed by the calf and the level of MHC I expression by specific cells. Thus, cellular material within potently-adjuvanted vaccines has the potential to generate alloreactivity, highlighting the need for better understanding of adjuvant activity.

POSTER NUMBER: 1012

Transient recruitment of inflammatory monocytes and early activation of lymphocytes in the draining lymph node of calves following injection of a saponin-based adjuvant

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Understanding the dynamics of skin-draining cells following injection is important for aiding vaccine design and delivery. Here, 500µg of a saponin-based adjuvant (Matrix-Q™, Novavax AB) was administered s.c. to 8 Norwegian Red dairy calves (8-9w old), resulting in a marked but transient recruitment of leukocytes to the draining lymph nodes (dLNs) 24h post injection. Recruited cells were predominately CD14+, constituting 30-40% of cells in the dLNs. The majority of CD14+ cells was CD16+, consistent with an intermediate monocyte phenotype (1, 2), and did not resemble dendritic cells or granulocytes. These cells have been described as inflammatory monocytes, associated with antigen transport to the LNs, differentiation into dendritic cells and the induction of Th1-responses (3). A significant increase of granulocytes and B-cells were demonstrated in the dLNs, whereas the relative numbers of T-cells and natural killer (NK) cells were reduced. All lymphocyte classes carried signs of activation, as CD69 was upregulated in B cells and NK cells, while CD25 was present on T- and NK cells. Most responses had approached the baseline state at termination (up to 96h). Except local oedema at the injection site and enlarged dLNs post mortem, no clinical adverse reactions were observed. Additional studies are warranted to evaluate efficacy and safety of this adjuvant in a vaccine context. Taken together, we have demonstrated that a saponin-based adjuvant induces a cellular response consistent with efficient antigen presentation in dLNs, particularly involving the recently described bovine inflammatory monocytes.

2. J. Hussen et al., PLoS. ONE. 8, e71502 (2013)

POSTER NUMBER: 1013

Functional genomics to define biomarkers of effective vaccination against Foot and Mouth Disease Virus

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High-throughput analyses allow viewing the complexity of biological processes at molecular level. Vaccines against foot-and-mouth disease virus (FMDV) are generally efficient and safe, but the immune correlates of protection induced are not precisely defined. Thus, we used functional genomics to identify a molecular signature associated with effective vaccination of cattle against FMDV. Cattle obtained from a FMVD-free zone were vaccinated with a
commercial vaccine or remained as naïve controls. Whole blood cells obtained from both groups of animals were stimulated in vitro with vaccine antigens and mRNA extracted from them. Genome-wide microarray-based transcriptional analysis evidenced 1109 differentially expressed genes, 344 overexpressed and 765 downregulated in vaccinated vs. naïve animals. Main altered biological processes were related to immunity but also to cell proliferation and trafficking. A set of vaccinated animals was challenged with live virus, and clinical evaluation evidenced protected and non-protected animals among them. Microarray-based transcriptional analysis of blood cells taken after the challenge showed 340 differentially expressed genes, including 275 over-expressed and 65 down-regulated genes in protected vs. non-protected animals. Based on the results obtained we have constructed a list of genes that can be used to screen a large population of vaccinated and non-vaccinated bovines, as well as vaccinated-and-protected and vaccinated-and-nonprotected animals to define a molecular signature of effective vaccination. Such a signature would be of enormous value as a highly cost-effective tool to evaluate new vaccines efficacy and to control and release new batches of vaccines to the field.

POSTER NUMBER: 1015

Immunogenicity of Leptospira interrogans outer membrane vesicles

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Leptospirosis, caused by Leptospira spp., is one of the most common zoonotic diseases in the US and throughout the world. Commercially available bacterins are only partially efficacious in that they protect against death, but not against disease. Protective immunity to Leptospira spp. require antibodies specific to outer surface proteins and/or adhesins of leptospires. Spirochetes produce membrane blebs or vesicles (OMVs) and OMVs have been shown to be good immunogens. In this study, we characterized leptospiral OMV components by liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis of and identified that the majority (58.1%) of proteins in the vesicles were cytoplasmic proteins (294 of 506), while 5 were extracellular proteins (0.99%), 11 were outer membrane proteins (2.17%), 14 were periplasmic proteins (2.77%), 48 were cytoplasmic/inner membrane proteins (9.49%) and 134 were unknown or having multiple locations (26.48%). Transmission electron microscopy (TEM) imaging showed OMVs are spherical bodies with a diameter of 50-200 nm. Vesicles were used to vaccinate hamsters. The results indicated that immunization with Leptospira OMVs induced significant protection against lethal challenge revealed by an enhanced humoral immune response, high survival rate and significantly reduced bacterial burden, all of which were reflected in decreased pulmonary, hepatic and renal lesions (p<0.05). To the best of our knowledge, this is the
first report showing that OMVs could be used as a novel vaccine formulation to protect hamsters against lethal challenge.

POSTER NUMBER: 1016

Effect of Bovine Viral Diarrhea Virus (BVDV) Strains on Bovine Monocyte-Derived Dendritic Cells (Mo-DC) and Mo-DC Phenotype

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Dendritic cells (DC) are important antigen presentation cells that monitor, process, and present antigen to T cells. Viruses that infect and replicate in DC can have a devastating impact on the immune system. In this study, the ability of bovine viral diarrhea virus (BVDV) to replicate and to effect the expression of MHCI, MHCII, and CD86 was measured. The adherent monocytes were isolated from PBMCs and differentiated into Mo-DC using bovine recombinant interleukin-4 (IL-4) and granulocyte-macrophage colony-stimulating factor (GM-CSF) and differentiatied to Mo-DC. Four strains of BVDV were used including the severe acute non-cytopathic (ncp) BVDV2a-1373; moderate acute ncpBVDV2a 28508-5; and an homologous virus pair [cytopathic (cp) BVDV1b-TGAC and ncpBVDV1b-TGAN]. The Cooper strain of bovine herpesvirus 1 (BHV1) was used as a control virus. Mo-DC were infected with one of the BVDV strains or BHV-1 and examined for virus replication, virus production, and the effect on MHCI, MHCII, and CD86 expression. The ability of monocytes to produce infectious virus was reduced as monocytes differentiated to Mo-DC, and was completely lost at 120 hours of maturation. Interestingly, viral RNA increased throughout the course of infection in Mo-DC, and the viral non-structural (NS5A) and envelope (E2) proteins were expressed. The ncp strains of BVDV were down-regulated while the cp strain up-regulated the expression of the MHCI, MHCII, and CD86. The study revealed that the ability of Mo-DC to produce infectious virus was reduced with its differentiation from monocytes to Mo-DC. Additionally, the study demonstrated that ncp BVDV down-regulated and cpBVDV up-regulated the expression of Mo-DC cell surface markers MHCI, MHCII, and CD86, which are important in the mounting of immune responses.

POSTER NUMBER: 1017

Evaluation of Different Vaccination Schemes by Quantification of Immunospecific Chicken Egg Yolk Antibodies

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Chickens are routinely vaccinated against a number of common avian diseases at breeding facilities before being transferred to egg-laying farms. However, vaccination programs vary greatly among different chicken farms and little is known about how different vaccination schemes potentially influence the resulting antibody titers against the individual vaccines and thus level of protection against the different viral diseases. It is well established that IgY levels in egg yolk are highly correlated with those in serum. Thus, based on IgY isolated from eggs from vaccinated hens, we investigated how different vaccination programs influence the concentration of total IgY, as well as vaccine-specific titers measured by direct ELISAs. Eggs from three different chicken farms with different vaccination programs were compared with eggs from unvaccinated SPF hens, which served as negative controls. Our preliminary data demonstrate a significant increase in total IgY levels in eggs from the vaccinated chickens. Antibodies with specificities against infectious bronchitis vaccine variants Ma5 and 4-91 could be detected in the egg yolk from all vaccinated chickens, and significantly higher concentrations of these antibodies were found in eggs from hens, which had been recently boosted. Easy access to eggs from chickens, which have received different vaccines, facilitates further investigation of potential synergistic or suppressive effects of different vaccination schemes on individual antibody responses. The results may have implications for future design of efficient poultry vaccination programs. The project is funded by the Danish National Advanced Technology Foundation (Ref. 088-2013-1)
Developing vaccines for *Fasciola hepatica* using omics technologies

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The liver fluke *Fasciola hepatica* is an economically important pathogen of livestock worldwide. Parasite control is reliant on the use of drugs, particularly triclabendazole (TCBZ), which is effective against multiple parasite stages. However, with the spread of parasites resistant to TCBZ novel control strategies, particularly vaccine development is now a major focus for *F. hepatica* research. The pursuit for a vaccine has focussed on understanding fluke biology, specifically the excretory-secretory (ES) proteins that act at the host-parasite interface and are involved in virulence and survival within the definitive host. The advancement of next generation technologies now offers an unbiased approach for the discovery of novel control strategies. Here we report the sequencing of the draft *F. hepatica* genome together with the generation of extensive transcriptome and proteomic datasets for the lifecycle stages in the mammalian host, including metacercariae, newly excysted juveniles (NEJ), juvenile and adult flukes. Analysis of this extensive dataset revealed the complexity of the ES proteins, including providing data regarding the gene structure for multi-gene families, such as the cathepsin cysteine proteases, a major component of the secretome. Furthermore, profiling of the differential expression of genes over lifecycle was also carried out, revealing stage-specific expression of several protein families. Integrated genomic-transcriptomic-proteomic analysis has also revealed potentially novel genes that show NEJ stage-specific expression. These investigations are vital to our understanding of fluke biology and can be exploited for vaccine development. In particular several proteins have been identified from this study for future vaccine development studies.

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Deletion of *relA* abrogates the capacity of *M. a. paratuberculosis* to establish a persistent infection

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Previous comparative studies in goats revealed deletion of *relA* but not *pknG* abrogates the capacity of *M. a. paratuberculosis* (*Map*) to establish a persistent infection. The immune response elicited by the mutant cleared infection. The objective of the present study was to extend the studies in calves and compare the proliferative response elicited by the *relA* deletion mutant (Δ*relA*) and *Map* using flow cytometry and quantitative reverse transcription real-time PCR (qRT-PCR). Six 3-day-old calves were divided into two groups. Three were vaccinated with Δ*relA* and 3 inoculated with wild type *Map*. The calves were challenged with *Map* 1 month later and necropsied 3 months post challenge. Three untreated calves were used as uninfected controls. Examination of tissues revealed the Δ*relA* mutant was immune eliminated. Bacterial load of *Map* was significantly reduced in the calves vaccinated with Δ*relA* and challenged with *Map* in comparison with calves inoculated and challenged with *Map*. A vigorous CD4 memory T cell response was detected at necropsy in PBMC from both infected groups. CD8 positive NK cells proliferated in the presence and absence of antigen stimulation in both treated groups but not in the uninfected group. IFN-γ, IL17, and IL22 gene expression were up-regulated with an associated increase in their transcription factors, Tbet and RORγt, in both treated groups. TGF-B, IL-10, and FoxP3 were not up-regulated, indicating no activation of regulatory T cells. The findings show that the immune response to Δ *relA* is clearly different than the response to *Map*. Further studies are needed to determine the immunological basis for this difference.

CD4+ T cells, γδ T cells and B cells are associated with non-responsiveness to vaccination in *Mycobacterium avium* subspecies *paratuberculosis* infection

Kumudika de Silva, Karren Plain, Douglas Begg, Auriol Purdie and Richard Whittington
Vaccination is one of the strategies used to control the spread of *Mycobacterium avium* subspecies *paratuberculosis* (MAP) infection in livestock. Gudair® is a widely-used vaccine in sheep and goats and is the only vaccine approved for use in sheep in Australia and New Zealand. This vaccine reduces mortality due to MAP-infection by up to 90% but some sheep remain infectious by shedding MAP in faeces, despite vaccination. In this study, using an experimental infection model in sheep, our aim was to assess differences in immune parameters between vaccinated MAP-exposed sheep in which the vaccine was effective compared to those in which it failed to protect against disease. We assessed immune parameters such as MAP-specific IFNγ, IL-10 and lymphocyte proliferative responses and serum antibody levels. At the end of the trial, 72% of non-vaccinated sheep and 24% of vaccinated sheep were infected, as defined by the detection of viable MAP in intestinal tissues when the trial was terminated at 49 weeks post exposure. There were significant differences in the proliferation of CD4⁺, B and γδ T-cells over time in vaccinated sheep in which the vaccine failed to protect against infection compared to the non-infected vaccinated sheep. There were no significant differences in the IFNγ response or serum antibody levels between the vaccinated infected and vaccinated non-infected sheep. These results emphasise the importance of lymphocyte subsets in protecting against MAP-infection, especially in vaccinated sheep, and that immune parameters other than the commonly used IFNγ and antibody tests are required when new assessing vaccines.

**Cell type-specific differences in β-glucan recognition and signalling in porcine innate immune cells**

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Vaccines often require adjuvants to trigger potent immune responses. β-glucans, the major component of the yeast cell wall, are a potential vaccine adjuvant. These carbohydrates exert their immunomodulating activities via several receptors, such as dectin-1 and complement receptor 3 (CR3). The role of these β-glucan receptors in the response of innate immune cells towards β-glucans is however still unresolved. Dectin-1 is considered as the main β-glucan receptor in mice, while recent studies in man show that CR3 is more important in β-glucan-mediated responses. This incited us to elucidate which receptor contributes to the response of innate immune cells towards particulate β-glucans in pigs. We show an important role of CR3 in β-glucan recognition, as blocking this receptor strongly reduced the phagocytosis of β-glucans and the β-glucan-induced ROS production by porcine neutrophils. Conversely, dectin-1 does not seem to play a major role in β-glucan recognition in neutrophils. However, recognition of β-glucans appears to be cell type-specific as both dectin-1 and CR3 are involved in the β-glucan-mediated responses in macrophages. Moreover, CR3 signalling through focal adhesion kinase (FAK) was indispensable for β-glucan-mediated ROS production and cytokine (TNFα, IL-1β, IL-8) production in neutrophils and macrophages, while the Syk-dependent pathway was only partly involved in these responses. We conclude that as for man, CR3 plays a cardinal role in β-glucan signalling in porcine neutrophils, while macrophages use a more diverse receptor array to detect and respond towards β-glucans. Nonetheless, FAK acts as a master switch regulating β-glucan-mediated responses in neutrophils as well as macrophages. Altogether, our results could lead to a rationale-based decision process to implement β-glucans as vaccine adjuvants.

**NanoStat™, an Oil-in-Water nanoemulsion (NE) technology for a rapid and timely response to the emergence of new infectious diseases and epidemics**

Ali Fattom¹, Vira Bitko¹, Tarek Hamouda¹, Paul Makidon², Roger Maes³, and Steve Gracon¹. NanoBio Corp., Ann Arbor, MI¹, University of Michigan², Michigan State University³  

Emerging infectious diseases including new viral infections often require a direct and rapid response. Traditional molecular approaches may be best suited for developing vaccines when urgency is not an issue. NanoStat™ technology is a potent antimicrobial adjuvant/delivery system for inactivating viruses to produce adjuvanted vaccines for intranasal or intramuscular immunization.  

We formulated RSV, HSV-2 and influenza viruses in NE and have shown that live virus can be rapidly inactivated within 30 min- 4 hours incubation. Using a NE-inactivated and formulated RSV, we demonstrated that vaccinating
cotton rats either IN or IM elicited potent immune responses and protected animals from challenge. Furthermore, antibodies elicited by NE-adjuvanted vaccine were 5-10 times more neutralizing compared to formalin-inactivated and Alum-adjuvanted RSV vaccine.

Similar results were obtained when HSV2 virus was inactivated/split, under optimized conditions, by NE. NE-HSV2 vaccine was shown to elicit protective immunity against acute and chronic infection following a live virus vaginal challenge in a guinea pig model. We applied this concept to feline Herpesvirus-1 (FHV-1) infection. A NE-FHV-1 inactivated /adjuvanted vaccine was injected 3 times, 3 weeks apart, into virus-free cats. The animals were challenged 3wks after the 3^{rd} immunization. Cats were scored for clinical symptoms for 3 weeks. All animals responded to the vaccine and were significantly protected compared to the unvaccinated control animals. These data demonstrate that NanoStat™ technology can be of significant value in development of veterinary vaccines under field conditions. A whole virus preparation can be inactivated/split via formulation in NE adjuvant and delivered instantly to the target animals, thus allowing for a rapid response to emerging epidemics.

POSTER NUMBER: 1023

Chicken interferon-inducible transmembrane (IFITM) proteins are functional and actively restrict avian viruses

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Interferon-inducible transmembrane (IFITM) proteins restrict the entry process and replication of several pathogenic viruses including SARS coronavirus, filoviruses, influenza A (IAV) and flaviviruses. These important interferon stimulated genes include IFITM1, -2, -3, and -5, that are present at a single locus in mammalian species. We have shown that the syntenic IFITM locus exists in chickens and that chicken IFITM (chIFITM) 2 and 3 are constitutively expressed in all tissues examined, whereas chIFITM1 is only expressed in the bursa of Fabricius, gastrointestinal tract, caecal tonsil, and trachea. IFITM3 is biologically functional and restricts cell infection by influenza A viruses, Infectious Bursal disease virus (IBDV) and infectious bronchitis virus (IBV) in the natural host. Furthermore, we show that knockdown of constitutive expression of IFITMs \textit{ex vivo} results in heightened infection by these viruses. Despite being highly divergent at the amino acid level, it appears that IFITMs of birds and mammals are able to restrict replication of viruses capable of infecting different host species, suggesting IFITM proteins may provide a crucial barrier for zoonotic infections. No prior characterisation of the role of chicken chIFITMs in restriction of avian viruses has been undertaken. While models animals have proved invaluable in elucidating fundamental immunological principles, the results often fail to translate target diseases in their natural hosts. In summary, elucidation of the chIFITM mediated viral restriction will prove crucial for designing new therapies for endemic and exotic, indeed zoonotic avian viruses, and provide new insights into chicken innate immunity. Poultry breeders will be able to select the IFITM allelic variants that confer enhanced levels of protection to both endemic and emerging pathogenic avian viruses into future breeding programmes.

This work was supported by grants from the Wellcome Trust (098051) & the BBSRC (BB/J016837/1 & BB/L003996/1).

POSTER NUMBER: 1024

Defining the function and role of bovine IL-17+ lymphocytes in protozoan infection

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Bovine protozoan infections such as Neospora caninum have been shown to induce strong IFN-gamma responses. These are often associated with both protective and pathological responses in the host. However the role of the novel inflammatory cytokine IL-17 is little understood in this economically important infection. In both human and murine experiment infections IL-17 has been linked with clearance of pathogens and host pathology, thus mirroring the role of IFN-gamma in bovine N. caninum infection. Using \textit{in vitro} infected macrophages to prime autologous CD4\textsuperscript{+} T-cells we found a strong relationship between IL-17 induction in CD4\textsuperscript{+} cells and reduction in cellular parasite load. These CD4 T-cells displayed hallmarks of Th17 cells, including expression of IL-23R and CCR6, as have previously been defined in other species. Derivation of gamma-delta IL-17 T-cells using established protocols showed a distinct profile to IL-17\textsuperscript{+} CD4\textsuperscript{+} T-cells, indicating a more transient nature. Nonetheless in a co-culture system IL-17\textsuperscript{+} gamma-delta T-cells were capable of inducing selective parasite death in infected autologous fibroblasts. Ultimately this was dependent on cell-cell contact suggesting a role for surface ligand/receptor interactions.

Our data to date would suggest an important role for IL-17 during the course of \textit{N. caninum} infection. Moreover the mechanism by which IL-17\textsuperscript{+} lymphocytes can specific death of parasite infected cells may provide into pathways for
future vaccination.

POSTER NUMBER: 1025

Immunopathologic Characterization of acute *Theileria parva* in Holstein calves


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East Coast fever (ECF) is a tick-borne disease of African cattle caused by the intralymphocytic apicomplexan parasite, *Theileria parva*. Infection results in cellular immortalization that is reversible with treatment. Clinical disease has a rapid onset, and over one million cattle die of ECF each year. However, the clinical disease course and pathologic changes associated with *T. parva* have been incompletely described. Lymphoproliferation and lymphocytolysis have been described, and animals succumb to ECF-induced pulmonary edema. Although infiltration of alveolar septae by transformed lymphocytes has been documented, the definitive pathogenesis of pulmonary edema in ECF has yet to be elucidated. A detailed understanding of disease pathogenesis, including a potential role of immune responses in lesion development is imperative for development of a vaccine for *T. parva*. To clarify disease pathogenesis, four Holstein calves were infected with a lethal dose of *T. parva* stabilate, and complete clinicopathologic characterization of infection performed. Clinical disease course was monitored via physical examination, lymph node cytology and complete blood counts. In all animals, lymphadenomegaly developed between days four and six post infection and *T. parva* schizonts were detected in lymph node aspirates one day later. Anorexia, dyspnea, leukopenia, and thrombocytopenia developed terminally. Postmortem gross, histologic, and immunohistochemical studies were completed on tissues from each animal. Histologic and immunohistochemical findings from this study significantly enhance the understanding of immunopathology in terminal ECF, and elucidate new avenues of investigation for vaccine development.

POSTER NUMBER: 1026

A Tale of Two Breeds: Dichotomy of Inflammasome Activation and Reactive Nitrogen Species Production

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Phagocytic cells, such as macrophages (MΦ) have the ability to kill pathogens via oxygen-dependent and independent mechanisms. The oxygen-dependent mechanisms rely on the generation of reactive oxygen and nitrogen species (ROS/RNS, respectively). ROS production has been shown to activate the inflammasome complex in MΦ leading to increased production of the pro-inflammatory cytokine Interleukin-1 (IL-1). Conversely RNS inhibits inflammasome mediated IL-1 activation, indicating a division between inflammasome activation and RNS production. In the present study MΦ from Brown Swiss (BS) cattle produce significantly more RNS and less IL-1 when compared to cells from Holstein Friesian (HF) cattle in response to bacterial or fungal stimuli. Furthermore, BS MΦ killed ingested Salmonella Typhimurium more efficiently, supporting anecdotal evidence of increased disease resistance of the breed. Inhibition of autophagy by 3-methyladenine (3-MA) stimulated IL-1 secretion in cells from both breeds, but more strongly in HF MΦ. Blocking RNS production by L-arginase completely abolished RNS production but dramatically increased IL-1 secretion in MΦ from BS. Sequence analysis of key pattern recognition receptors (TLR2 and Dectin-1) isolated from both breeds revealed polymorphisms in regions responsible for ligand binding and signal transduction indicating a mechanism for differences observed in macrophage stimulation. In addition differential expression of TLR2 on MΦ can be demonstrated by flow cytometry. Collectively these data suggest that the dichotomy of inflammasome activation and RNS production also exists for cattle and that differences observed between breeds may contain a genetic element.
Identification of CD8 cytotoxic T cell antigens of *Theileria lestoquardi*

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*Theileria lestoquardi* is a tick-borne parasite that infects blood cells of sheep and goat, resulting in high morbidity (100%) and mortality rates (46-100%). It is a major livestock disease particularly in Asia, the Middle East, and Africa. As part of an international effort to develop vaccines for ovine theileriosis, we undertook antigen screening. Five sheep were immunized for *T. lestoquardi* and used in this study. Thirteen putative *T. lestoquardi* genes, chosen based on immunogenicity of *T. parva* and *T. annulata* orthologues, were expressed in sheep fibroblasts and screened for autologous CD8 cytotoxic T lymphocyte (CTL) response by IFN-γ assays. Two genes encoding Tl8 (cysteine proteinase, 349 aa) and Tl9 (sub-telomeric ORF, 293 aa) were recognized by CTL from one of five sheep. Antigenic epitopes of Tl8 (aa position 241-252) and Tl9 (aa position 271-282) were identified through peptide scans using CTLs from the responsive sheep. Testing of different peptide sequence combinations of Tl8 241-252 and Tl9 271-282 was carried out to examine the contribution of residues to immunodominance of the epitopes. The peptide sequences identified here may provide a practical basis for rational vaccine design for *T. lestoquardi* infections.

This work is funded by EU Seventh Framework Programme for Research (FP7) grant number 245145.

Wide-range protection against avian reovirus conferred by vaccination with representatives of four defined genotypes

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Many isolates of the contagious avian reovirus have been characterized, mainly based on the sequence of their sigma C protein. These isolates have been classified into four genotypes. Currently available vaccines are of limited effectiveness, likely due to the existence of many variants. The aim of this study was to test the efficacy of a vaccine consisting of a mixture of prototypes (representatives) of the four defined genotypic groups of avian reovirus. The prototypes were selected based on their distance from the isolates within each genotype. All prototypes were found to be virulent. Antibodies produced against each of the prototypes neutralized all members of its genotype. Birds were then vaccinated with a mixture of the four prototypes. Results suggest that the 4-valent vaccine can prevent disease and confer broad protection against field isolates of avian reovirus.

Influence of Age on The Immune Response to Vaccine in Calves

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The aim of this research was to evaluate the influence of age on the immune response induced by vaccination. Were used 21 Holstein calves, which received six liters of colostrum derived from dams that were vaccinated to BoHV-1 (strain RLB103, thermosensitive, Cattle Master Gold FP5 + L5, Zoetis). Calves received 5 mL of the same vaccine
administered in the dams, and were divided into three experimental groups: prime vaccinated at 14 days (G1, n = 6), at three months (G2, n = 5) and at six months of life (G3, n = 5), and revaccinated 30 days after the first dose. Moreover, a control group was maintained with unvaccinated calves (CG1, CG2 and CG3, n = 5). Blood samples were obtained on the day of the first dose of the vaccine (M0); at 2nd dose (M1); and on the 30th day after the 2nd dose (M2). The immune response was assessed by serum neutralization and immunophenotyping of lymphocytes. GMTs of 194.1 and 112.20; 97.01 and 39.81; 27.86 and 63.17 for CG1 and G1 were obtained, respectively, at the moments M0 to M2. The CG2 and G2 presented GMTs of 6.96 and 19.95; 4.00 and 24.44 (P = 0.043); 2.30 and 6.06; 675.59 and 1.74 (P = 0.006), respectively, at the moments M0 to M2. CG3 and G3 showed GMTs of 3.03 and 12.13; 2.64 and 6.06; 675.59 and 1.74 (P = 0.006), respectively, at the moments M0 to M2. No differences were found among the ratios of lymphocytes subpopulations of CD21+, CD3+CD4+, CD3+CD8+ and CD3+WC1+, between control and vaccinated calves; however, the proportion of CD3+CD25+ cells was higher in G1 at T1 (0.016), G2 at T2, and G3 at T2 (0.032). The early vaccination of calves at 14 days stimulated the cellular immunity response, but the increase titers in neutralizing antibodies titers was obtained after the vaccination at three and six months of life.

POSTER NUMBER: 2004

Nanoparticle entrapped swine influenza virus peptides vaccine induces epitope specific T cell response in pigs

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Pigs are mixing vessels for the emergence of new swine influenza viruses (SwIV). The SwIV conserved peptides have the potential to elicit cross-protective response, but without the potent vaccine delivery system they are poorly immunogenic. Biodegradable poly(lactic-co-glycolic acid) (PLGA) nanoparticle (PLGA-NP) is a potent vaccine delivery system capable of presenting antigens to the immune system, and also possess the adjuvant property. In this study, Norovirus P particle M2e chimera and highly conserved four SwIV peptides were entrapped in PLGA-NP by double-emulsion method. Influenza antibody free 4-5 weeks old conventional pigs were vaccinated twice at two weeks interval intranasally and two weeks post-booster challenged with a SwIV (Sw/OH/24366/07) through intratracheal and intranasal routes; and 7 days later euthanized and determined the immune correlates and viral load. The PLGA-NP entrapped peptide vaccine received pigs had no fever in spite of comparable gross lung lesions compared to control virus-challenged animals. Interestingly, though the viral RNA copy numbers in BAL fluid was not significantly reduced in PLGA-NP vaccine group compared to control animals, the replicating infective virus was absent in NP vaccine received pigs. Immunologically, the difference in specific antibody, virus neutralizing and hemagglutination inhibition titers though higher in PLGA-NP vaccinated compared to control animals, the data was not statistically significant. But strikingly, the PLGA-NP vaccine received pigs had significantly increased frequencies of IFN-γ secreting CD3+CD4+CD8- cells, CD3+CD4+CD8- and CD3+CD4+CD8- cells in the lung mononuclear cells analyzed by flow cytometry. This data was consistent with the secretion of increased amounts of IFN-γ in the supernatant of in vitro stimulated lung mononuclear cells. Our data suggested that the PLGA-NP entrapped candidate SwIV peptides vaccine induced the viral epitope specific cell-mediated immune response in intranasally vaccinated pigs. This Project was supported by USDA-AFRI and OARDC, The Ohio State University to RJG and CWL.

POSTER NUMBER: 2005

Genome-based Approach to Discover Novel Anti-Cattle Tick Vaccine Antigens

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The cattle tick, *Rhipicephalus microplus*, is a livestock pest that imposes significant economic costs to farmers in tropical and semi-tropical regions of the world. We hypothesized that the tick's genome held the key to discovery of sustainable tick control technologies. Thus, we initiated the cattle tick genome sequencing project in 2003 and completed the sequencing and assembly in 2014. This project was accompanied by the concurrent application of reverse vaccinology to identify molecules for testing as candidate antigens to elicit a highly effective and protective immune response against *R. microplus* infestation in immunized animals. Vaccine discovery research efforts produced patented technology that is available for transfer and development by a commercial partner. Several
vaccine formulations have been tested in cattle, including recombinant whole and partial proteins produced in the yeast, *Pichia pastoris*, and recombinant DNA epitope vaccines.

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**POSTER NUMBER: 2006**

**Bovine gamma delta T Cells are a major regulatory T cell subset**

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A large proportion of bovine peripheral blood T cells express the gamma delta (γδ) T cell receptor. In addition, populations of γδ TCR⁺ T cells are found present within tissues such as the lung. A small subset of the γδ T cells, which express the workshop cluster 1 (WC1) scavenger receptor, have been shown to be involved in early priming events post-vaccination or following exposure to pathogens and to secrete significant levels of IFN-γ.

We present functional evidence of the role of bovine γδ T cells as potent regulatory and inhibitory lymphocytes. A subset of bovine γδ T cells spontaneously expressed IL-10 and these were induced to proliferate by contact with monocytes, lymph-migrating dendritic cells (DC) and lung DC. Proliferation of IL-10 expressing γδ T cells was also observed when DC were infected with modified vaccinia virus Ankara (MVA). The IL-10 expressing γδ T cells suppressed spontaneous expression of IL-2 and IFN-γ and induced both non-specific and antigen-specific αβ T cell anergy.

Our data suggest diverse roles for sub-populations of bovine γδ TCR⁺ T cells. While a small population of WC1⁺ γδ T cells appear immunostimulatory, we propose that γδ T cells have evolved to be a major suppressive T cell population in cattle.

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**POSTER NUMBER: 2007**

**Creating a live attenuated veterinary vaccine against schistosomiasis**

**Marina Harvie, Oliver Creagh, Najju Ranjit and Don McManus**

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Schistosomiasis is a parasitic infection caused by the trematode parasites from the genus *Schistosoma*. The disease is endemic in Africa, South America and parts of South East Asia and is a significant global problem. Praziquantel is the only effective drug treatment available however administration does not prevent re-infection and repeated dosages are required. *S. japonicum* is endemic to South East Asia and is unique in that it is a zoonosis; Buffalo and other bovines are heavily infected and act as an infection reservoir. Mathematical modelling suggests that vaccination of bovines, alongside mass drug administration and mollusicide will eliminate schistosomiasis in endemic areas.

A large proportion of bovine peripheral blood T cells express the gamma delta (γδ) T cell receptor. In addition, populations of γδ TCR⁺ T cells are found present within tissues such as the lung. A small subset of the γδ T cells, which express the workshop cluster 1 (WC1) scavenger receptor, have been shown to be involved in early priming events post-vaccination or following exposure to pathogens and to secrete significant levels of IFN-γ.

We present functional evidence of the role of bovine γδ T cells as potent regulatory and inhibitory lymphocytes. A subset of bovine γδ T cells spontaneously expressed IL-10 and these were induced to proliferate by contact with monocytes, lymph-migrating dendritic cells (DC) and lung DC. Proliferation of IL-10 expressing γδ T cells was also observed when DC were infected with modified vaccinia virus Ankara (MVA). The IL-10 expressing γδ T cells suppressed spontaneous expression of IL-2 and IFN-γ and induced both non-specific and antigen-specific αβ T cell anergy.

Our data suggest diverse roles for sub-populations of bovine γδ TCR⁺ T cells. While a small population of WC1⁺ γδ T cells appear immunostimulatory, we propose that γδ T cells have evolved to be a major suppressive T cell population in cattle.
A Novel Subunit Marker Vaccine Platform based on Whole Recombinant Yeast Kluyveromyces lactis - Full Protective Vaccination against Infectious Bursal Disease of Chickens

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Modern veterinary vaccines involve a whole catalogue of requirements. These include the necessity to rapidly develop vaccines against new emerging pathogens, the option to differentiate between vaccinated and infected animals (DIVA), a high stability and low costs.

Along these lines, we developed a novel type of subunit marker vaccines where individual viral proteins are stably expressed in the food grade yeast Kluyveromyces lactis. Following sterilization, the whole yeast material is applied for vaccination taking also advantage of the adjuvant character of K. lactis. K. lactis strains can be produced using industrial scale fermentation, and the vacuum dried material shows an extraordinary high stability. The recombinant yeast technology is broadly applicable for viral and bacterial pathogens.

We report on vaccination approaches against infectious bursal disease (IBD) of poultry using yeast strains that express defined quantities of the virus capsid forming protein VP2 of IBD virus. Full protection against a subsequent IBDV infection was achieved by subcutaneous inoculation of only milligram amounts of yeast material per chicken. Recombinant K. lactis was thus indicated as a potent tool for the induction of a protective immune response, achieving proof-of-concept as an efficacious anti-IBD subunit vaccine.

A recombinant multi-stage vaccine against paratuberculosis significantly reduces bacterial level in tissues without interference in diagnostics

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A new (FET11) recombinant vaccine against paratuberculosis was developed based on recombinant antigens from acute and latent stages of Mycobacterium avium subsp. paratuberculosis (Map) infection.

In two experiments 28 calves and 15 goats were orally inoculated with live Map in their third week of life and post-exposure vaccinated at different times after inoculation or with different vaccine constructs. In contrast to common whole-cells vaccination, the FET11 vaccine did not interfere with tests for paratuberculosis or bovine tuberculosis as no measurable antibody responses by ID Screen® ELISA, PPDj-specific IFN-γ responses or positive PPDa or PPDb skin tests developed in vaccinees. Antibodies and cell-mediated immune responses were developed against FET11 antigens, however. At necropsy 8 or 12 months of age, relative Map burden was determined in a number of gut tissues by quantitative IS900 PCR and revealed significantly reduced levels of Map and reduced histopathology. Diagnostic tests for antibody responses and cell-mediated immune responses, used as surrogates of infection, corroborated the observed vaccine efficacy: Five of seven non-vaccinated calves seroconverted in ID Screen® ELISA at 32 to 40 weeks p.i. indicating the progression of infection, while only four of 14 FET11 vaccinated calves seroconverted at 40-52 weeks p.i. Similarly, PPDj-induced IFN-γ responses increased over time in non-vaccinated calves, while FET11 vaccinated calves had significantly reduced PPDj IFN-γ assay responses from 40 to 52 weeks compared to non-vaccinated calves. These results indicate the FET11 vaccine can be used to accelerate eradication of paratuberculosis while surveillance or test-and-manage control programs for tuberculosis and Johne’s disease remain in place.

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Porcine chlamydia infections - new risks and possibilities

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Chlamydiaceae are obligate intracellular Gram-negative bacteria. Human C. trachomatis infections are often non-symptomatic but can also cause serious diseases including infertility (genital infections) and trachoma (ocular infections), the leading cause of preventable blindness worldwide. A vaccine against C. trachomatis is currently not
available but urgently needed. In addition, prevalence of the porcine chlamydia species, *Chlamydia suis*, is very high in pigs. *C. suis* is highly related to *C. trachomatis* and was recently detected in the eyes of slaughterhouse employees raising the question about the zoonotic potential of *C. suis*. Hence, analysing the host-pathogen interactions in pigs serves not only as a relevant model for *C. trachomatis* infections but also facilitates vaccine design against *C. suis* infections in pigs with the potential to improve animal welfare and to reduce the zoonotic risk according to the One Health initiative.

Therefore, the aim of this project is to establish and improve the pig model for studying ocular and genital *C. suis* and *C. trachomatis* infections and the resulting immune responses using state-to-the art techniques like multi-colour flow cytometry, confocal microscopy and RT-qPCR. As a first step, we used a porcine ocular cell line (VIDO R1) and primary porcine genital tract epithelial cells (GTECs) to compare the innate immune response of these cells after chlamydial infections. We compared the life cycle of *C. suis* with *C. trachomatis* and found several similarities (time course) of both pathogens but also interesting differences (e.g. shape of chlamydial inclusions). The porcine innate immune response mimicked the human immune response with an increased expression of IL1α, IL6, TNFα, TLR9, and MMP9. Future studies will address the adaptive immune response against chlamydial infections with a focus on the CD4+ T-cell immune response.

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**POSTER NUMBER: 2010**

**ELISpot Detection of FMDV-Specific Antibody Secreting Bovine B Cells During an Acute Virus Infection and Following Vaccination.**

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Foot-and-mouth disease virus (FMDV) causes a highly contagious disease of cloven-hoofed animals that has the potential to cause severe economic losses. Current vaccines confer protection remarkably early, after 7-10 days post vaccination. To track the immune response to vaccination, we have developed an ELISpot assay capable of detecting FMDV-specific antibody-secreting B cells (ASC) early in disease progression. This ELISpot assay also has the capacity to characterize the Ig isotype secreted by the FMDV-specific ASCs in peripheral blood. In addition, ASCs isolated from blood and lymph nodes proximal to the infection site have been characterized during FMDV disease progression. We also tested ASCs from cattle vaccinated with the recently licensed human Adenovirus 5 vectored vaccine for FMD by multiple routes of inoculation. Data presented here show the kinetics of anti-FMDV antibody secretion by peripheral blood B cells separated by Ig isotype in the response to FMDV. During infection and recovery, we detected both IgM and IgG, but not IgA isotypes as early as 3 days post infection. Different kinetics were observed following vaccination. The sensitivity of this ELISpot assay allows tracking of virus specific B cells revealing very early and rapid antibody responses to FMDV. These data indicate this technology will enhance understanding of the B cell response, thereby informing more effective vaccine design and development.

The work was supported by funding from the USDA, Agricultural Research Service, CRIS # 1940-32000-057-00D (WTG and JA) and an Inter-Agency Agreement between US Department of Homeland Security and USDA HSHQDC-09-X-00373 (WTG).

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**POSTER NUMBER: 2011**

**Increases of cells expressing PD-1 and PD-L1 in bovine leukemia virus infection and enhancement of anti-viral immune responses in vitro via its blockade**

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The inhibitory receptor programmed death-1 (PD-1) and its ligand, programmed death-ligand 1 (PD-L1) are involved in immune evasion mechanisms for several pathogens causing chronic infections. Blockade of the PD-1/PD-L1 pathway restores anti-virus immune responses, with concomitant reduction in viral load. To investigate the roles of bovine PD-1 and PD-L1, we analyzed the expression levels of PD-1 and PD-L1 in bovine leukemia virus (BLV) infection. The proportion of PD-L1 positive cells, especially among B cells, was upregulated in cattle with the late stage of the disease compared to cattle at the aleukemic infection stage or uninfected cattle. The proportion of PD-L1 positive cells correlated positively with prediction markers for the progression of the disease such as leukocyte number, virus load and virus titer whilst on the contrary, it inversely correlated with the degree of interferon-gamma expression. Blockade of the PD-1/PD-L1 pathway in vitro by specific antibodies upregulated the...
production of interleukin-2 and interferon-gamma, and correspondingly, downregulated the BLV provirus load and the proportion of BLV-gp51 expressing cells. These data suggest that PD-1/PD-L1 pathway induce immunoinhibition in disease progressed cattle during chronic BLV infection. Therefore, PD-L1 would be a potential target for developing immunotherapies against BLV infection.

POSTER NUMBER: 2029

Combining Immunizations with Routine Aerosol-based Vaccinations of Chickens
- A Refined Approach to Antibody Production

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The classical production methods for polyclonal antibodies involve multiple injections and blood samplings from rabbits or larger mammals in order to obtain sufficient amounts of antigen-specific antisera. We now consider a new approach to immunization, which makes the stressful and time consuming procedures of conventional production methods obsolete by combining immunizations with routine non-invasive vaccinations of chickens. Chickens present an attractive alternative to mammalian species used for the production of polyclonal antibodies with regards to both animal welfare and commercial interests. This is mainly due to the fact that ample amounts of antiserum may be isolated from the egg yolk, presenting a cost efficient and non-invasive source of polyclonal antibodies.

Chickens used for egg production in Europe are routinely vaccinated against a number of different pathogens through exposure to aerosolized live vaccines. By piggy-backing on the capsid of e.g. infectious bronchitis virus, we hypothesize that an immunogen will be able to pass the physical barriers of the airways and stimulate the adaptive immune system. As a result, vaccinated chickens will raise antibodies towards not only the vaccine virus, but also the virus-coupled immunogen, allowing the purification of immunogen-specific antibodies from the eggs of vaccinated chickens.

Exploiting routine aerosol-based vaccination of chickens for antigen administration is a refinement of conventional immunization procedures with potential impact on both animal welfare aspects and costs associated with commercial polyclonal antibody production.

Preliminary results from a proof-of-concept study will be presented.

POSTER NUMBER: 2012

Chicken IFNλ as an adjuvant

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Interferons (IFN) provide a critical first line of defence against viral infection in vertebrates. Moreover, the IFNλs, a recently identified group of mammalian IFN, has demonstrated antiviral potential in the treatment of viruses. With the growing concern over diseases, such as avian influenza, there is a pressing need for new antiviral strategies and the use of immune molecules, such as IFNλ, provides an attractive option for treating poultry by augmenting the host response to virus. With this in mind, we cloned and expressed the chicken orthologue of IFNλ (ChIFNλ) and assessed the biological activity and anti-viral potential of this avian cytokine. Analysis of the expressed recombinant ChIFNλ showed that, similar to the observations with mammalian IFNλ, ChIFNλ has viral inhibitory properties similar to that observed for type 1 IFN. Additionally, although ChIFNλ did show activity similar to type 1 IFN, ChIFNλ could be distinguished from type 1 IFN as it had dissimilar induction properties and was associated with the induction of different genes. Furthermore, we have also used ChIFNλ in in ovo and in vivo trials and have shown that ChIFNλ also has the capacity to augment vaccine responses. We were able to demonstrate that ChIFNλ was able to increase responses to CAV -5 fold and 10 fold for the primary and secondary immunization, respectively. The observed antiviral activity demonstrated by ChIFNλ supports its potential inclusion in therapeutic strategies directed against viral infections.
Lymphocyte responses depend on *Staphylococcus aureus* toxin production

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*Staphylococcus aureus*, a causative agent of mastitis, has no efficacious vaccine in the market. *S. aureus* vaccines eliciting antibody-based responses have had low success. An alternative way to induce immunity is to activate cellular components, such as mucosal immunity mediating IL-17 producing T helper (Th) 17 cells. We propose that decreasing toxin production of live *S. aureus* (LSA) by irradiation could increase cellular immune responses to vaccination. To test this, dendritic cells (DC) were loaded with irradiated (ISA) or LSA, and co-cultured with lymphocytes to measure cytokine mRNA levels. ISA induced lower levels of IL-4, and IFNγ mRNA transcripts compared to LSA, indicating decreased response from Th2 cells and Th1 cells respectively. ISA does not produce toxins, but still induces IL-17 responses at higher level than IL-4 and IFNγ. Based on this data, ISA has the advantage of stimulating cytokine response from Th17 cells while not stimulating the Th1 IFNγ production, which can negatively regulate IL-23 induced Th17 formation. This implicates structural components as potential vaccine antigens. To assess memory cell formation in response to the DC presented LSA and ISA, cells from dairy cows with no history (naïve) or with history (memory) of clinical *S. aureus* mastitis were activated in DC-lymphocyte co-cultures. Stimulation with ISA did not increase the number of CD8+ effector memory cells like the LSA did in naïve and memory animal cell cultures. The ability of LSA to produce toxins compared to ISA could cause the CD8+ effector memory cell formation. These data indicate that *S. aureus* structural components can favor Th17 polarized immune responses and secreted components favor CD8+ effector memory cells. Based on this, inclusion of structural and secreted components of *S. aureus* in vaccine design affects the resulting immune response as well as the type of memory cells produced.

Targeting NKT cells to enhance immunity: a novel swine model

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Natural killer T (NKT) cells are a lymphocyte population capable of potently stimulating innate and adaptive immune responses. They can be activated using therapeutic glycolipid antigens for multiple purposes, including as powerful adjuvants to improve vaccine responses against various infectious diseases. We hypothesize that NKT cell antigens have exciting potential for preventing microbial infections in NKT cell-expressing livestock species, including swine. Thus, our objective was to survey NKT cell frequencies and function amongst commercial pigs and to determine if porcine NKT cells can be therapeutically activated to enhance immunity against foreign antigens. For this, immune tissues from 85 pigs of mixed genetics were compared for NKT cell characteristics measured. Significant variation was found between individual pigs for all NKT cell characteristics measured. However, piglets within litters contained similar frequencies of NKT cells compared to piglets between litters, suggesting that the variation is partially due to genetic effects. All three agonists elicited HEL-specific cellular and humoral immune responses of varying quality, but only αGC increased the systemic concentration of NKT cells. For the first time, important information was generated about pig adjuvant responses to different varieties and dosage levels of NKT cell antigens, which demonstrates how NKT cells could be harnessed to protect swine from dangerous pathogens.

Supported by University of Florida’s Research Opportunity Seed Fund and IFAS Early Career Seed Fund awards. NIH Tetramer Core Facility provided OCH, C-glycoside and CD1d tetramers.

Immunogenetic analyses of swine responses to viral diseases

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Our goal is to understand genomic control of anti-viral disease responses focusing on the economically most important diseases of pigs: porcine reproductive and respiratory syndrome (PRRS) and porcine circovirus (PCV2). The PRRS Host Genetics Consortium (PHGC) was established to assess the role of genetics in determining pig resistance/ susceptibility to PRRS virus (PRRSV) infection, disease pathology and associated growth effects. We utilized a nursery pig PRRSV infection model with deep sampling for phenotypic analyses, extensive genotyping (60K SNPchip) and a shared database [http://www.animalgenome.org/lunney/](http://www.animalgenome.org/lunney/). We have completed 15 trials each using ~200 PRRSV-infected pigs. A genomic region on swine chromosome 4 (SSC4) was identified that is significantly associated with viral load (15%) and growth (11%) response following challenge with each of 2 different PRRSV isolates. The most recent trials involve complex challenges (PRRSV and PCV2) combined with PRRS vaccination, as well as field trials; each comparing pigs with different SSC4 genotypes. To address disease resistance mechanisms we probed serum protein expression (antibody and cytokine) and whole blood transcriptome (using microarrays and RNAseq) of PHGC pigs. We have verified proteins and genes that are differentially expressed in response to PRRSV infection and are probing this data for alternate control and regulatory networks. This data will help us identify new resistance pathways that may be used for new vaccines and biotherapeutics. Support: US National Pork Board, USDA ARS and NIFA, Genome Canada, Genome Alberta, pig breeding companies.

**POSTER NUMBER: 2017**

**Expression of PD-L1 on dog tumor cells and enhancement of IFN-γ production from tumor-infiltrating cells by PD-L1 blockade**

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An immunoinhibitory receptor, programmed death 1 (PD-1), and its ligand, programmed death ligand 1 (PD-L1), together induces the “exhausted” status in antigen-specific T cells and thus these molecules are involved in the immune evasion of some pathogens. In some chronic infections and tumor diseases, PD-L1 is expressed on the infected cells or tumor cells and it suppresses antigen-specific host immune responses. In order to develop a novel immunotherapy against chronic infections and tumors of dogs and cats, we chose tumors as a model disease and investigated the role of the PD-1 pathway in dogs.

At first, the canine PD-1 and PD-L1 genes were identified and the recombinant canine PD-1 and PD-L1 proteins were constructed. The binding of canine PD-1 with PD-L1 was observed by using these recombinant proteins. Importantly, anti-PD-L1 monoclonal antibody effectively blocked the binding of PD-1 with PD-L1 in a dose-dependent manner. Dog melanoma, mastocytoma, renal cell carcinoma and other types of tumors examined in this study expressed PD-L1, whereas some did not. Interestingly, anti-PD-L1 antibody treatment enhanced IFN-γ production from tumor-infiltrating cells. These results suggest that the canine PD-1/PD-L1 pathway is associated with the T cell exhaustion in dog tumors and that its blockade with antibody could be a new therapeutic strategy for dog tumors. Further investigations are needed to confirm the ability of anti-PD-L1 antibody to reactivate dog anti-tumor immunity in vivo, and its potential for the application to treatments of chronic infections in dogs has to be further discussed.

**POSTER NUMBER: 2018**

**Recombinant Newcastle Disease Viruses expressing swine and avian influenza hemagglutinins induce protective antibody responses in pigs and chickens**

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Newcastle Disease Virus (NDV) is an important avian pathogen. Currently available attenuated NDV strains are safe and induce protective immunity in poultry. The NDV genome (negative stranded, non-segmented RNA) can be
manipulated by reverse genetics to express additional genes. In mammals NDV replicates poorly and induces strong innate immune responses, resulting in high immunogenicity and increased safety. In addition, mammals generally have no pre-existing immunity against NDV.

We describe the generation of recombinant NDVs (La Sota strain) expressing the hemagglutinin from swine H3N2 and from avian H5N1 and H7N9 influenza viruses and the evaluation of immunogenicity and protection in pigs and chickens.

In swine, a recombinant NDV expressing the full length HA protein from A/swine/Texas/4199-2/98 (H3N2) induced specific anti-H3 antibodies and partial protection against challenge with the homologous strain. An improved NDV-swH3 vaccine candidate was generated by using a codon optimized HA sequence and by replacing the transmembrane and cytoplasmic regions by those of the NDV F protein (chimeric HA version), which resulted in enhanced expression levels. The optimized recombinant NDV-swH3 induced higher antibody titers and complete protection against challenge.

In poultry, we prepared recombinant NDVs expressing chimeric versions of the HA from a recent strain of highly pathogenic H5N1 avian influenza (clade 2.1) and from the H7N9 lineage recently emerged in China (A/Anhui/1/2013). Both recombinant NDVs expressed high levels of the recombinant HA protein and induced specific antibodies and complete protection against homologous challenge in chickens.

Our results show that recombinant NDVs expressing viral glycoproteins can induce strong immune responses in relevant livestock species that can be improved by strategies such as the use of codon optimized sequences and chimeric constructs. Therefore, recombinant NDVs are a promising platform for vaccine development against avian and mammalian veterinary pathogens.

Work partially funded by NIH contract HHSN266200700005C and DHS Grant 2010-ST-AG0001.

POSTER NUMBER: 2019

Vaccination with peptides of *Mycobacterium avium* subsp. *paratuberculosis* (MAP) reduces MAP burden of infected goats

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*Mycobacterium avium* subsp. *paratuberculosis* (Map) is the cause of paratuberculosis, a chronic enteritis of ruminants that is widespread worldwide. We investigated the effect of post-exposure vaccination with Map specific peptides in a goat model aiming at developing a Map vaccine that will neither interfere with diagnosis of paratuberculosis nor bovine tuberculosis.

Peptides were initially selected by two strategies 1) in silico selection of unique Map peptides (compared to other Mycobacteria) (n =51) and with predicted binding to 5 known bovine MHC class II molecules or 2) hydrophobic peptides unique to Map from selected proteins (n =68).

For vaccination, 23 MAP peptides (20 µg each) were selected and formulated with Montanide ISA 61 VG adjuvant. At age three weeks 10 goats were orally inoculated with 4x10E9 live Map and assigned to two groups of 5 goats each: 5 vaccinated (V) at 14 and 18 weeks post inoculation (PI) and 5 unvaccinated (C). At termination 32 weeks PI, Map burdens in 15 intestinal tissues and lymph nodes were determined by IS900 qPCR.

Of the 75 tissue samples from the 5 C goats only 5 samples were IS900 qPCR negative. In contrast, only 9 samples in total from 5 V goats were IS900 qPCR negative. All V goats responded with strong IFN-γ responses to peptides after vaccination while C goats were unresponsive. IFN-γ responses to PPDj were low in all goats at all times, except for one V goat that responded from 26 weeks PI and onwards. A single goat in the C group seroconverted in ID Screen ELISA at last sampling.

The results indicate that a peptide vaccine against Map can induce a protective immune response against paratuberculosis.

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POSTER NUMBER: 2020

Detection of Immunological Responses to BTB in African Wildlife

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Tuberculosis, caused by members of the *Mycobacterium tuberculosis* complex (*M. bovis, M. tuberculosis, M.*
**Evolutionary Characterization of Pig Interferon-inducible Transmembrane Gene Family and Member Expression Dynamics in Tracheobronchial Lymph Nodes of Pigs Infected with Swine Respiratory Disease Viruses**

Laura C. Miller, Zhihua Jiang, Yongming Sang, Gregory P. Harhay and Kelly M. Lager

Studies have found that a cluster of duplicated gene loci encoding the interferon-inducible transmembrane proteins (IFITMs) family have antiviral activity against several viruses, including influenza A virus. The gene family has 5 and 7 members in humans and mice, respectively. Here, we confirm the current annotation of pig IFITM1, IFITM2, IFITM3, IFITM5, IFITM1L1 and IFITM1L4, manually annotated IFITM1L2, IFITM1L3, IFITM5L, IFITM3L1 and IFITM3L2, and provide expressed sequence tag (EST) and/or mRNA evidence, not contained with the NCBI Reference Sequence database (RefSeq), for the existence of IFITM6, IFITM7 and a new IFITM1-like (IFITM1LN) gene in pigs. Phylogenetic analyses showed seven porcine IFITM genes with highly conserved human/mouse orthologs known to have anti-viral activity. Digital Gene Expression Tag Profiling (DGETP) of swine tracheobronchial lymph nodes (TBLN) of pigs infected with swine influenza virus (SIV), porcine pseudorabies virus, porcine reproductive and respiratory syndrome virus or porcine circovirus type 2 over 14 days post-inoculation (dpi) showed that gene expression abundance differs dramatically among pig IFITM family members, ranging from 0 to over 3,000 tags per million. In particular, SIV up-regulated IFITM1 by 5.9 fold at 3 dpi. Bayesian framework further identified pig IFITM1 and IFITM3 as differentially expressed genes in the overall transcriptome analysis. In addition to being a component of protein complexes involved in homotypic adhesion, the IFITM1 is also associated with pathways related to regulation of cell proliferation and IFITM3 is involved in immune responses.

**Innate Immune Recognition Pathways and Intracellular Signaling Triggered by the Protozoan Parasite Neospora caninum**

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Due to the high prevalence and economic importance of neosporosis, the development of safe and effective vaccines against *Neospora caninum* has been a priority in the field and it is crucial to limit vertical and horizontal transmission in natural hosts. The mechanisms underlying host resistance against this pathogen remains unclear and it has been the subject of intense study by our group. To unravel the initial host-parasite interactions, we have worked on the role of pathogen recognition receptors (PRRs) during innate immune recognition of *N. caninum*, with special emphasis on the triggered signaling pathways and effector molecules, as reactive oxygen species (ROS) and nitric oxide, using distinct experimental approaches that mimic natural exposure to the parasite, as oral infections.
and vertical transmission models in mice. Our in vivo and in vitro experiments demonstrate that PRRs are associated to the activation and evasion of host immune responses against *N. caninum* through production or inhibition of pro-inflammatory cytokines and effector molecules. Also, we have observed that this parasite manipulates pathways dependent on adaptor molecules and intracellular kinases in its favor, in order to downregulate the host’s innate immune responses. Taken together, these data may be useful for the development of prophylactic and therapeutic protocols against neosporosis.

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POSTER NUMBER: 2022

**Characteristics of the adaptive immune response to porcine epidemic diarrhea virus infection in pregnant sows**

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Porcine epidemic diarrhea virus (PEDV) emerged in the United States in 2013 and 16 months later had caused severe disease outbreaks in nearly 50% of all sow herds. Given the urgent need to develop diagnostic tools and immune countermeasures against PEDV, purified proteins were generated and used to characterize systemic, intestinal and mammary antibody responses in infected and recovering sows, and transfer of lactogenic immunity to piglets. The expected results of anti-PEDV immunity were based on results from a previously established cholera toxin model in which oral exposure was required for specific secretory IgA secretion into the intestinal lumen from B cells in the lamina propria. Because vaccination is not available for PEDV, live virus is routinely fed orally to induce protective immunity. Feedback-induced infection is characterized by viral shedding in feces regardless of the presence or absence of clinical signs. Sero-conversion is evident at 3 to 4 weeks after exposure, and shedding is resolved within 4 weeks. Oral infection of sows results in short-lived IgG anti-nucleocapsid antibody responses in serum, and substantial levels of anti-N IgG and IgA in colostrum, resulting in anti-N antibodies in piglet serum. Lactogenic antibodies were exclusively of the IgA isotype and were directed primarily against PEDV outer membrane proteins, consistent with findings from other enteric viral infections in which protection is dependent on IgA antibodies with neutralizing activity. The findings provide a solid foundation for a detailed characterization of protective immune mechanisms against PEDV.

POSTER NUMBER: 2023

**Analyzing the adverse side-effects of the Influenza A/H1N1 vaccine in health care staff in selected provinces of Afghanistan**

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In 2010 there was widespread public mistrust of the Influenza A/H1N1 vaccine in Afghanistan. To encourage vaccination, the Ministry of Public Health undertook a study to analyze the risks involved in vaccination. The purpose of this study was to demonstrate to the public that the adverse side-effects did not pose any serious health threat.

A total of 27,100 health care workers received the A/H1N1 monovalent vaccine in the four provinces of Kabul, Nangarhar, Balkh and Herat. OpenEpi was used to draw a randomized sample of 417 people for participation in this study, which was conducted over 4 weeks in March-April 2010. Interviewers were trained in questionnaire administration and assigned districts in the four regions. 370 people interviewed were included in the final study: 25% were female, 75% male; mean age was 36 years, and range was from 16-65. Standardization of injection procedure was ensured by using best practices of injection safety for vaccination.

53% of those interviewed reported pain at the injection site, 40% reported fever in the first three days after immunization, 39% reported body pain, 33% reported tiredness, 29% reported swelling at the injection site and 28% reported redness at the injection site. More females than males suffered adverse reactions; the rates varied across provinces, ranging from 79% of females in Balkh reporting adverse side-effects to 23% in Kabul.

While the results demonstrated that a high percentage of vaccine recipients experienced adverse side-effects, all were mild, non-life threatening and resolved within a few days. No serious lasting side-effects were reported. The results of this study were shared with over 20 governmental and non-governmental stakeholder institutions and
publicized through media interviews and announcements. As a result, reports from vaccination teams showed daily increases in the number of people vaccinated to the point at which Afghanistan suffered from a vaccine shortage.

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POSTER NUMBER: 2024

Interaction between vitamin D and IFN-γ pathways modulate bovine monocyte host defense responses.

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IFN-γ is a potent activator of bovine macrophage anti-mycobacterial activity and development of an IFN-γ response is correlated with protection against bacterial infection in cattle. Recently, TLR activation of an intracrine vitamin D pathway was shown to induce nitric oxide and β-defensin host defense responses in bovine monocytes; suggesting the hypothesis that actions of IFN-γ on bovine monocytes are mediated in part through the vitamin D pathway. The objective of this study was to investigate the interactions between the IFN-γ and vitamin D pathways on nitric oxide and β-defensin responses in bovine monocytes. Peripheral blood monocytes in three separate experiments were cultured for 12 h with IFN-γ (0-10 ng/mL) alone or in combination with 25-hydroxyvitamin D₃ (25D; 75 ng/mL) or 1,25-dihydroxyvitamin D₃ (1,25D; 4 ng/mL), and vitamin D pathway-related genes were evaluated by qPCR. Similar to TLR stimulation, IFN-γ induced a >20-fold increase in expression of CYP27B1, the gene that encodes the enzyme for conversion of 25D to 1,25D, and inhibited 1,25D-induced expression of CYP24A1, the gene encoding the vitamin D catabolic enzyme. IFN-γ induced iNOS gene expression also was 5-fold greater if monocytes were stimulated in the presence of 25D or 1,25D. In contrast, IFN-γ suppressed the β-defensin genes BNBD3, BNBD6, BNBD7, and BNBD10, but expression of each gene was at least 4-fold greater if monocytes were stimulated in the presence of 25D or 1,25D. In conclusion, IFN-γ activates the vitamin D pathway in bovine monocytes, and the IFN-γ and vitamin D pathways synergistically promote iNOS expression. Also, adequate 25D is necessary to prevent down-regulation of monocyte β-defensin expression by IFN-γ. These results suggest the critical actions of IFN-γ in bacterial defense mechanisms are compromised under conditions of vitamin D insufficiency in cattle.

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POSTER NUMBER: 2025

Identification and characterization of bovine programmed death-ligand 2 (PD-L2)

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BACKGROUND: In previous reports, we had indicated that the immunoinhibitory receptor programmed death (PD)-1 and its ligand, PD-L1 are involved in immune evasion mechanism of bovine chronic infection. However, no functional analysis of bovine PD-L2 has been reported in cattle. Thus, in this study, the molecular function of bovine PD-L2 was analyzed in vitro.

MATERIALS AND METHODS: Recombinant PD-L2 (PD-L2-Ig), which comprised an extracellular domain of bovine PD-L2 fused to the Fc portion of rabbit IgG1, was prepared based on the cloned cDNA sequence for bovine PD-L2. Flow cytometry analysis was used to confirm its binding to bovine PD-1 expression cells. Peripheral blood mononuclear cells (PBMCs) from cattle were cultured in the presence of PD-L2-Ig, and cell proliferation and production of IFN- in supernatants were measured to investigate the effect of PD-L2-Ig on bovine PBMCs.

RESULTS: Bovine PD-L2-Ig bound to bovine PD-1-expressing cells, and the addition of soluble bovine PD-1-Ig clearly inhibited the binding of PD-L2-Ig to membrane PD-1 in a dose-dependent manner. Cell proliferation and IFN-production were significantly enhanced in the presence of PD-L2-Ig in bovine PBMCs. Moreover, PD-L2-Ig significantly enhanced IFN- production from virus envelope peptides-stimulated PBMCs derived from bovine leukemia virus-infected cattle. Interestingly, PD-L2-Ig-induced IFN- production was further enhanced by treatment with anti-bovine PD-1 antibody.

CONCLUSIONS: These data suggest potential applications of bovine PD-L2-Ig as a therapy for bovine diseases.
A novel strategy for immunotherapy of paratuberculosis in cattle: regulation of T-cell response to *Mycobacterium avium* subsp. *paratuberculosis* by the blockade of immunoinhibitory receptors

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Paratuberculosis is a chronic enteritis of cattle caused by intracellular infection of *Mycobacterium avium* subsp. *paratuberculosis* (MAP). It is well known that Th1 response inhibits MAP proliferation in small intestine during the early subclinical stage. In the late subclinical and clinical stages, however, Th1 response is gradually exhausted. This T-cell exhaustion accelerates MAP proliferation and the progression of clinical disease, though the molecular mechanisms underlying T-cell exhaustion in paratuberculosis still remain unknown. Previous studies have shown that persistent antigen stimulation during chronic infection induces the upregulation of immunoinhibitory receptors such as PD-1 and LAG-3 on antigen-specific T cells and the exhaustion of T-cell effector functions through their inhibitory signals. Therefore, T-cell exhaustion is regarded as the common mechanism of immune evasion in chronic infections. In this study, we investigated the involvement of PD-1 and LAG-3 in the immune exhaustion of MAP-specific T cells in cattle infected with MAP.

Cattle were experimentally inoculated with MAP, and peripheral blood and gut-associated lymphoid tissues were collected at the late subclinical stage. Flow cytometric analysis revealed that the number of PD-1⁺CD8⁺ T cells was increased in ileal mesenteric lymph node of the infected cattle. In contrast, LAG-3 expression was upregulated on CD4⁺ and CD8⁺ T cells in peripheral blood of the infected cattle. Moreover, *in vitro* blockade of the LAG-3 pathway by a monoclonal antibody reactivated MAP-specific IFN-gamma response. Remarkably, LAG-3 blockade enhanced IFN-gamma production by MAP-specific CD4⁺ and CD8⁺ T cells. These results suggest that LAG-3 is dominantly involved in the immune exhaustion of MAP-specific T cells, and the reactivation of MAP-specific Th1 response by LAG-3 blockade could be a novel therapeutic strategy for the control of paratuberculosis in cattle.

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POSTER NUMBER: 2027

The Role of Prophylactic Treatment in Pregnant Sows and Piglets

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Veterinary pathogens have become a major problem. In swine, pathogenic diseases cause mild to chronic infections, abortions or death if untreated. The role of prophylactic treatment with multivitamins (daily), antiviral mix and antibacterial (single dose) for prevention and reduction of infections during pregnancy was evaluated in sows and their piglets.

Pigs were screened for absence of infections prior to prophylactic treatment. Bacteria and viral infection was detected at minimal levels in 5% of the flock, mostly in boars and treated accordingly. Sows on prophylaxis delivered healthy piglets whereas most untreated sows suffered abortion. Piglets from the treated sows were grouped into two: group I- given prophylactic treatment in the first week of life and repeated at week 3 and week 5, they survived with no sign of infection. Group II are those given no treatment, these piglets developed viral infections and died within 1 - 2 weeks of birth.

Prophylactic prevention of viral infections can reduce the incidence of morbidity and mortality in swine allowing for development of immune response to combat invading pathogens especially in young pigs with under-developed immunity. This will ultimately reduce zoonotic infections.

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POSTER NUMBER: 3001

Identification of peptides from foot-and-mouth disease virus structural proteins bound by bovine leukocyte antigen (BoLA) class I molecules

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Major histocompatibility complex (MHC) class I molecules are critical to immunity and regulate adaptive immune responses through the presentation of antigenic peptides to CD8⁺ T-cells. Polymorphisms in the peptide binding
region of class I molecules determine peptide binding affinity and stability during antigen presentation, and
different antigen peptide motifs are associated with specific genetic sequences of class I molecules. Understanding
bovine leukocyte antigen (BoLA; cattle MHC), class I allelic frequency and peptide-MHC binding specificity may
facilitate development of vaccines or reagents for quantifying the adaptive immune response towards intracellular
pathogens, such as foot and mouth disease virus (FMDV). Here, we describe the peptide-binding motif of four BoLA-I
peptide ligands using a combined approach of PSCPL derived position specific scoring matrix and neural network
based predictions (NetMHCpan). Predicted BoLA-I ligands were screened for pBoLA-I binding and the data was used
to update the NetMHCpan prediction server. The updated server was used to predict BoLA-I binding peptides within
FMDV structural protein P1 to BoLA-2*00801, BoLA-1*01901, BoLA-2*01201 and BoLA-4*02401. Peptide binders were
identified for all four molecules and for three of four molecules peptides were able to form stable pBoLA-I
complexes. In addition, the functional diversity of known BoLA alleles was predicted using the MHCcluster tool.
Ultimately, the data generated from this study will facilitate identification of MHC class-I restricted T-cell epitopes
from FMDV and provide insight into T-cell immunity following infection or vaccination.

POSTER NUMBER: 3002

Development and characterization of a monoclonal antibody specific for bovine CD209

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Dendritic cells (DC) play a central role in tailoring the immune response to pathogens. Effector activity is mediated
due to pattern recognition receptors (PRRs) that recognize pathogen associated molecular patterns (PAMPS).
C-type lectin receptors (CLR) comprise a group of PRRs that recognize a broad range of pathogens. CD209
(DCspecific ICAM3grabbing nonintegrin, DCSIGN) is a CLR expressed on DC that plays a critical role on DC function
and pathogen recognition. It facilitates DC migration to peripheral tissues and local lymph nodes and mediates T
Cell activation by binding ICAM-2 (CD102) and ICAM-3 (CD50). The absence of monoclonal antibody (mAb) to bovine
CD209 has limited the ability to characterize the phenotype and function of DC in cattle. To address this issue we
developed and used a mAb to CD209 to characterize the phenotype of CD209 expressing cells in bovine blood using
flow cytometry. Initial analysis has revealed the CD209 positive population in blood is comprised of multiple
phenotypically defined subsets.

The study was supported by the Department of Veterinary Microbiology/Pathology WSU Monoclonal Anti-body
Center.

POSTER NUMBER: 3003

Bm86 orthologs in Hyalomma marginatum as a vaccine candidate

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Ticks pose significant health problems to human and animals either by sucking blood or being a vector of many
parasitic, bacterial and viral diseases. Common preventive measures for ticks is to use acaracides but having disadvantages, the vaccination approach was introduced. Earlier vaccination studies carried out using the single antigen of Rhipicephalus (Boophilus) species were successful. The finding of the variation in vaccine efficacy among various tick species brought the necessity to reveal antigenic characters among them.

From the Hyalomma ticks collected from Turkey, gene encoding Bm86 ortholog was analyzed. The amplification of
partial gene allowed determining partial sequence of Bm86 ortholog that representing the portion of the full protein
after the removal of GPI anchor and signal peptide. This sequence was deposited to the GenBank (KF527438). The
sequence homology among the Hyalomma species was found to be between 90-99% but across the Rhipicephalus
(Boophilus) species was %62-63. In silico characterization of the protein was made. Partial Bm86 ortholog (Hm86)
sequence was cloned into E.coli and expressed protein was purified. Immunization of two rabbits with purified
antigen gave positive reaction in western blotting. The challenging of animals immunized with rHm86 antigen is planned to evaluate the efficacy of potential vaccine candidate.

POSTER NUMBER: 3004

The recombinant *Lactococcus lactis* oral vaccine induced protection against *Clostridium difficile* spore challenge in a mouse model

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The impact of *Clostridium difficile* infections (CDI) on healthcare is increasing. CDI represents a major cause of nosocomial antibiotic-associated diarrhea and colitis in animals and humans. It is important to develop effective vaccines for application in food producing animals and reduce contamination of animal products. Two potent cytotoxins, TcdA and TcdB are virulence factors of this pathogen and could be vaccine candidates. In the present study, we genetically engineered *Lactococcus lactis* to express non-toxic recombinant fragments derived from TcdA and TcdB C-terminal receptor binding domains (Tcd-AC and Tcd-BC) as an oral vaccine candidate. The immunogenicity of *L. lactis* oral vaccine delivery systems (LAC, LBC and the combination of both, LACBC) was compared with intramuscularly injected TcdA and TcdB C-terminal receptor binding domains (PAC, PBC and the combination of both, PACBC) expressed and purified from *E. coli*. After the *C. difficile* challenge, the control groups (unvaccinated) showed severe diarrhea symptoms and died at 2 and 3 days post-symptoms. All vaccinated groups (oral and intramuscular immunization) showed significantly reduced mortality rates, body weight loss and histopathologic lesions than the control groups (*p* < 0.05). Mice vaccinated with the recombinant protein Tcd-BC (group PBC) were totally protected in present experiment, however only 31% of mice vaccinated with *L. lactis* expressing Tcd-BC (group LBC) survived after challenge. Although, the survival rates after challenge reached 86% in groups PAC and PACBC, 75% in group LACBC and 65% in group LAC. After challenge, higher titers of IgG and sIgA were detected in vaccinated animals (*p* < 0.05). These results show the good potential of *L. lactis* as a delivery system to develop a cost-effective oral vaccine against CDI in animals.

POSTER NUMBER: 3005

Increased numbers of functional NK cells in pigs with Severe Combined Immune Deficiency (SCID) caused by natural mutations in the Artemis gene

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We have identified naturally occurring Severe Combined Immune Deficiency (SCID) disease in Iowa State University's inbred lines of Yorkshire pigs. SCID pigs lack B-cells, most T-cells (some gamma-delta T cells identified), but possess Natural Killer (NK) cells. This phenotype is caused by homozygosity or compound heterozygosity of two mutations in the Artemis gene. Interestingly, two human tumor cell lines, PANC-1 and A375-SM, survived after injection into SCID pigs (Basel et al. 2012). From this result, two important questions arose. Whether NK cells from SCID pigs can recognize human tumor cells, and whether these NK cells are being activated. NK cells are activated by interleukin (IL)-2 (produced by T-cells; may not be present in SCID pigs) or by IL-12 plus IL-18 (produced by macrophages and dendritic cells). We first determined whether normal porcine NK cells could recognize and kill human tumor cells. After activation with IL-2 *in vitro*, non-SCID NK cells (CD16+ SWC3a-) could kill PANC-1, A375-SM cells, and K562 cells (another human tumor line). We used the K562 cells to measure killing activity of NK cells isolated from non-SCID and SCID piglets at approximately 4, 11, 21, and 28 days of age. NK cells constituted an average of 40.2% of the peripheral blood mononuclear cell population in SCID pigs compared to 5.1% in non-SCIDs. Cells from both SCID and non-SCID pigs required activation with either IL-2 or IL-12/IL-18 to kill K562 cells *in vitro*. However, no significant differences between SCID and Non-SCID killing activity per NK cell were found. Thus, our current hypothesis is that the SCID NK cells are not being activated *in vivo* and suggests the SCID pig is a valuable genetic model for immunology and cancer.

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Porcine B cell Proliferation and Differentiation

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The B cell is the driving force behind the humoral immune response to infection. Terminal differentiation of B cells into immunoglobulin secreting plasma cells is crucial for the neutralization, opsonization, and complement fixation of many pathogens. CD21 enriched porcine B cells were activated in vitro with CD40L and then stimulated with various cytokines to screen for factors causing cellular proliferation and differentiation. IL-21 was a potent inducer of proliferation on activated B cells when compared to unstimulated cells and other purported stimulatory cytokines. Additionally, IL-21 induced the terminal differentiation of activated B cells to immunoglobulin secreting plasma cells. The addition of IL-4 and APRIL to CD40L and IL-21 treated B cells resulted in class switching to IgA and differentiation into IgA secreting plasma cells. These findings identify many essential components of the porcine humoral immune response that can be interrogated in vitro. Furthermore, these results set the stage for future delineation of anti-viral immune response in swine that will help advance the study of the porcine B cell roles in vaccinology, immunology, and immunotherapeutics.

Immunogenicity of an optimized synthetic consensus DNA vaccine for Porcine Epidemic Diarrhea Virus

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Background: Porcine epidemic diarrhea virus (PEDV), an Alphacorona virus, is highly infectious, causing acute diarrhea and dehydration in pigs. While adult pigs usually recover from infection, mortality rates in suckling piglets can reach 100%. PEDV has become of significant concern in several Asian countries and was first identified in the United States in 2013. Severe economic losses due to the more than 8000 confirmed cases in 31 states through September 2014 in the United States alone demonstrate the need for a safe and efficacious vaccine.

Methods: A synthetic DNA PEDV vaccine was generated using a consensus sequence of the PEDV spike protein (pPEDV-S). Female C57BL/6J mice were immunized three times at two week intervals with pPEDV-S by IM injection followed by electroporation. Splenocytes and sera were isolated two weeks after the last immunization to measure the cellular immune response by IFNγ ELISpot and the humoral immune response by ELISA.

Results: In comparison with the control pVax immunized group, pPEDV-S significantly increased the number of IFNγ producing cells and induced total IgG endpoint titers of 10^4 specific for the PEDV spike protein. Dominant T cell epitopes and dominant linear antibody binding domains within the PEDV spike protein were identified by epitope mapping with 15mer peptides overlapping by 11 amino acids.

Conclusions: pPEDV-S elicited both a strong T cell and antibody immune response in vaccinated mice. To our knowledge, this is the first consensus synthetic DNA based vaccine targeting PEDV to elicit both humoral and cellular responses. The use of a synthetic consensus sequence in this non live platform may improve the vaccine's efficacy across various circulating PEDV strains. Our data suggest that our novel pPEDV-S vaccine is a potential candidate for further study for possible vaccine development.

Marker Foot-and-Mouth Disease vaccine platform: A safe and potentially low cost option for global production of inactivated FMDV vaccines

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Chemically inactivated foot-and-mouth disease virus (FMDV) vaccines are effectively used to control FMD around the world. However, a major drawback in vaccine production is the fact that large quantities of infectious virulent FMD virus are necessary to produce vaccine antigen, with the associated risk of virus escape from manufacturing facilities or incomplete inactivation during the vaccine formulation process. A novel, antigenically marked FMDV-LL3B3D vaccine candidate developed by USDA ARS scientists, consists of a highly attenuated virus platform containing negative antigenic markers in the non-structural proteins 3Dpol and 3B. This vaccine platform allows for custom-design by virtue of the easy exchange of capsid coding sequences. In contrast to wild-type viruses, the recombinant FMDV-LL3B3D mutant virus induced no clinical signs of FMD and no shedding of virulent virus in cattle or pigs when inoculated as a live virus as part of the safety test for the platform. Cattle immunized with chemically inactivated FMD-LL3B3D vaccine were protected from challenge with parental virus. While this vaccine platform will use similar vaccine manufacturing technology as is currently used to produce FMDV vaccines worldwide, but with significantly lower biosafety risk. Two negative markers built into this vaccine, provide opportunities for the development of companion DIVA tests and allows for simplified downstream processing during manufacturing resulting in potential lower cost of goods. This platform, currently undergoing advanced development in the US, provides opportunities for safer and lower cost alternatives to current FMD vaccines in support of global control and eradication.

POSTER NUMBER: 3007

Use of Computational Bioinformatics to Improve the Humoral Immune Response Against PRRSV

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Infection with Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) causes one of the most economically important diseases of swine worldwide. PRRSV is a single-stranded positive sense RNA virus that belongs to the family Arterividae. It has one of the highest mutation rates known amongst all viruses, and is known to modulate host immune response by suppressing innate immunity and delaying the development of neutralizing antibody and T-cell responses. The goal of our research is to identify and improve upon neutralizing B-cell epitopes by using computational bioinformatics and structure modelling. To this end, different PRRSV strains were grouped into low (7), intermediate (3) and high pathogenic strains (11). ABCpred, BCpred and Bayes B bioinformatics programs were used to predict linear B-cell epitopes in GP3, GP4 and GP5 (structural glycoproteins of PRRSV) with settings of predicted overlapping peptide length of 20 amino acids (chose from 16-20), score threshold of 0.900 for BCpred and 0.55 for ABCpred and specificity of 75%. Non-overlapping epitopes with consensus of at-least two programs were selected. Peptides were synthesized accordingly and used to generate polyclonal sera in rabbits, which then were used against homologous and heterologous strains to identify epitopes with high neutralizing potential for further vaccine development.

The predicted epitopes in glycoproteins; for instance 39-70 in GP4 were then evaluated in structure prediction models to identify potential binding sites. For example, the porcine protein CD163 (UniProtKB entry Q2VL90), one of the two known receptors for PRRSV, is made up of 9 Scavenger Receptor Cysteine-Rich (SRCR) domains. SRCR domains 5 and 6 are believed to be vital for infection of porcine cells and thus represent important targets for neutralizing antibodies. Accordingly, the sequence spanning SRCR domains 5 and 6 in CD163 was submitted to the I-TASSER server for structure modelling. In addition, the PPiPP server was used to predict interacting residues between CD163 and its binding partner, GP4. PPiPP predicted high compatibility between the purported epitopic region of GP4 (residues 39-70) and residues of SRCR domains 5 and 6 that localize to the middle section of the CD163 structural model. Additionally, various tools were used to predict features of the GP4 protein. The purported epitope of GP4 was characterized by a high degree of predicted solubility and disorder, suggesting that it may be a disordered loop on the surface of GP4. Further, a sequence logo for this region of GP4 was generated by Weblogo, using complete GP4 sequences in UniProtKB. The analysis shows this loop to be hypervariable, which is consistent with the theory that neutralizing epitopes are under increased pressure to evolve.

POSTER NUMBER: 3012

Type VI Secretion System is involved in Mammary Pathogenic Escherichia coli virulence.
Mastitis, infection and inflammation of the mammary gland, is a well-known problem in the dairy industry, affecting cows worldwide and causing considerable financial losses. Multiple bacterial species have been identified as a causative agent in mastitis and *E. coli* is often involved. Despite many years of mastitis research, no efficient measures exist to prevent or treat the disease, and only little is known about specific virulence factors of the bacteria.

Our long-term goal is to understand the molecular mechanisms of host-pathogen interactions in the mammary gland and relate these mechanisms to disease processes and pathogenesis. The objective here was to identify and explore virulence factors of mammary pathogenic *Escherichia coli* (MPEC), in hope that understanding these mechanisms will lead to development of novel tools to combat *E. coli* mastitis.

Using genome sequencing and analysis of six MPEC strains, we found that type VI secretion system (T6SS) gene clusters are present in all six strains. Furthermore, using unbiased screening of MPEC strains for reduced colonization, fitness and virulence in our murine mastitis model, we have identified in MPEC P4-NR strain a new pathogenicity island encoding the core components of T6SS and its hallmark effectors Hcp, VgrG and Rhs. Next, we have shown that specific deletions of T6SS genes reduced colonization, fitness and virulence in lactating mouse mammary glands.

Based on our results we hypothesize that T6SS is an important virulence mechanism of MPEC. To our knowledge, we are the first to describe relevance of T6SS in the pathogenesis of mastitis. We intend to further validate our findings in lactating dairy cows and MPEC field strains and to study the molecular mechanisms of T6SS associated with MPEC virulence. The identified mechanism may provide new targets for diagnostic, preventive and therapeutic intervention.

This research was funded by BARD IS-4281-10R.

POSTER NUMBER: 3013

**Immune memory resilience, a new way of evaluating adjuvants**

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Adjuvants are traditionally assessed for their ability to enhance or modulate immune responses to a vaccine antigen, as measured by their capacity to induce strong primary immune responses, both cellular and humoral. More recently, the ability of adjuvants to induce immune memory responses that are long-lived and can effectively be boosted has also been assessed. Here we propose a novel way of evaluating adjuvants, based on their ability to induce immune memory responses that are resilient to manipulation by pathogens.

In most cases vaccines' effectiveness rely on the induction of immune memory responses, which are subsequently recalled during the early stages of infection. For many pathogens the recall of immune memory responses represent a real challenge to their survival resulting in significant evolutionary pressures on pathogens. As a result some pathogens have developed immuno-modulatory properties in an attempt to circumvent immune destruction. These mechanisms include manipulation of the recall response away from protective immunity. Hence there is a need to assess and optimize adjuvants for their ability to induce resilient immune memory responses, able to withstand such manipulation.

Using a combination of adjuvants and model antigens we have developed methods of measuring immune memory resilience using both a transgenic mouse model and a lymphatic cannulation model in sheep. The surprising results suggest that while it is possible to induce resilient immune responses in a transgenic mouse model by differentiating T cells with cytokines *in vitro*, a single injection of an adjuvanted vaccine *in vivo* in a sheep is insufficient to induce resilient immune memory in the local draining lymph node. Hence, induction of resilient immune memory responses is not trivial and should be a major consideration in designing novel vaccines and vaccination protocols.

POSTER NUMBER: 3014

**Chemokine CCL19 as an indicator of vaccine efficacy in nasal vaccination of rainbow trout (Oncorhynchus**
Chemokines are a large group of small proteins, which have fundamental immunological roles in migration, activation, and differentiation of leukocytes. The chemokine (C-C motif) ligand 19 (CCL19) is known to be crucial both for lymphoid cell trafficking and for the structural organization of lymphoid tissues in mammals. Recently, CCL19 has been found in teleost fish but its function remains enigmatic. Previous studies in our laboratory showed 100% protection in nasally vaccinated trout 7 days post immunization with a clear induction of CCL19 in the olfactory organ. The aim of this study is to evaluate CCL19 as a marker for vaccination success in rainbow trout using a live attenuated infectious hematopoietic necrosis virus (IHNV) vaccine. Rainbow trout were vaccinated nasally (I.N), by short immersion, long immersion or intramuscular (i.m) injection and olfactory organs sampled 1, 4 and 7 days following vaccination. CCL19 expression in the olfactory organ was examined by qPCR. Nasal vaccination with IHNV vaccine significantly increased the CCL19 expression levels in trout olfactory organ at all four sampling points with i.m injection showing a delayed and weaker response. The highest expression of CCL19 was observed in I.N and 2 hours long immersion 4 days post vaccination, with about 70 and 4 fold increase, respectively. Short immersion, known to elicit weak protection against IHNV, only induced a 2-fold induction of CCL19 expression at day 4. Our data suggest a critical role for CCL19 chemokine in trout nasal immunity. Therefore, CCL19 might be used as an indicator of vaccine efficacy in nasal vaccine trials.

POSTER NUMBER: 3015

Investigation of Immune Response through Flow Cytometry and Cytokines Expression on Going of Experimental Infection With Classical Swine Fever Virus (CSFV)

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Purpose: Classical swine fever (CSF) is a dreadful disease and is one of the greatest risks for the swine industry worldwide. The objective of this work was to investigated the mechanisms of interaction between CSFV and the immune system. We especially investigated and studied the immunological mechanisms associated with the immune response of vaccinated and unvaccinated pigs respect to a live attenuated vaccines. For this reason we used the FACS analysis results and the cytokine quantitative production.

Methods: Two group of pigs were involved for five weeks; fifteen days after vaccination both groups were infected with ISS 60 CSFV strain. At interval, blood samples (b.s.) were collected to detect antibody production, virus replication, cytokines expression. At the same time b.s. were collected to analyse the lymphocyte subpopulations CD3, CD21, CD4, and CD8 using a BD FACSCalibur.

Results: During the experiment we studied the immune response of porcine leukocytes populations in the acute infection of pigs vaccinated and unvaccinated caused by virulent strain of CSFV (ISS 60). Unvaccinated pigs showed initial followed by severe leukopenia that lead to death the animals. In the other group of vaccinated animal we observed an important protective immune response and the animals survive.

Conclusion: The "home made" live attenuated vaccine produced in our Institute (IZSUM) demonstrated safety and high protection also in case of infection with virulent CSFV strain. A full and completely knowledge of immune response to CSFV virus is important to better understanding the host defence mechanisms and it is essential to develop a new generation of CSFV vaccines.

This study was supported by a research grant from the Italian Ministry of Health (IZSUM 02/10 RC).
Immunization of cattle against buffalo-derived *Theileria parva* with a live sporozoite vaccine

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Sustainable increases in livestock productivity and maintenance of wildlife are essential to the economic and social development of rural Africa. Modern conservation efforts are aimed at integrative management of wildlife and livestock thus development of more effective control methods for wildlife-derived livestock diseases is required.

In Africa, a disease of major interest in this context is caused by infection with *Theileria parva*, an apicomplexan protozoan parasite which is transmitted by *Rhipicephalus appendiculatus*, the brown ear tick. *T. parva* infects both cattle and African buffalo (*Syncerus caffer*); infection of cattle with buffalo-derived parasites results in Corridor Disease (CD), characterized by low levels of parasitized leukocytes. *T. parva* parasites which circulate in buffalo appear to be more heterogeneous than those in cattle.

The Muguga Cocktail (MC) vaccine protects against experimental challenge with cattle-derived *T. parva* isolates and in the field where cattle predominate. The hypothesis of this study was that a functional difference in the buffalo-derived parasite will lead to failure of the MC. Groups of vaccinated and control calves were introduced into an area only grazed by buffalo and where *T. parva* challenge is known to occur and monitored.

The study solidly demonstrated that MC does not protect cattle against natural field challenge with buffalo-derived parasites on a ranch endemic with *Theileria*. Additionally, differences in disease manifestation were seen in the buffalo-derived parasite infection in comparison to cattle-derived parasite infection. The authors suggest this is due to a functional difference between the cattle- and buffalo-derived parasites.

A novel viral immune evasion mechanism suppresses the PI3-kinase signaling pathway by induction of expression of PTEN in B cells.

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Infectious agents have evolved a variety of mechanisms to evade the immune system in many different species. One such mechanism manifests as suppression of antibody responses during and following acute infection by viruses such as Cytomegalovirus, EBV, and Friend virus. Although this evasive mechanism has been recognized for more than 40 years, the underlying molecular mechanisms are unknown.

Here we report that during infection by three different viruses, murine gammaherpesvirus 68, mouse cytomegalovirus, and Friend virus, the ability of B cells in infected animals to transduce biochemical signals upon B cell receptor (BCR) crosslinking is altered. While certain receptor proximal events are intact, such as Syk phosphorylation, calcium mobilization is blunted and, in particular, phosphorylation of signaling proteins downstream of the PI3K pathway is reduced. Analysis of negative regulators of BCR signaling revealed that B cells from infected mice express increased PTEN. PTEN is an inositol phosphatase that inhibits the PI3K pathway. In support of a central role for PTEN upregulation in mediating the suppression of B cell responses during infection, B cells deficient in PTEN are not susceptible to infection-induced suppression. Furthermore, ectopic expression of constitutively active PI3K in B cells restores the ability to mount antibody responses in an infected environment. Finally, PTEN upregulation in the absence of infection mimics the effect of infection on the antibody response. Thus, PTEN upregulation during infection is both necessary and sufficient to mediate infection-induced suppression.

Together these results identify a novel mechanism by which B cells are suppressed during acute infections.
Secreted proteases of liver fluke: facilitators of parasitism and potential vaccine targets

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Parasites secrete proteases that act in several ways to facilitate parasitism. The liver fluke protease, *Fasciola* secretes cathepsin proteases of the B and L class. Various proteases are secreted at different stages of the life cycle in the definitive host, and have some common targets. Cathepsin B is predominately expressed in metacercariae and in the newly excysted juvenile stage, while different isoforms of cathepsin L are secreted throughout the life cycle.

We have identified a cathepsin L protease (catL5) that in adult fluke comprises 5-10% of the proteolytic activity in E/S. We have previously examined the fine specificity of this protease and established that it has an unusual specificity at the S2 subsite position, which is a key subsite for determining substrate specificity. CatL5 can efficiently accept aspartic acid in this position, which neither of the two major proteases in E/S (L1 and L2) can. Molecular modeling indicates that a glycine at amino acid 163 in catL5 may be crucial to this acceptance. The unusual specificity, coupled with the relatively small proportion of catL5 secreted, argues for a specific target.

In addition to proteases, there are other factors secreted into E/S which have been shown to modulate the host defence system. One of these, HDM, is secreted as a precursor, which needs to be cleaved prior to being active. Investigation of the cleavage site reveals that the amino acid in the S2 position is Asp, so it may be that this is a target of the catL5 protease. We have confirmed that this protease does indeed cleave the precursor.

Given that the secreted proteases perform vital functions for the parasite, they may be key drug and/or vaccine targets. We have previously shown in rats that a combination vaccine (cathepsin L5/cathepsin B) significantly reduced parasite numbers after challenge. Therefore, these proteases may be targets for generating protective host immunity.

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Complex synthetic minigene vaccines: An alternative approach to antigen discovery

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Development of vaccines against complex intracellular pathogens expressing thousands of proteins is complicated by the requirement to molecularly clone and screen large numbers of pathogen-derived candidate antigens. This bottleneck has dramatically limited the development of subunit vaccines for complex pathogens such as *Theileria parva*, *Coxiella burnetii*, *Anaplasma marginale* and others. To address this bottleneck, we have performed proof-of-principle experiments in mice testing a rapid synthetic “minigene” technology for highly parallel synthesis of multi-antigen DNA vaccines. We utilized microarray-based oligonucleotide synthesis technology and ligation-independent cloning to rapidly synthesize two DNA vaccines, each encoding the complete peptide compliment of 50 secreted or transmembrane *Plasmodium yoelii* proteins. Naïve balb/c mice were vaccinated three times with a single vaccine using biolistic particle delivery (gene gun) and screened for responses against vaccine encoded antigens. Vaccination alone induced responses against four novel antigens. Unvaccinated mice treated with a single dose of radiation attenuated sporozoites mounted a T cell response against only one of the four novel targets, while three targets could be primed by minigene vaccination and recalled with attenuated sporozoites. Protection studies with these antigens are ongoing. These data show that rapidly produced, complex experimental DNA vaccines are capable of identifying multiple subdominant T cell antigens. We believe that this approach represents a high-throughput system for discovery of vaccine subunits that cannot be identified by conventional screening of animals exposed to attenuated pathogens.

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POSTER NUMBER: 3020
Investigation of Humans, Horses and Mosquitoes for Exposure to West Nile Virus in Southwestern Nigeria

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Presence of West Nile virus (WNV) infection in humans, horses and mosquitoes in southwestern Nigeria is uncertain. Seventy nine consenting participants, who visited two health facilities in Ibadan, Nigeria between June, 2011 and October, 2012 were sampled. Blood smears and serum from each patient were subjected to malaria parasite (MP) microscopy, Widal test (WI) and total anti-WNV antibody (Ab) ELISA. In addition, 145 horse sera were tested using cELISA and IgM-ELISA; a subset of these was tested using PRNT50. A total of 150 mosquito pools (1,390 females) collected with BioGent and CDC light traps from horse stables were studied using real time RT-PCR with WNV-specific primers and probes. All tests were performed at AHVLA, UK. Humans (mean age: 32.7 years [95% CI: 29.6-35.8 yrs]) recorded 24.1% [95% CI: 14.7-33.5%], 40.5% [95% CI: 29.7-51.3%] and 79.7% [95% CI: 70.8-88.6%] positivity rates for MP, WI and WNV Ab tests respectively while 91.1% [95% CI: 84.8-97.4%] tested positive for at least one pathogen. Age was associated (P=0.001 and 0.009) with WNV Ab positivity which increased with age. The 6 (7.6% [95% CI: 1.8-13.4%]) participants positive only for WNV Ab were considered patients with “fever of unknown origin”. In horses, high rates of anti-WNV Ab prevalence were observed in all locations with a mean level of 90.3% [95% CI: 85.5-95.1%]. None of the horses had detectable anti-WNV IgM. It was observed that 50.0% of tested sera were positive for anti-WNV neutralizing Ab (PRNT50 titre of 1:10 to 1:320). The sera that were negative by PRNT50 but positive by cELISA possibly contained non-neutralizing Ab. Humans and horses in the study areas had clear evidence of exposure to WNV with high prevalence rates and protective (neutralizing) Ab in horses. However, there was no evidence of WNV RNA in all the mosquitoes tested. The high WNV Ab prevalence in humans suggests inclusion of WNV infection among the differential diagnoses of febrile illnesses in southwestern Nigeria while high prevalence among horses could explain the absence of reports of WNV-outbreak/encephalitis in this species.

POSTER NUMBER: 3021

The role of a growth factor like protein from Fasciola hepatica in host immunomodulation

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Fasciola hepatica is a major trematode parasite throughout the globe causing massive economic losses and resulting in welfare issues for infected animals. Triclabendazole is the current drug of choice but there is pressure on its continued use as increasing drug resistance is reducing its efficacy. The most sustainable route for parasite control is through vaccination, however key to this is an understanding of the complex mechanisms F. hepatica uses to control its hosts immune responses.

We have identified and cloned a novel growth factor, which we termed F. hepatica Transforming Like Molecule - FhTLM. FhTLM displays a restricted expression pattern across the parasite lifecycle which may underlie a dual use by the parasite. Stimulation of bovine whole blood and PBMCs indicates that FhTLM is a strong inducer of IL-10. Furthermore, we believe it be important in the modulation of lymphocyte proliferation and IFN-gamma production. FhTLM is able to bind to and initiation signal transduction as judged by a luciferase reporter system transfected into host cells. Further investigation of this signalling pathway will help to inform our use of FhTLM as vaccine candidate.

POSTER NUMBER: 3022

Immune responses to a candidate universal influenza vaccine in pigs

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Influenza is a major health concern both in humans and livestock. Swine influenza is one of the most important primary swine respiratory pathogens and in addition a zoonotic threat. The major obstacle in combating influenza is the rapid evolution of the virus. Existing inactivated vaccines against swine influenza are strain specific and do not protect against new strains. Therefore, a cross-protective “universal vaccine” would be an enormous advantage to prevent influenza virus infection in pigs and to reduce the zoonotic threat.

S-Flu is an experimental pseudotyped influenza virus, based on the suppression of haemagglutinin, so that S-Flu can infect cells and express the viral core proteins, but cannot replicate (Powell T et al, J Virol 2012). A high degree of cross protection, comparable to Flumist, has been demonstrated in mice and ferrets immunised with S-Flu against H1, H3, H5, H6 and H7 Flu strains.

We evaluated immune responses in Babraham pigs to the S-Flu vaccine, administered intra-nasally, by aerosol or intra-tracheally. We measured local and systemic T cell responses using proliferation, intra-cytoplasmic staining and IFN gamma ELISPOTs. Flu specific antibody responses were analysed in BAL fluid and blood. The most efficient route for inducing T cell, Ab responses and optimal protection by S-Flu in pigs will be discussed.

**Staphylococcus aureus** IsdA and ClfA - cholera toxin A2/B fusions as mucosal mastitis vaccines

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Mastitis is a common and costly bacterial infection in the udder that affects dairy cattle worldwide. Infection with *Staphylococcus aureus* can spread through herds and cause chronic mastitis that is difficult or impossible to treat. An effective *S. aureus* vaccine would improve animal health and reduce agricultural dependence on antibiotics. *S. aureus* matrix binding proteins, such as the iron-regulated surface determinant A (IsdA) and clumping factor A (ClfA), are conserved adhesins that are promising vaccine candidates. We have previously reported the construction of an IsdA-cholera toxin A2/B fusion (IsdA-CTA2/B) and the ability of this vaccine to stimulate antigen-specific responses after mucosal delivery in mice. Here we report the construction of ClfA-CTA2/B and further characterization these molecules as bovine vaccines. A small immunogenicity trial was performed using 150 g of purified IsdA-CTA2/B or IsdA alone and delivered to cows intranasally. Humoral responses to IsdA were assessed by ELISA on serum, milk, and nasal wash. ClfA-CTA2/B was constructed and purified from the *E. coli* periplasm, and a larger scale immunogenicity trial using intranasal IsdA-CTA2/B + ClfA-CTA2/B at dry off is currently underway. To further evaluate IsdA as a vaccine candidate, we also analyzed the expression and sequence of IsdA from over 100 clinical samples from two local Idaho dairies. Blood and milk samples were collected and assayed by ELISA for anti-IsdA responses. *S. aureus* from these samples was tested by PCR for the presence or absence of isdA and clfA, and the variable region of isdA was sequenced. Our results support the conclusion that CTA2/B fusions can stimulate antigen-specific responses in cows after intranasal delivery. In addition, isdA is conserved in bovine *S. aureus* and *S. hemolyticus*, and is expressed during clinical infection. These studies support the continued exploration of mucosal vaccines containing IsdA and ClfA in a multivalent bovine *S. aureus* vaccine.

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**Inability of Severe Combined Immune Deficient (SCID) pigs to control IAV replication despite innate immune activation**

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We previously identified a line of SCID pigs that lack B and T cells. This line may be a useful model for testing influenza A virus (IAV) vaccines following adoptive transfer of specific immune cells. To develop this model, we
investigated whether innate immunity can control IAV infection in the absence of antibody production, T cell killing of infected cells, or T cell regulation.

Litter matched SCID and normal piglets were intranasally inoculated with IAV or PBS, with 5-8 animals per treatment combination. Rectal temperatures were similar for all animals across the seven-day experiment. Viral titers in nasal secretions (NS) were similar between SCIDs and controls on 1, 3 and 5 days post-infection (dpi), but at 7 dpi SCID pigs had 2 logs higher virus in NS and 3 logs higher virus in bronchial-alveolar lavage fluid (BALF), whereas normal pigs had begun clearing IAV. The 7 dpi BALF titers were supported by IAV antigen detection by immunohistochemistry in the SCID challenged pigs. Conversely, IAV-infected SCID pigs had significantly less microscopic pathologic changes in the lung. Interferon-alpha, interferon-gamma, IL-1beta and IL-6 in the BALF were elevated in IAV-SCID pigs, indicating a response by the innate immune system in SCID pigs.

The lack of T and B cells in SCID pigs resulted in the inability to control viral replication, indicating that IAV clearance requires support from the adaptive immune system. Further study is required to determine if the SCID pigs can eventually clear IAV infection, albeit at a slower rate than controls.

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**POSTER NUMBER: 3026**

**Bioinformatics prediction of swine MHC class I epitopes from Porcine Reproductive and Respiratory Syndrome Virus**

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Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) causes one of the most important diseases in all swine producing countries. The infection has a high impact on animal welfare, food safety and production economics.

PRRSV possesses multiple immunoevasive strategies, from suppression of the host cell antiviral machinery, to the deceptive induction of a non-neutralizing antibody response through decoy antigen presentation. This, combined with a very high mutation rate, has hampered the development of safe and effective vaccines.

With the overall aim to design a vaccine that induces an effective CTL response against PRRSV, we have taken a bioinformatics approach to identify common PRRSV epitopes predicted to react broadly with predominant swine MHC (SLA) alleles. First, the genomic integrity and sequencing method was examined for 334 available complete PRRSV type 2 genomes leaving 104 strains of high quality. For each strain, a library of all possible 9- and 10-mer peptides was generated considering the known ribosomal frame shift sites and sites for post translational cleavage.

All peptides were in silico analyzed for binding affinity to either of five common SLA class I alleles. A quantitative rank score was generated for each peptide by combining two algorithms based on the NetMHCpan neural network and lab determined SLA binding affinity of each amino acid at any position in the peptide, respectively.

Peptides with a rank score above a predefined threshold were further analyzed by the PopCover algorithm, providing a final list of 54 epitopes prioritized according to maximum coverage of PRRSV strains and SLA alleles.

This bioinformatics approach provides a rational strategy for selecting peptides for a CTL-activating vaccine with broad coverage of both virus and swine diversity. The immunogenicity of the selected peptides is in the process of being verified in vivo.

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**POSTER NUMBER: 3027**

**Differences in inflammasome activation between cow breeds impact on bacterial killing**

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There is strong evidence that high yielding dairy cows are extremely susceptible to infectious disease, and that this has severe economic consequences for the industry and welfare implications for the cows. Furthermore, there is a genetic component to the innate immune response which is an important determinant in how severely individual cows are affected. Control of infections by existing management and veterinary interventions is difficult. An alternative approach is to use recent advances in genomics to identify differences in genes associated with the key pathways which need to be activated to mount an effective innate immune response. Here, we present data that the ability of innate immune cells derived from different cow breeds to mount an appropriate innate immune response to bacterial infections is severely impacted on by difference in inflammasome activation. Indeed, whereas in innate immune cells derived from some breeds, a direct bacterial killing mechanism with ROS and RNS dominated, the same cells derived from other breed mainly produced a pro-inflammatory response. This response pattern could be shifted by blocking NO/ROS production by either blocking iNOS function or activating the inflammasome and thus caspase-1 activity. Our data may provide some easy assays to link innate immune system phenotypes with host genotypes by identifying host correlates of a protective innate immune response. This could potentially aid the identification of in vitro predictors of an appropriate innate immune response that could help to facilitate the decision making process to identify candidate genes for breeding to increase natural resistance.

POSTER NUMBER: 3010

An Immunological Profile of 79 Beef Calves at 1 to 4 Days of Age

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Few available data describe immunity in large populations of conventionally reared neonatal beef calves; this leads to assumptions about neonatal bovine immunity that may be incorrect. As part of a study evaluating the response of calves to different prime-boost vaccination strategies, blood was collected from 79 crossbred beef calves at 1 to 4 days of age for assessment of humoral and cell mediated immune responses. This offered a multi-parameter assessment of the neonatal immune response. Total serum IgG and total nasal secretion IgA was measured by ELISA. Peripheral blood mononuclear cells (PBMC) were exposed to Con A or staph enterotoxin B (SEB), and proliferative responses were measured by H3-thymidine uptake; expression of CD25 by CD4, CD8, and γδ T cells was measured by flow cytometry. Secretion of interferon gamma (IFN-γ) by PBMC exposed to Con A was measured by ELISA. Mean (+/- SD) birth weight of calves was 88 +/- 1.1 lb (40 +/- 0.5 kg). Mean (range) serum IgG was 8.81 (3.2 - 19.0) g/dl; mean (+/- SD) nasal IgA was 316 +/- 17 ng/ml. H3-thymidine uptake by PBMC was strongly stimulated by SEB (4550 +/- 2937 cpm), and less strongly stimulated by Con A (900 +/- 1398 cpm), as compared to unstimulated PBMC (64 +/- 43 cpm). Con A stimulated PBMC secreted IFN-γ (24 +/- 125 ng/ml), whereas IFN-γ was not measureable in supernatants of unstimulated PBMC. Flow cytometry revealed that unstimulated PBMC contained a small fraction of blast cells (about 7%) and few CD25+ cells (less than 5% above background). In contrast, PBMC exposed to SEB had about 30% blast cells and almost 50% of the blast cells expressed CD25. Most of the observed blast cells were WC-1+. Neonatal calf PBMC mounted strong responses to stimulation, but the responses were variable among calves.

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